

IMPROVING FOOD SAFETY THROUGH A ONE HEALTH APPROACH

WORKSHOP SUMMARY

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and Alison Mack, *Rapporteurs*

Forum on Microbial Threats

Board on Global Health

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this report:

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The Forum on Emerging Infections was created by the Institute of Medicine (IOM) in 1996 in response to a request from the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). The purpose of the Forum is to provide structured opportunities for leaders from government, academia, and industry to regularly meet and examine issues of shared concern regarding research, prevention, detection, and management of emerging, reemerging, and novel infectious diseases in humans, plants, and animals. In pursuing this task, the Forum provides a venue to foster the exchange of information and ideas, identify areas in need of greater attention, clarify policy issues by enhancing knowledge and identifying points of agreement, and inform decision makers about science and policy issues. The Forum seeks to illuminate issues rather than resolve them. For this reason, it does not provide advice or recommendations on any specific policy initiative pending before any agency or organization. Its value derives instead from the diversity of its membership and from the contributions that individual members make throughout the activities of the Forum. In September 2003, the Forum changed its name to the Forum on Microbial Threats.

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Workshop Overview¹

*“Between animal and human medicine there is
no dividing line nor should there be.
The object is different but the experience obtained
constitutes the basis of all medicine.”*
—Rudolf Virchow (1958)

IMPROVING FOOD SAFETY THROUGH A ONE HEALTH APPROACH

The daily activity of producing, preparing, and consuming food directly links our health with the health of the planet in both direct and indirect ways. Over the past century, the distance between “farm” and “fork” has gone global such that the ingredients in a single meal may be obtained from numerous “local” and “global” sources. Food production and distribution for the developed world takes place across vast and complex global networks in increasingly shorter timescales. As consumers, many of us fail to recognize that our local and domestic food supplies are part of an increasingly interconnected, globalized, food production system.

The U.S. food supply comprises thousands of types of foods and food components—many grown and processed outside of the borders of the United States—as illustrated in Figure WO-1, “the well-traveled salad.” The well-traveled salad’s 10 ingredients originate in more than 37 countries. The increasingly global nature of both domestic and local food supplies underscores the need for a comprehensive One Health approach to food safety, as even common and “whole” ingredients may travel across the world before they reach the table. The

¹ The planning committee’s role was limited to planning the workshop. The workshop summary has been prepared by the workshop rapporteurs (with the assistance of Katherine McClure, LeighAnne Olsen, Rebekah Hutton, and Pamela Bertelson of the staff of the IOM’s Forum on Microbial Threats) as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants and are not necessarily endorsed or verified by the Forum or the Institute of Medicine. They should not be construed as reflecting any group consensus.



FIGURE WO-1 The well-traveled salad. To view an interactive version of this infographic on your computer or to download the static version of this image, visit <http://iom.edu/Activities/PublicHealth/MicrobialThreats/2011-DEC-13.aspx>. To use your smartphone to link directly to the interactive version use the QR code.

health of humans, animals, and crops plays a pivotal role in ensuring the safety of the world's food supply.

Globalization of the food supply has created conditions favorable for emergence, reemergence, and spread of food-borne pathogens and has compounded the challenge of anticipating, detecting, and effectively responding to food-borne threats to health. In the United States alone, food-borne agents cause approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths each year (Scallan et al., 2011b). This figure likely represents just the tip of the iceberg, because it fails to account for the broad array of food-borne infections that run the gamut from asymptomatic to serious disease with complications such as renal failure and death² or for the wide-ranging repercussions they can have for consumers, government, and the food industry—both domestically and internationally.

Most food-borne illnesses are preventable. The interconnectedness of individual, regional, and global public health; the health of the planetary environment(s); and billions of food animals and wildlife would suggest the need for a new paradigm—one that shifts away from a reactive to a more anticipatory, proactive approach to food safety. Such a prime example might be captured in a “One Health” approach to food safety—which has been defined as “the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals and the environment” (AVMA, 2008).³ Were such an approach to be implemented for food safety, it may hold the promise of harnessing and integrating the expertise and resources from across the spectrum of multiple health domains including the human and veterinary medical, and plant pathology, communities with those of the wildlife and aquatic health and ecology communities.

Statement of Task

Such transdisciplinary synergies could reveal important insights into sources, reservoirs, and factors underlying emergence of infectious diseases; trace and disrupt pathways that lead to food contamination; and contribute to creating systems needed to anticipate and prevent adverse health impacts associated with emergence and spread of novel, emerging, or reemerging food-borne diseases. On December 13 and 14, 2011, the Institute of Medicine's (IOM's) Forum on Microbial Threats hosted a public workshop that examined the potential of a “One Health” approach to improve the safety of the food supply domestically and

² For the purposes of this workshop summary report, food-borne illness refers to a broad group of illnesses that are caused by the consumption of food contaminated with viruses, bacteria, or parasites that are pathogenic in susceptible human hosts (Tauxe et al., 2010). Food-borne illness is also referred to as food-borne disease, food-borne infection, or food poisoning.

³ There are many, many definitions for “One Health.” The definition from the American Veterinary Medical Association (AVMA) is being used for convenience.

globally. Through invited presentations and discussions, workshop participants explored existing knowledge and unanswered questions on the nature and extent of food-borne threats to health, and considered the structure of food systems, the spectrum of food-borne threats, and the particulars of illustrative case studies. Participants also reviewed existing research, policies, and practices to prevent and mitigate food-borne threats and identified opportunities to implement and strengthen practices informed by One Health throughout the global food system.

Organization of the Workshop Summary

This workshop summary was prepared by the rapporteurs for the Forum’s members and includes a collection of individually authored papers and commentary. Sections of the workshop summary not specifically attributed to an individual reflect the views of the rapporteurs and not those of the members of the Forum on Microbial Threats, its sponsors, or the IOM. The contents of the unattributed sections of this summary report provide a context for the reader to appreciate the presentations and discussions that occurred over the 2 days of this workshop.

The summary is organized into sections as a topic-by-topic description of the presentations and discussions that took place at the workshop. Its purpose is to present information from relevant experience, to delineate a range of pivotal issues and their respective challenges, and to offer differing perspectives on the topic as discussed and described by the workshop participants. Manuscripts and reprinted articles submitted by some but not all of the workshop’s participants may be found, in alphabetical order, in Appendix A.

Although this workshop summary provides a description of the individual presentations, it also reflects an important aspect of the Forum’s philosophy. The workshop functions as a dialogue among representatives from different sectors and allows them to present *their* views about which areas, in their opinion, merit further study. This report only summarizes the statements of participants at the workshop over the course of 2 consecutive days. This workshop summary is not intended to be an exhaustive exploration of the subject matter nor does it represent the findings, conclusions, or recommendations of a consensus committee process.

Recent Food-Borne Outbreaks: The Changing Nature of the “Threat”

Recent, well-publicized, national and international outbreaks⁴—discussed in greater detail in Box WO-3, “The Changing Nature of the Threat” (found on pages 36-43)—of food-borne illnesses and death illustrate their far-reaching

⁴ In public health practice, a food-borne disease outbreak is defined as the occurrence of two or more cases of similar illness resulting from the ingestion of a common food (CDC, 2012).

public health and economic consequences. Today, the ecological context of food encompasses the planet, as food commodities are traded across the globe and the ingredients in a single meal may be obtained from hundreds of sources in dozens of countries. Multistate and multicountry outbreaks of food-borne morbidity and mortality linked to *Listeria* in cantaloupe; *Salmonella* spp. in eggs, ground turkey, and ground beef; and *Escherichia coli* in bean sprouts are but some of the most recent examples of a growing threat to health, trade, and local economies.

Listeria Contamination of Cantaloupe

One of the largest and deadliest multistate outbreaks of listeriosis in the United States occurred in late summer of 2011. The incident marked the first time that *Listeria* contamination had been linked to whole cantaloupe and one of the few times it had been linked to fresh produce (Figure WO-3-3) (CDC, 2011g). As of November 1, 139 individuals⁵ had become ill after being infected with the outbreak strain of *Listeria*; 29 deaths and 1 miscarriage had also been attributed to infection (CDC, 2011f). In response to the Centers for Disease Control and Prevention (CDC) outbreak investigation, the cantaloupe producer, Jensen Farms of Holly, Colorado, announced a voluntary recall of the 300,000 cases of cantaloupes produced between July 29 and September 10 (CDC, 2011f; FDA, 2011c). The recall included 1.5 to 4.5 million melons that were distributed at supermarkets and chain stores in at least 28 states.

Salmonella Enteritidis Contamination of Chicken Eggs

In late 2010, an outbreak of *Salmonella* Enteritidis infections led to the recall of more than half a billion shell eggs (CDC, 2010). More than 1,900 people in 11 states became ill, and epidemiological investigations traced the source of the outbreak to eggs supplied by two Iowa egg farms: Wright County Egg and Hillandale Farms. Environmental samples confirmed the presence of the outbreak strain on both farms. A contaminated feed mill provided a connection between these two farms, as Wright County Egg used finished feed from this mill to raise the flocks of egg-laying hens that populated all of the Wright County Egg and Hillandale Farms facilities in Iowa (FDA, 2010a). In August 2010, Wright County Egg and Hillandale Farms conducted nationwide voluntary recalls of shell eggs. Recalled eggs had been packaged under a dozen different brand names and distributed to grocery distribution centers, retail grocery stores, and food-service companies located in 22 states and in Mexico (FDA, 2010a). *Salmonella* Enteritidis contamination is not limited to large, industrial-scale, egg producers. In October 2011, an outbreak of *Salmonella* Enteritidis in Minnesota was traced to eggs produced by the Larry Schultz Organic Farm in Owatonna. These eggs

⁵ The mean age of all people infected was 78.

were subsequently distributed to restaurants, grocery stores, food wholesalers, and co-ops in Minnesota, Wisconsin, and Michigan and sickened at least six individuals (Food Safety News, 2011).

Salmonella Heidelberg in Ground Turkey

Between March and September 2011, at least 136 persons from 34 states were infected with the outbreak strain of *Salmonella* Heidelberg (USDA, 2011a). On July 29, the U.S. Department of Agriculture's (USDA's) Food Safety and Inspection Service (FSIS) issued a public health alert about the potential association of these illnesses with the consumption of ground turkey (USDA, 2011a). The outbreak strain of *Salmonella* Heidelberg is resistant to several commonly prescribed antibiotics, such as ciprofloxacin, ceftriaxone, and trimethoprim-sulfamethoxazole. This antibiotic resistance may be associated with an increase in the risk of hospitalization or possible treatment failure in infected individuals (CDC, 2011b). Ill persons range in age from less than 1 year to 90 years old, with a median age of 23 years (CDC, 2011b).

Epidemiological and traceback investigations, as well as in-plant findings, determined a link between disease outbreak and ground turkey products produced by the Springdale Arkansas establishment of Cargill Meat Solutions (USDA, 2011a). On August 3, 2011, Cargill recalled approximately 36 million pounds of fresh and frozen ground turkey products (CDC, 2011b). In addition to the recall, Cargill addressed conditions in the processing facility. The plant where the turkey was processed was completely disassembled, steam-cleaned, treated with an antibacterial wash, and equipped with the most current monitoring and sampling system. Unfortunately, less than a month later, another 185,000 pounds of turkey—produced at the same factory—was recalled with the same strain of *Salmonella* (CDC, 2011b).

E. coli O104:H4 Contamination of Fenugreek Seeds

Outbreaks of food-borne diseases increasingly span multiple states and countries, and recall efforts can shut down global markets of entire product lines. The outbreak of a rare strain of *E. coli* O104:H4, first identified in northern Germany in May 2011, resulted in 4,321 outbreak cases, including 3,469 cases of Shiga toxin-producing *E. coli* and 852 cases of hemolytic-uremic syndrome (HUS), and 53 deaths had been reported in 14 European countries, the United States, and Canada⁶ when the epidemic was declared to be over at the end of July 2011

⁶ The majority of illnesses associated with this outbreak were reported in Germany and France. Cases were also reported in Austria, Canada, the Czech Republic, Denmark, Greece, Luxembourg, the Netherlands, Norway, Poland, Spain, Sweden, Switzerland, the United Kingdom, and the United States. Cases outside of Germany and France are suspected to be travel-related or incidences of secondary spread of infection by those who had recently travelled to the affected area in Germany.

(Buchholz et al., 2011; Burger, 2011; Robert Koch Institute, 2001; WHO, 2011). Confusion over the source of the outbreak caused economic losses and political frictions that transcended national boundaries and continue to this day. The European Union approved U.S.\$287 million in emergency aid for European vegetable farmers affected by the crisis—a sum estimated to be a mere fraction of actual losses (Marucheck et al., 2011).

THE GLOBAL FOOD SYSTEM

Globalization of the food supply has served to expand the range of food-borne pathogens as well as to amplify health and economic impacts of a single contamination incident. Production, processing, and distribution of food increasingly takes place across vast and complex networks—each part or pathway of which must be working optimally—without the introduction of contaminants and/or adulterants that could taint the final product(s).

The U.S. food supply is composed of thousands of types of foods,⁷ much of it grown and processed elsewhere (Figure WO-2). The increased distance between the sources of production and consumption is a global phenomenon; more than two-thirds of countries are now net importers of food (Buzby et al., 2008). In 2010, the United States imported an estimated 10 to 15 percent of all food consumed by U.S. households, including more than three-quarters of the fresh fruits and vegetables and more than 80 percent of fresh or frozen fish and seafood (FDA, 2011a). Upon arrival, these products—along with domestically produced foodstuffs—are typically distributed across the country from central facilities. The meat prepared and consumed at a typical American table, for example, has traveled 1,000 miles from its farm (or farms) of origin (Chalk, 2004).

Innovations such as refrigeration, transportation (air, sea, and land), and instantaneous communication support food distribution systems that can rapidly transport perishable goods, provide just-in-time restocking of non-perishable items, and take advantage of economies of scale (ERS, 2001; FDA, 2011a). These innovations have also linked U.S. food safety concerns to conditions in the more than 200 countries and territories from which the United States imports food (IOM, 2010b). An estimated 200,000 overseas facilities are registered with the Food and Drug Administration (FDA) to sell foods to the United States, and there are likely substantial variations in the sanitation and hygiene practices at these facilities (Taylor, 2009). Screening processes at the more than 300 U.S. ports of entry identify and reject contaminated or damaged goods; yet, just barely 1 percent of all foods imported into the United States are subjected to border inspections (CRS, 2009). This reality along with the complexity of food distribution

⁷ According to the Food Marketing Institute, the average number of items stocked by U.S. grocery stores is approximately 39,000 (FMI, 2010). In the 1950s, U.S. grocery stores stocked an average of 300 food items (Dupont, 2007).

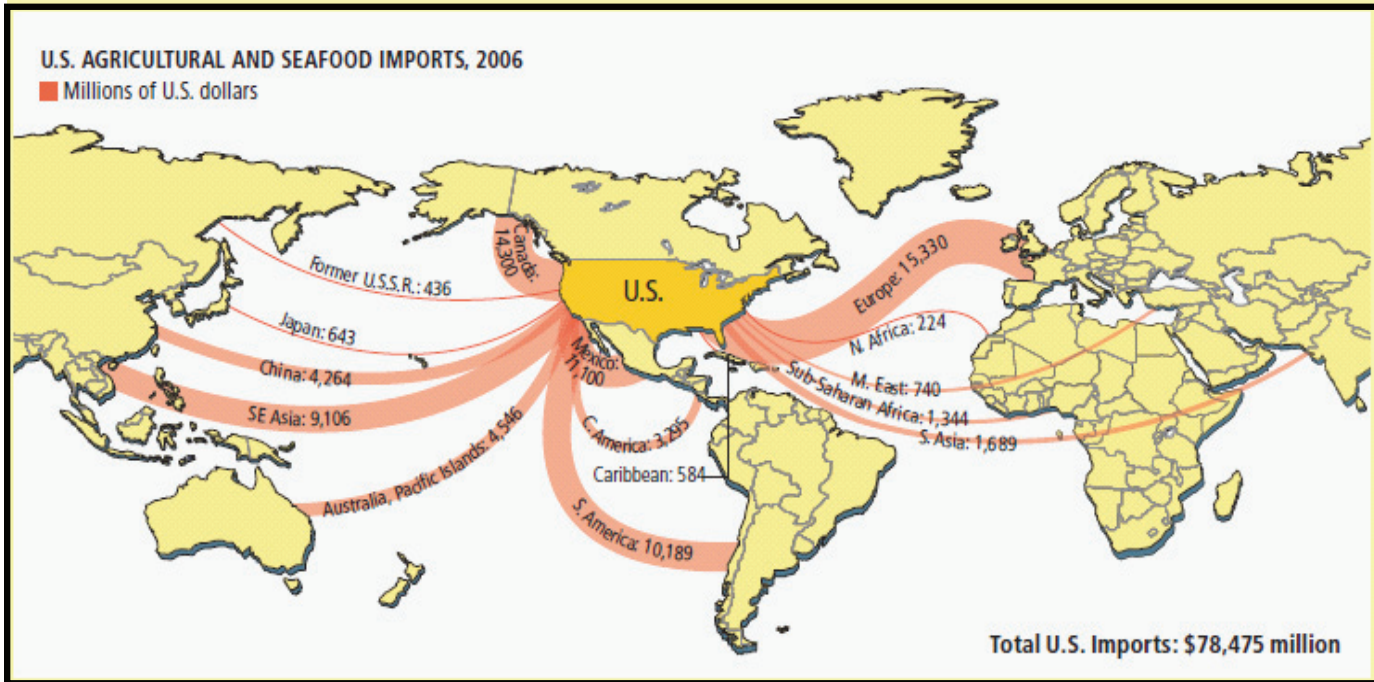


FIGURE WO-2 U.S. agricultural and seafood imports (millions of U.S. dollars).
SOURCE: George Retseck and Lucy Reading-Ikkanda for *Scientific American* magazine in Fischetti (2007).

makes food extremely vulnerable not just for inadvertent microbial and chemical contamination but also for potential intentional or bioterrorist activities.

Emerging Food-Borne Diseases and the One Health Paradigm

The workshop opened with a keynote presentation by two speakers, Lonnie King of The Ohio State University (Dr. King's contribution to the workshop summary report may be found in Appendix A, pages 218-225.) and Peter Daszak of EcoHealth Alliance (Dr. Daszak's contribution to the workshop summary report may be found in Appendix A, pages 130-140.). They discussed the convergence of factors leading to the global emergence of food-borne diseases and defined the principles of One Health, which they characterized as a paradigm for addressing the complex problem posed by these conditions and diseases.

King, referring to the Forum on Microbial Threat's longstanding "convergence model" of factors influencing infectious disease emergence (IOM, 2003), characterized the spectrum of global threats to food safety and why diseases emerge (illustrated in Figure WO-3) as a "perfect microbial storm."

King went on to discuss the many factors that influence the complex interactions among host, pathogen, and environment that can lead to the emergence or reemergence of infectious diseases (IOM, 1992, 2003; and illustrated in Figure WO-3). Several environmental factors are of particular relevance in driving emergence and spread of food-borne pathogens, including, but not limited to the following:

- *Intensive agricultural practices.* In the drive for efficient production, practices such as raising and transporting large livestock herds, flocks of birds, or schools of fish or shellfish in close quarters create ideal conditions for disease emergence and spread (King, 2004).
- *Increased interactions between humans, domestic animals, and wildlife.* Often caused by habitat destruction, changing land-use patterns, and hunting of animals for food or for the food trade, increased contact between humans, animals, and their associated microbes also increases the potential for pathogen transmission between animal species or between humans and animals (Pike et al., 2010).
- *Environmental "commons" such as water.* Contamination of common resources distributes and increases both the risk of pathogen emergence and chemical contaminants and can be spread across different farms, regions, states, and nations.

As previously discussed, approximately 48 million cases of food-borne illness occur annually in the United States—1 for every 6 residents (CDC, 2011a; Scallan et al., 2011a). Extrapolating that figure to a global scale, King estimated that at least 1 billion cases of food-borne disease arise annually—a largely silent

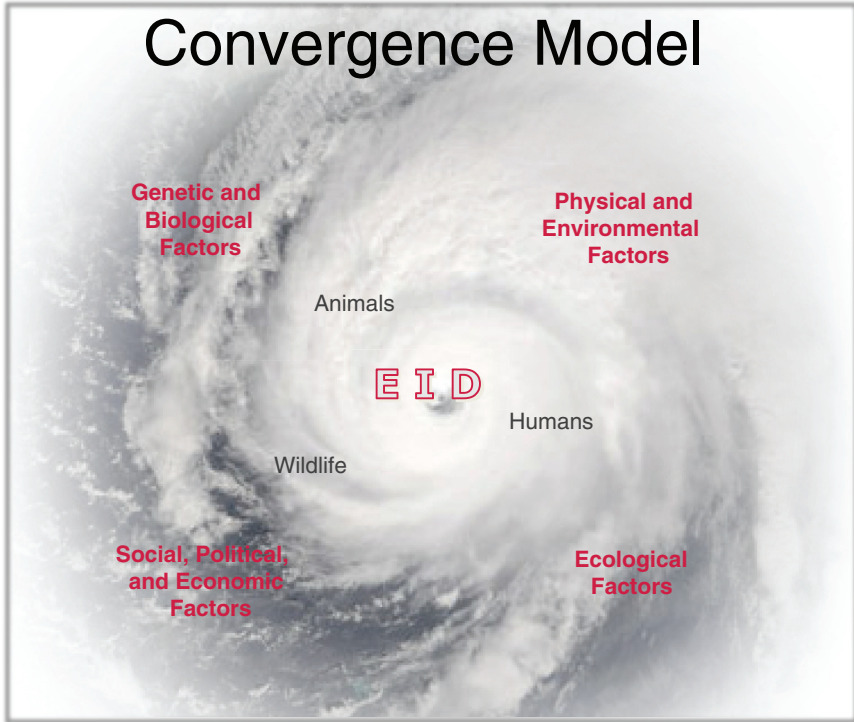


FIGURE WO-3 The convergence model.

SOURCE: King (2011).

“raging epidemic.” Moreover, as Daszak observed, significant emerging viral diseases such as HIV/AIDS⁸ and severe acute respiratory syndrome (SARS)⁹ should be characterized as food-borne pathogens, in view of the fact that their introduction into humans and subsequent transmission is intimately linked to the provision of food. These include a large number of viruses that have jumped from wildlife or livestock into humans who hunt for bush meat (HIV/AIDS) or who butcher and process exotic and domesticated animals in wet markets¹⁰ (Rasko et al., 2011).

⁸ Emergence of HIV and Ebola hemorrhagic fever is likely associated with the butchering and percutaneous and mucous membrane exposure to blood and body fluids of nonhuman primates hunted for food in Sub-Saharan Africa.

⁹ The SARS outbreak was associated with the trade of a small carnivore, the palm civet, sold for human consumption in Guangdong Province, China. Subsequent investigations found the virus in other wild animals sold in Guangdong’s markets as well as domestic cats. Human infection was the direct result of contact with these animals. The virus was later determined to be of bat origin.

¹⁰ A wet market is generally an open food market. The main characteristics of the market have traditionally been associated with a place that sells live animals out in the open. The collection may

Trends Threatening Food Safety

Several workshop presentations addressed the complex and interconnected factors influencing food safety, among them the following key trends introduced by King and Daszak. Several of these trends have been discussed in depth in previous Forum workshop summary reports, including *Addressing Foodborne Threats to Health* (IOM, 2006), *Infectious Disease Movement in a Borderless World* (IOM, 2010c), and *Antibiotic Resistance: Implications for Global Health and Novel Intervention Strategies* (IOM, 2010a).

Growth, migration, and aging of human populations As depicted in Figure WO-4, the overwhelming majority of global population growth is occurring in developing countries. An estimated 1 billion people reside in periurban slums, which, King noted, are home to the fastest-growing human populations; by 2020 their numbers are expected to increase by 50 percent (UN, 2006). These areas are potential hotspots for infectious disease emergence, including water- and food-borne diseases, he observed.

At the same time, human migration from rural to urban settings is just one facet of the more general phenomenon of increased migration—of humans, animals, plants, and diseases, King continued. “More than 1 billion people cross international borders every year, often bringing their food with them,” he stated. Meanwhile, populations in developed countries such as the United States are aging and, therefore, increasingly vulnerable to illness associated with consumption of foods tainted by food-borne pathogens.

Globalization of food trade We live in a world of “collapsed space,” King observed, and it is becoming increasingly smaller, faster, and more interconnected. Vast amounts of food and food products move around the world, as he and several other workshop speakers observed. The global nature of food supply chains is reflected in the United States, he said, where approximately 75 percent of processed food items contain ingredients from another country.¹¹ Upon arrival, these products—along with domestically produced raw and finished foodstuffs—are typically dispersed hundreds or thousands of miles across the country from central distribution or processing facilities. Food distribution networks are designed to rapidly move perishable goods, to provide just-in-time restocking of nonperishable items, and to take advantage of economies of scale (Sobel, 2005). Unfortunately, he added, there is a “disconnect between health and commerce”

include poultry, fish, reptiles, and pigs. Depending on the region, animals are usually caged and killed for live preparation. Fresh fruits and vegetables are also available. Wet markets generally include butcher shops and fish markets, which are in a separate section from the fruit and vegetable stalls. (University of Hong Kong Social Mapping Project: <http://www.wix.com/geog3414/geog3414-wet-market>; accessed April 24, 2012).

¹¹ On an annual basis, this country imports more than 75 percent of its fresh fruits and vegetables and more than 80 percent of its seafood (FDA, 2011a).

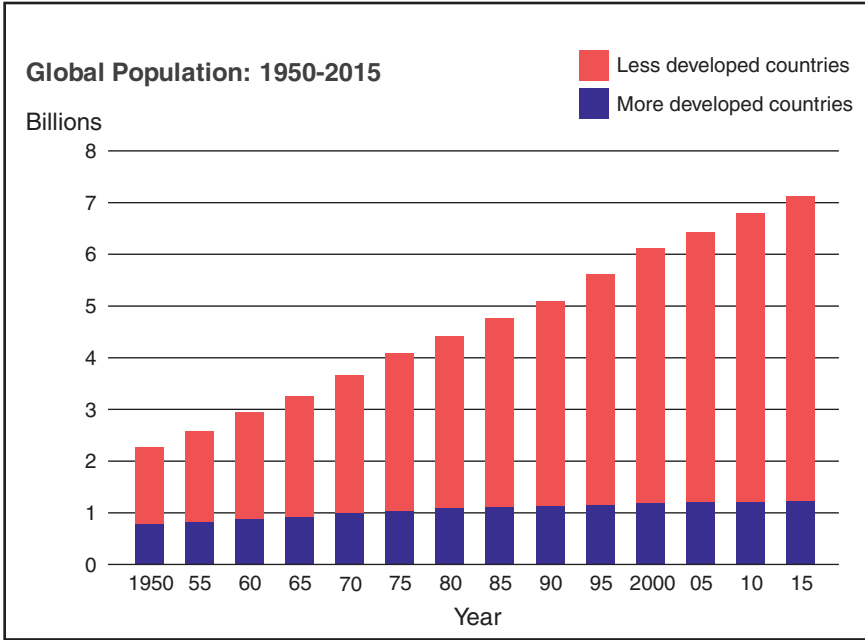


FIGURE WO-4 Trends in global population: 1950-2015.
SOURCE: King (2011).

and, as a result, “real concern about the vulnerability of these remarkable food systems to unintentional natural or even intentional introduction of pathogens and contaminants.”

Increased meat consumption Since 1983, meat consumption has risen steadily in developed countries and steeply in developing countries. As illustrated in Figure WO-5, this exponential growth in the developing world is expected to continue through the next decade. In 2010, nearly 30 billion food animals were produced to help feed the world’s 7 billion people, King reported. If the demand curve for animal protein continues to grow as projected—by more than 50 percent over the next two decades—another 15 billion animals will be needed to feed the world’s estimated population of 9 billion people.

Expansion of the human–animal interface All three trends described above have led to increased contact between humans and animals. Humans migrating from rural areas to urban centers bring their domestic animals such as poultry, swine, and cattle along with them. Eventually, King observed, all agricultural activity will shift toward urban areas. Meanwhile, the expanding human popula-

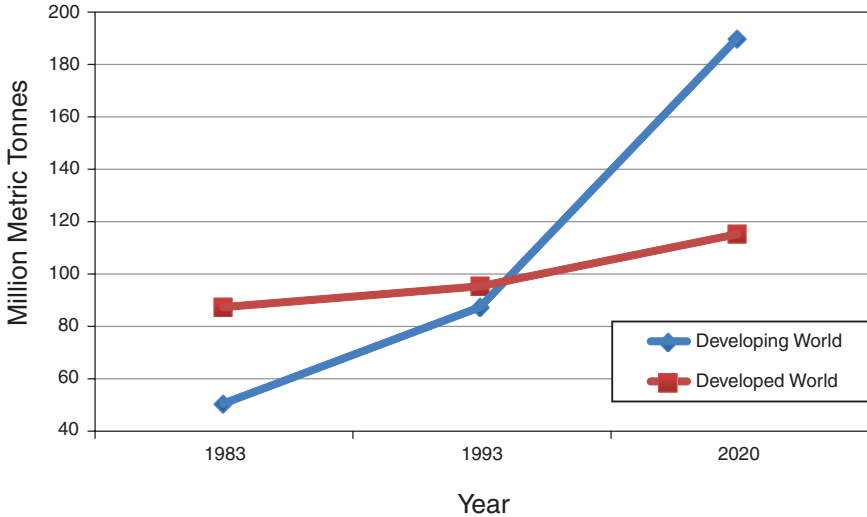


FIGURE WO-5 World meat consumption, 1983-2020.
SOURCE: King (2011).

tion’s demand for meat drives increased contact between hunters and wildlife, as well as the intensification of livestock production. “We have never experienced the intensity and scope of the human–animal interface that we observe today,” he stated. “This is, I think, the great possibility for emerging zoonoses, and certainly food-borne illnesses and rapid changes in our environment.” As illustrated in Figure WO-6 on why diseases emerge, it is essential to understand how pathogen behavior changes in response to environmental upheaval, such as the transition to intensive agriculture, he said.

“What we have now is an incredibly difficult system, a mixture of very intensively farmed production animals in developed countries, with a huge global connectivity,” Daszak added. At the same time, in some parts of the world, and in increasingly remote areas, wildlife continues to be hunted, in increasingly remote areas, he said, “so it really is no surprise that we’re seeing new pathogens that have a higher and higher impact and are emerging at a growing rate.”

Addressing the “Wicked Problem” of Food Safety with a One Health Paradigm

King introduced the concept of the “wicked problem,” as defined in Box WO-1, and explained why the quest for safe food in a globalized environment fits that definition. The term “wicked problems”—referring to problems that arise in complex and interdependent systems and that are difficult or impossible to solve because of incomplete, contradictory, changing, or incomprehensible

Why Diseases Emerge

Genetic and biological factors

- Microbial adaptation and change
- Human susceptibility to infection

Physical environmental factors

- Climate and weather
- Economic development and land use

Ecological factors

- Changing ecosystems
- Human demographics and behavior

Social, political, and economic factors

- International travel and commerce
- Poverty and social inequity
- War and famine
- Lack of political will
- Intent to harm



FIGURE WO-6 Why diseases emerge.
SOURCE: King (2011).

BOX WO-1 Wicked Problems

Wicked problems often arise as organizations face constant or unparalleled change, and in social contexts featuring numerous stakeholders with diverse opinions. The problem of food safety fits this description and displays the following characteristics that define a wicked problem:

- complex and tangled;
- unprecedented and unique, unrelated to past experiences;
- difficult to define and enigmatic;
- having many possible solutions, none of which involves an either/or, yes-or-no choice;
- one for which any solution may generate unexpected consequences;
- threatening; and
- often a symptom of another problem.

SOURCES: Ackoff (2008); King (2011).

requirements—surfaced in the social sciences during the 1960s and was formally defined in the social policy literature a decade later. The concept subsequently has been generalized to other disciplines, such as economics, environmental science, politics, and business (Ackoff, 2008).

Wickedness, he said, does not refer to the difficulty of such problems, but to their inability to be solved by standard approaches. “We have made some really good progress in food safety, without question, but we continue to come back with problem after problem, and new problems emerge,” King observed. He went on to note that it may be time “to think about whether these traditional processes and the way we operate still resolve these difficult and emerging problems.”

Traditional approaches for ensuring food safety are rooted in principles of medical training and education that attempt to define a problem, make a diagnosis, and prescribe a treatment, King explained. A One Health paradigm recognizes the interconnectedness of people, animals, and the environment and emphasizes disease prevention. As discussed in greater detail in King’s contributed manuscript in Appendix A (see pages 218-225), One Health is the collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and our environment. The scale and complexity of food safety issues demand that scientists, researchers, and others move beyond the confines of their own disciplines, professions, and mindsets and explore new organizational modes of team science; a One Health concept embodies this declaration. The scope of One Health is impressive, broad, and growing. Much of the recent focus of One Health has been limited to emerging infectious diseases, yet the concept clearly embraces environmental and ecosystem health, social sciences, ecology, non-infectious and chronic diseases, wildlife, land use, anti-microbial resistance, biodiversity, and much more.

While these components are appreciated within our understanding of the broad dimensions of health, they also add to the complexity of One Health and the difficulty in implementing strategies, building effective coalitions, and mobilizing scientific communities who embrace One Health yet who have been trained and think in much narrower scope and scale. Although there may be disagreement on the exact definition of One Health there is broad consensus that a new framework for preventing food-borne diseases is essential rather than the alternative of constantly responding to them reactively.

The concepts expressed as One Health are not new but are predicated on the discoveries of Louis Pasteur in the late 19th century and were widely accepted before the advent of specialized medicine, King observed. He speculated that these concepts have “re-emerged” as One Health because they place the problem of infectious disease emergence within ecosystems, a relationship championed by the late Nobel Laureate Joshua Lederberg, a founding member of the Forum on Microbial Threats. In his essay “Infectious History,” Lederberg observed that “an axiomatic starting point for progress [against emerging infectious diseases] is the simple recognition that humans, animals, plants, and microbes are cohabi-

tants of this planet. That leads to refined questions that focus on the origin and dynamics of instabilities within this context of cohabitation. These instabilities arise from two main sources loosely definable as ecological and evolutionary” (Lederberg, 2000).

Taking a One Health approach to food safety is an example of changing paradigms, as described by philosopher of science Thomas Kuhn in his seminal work, *The Structure of Scientific Revolutions* (Kuhn, 1996), according to King. With regard to the science of food safety, we have reached an era when old models are failing, but new models have yet to be created; a time when basic assumptions must be questioned and changed. Table WO-1 lists several key parameters underlying the paradigm shift to One Health.

Such changes need not be led by the scientific community. King observed that, in the case of food safety, the paradigm shift to One Health may be consumer-driven. Indeed, he continued, One Health should be considered in terms of its economic benefits to stakeholders, and its value judged according to evidence of its superiority to current approaches to food safety, or to alternative models. “The evidence has to be based on metrics of reduced costs, reduced or elimination of cases and deaths, [and greater] effectiveness,” he said.

TABLE WO-1 Understanding the One Health Paradigm for Food Safety

Dimension	From	To
Problem solving	Specific, technical solutions that exist	Managing complex dilemmas and wicked problems
Perspective	Fragmented and siloed	Systems approach, integrated and holistic
How work is done	Individual and often isolated	Collaborative and across disciplines and professions
With whom work is done	Without partners	Partners; government, industry, academe, and public/consumers
Where work is done	Focus on human illness	Closest to origin of infection or contamination
What we work on	Single domain	Human, animal, and environmental health domains
Surveillance and information	Limited to human health and disconnected from other domains	Food, animals, environment, and peoples; shared data
Time line	Reactive and emphasis on treating disease	Proactive, preventive, and anticipatory

SOURCE: King (2011).

“In many of its current forms, the concept of One Health is long on visionary scope and maddeningly short on tangible specifics and short term action steps for implementation.”

—Peter Rabinowitz (2010)

Key Challenges and Questions

Many workshop participants, in the discussion that followed the keynote presentations of King and Daszak, focused on the challenges and questions to be addressed in pursuing a One Health approach to preventing food-borne diseases. The following issues, summarized below, were identified by many participants as significant barriers to this goal:

- Public health agencies have yet to adapt to globalization, which demands that they collaborate and cooperate to reduce the burden of food-borne disease.
- Regulation involves negotiating national and regional differences in approaches to food safety.
- The “stovepiped” state of scientific training, research, and funding inhibits interdisciplinary and transdisciplinary research and collaboration.
- There is a need to train medical, veterinary, and public health professionals in One Health precepts.

Many workshop participants suggested that the questions captured in bullet points below might stimulate new ways of thinking about the process of adopting a One Health approach to food safety:

- What are the greatest threats to the global food supply, and which of these threats are most amenable to intervention?
- Despite the “wickedness” of emerging food-borne diseases, can promising “control points” be identified that will increase the likelihood of predicting or preventing potential outbreaks? Can one elucidate ecological rules that govern disease emergence?
- What novel approaches might be taken to increase “upstream” surveillance of food-borne diseases and their associated risk factors?
- What incentives might increase participation by the food industry in such efforts?
- What are the key scientific questions from the One Health perspective that should be pursued but which are not currently given sufficient attention?
- What metrics must be developed to evaluate the effectiveness of interventions based on One Health?

These challenges and questions laid the foundation for ongoing discussions throughout the 2 days of the workshop.

Overview of the Global Food System

Will Hueston, of the University of Minnesota, began his presentation on the global food system with a brief history of human food systems, from the time of hunter-gatherers to today's complex, interdependent, globalized world in which, he said, "everyone trades food." (Dr. Hueston's contribution to the workshop summary report can be found in Appendix A, pages 189-198.) According to Hueston, food systems emerged with the dawn of civilization when agriculture, including the domestication of animals, set the stage for permanent settlements. Inhabitants could grow more crops and raise more animals than necessary to feed those who tended them. This changed human culture; unlike earlier hunter-gatherers, agriculturalists did not need to be in constant motion to find new sources of food. Cultivating grain allowed for drying and storage of some of the harvest for later consumption. Different grain cultures emerged in each of the cradles of civilization—maize in Mexico, rice in China, and wheat and barley in the Middle East. The ability to produce a surplus of grain also set the stage for the development of art, religion, and government.

Hueston observed that, since agriculture began, food systems have constantly evolved, with each change bringing new advantages and challenges and ever-greater diversity and complexity. In the early 1900s people in the United States bought mostly unprocessed foodstuffs from local producers to be prepared and consumed in the home (CAST, 2004). More than a century later, one hamburger from Burger King® can contain ingredients from approximately 200 suppliers located throughout the United States and around the world (Scholl, 2005). And this is just one of the many food choice options available to more than 8 million customers served each day at more than 11,000 Burger King outlets worldwide (Scholl, 2005). Figure WO-7 illustrates both the breadth and the intricacy of current supply chains, through the example of the "inputs" and ingredients for the creation of a classic "megaburger."

Each of the ingredients listed may come from multiple sources and multiple countries, depending upon the ingredient, time of year, and price of the commodity. Hueston predicted that the future will bring even longer and more complex food supply chains, in part because of the increasingly urbanized global population, and also in response to consumer demand in terms of purchasing power combined with a desire to purchase any kind of food year-round.

Moreover, as illustrated in Figure WO-8, vast—and, in some cases, unknown—numbers of farms and livestock operations, processors, packers, shippers, and retail outlets comprise the current global food system, upon which the U.S. food supply¹² increasingly depends. This complex, dynamic web of relationships is prone to the sorts of "wicked problems" described by King.

¹² Altered dietary habits, higher living standards, and lifestyle changes have contributed to changing patterns of food consumption (ERS, 2001, 2005). In a later presentation, David Acheson, of Leavitt Partners, LLC, stated that approximately 15 percent of the food currently consumed in the United

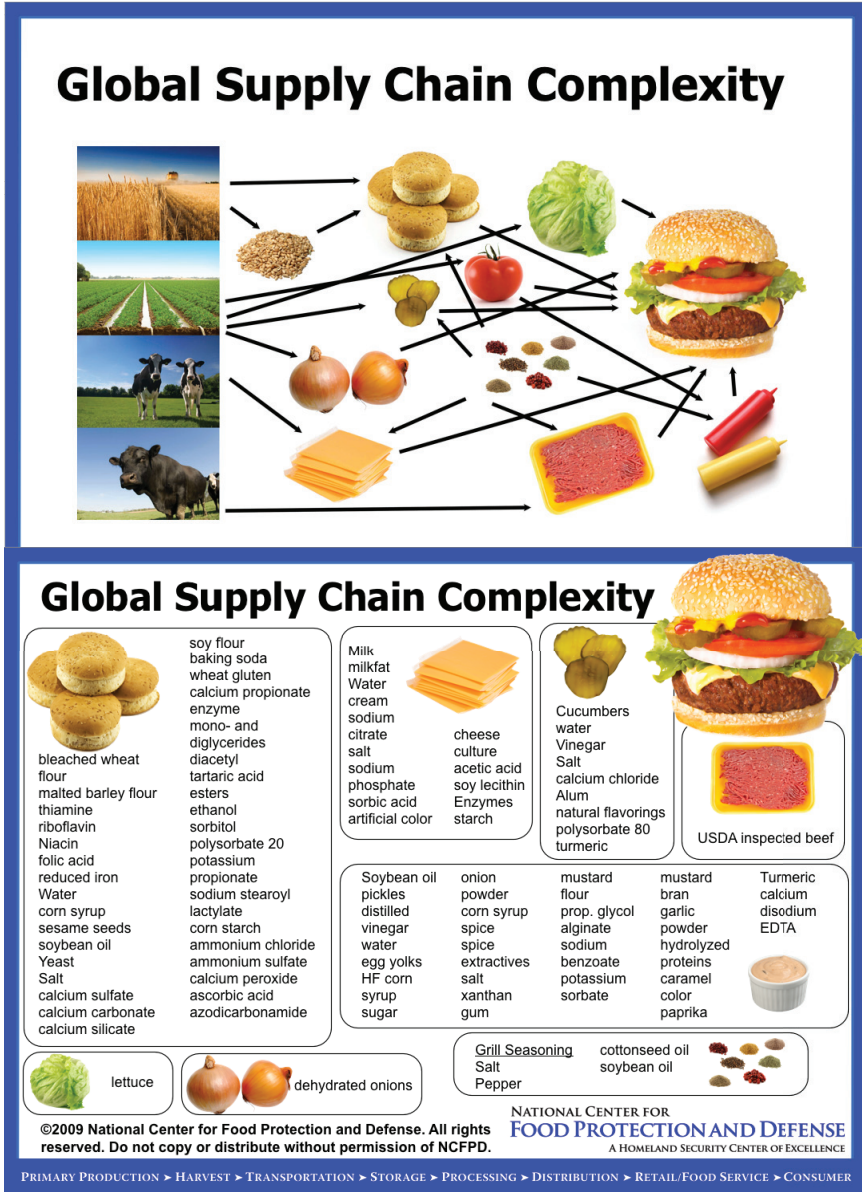


FIGURE WO-7 Global supply chain complexity: Origin and contents of a generic “megaburger.”

SOURCE: Shaun Kennedy, Director, National Center for Food Protection and Defense, University of Minnesota, as cited by Hueston (2011).

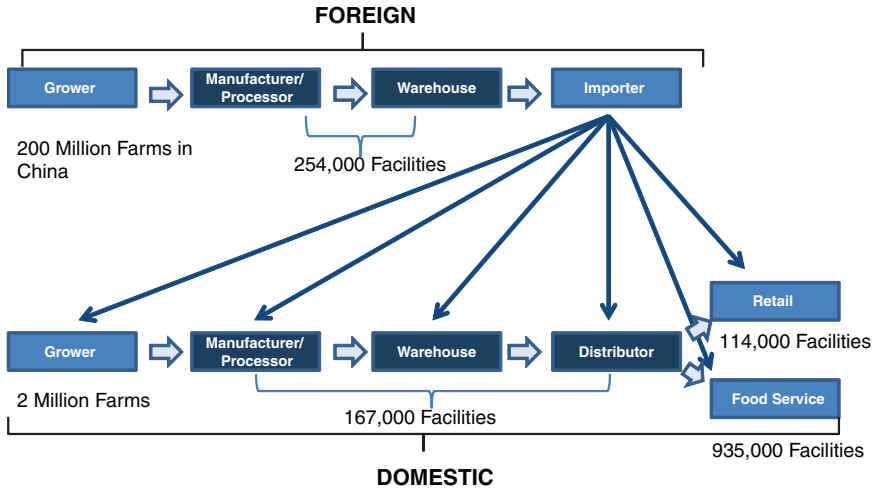


FIGURE WO-8 The global U.S. food supply: Many components.
SOURCE: Acheson (2011).

Hueston insisted that there is no *single* global food system but rather a multitude of interdependent food systems driven by the diverse needs of different countries and populations. These interconnected systems are also affected by environmental conditions and advancements in technology, he observed. “There is no best system,” he said, “and . . . every success in improving one food system perturbs the whole system of systems and changes the nature of [global] food safety problems.”

Hueston identified some of the characteristics of this “system of systems” and trends of particular relevance to One Health and the future of food safety:

- 1. Continuous and dynamic change:** Food systems adapt to a host of factors, including trade patterns, population growth, political upheaval, social instability, and advances in technology. The global “system of systems” exhibits properties that are not predictable from its individual subsystems; for example, a small, local perturbation may have a large effect at a global level, or it may have a proportional effect, or none at all.
- 2. Panarchy:** Exponential growth in connectedness and efficiency makes systems less and less resilient, which inevitably leads to collapse. Afterward, systems return to a state of greater resilience, with fewer connections and less efficiency. This model could describe the peril of food

States is imported; this includes more than 70 percent of seafood and 50 percent of fresh produce sold in this country. Over the past decade, the amount of food importation into the United States grew by more than 10 percent per year.

systems dependent entirely on “just-in-time” supply chains; the more interconnected and efficient they become, the greater their vulnerability to failure at multiple points.

3. **Demand-driven economy:** “Big-box” stores, multinational fast-food chains, and large processors compete to meet consumer demands—including the demand for safe food—at the lowest possible price. As a result, coalitions of companies are setting standards for food safety ahead of governments and international organizations.
4. **Culture clash:** Countries and cultures differ in assigning responsibility for food safety. In many developing countries, Hueston observed, “they cook the heck out of everything . . . [so] there is no microbial food safety threat.” In such cultures, consumers are assumed to be responsible for the safety of their food.

Workshop participants considered another consumer demand trend in subsequent discussion—foods that are locally raised by small (often organic) producers. “There is pressure in a number of states to expand the exclusion of small producers from any and all food safety regulation,” Hueston noted; such exemptions already exist for small producers of meat and poultry. “I applaud the enthusiasm and commitment of the individuals involved, and I am horrified at the lack of knowledge of basic sanitation,” he said. “Public health interventions that have been successful over the years in reducing the likelihood of food-borne illness are now called into question,” Hueston observed. “When we no longer see the problem, then we don’t think the problem exists. It’s the curse of high health status.”

Dr. Robert Tauxe, of the CDC, identified the desire for locally sourced food as arising from a need to know who is responsible and accountable for food safety. (Dr. Tauxe’s contribution to the workshop summary report can be found in Appendix A, pages 307-331.) “I depend entirely on the people who produced it to make sure it’s safe, so I have some comfort at least, if I know who they are,” he said. He urged the food industry to consider satisfying that need by providing information to consumers as to the origins of their products and ingredients. “Maybe that captures some of that market interest and increases the safety of all,” he concluded.

Given these conditions, we must accept that no one system can make food unfaillingly safe, and that the problem of food safety cannot be understood in its entirety, Hueston argued. While we need to act to make food safer, we also need to recognize that every action we take perturbs the system, he continued; that will require systems thinking, shared leadership among all stakeholders, and a holistic view of public health and its relationship to the health of ecosystems, economies, and societies.

Hueston also observed that such a multifaceted approach is consistent with the One Health paradigm. He also noted that similar thinking informed the definition of health adopted by the World Health Organization (WHO) at its in-

ception in 1946 as a “state of complete physical, social, and mental well-being, and not merely the absence of disease or infirmity.”¹³ The WHO and the Food and Agriculture Organization (FAO) of the United Nations (Joint FAO/WHO Food Standards Programme) jointly defined food safety as “all the conditions and measures necessary during production, processing, storage, distribution, and preparation of food to ensure that it is safe, sound, wholesome, and fit for human consumption [*sic*].”¹⁴

“We need to move from finger-pointing to shared leadership,” Hueston asserted. He envisioned a new model of partnership that engages the food industry through a flexible and realistic regulatory system. “Voluntary compliance [with food safety standards], building a trusting relationship between the food industry and public health, has a much higher likelihood of achieving prompt action early in an epidemic and preventing illness and saving lives,” he concluded. “This isn’t something that’s going to be solved by regulation.” Partnership between government and industry, a central theme of workshop discussion, is further considered in the final two sections of this overview.

COMMON FOOD-BORNE PATHOGENS IN THE UNITED STATES

More than 250 pathogens and toxins are known to be transmitted by food, and this list continues to grow steadily, Robert Tauxe reported. Table WO-2 lists food-borne pathogens identified since 1970, which include several nonbacterial organisms.

In the United States, the food-borne pathogens *Campylobacter*, *Clostridium perfringens*, *E. coli*, *Listeria monocytogenes*, Norovirus, *Salmonella* spp., and *Toxoplasma* account for more than 90 percent of all symptomatic food-related illnesses with a known cause. These are briefly discussed in Box WO-2.

¹³ The Constitution of the WHO (1946) states that good health is a state of complete physical, social, and mental well-being, and not merely the absence of disease or infirmity. Health is a resource for everyday life, not the object of living, and is a positive concept emphasizing social and personal resources as well as physical capabilities. Health is a fundamental human right, recognized in the Universal Declaration of Human Rights (1948). It is also an essential component of development, vital to a nation’s economic growth and internal stability. Along with the traditional and unequivocal arguments on social justice and the importance of health, it is now accepted that better health outcomes play a crucial role in reducing poverty. There is also increased understanding of how health fits into a wider cross-sectoral, cross-border, and globalized framework. Source: <http://www.who.int/trade/glossary/story046/en/index.html>.

¹⁴ The Codex Alimentarius Procedural Manual states that food hygiene “comprises conditions and measures necessary for the production, processing, storage and distribution of food designed to ensure a safe, sound, wholesome product fit for human consumption” (FAO/WHO, 2001; <ftp://ftp.fao.org/docrep/fao/005/Y2200E/Y2200E00.pdf>).

TABLE WO-2 Many of the Major Food-Borne Pathogens in the United States Were Only Recently Characterized

Bacteria	Viruses
<i>Bacillus cereus</i>	Astrovirus*
<i>Brucella</i> spp.	Hepatitis A virus
<i>Campylobacter</i> spp.*	Norovirus*
<i>Clostridium botulinum</i>	Rotavirus*
<i>Clostridium perfringens</i> *	Sapovirus
<i>E. coli</i> (STEC) O157	
<i>E. coli</i> (STEC) non-O157*	
<i>E. coli</i> other diarrheogenic (not STEC or ETEC)*	Parasites
Enterotoxigenic <i>E. coli</i> (ETEC)	<i>Cryptosporidium</i> *
<i>Listeria monocytogenes</i> *	<i>Cyclospora cayetanensis</i> *
<i>Mycobacterium bovis</i>	<i>Giardia intestinalis</i> *
<i>Salmonella</i> spp. nontyphoidal	<i>Taenia saginata</i>
<i>Salmonella enterica</i> Serotype Typhi	<i>Taenia solium</i>
<i>Shigella</i> spp.	<i>Toxoplasma gondii</i> *
<i>Streptococcus</i>	<i>Trichinella</i> spp.
<i>Streptococcus</i> spp. group A, foodborne	
<i>Vibrio cholerae</i> , toxigenic (O1 and O139*)	
<i>Vibrio vulnificus</i> *	
<i>Vibrio parahaemolyticus</i> *	
<i>Vibrio</i> spp., other	
<i>Yersinia enterocolitica</i> *	

NOTE: Pathogens that have emerged or been recognized as predominantly food-borne in the past 40 years are indicated with an asterisk (*).

SOURCE: CDC (2011h); Tauxe (2002).

Recent Food-Borne Disease Outbreaks: Patterns of Emergence and Lessons Learned

Even in the industrialized world, food-borne illness is a relatively common phenomenon. The true incidence of food-borne illness is unknown because of a combination of factors. A case of food-borne illness is only reported to a health department if a person has become ill, has sought medical care, and has undergone diagnostic testing that has revealed evidence of a pathogen in stool or other specimen. Diagnosed cases are therefore likely to represent only a small fraction of the cases of food-borne illness that actually occur. It is likely that many people do not seek medical attention for symptoms of food-borne illness. Moreover, the diagnosis of some food-borne diseases is difficult, if not impossible, as illustrated by the fact that “unrecognized agents” account for 81 percent of all U.S. food-borne illnesses and hospitalizations and 64 percent of deaths (Mead et al., 1999; Scallan et al., 2011a, 2011b). In developing countries, where food safety presents even greater challenges, food-borne disease is a daily fact of life and a significant cause of death due to diarrheal illness (Mead et al., 1999).

BOX WO-2 The Seven Most Common Food-Borne Pathogens in the United States

Campylobacter

Campylobacter spp. is one of the most common causes of diarrheal illness—responsible for approximately 850,000 illnesses, 8,500 hospitalizations, and 76 deaths in the United States each year (Scallan et al., 2011b) (Figure WO-2-1). Guillain-Barre syndrome, an acute paralytic illness that may leave chronic deficits, can follow *Campylobacter* infections. *Campylobacter* spp. are part of the normal intestinal flora of a wide variety of healthy domestic and wild animals^a and are often found associated with bodies of water such as water troughs and streams. Most cases of campylobacteriosis are associated with eating raw or undercooked poultry meat or from cross-contamination of other foods by these items; outbreaks of *Campylobacter*-associated disease are also linked to unpasteurized milk or contaminated water.

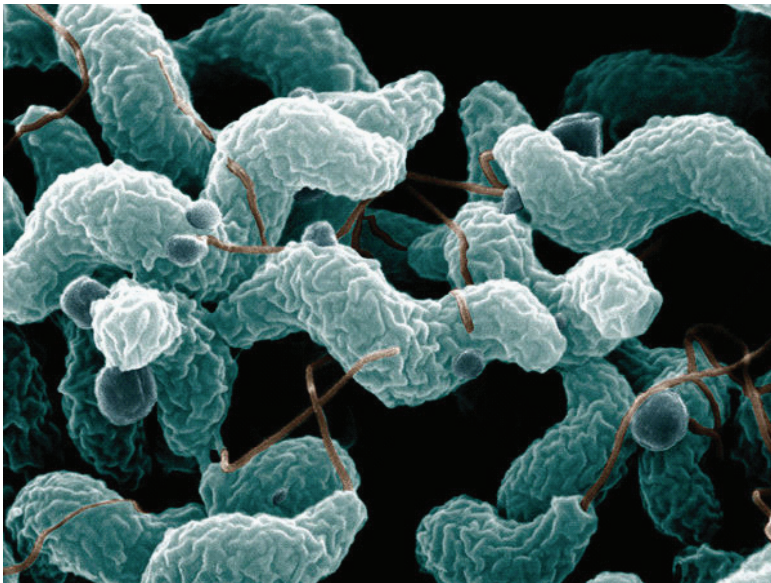


FIGURE WO-2-1 Scanning electron microscope image shows the characteristic spiral, or corkscrew, shape of *Campylobacter jejuni* cells.

SOURCES: De Wood, Pooley, U.S. Department of Agriculture, Agricultural Research Service, Electron Microscopy Unit.

^a Including cattle, sheep, goats, pigs, chickens, ducks, geese, wild birds, dogs, cats, rodents, and marine mammals.

Clostridium perfringens

Clostridium perfringens is a spore-forming bacterium that produces a toxin estimated to cause nearly a million cases of food-borne illness, 440 hospitalizations, and 26 deaths in the United States each year (Scallan et al., 2011b) (Figure WO-2-2). This organism is found in many “external” environments, as well as in the intestines of humans and animals, and commonly on raw meat and poultry, as well as in gravies and in dried or pre-cooked foods. *C. perfringens* spores can survive high temperatures. Spores germinate during cooling and storage at temperatures from 68°F to 140°F (20°C to 60°C). If food is served without reheating to kill bacteria, live bacteria may be eaten and cause infection.

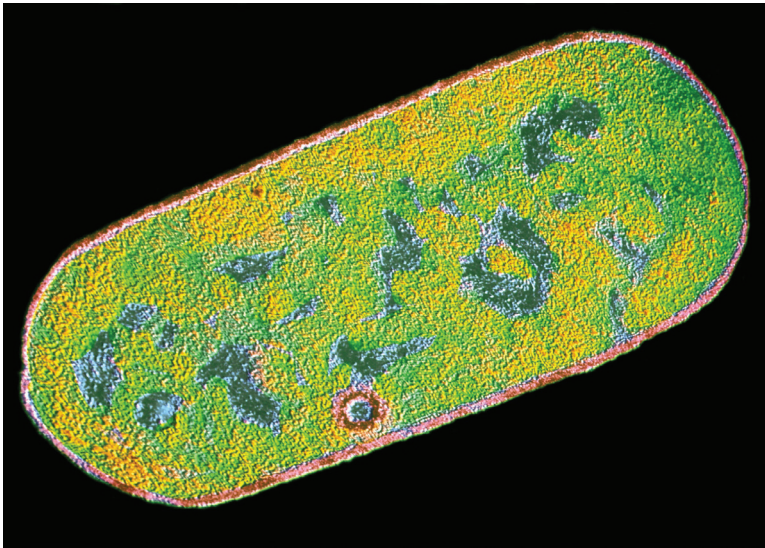


FIGURE WO-2-2 *Clostridium perfringens* bacterium. Colored TEM. Magnification 43,000x.

SOURCE: CNRI/Science Photo Library.

Escherichia coli

Escherichia coli comprise a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can be pathogenic to humans, including Shiga toxin-producing *E. coli* (STEC). The most commonly identified STEC in North America is *E. coli* O157:H7 (Figure WO-2-3). O157 was first identified in 1982 in outbreaks of severe bloody diarrhea in North America. STEC live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk. Other kinds of animals, including pigs and birds, sometimes pick up STEC from the environ-

continued

BOX WO-2 Continued

ment and may spread it (CDC, 2011c). Today almost any food vehicle in contact with ruminant feces is a potential exposure source, including vegetables, sprouts, fruits, meat products, juices, and milk. Drinking, recreational, and bathing waters may be fecally contaminated. Novel transmission routes for outbreaks continue to arise.

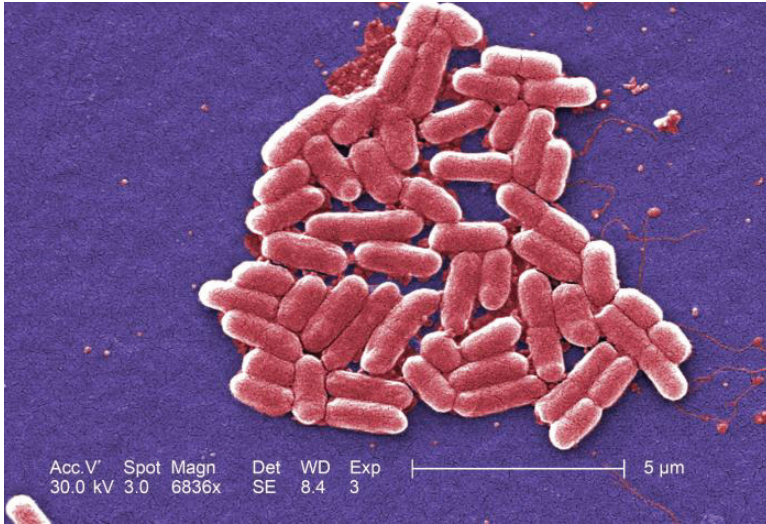


FIGURE WO-2-3 This colorized scanning electron micrograph (SEM) depicts a number of *Escherichia coli* bacteria of the strain O157:H7 (Magnification 6,836x). SOURCE: Janice Haney Carr, CDC Public Health Image Library (10068).

Listeria monocytogenes

Listeriosis—a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*—is a relatively rare disease with a high mortality rate (20 to 30 percent) that makes it one of the deadliest food-borne threats (CDC, 2011i; Weinstein, 2011) (Figure WO-2-4). The bacterium is found in soil and water and is carried asymptotically by numerous animal species. The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in foods that become contaminated after cooking or processing (CDC, 2011i). *L. monocytogenes* is considered an opportunistic pathogen and causes disease in older adults, pregnant women, newborns, and adults with weakened immune systems (CDC, 2011i). Infections in pregnant women can be devastating to the fetus, resulting in miscarriages, stillbirths, and birth defects. Unlike many other food-borne pathogens, *Listeria* multiplies in cold environments such as refrigerators (Jemmi and Stephen, 2006). It can quickly spread in damp

buildings, dripping off pipes or ceilings onto food. Once *Listeria* bacteria get into a food-processing factory, they can live there for years, sometimes contaminating food products (Jemmi and Stephen, 2006).

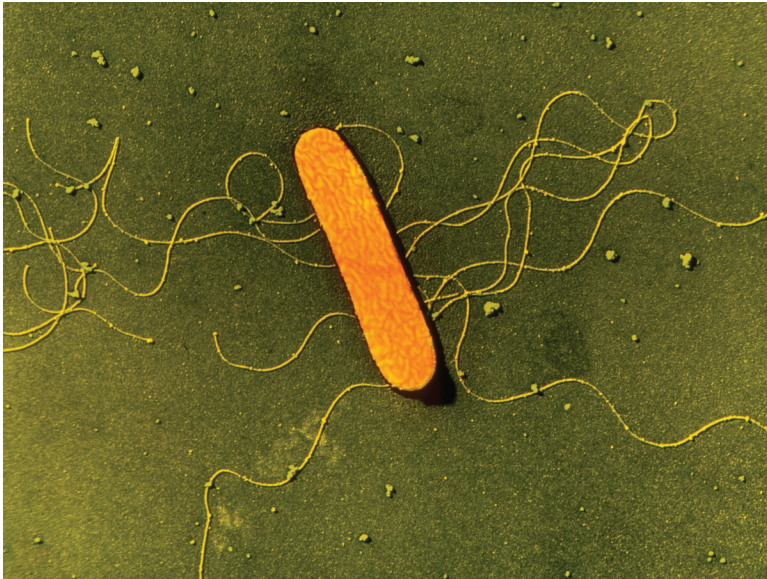


FIGURE WO-2-4 False-color transmission electron micrograph (TEM) of a single flagellate bacterium, *Listeria monocytogenes*. Magnification 5,700x at 35 mm size, 8,000x at 6 x 4.5 cm size.

SOURCE: A.B. Dowsett/Photo Researchers, Inc.

Noroviruses

Noroviruses are the most common source of gastroenteritis outbreaks in the United States, causing nearly 21 million gastrointestinal illnesses annually (Desai et al., 2011) (Figure WO-2-5). Fecal–oral spread is the primary mode of transmission. The virus’s abilities to withstand a wide range of temperatures (from freezing to 60°C) and to persist on environmental surfaces and food items contribute to rapid dissemination, particularly via secondary spread (via food handlers or to family members) (Glass et al., 2009). Food can be contaminated at the source (via contaminated water) or during preparation (Glass et al., 2009). Recent evidence suggests the possibility of animal reservoirs, but direct zoonotic transmission appears to be rare. Some noroviruses have been identified in animals—such as pigs and cattle—but none of these strains has yet been

continued

BOX WO-2 Continued

detected in humans^b (Glass et al., 2009; Koopmans, 2008). While usually associated with cruise ships, a recent CDC study reports transmission of norovirus among National Basketball Association players and staff during the winter 2010-2011 season (Desai et al., 2011).

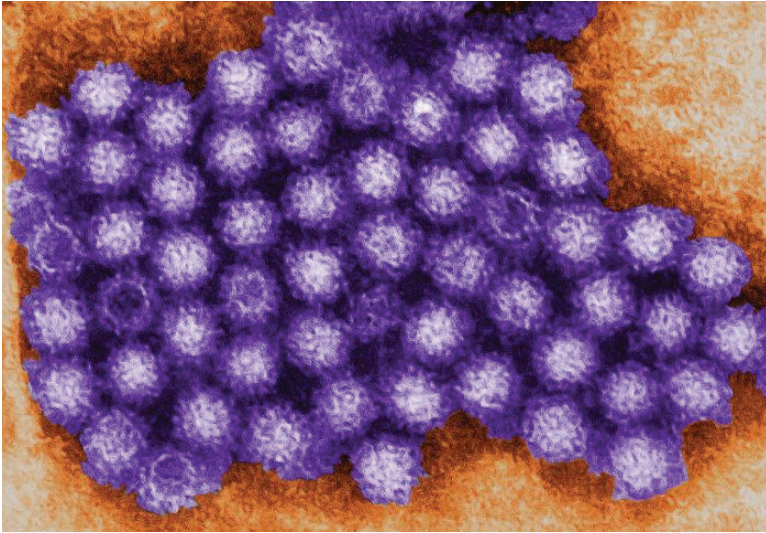


FIGURE WO-2-5 Transmission electron micrograph of norovirus virions.
SOURCE: Charles D. Humphrey/CDC Public Health Image Library (10708).

Salmonella

Salmonella is the leading bacterial cause of food-borne illness in the United States. The CDC estimates that more than 1 million people in the United States contract *Salmonella* each year, with an average of 19,000 hospitalizations and 380 deaths (Scallan et al., 2011b) (Figure WO-2-6). *Salmonella* live in the intestines of most livestock and many wild animals. *Salmonella* infection usually occurs when a person eats food contaminated with the feces of animals or humans carrying the bacteria. *Salmonella* outbreaks are commonly associated with eggs,

^b Humans are believed to be the only host for human norovirus, but several genogroups (GI and GIV) contain both human and animal strains, raising the possibility of zoonotic transmission.

meat, and poultry, but these bacteria can also contaminate other foods such as fruits and vegetables.^c

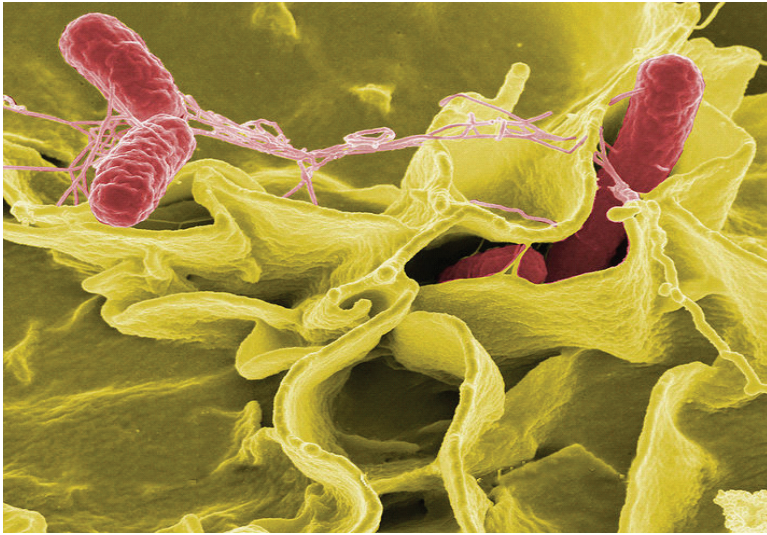


FIGURE WO-2-6 Negatively color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells. SOURCE: National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Toxoplasma gondii

Toxoplasma gondii is one of the world's most common parasites (Figure WO-2-7). Although cats are the only known host in which the parasite can com-

^c More recently, the CDC has reported a total of 258 persons infected with the outbreak strain of *Salmonella* Bareilly (247 persons) or *Salmonella* Nchanga (11 persons) from 24 states and the District of Columbia. The numbers of ill persons with the outbreak strain of *Salmonella* Bareilly identified in each state are as follows: Alabama (2), Arkansas (1), California (2), Connecticut (9), District of Columbia (2), Florida (1), Georgia (10), Illinois (23), Louisiana (3), Maryland (24), Massachusetts (27), Mississippi (2), Missouri (4), Nebraska (1), New Jersey (25), New York (39), North Carolina (4), Pennsylvania (20), Rhode Island (6), South Carolina (3), Tennessee (2), Texas (4), Virginia (16), Vermont (1), and Wisconsin (16). Thirty-two ill persons have been hospitalized, and no deaths have been reported. Collaborative investigation efforts of state, local, and federal public health agencies indicate that a frozen raw yellowfin tuna product, known as Nakaochi Scrape, from Moon Marine USA Corporation is the likely source of this outbreak. <http://www.cdc.gov/salmonella/bareilly-05-02> (accessed May 3, 2012).

continued

BOX WO-2 Continued

plete its life cycle, this parasite can use almost all warm-blooded vertebrates—including humans—as hosts. *T. gondii* infections are estimated to cause approximately 87,000 illnesses, 4,400 hospitalizations, and 330 deaths each year in the United States, making it the second leading cause of food-borne mortality in the United States and the third leading cause of food-borne hospitalizations (Scallan et al., 2011b). The most common sources of *Toxoplasma* are undercooked meat, animal feces, and transmission from mother to unborn child. While most people infected with *Toxoplasma* experience no symptoms, unborn children (who contract it from their mothers) and adults with compromised immune systems risk serious side effects. An estimated 22.5 percent of the U.S. population over the age of 12 has been infected with *Toxoplasma*. For some countries, this figure is as high as 95 percent.

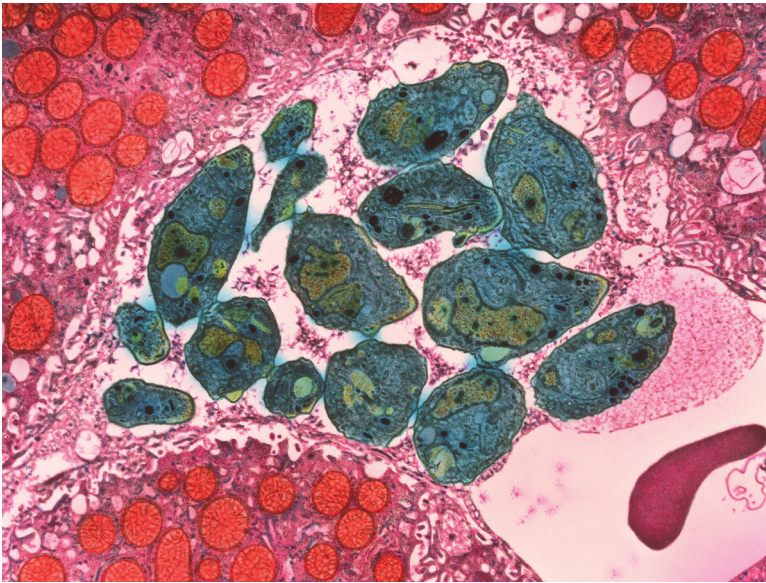


FIGURE WO-2-7 Colored transmission electron micrograph (TEM) of *Toxoplasma gondii* parasites (green), cause of toxoplasmosis. This unicellular parasite is seen here in liver tissue (pink). Magnification: 12,000x.

SOURCE: Moredum Scientific, Ltd./Photo Researchers, Inc.

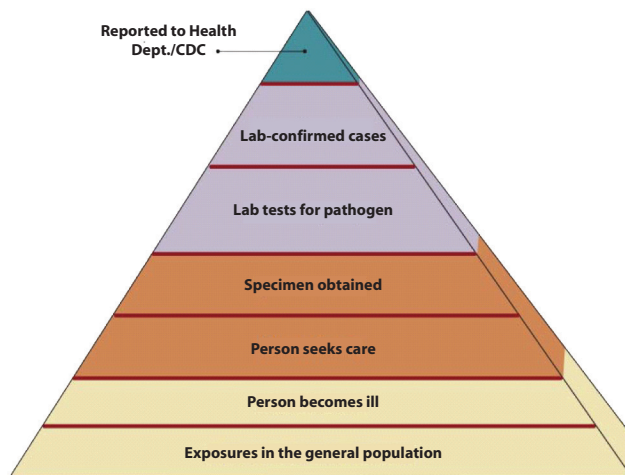


FIGURE WO-9 The true burden of food-borne disease remains unknown. SOURCE: CRS, 2010. Adapted from CDC, “FoodNet Surveillance—Burden of Illness Pyramid,” http://www.cdc.gov/FoodNet/surveillance_pages/burden_pyramid.htm.

Be that as it may, food-borne disease is a persistent and evolving threat to global health. These diseases occur daily, in all countries—from the least to the most developed—and are caused by consumption of foods and food components contaminated with a variety of microorganisms. According to the CDC, more than 250 different food-borne diseases have been identified (CDC, 2011j). The health impacts¹⁵ associated with these diseases can be acute or long term, including episodes of mild to severe diarrheal illness, kidney failure, chronic arthritis, brain or nerve damage, and death (CDC, 2011j). The health burden of these illnesses is substantial,¹⁶ but because many cases are often not reported to health officials, the true health impact of food-borne illness is unknown¹⁷ (Figure WO-9). Outbreaks of disease also cause billions of dollars in health care–related and industry costs annually (CDC, 2011k).

Beyond the health effects of infection, food-borne illness can also cause substantial economic hardships. *Salmonella* infections cause approximately 1 million food-borne infections and cost US\$365 million in direct medical expenditures annually. The societal cost of a single fatal case of *E. coli* (STEC) O157 infec-

¹⁵ The most severe cases tend to occur in the very old, in the very young, in those who have compromised immune system function, and in healthy people exposed to a very high dose of an organism (CDC, 2005).

¹⁶ Seventy percent of the 2.2 million deaths that occur each year due to acute diarrheal disease are associated with either water- or food-borne contamination (WHO, 2007).

¹⁷ The WHO launched an initiative in 2007 to provide better estimates of the global burden of food-borne disease. See http://www.who.int/foodsafety/foodborne_disease/ferg/en/.

tion has been estimated at US\$7 million (Frenzen et al., 2005). The USDA estimates costs associated with medical expenses and losses in productivity due to missed work and premature deaths attributed to five major types of food-borne pathogens (*Campylobacter*, *E. coli* O157:H7, Shiga toxin-producing strains of *E. coli*, *Listeria monocytogenes*, and *Salmonella* spp.) at US\$6.9 billion annually (Crutchfield and Roberts, 2000).

Several workshop presentations described the unfolding investigation, and analysis, of recent food-borne disease events that have informed a One Health view of food-borne disease emergence. To introduce this topic, Tauxe provided both an overview of domestic trends in food-borne disease and a review of recent progress toward reducing that threat.

Food-Borne Illness Trends in the United States

Tauxe illustrated the consequences of a health threat he called “common, costly, and preventable” with the following statistics (Scallan et al., 2011a, 2011b):

- Each year, an estimated 48 million Americans—1 out of every 6—become sick after eating contaminated food. Of them, 128,000 are hospitalized, and 3,000 die. The domestic burden of disease associated with six major food-borne pathogens is shown in Figure WO-10.
- Approximately 1,200 food-borne outbreaks occur annually in the United States.
- *Salmonella* infections alone cost the United States US\$2.8 billion.
- *Preventing* a single fatal case of *E. coli* O157 infection would save an estimated US\$7 million.

“Each one of these required a public health response somewhere, and almost all of them were identified in the course of public health investigations of outbreaks,” he observed. Many of these organisms (e.g., *Campylobacter*, *E. coli* O157:H7, *Salmonella* spp., *Vibrio* spp., and *Yersinia enterocolitica*) have animal reservoirs and live primarily as commensals or colonists that do not appear to cause illness in nonhuman hosts, he added.

As illustrated in Figure WO-11, between 2003 and 2008, 1,565 outbreaks associated with single foods were reported to the CDC. Both foods of animal origin and produce are important food vehicles in these outbreaks. Tauxe noted that since 2006 food-borne outbreaks have been associated with the following food items not previously identified in the United States as vehicles for food-borne disease. Nearly half of these items were imported, he added, and nearly all of them either consist partly or entirely of plant-based foods, including produce, nuts, seeds, flour, or spices:

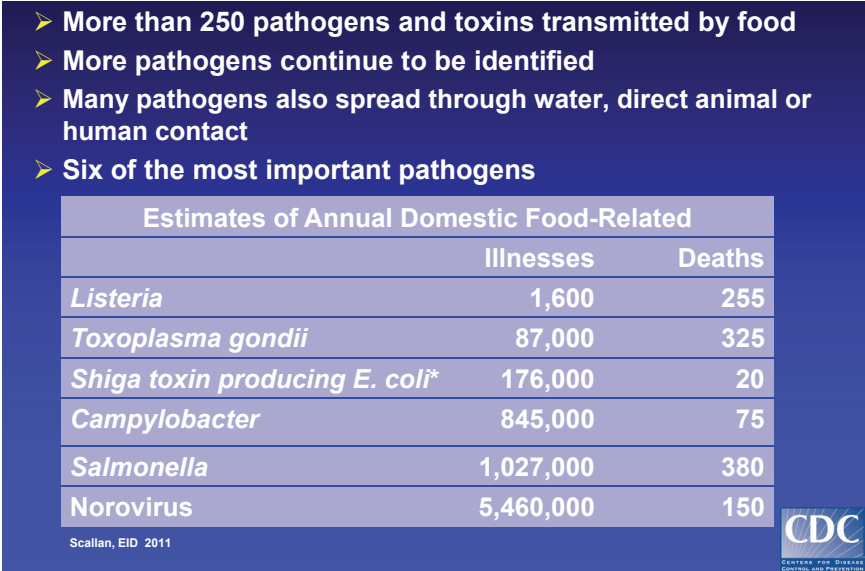


FIGURE WO-10 Many different pathogens and toxins.
 SOURCES: Tauxe (2011); from Scallan et al. (2011a, 2011b).

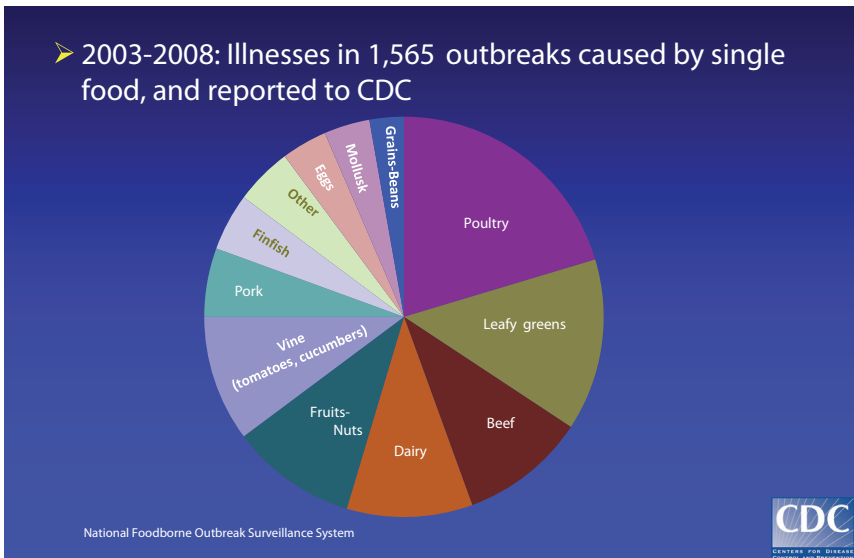


FIGURE WO-11 Foods implicated in outbreaks.
 SOURCE: Tauxe (2011).

- bagged spinach
- carrot juice
- peanut butter
- broccoli powder on a snack food
- dry dog food
- frozen pot pies
- canned chili sauce
- hot peppers
- white and black pepper
- raw cookie dough
- hazelnuts
- fenugreek sprouts
- papayas
- pine nuts

In subsequent discussions of food-borne disease trends, workshop participants also considered the threat of food-borne contaminants, such as mycotoxins and aflatoxins,¹⁸ which may cause long-term, chronic health problems in both people and animals—in contrast to the acute symptoms of food-borne infections. Such problems are known to exist but are very difficult to study, Tauxe observed. “Mycotoxins, particularly in the developing world, have been a recurrent issue when there’s famine, when there’s food shortage,” he said. “When the only thing left to eat is moldy corn, that’s what you eat.”

Research on the food safety implications of mycotoxins and aflatoxins is a potential arena for One Health, Hueston noted. “The veterinary profession and animal scientists have done a lot more work on [the health effects of these compounds], because it has direct impact on animal production,” he said. Combining their knowledge with the expertise of plant pathologists in a cross-disciplinary, cross-sectoral approach to food safety has “huge potential,” he declared.

Some food-borne infections may also have enduring consequences, Tauxe added. “About 11 percent of the U.S. population has antibodies to toxoplasmosis, which probably means they have cysts in them, and some of those are in their brains,” he stated. “What is that long-term effect? I don’t think we know.”

Many recent disease outbreaks reflect the changing nature of food-borne threats to health. These case studies underscore the vital connections between human, animal, and environmental health, and how changes in ecology or technology can drive the emergence or reemergence of food-borne pathogens by connecting “a potential pathogen with the food chain” (Tauxe et al., 2010). A deeper understanding of the ecology of food-borne pathogens and the root causes of their

¹⁸ Mycotoxins and aflatoxins are naturally occurring toxins produced by fungi, which may be present in moldy grains such as corn or rice, and in peanuts. Aflatoxins are known to cause cancer in some animals, and mycotoxins have been associated with several cancers in humans (e.g., liver cancer, esophageal cancer). Sources: <http://www.medterms.com/script/main/art.asp?articlekey=26613>; <http://www.medterms.com/script/main/art.asp?articlekey=10796>.

emergence and spread through the food system will enhance our capabilities to anticipate and prevent future emergence events.

Wake-up Calls: Case Studies of Food-borne Illnesses

Recent incidents of food-borne illness (discussed in greater detail below) that have received widespread attention illustrate the breadth and depth of potential threats from microbial food adulterants. In 1984, cult members in Oregon contaminated local salad bars with *Salmonella typhimurium* with the intent of influencing an election by incapacitating voters; a limited “trial run” of their plan sickened more than 700 people (Torok et al., 1997). In 1996, a worker in a large Texas medical center laboratory deliberately infected at least 12 coworkers with *Shigella dysenteriae* by leaving tainted pastries in their break room.

More devastating casualties have resulted from inadvertent food contamination. In 1994, approximately 224,000 people across a widespread area of the United States were infected with *Salmonella* Enteritidis from ice cream that was contaminated following pasteurization (Sobel et al., 2002). More than 7,000 Japanese children became ill with *E. coli* O157:H7 in a 1996 outbreak that originated in radish sprouts in school lunches (Sobel et al., 2002). Contaminated clams caused a 1991 outbreak of hepatitis A in China that affected more than 300,000 people and is perhaps the largest known food-borne epidemic (WHO, 2002). Despite the fact that an excellent vaccine for hepatitis A was licensed more than a decade ago, hepatitis A virus contamination of imported vegetables recently resulted in a large epidemic with many hundreds of cases and three deaths in the United States. This resulted from accidental contamination of the foodstuff with the virus; purposeful contamination could be substantially more devastating.

In recent years, special concern has been raised about the safety of fresh fruits and vegetables following several incidents of food-borne illness associated with produce. Fruits and vegetables have been associated with an increasing proportion of outbreaks; however, this trend has probably been influenced by the increased consumption of raw produce and by the advent of better surveillance techniques (Wang and Moran, 2004). In particular, recent outbreaks caused by the coccidian parasite *Cyclospora cayetanensis* and by hepatitis A virus bear examination as object lessons in the etiology, transmission, surveillance, diagnosis, and control of produce-associated illness.

Large-scale, centralized, food-processing operations followed by broad product distribution pathways create additional vulnerabilities in the food supply (ERS, 2005; Maki, 2009). The “bundling” of large quantities of single ingredients or mixing dozens of ingredients of various origins into a single batch can amplify the effects of a single contamination event. It has been estimated that just one infected beef carcass can lead to the contamination of 8 tons of ground beef; and the origin of a single lot of hamburger processed at one plant can be traced to more than 400 individual animals from six states (Nestle, 2003). These scenarios are reflected in the following real-world incidents of large-scale food contamination below, and in Box WO-3:

BOX WO-3

Recent Food-Borne Outbreaks: The Changing Nature of the “Threat”

As demonstrated in the case examples below, many recent outbreaks of disease reflect the changing nature of food-borne threats to health. These case studies underscore the vital connections between human, animal, and environmental health, and how changes in ecology or technology can drive the emergence or reemergence of food-borne pathogens by connecting “a potential pathogen with the food chain” (Tauxe et al., 2010). A deeper understanding of the ecology of food-borne pathogens and the root causes of their emergence and spread through the food system will enhance our capabilities to anticipate and prevent future emergence events.

Escherichia coli

Escherichia coli is a large and diverse group of bacteria that are present in the environment and as commensal^a organisms in a wide range of animals, including humans (Garcia et al., 2010). Most strains of *E. coli* are harmless. Other strains have acquired characteristics, such as the production of toxins, which make them pathogenic to humans^b (CDC, 2011c). Transmission of *E. coli* occurs when food or water that is contaminated with feces of infected humans or animals is consumed. Contamination of animal products often occurs during the slaughter and processing of animals (Garcia et al., 2010). The use of manure from cattle or other animals as fertilizer for agricultural crops can contaminate produce and irrigation water (Garcia et al., 2010). *E. coli* can survive for long periods in the environment and can proliferate in vegetables and other foods.

Shiga toxin-producing *E. coli* (STEC) are particularly notorious food-borne pathogens. STEC infection can cause episodes of mild to severe diarrhea, and 5 to 10 percent of infections develop into hemolytic-uremic syndrome (HUS)—a severe complication marked by profuse bleeding that can lead to kidney failure and death (CDC, 2011c). STEC strain O157:H7 is estimated to cause 63,000 illnesses, 2,100 hospitalizations, and 20 deaths each year (Scallan et al., 2011b). The principal reservoir for this zoonotic pathogen is the intestinal tract of cattle, but other animals may also serve as reservoirs. O157:H7 emerged as a significant public health threat in 1982 during two outbreaks of disease that investigators associated with the consumption of undercooked ground meat. A wide variety of foods, including fresh produce, have since served as a vehicle for *E. coli* O157:H7 outbreaks.^c Some

^a Organisms in a mutually symbiotic relationship where both live peacefully together while not being completely dependent on one another.

^b Researchers have associated intestinal disease with six different mechanisms or “pathotypes”: enteropathogenic *E. coli* (EPEC); enterohemorrhagic *E. coli* (EHEC, also known as Shiga toxin-producing *E. coli* [STEC] and formerly referred to as verotoxin-producing *E. coli* [VTEC]); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAaggEC); enteroinvasive *E. coli* (EIEC); attaching and effacing *E. coli* (A/EEC).

^c Food producers must report the presence of *E. coli* O157:H7 to health authorities. There are more than 100 “non-O157” STEC strains, and 6 of these strains cause up to two-thirds of

recent outbreaks include contamination events involving spinach and fenugreek bean sprouts.

***E. coli* O157:H7 contamination of spinach.** In 2006, investigators linked at least 205 illnesses and 5 deaths to the consumption of fresh spinach contaminated with *E. coli* O157:H7 (Weise and Schmit, 2007). In response to the growing outbreak—which included cases across 26 states and Canada—FDA advised consumers to stop eating all uncooked, fresh spinach, or products containing uncooked spinach^d (Calvin, 2007). Epidemiological studies traced the contamination to a single shift at a Natural Selections Foods processing plant in San Juan Batista, California, which had produced 42,000 bags of pre-washed and ready-to-eat baby spinach (Weise and Schmit, 2007). Based on isolates from contaminated produce from sick consumers, investigators matched the outbreak strain to environmental samples from a single field in central California. Organic spinach grown on this 2.8-acre plot was surrounded by an 8,000-acre plot of land primarily dedicated to cattle grazing (Jay et al., 2007). Environmental sampling revealed the presence of the outbreak strain in river water and the feces of cattle and wild pigs less than 1 mile away from the spinach field (Figure WO-3-1) (Berger et al., 2010; Jay et al., 2007). Because the contamination event occurred before the start of the investigation, the precise means by which the bacteria were transmitted to the spinach field remain unknown (Garcia et al., 2010).

***E. coli* O104:H4 contamination of fenugreek seeds.** In 2011, a rare strain of *E. coli* O104:H4 caused the second largest and the deadliest outbreak of *E. coli*-associated disease ever recorded. Between May 21 and July 22, 2011, more than 4,000 people became ill—in 16 countries—and 50 individuals died (Rasko et al., 2011) (Figure WO-3-2). By the time the outbreak ended in early July (2011), there were reports of more than 4,000 illnesses, 800 cases of HUS, and 50 deaths in Germany and 15 other countries (Blaser, 2011).

The outbreak was unusual because of the high proportion of adult patients (~25 percent) with HUS and the frequent development of neurological symptoms in these patients (Frank et al., 2011a). Research suggests that these clinical characteristics were due to the unique combination of traits carried by the pathogen, which included features typical of enteroaggregative^e *E. coli* and the capacity to produce Shiga toxin (Frank et al., 2011a). This strain also has a distinct set of additional virulence and antibiotic-resistance factors^f (Rasko et al., 2011).

continued

associated illnesses. As of March 2012, these “big six” non-O157 STEC serotypes will also be tested by certain food producers, such as beef producers. Food products contaminated with these bacteria will need to be destroyed or cooked to kill the bacteria (USDA, 2011a).

^d The resulting drop in sales and consumer confidence in the fresh spinach industry cost the \$3.5 billion dollar industry more than \$350 million (Weise and Schmit, 2007).

^e Enteroaggregative *E. coli* infections are common in humans, but no animal reservoir has been described (Rasko et al., 2011).

^f The strain produces extended-spectrum beta-lactamase (ESBL) enzymes and other factors that render it resistant to at least a dozen antibiotics in eight different drug classes.

BOX WO-3 Continued

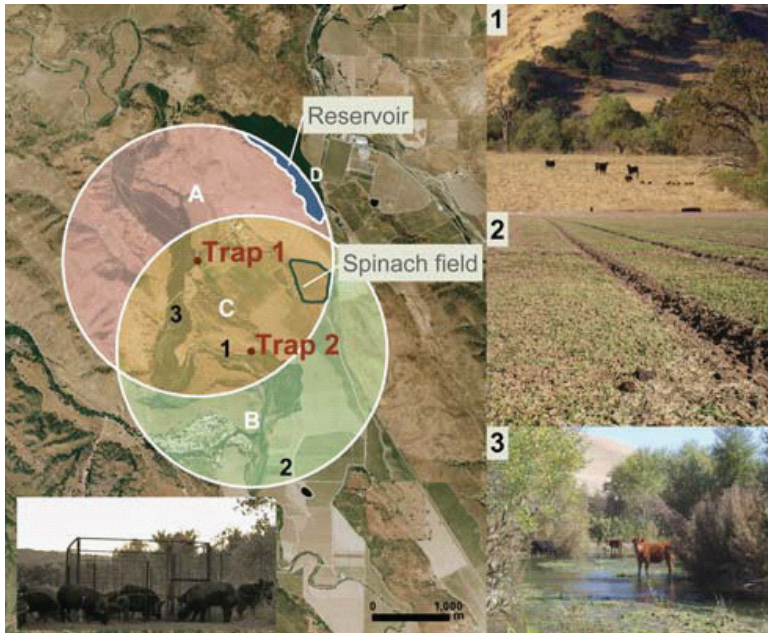


FIGURE WO-3-1 Left: Aerial (~15 km²) photograph of ranch A showing overlapping circular buffer regions around feral swine trap 1 and trap 2 (San Benito Crop Year 2006; Image Trader, Flagstaff, Arizona). The radius for the buffer (1.8 km) is the circumference of the mean home range for feral swine in mainland California. Estimated density = 4.6 swine/km² and total area = (A + B + C) – D = 14.8 km². Areas A, B, and C, combined with counts of individual feral swine from October through November 2006, were used to calculate the average population density. Bottom left: digital infrared photograph of feral swine at trap 1. Right: potential risk factors for *E. coli* O157:H7 contamination of spinach at ranch A: (1) feral sow and piglets sharing rangeland with cattle; (2) feral swine feces, tracks, and rooting in a neighboring spinach field; and (3) cattle in surface water. SOURCE: Jay et al. (2007).

Investigators initially identified fresh produce—including leafy greens, tomatoes, and cucumbers as likely sources of the outbreak (Frank et al., 2011b). Traceback studies of disease clusters in five German provinces that were affected early in the outbreak pointed to sprouts produced by an organic grower in Lower Saxony (Kupferschmidt, 2011). A smaller, second wave of illnesses around the French city of Bordeaux also resulted from the consumption of sprouts, and patient isolates from both outbreaks were identical (EFSA, 2011b). It was later discovered that sprout seeds associated with both outbreaks had a common origin

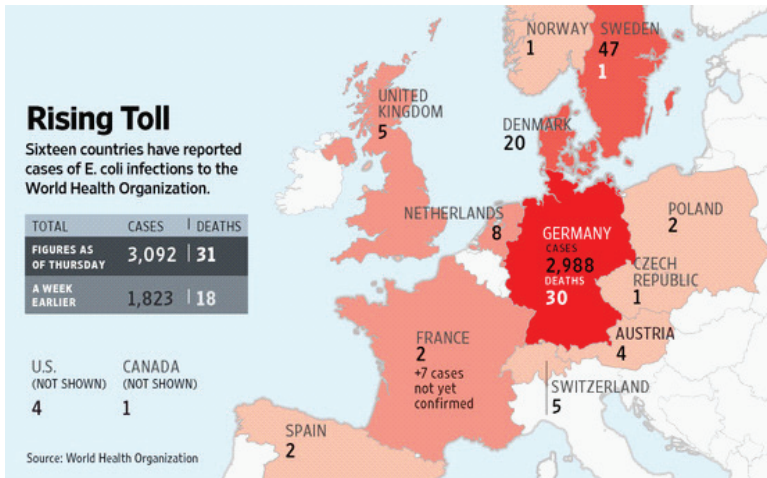


FIGURE WO-3-2 Incidence of HUS. Sixteen countries reported cases of food-borne illness or death associated with the 2011 *E. coli* O104:H4. The numbers of cases and deaths noted in this figure reflect the outbreak statistics as of June 9, 2011. SOURCES: Reprinted by permission of the Wall Street Journal. Copyright 2011, Dow Jones & Company, Inc. All Rights Reserved Worldwide.

in a 16.5-ton shipment of fenugreek seeds from Egypt (McKenna, 2011). Upon the shipment’s arrival in Germany in 2009, various distributors in Germany and other European countries subdivided, packaged, repackaged, and widely distributed these seeds as part of thousands of packets of “seed mixes” (McKenna, 2011). Despite extensive recall efforts, the complex chain of packaging and distribution may mean that contaminated seeds could remain on store shelves until their expiration date in 2014 (McKenna, 2011). The pathogen was not isolated from any remaining batches of the suspect seeds,^g and questions remain as to the source and reservoir of the contaminating pathogen (EFSA, 2011a).

Listeria monocytogenes

Listeria monocytogenes is a bacterium that is widely distributed in nature. It is commonly found in soil, surface water, plants, and foods and is carried by a variety of animals.^h Most infections are acquired by ingestion of contaminated food

continued

^g It is possible that contaminated seeds were no longer in stock when sampling took place, or even if present were contaminated at a level that made isolation of the organism impossible (EFSA, 2011b).

^h In addition to humans, at least 42 species of wild and domestic mammals and 17 avian species, including domestic and game fowl, can harbor *Listeria*. *Listeria* has also been isolated from crustaceans, fish, oysters, ticks, and flies. <http://textbookofbacteriology.net/themicrobialworld/Listeria.html>.

BOX WO-3 Continued

or feed, and infected animals can shed the bacterium in feces, milk, and uterine discharges (Jemmi and Stephan, 2006). In humans, *Listeria* infection can result in the relatively rare but dangerous disease Listeriosis, which has a case fatality rate of approximately 20 percent.ⁱ Disease primarily affects the very young or old and pregnant women, but it can also affect healthy individuals (CDC, 2011i). *Listeria* is well adapted to food-processing and storage environments. It can grow and multiply at low “refrigeration” temperatures and establish persistent infections on food-processing equipment (Ghandhi and Chikindas, 2007).^j *Listeria* is killed by pasteurization and cooking; however, in some ready-to-eat foods contamination may occur after factory cooking but before packaging. Deli meats, hot dogs, unpasteurized milk, and soft cheeses are common sources of *Listeria* infections (CDC, 2011i).

Listeria contamination of cantaloupe. As discussed earlier in this volume, one of the largest and deadliest multi-state outbreaks of listeriosis in the United States occurred in late summer of 2011. The incident marked the first time that *Listeria* contamination had been linked to whole cantaloupe and one of the few times it had been linked to fresh produce (Figure WO-3-3) (MMWR, 2011). As of December 2 (2011), 146 individuals had become ill after being infected with the outbreak strain of *Listeria*; 29 deaths and 1 miscarriage had also been attributed to the infection (CDC, 2011f). In response to the CDC outbreak investigation, the cantaloupe producer, Jensen Farms of Holly, Colorado, announced a voluntary recall of the 300,000 cases of cantaloupes harvested and produced between July 29 and September 10 (CDC, 2011f; FDA, 2011c). The recall included 1.5 to 4.5 million melons that were distributed at supermarkets and chain stores in at least 28 states.

Federal officials found four separate strains of *Listeria* on contaminated cantaloupes and equipment in the packing shed of the Colorado farm (CDC, 2011g; FDA, 2011b). FDA inspectors cited unsanitary conditions—such as old, corroded, and difficult-to-clean equipment and standing pools of water—and the absence of processing steps to cool the melons before cold storage as likely contributors to contamination (FDA, 2011b, 2011c). The bacterium was not found on fruit or soil in the fields, so questions remain as to the initial source of contamination.

Norovirus

Noroviruses^k cause the majority of acute viral gastroenteritis cases worldwide, including an estimated 5.4 million cases, 14,000 hospitalizations, and 149 deaths in the United States annually (Scallan et al., 2011b). Recent improvements to diagnostic techniques have allowed researchers to describe the signifi-

ⁱ Scallan et al. estimate that *Listeria monocytogenes* causes on average 1,591 episodes of domestically acquired food-borne illnesses, 1,455 hospitalizations, and 255 deaths annually in the United States (Scallan et al., 2011b).

^j *Listeria monocytogenes* may grow in biofilms that protect them against environmental stress and can be isolated from surfaces after cleaning and disinfection (Ghandi and Chikindas, 2007).

^k Also called Calcivirus, Norwalk-like virus, small round structured viruses (SRSVs).

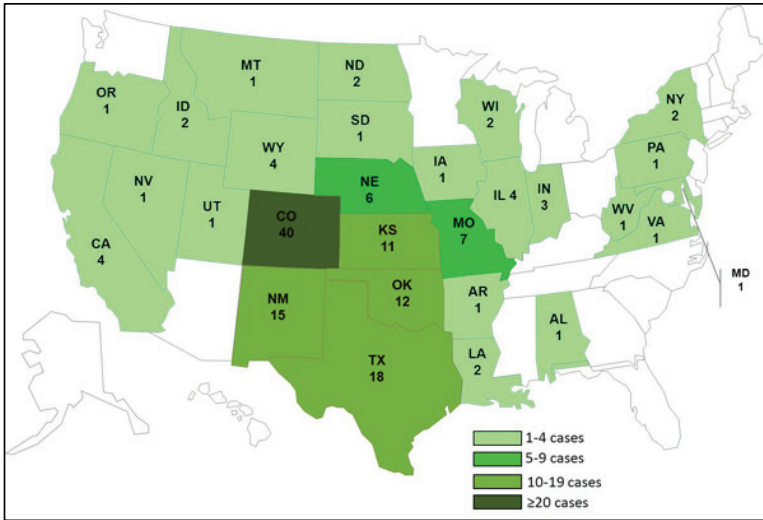


FIGURE WO-3-3 Persons infected with the outbreak-associated strains of *Listeria monocytogenes*, by state, n= 146 for whom information was reported to CDC on December 2, 2011. A total of 146 persons infected with any of the four outbreak-associated strains of *Listeria monocytogenes* were reported to CDC from 28 states. The number of infected persons identified in each state was as follows: Alabama (1), Arkansas (1), California (4), Colorado (40), Idaho (2), Illinois (4), Indiana (3), Iowa (1), Kansas (11), Louisiana (2), Maryland (1), Missouri (7), Montana (1), Nebraska (6), Nevada (1), New Mexico (15), New York (2), North Dakota (2), Oklahoma (12), Oregon (1), Pennsylvania (1), South Dakota (1), Texas (18), Utah (1), Virginia (1), West Virginia (1), Wisconsin (2), and Wyoming (4).

SOURCE: Centers for Disease Control and Prevention; National Center for Emerging and Zoonotic Infectious Diseases (NCEZID); Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)

cant contribution of this highly infectious RNA virus to the burden of food-borne illness—particularly as the cause of numerous outbreaks of food-borne disease in community settings such as nursing homes, hospitals, the military, and cruise ships (Estes et al., 2006; Glass et al., 2009).¹ Humans are likely to be the primary

¹ Among the 232 outbreaks of norovirus illness reported to the CDC from July 1997 to June 2000, 57 percent were food-borne, 16 percent were due to person-to-person spread, and 3 percent were water-borne; in 23 percent of outbreaks, the cause of transmission was not determined. Among these outbreaks, common settings included restaurants and catered meals (36 percent), nursing homes (23 percent), schools (13 percent), and vacation settings or cruise ships (10 percent) (CDC, <http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus-factsheet.htm>).

continued

BOX WO-3 Continued

reservoir^m for several norovirus strains, and transmission of the virus between individuals can occur in a variety of ways—via ingestion of contaminated food and water, person-to-person contact, or fecal–oral or aerosol spread (Koopmans and Duizer, 2004). Prevention of infection is difficult because these viruses can persist on environmental surfaces and food items. Comparison of norovirus sequences collected from around the world over the past decade have raised the possibility that pandemic strains of norovirus are spread through foods sold internationally, or through person-to-person contact when travelers carry the virus (Glass et al., 2009; Verhoef et al., 2011).

Norovirus outbreaks and cruise tourism. Organized much like large, floating hotels, cruise ships provide ideal conditions for the introduction and the rapid, global spread of norovirus infection. Thousands of passengers from different geographic areas are transported in close quarters to multiple destinations around the world. Passengers and crew often disembark at multiple ports throughout the cruise where they can sample the local foods and culture (Figure WO-3-4).

Cruise ships account for 10 percent of all reported outbreaks of norovirus in the United States (CDC, 2011). With the average carrying capacity of a cruise ship now exceeding 2,500 passengers and crew, these outbreaks often affect a large number of people. In 2010, outbreaks of diarrhea and vomiting among passengers and crew on the Celebrity Cruise ship “Mercury” occurred during three consecutive sailings. More than 10 to 22 percent of the passengers and 2 to 4 percent of the crew fell ill during each trip, resulting in a total of 1,058 cases of illness over the course of a month.ⁿ These outbreaks also have “off-ship”

^m Within the norovirus genus, there are two branches represented by animal strains, with bovine viruses in GII and murine noroviruses in GV. The GII and GIV genogroups contain both human and animal strains. This raises questions about zoonotic transmission. To date, there is little evidence for direct zoonotic transmission, but because mixing of genes from human viruses (by virus recombination) within a genogroup has been observed, the question arises whether it could also happen in recombination events with animal strains (Koopmans, 2008).

ⁿ <http://www.cdc.gov/nceh/vsp/surv/GIlist.htm#years>.

- In 1994, 138,000 gallons of ice cream were contaminated by *Salmonella*. This “single batch” of ice cream was consumed by individuals in 15 states, where it sickened an estimated 225,000 individuals (Hennessy et al., 1996).
- In 1996, 1,465 persons in 20 states, the District of Columbia, and two Canadian provinces became ill after consuming fresh raspberries that were imported from Guatemala and infected with the parasite *Cyclospora cayatanensis* (Tauxe, 2002). Following several additional outbreaks in 1997, Guatemalan producers temporarily suspended raspberry exports to the United States, which resulted in more than US\$10 million in losses for growers in this region (ERS, 2001).

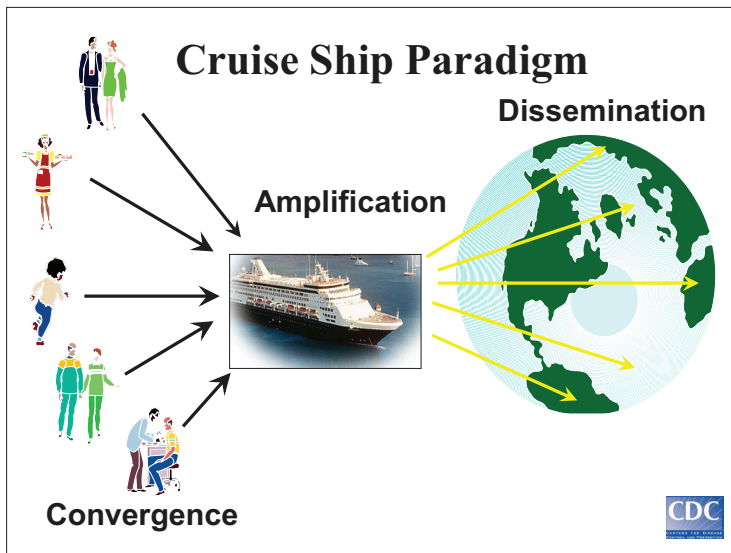


FIGURE WO-3-4 Cruise ships provide ideal conditions for the amplification and spread of infectious diseases.
SOURCE: Marty Cetron, CDC.

community-wide consequences, contributing to disease dissemination at ports of call (IOM, 2010c).

Infection can thus be introduced to the cruise ship environment in a variety of ways: by passengers or crew infected before embarkation; with food items contaminated before loading; by persistently contaminated environmental surfaces; or after ships dock in countries where sanitation might be inadequate—either through contaminated food or water, or via passengers that have been infected while ashore (Hall et al., 2005).

- In 2003, a series of hepatitis A outbreaks resulted in 1,000 cases of illness across multiple states and 3 deaths. The outbreaks were linked to green onions imported from four farms in Mexico where hepatitis A is endemic (FDA, 2003; IOM, 2006). FDA subsequently banned imports from these farms.
- In 2008, 1,450 individuals in 43 states and the District of Columbia became ill from salmonellosis and two patients died after consuming jalapeño and serrano peppers imported from Mexico. Investigations traced the contaminated peppers to one farm in Mexico, but the source of contamination is unknown (Maki, 2009).

- In 2009, *Salmonella* contamination of peanuts and peanut products led to one of the largest product recalls in U.S. history. More than 714 people in 46 states were sickened in this outbreak and 9 individuals died (Cavallaro et al., 2011). Investigators traced the contamination to a single facility that produced peanuts, peanut butter, and peanut paste. Because more than 200 companies used these foodstuffs as ingredients in a variety of other products,¹⁹ the recall extended to more than 3,900 products (Cavallaro et al., 2011).

Recent Efforts to Reduce the Threat of Food-Borne Disease

Tauxe divided the “farm-to-table” continuum into three stages in which risk for food-borne disease can be reduced: production, processing, and final preparation and cooking. Although, as he later noted, most food-borne pathogens are heat-labile and therefore can be inactivated by cooking, an increasing proportion of outbreak-associated foods are uncooked (e.g., produce), requiring attention to earlier stages in their procurement; other foods, including meats, are frequently cooked or served at temperatures insufficient to inactivate pathogens. Since a 1993 outbreak associated with hamburgers purchased from a fast food chain resulted in more than 500 laboratory-confirmed infections with *E. coli* O157:H7 and at least 4 deaths (CDC, 1993), several interventions have been introduced to reduce the contamination of beef during processing and in the retail and restaurant industries (FSIS, 2002).

Many recent food-borne outbreaks have been identified through PulseNet (CDC, 2011e), the national network for molecular surveillance of bacterial enteric infections, Tauxe explained. Established in 1996, PulseNet connects state health departments, city health departments, and laboratories of the CDC, FDA, and the USDA’s FSIS, all of which collect genetic information on food-borne pathogens from infected people, foods, and animals and submit it electronically to a common database, so that sudden increases in a particular subtype can be flagged and investigated. This network is in turn linked with similar databases created by the Public Health Agency of Canada (<http://www.nml-lnm.gc.ca/Pulsenet/index-eng.htm>), by U.S. veterinarians,²⁰ and by the food industry. A similar collaborative program coordinated by the CDC, FoodNet, conducts active surveillance for several major food-borne pathogens to measure burden and track trends over time (CDC, 2011b). Figure WO-12, which depicts trends in infections caused

¹⁹ Other products included brownie products, cake and pie products, candy products, cereal products, cookie products, cracker products, prepackaged meals, snack mix products, ice cream, pet food, and topping products (Maki, 2009).

²⁰ USDA VetNet commenced in March 2004. The objectives of USDA VetNet are to determine PFGE (Pulse Field Gel Electrophoresis) patterns of *Salmonella* isolates submitted to the National Antimicrobial Resistance Monitoring System (NARMS), compare USDA VetNet and PulseNet’s PFGE patterns, and to use the comparative data for surveillance and investigation of food-borne illness outbreaks. Source: http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=199378.

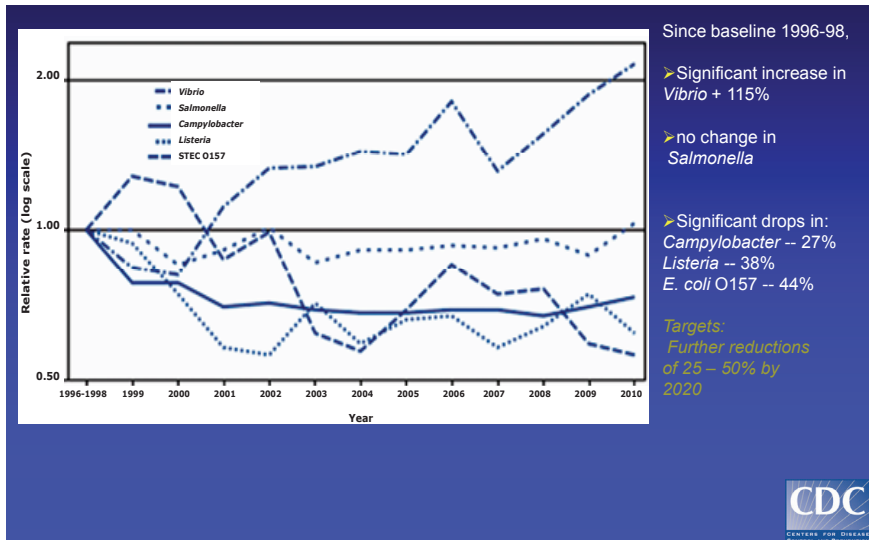


FIGURE WO-12 Fifteen years of progress in prevention: Trends in food-borne diseases, Foodnet, 1996-2010.

SOURCE: Tauxe (2011).

by some of these pathogens over the past 15 years, reveals significant decreases in *Campylobacter*, *Listeria*, and *E. coli* O157 cases, little change in *Salmonella* cases, and a significant increase in *Vibrio* cases.

Tauxe attributed the significant subsequent decrease in *E. coli* infections to these measures, which he said were achieved through a combination of regulatory, industry, and public health efforts. In a later discussion of the response to the threat of *E. coli* O157:H7 in meat, speaker Cathie Woteki, Chief Scientist and Under Secretary for Research, Education, and Economics at the USDA, attributed the subsequent decline in such illnesses in part to the introduction of Hazard Analysis and Critical Control Point (HACCP) systems²¹ and their implementation by the ground beef industry. “HACCP by its nature is a holistic approach to an environment where food is processed,” she said.

Meanwhile, Tauxe observed, few measures have been taken to prevent food-borne infections at the level of production, a stage emphasized by One Health. Returning to the *E. coli* O157:H7 example, Tauxe noted that several production-stage interventions against food-borne disease—including two vaccines and a

²¹ HACCP systems are science-based, systematic protocols for identifying hazards to food safety that arise in the course of processing a specific food, as well as measures for the controlling these hazards. HACCP is intended to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. Source: <http://www.fao.org/DOCREP/005/Y1579E/y1579e03.htm> (accessed June 27, 2012).

promising feed additive—had been developed, but not adopted, in the United States. This is not due to a technical impasse, Tauxe stated, but to challenges in gaining regulatory approval—as well as the need for incentives to offset costs to producers—for animal products that benefit human health.

On the other hand, Tauxe recognized farm-based control efforts in other countries that have significantly reduced poultry-associated *Campylobacter* and *Salmonella* infections (for example, see the subsequent discussion of Danish efforts to reduce antimicrobial-resistant infections). In Iceland, all chicken flocks are tested for *Campylobacter*, he reported; those that test positive must be used only to produce frozen meat, considerably reducing potential profits. “The year after [this measure] was introduced, domestic *Campylobacter* dropped 70 percent in Iceland,” Tauxe said (Tustin et al., 2011). He also described a voluntary program of flock sanitation, hygiene, and vaccination that dramatically reduced *Salmonella* in egg-layer and broiler breeder flocks in the United Kingdom, and noted that similar steps are being considered in the United States (DEFRA, 2008).

Progress over the past 15 years in reducing the risk of food-borne disease has largely resulted from improvements in post-slaughter or post-harvest practices, Tauxe concluded. “We are very pleased to see that *E. coli* O157 is essentially half of what it was in the 1990s,” he said, “but contamination often starts before harvest or slaughter. Interventions in food animals exist. They have worked in other countries. Implementing them may depend on getting the incentives right.”

*Enterohemorrhagic*²² *E. coli* (EHEC) O104:H4

Reinhard Burger, of the Robert Koch Institute, discussed the largest outbreak of hemolytic-uremic syndrome (HUS) ever reported in the world, caused by a Shiga toxin-producing strain of *E. coli*, that occurred during the summer of 2011 (Buchholz et al., 2011). (Dr. Burger’s contribution to the workshop summary report can be found in Appendix A, pages 115-130.) “The events this (past) summer in Germany show how rapidly—literally over a weekend—an infectious agent can develop into a major health threat for a whole country,” he said of the outbreak, which was focused in Germany but which also affected several other European countries, the United States, and Canada. The outbreak, which resulted in many severe cases of illness and dozens of deaths, caused fear and changed basic eating habits among consumers, and had enormous economic consequences for farmers, he reported. “It was literally a tragedy for many people,” he concluded. “We should learn from this critical event.”

²² Enterohemorrhagic strains of *E. coli* (EHEC) produce compounds known as Shiga toxins because of their similarity to those produced by another enteric pathogen, *Shigella dysenteriae* Type 1. EHEC is transmitted to humans primarily through consumption of contaminated foods, including raw or undercooked ground meat products, raw milk, and contaminated raw vegetables or greens. Most people with EHEC infections recover within 10 days, but up to 10 percent of patients—especially young children and the elderly—develop HUS, a potentially life-threatening condition (WHO, 2011).

Approximately 4,321 people became infected with EHEC during this outbreak, of which more than 850 developed HUS—a much higher rate of progression to this life-threatening condition than is typical of such infections—and 53 died, Burger reported. Also atypical was the population affected by the disease, which is usually limited almost exclusively to children: 90 percent of the outbreak infections occurred in adults, among whom there was a preponderance of young females, he said.

By the time the local authorities in Hamburg recognized that an outbreak was occurring and alerted Burger and his colleagues, the epidemic had already peaked, he said—a conclusion they reached after conducting a comprehensive range of epidemiological studies. “The first call came on Thursday, May 19,” he recounted. “The next day, the first team went to Hamburg, discussed it. We informed other agencies. On Sunday, we reported to the early-warning response system and gave the first interview that vegetables may be involved. On Tuesday, four days later, we had the first official press conference. On Wednesday, the pathogen was identified” (Frank et al., 2011a, 2011b).

At a press conference that day, the Robert Koch Institute warned against the consumption of raw tomato, cucumber, and salad in northern Germany, based on their early findings, Burger recounted. “The next day, most newspapers wrote ‘from northern Germany.’ Of course, this caused major concern with all the farmers, and the economic consequences were immediately clear.” Later, without consulting Burger or his colleagues, a German Minister of Health in Bremen associated Spanish cucumbers with the source of the outbreak, he said, causing an immediate drop in the sale and import of Spanish cucumbers along with frictions between Spain and Germany. The Spanish farmers who suffered from this mistake were eventually compensated for their losses. These circumstances led Burger to wonder aloud, “How do you communicate the risk—and also the uncertainty—in such exceptional situations? The demand for information was enormous,” he observed. “To inform reliably, to the best of present knowledge, without losing credibility and convincing the people that it’s appropriate, this was a challenge, which one should really be aware of in advance of such crisis situations.”

Cohort studies of groups of people who became ill (such as a team from a Swedish company who stayed a short time in Germany, so it could easily be determined where they stayed and what they ate) helped identify sources of contaminated food items, Burger recalled. Ten such cohorts, comprising 168 people, were found to have eaten at a particular restaurant within the likely time of infection, he explained; the 31 people among them who developed bloody diarrhea or HUS within 14 days of their visit to the restaurant were questioned as to what they had eaten, and the common ingredient in every meal (as identified by the chef) was found to be sprouts (Buchholz et al., 2011).

However, Burger continued, because bean sprouts are often a mixture, the specific type of sprout involved in this outbreak remained unknown.

Then unexpected help came in the form of another outbreak near Bordeaux, France, which involved one type of sprouts, grown from fenugreek (*Trigonella foenum-graecum*) seeds by individuals in their own homes. Epidemiological investigation linked a single Egyptian supplier of fenugreek seeds to both French and German outbreaks (Buchholz et al., 2011).

This discovery did not surprise speaker Michael Doyle, of the University of Georgia, who in his prepared remarks showed electron micrographic evidence that fecal bacteria can enter cracks in the hard coats of seeds and flourish inside (Michino et al., 1999; Scallan et al., 2011a). (Dr. Doyle's contribution to the workshop summary report can be found in Appendix A, pages 140-175.) Many food microbiologists consider sprouts to be one of the most hazardous of foods, he observed. Since 1988, dozens of sprout-associated food-borne disease outbreaks caused by *Salmonella* and *E. coli* have been reported, he stated. As a result, FDA has recommended that pregnant women, the elderly, and immunocompromised women should not consume raw sprouts—and in his opinion, Doyle added, neither should anyone else.

The pathogen responsible for the 2011 outbreak, EHEC O104:H4, was isolated from patients for characterization but has yet to be isolated from sprouts or seeds, Burger reported. It is a rare serotype, previously identified only a few times in humans and never in animals (Bielaszewska et al., 2011). This observation allowed epidemiologists to rule out meat and dairy products as possible vehicles of this outbreak. Unexpectedly, he noted, in addition to expressing Shiga toxin, EHEC O104:H4 was found to be antibiotic resistant because of the expression of ESBL (Rasko et al., 2011). Therefore, it has been hypothesized that this strain acquired its increased virulence from two independent events (Brzuszkiewicz et al., 2011). "It's obviously a virulence combination of two different *E. coli*," Burger concluded. When asked in the subsequent discussion about the possibility that this outbreak resulted from an intentional release of the pathogen, Burger replied that this scenario had been considered but was dismissed as unlikely. Only one researcher was in possession of the outbreak strain prior to this event, he said, and it is doubtful that the strain could have been produced independently.

As illustrated schematically in Figure WO-13, the supplier of the tainted fenugreek seed distributed more than 15,000 kilograms of seed from the same lot (lot number 48088) to companies throughout Europe, which in turn distributed it further, including to people who grow their own sprouts at home, Burger said. Given the enormous interconnectedness of the distribution and supply chain for just one lot of fenugreek seeds it is unlikely that all the contaminated seed has been removed from the supply chain with hundreds of distributors. Furthermore, *E. coli* can survive on seeds for years, serving as a potential source for future infections. The pathogen can also be shed from infected individuals for more than 6 months, and it may be possible that some infections persist, creating carriers. Secondary infections—of the sort that have already been identified within households, hospitals, and laboratories—may also continue into the future, he

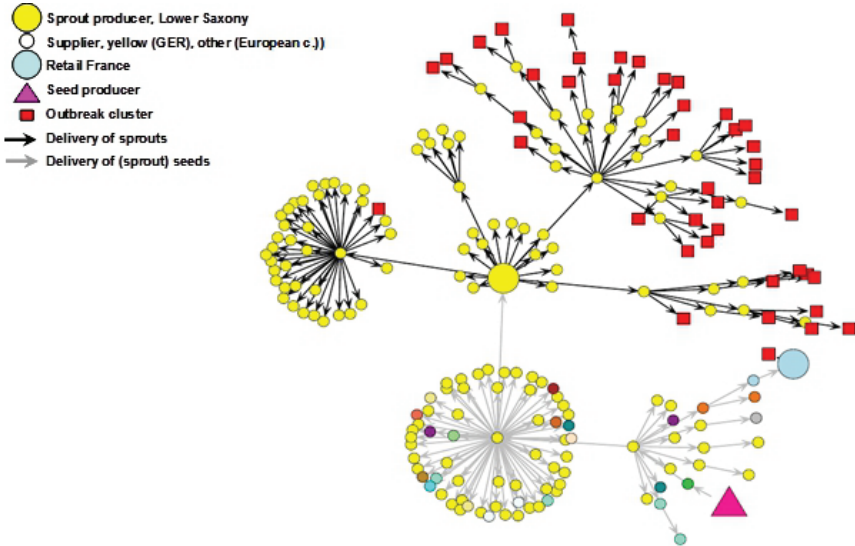


FIGURE WO-13 EHEC outbreak 2011: Investigation of the outbreak along the food chain. SOURCES: Published by B. Appel, G.-F. Böhl, M. Greiner, M. Lahrssen-Wiederholt and A. Hensel, BfR, Federal Institute for Risk Assessment Max-Dohrn-Str. 8-10, 10589 Berlin; Burger (2011); Weiser et al. (2011).

observed. At a press conference at the end of July (2011), Burger declared that the outbreak had ended. However, he added, “there are still one or two cases per month, always secondary cases connected to previous cases.”

Nipah Virus in Malaysia and Bangladesh

The emergence of Nipah virus (NiV) in Malaysia and Bangladesh provides particularly deadly examples of the many routes of zoonotic disease transmission that are associated with the food system. The animal reservoir for this paramyxovirus is fruit bats of the genus *Pteropus* (Halpin et al., 2011). In the 1990s, the development of large commercial pig farms in Malaysia expanded agricultural lands into the natural habitat of the fruit bat. The resulting increase in interactions between swine and fruit bat populations—including materials contaminated with the saliva or urine from fruit bats—led to an outbreak of disease in swine and humans (Epstein et al., 2006). Although unknown to science before this outbreak, NiV had been circulating in fruit bats for several decades (Epstein et al., 2006).

Exposure of this virus to large numbers of swine facilitated the amplification of NiV in the respiratory tracts of swine and the infection of farm workers

(Epstein et al., 2006). From September 1998 through May 1999, 283 human cases of NiV infection were reported in peninsular Malaysia and Singapore, and most of these patients had come into contact with sick pigs, as illustrated in Figure WO-14 (Chew et al., 2000; Chua, 2003; Luby et al., 2009; Parashar et al., 2000; Paton et al., 1999).

The Malaysian Nipah outbreak ended following the culling of more than 900,000 pigs (Uppal, 2000). This action, plus the loss of market for Malaysian pork in response to the outbreak, decimated the Malaysian swine industry. There have been no new cases of Nipah virus reported in Malaysia or Singapore since the 1998 to 1999 outbreak (Epstein et al., 2006). Between 2001 and 2008, recurrent NiV outbreaks in Bangladesh have caused at least 135 human infections and 98 deaths (Luby et al., 2009).

First recognized as the result of a 1999 outbreak in Malaysia, Nipah virus has since been more frequently associated with Bangladesh and adjacent areas of India, where many outbreaks over the past decade have resulted in more than 250 cases and nearly 200 deaths, according to speaker Steve Luby of the CDC (Luby et al., 2006, 2009). (Dr. Luby’s contribution to the workshop summary report can be found in Appendix A, pages 271-298.) The Malaysian outbreak, which claimed more than 100 lives—about 40 percent of those known to have been infected with the virus—was first traced to direct contact with infected pigs, which in turn were likely infected by bats living in forested areas close to large commercial pig farms (Epstein et al., 2006).

“We think that the bats were eating fruit, including the fruit from trees that had been intentionally planted near the piggeries to provide food for the pigs, as well as for separate agricultural production,” Luby explained. “Partially

Malaysia Nipah Outbreak



- September 1998 – May 1999
- 283 human cases of acute encephalitis
 - 109 deaths
 - Case fatality ratio 39%
- Paul Chua isolated a novel paramyxovirus from a patient in Sungai Nipah village

FIGURE WO-14 Malaysia Nipah outbreak.

SOURCE: Luby (2011); Adapted from CDC.

eaten saliva-contaminated fruit, as well as bat urine, would be dropped into the piggeries. The pigs became sick. We had a big pig outbreak, and then eventually this went on to people.” Mango and pork production had skyrocketed in Malaysia in the years preceding the outbreak, he said, bringing together bats carrying Nipah virus, pigs, and humans.

Uncovering the story of Nipah virus transmission in Bangladesh proved to be much more complicated than in Malaysia, Luby explained. Beginning with the first reports in early 2005, Nipah cases in Bangladesh tended to be clustered in space and time. A case-control assessment of a broad range of possible exposures shared among the first 12 cases of viral encephalitis (among which all but 1 died) revealed that these people were far more likely to drink raw palm sap than were healthy controls (Luby et al., 2006).

Date palm sap collection, which occurs from late November through March in Bangladesh, involves cutting into and shaving the tree so that the sap, which rises overnight, flows into clay pots hung beneath the cuts, he said; the pots are gathered early in the morning. The collection of date palm sap is illustrated in Figure WO-15. Most of the sap is then cooked into molasses, but some is sold immediately as a drink and is considered to be a delicacy.

One of the fatal cases, in 2005, was the son of a date palm sap collector. His family reported having heard bats in the trees from which they were collecting sap, and they had found bat excrement on some of the collecting pots, Luby said. Several days before the outbreak, the family sent date palm sap to nearby relatives; three people in that household were also among the cases in that outbreak. “As we sorted this out, we said, we’re epidemiologists, so we’re going to put this in an epidemiology journal and we’re going to talk about food-borne transmission of Nipah virus,” Luby recalled—but their conclusion was questioned by microbiologists, who noted that the virus had never been found in food, and wanted

- Late November through March

- Sap harvesters cut a tap into the tree
 - In the evening they place a clay pot under the tap
 - Each morning the pot is removed
- Most sap is made into molasses
- Some sold fresh early in the morning
 - A local delicacy



FIGURE WO-15 Date palm sap collection.

SOURCE: Luby (2011).

the words “Evidence for” prefacing the title, “Foodborne transmission of Nipah virus, Bangladesh” (Luby et al., 2006).

As Nipah recurred in Bangladesh, Luby and his colleagues continued to collect epidemiological evidence linking Nipah infection to date palm sap consumption. “We knew that *Pteropus* bats occasionally shed Nipah virus RNA in their saliva,” he recounted. “We knew that if you put Nipah virus into fruit juice, it would survive for days at 22 degrees. We knew date palm sap had been implicated in outbreak investigations, and we knew that it was almost impossible to isolate the virus in the sap. By the time we knew of an outbreak, by the time we implicated sap, by the time we occasionally could figure out which tree it came from, this would have been weeks since the transmission event. So, yes, we looked for Nipah virus in sap, and we never found it.” Then, a veterinarian colleague suggested a different approach: using infrared cameras to monitor nocturnal bat activity around sap collection sites (Khan et al., 2011; Rahman et al., 2011). “Sure enough, we could see bats coming in,” Luby said; a typical tree would get 49 bat visits, during which they drank sap an average of 29 times. The experiment to monitor the nightly visitations of bats to drink date palm sap is presented in Figure WO-16.

This discovery prompted Forum member Gerald Keusch, of Boston University, to remark on the growing recognition of the role of bats as carriers of infectious diseases, and to suggest that epidemiological surveillance should be conducted on bats to identify prospective human pathogens. Forum member Fred Sparling, of the University of North Carolina at Chapel Hill, noted that this idea

- Identified tree 500 meters from *P. giganteus* roost
- Mounted silent infrared wildlife camera
- 49 bats visited the tree
 - 26 drank sap from the shaved part of the tree



Photo by Salah Uddin Khan

FIGURE WO-16 How often do bats visit date palm trees to drink their sap?

SOURCE: Luby (2011). Photo by Salah Uddin Khan.

spurred a recent metagenomic analysis of viral sequences present in the feces of North American bats (Donaldson et al., 2010). The authors identified a wide variety of both known and novel viral sequences, suggesting that bats encounter and disseminate a large assortment of viruses capable of infecting many different animals, insects, and plants in nature.

“The molecular evolution of the Nipah virus suggests that it coevolved with the bat over the course of the last 10,000 years,” Luby stated. Bats carry both Nipah and Hendra viruses, apparently asymptotically. Occasional human infections—which have probably occurred whenever human and bat populations have been in close contact—represent “collateral damage” in the co-evolution of bats and these viruses, he explained; this relationship is only now being recognized due to advances in epidemiological surveillance and global communications.

Transmission pathways associated with Nipah and Hendra viruses are clearly complex and much remains to be understood about them, Luby observed. Their epidemiological investigations in Bangladesh have identified drinking fresh date palm sap as the most frequent pathway of viral transmission from bats to humans (Luby et al., 2009). Other outbreaks arose when bats transmitted the virus to domestic animals (as occurred in Malaysia; in this case, the vehicle was sometimes date palm sap fed to animals). About half of all Nipah infections in Bangladesh resulted from person-to-person transmission, he added; in many of these instances, people who were caring for infected relatives themselves became infected, producing clusters of disease.

Luby observed that since farms in Bangladesh tend to be small, in contrast to the large commercial pig farms where Nipah emerged as a widespread zoonosis in Malaysia, the Nipah outbreaks in Bangladesh have tended to be localized. In the course of investigating transmission to humans through domestic animals, Luby and coworkers discovered that some cattle and goats living near outbreak areas several years later—especially those that were regularly exposed to bats—had antibodies against both Nipah and Hendra viruses. They were also found to have apparent cross-reactivity against another unknown virus of the same (Henipah) family. Bats shed Nipah virus intermittently, he noted. Research is under way to determine factors that influence periodic viral shedding.

Luby and coworkers have also attempted to devise methods to prevent bats from contaminating date palm sap. One method, already used in parts of Bangladesh, involved applying lime to trees around the collection sites, but this did not deter bats from drinking sap, as infrared photographs revealed. A physical barrier proved more successful, he reported; bats did not visit trees with “skirts” made of polythene, or of bamboo and other readily available materials, whereas control trees received thousands of bat visits (Khan et al., 2011). Attempts to get palm sap harvesters to adapt this technology have received mixed results, he observed. “People are willing to try it for a while, particularly on that minority of trees that they are interested in drinking fresh sap out of,” he said. “But we are concerned

about long-term acceptability and uptake, and about how we would roll this out to all producers. These are issues that [we] are still studying.”

The emergence of Nipah virus offers several relevant lessons to One Health and food safety, Luby observed. First, it illustrates that food is produced in the environment, and so shares environmental pathogens, he said. Second, spillovers of virus to humans occur through a confluence of multiple factors in a complex, dynamic system. Third, while Nipah and related viruses have had little impact on the United States to date, their potential for both genetic instability and respiratory transmission bear watching. “It is certainly conceivable that somebody, while incubating the illness, could step on an airplane,” he acknowledged. The discovery of cross-reactive antibodies in domestic animals suggests the existence of additional henipaviruses that may present emerging threats to human health.

Finally, Luby added, “I think the whole process of working on this for several years also illustrates the value of interdisciplinary research and what we call the public health cycle, the idea that we are doing surveillance for serious disease. When we find it, we do outbreak investigations. We work to identify risk factors, to mount interventions, to evaluate those interventions, even when those evaluations are not quite as resoundingly successful as we would like.”

Challenges in Food-Borne Pathogen Detection

One workshop participant noted in discussion, that in the case of both the German EHEC outbreak and the Nipah virus epidemic in Bangladesh, investigators were unable to isolate the pathogen from the suspected food source. This observation led to a discussion of sampling and testing strategies for food-borne pathogens.

“It’s actually not a typical event to identify the organism in the implicated food,” Tauxe stated. There are several reasons for this, he explained: food is transient, food-borne organisms are transient within foods, and many food-borne pathogens are resistant to extraction and culture from food sources. Thus, he said, “over the last decades, one of the most important advances we have been able to make is to get regulatory action to occur, industry action to occur if we have strong epidemiologic implications and a traceback to a particular source, without necessarily requiring that [the causative agent] be isolated from the food. If we [were to] require that, there will be far less protective action.”

“Prompt regulatory action” is an oxymoron, Hueston responded. “Regulatory action requires that you meet an administrative law level of evidence . . . [whereas] voluntary compliance, building a trusting relationship between the food industry and public health, has a much higher likelihood of achieving prompt action early in an epidemic and preventing illness and saving lives.”

This situation underscores the importance of integrating all kinds of evidence in the course of investigating food-borne disease, Luby argued. “We need to look broadly at the whole story we’re telling in order to reach reasonable scientific

inference,” he said. “I think that’s part of what the United States has done in terms of moving to regulatory action in the absence of microbiological confirmation in source food.”

Given the rapid evolution of molecular technologies and the continued lag in capability of recovery of food-borne pathogens by cultivation, one should expect that molecular and sequence-based data will be increasingly used to link exposures to causative agents and hosts, observed Forum chair David Relman, of Stanford University. The benefits of this approach include improved sensitivity and more information, but there are also drawbacks. While whole-genome sequencing technologies will be able to detect extracted DNA, it will be difficult to pinpoint which organism in a complex mixture of genes and gene fragments revealed by such techniques is the actual causative agent of the epidemic.

“If you just look at the sequence data in isolation, as you suggest, it is actually very hard to interpret,” Luby agreed. “But if we know that we are dealing with an implicated food, if we have anecdotal evidence, if we have epidemiologic evidence . . . then you are really looking at your microarray data very differently,” he added. This implies a greater need for interdisciplinary collaboration, he continued. “I see it as not just an internal bioinformatics microbiological issue, but more broadly, whether we can tell coherent stories.”

Tauxe noted that the CDC has used molecular subtyping of food-borne pathogens for many years as part of public health surveillance of food-borne diseases to detect and investigate outbreaks that would otherwise be missed. Now, concerns have arisen regarding the transition in the tests that clinical laboratories will use to diagnose these infections from isolating the organism in culture to diagnosis based on detecting antigen or sequence. “When rapid culture-independent diagnostic tests come into play and diagnosis is made on the basis of something that doesn’t yield a culture, we will lose what we have now, unless we can replace that with something that is also sequence-based, that also depends on the same sample, and perhaps something that can even be integrated with clinical diagnosis,” he said. “We’re going to be in a transition period . . . [during which] we are going to still depend on routine microbiology for our surveillance and our testing of foods and so forth, because we need the specificity of the sub-typing we get from [culturing] the living organism. But . . . new methods have to be developed that are going to let us move to a probably swifter and probably finer-grained system of surveillance in the future.”

“Food-borne pathogens are ubiquitous,” Hueston observed. “Everybody in this room will be exposed to food-borne pathogens today. If you come up with a fancy enough test and test for a large enough number of pathogens, you’re going to find them in everything.” Moreover, he asserted, “we can’t test our way to food safety.” Instead, he advised, people from a range of disciplines—epidemiologists, food scientists, and business people—who understand the constellation of factors that result in food-borne disease should devise strategies to minimize risk, he said; that would be One Health in action. “The vast majority of the food-borne

outbreaks of which I'm aware have a huge human component," Hueston added. "We are completely underestimating and missing the opportunity to work effectively with the human component."

Food-Borne Vehicles and Pathogens: Illustrative Challenges

Workshop presentations describing the roles of plant products as vehicles associated with food-borne disease, viruses as food-borne pathogens, and antimicrobial resistance as a driver of food-borne infections offered insights into many of the critical challenges to be addressed, and opportunities to employ a One Health paradigm.

Plant Foods

As described earlier, this country imports more than 75 percent of its fresh fruits and vegetables, annually (FDA, 2011a). Upon arrival, these products—along with domestically produced foodstuffs—are typically distributed hundreds or thousands of miles across the country from central distribution or processing facilities. Food distribution networks are designed to rapidly transport perishable goods, to provide just-in-time restocking of non-perishable items, and to take advantage of economies of scale (Sobel, 2005).

This system of multiple food "inputs" of diverse—and frequently foreign—origin, quickly dispersed over an elaborate network of processors, distributors, and purveyors to a public with increasingly broad tastes and immense purchasing power, is staggering in its scope, scale, and complexity. It also represents a vehicle for rapid and widespread distribution of food-borne disease, a situation that may delay recognition of an outbreak and impede timely identification of the source (Sobel, 2005). Even more challenging, the U.S. food supply offers countless opportunities for intentional contamination, many of which would be difficult to trace back to their "origin" because of the intricacies of food production and distribution networks.

Both Tauxe and Doyle discussed the nature, scope, and environmental sources of plant food-borne disease. As previously noted by Tauxe, produce and other plant-associated products are important vehicles of food-borne illness in the United States. According to Doyle, about one-third of produce-associated outbreaks of food-borne disease are attributed to leafy greens contaminated with norovirus (discussed in greater detail below) or with EHEC (WHO, 2011). Following leafy greens, melons (mainly cantaloupe) and tomatoes are the next most common outbreak-associated plant food vehicles, he said; the contaminant in these cases is often *Salmonella*. Doyle also noted the growing importance of spices as a vehicle for food-borne disease. "Seventy-five percent of our spices come from eight countries," he reported. "These are developing countries, and

if you saw how these things were grown, harvested, dried, and transported you might not eat spices anymore.”

Food-borne pathogens generally make their way onto plant surfaces through direct or indirect contact with animal manure or human feces, Doyle stated. He noted that several different species of food-borne pathogens (e.g., *Salmonella*, *Campylobacter*, EHEC, *Cryptosporidium*, and *Listeria*) are naturally and harmlessly present in the digestive tracts of animals and shed in their feces, in which pathogens can survive for months to years. A recent study in the United Kingdom found that fresh and stored manure samples from cattle, swine, poultry, and sheep frequently contained EHEC, *Salmonella*, and/or *Campylobacter* (Hutchison et al., 2004). In 1997, 5 tons of animal manure were produced for every person living in the United States, he reported.

Plant-derived foods become contaminated with pathogens by one of four primary routes, Doyle explained:

- as a result of wildlife incursion into growing areas;
- through the use of contaminated irrigation or processing water;
- through the use of human or animal feces as soil amendments; or
- from infected humans who handle the food (e.g., food handlers infected with food-borne viruses, as discussed below).

Numerous studies suggest that pathogens can easily travel any of these routes, Doyle observed. A wide range of animal species—including feral pigs and boars, deer, coyotes, rabbits, skunks, rodents, birds, reptiles, and insects—have been found to function as carriers for various food-borne pathogens.

Plants frequently come into contact with pathogens through contaminated water. “You would be surprised how prevalent *Salmonella* and *E. coli* O157 can be in environmental water sources,” Doyle observed. Among the results of several studies of both domestic and foreign environmental water sources he presented, Doyle noted that nearly 60 percent of river water tested in Canada, and 80 percent in the state of Georgia, was positive for *Salmonella*. EHEC, though not found as frequently as *Salmonella* overall, was nonetheless detected in up to 15 percent of ponds and creeks tested in Brisbane, Australia, he reported. Protozoan parasites, including *Cryptosporidium* and *Giardia*, have been found in high concentrations in irrigation water in Mexico and the United States. Doyle observed that global trade in produce means that local water problems can result in food-borne disease anywhere in the world.

Doyle suggested that reducing the fecal shedding of harmful microbes by animals should be a focus of a One Health strategy to improve food safety. This is especially important given the phenomenon of so-called super-shedders: the 10 percent or so of cattle that excrete the majority of *E. coli* O157:H7, he said. “Those are the ones we have to either vaccinate or use probiotics or a variety of

other practical interventions that can be applied on the farm,” he observed. In addition, animal waste must be managed to improve containment and control of pathogens, according to Doyle, and to ensure that they are inactivated before manure or its by-products are applied to soil.

There are also many pathways by which pathogens can enter the interiors of plant tissues, including fruits and seeds, Tauxe pointed out, either passively or actively. Once internalized, these microbial contaminants can persist or multiply; they cannot, however, be washed off or inactivated by surface treatments. “A bruise on a tomato is a great place for *Salmonella* to grow,” he said. “So a bruise is actually not just a quality issue; it’s a safety issue.”

Pathogens may be passively internalized during produce processing, Tauxe said. This occurred in 1999, when mangoes imported to the United States from Brazil were treated to kill possible Mediterranean fruit fly by dipping them in hot water, after which they were chilled in a cold-water bath. “The problem was, the cold water was not treated, nor potable nor, in fact, clean,” he stated; rather, it was contaminated with *Salmonella* Newport, which infected 78 people in 13 states (Penteado et al., 2004; Sivapalasingam et al., 2003). “If you take a hot fruit and put it in a cold bath, the internal spaces contract . . . and the fruit takes up fluid through the stem scar, the calyx, or other pores, and any bacteria in the water are drawn in,” he explained. “This general phenomenon has been demonstrated for a variety of fruits.”

Active internalization of pathogens into plants can also occur, Tauxe continued. For example, he noted, an electron micrographic study of *Salmonella* distribution on fresh lettuce leaves shows that the bacterial cells are distributed randomly over the leaf surface during the night, while in daylight, they are concentrated near the stomata, where metabolic products of photosynthesis are released (Kroupitski et al., 2009). Stomata typically close in response to bacterial flagella, he pointed out, but *Salmonella* and other bacteria, including *E. coli* O157:H7, can manipulate leaf stomata to open them and get inside the plant tissue as illustrated in Figure WO-17 (Melotto et al., 2006; Saldaña et al., 2011).

The presence of a specific stoma-opening factor—which apparently serves no role in the pathogen’s animal hosts—raises the possibility that some enteric pathogens have a two-host life cycle, involving both plants and animals, Tauxe said. While herbivores are generally considered to be a reservoir for enteric pathogens, plants may be part of the cycle if they are colonized by pathogens excreted by herbivores. “That might make evolutionary sense, because the plant, producing edible materials, is then eaten by the herbivore, and if the bacteria can ride this cycle, then they can move around and they can colonize the next generation of plants and the next generation of herbivores,” he observed. A better understanding of this relationship may present new opportunities to interrupt pathogen transmission “upstream” of human consumption of either plant- or animal-derived food, he concluded.

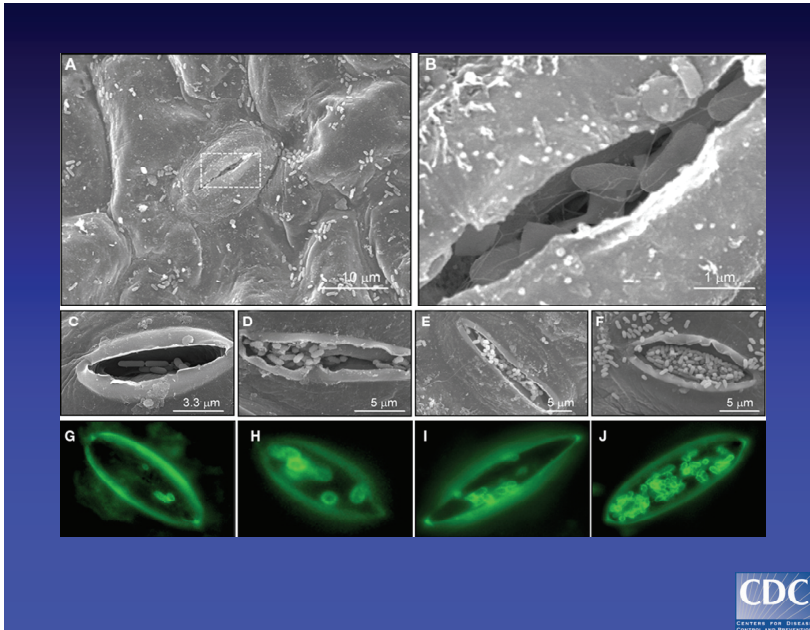


FIGURE WO-17 Bacteria manipulate leaf stomata and get inside. (A) Scanning electron micrograph showing bacteria on leaf epidermis at 6 h of infection. (B) High magnification of boxed area in (A) showing flagellate bacteria internalized in the stomata. (C-F) Micrographs (60X) of time-course EDL933 infection experiments between 3, 6, 12, and 24 h showing progressive association of bacteria with stomata. (G-J) Same experiment as before employing IFM and anti-0157 antibodies to stain bacteria (green).

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Food-Borne Viruses

According to speaker Marion Koopmans, of Erasmus University in Rotterdam, Netherlands, there is evidence for food-borne transmission for members of many virus families (Duizer and Koopmans, 2008). (Dr. Koopmans' contribution to the workshop summary report can be found in Appendix A, pages 225-251.) As shown in Figure WO-18, viruses tend to follow one of three routes to cause food-borne disease:

- through infected food handlers;
- through contamination during production (e.g., of irrigation water); and,
- through zoonotic transmission from an animal reservoir (e.g., a wild animal, consumed as bush meat).



FIGURE WO-18 Grouping of (potential) food-borne viruses.

SOURCE: Microbial Risk Assessment Series: Viruses in Food (WHO, 2008); Tomato image: istock ©Mark Penny; Contaminated water: istock ©Claes Torstenson; Necropsy: FAO Animal Production and Health Commission for Asia and the Pacific.

“The common theme for all of these is really how little we know,” she observed, asserting that the prevalence and burden of food-borne viral disease is vastly underestimated because of the lack of systematic surveillance of viral food-borne outbreaks, and a general lack of knowledge of these viruses within the food sector and of the threat they pose.

Despite evidence that viruses rank among the top causes of diarrheal disease, there is no systematic testing of patients for these viruses, Koopmans stated. Food-borne viral disease outbreaks that are recognized as such represent the “tip of the iceberg,” she asserted. “Not only do we need to have people with [gastro-intestinal] illness tested and notified, but they also need to think about the potential for food as a source of their illness,” she said. “We need the [suspected] food tested as well to get conclusive evidence. That hardly ever happens.”

Noroviruses, the most common viral cause of diarrhea, infect 1 in 20 people each year, Koopmans estimated. Infected people—and also infected wild and domestic animals—shed large amounts of virus through the gastrointestinal tract. Because the virus is not effectively removed through sewage treatment systems, it can go on to contaminate seafood and crops that come into contact with recycled water. Koopmans observed that among European shellfish-growing areas that are graded according to their influx of sewage, Grade A areas—those that have a very rare influx of sewage—are virtually nonexistent.

While noroviral disease is relatively common, Koopmans observed, its symptoms are mild compared with hepatitis A, which causes the vast majority of mortality associated with food-borne viral disease (Scallan et al., 2011b). In an effort to better understand patterns of emergence of noroviruses, Koopmans and colleagues established an informal global surveillance network, *NoroNet*, in 2006.²³ The researchers have observed that there are two main types of norovirus outbreaks reported: those that occur seasonally in nursing homes and other health care settings, which are typically caused by a single viral genotype, and all other outbreaks (including those associated with food), which appear to be caused by an evolving mixture of viral genotypes (Kroneman et al., 2008; Siebenga et al., 2010; van Asten et al., 2011). The public health impact of noroviral disease may, in fact, be underestimated. Data collected by *NoroNet* suggests that recently introduced noroviral variants cause increased outbreak activity and more severe disease, Koopmans reported.

The evident frequency of recombination among food-borne viruses—demonstrated, for example, in the results of multi-year analyses of viruses from shellfish samples—is a reason for concern, Koopmans argued. “Particularly with these sewage-contaminated food-borne outbreaks, we run the risk of generating more diversity through recombination,” she warned. “We should look at this as a warning sign. These are relatively mild viruses, but this is going on all the time.”

Although lack of diagnosis and reporting makes food-borne virus outbreaks difficult to investigate as they unfold, mining of molecular data on viral genotypes permits retrospective detection of clusters of outbreaks linked to common, internationally distributed food sources, Koopmans explained (Verhoef et al., 2011). At the present time, epidemiological and molecular surveillance of food-borne viral outbreaks is insufficient to permit their early detection, she said, but this should be a goal for the future. In the meantime, once an outbreak is detected, these methods may be useful in warning potential consumers of infected food preserved by freezing or drying.

“I feel that food-borne transmission of viruses is very common, but it’s rarely diagnosed,” Koopmans concluded. She urged increased international efforts to exchange molecular and epidemiological information to enable the sequence-based linking of clusters of viral enteric disease, and thereby to track global food-borne outbreaks—outbreaks that threaten to produce more virulent viruses through recombination.

Ultimately, Koopmans added, “I think we need to start moving away from individual surveillance systems for individual pathogens and really think through what the fecal flows and the produce flows are, what smart sampling is, and . . . use the developing technologies to not just look for a single pathogen, but whatever is around there quantitatively.” For example, she noted, researchers have

²³ <http://www.noronet.nl/noronet/>.

mapped networks of hospitals to determine which experience the highest rates of patient exchanges—an important risk factor for the transmission of drug-resistant microbes; a similar analysis might also reveal risks for food-borne outbreaks, she speculated.

Antimicrobial-Resistant Pathogens

The general phenomenon of antimicrobial resistance (AMR) has been widely discussed, including by this Forum (IOM, 2010a). According to speaker Henrik Wegener, of the Technical University of Denmark, AMR represents another “wicked problem” of the sort previously described by King: a complex and not entirely predictable system of transmission of resistance genes among animals, humans, and the environment. (Dr. Wegener’s contribution to the workshop summary report can be found in Appendix A, pages 331-349.) Food figures prominently in this system, because the use of antimicrobials in low doses as a growth promotant in food animals acts as a chemical driver for AMR, Wegener observed. “There is lifelong exposure of animals and other factors that certainly enhance the selective pressure favoring the emergence of resistance,” he concluded.

This selective pressure operates well beyond the bodies of livestock treated with low-dose antimicrobials, Wegener pointed out. One hundred percent of Vietnamese shrimp farms uses ciprofloxacin. Fluoroquinolone concentrations in sediments and surface waters may reach $>4,000\mu\text{g}/\text{kg}$ (Thuy et al., 2011). All kinds of bacteria inhabit these ponds, including those present in the manure of terrestrial animals (such as chickens) that is fed to the shrimp, he reported. “Nobody can know where this leads,” he observed.

Agricultural and aquacultural systems for raising food animals are vertically integrated, Wegener explained. As illustrated in Figure WO-19, “just a few thousand animals in the top of a breeding pyramid become trillions of eggs or trillions of broilers or trillions of slaughter pigs at the bottom of this pyramid,” he said. “That is what has happened with *Salmonella* Enteritidis, where the unwillingness to sacrifice a few thousand pedigree birds led to millions and millions of human cases. These things have to be addressed from the top down if we want to really control them.”

In Denmark, this was accomplished by instituting serological surveillance of egg producers in 1997, an intervention Wegener described as simple and inexpensive. Flocks found positive for the pathogen are either culled and repopulated, or they are used solely to produce heat-processed eggs. After these practices were instituted, along with a similar program of surveillance and eradication of infected broiler flocks, Denmark experienced a significant decline in human *Salmonella* infections. However, he noted, these results would not have been so effective if Denmark imported more meat and eggs or breeding poultry and livestock, which may also carry pathogenic bacteria and viruses, including those that are drug-resistant.

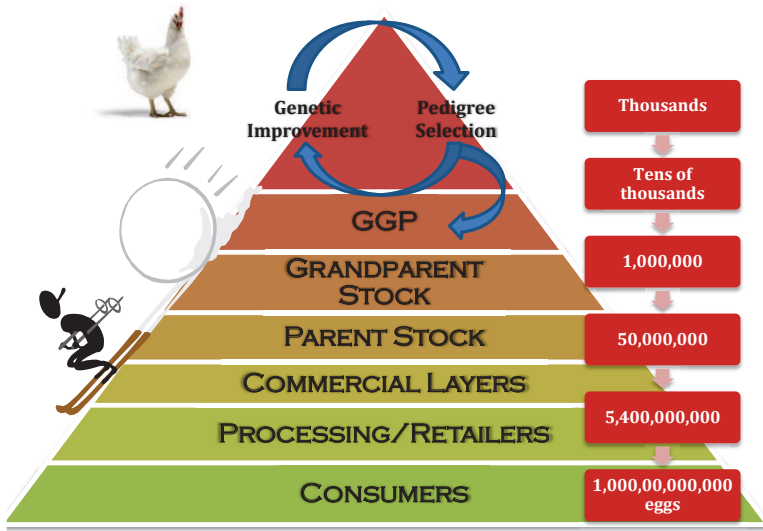


FIGURE WO-19 Pyramids and snowball effects.
 SOURCE: Wegener (2011).

Additional contributors to the threat of food-borne AMR pathogens include the use of critical human antibiotics for animal therapy and growth promotion and the overuse of all antimicrobials—encouraged by their easy acquisition by livestock producers, as well as by the significant profit veterinarians receive from selling antimicrobials, Wegener said.

Integrated surveillance for AMR Building on the success of surveillance to reduce the incidence of salmonellosis, and out of concern for the increasing emergence of resistant strains of *Salmonella* and *Enterococci*, Denmark instituted an integrated surveillance system for AMR, called DANMAP (DANMAP, 2012; Hammerum et al., 2007), along with a complementary surveillance program for antimicrobial usage (Rodo et al., 2011), called VETSTAT (Stegé et al., 2003). DANMAP monitors antimicrobial resistance, through systematic sampling and testing of bacterial isolates, from humans, food, and food animals. It includes human and animal pathogens, as well as indicator bacteria. The results are published annually in a report, which can be found online, according to Wegener. A schematic of surveillance inputs to DANMAP is illustrated in Figure WO-20.

The part of DANMAP that monitors antimicrobial usage in animals is called the VETSTAT program. Started in the year 2000, it monitors the use of prescribed antimicrobials in animals at a very detailed level. According to Wegener, for each record in the database, VETSTAT has information on:

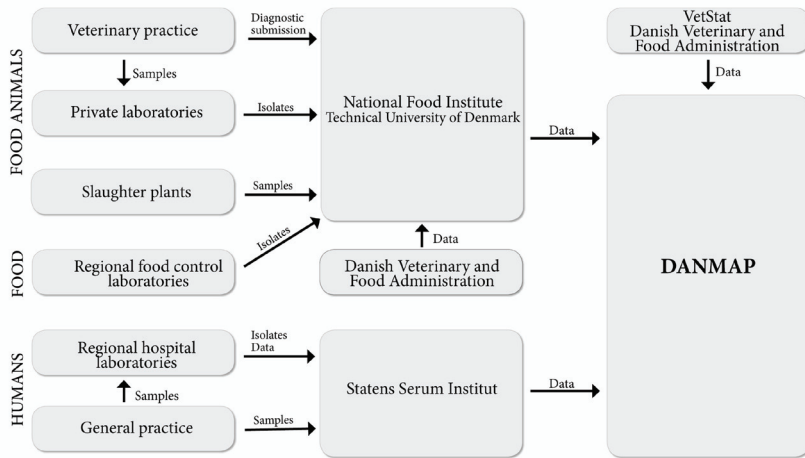


FIGURE WO-20 DANMAP: Integrated surveillance of antimicrobial resistance and antimicrobial usage in Denmark.

SOURCE: DANMAP, Danish Integrated Antimicrobial Resistance Monitoring and Research Program; National Food Institute, Technical University of Denmark (DTU); Wegener (2011).

- farm ID
- animal species
- age group
- date
- drug ID
- drug quantity
- disease category
- prescriber ID

Antimicrobial usage data collected by VETSTAT also supports Denmark’s “yellow card” system, which identifies high-usage swine producers, warns their veterinarians, and encourages reduction in usage within a 9-month period. “If they don’t do that, they may get a visit from the district veterinarian’s office,” Wegener explained; however, there are no defined consequences for ignoring the “yellow card.” Nevertheless, he said, this measure has been associated with a 20 percent reduction in antibiotic use since it was instituted in 2010. Efforts are under way to implement integrative surveillance of AMR and antimicrobial usage throughout Europe and also at the global level, through the WHO’s Advisory Group on Integrated Surveillance of Antimicrobial Resistance,²⁴ he reported.

Based on his experience with DANMAP and VETSTAT, and in recognition

²⁴ http://www.who.int/foodborne_disease/resistance/agisar/en/.

of the challenges of extending Denmark's success to the regional and global levels, Wegener suggested that systems for the integrated surveillance of AMR meet the following criteria:

- systematic sampling, harmonized laboratory methods, and good data management;
- detailed information on pathogen sample origin and antimicrobial usage;
- sub-typing of bacterial isolates and molecular characterization of resistance genes;
- collaboration and coordination among all parties, including data sharing and comparison; and
- establishment of a solid basis for further detailed investigation of specific questions.

Upstream interventions for AMR Wegener described a range of strategies Denmark has implemented to reduce the emergence and spread of AMR through the food chain (Aarestrup et al., 2008; Wegener, 2006). In 1995, the country passed legislation to limit profits to veterinarians on the sale of antimicrobials to 5 percent of their cost, Wegener stated. At the same time, routine prophylaxis—the use of antimicrobials to treat animal flocks without a disease history—was also outlawed. During the year after these changes were made, veterinary antibiotic use in Denmark declined by more than 30 percent, he reported (Wegener, 2006).

Additional voluntary actions further limited antimicrobial use in Danish food animals. In 1999, Danish swine producers voluntarily terminated the use of in-feed fluoroquinolones. Despite the voluntary termination of the use of this class of antibiotics, Wegener reported, usage of the drug slowly increased thereafter. To address this problem, in 2002, veterinarians were barred from administering fluoroquinolones to animals unless no alternative treatment existed. This requirement effectively reduced fluoroquinolone usage and, with it, the frequency of fluoroquinolone-resistant *E. coli* in humans, he said. Despite the loss of several growth-promoting antimicrobials over the past two decades, Wegener noted that the Danish swine industry has experienced increased productivity over this period (Aarestrup et al., 2010). More recently, Danish swine and cattle producers have voluntarily agreed to eliminate the use of cephalosporins. The effects of this change on both human and animal health have yet to be determined.

The Danish food animals consume less than 20 percent of the amount of antimicrobials used by U.S. producers to yield the same amount of meat, Wegener stated. Organic meat producers in Denmark use a further 10-fold less than conventional ones, at an apparent productivity loss of only 10 percent. “Maybe if you had spent as much science on improving a production system like this, we could have 100 percent productivity, but make do with one-tenth of the antibiotics,” he speculated.

Adopting—or ignoring—the Danish model In the discussion session that followed Wegener’s presentation, Forum member Jeffrey Duchin, of the University of Washington, noted that the documented success of restrictions on the use of antibiotics in poultry and livestock in Denmark (as well as in other countries) has done little to influence U.S. policies. “It makes me a little bit cynical about our ability to take action on other food safety issues, when we . . . [fail to act on evidence] . . . that is as far advanced as the data and the research on antimicrobial drug resistance,” he confessed. This situation may be changing. In a recent development, a federal court judge in late March 2012 ordered the FDA to take action on its own 35-year-old rule that would stop farmers from mixing popular antibiotics into animal feed, a practice which is widely believed to have led to a surge in dangerous, drug-resistant bacteria (Perrone, 2012).

“I think the lesson from my own country is that you can never have complete evidence of anything, and at some point in time, you should intervene and then learn,” Wegener observed. “For many of the interventions that we have done, we had no evidence that they would work before we intervened. We intervened based on, say, best scientific evidence and common microbiology sense. Then you have a huge experiment with that entire production system, and you evaluate it and you change your program or your policies if you find out that it’s not working as you expected.” These kinds of interventions are more possible in Denmark because the scientific culture is far less polarized than in the United States, he added. “We don’t really see industry and government as being opposites and in opposition to each other. It is more based on a culture of agreeing to a common problem and then trying to agree to a solution and then moving along.”

Although Wegener stated that these decisions were made primarily to satisfy risk-averse Danish consumers, Hueston observed that Denmark “made a decision decades ago to focus on their export market, as they should.” Daszak added that Denmark’s policies had allowed it to gain a market-share advantage—a strategy that would not be as effective in the United States, which does not export the majority of the meat it produces.

Within the United States, much of the discussion of the use of antibiotics in animals has been limited to two alternatives—bans or unrestricted use—despite the fact that a myriad of options exist for managing the associated risk, Hueston asserted. “There is a need for antibiotics, but we don’t need to misuse and over-use,” Wegener agreed. “I would just like to see concerted movements toward trying to find out how low we can go,” he continued, and he encouraged Europe and the United States to agree to a strategy of reduction in the use of antibiotics that does not compromise productivity—and to document their progress so as to influence future policy in the rapidly expanding markets of Southeast Asia, China, and Africa.

Approaches to Food-Borne Disease Surveillance, Detection, and Response

A central tenet of a One Health approach is its focus “upstream” of disease outbreaks, ideally in order to prevent them from occurring in the first place. When that is not possible, however, the same perspective may enable researchers to anticipate disease emergence and to detect outbreaks as early as possible. Workshop participants discussed several such efforts, including computer-generated predictions of “hot spots” for disease emergence, the development of digital epidemiology methods for early outbreak detection, the use of sequence information to identify novel pathogens, and the implementation of test-and-hold strategies to detect food-borne contamination before it reaches consumers.

Predicting Food-Borne Disease Emergence

Nipah and avian influenza outbreaks Although neither the timing nor the pathogen type involved in food-borne disease events can be accurately predicted, it is increasingly possible to identify likely outbreak scenarios that can be used to target surveillance efforts for specific food-borne diseases, as well as for food-borne disease in general, Daszak observed. Returning to the emergence of Nipah virus in Malaysia, Daszak described how he and his colleagues used a One Health approach to analyze the livestock production system and surrounding ecosystem in order to understand how the outbreak happened. After unraveling the story of the virus-carrying bats messily eating mangoes among the pigsties, the researchers attempted to determine why the outbreak occurred when and where it did.

Nipah virus has probably been circulating in bats for millions of years, Daszak said, so why did it suddenly emerge on this particular pig farm in 1999? To answer this question, the researchers constructed a mathematical model to simulate transmission dynamics of the virus within the index farm’s pig population, based on evidence that the virus was introduced repeatedly to pigs there over a 2-year period prior to the human outbreak (Pulliam et al., 2012). The result suggests that Nipah emerged in Malaysia when the intensive farming of pigs reached a certain threshold, with “conditions that allowed the virus to keep ticking over,” he explained. In this model, it was assumed that the index farm was not biosecure, he continued, and that bats repeatedly visited, allowing the virus to be reintroduced multiple times into the pig population. The particular, highly compartmentalized structure of the farm created the perfect condition for Nipah to exist.

“Using computer models allows you to re-create epidemics and, to some extent, predict issues around future emergence of that pathogen,” Daszak observed. Based on their findings, the investigators advised the Malaysian government on ways to reduce the risk of future human Nipah outbreaks associated with pig farms. One is to avoid raising pigs near fruit trees of species that are particularly attractive to bats, which include mango, durian, and papaya, he said; the other is

to optimize farm size. “What happened with the outbreak was that the virus got into a really big, highly structured farm and persisted. Then, when people started dying . . . everybody started to sell the pigs. They sold them to the fattening farms . . . ready to send on to Singapore. That’s when the outbreak expanded,” he explained, because in these small farms more people were exposed to each infected animal. Under those circumstances, he concluded, “as your farm size decreases, the risk of getting infected actually increases.”

Taking a similar approach, Daszak and colleagues modeled the transmission dynamics for highly pathogenic avian influenza (HPAI) to identify potential hot spots for outbreaks. Previous studies suggested that the practice of double-cropping rice, which attracts HPAI-carrying ducks to the fields to feed throughout the year, increases the potential for viral crossover into pigs and, eventually, to humans (Martin et al., 2011). Daszak and coworkers attempted to build on this work to understand the role of farm size and connectivity as risk factors for avian influenza transmission. Using an outbreak simulation model, as depicted in the cartoon in Figure WO-21, they found that HPAI viral introductions to small “backyard” farms posed a relatively high risk for human infection; however, as farm size increases, outbreaks that do occur last longer.

“The worst-case scenario for avian influenza to persist is when you have a mixture of backyard and large-scale farming,” Daszak observed. Although very

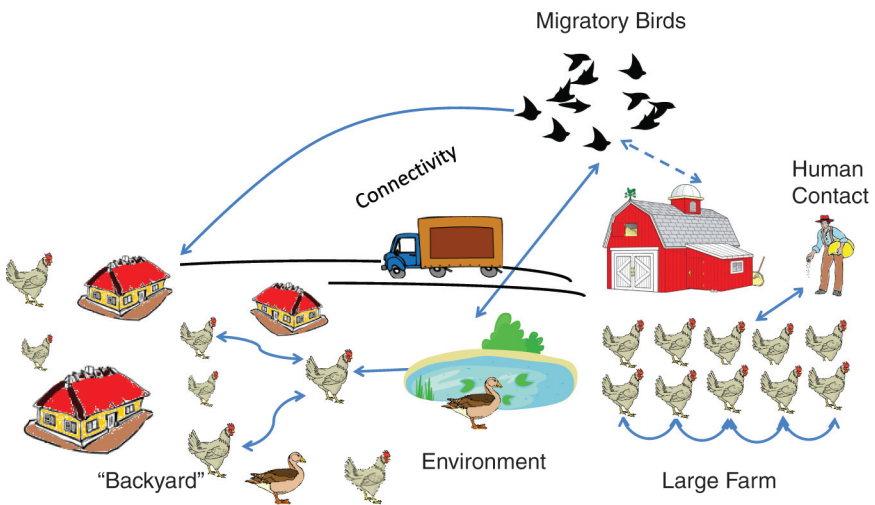


FIGURE WO-21 A schematic representation of how farm size can affect risk of avian influenza emergence. Highly pathogenic avian influenza has trouble persisting on large farms, where it is rapidly noticed and birds culled. Mathematical models show that the virus persists much better when both large and small farms co-exist.

SOURCE: Image provided courtesy of L. Mendiola and P.R. Hosseini, EcoHealth Alliance.

large farms tend to be more biosecure than backyard operations, the virus seems able to circulate between large- and small-sized farms, persisting longer than if only one farm system existed. Thus, he concluded, a mixture of backyard farms with less-than-secure large farms raises the risk for human transmission of HPAI.

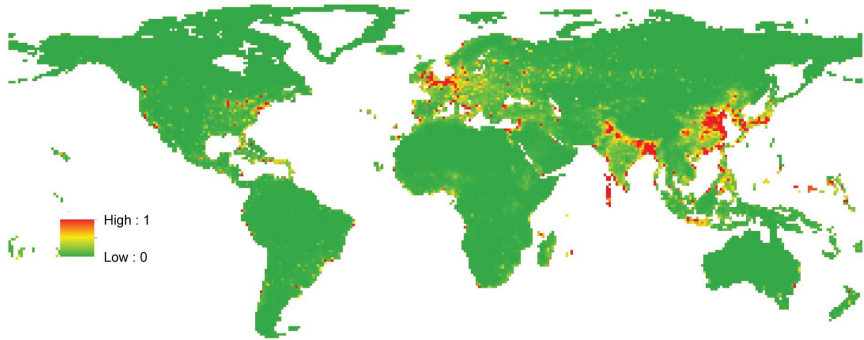
Identifying food-borne disease hotspots Daszak and colleagues developed a database of every infectious disease that has emerged over the past five decades in order to develop more generalized rules for predicting where infectious diseases are likely to emerge (Jones et al., 2008). He reported that a significant proportion of these diseases are driven by food-borne transmission, and that most were caused by bacteria; however, the number of viral emerging diseases, particularly zoonoses, has increased in recent years. The main drivers associated with food-borne diseases are increasing technology and industry, travel, commerce, and human susceptibility, he said.

“When we know all the drivers of food-borne infections, we can map those out spatially, analyze the presence or absence of prior outbreaks, and try and get at the map of where food-borne pathogens will emerge in the future,” Daszak continued. As illustrated in Figure WO-22, hotspots for food-borne pathogen emergence are concentrated in the tropics, including the increasingly population-dense areas of South and Southeast Asia, and also in parts of Europe and North America. When an additional driver of infectious disease emergence, land-use change—a proxy for broad-scale deforestation and agricultural development—is incorporated into this analysis, “parts of Latin America light up and parts of Southeast Asia become less important, where land-use change has already had its impact,” he observed. Further, if travel and trade out of hotspot areas are taken into consideration, he said, “what you see is an incredible risk from the rapidly developing areas of the planet, where there’s a lot of export, a lot of import. If you follow the trade routes, it all points to the developed countries that import these products.” This knowledge can help focus surveillance efforts, he added.

The EcoHealth Alliance, through a collaboration with the university of California, Davis, and the U.S. Agency for International Development (USAID) called Deep Forest,²⁵ is analyzing the effects of deforestation and agricultural development in three regions: the Brazilian Amazon near Manaus, the Bwindi Impenetrable Forest in Uganda, and the Maliau “Lost” Basin and Kinabatangan River in Borneo. Within these areas, each of which encompasses a gradient ranging from primary forest to rural farmland to urban landscape, researchers will attempt—through a combination of metagenomic techniques and interviews

²⁵ Launched in 2011, Project Deep Forest builds on EcoHealth Alliance scientists’ work, which shows that deforestation threatens global health by leading to the emergence and spread of new diseases. Initially the project focuses on the tropical forests of Brazil, Uganda, and Malaysia, identifying health threats to people and wildlife in the communities closest to these forests, and working to prevent their possible spread to nearby urban areas and ultimately around the world. http://www.ecohealthalliance.org/writable/publications/annual_gala_invitation_2012.pdf (accessed April 3, 2012).

Food-borne EID Hotspots map



Relative risk of food-borne EID events, based on Jones et al. (2008). Human population density and human population growth, were the most important variables.

Food-borne EID Hotspots map: Including land use change



Relative risk of food-borne EID events, additional drivers included the change in area of pasture and crop between 1900 – 2000 (modified from Jones et al., 2008).

Human population density, mammal diversity and the land use change were the most important variables.

FIGURE WO-22 Hotspots for food-borne pathogen emergence.

SOURCE: Jones et al. (2008).

of residents about their interactions with wildlife—to measure both the number of unknown pathogens of interest carried by representative wildlife species and the amount of human–animal contact, Daszak said. Using that information, the researchers hope to answer the following questions:

- How many viruses are out there?
- What animals carry them?
- What is the risk of them emerging?
- How can we stop them from emerging?
- Can we use the health angle to reduce deforestation?

The ultimate goal of the Deep Forest project is to elucidate precisely how agricultural change drives disease emergence, Daszak stated. “Once we have done that, we can start to work with local people and say [to them], ‘you’re at risk of a new, emerging disease. There are ways you can change behavior . . . that are cheap, cost-effective, and will save your health, and you can still make money,’” he concluded.

Wildlife and Food-Borne Disease

Wildlife are known to transmit a variety of food-borne diseases to humans through multiple routes, the most direct of which is through human consumption of wild animals, speaker William Karesh, also of EcoHealth Alliance, observed. (Dr. Karesh’s contribution to the workshop summary report can be found in Appendix A, pages 207-217.) While this description conjures images of hunters eating wild prey—a focus of Karesh’s work in wildlife conservation and ecology—he also reminded the audience that nearly half of all seafood consumed is wild caught and therefore fits the description of “wildlife.” Seafood has been characterized as an important source of emerging food-borne diseases (Broglia and Kapel, 2011; Nawa et al., 2005).

Karesh and his colleagues have long engaged hunters around the globe to participate in the surveillance of wildlife for infectious diseases. Before wildlife-associated emerging infectious diseases such as SARS, monkeypox, or avian influenza commanded headlines, EcoHealth Alliance was examining connections between wildlife and infectious diseases in such settings as logging camps and bush meat markets, he said. People in logging camps, with populations numbering into the thousands, are essentially hunter-gatherers who must obtain all of their food from the surrounding forest. Bush meat markets, a common food supply system for much of the world, yield approximately 1 billion kilograms of meat per year in central Africa alone, according to Karesh. The mass culling of wildlife is not a sustainable system for providing food, he insisted; therefore, strategies to replace these practices could simultaneously reduce the risk of food-borne disease and conserve wildlife.

Using the case of the emergence of monkeypox²⁶ in the United States as an example, Karesh noted that food insecurity in one part of a world linked by global trade can prefigure outbreaks of infectious diseases in places where food is plentiful. Thus, he concluded, we should be concerned about food safety everywhere and investigate food-borne diseases wherever they occur. In Africa, where EcoHealth Alliance has engaged with hunters and their communities to assist in the surveillance of emerging infectious diseases, the organization has also worked to educate people about food safety. “You work with the hunters and tell them what’s safe to hunt and what’s not safe to hunt, what should they bring back to the village, what not to bring back, to cook your food and wash your hands,” he explained. “You work with the suppliers and the consumers.”

These efforts may have paid off, as there have been no human cases of Ebola hemorrhagic fever in northern Congo since 2005, Karesh stated, despite the fact that the disease continues to circulate in wildlife in that region. He attributed this success to the development of an “honest, multi-stakeholder dialogue” as a foundation for intervention. Karesh described additional EcoHealth Alliance projects that reflect this same approach and alignment with the One Health paradigm. These include collaboration with the CDC to identify pathogens present in bush meat imported into the United States in order to begin to estimate associated risks for infectious disease.

A similar effort is needed to estimate the volume of illegal wildlife (e.g., exotic pets) imported into the United States, and their associated risks, he continued (Karesh et al., 2005). “There is no financial support for CDC or USDA or FDA to really do inspections,” Karesh observed. “It’s very hard to do a risk analysis when you don’t have any data to do the analysis on, but we do see this stuff coming in every day. It’s probably a threat to livestock. It’s probably a threat to human health. It’s certainly a threat to wildlife because it’s depleting wildlife resources. Probably one of the biggest threats to wildlife conservation left in the world today, [along with] habitat destruction . . . [is] the illegal wildlife trade.” In the meantime, EcoHealth Alliance works to educate consumers about how to pick a safe pet, through their PetWatch website.²⁷

EcoHealth Alliance also participates in a project sponsored by USAID known as PREDICT (USAID, 2009), which seeks to improve monitoring of infectious disease emergence in wildlife. Its strategies include a range of efforts to better target infectious disease surveillance, such as geospatial risk modeling (Jones et al., 2008), determining routes of transmission, identifying animal species most likely to transmit infectious diseases, and conducting Internet surveillance for outbreak cues. PREDICT is also building surveillance capacity in hotspots for disease emergence to increase the possibility of early detection and effective containment.

²⁶ Humans initially acquired monkeypox when Africans consumed infected rats, having nothing else to eat, Karesh stated. Monkeypox was introduced into the United States in 2003, in a shipment of small mammals from Ghana intended for sale as exotic pets (CDC, 2008).

²⁷ <http://www.petwatch.net/>.

As Karesh noted, all of these strategies are applicable to food-borne diseases, as a subset of emerging infectious diseases (Cardoen et al., 2009).

Detecting Viral Outbreaks

Speaker Nathan Wolfe, of the Global Virus Forecasting Initiative (GVFI) and Stanford University, invoked John Snow’s map of the 1854 London cholera epidemic (UCLA Department of Epidemiology, 2012), the “ghost map” reproduced in Figure WO-23, which led Snow to conclude that cholera was a water-borne disease, and thereby to the means to stop its deadly spread. (Dr. Wolfe’s contribution to the workshop summary report can be found in Appendix A, pages 349-362.) Among computer scientists, that map is known as the first geographic information system, and despite the technological advances that have occurred

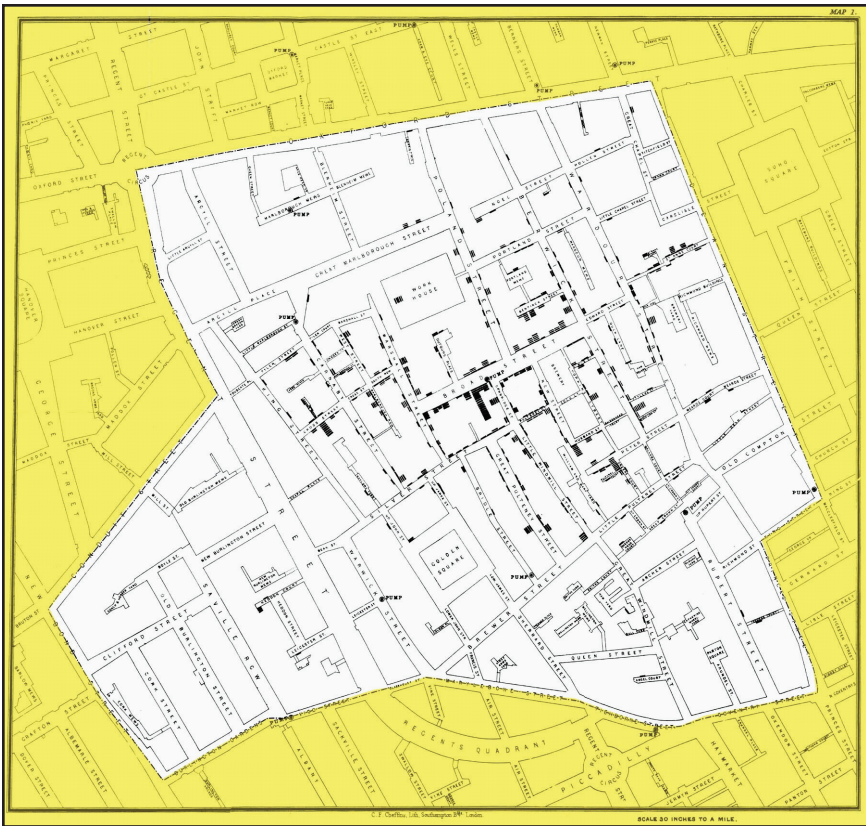


FIGURE WO-23 John Snow’s map of the 1854 cholera epidemic in London.
SOURCE: Cheffins (1854).

since then, epidemiologists continue to investigate outbreaks much as Snow did, Wolfe observed. Therefore, he continued, when considering novel ways to detect food-borne diseases, “it’s worth our asking ourselves, if John Snow was in the audience today, how would he be thinking about these problems?”

Wolfe proceeded to describe several innovative approaches to detecting the emergence of infectious diseases, focusing on food-borne viral outbreaks. Viruses frequently jump from wild animals to domesticated animals and human hosts, but only by degrees, and often unsuccessfully—as illustrated in Figure WO-24—a phenomenon he referred to as “viral chatter.” Only occasionally do viruses become exclusively adapted to a human host, he said, but until recently, these relatively rare events have commanded researchers’ attention and have prevented them from understanding—and therefore predicting—how infectious diseases emerge. Consequently, for the past decade, GVFI has focused on human populations most exposed to wild animals, such as people living in central Africa, where HIV is thought to have emerged—as a food-borne illness, he asserted. “This is definitely a virus which was associated with the hunting and butchering of chimpanzees and the contact of food handlers with these sorts of viruses,” he explained. “Whenever we are going to have an interface with this sort of diversity

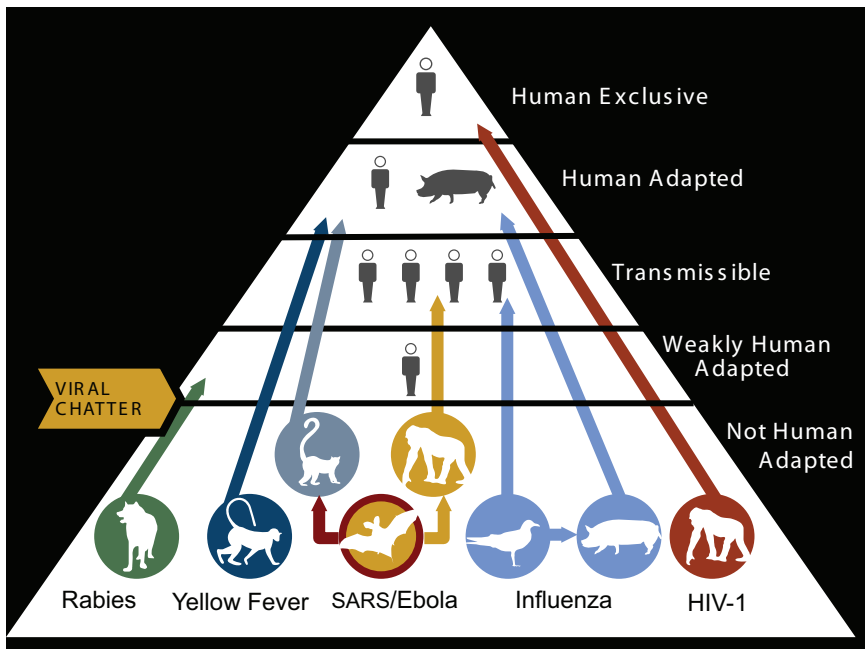


FIGURE WO-24 Human viruses have animal origins.
SOURCES: Wolfe (2011); adapted from Wolfe et al. (2007).

of animal viruses, we are going to have the opportunity for these new viruses to enter into human populations and spread.”

In order to monitor the transition of viruses from animals into humans at the interface between their populations, Wolfe said that GVFI works with partners to establish collection sites and collaborations in many parts of the world; this includes the creation of laboratories, as well as training programs to staff these facilities, throughout central Africa and parts of Asia. “The basic idea is to move pathogen discovery from . . . ivory tower laboratories to places where we have this biological diversity,” he explained. For example, he said, they have enlisted volunteers who have high levels of contact with the blood and body fluids of wild animals, such as hunters and workers in wet markets, to spot filter paper with samples of their own blood, as well as blood from the animals with which they come into contact.

GVFI has amassed a large collection of these specimens from humans and more than 140 animal species, Wolfe reported. “In the last twelve years we’ve assembled some of the most comprehensive sample sets of human [$>120,000$] and animal [$>60,000$] blood spot collections in the world,” he stated. These resources allow the researchers to monitor the flow of viruses into the human population, and even to witness the moment at which an outbreak is born, he said; for example, they were able to detect the crossing of simian foamy viruses (SFVs) from gorillas and mandrills into the humans who hunt and butcher them (Wolfe et al., 2004). Daszak also made note of these retroviruses, observing that they “could be the next HIV, coming in through the food system just like HIV did.”

Wolfe and colleagues conducted similar analyses of human T-lymphotropic viruses (HTLVs) types 1 and 2, which originated independently and are related to simian T-lymphotropic viruses STLV-1 and STLV-2, respectively (Wolfe et al., 2005). HTLV-1 and HTLV-2 are pandemic viruses that infect between 5 million and 20 million people worldwide, causing severe disease in a percentage of those individuals, he reported. They found that central Africans who had contact with the blood and body fluids of non-human primates (e.g., through hunting, butchering, and pets) were infected with a wide variety of HTLVs, including two previously unknown retroviruses (HTLV-3 and HTLV-4). In addition to revealing new levels of HTLV diversity and suggesting that human exposure to non-human primates contributes to HTLV emergence, these findings also indicate that cross-species transmission is not the rate-limiting step in pandemic retrovirus emergence; rather, they show that it may be possible to predict and prevent disease emergence by surveillance of populations exposed to animal reservoirs, as well as through interventions to reduce human exposure to non-human primates.

GVFI has also been involved in a collaborative effort in the Democratic Republic of the Congo (DRC) to study the transmission of human monkeypox virus (Rimoin et al., 2010). Thirty years after mass smallpox vaccination campaigns ceased, rendering the population increasingly immunologically naïve to orthopoxviruses including monkeypox virus, the incidence of monkeypox

infection has dramatically increased in rural areas of the DRC. Using filter paper–based blood samples from people exposed to wildlife through hunting or meat-handling, as previously described, GVFI researchers will be able to determine whether monkeypox virus emerged in a similar fashion to SFV and the HTLVs.

In addition to their retrospective analyses of food-borne disease emergence, GVFI investigators are exploring the detection of outbreaks in “real time” through the application of digital epidemiology. The global expansion of cellular phone usage and social networks is producing a wealth of data that can be mined for both content and location, Wolfe observed; this is the basis for GVFI’s EpidemicIQ project, which queries a variety of open-source and proprietary data feeds (e.g., blogs, Twitter feeds) to detect patterns of illness in their content and combine this with geolocation. These methods are capable of detecting outbreaks well before they are announced, he continued; they can also permit estimation of the outbreak’s impact and identification of risk factors associated with infection.

Digital epidemiology transcends the division between epidemiological and microbiological investigation of food-borne outbreaks, Wolfe observed. This approach has the potential to detect infectious outbreaks early, based on sequence data that represent both host susceptibility and the genetic diversity of various populations of microorganisms, and on indirect information gleaned from data sources, in order to prevent or limit their spread.

Microbe Hunting

Advances in technology are rapidly expanding the viral sequence database. According to speaker W. Ian Lipkin, of Columbia University, the vast majority of the estimated 1 million viruses carried by vertebrate animals have yet to be identified (Morse, 1993). (Dr. Lipkin’s contribution to the workshop summary report can be found in Appendix A, pages 251-271.) Thus, a major challenge facing today’s microbe hunters is the need to discriminate among this vast array of potential pathogens—in Lipkin’s case, viruses—to identify the causative agent of a specific outbreak. Researchers can take a number of different approaches to pathogen identification Lipkin noted; these are summarized in Figure WO-25 (Lipkin, 2010).

Classical methods involve culturing the pathogen, a critical step in fulfilling Koch’s postulates,²⁸ while molecular techniques for detecting pathogen sequences—which have provided a wealth of information—frequently lead to the discovery of microbes that cannot be propagated *in vitro*, or for which no animal model systems have been developed, he observed. Lipkin presented numerous

²⁸ Koch’s postulates, which establish the causal relationship between pathogen and disease, stipulate that the pathogen is present in every case of the disease, specific for that disease, and can be propagated in culture and capable of replicating the original disease upon inoculation into a naïve host (Koch, 1891).

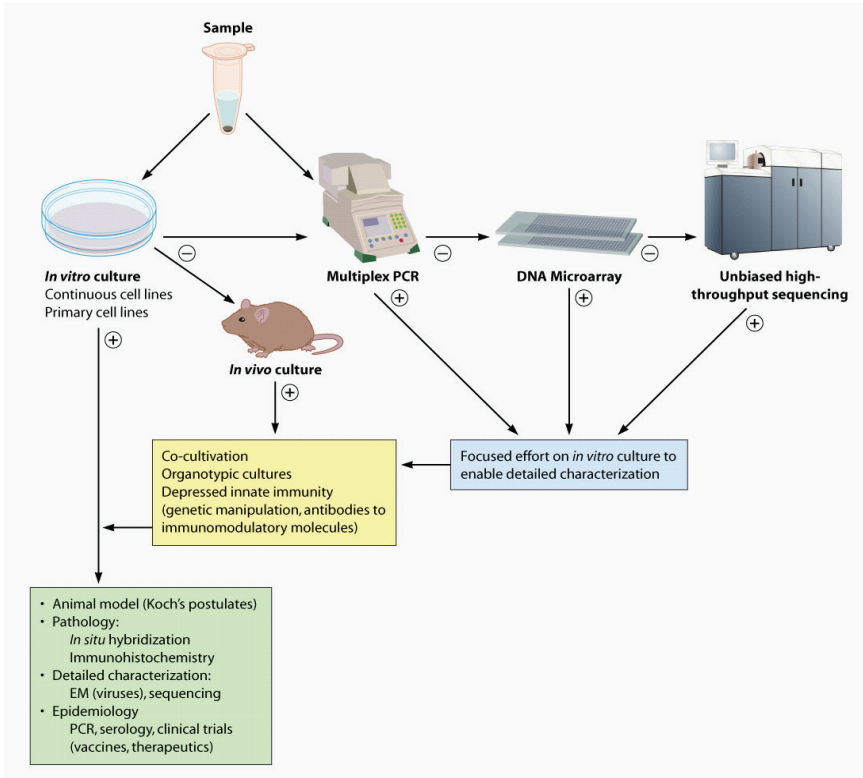


FIGURE WO-25 A staged strategy for pathogen discovery.

SOURCE: *Microbiology and Molecular Biology Reviews*, 2010, 363-377, doi: 10.1128/MMBR.00007-10 and reproduced with permission from the American Society for Microbiology.

illustrative examples, gleaned from his own experience, of the application of a range of molecular methods for pathogen discovery, described in Box WO-4, to the investigation of infectious outbreaks that appear to be caused by novel infectious agents.

In one case, when Lipkin and colleagues used MassTag PCR (see Box WO-4) to investigate an outbreak of influenza-like illness in New York State, they discovered a novel rhinovirus was the likely cause of this outbreak (Lamson et al., 2006). Follow-on studies implicated these viruses not only in influenza-like illnesses but also in asthma, pediatric pneumonia, and otitis media. He noted that a similar study implicated rhinoviruses and enteroviruses as the cause of influenza-like illness during the summer months in New York City at the time that H1N1 was circulating (Tokarz et al., 2011); another determined that high case fatality

BOX WO-4 Molecular Methods for Pathogen Discovery

Singleplex Assays

The most common singleplex assays employed in clinical microbiology and microbial surveillance are polymerase chain reaction (PCR) assays, wherein DNA strand replication results in either the cleavage or release of a fluorescence-labeled oligonucleotide probe bound to a sequence between the forward and reverse primers. Nested PCR, in which two amplification reactions are pursued sequentially with either one (hemi-nested) or two (fully nested) primers located 3' with respect to the original primer set, may be more sensitive than fluorescent reporter dye singleplex assays. However, because the original reaction vessels must be opened to add reagents for the second, nested reaction, the risk for contamination is high, even in laboratories with scrupulous experimental hygiene.

Multiplex Assays

Signs and symptoms of disease are rarely suggestive of a single agent, particularly early in the course of an illness. Multiplex assays may be helpful in such situations because they may be used to entertain many hypotheses simultaneously. The number of candidates considered ranges from 10 to 100 with multiplex PCR, to thousands with microarrays, to the entire tree of life with unbiased high-throughput sequencing. In multiplex assays many genetic targets compete for assay components (e.g., nucleotides, polymerases, and dyes), in some instances with variable efficiencies. Thus, multiplex assays tend to be less sensitive than singleplex assays.

Multiplex PCR Assays

Gel-based multiplex PCR assays, wherein products are distinguished by mass, can detect as many as 10 distinct targets. Two platforms that combine PCR and mass spectroscopy (MS) for the sensitive, simultaneous detection of several targets have been established. The Ibis T5000 biosensor system uses matrix-assisted laser desorption–ionization MS to directly measure the molecular weights of PCR products obtained in an experimental sample and to compare them with a database of known or predicted product weights. MassTag PCR uses atmospheric pressure chemical ionization MS to detect molecular weight reporter tags attached to PCR primers. Syndrome-specific MassTag PCR panels have been established for the detection of viruses, bacteria, fungi, and parasites associated with acute

associated with H1N1 in Argentina was not caused by a more virulent influenza virus, but by co-infection with *Streptococcus pneumoniae* (Palacios et al., 2009). These findings illustrate the potential pitfalls in prematurely narrowing an outbreak investigation to one pathogen, or even a single class of pathogens, he observed.

Sequencing technologies to identify potential pathogens are fast, inexpensive,

respiratory diseases, diarrheas, encephalitides/meningitides, and hemorrhagic fevers. The Bio-Plex (also known as Luminex) platform employs flow cytometry to detect PCR amplification products bound to matching oligonucleotides on fluorescent beads. Assay panels that allow the detection of up to 50 genetic targets simultaneously have been developed.

Microarrays

Microarray technology runs the gamut from assays that comprise hundreds to those comprising millions of probes. Probes can be designed to discriminate differences in sequence that allow virus speciation or to detect thousands of agents across the tree of life. Arrays comprising longer probes (e.g., >60 nt) are more tolerant of sequence mismatches and may detect agents that have only modest similarity to those already known. Two longer probe array platforms are in common use: the GreeneChip and the Virochip. Although they differ in design, both employ random amplification strategies to allow an unbiased detection of microbial targets.

Unbiased High-Throughput Sequencing

The power of unbiased high-throughput sequencing has enabled unique advances in microbial surveillance and discovery. Applications include metagenomic characterization of environmental and clinical samples, rapid and comprehensive sequence analysis of microbial strains and isolates, and pathogen discovery. Unlike cPCR or array methods, whereby investigators are limited by known sequence information and must choose the pathogens to be considered in an experiment, high-throughput sequencing can be unbiased and allow an opportunity to inventory the entire tree of life.

After amplification and sequencing, raw sequence reads are clustered into non-redundant sequence sets. Unique sequence reads are assembled into contiguous sequences, which are then compared to databases using programs that examine homology at the nucleotide and amino acid levels using all six potential reading frames. However, because a truly novel pathogen might elude this level of analysis, researchers are exploring ways in which insights into the identity of agents may be determined by features such as nucleotide composition or predicted secondary or tertiary structures.

SOURCE: Excerpted from *Microbiology and Molecular Biology Reviews*, 2010, 363-377, doi: 10.1128/MMBR.00007-10 and reproduced with permission from the American Society for Microbiology.

and likely to become increasingly so, which begs the question of how best to analyze the volumes of data these methods generate, Lipkin stated. “The traditional methods that people use are alignment-based strategies,” he explained. “You look for similarities between what you’ve found and what is known at the nucleotide level and at the protein level.” Other approaches to investigating virus-like

sequences not identifiable through alignment include nucleotide composition and order analysis—methods Lipkin likened to cryptography—to identify sequences as belonging to a specific viral genus or family or to infer the infected host species. For example, he said, viral sequences obtained from fecal material may originate from humans, animals or plants consumed by humans, or even from plant material eaten by an animal that the human later consumed. Sometimes, however, putative outbreaks turn out to be caused by non-infectious agents, Lipkin reported. One such event occurred among workers at a pork processing plant who developed severe peripheral neuropathy (Holzbauer et al., 2010). All of the affected workers, whose task it was to extract pig brains with high-pressure air hoses, were not protected from exposure to brain tissue by facemasks or skin covering, he said. However, because the disease did not spread beyond these workers, it seemed unlikely to be infectious; eventually, it was confirmed that the workers were suffering from a previously described autoimmune reaction, not an infectious disease, he reported.

Another episode of pathogen “de-discovery,” described in detail in a recent review by Lipkin, involved evidence that contributed to discrediting a proposed causal relationship between the measles, mumps, and rubella (MMR) vaccine and the development of autism (Hornig et al., 2008). According to the discredited model, measles virus present in the vaccine was hypothesized to alter the permeability of the intestinal lumen to neuroactive molecules that passed, via the circulatory system, to the brain; however, Lipkin and coworkers found little evidence for the presence of measles virus RNA in the intestinal lumen of children with autism and gastrointestinal disturbances (case) or gastrointestinal disturbances alone (control), and no differences between case and control groups. They did discover intriguing differences between children with autism and gastrointestinal dysfunction, and children with gastrointestinal dysfunction alone, in levels of enzymes that break down complex sugars and of transporters that carry simple sugars from the lumen of the intestine into the systemic circulation. These differences were associated with differences in the microflora in the intestines of the two groups of children that may prove to be clinically significant.

Lipkin also provided a brief review of a number of novel viruses his group has recently identified:

- The first filovirus found to be endemic to Europe (Negredo et al., 2011). The virus is an intermediate between the Marburg and Ebola viruses, which suggests that many more filoviruses have yet to be discovered. The virus’s host is a widely distributed bat species, *Miniopterus schreibersii*.
- Canine hepatitis virus, also present in horses, the closest relative to human hepatitis C virus, which infects more than 200 million people worldwide (Kapoor et al., 2011).
- An influenza virus that infected and killed ringed seals in Alaska (Nolen, 2011) and New England (NOAA, 2011a) in 2011. The H3N8 influenza virus had previously been isolated from dogs and birds, and was appar-

ently transmitted from marine birds to seals, Lipkin said (NOAA, 2011b). It has the potential to jump to domestic animals and perhaps, even to humans, he added.

One Health Approaches to Food System Biosecurity

The number of food-borne disease outbreaks is increasing in frequency, yet the myriad approaches to food safety used around the world make it difficult to implement a unified, risk-based approach to managing and controlling these hazards (Coker et al., 2011; Karesh et al., 2005). Food and agricultural systems have become so complex and extensive in size that food safety hazards have the potential to cause extensive and far-reaching damage to human and animal health. The changing nature of food-borne pathogens further compounds efforts to keep pace with this “wicked problem.” The complexity of maintaining food system biosecurity also makes it a natural place to apply a One Health approach, which focuses on upstream factors such as animal health and ecological disturbances (Figure WO-26).

The global food system depends on the ability to safely trade food-related goods and services. Food and water are major pathways for the introduction and spread of emerging and reemerging infectious diseases (World Bank, 2010). Ensuring their health is a means of maintaining a safe and adequate food supply, but it requires expertise from multiple disciplines in order to comprehensively evaluate current approaches to managing and preventing disease outbreaks. The complex nature of the global food supply chain thus increases the importance of a cross-disciplinary approach to examining food-borne zoonoses (e.g., *Salmonella*, *E. coli*, etc.) linked to livestock production (Coker et al., 2011). Disease outbreaks in animals and contamination events erode confidence in international trade while simultaneously exerting economic consequences associated with reporting adverse health events linked to a food item.

While globalization has increased the need for efficient, effective, coordinated, and comprehensive responses to zoonotic diseases, food-borne disease outbreaks, and detrimental changes to the environment, stakeholders in relevant sectors have continued to operate in relative isolation without considering the obvious links that One Health underscores (Figure WO-27). Rapid disease transmission across borders and between humans and animals has ramifications for health, international trade, international development, and the global economy (WHO, 2011). The past outbreaks of H5N1 and H1N1 influenza viruses were important events that helped focus international attention on One Health. The lessons learned from these epidemics have applications for One Health to improve food safety, including the need for integrated microbial surveillance across health domains, readily sharing information and data including the private sector, and building the capacity and infrastructures for both public and animal health.

Several workshop speakers discussed the important implications of a One

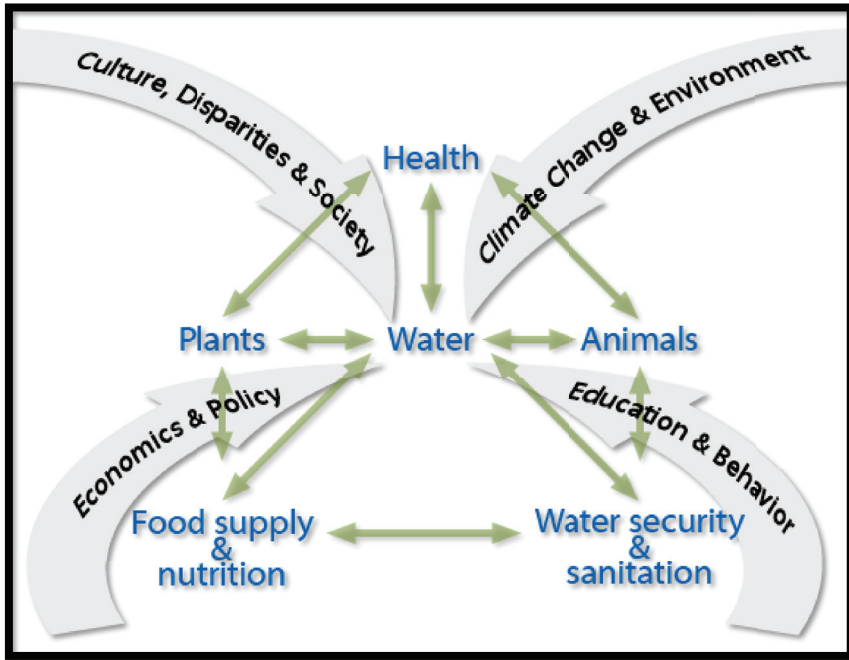


FIGURE WO-26 A One Health approach recognizes the interconnection between humans, plants, animals, water, and the environment as it relates to health problems. SOURCE: University of California Global Health Institute (<http://www.ucgh.i.universityofcalifornia.edu/images/one-health-chart.png>).

Health paradigm for food system biosecurity. Their presentations focused on the essential role of surveillance in understanding the relationships between food-borne diseases and ecosystems, and in using that knowledge to anticipate, detect, and respond to risk in a range of different contexts. Such complex efforts demand the involvement and coordination of multiple stakeholders—a challenge that is only beginning to be met, and one that ultimately will require organizational and institutional changes.

One Health in Australia: The Biosecurity Continuum

While acknowledging that common factors drive the emergence of infectious diseases, including those that are food-borne, speaker Martyn Jeggo, director of the Commonwealth Scientific and Industrial Research Organisation (CSIRO)²⁹ Austra-

²⁹ CSIRO is Australia's national science agency.

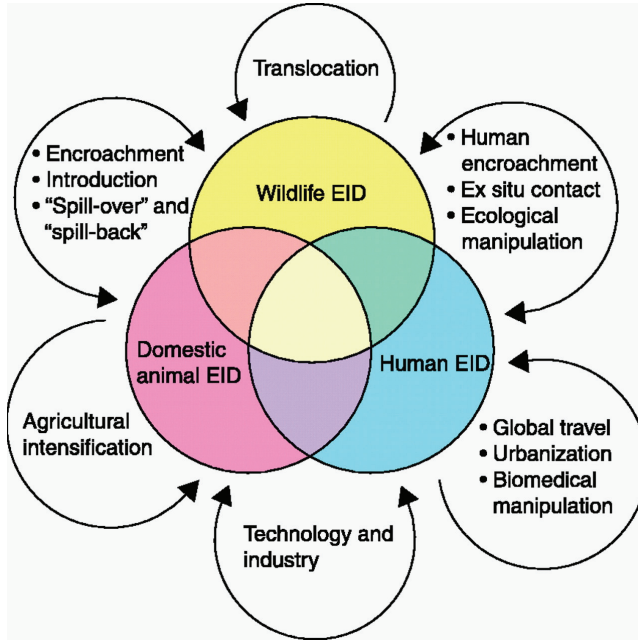


FIGURE WO-27 The “host–parasite” continuum. The host–parasite ecological continuum (in this context “parasites” include viruses and parasitic prokaryotes). Most emerging diseases exist within a host and parasite continuum between wildlife, domestic animal, and human populations. Few diseases affect exclusively any one group, and the complex relations between host populations set the scene for disease emergence. Examples of emerging infectious diseases that overlap these categories are canine distemper (domestic animals to wildlife), Lyme disease (wildlife to humans), cat scratch fever (domestic animals to humans), and rabies (all three categories). Arrows denote some of the key factors driving disease emergence.

SOURCE: From Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* 287(21):443-449. Reprinted with permission from AAAS. <http://www.sciencemag.org/content/287/5452/443.full.html>.

lian Animal Health Laboratory, noted that food safety tends to be managed quite differently from infectious disease control in Australia (and indeed throughout the world), and that these differences are likely to persist. (Dr. Jeggo’s contribution to the workshop summary report can be found in Appendix A, pages 198-206.) “From an infectious disease point of view we’re concerned with the effect of the disease on the host [which may be an animal or plant], whereas from a food safety point of view we’re primarily concerned with the impact [of adulterated food] on humans,” he noted. Nevertheless, Jeggo continued, Australia has embraced the One Health

paradigm in its emphasis on biosecurity, which he defined as “the protection of the economy, the environment, social amenity, and human health from the negative impacts associated with the entry, establishment, or spread of animal or plant pests and diseases, or invasive plant and animal species.”

Australia divides its efforts to manage biosecurity risk among preborder, border, and postborder activities, Jeggo explained. Preborder activities include epidemiological intelligence, risk analysis, and efforts to address offshore risks. Postborder activities include surveillance, detection, and response to biosecurity threats that have not been excluded by measures such as inspection and quarantine (Commonwealth of Australia, 2008). Although more than half of Australia’s investment in biosecurity currently supports activities at its border, he said, “we actually need to focus a lot more on our postborder activities.” Moreover, while compartmentalizing biosecurity activities relative to the border has been convenient, “we now recognize that if we’re going to be effective, it needs to be managed as a continuum,” Jeggo noted.

Australia’s federal government is primarily responsible for preborder and border biosecurity, whereas the states and territories implement postborder activities. Recently, in response to resource limitations, states and territories have sought support from the federal government and from industry in order to strengthen postborder biosecurity efforts, Jeggo said.

“We do have a very extensive National Animal Health Surveillance System, but we need to improve it,” he observed. “We have a very strong relationship with industry, and industry recognizes that [disease] poses a risk to trade and . . . local production. We’ve now got a strong dialogue going on, with industry prepared to seriously invest in this area.” However, he added, partnership with industry “comes with the underlying understanding that industry will also want to be involved in at least influencing the decision-making process.”

Australia recognizes that the One Health approach is essential to managing both food safety and infectious disease risks, Jeggo concluded. “It is clear to all of us that if we work together across that continuum of wildlife, animal health, and human health, we should deliver better outcomes,” he said, but he noted that actual evidence for that conclusion is lacking—and that it is necessary to support further efforts.

Jeggo also suggested that intergovernmental organizations that in the past “paid lip service to One Health” should continue to undertake organizational changes necessary to implementing interdisciplinary approaches to food safety. “We need to create divisions, departments, institutes of One Health where we can actually get a genuine partnership going on, [and] where resource allocation will drive the cultural changes that we need,” he insisted. “Organizational change [will] drive what we really want to achieve, and that is a genuine One Health approach.”

One Health in Canada: Integrated Surveillance

As in Australia, responsibility for food safety in Canada is divided among federal and provincial or territorial agencies, according to speaker Rainer Engelhardt, of the Public Health Agency of Canada. (Dr. Engelhardt's contribution to the workshop summary report can be found in Appendix A, pages 176-188.) Following an international expert consultation on One Health held in Winnipeg in 2009 (Public Health Agency of Canada, 2009), Canada "took up the gauntlet of One Health," he said, developing a strategic framework for ongoing efforts toward risk identification, assessment, and avoidance.

"Our current approach in the country is to look at One Health in the food safety context in the multiple dimensions—how to optimize health programs, targeting science and research, more integrated surveillance, enhancing food safety epidemiology, risk assessment, inspection and regulation," Engelhardt explained. To be effective will require collaboration among a broad range of agencies at the federal and provincial/territorial levels, as well as partnership with other countries and with non-governmental agencies, such as the WHO, he added.

Addressing AMR (an issue previously discussed in the subsection titled *Antimicrobial-Resistant Pathogens*) is a focus of Canada's One Health approach to food safety, and surveillance is the keystone of these efforts, Engelhardt said. Canada has established two primary complementary surveillance systems, which he described in detail: the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and the National Integrated Enteric Pathogen Surveillance Program (C-EnterNet). Information from these sources is further integrated with data provided by PulseNet (CDC, 2011d), by the Global Public Health Information Network (Public Health Agency of Canada, 2004), and by parties to the International Health Regulations (WHO, 2008).

CIPARS (Public Health Agency of Canada, 2007) is modeled after DANMAP (DANMAP, 2012) and also after NARMS (FDA, 2012), Engelhardt said. He explained that CIPARS monitors trends in antimicrobial use, as well as antimicrobial resistance in selected bacteria (particularly *Salmonella* and *E. coli* among food-borne pathogens), at major points along the "farm-to-fork" continuum. CIPARS is intended to enable the timely national and international dissemination of surveillance data, and its accurate comparison to similar data collected by other countries, he said. He also noted that in 2005, information from CIPARS linking the use of the antimicrobial ceftiofur in poultry to the development of resistance in humans and animals led to a voluntary ban on the use of the drug by the poultry industry.

Engelhardt described C-EnterNet, which was modeled to some extent on the CDC's FoodNet surveillance system (CDC, 2011c), as "an integrated program designed to monitor human infectious enteric illness in order to inform food and water safety policy." Through surveillance, C-EnterNet (currently a pilot program run at only two sentinel sites in Ontario and British Columbia but slated to be expanded soon to additional sites in Canada) detects changes in trends of the human

enteric disease incidence and pathogen exposure levels from food, animals, and water. These data sets are then analyzed to determine the proportion of cases due to water, food, or animal contact and thereby to identify statistically significant risk factors for enteric illness. For example, C-EnterNet analyses revealed that nearly one-third of reported cases of enteric disease (involving both food-borne and non-food-borne pathogens) were travel-related; such information has been used to develop advice for both travelers and physicians, he said.

“It’s important to have the CIPARS system and the C-EnterNet system work congruently,” Engelhardt observed. “The CIPARS side brings into play information on antimicrobial use and relevant elements of animal husbandry and management . . . [while] C-EnterNet looks at the inputs from the social/cultural and natural environments . . . [and] economic and trade considerations.” Together, they provide Canada with a national structure for integrated surveillance, he concluded.

Echoing remarks by Karesh and Jeggo, Engelhardt noted that present applications of the One Health paradigm to the complex problem of ensuring a safe food supply are implemented piecemeal, and their integration constitutes a work in progress. The programs and strategies described by these three speakers focus mainly on specific environmental interfaces critical to addressing food-borne disease, but as Engelhardt observed, “as far as the full operationalization of One Health is concerned, we’re not there yet. I think we see how to do it, but we’re not yet fully committed, especially institutionally to implementing the concept.”

One Health in the United States

Publication of Upton Sinclair’s *The Jungle* (1906) led to the passage of the first food safety law in the United States in the early 1900s; today, 15 federal agencies are responsible for executing the more than 30 laws that direct food inspection in the United States (GAO, 2004, 2005). A One Health approach to food safety emphasizes the *sharing of relevant information among disparate organizations, unifying organizational mandates among human, animal, and environmental health professionals, and integrating local national and international surveillance networks*. Although there has been limited interaction between human and veterinary health professionals, the implementation of a One Health approach could have numerous applications in the prevention of food-borne illness. Many scientific, regulatory, and surveillance organizations have begun to adopt a One Health approach to their programs,³⁰ but in many cases they have faced barriers to implementation (Atlas et al., 2010; Karesh et al., 2005; World Bank, 2010). *Currently, there is no single robust system in*

³⁰ For example, the U.S. Secretary of Agriculture is working to manage interagency cooperation in the area of One Health by creating the USDA One Health Multiagency Coordination Group (USDA, 2011b).

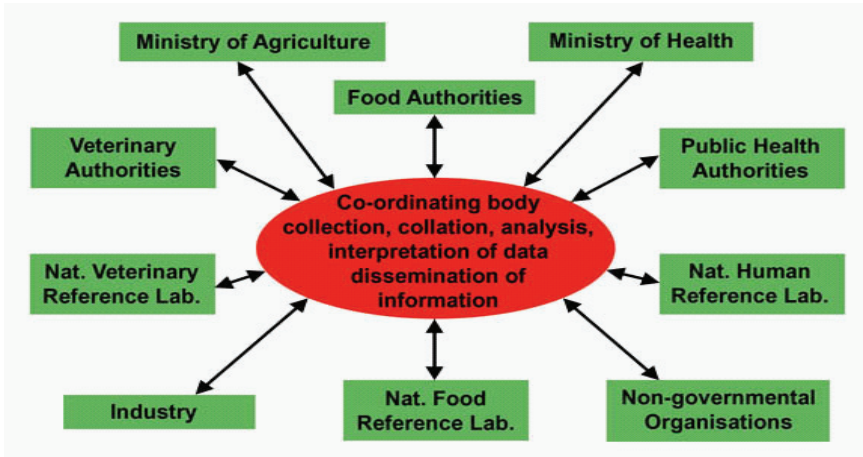


FIGURE WO-28 Schematic presentation of the collection, collation, analysis, and interpretation of surveillance data and the subsequent dissemination of information to all the major stakeholders in food safety. There is currently no single organization responsible for coordinating surveillance data.

SOURCE: Adapted from Wong et al. (2004).

place that embraces this approach,³¹ as illustrated in Figure WO-28 (Atlas et al., 2010, emphasis added).

Given the resource limitations that most governments face, adopting an efficient system that eliminates redundancy, maximizes benefits to public health, and reduces health risks would allow resources to be allocated in a way that provides the greatest benefit to the public. Yet, no single multilateral organization or government agency has a mandate to pursue policies or collect data related to disease spread based on a One Health approach (Karesh et al., 2005). Nearly all of the outbreaks discussed in this workshop are preventable when measures are taken to prevent, detect, and remove contaminants. Through collaboration of producers, processors, retailers, and consumers, interventions and systemic changes at multiple points along the food safety spectrum can dramatically reduce occurrences of food-borne illness (Taylor, 2002; Wegener, 2006).

³¹ The NARMS is a shared project among FDA, the USDA, and the CDC that is a good example of sharing information across agencies. In addition, the CDC has had an integrated strategy in place in a system to monitor West Nile that includes animals, mosquitoes, and people that has been successful, albeit not in food safety. The National Biosurveillance Advisory Committee issued a report to the Director of the CDC titled *Improving the Nation's Ability to Detect and Respond to 21st Century Urgent Health Threats* that recommends the need to have a more integrated surveillance strategy for the United States that includes animal populations and food (http://www.cdc.gov/about/advisory/pdf/NBASFinalReport_April2011.pdf).

FDA, One Health, and the U.S. Food Safety Modernization Act The January 2011 passage of the FDA Food Safety Modernization Act (FSMA)³² has increased FDA's role in food safety regulation, prevention of contamination, and import oversight, as well as its power to issue recalls; however, funding of these mandates remains uncertain (Stewart and Gostin, 2011). With its emphasis on prevention, rather than reaction, and its risk-based framework for inspections and regulation, the FSMA aligns FDA's food safety practices with core public health tenets, as well as with recent IOM recommendations (IOM, 2010b).

Michael Taylor, FDA's deputy commissioner for foods, described how the FSMA reflects the principles of One Health in his keynote address to the workshop. Calling the One Health perspective "indispensable to the goal of preventing food-borne illness," he stressed that One Health is central to FDA's overall approach to improving food safety, not just to implementation of the FSMA. For example, he said, the Office of Foods, which he directs, was created to integrate the work of FDA's Center for Food Safety and Applied Nutrition (CFSAN) and its Center for Veterinary Medicine (CVM). "The regulatory activities of CVM cut across to the human food safety side, whether it's dealing with the residues of animal drugs and animal feed additives in edible tissue of animals, [or with] the antimicrobial resistance issue . . . [or] with the issue of food animal shedding of pathogens," he explained. Resource allocation and budgeting for both Centers are integrated and guided by risk-based decision making aimed at "getting the most public health bang for the buck," he said.

The Office of Foods has also established a Science and Research Steering Committee, consisting of science and laboratory directors of CFSAN and CVM, as well as research directors from FDA's Office of Regulatory Affairs, which encourages integrated food safety research and methods development, Taylor continued. Experts from these agencies are also mounting a combined effort to implement key elements of the FSMA, which he called "a remarkable public policy breakthrough."

FDA's food safety program did not arise from an overarching vision, but instead consisted of a set of statutory provisions that had evolved in response to crises that arose over the course of the past century, Taylor explained. With the FSMA, Congress recognized the advantages of developing an integrated, whole-system approach to food safety—a view consistent with One Health, he observed. Specifically, the FSMA

- mandates an examination of the entire food system, from farm to table;
- emphasizes evidence-based risk reduction;
- includes both human food and animal feed; and
- recognizes the significant role and the inherent risks of international trade with regard to food safety.

³² FDA Food Safety Modernization Act, Public Law 111-353, 124 Stat. 3885.

Four illustrative issues In order to illustrate the influence of One Health in guiding FDA food safety policy, Taylor described the agency's approach to ensuring the safety of produce, eggs, and pet foods, as well as their efforts to reduce the risk of AMR.

As directed by the FSMA, FDA is in the process of establishing regulatory standards for growing practices on the farm to deal with the problem of the microbial contamination of produce and resulting food-borne illness, Taylor stated; these standards will address issues such as the microbial quality of water and the means to protect water supplies from contamination, and they will define the responsibility of the grower to prevent food contamination. However, there is also a need to encourage primary prevention of pathogens entering the food system, he added; to that end, FDA partners with the USDA, which in turn collaborates with the livestock industry, to develop on-farm practices and interventions to reduce pathogen loads in animals that could contaminate produce.

In 2009, FDA issued an egg safety rule intended to reduce the transmission of *Salmonella enteritidis* to eggs from infected laying hens (FDA, 2009). *Salmonella* infections are often spread to chickens in such facilities by rodents or birds, he noted. The rule stipulates that laying hens be separated from other animals that could potentially transmit infection. Applying this rule systematically and comprehensively will reduce the burden of salmonellosis in this country, he asserted.

The 2007 contamination of pet foods with melamine (FDA, 2010b), which caused more than 100 pet deaths amongst nearly 500 cases of kidney failure (Associated press, 2007) in the United States, catalyzed political action for pet food safety, Taylor observed. The agency responded by proposing rules governing the safety of pet food; recent incidents of human illness caused by *Salmonella*-contaminated pet food treats have also been taken into account in these proposed FDA rules, he said.

No issue captures the importance of understanding the link between the health of animals and humans as does the threat of AMR, Taylor observed. In 2010, FDA released a draft guidance document discussing the significant public health challenge posed by AMR and describing FDA's proposed strategy for addressing this issue, which includes phasing out antibiotic use for food animal production, feed efficiency, and growth promotion, as well as requiring veterinary supervision of the use of medically significant antibiotics. Several major food retailers and fast food chains have already made the decision not to buy meat from animals treated with medically significant antibiotics, he noted.

FDA's voluntary antibiotic phase-out strategy was informed by discussion with drug companies, the veterinary community, and the animal production industry, Taylor said. "We don't take the regulatory options off the table, but we are embarked in a very active dialogue with key elements of that community to pursue this phase-out strategy," he said, and that includes identifying and evaluating the remaining valid prevention or treatment uses of antibiotics in food-producing animals.

Taylor's description of FDA's approach to addressing AMR sparked considerable discussion among workshop participants, who had already considered Denmark's approach to that issue (see the subsection titled *Antimicrobial-Resistant Pathogens*). When asked what progress FDA had made toward assessing antimicrobial usage by animal type and geographic region, which would provide information necessary to establishing a baseline and monitoring responses to the proposed phase-out, Taylor acknowledged, "When it comes to really understanding in detail the patterns of usage by animal, by amounts, by region, we don't have that information." Moreover, he said, it is unclear whether FDA has the authority to collect such information. However, while such data are essential to science-based interventions, he stated his belief that they were not needed in order to support the more judicious uses of antimicrobials.

In the discussion that followed Taylor's remarks, King asked Taylor if he thought that U.S. government agencies, such as FDA, the USDA, and the Environmental Protection Agency, might coordinate their policies and regulatory activities under a One Health framework. Taylor expressed doubt that the lack of a conceptual framework was keeping these agencies from working together; he was more inclined to attribute "classic institutional organizational behavior issues" such as the creation of specialized "silos" of expertise and "turf" that must be funded and defended. However, he also noted that FDA was taking several steps to encourage collaboration, such as research under way with the CDC on ways to identify specific foods and pathogen-food combinations as causes of food-borne disease outbreaks.

The FMSA stipulates that the CDC's conduct of food chain surveillance should fulfill the needs of consumers, FDA and other state and local regulatory agencies, and the food industry, Taylor continued. In addition, he contended, such surveillance should be designed not only to generate data, but also to derive the greatest possible value from the data collected. Pursuing this goal as a collaborative effort is currently difficult, because the CDC and FDA budgets are separately funded, he added. "Ideally," he observed, "on cross-cutting subjects like surveillance you'd actually have an integrated budget initiative approach," but unfortunately, the appropriations process does not encourage it.

Keusch asked whether, given that more than 40 years of discussion and recommendations to reduce AMR had not produced significant regulation, there could be any reason for optimism on this issue. Nevertheless, he suggested that the possibility of creating partnerships with industry in which antimicrobial usage could be monitored and evaluated, and among which data on AMR were shared, might offer a glimmer of hope. Taylor agreed, and he observed that such partnerships would expand access to data collected by various companies on the distribution of microbial pathogens throughout the food system. Under the FSMA, FDA will examine privately conducted food safety audits of companies seeking accreditation in food safety, he added. "That's an enormous body of information that could be very valuable to us," he said, "but only if we have

an information system that permits us to put it together and analyze it and take advantage of it.”

In response to a question from Duchin, Taylor stated that FDA has not set a quantitative goal for reducing the non-therapeutic use of antimicrobials in animals. Rather, he said, FDA’s strategy represents a shift from the uncontrolled use of antimicrobials to controlled and monitored use, and the consequent monitoring of impact. The FSMA directs FDA to identify the most significant food-borne hazards across the food supply, and to implement measures to minimize those hazards, he further explained. “There will no doubt be some opportunities to set perhaps some quantitative benchmarks as performance standards,” he said. For example, he noted, FDA has long regarded *Salmonella* in ready-to-eat foods as an adulterant; in that case, he said, “the performance standard is we don’t want any.” But in many cases, the specific practices and verification tests needed to minimize risk from a given food-borne pathogen remain to be determined.

Another concept in need of definition is the “non-therapeutic” or “preventive” use of antimicrobials, as several workshop participants observed. In a forthcoming guidance statement, FDA defines as “medically important” those antimicrobials that are targeted to specific pathogens and are demonstrated to have prevented disease as having legitimate preventive use, Taylor explained; once this guidance is released, FDA will establish practices based on this definition and informed by dialogue with industry. Similarly, FDA has compiled a list of antimicrobials it considers to be “medically important,” but he noted that the list needs to be revised and updated (FDA, 2010b, 2011b).

Research agenda The overarching challenge in improving food safety through One Health is to bring interdisciplinary science to bear to implement interventions of proven benefit, Taylor observed. That cannot be achieved without cutting across organizational lines within FDA and among federal agencies, and forming both interstate and international partnerships, as well as interdisciplinary collaborations, he concluded.

Significant scientific questions remain to be answered before the benefits of many potential interventions to improve food safety can be evaluated, Taylor noted. For example, standards for the use of raw manure on crops, and for the microbial quality of irrigation water, cannot be set without detailed knowledge of pathogen survival under various environmental conditions; FDA is engaged with the USDA and other government agencies, with the food industry, and with academic researchers to gain the understanding necessary to set evidence-based standards, he said.

Methods development is another crucial area of food safety research, Taylor continued. The increasing role of verification testing among food processors and purveyors, and the enhanced role of microbial testing as performed by FDA, is driving demand for fast and reliable diagnostic methods, he said. While there is no FDA approval process for the use of testing technologies by the food indus-

try, he claimed that there is less a need for formal guidance in this area than there is for harmonization—and modernization—of testing methods used in all sectors.

Taylor noted that a key partner in the dissemination and implementation of research and regulation to improve food safety is the USDA's Cooperative Extension System.³³ For example, he said, FDA has formed a produce safety alliance in partnership with the USDA and the National Association of State Departments of Agriculture with the goal of educating and guiding small growers and food producers in best practices for food safety. The use of the Cooperative Service and its array of educational and technical resources will be critical to implementing the FSMA throughout the community of food growers, and particularly among smaller operations, he observed.

USDA, One Health, and Food Safety Research Speaker Cathie Woteki directs four agencies within the USDA that participate in food safety research: the Agricultural Research Service, the Economic Research Service, the National Agricultural Statistics Service, and the National Institute of Food and Agriculture, an extramural agency that supports research and education programs and extension. (Dr. Woteki's contribution to the workshop summary report can be found in Appendix A, pages 362-368.) She noted that three additional USDA agencies have food safety responsibility: the Animal and Plant Health Inspection Service, the FSIS, and the Forest Service.

Woteki's presentation focused on the importance of food safety research and the USDA's contribution to the field, which is increasingly aligned with One Health. She characterized the USDA as an organization where expertise in animal health and science, human food safety and nutrition, wildlife ecology, plant and crop science, and economics come together in one place: fertile ground for establishing a One Health approach, which has evolved out of the department's efforts to plan for pandemic influenza. "This comprehensive approach is going to improve global capabilities to detect, prevent, prepare for, and respond to emerging diseases, pandemic threats, and other issues in the human, animal, and ecosystem interface," she stated. "By applying the One Health principles, it's our hope at USDA to encourage a synergy of ideas, reduce our program redundancy, and apply this holistic approach ultimately to improving global health, whether it's human health, animal health, or the health of the environment."

"Research is often a silent partner in food safety," Woteki observed. She noted that while outbreaks raise public consciousness about the importance of food safety and outbreak investigation, research programs are crucial to the identification of novel food-borne threats. At the USDA, she continued, "we monitor the food illness epidemiological data to identify emerging threats. We work closely

³³ Each U.S. state and territory has a state office of cooperative extension at its land-grant university and a network of local or regional offices. These offices are staffed by one or more experts who provide useful, practical, and research-based information to agricultural producers, small business owners, and the general public (<http://www.csrees.usda.gov/Extension/>).

with our research partners to develop tests and new intervention approaches that work in a regulatory setting, as well as to develop intervention strategies to reduce risk throughout the food chain.” Woteki highlighted several such contributions, including the following:

- Agricultural Research Service research on high-priority national and international food-borne pathogens and contaminants, together with pathogen sources and reservoirs, detection methods, and post-harvest processing.
- A collaborative effort between the USDA and the CDC to develop a national swine influenza virus (SIV) surveillance pilot program to better understand the epidemiology of SIV infections and to improve diagnostic tests, preventive management, and vaccines for swine and humans (Sivapalasingam et al., 2003). This program was instrumental in implementing surveillance during the 2009 H1N1 influenza pandemic, Woteki noted.
- The funding of extramural research and education through the National Institute of Food and Agriculture (NIFA), including a joint program with FDA in 2009 to solicit research focused on integrating food system signals (e.g., clusters of illnesses reported by government authorities or problems identified through routine testing) with innovative technologies (e.g., geospatial analysis) to detect product contamination. NIFA also recently awarded a very large integrated grant to facilitate research on norovirus (see previous subsection titled “Food-Borne Viruses”), Woteki reported.

Two formidable challenges threaten the continued advancement of food safety research in the United States, according to Woteki. First, limited public funding, which tends to support basic and “public goods” research (as compared with private-sector research, which favors product development), constrains not only the improvement of food safety but also the overall productivity of the food system (Heisey et al., 2011), she argued. Second, she noted that the production of agricultural scientists in disciplines relevant to food safety has been flat for many years. “There are, according to the private sector, very good jobs that are going vacant because we’re not producing the well-trained scientists to fill them,” she said.

One Health in Practice: Regulations, Research, and Industrial Applications

Several speakers attested to the influence of the One Health paradigm in shaping regulations, research agendas, and industry practices to improve food safety. Each of the presentations summarized below identified ways in which government agencies, food companies, and sectors of the food industry have looked across the food chain to identify opportunities to minimize risk of food-borne disease. However, as many workshop participants noted, most of this activity

has occurred within industries and agencies, leaving vast untapped potential for transdisciplinary, transagency, and trans-sectoral collaboration.

The Role of Industry

Globalization of the international food supply has brought an increasing variety of foods to the global marketplace as well as reduced food costs; it has also led to recent food-borne outbreaks covered by the media (see Box WO-3). The trade-off has been an increased risk for food-borne illnesses (IOM, 2006), as well as increased bureaucracy among the agencies responsible for monitoring food safety and responding to outbreaks. Ensuring that all parts of the global food supply system function properly is critical to keeping the food supply safe.

Ultimately, consumer safety is the responsibility of industry. Technological advances have dramatically improved food safety; however, they do not necessarily represent advancement in prevention. Risk management is complicated not only by the numerous points where contamination may occur, but also by the diversity of food supply chains. Food producers face multiple risks. Whether microbiological or chemical in nature, these risks can be the result of poor sanitation, contaminated water, purposeful adulteration of products for economic gain (e.g., melamine in milk powder), non-adherence to best practices, or even intentional contamination. Unlike the United Kingdom, Canada, and many other countries, the United States uses a “risk-by-risk” approach to food safety rather than a comprehensive and unified preventive system (IOM, 2009).

The private sector, working synergistically with the public sector, must be able to develop and establish food safety protocols even in the absence of a specific law or regulation. A number of companies have recognized the value of going above and beyond mandated regulations in order to bolster consumer confidence. The cost of recalls and the damage that association with a food-borne outbreak can do to an industry are strong incentives for private-sector regulatory compliance.

It is in every country’s best interest that regulatory agencies collaborate with industry and incentivize improvements to food safety systems. Countries also have their own “brand and reputation” to preserve. When countries are linked to food-borne illness, it is extremely difficult to rebuild consumer confidence. By collaborating, it is likely that industry compliance would increase, and regulatory agencies would be able to decrease inspections. Increased collaboration would also allow regulators to make better risk-management decisions (IOM, 2009).

Industry Response to Food-Borne Disease Risks:

Costco’s Approach to Food Safety

Food represents a significant proportion of sales by Costco Wholesale Corporation, the third-largest retailer in the United States and the eighth-largest in the world, according to speaker Craig Wilson, the company’s vice president and

general merchandising manager of quality assurance and food safety. Costco has a comprehensive program of microbial food testing, he said, that is guided by the premise that prevention beats the alternatives.

Every food product sold by Costco must conform to microbiological Quality Assurance/Quality Control (QA/QC) criteria, Wilson stated, including total aerobic plate count and measures of coliform bacteria, *E. coli* species, EHECs, *Salmonella*, and *Listeria*. Until each lot of a given food product passes these and other tests, including X-ray analysis for metals and other solid contaminants, it is withheld from distribution. This test-and-hold policy has reduced the number of recalls for several products, including bagged ready-to-eat salads and meat, he observed.

Costco also investigates food safety practices at its food suppliers. For the past decade, every supplier's facility has been required to have a HACCP system in place. In addition, Costco insists that suppliers prove that they know the origins of every ingredient they use. Why go to these lengths? "We want to ensure that the processes are validated," Wilson explained. "We want to document due diligence . . . [and] to minimize recalls," he continued. "We never want you to go into a Costco and even think about food safety."

While he agreed with Hueston's earlier assessment that testing in and of itself does not ensure food safety, Wilson stated that testing is an important way to gauge process control by food suppliers, as well as to determine whether interventions taken to improve food safety are effective. "We do a lot of food safety audits," he said; these involve not only checking the microbial specifications of the product, but also determining how well the suppliers themselves perform these tests and keep their records, and how they perform in a mock recall of their product. "We want to inspect what we expect," he concluded.

Despite these measures, Costco has inevitably experienced recalls. Food safety will never be absolute, Wilson said; however, the numbers of recalls can be continually driven down. He noted a number of factors limiting improvement in food safety, including the fact that a proven intervention, food irradiation, has yet to gain public acceptance; nevertheless, he expressed certainty that "its time is coming" (see further discussion of food irradiation in the subsection entitled *Industry's Performance*). Similarly, in an effort to address the issue of antimicrobial resistance, Costco also offers—and expects to expand—a range of antibiotic-free meat products.

Industry Perspective: Cargill's Approach to Ensuring the Safety of the Global Food System

Mike Robach, of Cargill Incorporated, offered the perspective of an international food company with interests that span the entire food system. (Mr. Robach's contribution to the workshop summary report can be found in Appendix A, pages 298-307.) The company's 1,200 facilities in 66 countries are united by a set of core operating principles, including prevention-based, third

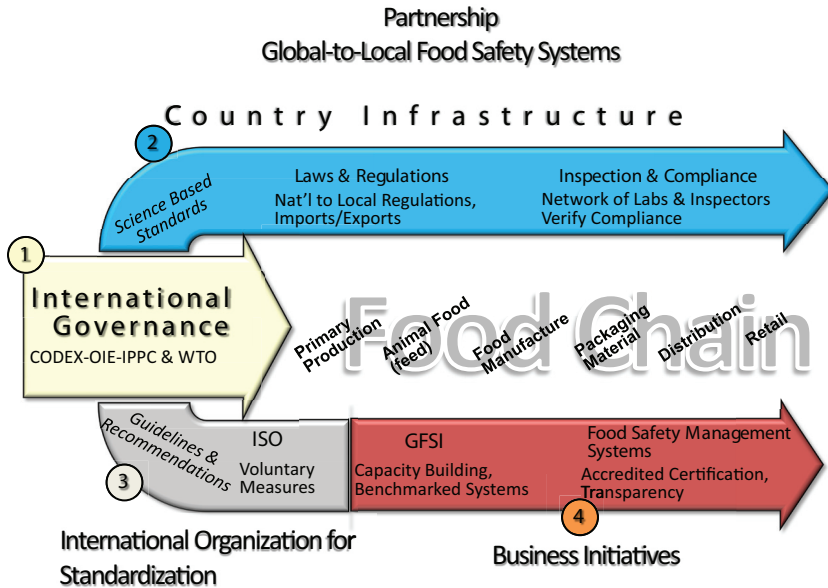


FIGURE WO-29 Global-to-local food safety systems.
SOURCE: Cargill; Robach (2011).

party-accredited food safety standards, which he described as “critical to our success.” This approach integrates with a broad vision of partnership in managing food safety that incorporates international governance and standardization, national governance, and business initiatives, depicted in Figure WO-29.

As discussed by Robach, the key elements of safety systems across the food chain include

1. international governance, including the Codex Alimentarius (Codex; www.codexalimentarius.net/web/index_en.jsp), the World Organisation for Animal Health (www.oie.int/), International Plant Protection Convention (www.ippc.int/), and the World Trade Organization (www.wto.org/);
2. country infrastructure (including laws, regulations, and their enforcement at all levels of governance) founded on science-based standards;
3. guidelines and recommendations issued by the International Organization for Standardization, which include voluntary standards and implementation procedures for food safety accreditation, audits, and management systems; and
4. business initiatives, including the Global Food Safety Initiative, a multi-stakeholder group that has developed guidance and benchmarks for food safety systems based on Codex.

According to Robach, Cargill's food safety policy is based on the Codex Alimentarius (often known simply as the Codex), a collection of international food standards maintained since 1961 by a commission comprised of members of the FAO and the WHO (Joint FAO/WHO Food Standards Programme, 2006). Codex specifies a comprehensive program of food safety policies and procedures, including management responsibility, resource management, hazard analysis, traceability, and validation. Among the many programs Cargill has developed to meet these standards, Robach described two illustrative examples: the management of purchased materials and measures taken to prevent cross-contamination of foods.

Cargill expects the more than 400 external manufacturers that produce products on the company's behalf to meet the same food safety standards as its own manufacturing plants, Robach stated. To evaluate the suitability and compliance of external manufacturers, Cargill uses a risk assessment model that scores the risk inherent to the materials being supplied, as well as the capability of the supplier to manage that risk. The company carefully scrutinizes and works intensively with the small percentage of suppliers judged to be high risk, he said.

Most Cargill facilities employ environmental monitoring in order to prevent cross-contamination of foods, Robach reported; in facilities where contamination with *Salmonella* and *Listeria* are deemed likeliest to occur, there are specific control programs in place for those pathogens, he added. He noted that a decision tree, used in every facility to support accurate risk assessment, encourages Cargill's employees to think beyond the manufacturing process to the rest of the food chain. "The more preventative measures we can have in place around the world, the more assurance we're going to have of an abundant, safe food supply," he stated. "It builds confidence in food safety, enhances global trade. It enhances food security."

In subsequent discussion, several participants took up the more difficult challenge of imposing food safety standards on small-scale suppliers in developing countries, where, for example, shrimp might be raised in high concentrations of antibiotics or toxic chemicals. In an attempt to avoid or ameliorate such problems, Cargill partners with major customers who buy products from these markets to build better capacity and educate growers and suppliers about food safety, Robach said.

The One Health approach at Cargill hinges on global partnerships, Robach observed. "We work closely not only with our supply chain and our competitors in the industry, but also with our customers and with the regulatory agencies," he said, adding that the company also works closely and shares information with the CDC. "Working with academia, consumer groups, government, and industry is the path forward," he continued. "We've got to work together."

Nevertheless, Robach concluded, "business shoulders the responsibility for safe food. I know a lot of times government thinks they have the responsibility. They don't. We do. It's our product. It's our brand. They're our customers. We

want to work together, and we want to work collaboratively. But at the end of the day, we're the ones who have the responsibility, and we accept that."

Industry Perspective: Fresh Produce

Although most food-borne illness is in theory preventable, the especially vulnerable fresh produce sector does not yet have the tools to eliminate such risk, according to speaker David Gombas, of the United Fresh Produce Association. An estimated 1 billion servings of fresh produce—a category that comprises more than 300 different foods—are consumed in the United States each day, he noted. These foods originate from more than 100,000 farms in the United States and many times that number of foreign farms, he continued, with the largest operations contributing the majority of fresh produce sold.

The produce industry's primary food safety tool is prevention, Gombas stated. There is no "kill step" that effectively removes all pathogens from produce while preserving its "fresh" status. "While we are very good at getting rid of 90 to 99 percent of the contamination that could be on fresh produce, there is always going to be some residual number of organisms that are able to hide away," he said. "Therefore, we strive at every point in the supply chain to prevent contamination from occurring, and we're not always successful."

When prevention fails and a produce-associated outbreak of food-borne disease occurs, it is frequently difficult to discern its cause—and therefore to avoid a recurrence, Gombas observed. For example, he noted, all *Listeria* cases so far have been linked to processing, yet the pathogen's primary habitat is in the field. He also questioned as speculative the interpretation of recent investigations of salmonellosis linked to hot peppers and papayas and of *E.coli* O157:H7 linked to strawberries, and in particular that of the 2006 outbreak of *E. coli* O157:H7 linked to bagged spinach, which had previously been described by King, Tauxe, and Doyle. "At the end of the day we really don't know what happened in this incident, and in many of the other incidents we don't know what the vector was either," he concluded.

According to Gombas, the most likely sources and vectors of produce contamination, as identified in FDA guidance (FDA, 1998) are

- water (in all its forms),
- workers,
- surface contact (e.g., equipment, containers, utensils),
- animals (domestic and wild),
- soil amendments,
- prior land use,
- adjacent land activities, and
- cross-contamination.

These factors are well known and carefully considered by the produce industry, he asserted. Improving on this general approach will require identifying the actual risk factors at each stage of produce growing and processing. In particular, he noted, risk factors for preharvest produce contamination—those associated with water, animals, soil amendments, and land use—are not well understood. He demonstrated this point with a lengthy series of unanswered questions about the actual and comparative risks of various agricultural practices (Can manure be safely composted? Can some crops be safely amended with manure? Which wild animals pose the greatest risks as vectors of food-borne disease? What precautions should be taken if animal droppings are found in a field?). Much is known about risk factors for food-borne disease, he concluded, but very little is known about what is actually safe.

Recognition of these risk factors has inspired an escalation in food safety standards based on fear, rather than on science, which consume resources that might be better spent to improve the overall healthfulness of food, Gombas suggested. “You’ve got a limited number of dollars to spend on the quality control and food safety of these fresh produce items,” he observed. “There are consequences to the escalating food safety standards. There are also consequences on conflicting [food safety] audit standards, conflicting training messages, industry and consumer confusion.”

The answer to this dilemma is research to determine the actual risks associated with every step of food processing, Gombas argued. “We need the research based on real-world conditions . . . [because] produce is grown in a completely uncontrolled environment,” he said. “We need to be able to understand what those environmental [risk] factors are and what influence they have on the survival of the pathogen. We need to know what’s really happening, not what could be made to happen [in the laboratory]. And it has to be solution-directed research. We don’t need more basic research on potential pathogens, potential risk factors because we’ve got plenty enough right now that we don’t have answers for.”

David Acheson, of Levitt Partners, LLC, agreed that specific measurements of risk factors are necessary to improving food safety. For example, he noted, it was once assumed that a person would need to consume approximately 1 million *Salmonella* bacteria to become ill—until precise measurements were made, which reduced the “dose” to only 25 organisms. “Funding agencies need to change their metric and put the money where the tough questions are, and not on the easy lab stuff,” he declared.

Fletcher responded by pointing out several factors that make field-based research difficult. Detecting tiny amounts of naturally occurring pathogens in the field—amounts that could nevertheless pose a health threat—is currently impossible, she noted. Researchers are typically constrained (both legally and financially) from inoculating virulent bacteria in the field, she added, and attenuated strains may not accurately reflect pathogen behavior. “How can we do the experiments in a way that is meaningful?” she asked.

Forum member David Rizzo, of the University of California, Davis, wondered who would fund and perform such field experiments. Applied work is not favored by granting agencies, he observed, and field experiments tend to be performed by agricultural extension agents, whose positions have been cut in the name of deficit reduction. Gombas replied that his organization had advocated for a program to fund such research jointly with industry as part of the 2008 Farm Bill, but that it is only now beginning to live up to its promise. “With the 2012 Farm Bill coming along very soon, we’re hoping to put some fixes in place that will get the money to those individuals that can do the work that we need to have done,” he said.

Food Safety Trends: Implications and Possibilities for the Future

In a presentation titled “How Well Are Food Companies Addressing Microbiological Safety Issues?” Acheson disputed the common perception—based on increasing numbers of reported outbreaks and product recalls—that food is becoming less and less safe. On the contrary, he insisted: recalls are good, because they show that the food safety system is working.

That system has been strengthened by several recent improvements over the past two decades, including the ability to link food with disease and to detect lower levels of chemical adulterants, Acheson reported. In addition, greater fidelity of epidemiology, aided by improvements in genetic testing, enable quicker and more accurate outbreak investigations.

Nevertheless, the increasing importation of food presents obvious challenges to maintaining a safe domestic food supply—a task rendered even more difficult by shifting expectations among consumers, Acheson observed. Concerns regarding the intentional adulteration of food, whether it is done for profit or as an act of terrorism, are well-founded, he acknowledged. However, he observed, those worries often accompany the unrealistic expectation that all foods available to Americans will be unfailingly safe; when outbreaks inevitably occur, consumers blame food producers, causing damage to their businesses and brands. He also noted that American consumers increasingly want to buy local and unprocessed food, free of chemical pesticides and fertilizers.

The news media profoundly influences how the U.S. consumer views food safety and offers unmatched potential to educate the public about food-borne illness, Acheson said. Unfortunately, he added, both the corporate news media and social media outlets respond rapidly (and sometimes hastily) to food-borne disease events and are vulnerable to bias, selective reporting, and a tendency to seek blame.

In an attempt to answer the question he posed in his presentation’s title, Acheson reviewed trends in annual numbers of food recalls and Warning Letters³⁴

³⁴When it is consistent with the public protection responsibilities of the agency and depending on the nature of the violation, it is FDA’s practice to give individuals and firms an opportunity to take voluntary and prompt corrective action before it initiates an enforcement action. Warning Letters are

issued by FDA, as well as in rates of laboratory-confirmed infections with important microbial pathogens. There was a “massive” increase in FDA-reported recalls of contaminated foods in 2009-2010—many of them due to *Salmonella*—and the same trend is likely for 2011, he reported. However, he contended, most recalls are triggered by testing and process control analysis by the food industry detects contaminants, and thus before outbreaks occur. Similarly, in 2010 the number of warning letters issued by FDA nearly doubled as compared with previous years, he said; he believes this reflects both increased enforcement and vigilance by that agency, as well as a lower bar for issuing such letters. Finally, many microbial pathogens (*Vibrio* spp. and *Salmonella* excepted) have been associated with decreasing numbers of food-borne outbreaks in the United States over the past 15 years—despite increased capacity to detect and investigate food-borne illness, and an increasingly vulnerable population (due to aging and compromised immunity). All three trends suggest that the food industry is doing a good job of controlling food-borne pathogens, he concluded.

Industry leaders are pursuing a range of strategies to continue to improve food safety, Acheson said; these measures include better tracking of the materials they use and the products they sell, and the use of process controls such as good manufacturing practices, judicious testing, and system monitoring. Unfortunately, he noted, these advancements are not yet feasible for many smaller companies that, collectively, play a significant role in the U.S. food supply. Further improvement in the overall safety of the U.S. food supply is also limited by consumer aversion to technological solutions such as irradiation, he observed; conference participants pointed out additional drawbacks to food irradiation, including cost (in the case of leafy greens, according to Gombas) and aesthetics (in the case of ground beef, which—according to Robach—has been said to [smell] like a wet dog).

Strategic Partnership with Industry

Although the ability to link food with disease continues to increase, capacity to respond to such information remains limited, King observed, leading him to wonder whether industry could help bridge this widening gap by leading adoption of the One Health paradigm. Robach provided an example of such leadership: a recent voluntary recall of ground turkey, prompted by Cargill’s discovery that its product was contaminated with *Salmonella*. “It was through a series of pieces of

issued to achieve voluntary compliance and to establish prior notice. The use of Warning Letters and the prior notice policy are based on the expectation that most individuals and firms will voluntarily comply with the law. The agency position is that Warning Letters are issued only for violations of regulatory significance. Significant violations are those violations that may lead to enforcement action if not promptly and adequately corrected. A Warning Letter is the agency’s principal means of achieving prompt voluntary compliance with the Federal Food, Drug, and Cosmetic Act (the Act). For more information please see FDA, Regulatory Procedures Manual 4-1; Warning Letters. <http://www.fda.gov/ICECI/ComplianceManuals/RegulatoryProceduresManual/ucm176870.htm> (accessed April 5, 2012).

information that we collected rather serendipitously from different sources combined with information that we had internally that led us to the conclusion that our product was likely associated with some illnesses that were being reported,” he said; Cargill initiated the recall without prompting from the USDA.

This incident illustrates the need for better and clearer lines of communication between the public health community and industry, Robach observed. For example, he said, combining the CDC’s preliminary epidemiological information with industry’s knowledge of supply chains could reveal potential sources or vectors associated with food-borne disease clusters early in their investigation. He stressed that two-way communication—now a relative rarity—is essential to such strategic partnerships.

“There’s a tendency from the regulator side to want it all wrapped up in a nice little bow, and then take it to the food industry and say, ‘We’ve got you,’” Acheson observed. “That’s not the way forward because we all know from our experiences in the public sector that taking these disparate facts and connecting the dots takes a lot of footwork and . . . dollars.” Instead, he encouraged regulators to establish a trust-based relationship with industry in order to collaborate in solving food-borne disease problems.

Gombas’ long list of unanswered research questions toward defining “what is safe?” for produce reminded Tauxe of similar questions posed by ground beef producers following the previously described 1993 outbreak of *E. coli* O157:H7 (see the earlier section *Food-Borne Disease Trends in the United States*). At that time, key safety questions were addressed by business leaders, who set aside competition to develop practices that could benefit the industry as a whole, he recalled. “Theirs was actually a very practical approach, not an enormous, high-level research approach,” he observed. Nevertheless, he added, their efforts produced a substantial reduction in *E. coli* O157:H7 infections without devastating the ground beef industry. Could the produce industry adopt that model, Tauxe wondered?

Such efforts are under way, Gombas said; they include partnering with FDA to develop guidance statements for produce growing and processing, along with programs to support grower adoption of recommended practices. “The industry has gotten together in many of these cases and has established what the risk factors are and what are the best mitigations we know today,” he concluded.

The Future of One Health

As the workshop drew to a close, King presented a summary of strategic actions identified by individual workshop participants that could advance the cause of improving food safety with One Health beyond mere awareness of its promise, and into action. These steps include the following:

- presenting a sufficiently compelling case for the One Health paradigm that is expressed in training and education programs;

- conducting outcomes research to demonstrate the economic advantages of One Health;
- embracing One Health as an opportunity for organizational change, directed toward cross-disciplinary education and collaboration; and
- designing research prototypes for proof-of-concept validation of One Health principles as applied to food safety in the developing world, and also to public–private partnerships between government and the food industry.

Finally, King emphasized the importance of a unified effort to advance the One Health paradigm. As the breadth of workshop presentations demonstrated, many stakeholders in the global food system have recognized the promise of One Health and are exploring its strategic adaptation; however, he continued, these activities are largely independent of and isolated from each other. “There’s already a concern that these different pieces of One Health are already competing and going their different ways,” he observed, and, in so doing, undermining One Health principles of cooperation and collaboration. “Somehow before we get too far along there needs to be a unification of these efforts, and to rethink this in a way that will be effective and worthwhile,” he concluded.

As discussed earlier in this chapter, prevention is the chief means for achieving food safety, but preventing food-borne outbreaks will require a much broader approach than currently exists (Karesh et al., 2005). A risk-based food safety approach is the underpinning of a strong food safety system that is able to prioritize risks and allocate limited resources where they will be the most effective. Moving away from many of the current practices to a system that allows agencies, the private sector, and other third parties to share responsibility for maintaining a safe food supply will help to eliminate regulatory gaps as well as reduce resource burden (IOM, 2010b; Stewart and Gostin, 2011).

A key component of prevention will be the ability to use data to anticipate where outbreaks are likely to occur. Shifting to a proactive food safety approach will require governments to implement research-based interventions through regulation and education that will produce the greatest reduction in disease burden at the lowest cost. Such transformations will require a substantial “sea change” in philosophy—moving away from a top-down approach to public health and toward cooperative, interdisciplinary strategies for disease prevention; this is the essence of the One Health principles.

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Appendix A

Contributed Manuscripts

A1

**EHEC O104:H4 IN GERMANY 2011: LARGE OUTBREAK
OF BLOODY DIARRHEA AND HAEMOLYTIC
URAEMIC SYNDROME BY SHIGA TOXIN-PRODUCING
E. COLI VIA CONTAMINATED FOOD**

Reinhard Burger^{1,2}

In the summer of 2011 Germany experienced one of the largest outbreaks of a food-borne infection caused by enterohaemorrhagic *Escherichia coli* (EHEC) with the serotype O104:H4. A large number of cases with bloody diarrhea and haemolytic uraemic syndrome (HUS) occurred. Never before was such a high rate of HUS cases observed in an outbreak caused by a food-borne pathogen. The events in Germany caused by EHEC O104:H4 in the summer of 2011 show dramatically how rapidly an infectious agent is able to develop into a major health threat for a whole country. The outbreak caused widespread concern among the population, turning soon into fear. People expecting safe and healthy food felt threatened. It changed the eating habits of the majority of the population, and it had enormous economic consequences, particularly for farmers producing salad ingredients. It resulted in a large number of seriously ill patients and in a substantial number of deaths. The burden of disease and the economic consequences

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have made it a tragedy for many. It is important to analyse this outbreak scientifically in order to learn from this unique event and to be prepared for comparable infections in the future. In particular, all the steps regarding detection of cases, diagnostic procedures, identification of vehicle and origin, and infection control measures, all the way to therapy, should be reflected carefully. Usually, even experienced physicians encounter only a few cases of EHEC-induced HUS in adults in their whole career. Therefore, the large number of cases in Germany represents a valuable source of information for future epidemics.

This manuscript summarises the work of the HUS investigation team of the Robert Koch Institute (RKI) and gives an overview of the work done by the colleagues in the Department of Infectious Disease Epidemiology at the RKI (G. Krause, C. Frank, D. Werber, K. Stark, and U. Buchholz), the Department for Infectious Diseases (M. Mielke and A. Fruth), and the RKI-Consultant Laboratory for HUS/EHEC at the University of Münster (H. Karch). Many additional colleagues were involved.

Epidemic Profile and Development of the Outbreak

The extent of the outbreak becomes apparent by comparison with the average annual numbers of EHEC cases or HUS in Germany. In previous years about 1,000 patients per year were identified, with a median age of about 5 years. Of these patients about 70 per year developed HUS, with a median age of about 2 years (Frank et al., 2011a). In the outbreak from May to September 2011, approximately 3,000 EHEC cases were observed with a median age of 46 years, 58 percent of those patients were female, and 18 deaths were observed among the EHEC patients (0.6 percent). An additional 855 EHEC patients who developed HUS were identified (Frank et al., 2011b). This represents more than 20 percent of the total number of patients (3,842). The large majority of these patients were adults, the average age was 42 years, 68 percent of the HUS cases were female, and 35 deaths were observed among the HUS patients (4.1 percent). The total death toll was 53 patients (Figure A1-1).

Analysis of the incidence of HUS by the likely county of infection revealed that northern Germany was mainly affected. The same is true for cases with travel history; also for these patients the county of residence at the time of infection was northern Germany. Most cases were observed in the states of Schleswig-Holstein, Mecklenburg-Western Pomerania, Hamburg, Bremen, and Lower Saxony. Later in the epidemic, cases were found in all of the 16 German states. The incidence in the five northern German states varied from 1.8 to 10 cases per 100,000 persons. All other states had incidence rates with less than 1 case per 100,000 persons (Frank et al., 2011b; Wadl et al., 2011).

A substantial number of EHEC or HUS cases occurred also internationally during this time, particularly in the European Union, but also a few cases in the United States and Canada. Particularly affected was Sweden with 35 EHEC

Outbreak cases	N	Age (median)	% Female	Deaths
EHEC	2.987	46 years	58%	18 (0,6%)
HUS	855	42 years	68%	35 (4,1%)
	>20%	90% adults!	> ♀	
Total	3.842			53

Comparison with previous years

EHEC: ~1,000 illnesses/ year median age: 5 years

HUS: 65 illnesses/ year median age: 2 years


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FIGURE A1-1 Total number of EHEC and HUS cases and associated deaths during the outbreak of EHEC O104:H4 in summer 2011 in Germany and comparison to an average year.

and 18 HUS cases including one fatality, Denmark with 15 EHEC and 10 HUS cases, and France with 10 EHEC and 8 HUS cases. Single cases were found in 12 additional European countries. In the United States 2 EHEC and 4 HUS cases were identified with one fatality, and Canada had a single EHEC case. An epidemiological analysis revealed that—with two exceptions—all cases in this outbreak of EHEC or HUS found internationally were directly or indirectly associated with a visit to Germany during the weeks of the outbreak. Most of these patients visited northern Germany for a shorter or longer period of time during the peak of the outbreak.

The RKI was notified about the outbreak by a phone call from the local health authority of the state of Hamburg on May 19, 2011. Immediately (i.e., the next day), the RKI sent a substantial team of experts to Hamburg in support of the local colleagues. The subsequent epidemiological analysis revealed in retrospect that the outbreak had in fact started at the beginning of May and reached the peak of cases on May 22, 2011 (Figure A1-2). Thus, there was an obvious and substantial notification delay (Altmann et al., 2011). Up to the moment of notifying the RKI, a large proportion of the infections had already occurred. After May 22 both the reported number of EHEC gastroenteritis and the number of HUS cases decreased (Wadl et al., 2011).

The team of epidemiological specialists sent to Hamburg started right away with initial explorative interviews. The team size was enlarged in the next days

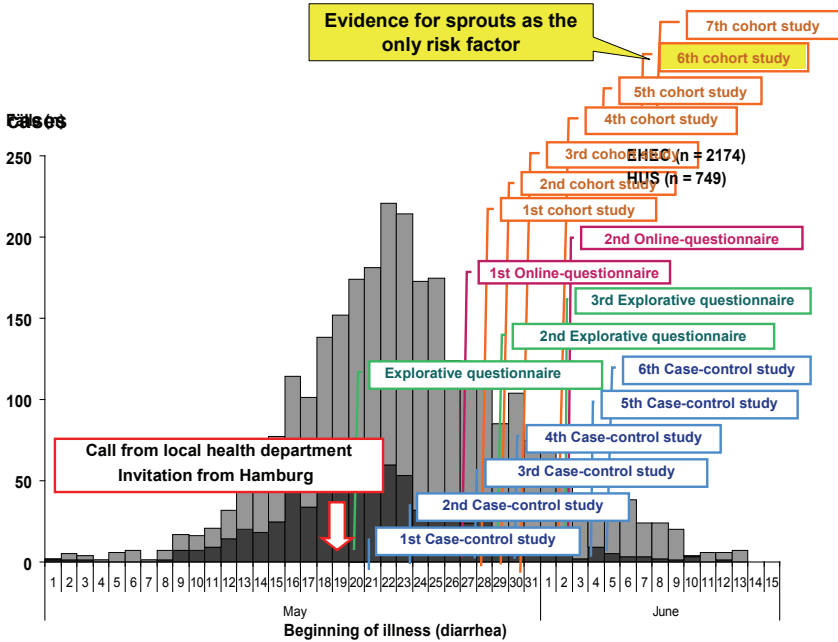


FIGURE A1-2 Epidemiological curve of EHEC (gray) and HUS cases (dark) and overview of epidemiological studies performed by the Robert Koch Institute for identification of sprouts as the vehicle of transmission.

SOURCE: Robert Koch Institute.

(up to 15 members), and a substantial number of case-control studies, additional explorative interviews, and cohort studies were started. As early as May 21 (i.e., 2 days after the RKI was notified), the first qualitative evidence for the role of vegetables was obtained. Raw milk products or products from raw meat, which frequently represent a source of infections with EHEC, had already been ruled out as the origin of infection in this outbreak. On May 22 the corresponding information was submitted to the European Early Warning and Response System and to the World Health Organization. Local public health authorities were warned, and initial interviews were given to the German press. During the next few days, information was provided on the website and in a series of press conferences and interviews. On May 25 (i.e., 5 days after the outbreak), the pathogen was identified from patient samples as EHEC O104:H4 by the RKI-Consulting Laboratory for HUS in Münster and the National Reference Centre laboratory for bacterial enteric pathogens at the RKI (Buchholz et al., 2011).

After a number of telephone conferences, the RKI together with the Federal Institute for Risk Assessment and the Federal Office of Consumer Protection

and Food Safety conducted a press conference advising on food consumption. Advice was given not to consume raw tomatoes, cucumbers, and salad *in* northern Germany. This recommendation was based on the increased risk of illness after consumption of these raw salads in northern Germany. Unfortunately, the majority of the press reported this advice as warning against salad *from* northern Germany.

Once the magnitude of the outbreak became apparent, the RKI immediately established a website providing all details about the infectious agent, updated as they developed, both for the medical specialists and microbiological laboratories in Germany and abroad and for the general public. Data sheets on the infectious agent and frequently asked questions, sometimes updated several times a day, proved to be an important source of information.

After mid-June 2011 only single cases of HUS occurred. On July 26 the RKI declared in a press conference the end of the outbreak because no new cases clearly associated with the outbreak had been reported for 3 consecutive weeks since the last newly reported illness on July 4.

Identification of the Infection Vehicle

In addition to the explorative interviews and case-control studies, cohort studies in disease clusters proved to be particularly helpful. Beginning on June 1, more than 30 cohorts were investigated in order to identify the vehicle of infections and to identify further cases. Particularly useful were cohort studies of travel groups that included international visitors or tourist groups from abroad. Here a close cooperation with foreign health authorities was instrumental. For a number of travel groups the length of stay, the particular location, and food consumption could be reconstructed in detail. Also, cluster analysis of patients associated with food consumption in different restaurant-associated outbreaks provided information. An analysis of billing data of guests at an affected canteen provided further data. In these studies a detailed investigation was performed using ordering information and additional details documenting the consumption as revealed by the corresponding bills. The most substantial evidence regarding the vehicle of infection was obtained by a so-called recipe-based restaurant cohort study (Buchholz et al., 2011).

Sprouts as the Responsible Vehicle of Infection

In the course of the epidemiological analysis it became obvious that patient memory is not a reliable source of information. This proved to be particularly true because in these EHEC/HUS patients not only symptoms of gastrointestinal infection and impaired kidney function were observed but also major neurological symptoms, preventing reliable interviews. Therefore, the recipe-based restaurant cohort study was designed to obtain information independent of a functioning patient memory (Figure A1-3).

Table 3. Relative Risk of Infection Associated with Sprouts and Other Raw Food Items in Univariable Analysis.

Food Item	Total Subjects Evaluated	Subjects Exposed (Percent of Cohort)	Cases among Subjects Exposed (Attack Rate)	Subjects Not Exposed (Percent of Cohort)	Cases among Subjects Not Exposed (Attack Rate)	Relative Risk (95% CI)	P Value
	no.			no. (%)			
Sprouts	152	115 (76)	31 (27)	37 (24)	0	14.23 (2.55–∞)	0.001
Tomatoes	152	50 (33)	14 (28)	102 (67)	17 (17)	1.68 (0.77–3.62)	0.18
Cucumbers	152	50 (33)	14 (28)	102 (67)	17 (17)	1.68 (0.77–3.62)	0.18
Chinese cabbage	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17
Radicchio	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17
Lettuce	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17

Source: Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med* 2011. DOI: 10.1056/NEJMoa1106482.

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FIGURE A1-3 Recipe-based restaurant cohort study of the Robert Koch Institute reveals risk for infection associated with the consumption of sprouts.
SOURCE: Taken from Buchholz et al., *NEJM*, 365, 1763 (2011).

Ten cohorts with a total of 168 guests of a given restaurant in the city of Lübeck in Schleswig-Holstein were identified. All persons had dinner at the same restaurant between May 12 and 16. Eighteen percent of the guests consuming food at this restaurant showed bloody diarrhea or EHEC/HUS within 14 days (31 persons). All persons were questioned about which meals they ordered, using photos of the dishes as a reminder. Booking details and billing documents were utilized. Using these consumption data from the individual guests, the chef of the restaurant was interviewed about the detailed ingredients of each dish ordered by the guests. This included not only the major ingredients of each dish itself but also elements used for decoration of the dish or of the salad served separately. This approach provided reliable information about which food ingredients each guest had actually ordered and eaten. This interview technique and analysis had the major advantage that it was no longer necessary to depend on the memory of the guests to find out what they had eaten. Additional verification was obtained through photos taken at the table by a number of groups. These photos confirmed the details given for the nature of the ordered dish and its contents.

In univariate analysis the relative risk of disease was 14.2 times higher for persons eating sprouts compared to that of persons not eating sprouts (Buchholz et al., 2011). All 31 patients with EHEC/HUS had consumed sprouts. None of the guests who did not consume sprouts became ill. Based on these cohort studies, in a joint press conference of the RKI with the food safety authorities on June 10 the public announcement was made that sprouts were the vehicle of infection. The

earlier warning against the consumption of salad was now focused on a warning against consumption of the salad ingredient sprouts.

Origin of Bacterial Contamination of the Sprouts

The more than 40 clusters within this outbreak were analyzed for a common denominator. The federal authorities responsible for food safety in Germany (the Federal Institute for Risk Assessment and the Federal Office of Consumer Protection and Food Safety) performed an intensive forward-backward tracing of the food supply chain of the various cluster locations (Figure A1-4). Through one or several distributors and intermediates, all clusters turned out to be connected to a specific food enterprise producing sprouts commercially. All infections within this outbreak in the state of Lower Saxony had in common that originally the supply of sprouts came from this single food enterprise.

Two clusters of infection independent of the outbreak in Lower Saxony provided information on the origin of the sprout contamination (Appel et al., 2011). Both clusters had definitely no connection to the sprout producer in Lower Saxony. One cluster consisted of so-called self-sprouters (i.e., consumers who grow their

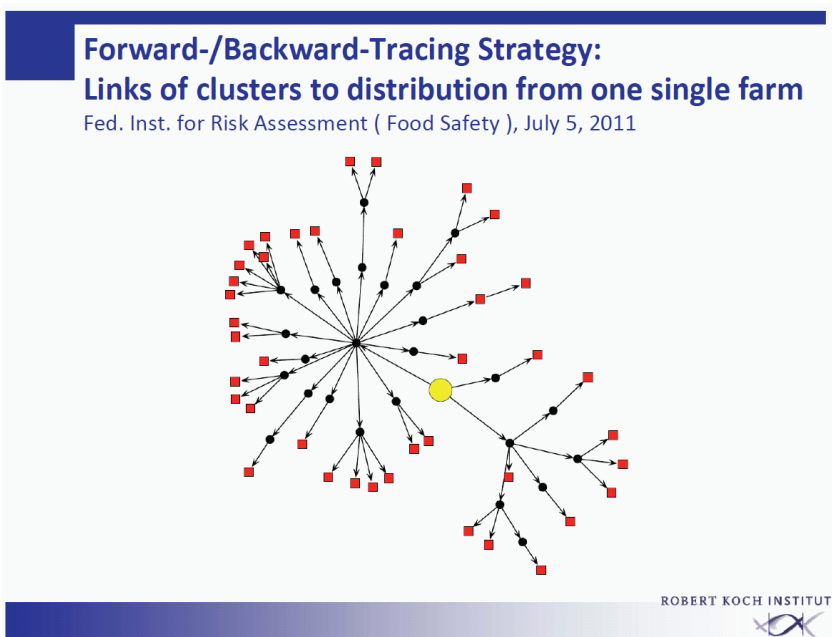


FIGURE A1-4 Trading network reveals linkage of 41 identified outbreak clusters. Supply chain of contaminated sprouts leads to one single sprout producer farm in Lower Saxony. SOURCE: Modified from Buchholz et al., NEJM, 365, 1763 (2011).

own sprouts at home from seeds provided by commercial suppliers). The second source of information was a small outbreak comprising 15 cases in the area of Bordeaux in France in mid-June. Detailed and labor-intensive tracing of the delivery channels revealed that the only common feature of the seeds used for growing sprouts in the food enterprises in Lower Saxony, in Bordeaux, and in the private households with the home-grown sprouts was a given lot of fenugreek seeds originating from Egypt. Fenugreek seeds (*Trigonella foenum-graecum*) are frequently used for the production of sprouts. The seeds are also used in many other food products (e.g., spices, cheese, and even tea) because of their very aromatic taste and intensive smell. The seeds are small (4-5 mm) and have a peanut-like colour.

Through a number of intermediates located in different countries this seed lot had been delivered to these three outbreak locations. No other common ingredient used for the production of sprouts was identified. This was clear evidence that contaminated seeds used for sprout growing were responsible for the outbreak (Appel et al., 2011). By nature, the epidemiological evidence is indirect or circumstantial but it explained the distribution of infections. The corresponding lot of fenugreek seeds was removed from the market. It is difficult to verify how complete this removal was.

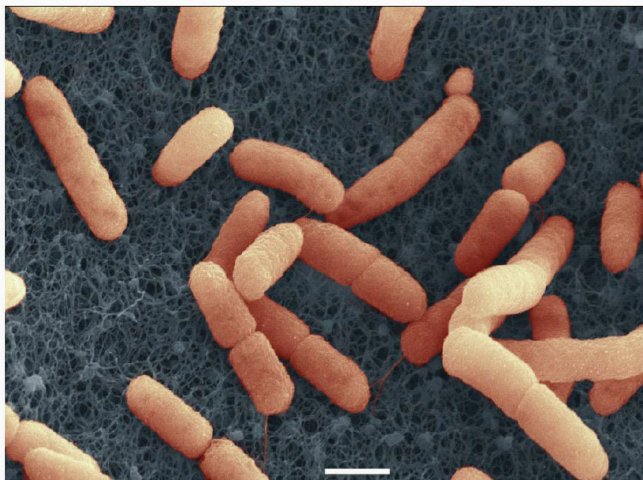
“Stealth Food”

When the affected patients were interviewed initially during the first weeks of the outbreak, it became obvious that people do not remember in detail what they ate 1 or 2 weeks ago. Only in retrospect, after the second or third interview together with reports in the press, did they realize and remember that their dishes had in fact contained sprouts. Similar phenomena had been observed internationally in other outbreaks. In 2008, jalapeno chili peppers were contaminated with *Salmonella Saintpaul* in the United States. Chili peppers are used as an ingredient in tomato sauce-like salsa. The consumers were not aware that one of the spicy ingredients was chili peppers and, when interviewed, denied consumption of this food item, thereby delaying the identification of the vehicle. The identification of sprouts as a source in Germany within less than 3 weeks was quite rapid. The identification of the chili peppers took about 7 weeks. In another outbreak in 1996 with radish sprouts causing an outbreak of EHEC O157 in Japan, 7 weeks were required for the detection of the outbreak and 4 weeks to identify its source.

Microbiological Characterization of EHEC O104:H4

Once the outbreak had been recognized, EHEC O104:H4 was rapidly isolated from stool specimens of affected patients within a few days (Figure A1-5) (Askar et al., 2011, Bielaszewska et al., 2011). This is a rare serotype that had not been described previously in animals. As a rule, faecal contamination by ruminants is responsible for EHEC infections through vegetables or through

Microbiological characterisation of EHEC O104:H4



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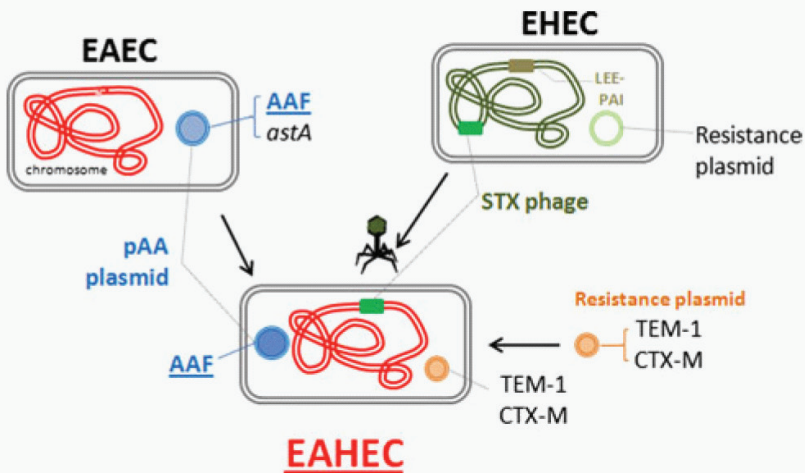
FIGURE A1-5 Electron micrograph of EHEC O104:H4.
SOURCE: Laue, Robert Koch Institute.

food products derived from animals (milk, meat). The usual EHEC strains (e.g., EHEC O157) are found in faeces of ruminants. EHEC O104:H4 has only rarely been identified previously in human beings (in a total of seven patients). A closely related EHEC strain, HUSEC041, was identified in 2001 by the laboratory of Karch at the University of Münster, Germany. Later, a few cases were identified in Korea in 2006, in Georgia in 2009, and in Finland in 2010.

A detailed microbiological characterization of EHEC O104:H4 was performed at the National Reference Centre for Gastrointestinal Bacteria at the RKI and the RKI-Consultant Laboratory of Karch in Münster (Bielaszewska et al., 2011; Brzuskiwicz et al., 2011). From the virulence markers, the outbreak strain was negative for Shiga toxin 1 and positive for Shiga toxin 2 (variant *vtx2a* of Shiga toxin 2). It was negative for Intimin (*eae*) and also negative for enterohaemolysin (*hly*). Macrorestriction analysis (pulsed-field gel electrophoresis) with a number of selected isolates obtained from various areas of Germany showed the same pattern, indicating early that the corresponding patients were all affected by one and the same outbreak event.

Surprisingly, the outbreak strain showed virulence characteristics of enteroaggregative *E. coli* (EAEC). It had the typical EAEC virulence plasmid with adhesion fimbriae type AAF/I. This virulence plasmid has not been described previously in EHEC isolates. All other previously identified EAEC or Shiga toxin-producing *E. coli* (STEC)/EAEC O104:H4 had AAF/III fimbriae. Subsequent sequencing revealed strong homology to an enteroaggregative *E. coli* (EAEC 55989). Obviously, the outbreak strain EHEC O104:H4 represents a virulence combination of two different pathogens. The origin of this outbreak strain with the characteristics of two different pathogens remains unclear for the time being. It is unclear whether the new EHEC O104:H4 pathotype had developed from two separate ancestors by horizontal gene transfer, leading to the observed acquisition of virulence factors (Figure A1-6) (Brzuszkiewicz et al., 2011; Mellmann et al., 2011; Rasko et al., 2011). A number of mobile genetic elements can transfer traits in *E. coli* like the Stx-bacteriophage found in EHEC strains. Alternatively, an evolutionary model is discussed, postulating a common progenitor of EAEC

Proposed scheme of the origin of the new *E. coli* pathotype



(Brzuszkiewicz, E et al. Arch. Microbiol., Doi 10.1007, June 2011)

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FIGURE A1-6 Putative origin of the EHEC outbreak strain as a combination of virulence traits derived from two different ancestors.

SOURCE: Brzuszkiewicz et al. (2011).

55989 and EHEC O104:H5 developing into two lines, each losing or acquiring virulence factors. The second explanation is favoured by the group from Karch, University of Münster.

The continuously updated EHEC datasheet on the RKI website summarized all known characteristics of the pathogen and suggested the proper microbiological diagnostic procedures.

ESBL Resistance Phenotype

The microbiological characterization revealed a resistance unusual for intestinal *E. coli*. The outbreak strain had an extended-spectrum β -lactamase (ESBL). This is an unusual property of intestinal *E. coli*. This resistance phenotype allowed efficient diagnostics of the outbreak strain. It permitted the use of the corresponding selective media for a targeted search in clinical samples, facilitating a rapid diagnosis. Colonies on an ESBL-agar plate were further characterized with multiplex polymerase chain reaction screening for genes of Shiga toxin 1 and 2 and Intimin.

Absence of Direct Microbiological Evidence for Contamination of Seeds with EHEC O104:H4

The identification of seeds as the source and sprouts as the vehicle of infection relied on sophisticated and elegant epidemiological analysis (i.e., indirect evidence). Direct microbiological evidence has not been obtained so far (Aurass et al., 2011). Intensive bacteriological screening of the fenugreek sprouts and seeds was performed. A large number of samples were also taken at the production site of the sprouts, including the water supply or waste water. All attempts to identify the outbreak strain on seeds or sprouts or in the samples obtained at the production site failed. Sampling sprouts in households with EHEC cases was successful in one or two cases. However, these results were more than questionable. One positive result was obtained from a single box of sprouts originating from the incriminated producer. However, it had already been opened in a household with EHEC cases and might simply have been contaminated by the handling. In another example the outbreak strain was identified in salad samples found in a trashcan days after disposal. Also here, the causal connection is unclear.

One reason for the failure to identify the outbreak strain through bacteriological screening may be the enormous size of the incriminated fenugreek seed lot. The lot size was around 15,000 kg. If only a minor part of this lot had indeed been contaminated, searching for contaminated seeds would resemble the search for a needle in a haystack. In addition, on the same day, the sprout-producing enterprise received another lot of seeds from the same seed distributor. The incriminated lot had been distributed to 70 different companies, 54 of them in Germany and 16 of them in 11 European countries (Appel et al., 2011). How-

ever, despite the two additional independent clusters (home-grown sprouts and the cluster in France; see above), no obvious other outbreaks were recognized. Despite all efforts to remove the incriminated lot from the supply chain, it is difficult to estimate how effective and complete this removal has been. Especially in private households, growing sprouts from small aliquots of seeds could lead to new infections. It is known that *E. coli* can survive on dried seeds for longer periods of time, potentially for years.

Incubation Time and Shedding Time

Detailed analysis revealed a median incubation time of 8 days. The maximum was 18 days. Seventy-five percent of the patients developed clinical symptoms after 10 days. Some of the patients showed a shedding of the pathogen for an extensive period. A few patients shed the pathogens for up to 8 months. It remains to be determined whether shedding might even be longer and whether a carrier status may develop. For enteroaggregative *E. coli* this extensive shedding period is not unexpected. It is known that aggregative bacteria adhere more strongly and remain in the gastrointestinal tract for longer periods of time. A close collaboration with the local health authorities proved to be important in the analysis of this outbreak (e.g., for these shedding studies) (Robert Koch Institute, 2011).

Secondary Infections

Even after the end of the outbreak had been announced, recommendations were made to enforce the standard hygiene rules, regarding both personal and hand hygiene and in particular kitchen and food hygiene. This included the recommendation to always clean kitchen utensils carefully when preparing food intended for raw consumption. A small number of secondary infections were observed, predominantly consisting of household members of patients. Therefore, stringent adherence to hygienic practices was strongly suggested in those households where EHEC patients or persons with diarrhea were present.

Single nosocomial infections occurred in hospitals (coloscopy). Transmission also occurred through the preparation or distribution of food. Also several laboratory infections were found. Therefore, raised awareness of the risk of infection was also emphasized in public announcements during the months after the official end of the outbreak.

Communication

The RKI made great efforts to inform the medical experts and the public health service and the professional societies (clinical and microbiological) about details of the outbreak in a very timely fashion. During the outbreak, at least daily updates were distributed by e-mail. The Internet proved to be the most important

tool for distribution of information. Usually visits to the RKI homepage result in 4 to 6 million page uses per month. During the outbreak months, May and June 2011, the numbers increased to 16.5 and 17.9 million, respectively. The information provided also included outbreak case definition, forms concerned with sample reporting, diagnostic procedures, information on hygienic measures, etc.

When a whole country is concerned about the safety of its food, the risk communication is important. It proved be helpful to clearly and reliably state the current knowledge and the known risks and their prevention. Also lack of knowledge or uncertainty should be stated clearly, as well as the point in time when new information might be expected. This is important in order to maintain public confidence in recommendations. Farmers requested information because a substantial number of farms suffered economically and were in danger of going out of business.

Conclusion

This outbreak of EHEC infections was the largest recorded outbreak of a bacterial infection observed in Germany in many decades. The enormous rate of HUS cases makes it the largest outbreak of HUS worldwide. It revealed how rapidly a food-borne pathogen can spread and cause serious illness and death. It demonstrates the importance of proper surveillance systems in order to detect an outbreak early and of a rapid reporting system in notifying the corresponding health authorities, in this case the RKI in Germany. According to the specifications of the German Infection Protection Act, a rapid report by the physician or the diagnostic laboratory to the local health authorities is required. In retrospect, between the onset of the disease, the visit to the doctor or hospital, diagnosis, and the report to the local health authority and subsequently to the state authorities and finally to the RKI, a substantial period of time passed, varying from a few days up to several weeks. Measures were taken to improve reporting and to prevent the notification delay. In the analysis of outbreak clusters a close cooperation of health authorities and food safety authorities and a rapid exchange of information is necessary.

The origin of the outbreak strain and how the seeds were contaminated remain unclear. It also remains to be determined whether EHEC O104:H4 will have a reservoir, in human beings, in animals, or in the environment. There is no evidence today that EHEC O104:H4 has become endemic anywhere in humans, animals, or in the environment in Germany. After the sprouts had been identified as the vehicle of this outbreak and after the sprout distribution ended, no further outbreak clusters were identified to be associated with the consumption of sprouts. It is unclear how frequently EHEC is present on sprouts, which are often consumed raw and represent a particularly vulnerable food for bacterial contamination. A rapid and sensitive EHEC diagnostic should also be available in routine diagnostic laboratories in order to identify outbreak events early and

reliably. Detailed subtyping should predominantly be performed in specialized laboratories, also in such an outbreak situation. It seems appropriate to observe these aspects or questions also in the future.

The outbreak had enormous consequences, not only for the patients affected but also economically because of strongly reduced trade in salads and salad ingredients. Spanish cucumbers had been discussed by a local health authority as a potential source of the pathogen. This assumption was not confirmed by laboratory analysis, and attempts to show a connection to the outbreak strain failed; however, it affected the sale and led to a major drop in the consumption and export of Spanish vegetables. Farmers in a number of vegetable-exporting countries were in turn compensated by the European Union in the amount of 220 million Euros for this loss in income.

In summary, the events in Germany during the summer of 2011 revealed the importance of functioning public health institutions, both at the county and state level and at the federal level.

A final detailed report of the EHEC O104:H4 outbreak in Germany is available through the RKI website (http://edoc.rki.de/documents/rki_ab/reQHS31jDrGxc/PDF/23NXL3JomOyAA.pdf) in an English version.

Declaration of Interest

The author declares no conflict of interest and has received no payment in preparation of this manuscript.

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A2

ONE HEALTH AND HOTSPOTS OF FOOD-BORNE EIDS

C. Zambrana-Torrel, *K. A. Murray*, and *P. Daszak*³

Summary

In this section, we focus on a One Health approach to food-borne emerging infectious diseases (EIDs), their causes, global patterns, and the drivers of their emergence. First, we review two case studies that show the complexity of food-borne pathogen emergence across the One Health domain. Second, we examine the composition of food-borne diseases with respect to their causal agents (pathogen type), their association with pathogens of zoonotic origin, and their apparent disassociation with pathogens that show drug resistance. Third, we analyze the socioeconomic, environmental, and ecological drivers of food-borne EID events. Finally, we use published, spatially explicit information on the drivers of disease emergence to produce a preliminary “hotspot” map that reveals the epicentres, or hotspots, of food-borne EID events globally.

Introduction

One Health’s focus on the intersection of human, domestic animal, and environmental health is ideally suited to managing emerging zoonoses. However, the patterns of emergence are complex and poorly understood and for food-borne infections may involve multiple pathways. Food-borne infections can include directly transmitted or vector-borne diseases, for example, Rift Valley fever (Arzt et al., 2010). Single strains of drug-resistant microbes can infect livestock, wildlife, and humans (e.g., *E. coli* O157:H7) (Hughes et al., 2009; Nielsen et al., 2004; Rahn et al., 1997). Finally, viral pathogens that originate in wildlife may be driven to emerge by the intensification of livestock production (Pulliam et al., 2011) or by contamination of bush meat (Wolfe et al., 2005) or other food sources

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(Khan et al., 2011). Our ability to predict the emergence of food-borne infections is hampered by this complexity. However, recent efforts to analyse disease emergence (Jones et al., 2008; Taylor et al., 2001) have provided a strategy that can be adapted to analyzing the origins of food-borne infections.

Following our first efforts to predict global patterns of disease emergence (Jones et al., 2008), we have continued to compile data on human EID events and their drivers under the aegis of the U.S. Agency for International Development-funded Emerging Pandemic Threats PREDICT project (Daszak, 2011). In the updated database, when the EID events are classified according to their disease transmission modes (Figure A2-1), we find that food-borne pathogens are responsible for 14.9 percent of known EIDs.

In this section, we focus on food-borne EIDs, their causes, global patterns, and the drivers of their emergence. First, we review two case studies that show

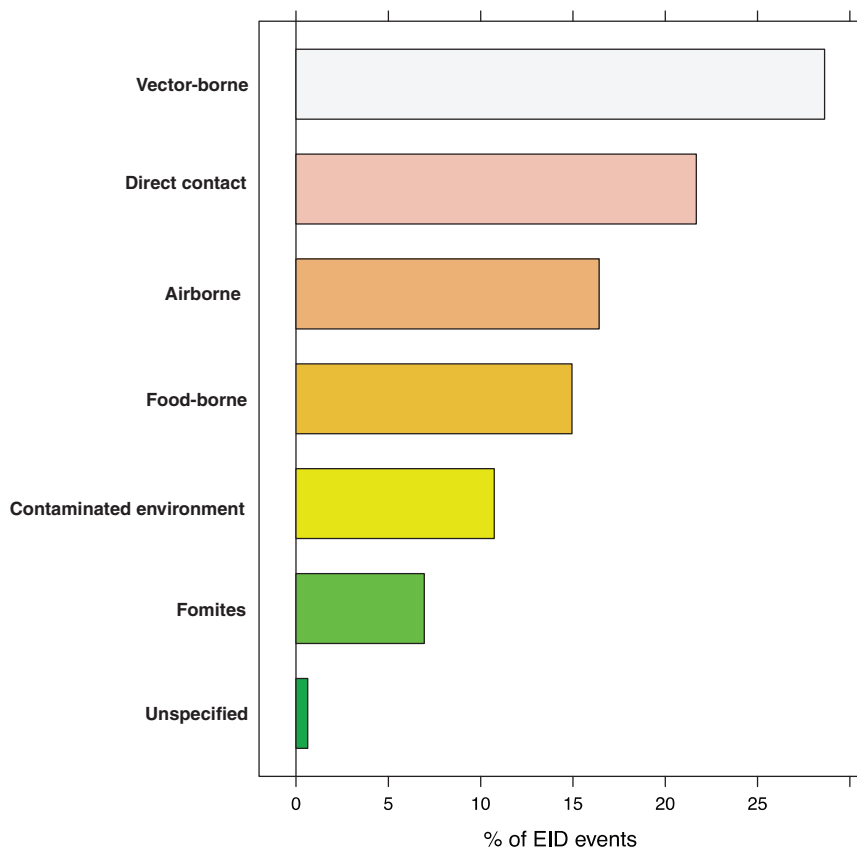


FIGURE A2-1 Proportion of EID events categorized by transmission mode.

the complexity of food-borne pathogen emergence across the One Health domain. Second, we examine the composition of food-borne diseases with respect to their causal agents (pathogen type), their association with pathogens of zoonotic origin, and their apparent disassociation with pathogens that show drug resistance. Third, we analyze the socioeconomic, environmental, and ecological drivers of food-borne EID events. Finally, we use published, spatially explicit information on the drivers of disease emergence to produce a preliminary “hotspot” map that reveals the epicentres, or hotspots, of food-borne EID events globally.

Food-Borne, Wildlife-Origin Pathogens: Two Case Studies

Nipah virus (NiV) is a paramyxovirus that first emerged in Malaysia in 1999, causing encephalitis with a 40 percent case fatality rate in humans (Chua et al., 2000). The virus originated in fruit bats of the genus *Pteropus* but was first transmitted to domestic pigs, which amplified the virus via a rapidly spreading respiratory infection. Subsequent transmission to people occurred via droplets or fomites contaminated with pig saliva. The initial spillover of NiV seems to have occurred when fruit bats fed on mango and other fruit trees planted next to pigsties at the index farm as a source of additional income and to increase shade. The question remained: Why did it suddenly emerge in this pig farm and not in pig farms 20 years earlier or 20 years later?

To answer this question we analyzed pig production and the age structure of NiV dynamics within the index farm population (Pulliam et al., 2011). We produced a mathematical model, parameterized with detailed data from the index farms and other similar farms still in existence in Malaysia today. This model allowed us to re-create the conditions of the farm when NiV first emerged and to test hypotheses on the drivers of its emergence. Our analyses suggest that repeated introduction of NiV from bats changed infection dynamics in pigs. Initial viral introduction produced an epizootic that drove itself to extinction within 1 to 2 months. Subsequent introduction into a now partially immune population, coupled with the gradual loss of maternal antibodies in pigs born to sows infected in the initial outbreak, led to ideal conditions for pathogen persistence and a prolonged window of spillover to people and regional spread as infected pigs were sold. The structured, compartmentalized nature of the index farm was critical to the emergence of NiV and was a product of agricultural intensification.

A similar scenario surrounds the emergence of highly pathogenic influenza A/H5N1. This virus is able to infect wild waterbirds, domestic poultry, and humans, and its emergence is linked to both intensive production of poultry and the patterns of rice farming within Southeast Asia. When rice is double-cropped, it attracts ducks throughout the year and allows greater potential for new strains of influenza to cross over into pigs and for subsequent crossover of those strains (Gilbert et al., 2008). Analysis of the patterns of double-cropping in Southeast Asia shows that it is possible to predict the risk of its presence throughout

the region based on the type of agricultural system (Gilbert et al., 2008). Poultry production in this region includes large intensive and small “backyard” farms, all connected via trade routes into markets and through the supply of breeding stock and their contact with wild birds. We have used a similar modeling approach for A/H5N1 to examine how farm size and connectivity matter as risk factors for the emergence of avian influenza. Our modeling shows that both factors interact to produce specific conditions conducive to outbreaks. When the vast majority of farms are of small size, outbreaks occur more frequently and last longer, but they involve few individual birds and therefore have a lower risk of infecting people. When farms are poorly connected these outbreaks die out because of stochastic factors. When large intensive farms predominate, outbreaks are very few in number, but their duration is relatively short because so many birds die in such a short space of time that the cause is rapidly recognized and the farm culled. The peak in duration and intensity of outbreaks occurs when there is a mixture of intensive and backyard farming. These are the conditions that occur most commonly in Southeast Asia because of the rapid growth of some economies and efforts to intensify poultry production.

Causes, Patterns, and Drivers of Food-Borne EIDs

How important are food-borne infections in the context of global disease emergence events? Going back to Figure A2-1, approximately 15 percent of human EID events are associated with food-borne transmission pathways. With 475 EID events in the updated database, this translates to 71 separate food-borne EIDs, at an average emergence rate of just under one completely new, previously unknown EID event per year reported globally.

When broken down by causal pathogen type (Figure A2-2), food-borne EIDs are usually bacterial in origin, with smaller proportions of protozoan and helminth-driven diseases. While bacteria are also the major causes of EIDs associated with the contaminated environment and fomites, food-borne EIDs are generally more common and therefore account for the highest number of EIDs of bacterial origin (50) among all of the transmission modes. Hence, when bacteria are the causal agent implicated in EID events, they are more likely to be food-borne than of any other transmission mode. In contrast to the other transmission mode groups, food-borne EIDs are very rarely viral, accounting for only one (1.4 percent) food-borne EID (hepatitis A) compared to ~20 to 45 percent (average 30.9 percent) in the other groups. However, many viral pathogens (e.g., NiV and H5N1) are considered simply zoonotic because the role of food-borne transmission is either less well known or less well understood.

Our analyses suggest that the vast majority of food-borne EIDs are indeed zoonotic; in fact, an even higher proportion of food-borne EIDs are zoonotic (84.5 percent) than the background rate of all EIDs in the updated database (62.3 percent) and of any other transmission mode (Figure A2-3). Clearly, patho-

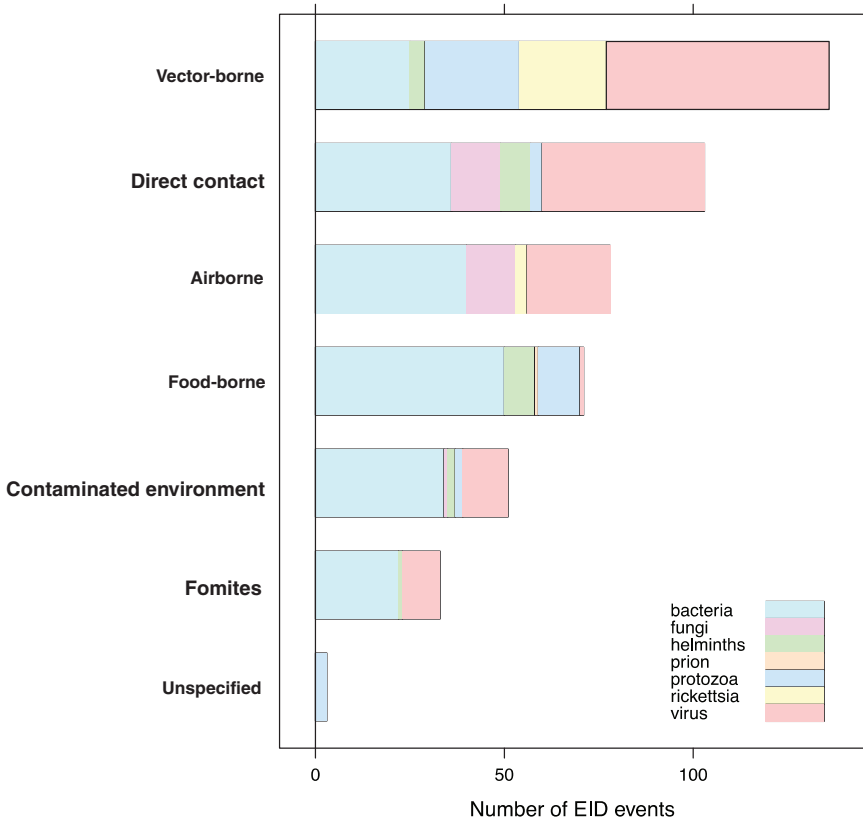


FIGURE A2-2 Number of EID events per transmission mode classified by pathogen type.

gens from animals entering the food-production chain are of significant concern for their potential to become EIDs.

One of our earlier findings (Jones et al., 2008) was that a majority (54.3 percent) of human EIDs were bacterial/rickettsial in origin, reflecting a large number (20.8 percent of all EIDs) of new drug-resistant pathogen strains. We show above that if an EID was identified as being caused by bacteria, it was most likely to be food-borne, and similarly if an EID was linked with food it was most likely to be bacterial than of another transmission mode. Given the propensity of bacteria to develop drug resistance, and the abundance of food-borne infections of bacterial origin, is there any evidence that food-borne pathogens are contributing to new drug-resistant diseases?

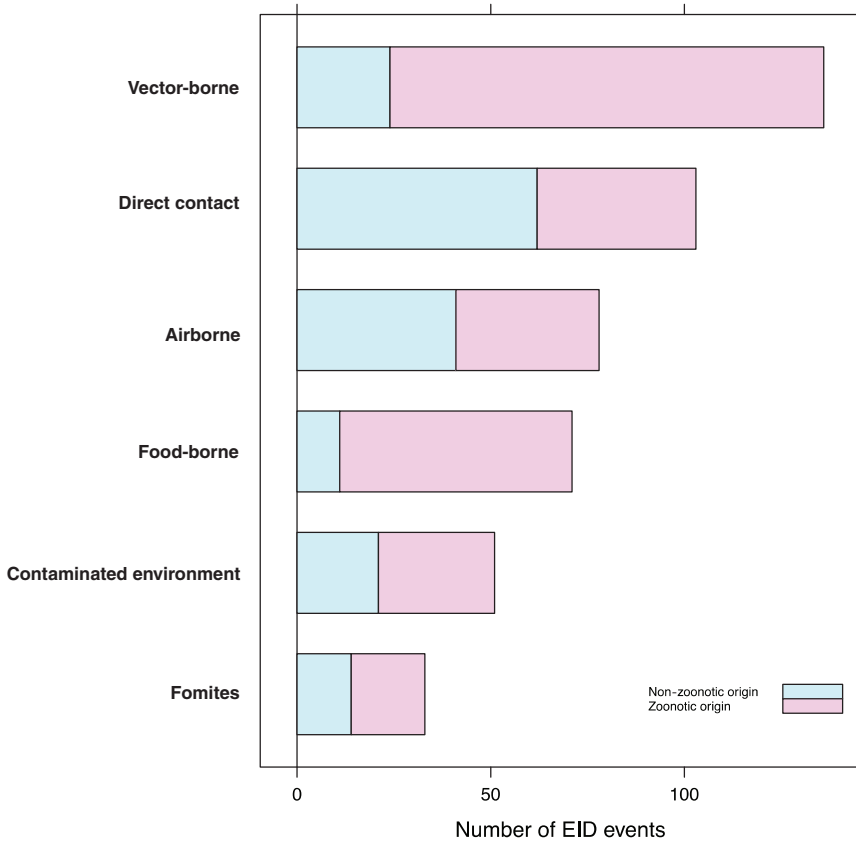


FIGURE A2-3 Number of EID events per transmission mode categorized by zoonotic origin.

Perhaps surprisingly, the answer is no: when EID events are split into categories reflecting the presence or absence of drug resistance (ignoring for a moment the secondary split on whether the pathogen was zoonotic or not), food-borne pathogens are very unlikely to be drug resistant (Figure A2- 4). Although it is true that drug resistance is relatively infrequently observed across most transmission modes (the exception being fomite-associated EIDs), resistance is particularly infrequent in food-borne (as well as vector-borne) EIDs. Hence, even though bacteria are quite likely to cause food-borne EIDs and bacteria also cause the majority of new drug-resistant diseases, this is quite unlikely to occur together, resulting in very few drug-resistant food-borne EIDs. Why is drug resistance not more common in this group?

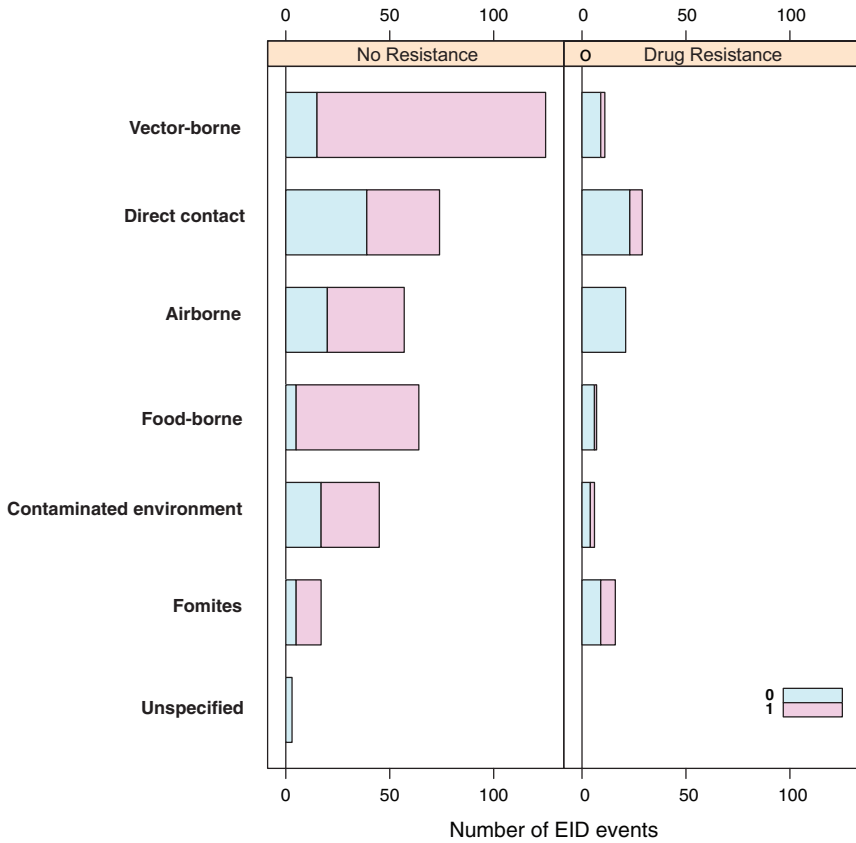


FIGURE A2-4 Proportion of drug-resistant and nonresistant EID events of zoonotic (1), or non-zoonotic origin (0).

The answer may be related to whether the causal agent is zoonotic or not. Generally speaking, there is a low frequency of zoonotic EIDs that exhibit drug resistance (6.0 percent), regardless of the transmission mode (Figure A2-4). Non-zoonotic EIDs are far more likely to be associated with drug resistance (40.9 percent), again across all groups. This is consistent with the idea that new drug-resistant pathogens result from selection on our own circulating pathogens by the routine use of antimicrobial drugs, and not on the pathogens circulating in the food industry that originate in animals. In other words, even though most food-borne EIDs are caused by bacteria, which generally show high potential for becoming drug resistant, the fact that most food-borne EIDs are zoonotic means that the group is quite unlikely to experience strong selection pressure from

routine drug administration in human patients. Obviously there are limitations to this type of analysis, particularly in how extensive the data are, but it is clear that this issue is an important target for future research.

Finally, what are the drivers of food-borne pathogens, and are they an ongoing concern for their EID potential? As we have seen, food-borne EIDs are common, usually zoonotic, usually bacterial, and not likely to exhibit drug resistance. So what factors are driving them to emerge? What factors are allowing them to enter and circulate within the food-production system to subsequently cause disease in humans? In Figure A2-5, we analyse the underlying drivers listed in a previous Institute of Medicine report (IOM, 2003) for food-borne EIDs and find that the vast majority of food-borne EIDs are associated with “technology and industry,” and to a lesser extent with “international trade and commerce” and “human susceptibility to infection.” This is consistent with previous studies that have suggested that outbreaks of food-borne infections are likely to be associated with changes in livestock production and centralization of slaughtering

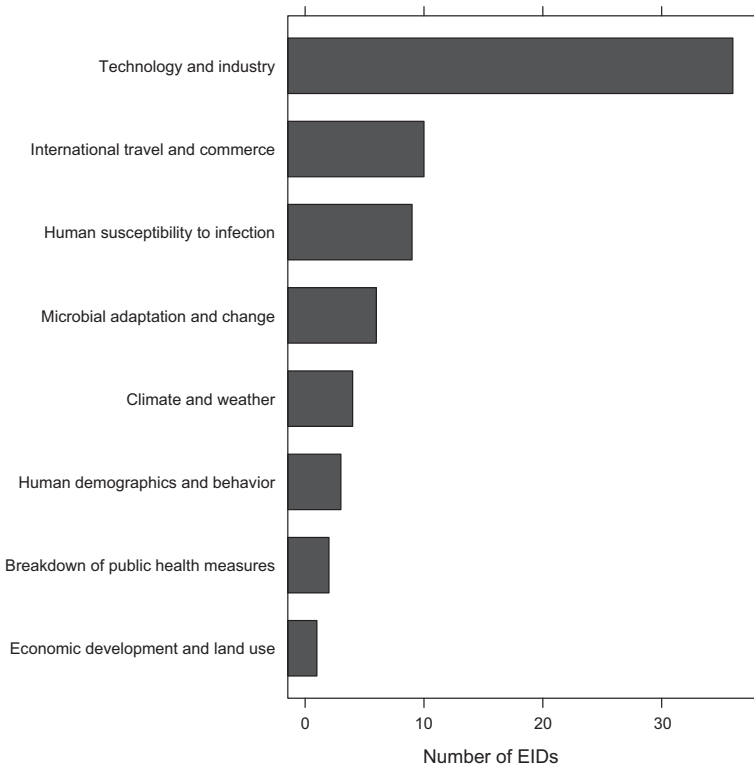


FIGURE A2-5 Association of food-borne EIDs with other drivers.
 SOURCE: Following IOM (2003).

and processing (IOM, 2003; Tauxe, 1997). As a result of these analyses, we can hypothesize that the global distribution of food-borne EIDs is driven by a process of intensive production of livestock and food, not simply the number of livestock produced in a region.

Food-Borne EID Hotspots

Our previous approach to predicting the future geographic origins of new EIDs (Jones et al., 2008) can be adapted for food-borne EIDs. This approach involves identifying the geographic and temporal origins of previous disease emergence events and correcting them for surveillance biases. We then identify correlations between these and purported socioeconomic (demography, travel, trade), environmental (climate, land cover), and ecological drivers (biodiversity, species interactions). Considering all EIDs together, these models suggest that surveillance should be directed toward regions of high biodiversity and dense human populations, which mainly occur in tropical and subtropical latitudes (Jones et al., 2008). When we adapt this approach to food-borne EID events and use the same drivers as in our earlier analysis, human population density and human population growth emerge as the most important in the emergence of novel food-borne outbreaks (Figure A2-6). This suggests that rapidly developing regions are the sites where most novel food-borne pathogens will emerge in future. This may appear to be in conflict with Figure A2-5; however, this is because the spatial analyses have so far been limited primarily by the availability of relevant spatial information. Human population density and growth are likely to be meaningful proxies for a range of other mechanistically more relevant drivers. One of our main goals more recently has thus been to improve our database of detailed drivers. We have, for

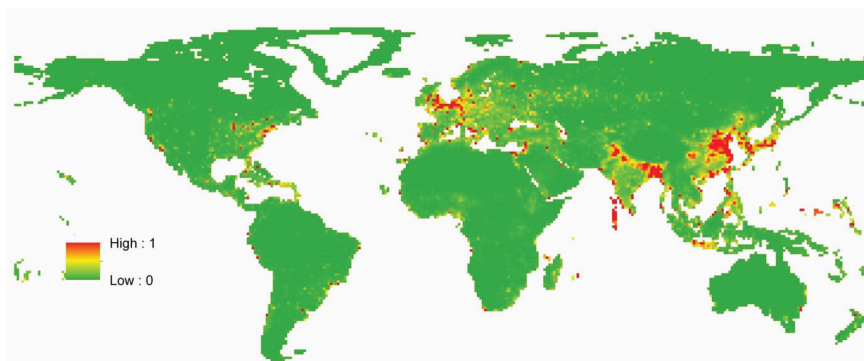


FIGURE A2-6 Relative risk of food-borne EID events, based on Jones et al. (2008). Human population density and human population growth are the most important variables. SOURCE: Reprinted by permission from Macmillan Publishers Ltd: Nature, (Jones et al., 2008).

example, begun to include spatial information on land-use change (cropping and pasture) and livestock density (including cattle, pigs, buffalo, goats, and sheep) into the predictive models. We are currently validating these new data sources for use in future models.

We conclude that food-borne EIDs are a common and important group within emerging diseases that emerge through complex pathways involving wildlife, livestock, and humans. They are therefore ideal candidates for a One Health approach but have rarely been considered in this way previously. Our analyses show that the majority of food-borne EIDs (1) are bacterial; (2) are, if bacterial, more likely to be food-borne than of any other transmission mode; (3) are zoonotic; (4) do not tend to be associated with drug resistance, perhaps because zoonotic pathogens in general show little tendency to become resistant; and (5) are driven by changes in human food-production systems, including intensification and centralization as human populations grow larger and more dense.

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A3

PLANT FOOD SAFETY ISSUES: LINKING PRODUCTION AGRICULTURE WITH ONE HEALTH

*Marilyn C. Erickson and Michael P. Doyle*⁴

During the past decade, fruits and vegetables have become leading vehicles of food-borne illnesses. Furthermore, many plant-based foods and ingredients, not previously considered a risk, have been associated with food-borne disease outbreaks. Most of the pathogens that have been identified as causative agents in these illnesses or outbreaks are enteric zoonotic pathogens that are typically associated with animal hosts. Transmission of zoonotic pathogens from animals to plant systems occurs by a variety of routes, but the initial contributing factor is the discharge of animal manure into the environment. Using a “One Health” approach that focuses on animal, human, and environmental health concurrently can provide practical and effective interventions for reducing the incidence of such outbreaks. This paper addresses this concept by providing recent food-borne disease outbreak data related to fruits and vegetables, delineating findings regarding the prevalence of pathogens in animal manures and describing the vehicles that transmit pathogens from manure to produce fields, and discussing the merits of reducing pathogen transmission through interventions that would not adversely affect the health of the environment or animals.

Outbreaks and Illnesses Associated with Fresh Fruits and Vegetables

Food-borne illnesses have been a persistent challenge to public health and are now being detected with greater frequency largely because of enhanced surveillance systems that have been implemented in many countries. These enhanced surveillance systems have during the past decade revealed that the proportion of total outbreaks attributed to produce is significant (Lynch et al., 2009) but varies with the country. For example, only 4 percent of all food-borne outbreaks

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reported in Australia from 2001 to 2005 were attributed to fresh produce (Kirk et al., 2008); similarly, in Canada, between 1976 and 2005, 3.7 percent of 5,745 outbreaks with a known vehicle of transmission were attributed to produce (Ravel et al., 2009). However, in contrast, data from the Centers for Disease Control and Prevention (CDC) identified produce as either the first or second leading vehicle in food-borne disease outbreaks attributed to a single commodity within the United States for the period 2006-2008 (Table A3-1). Furthermore, outbreak surveillance data of produce items compiled by the CDC during the period 2000-2009 revealed that leafy greens were the most common item associated with food-borne disease, followed by tomatoes and cantaloupes (Table A3-2). Moreover, attribution risk rankings of fresh produce-associated outbreaks in the United States identified enterohemorrhagic *Escherichia coli* in leafy greens as the leading pathogen-produce vehicle combination, followed by *Salmonella* spp. in tomatoes, and *Salmonella* spp. in leafy greens (Anderson et al., 2011). Further differentiation of vehicles of produce-associated outbreaks that occurred in the United States during the period of 1998-2008 revealed that fresh-cut produce accounted for 56 percent, 36 percent, and 17 percent of the outbreaks attributed to leafy greens, tomatoes, and melons, respectively (Sneed, 2010).

An evaluation of selected produce-associated outbreaks that occurred during the past 5 years revealed several common features (Table A3-3). These outbreaks often were multistate or multinational in nature and reflect the large areas to which the foods are distributed. With imports accounting for nearly 39 percent of fresh fruits and 14 percent of fresh vegetables in 2005 (Johnson, 2012), improved sampling and pathogen testing of produce at the borders of the United States offers one barrier for reducing the likelihood of contaminated produce

TABLE A3-1 Food-Borne Disease Outbreaks Attributed to a Single Commodity by Leading Food Vehicles, 2006-2008

Year	Rank	Food vehicle	Outbreaks (%)
2006	1	Produce	23.5
	2	Meat	19.3
	2	Fish	19.3
	4	Poultry	14.4
2007	1	Meat	23.0
	2	Produce	22.6
	3	Fish	17.4
	4	Poultry	17.0
2008	1	Produce	27.5
	2	Meat	23.4
	3	Poultry	14.7
	4	Fish	13.8

SOURCE: CDC (2009a, 2010c, 2011e).

TABLE A3-2 Number of Outbreaks (illnesses) Reported Between 2000 and 2009 in the United States That Were Associated with Selected Fresh Produce Items as a Function of their Etiology^{a,b}

Produce item	Bacterial agents				
	<i>Salmonella</i> spp.	<i>Escherichia coli</i> O157:H7 ^c	<i>Shigella</i> spp.	<i>Campylobacter jejuni</i>	Other ^d
Cabbage	1 (8)	1 (41)			2 (68)
Lettuce	10 (456)	14 (364)	1 (4)	2 (16)	3 (114)
Spinach		2 (223)			1 (6)
Sprouts	12 (441)	4 (46)			1 (20)
Herbs	3 (70)				
Leafy green salads	23 (997)	15 (280)	7 (190)	7 (42)	10 (145)
Coleslaw	1 (26)				4 (22)
Peppers	4 (1,643)			1 (5)	2 (17)
Tomatoes	25 (1,867)		1 (886)	1 (13)	2 (10)
Cantaloupe/melons	19 (1,180)	1 (5)	1 (56)		1 (55)

^a Data compiled from the CDC website on outbreak surveillance (http://www.cdc.gov/outbreaknet/surveillance_data.html).

^b Outbreaks/illnesses attributed to each pathogen group includes both confirmed and suspected.

^c Includes other Shiga toxin-producing *Escherichia coli*.

^d Includes where multiple bacterial pathogens have been found and cases involving the agents of *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, *Listeria monocytogenes*, and *Staphylococcus aureus*.

SOURCE: CDC.

from entering the retail sector. However, better implementation both domestically and abroad of best food safety practices for producing and processing fruits and vegetables would have even more impact on reducing pathogen contamination and the likelihood of produce-borne illnesses. This approach would address a significant contributing factor associated with several recent produce outbreaks, which is that contamination occurs on the farm where production and processing can occur. For example, in a multistate outbreak of listeriosis in 2011 that resulted in 34 deaths and was the most deadly food-borne outbreak in the United States since 1924, four outbreak-associated strains of *Listeria monocytogenes* were traced back to whole cantaloupes and packing equipment on Jensen Farms in Colorado (CDC, 2011c). In another 2011 outbreak, fenugreek seeds that were likely contaminated with fecal matter led to the largest outbreak in the number of cases of hemolytic uremic syndrome (22.3 percent of 4,075 total cases) ever reported in the world (WHO, 2011).

Surveillance of Pathogens in Retail Produce

A number of studies have been conducted to determine the prevalence of enteric pathogens on fruits and vegetables, and the results varied with respect

Viral agents		Other agents		
Norovirus	Hepatitis A	Protozoan parasites	Unknown	Total
3 (78)	1(16)		3 (16)	11 (227)
39 (999)	1 (22)		10 (60)	80 (2,035)
			3 (9)	6 (238)
			1 (2)	18 (509)
		1 (592)	1 (20)	5 (682)
257 (8,520)		3 (47)	114 (1,419)	436 (11,640)
20 (676)		1 (8)	1 (11)	27 (743)
			1 (2)	8 (1,667)
15 (399)	1 (23)			45 (3,198)
12 (502)			6 (79)	40 (1,877)

to the country of origin and the target pathogen. For *Salmonella*, there was for most developed countries a very low prevalence in cabbage, lettuce, and mixed salads, whereas higher prevalences were observed for developing countries where agricultural production and hygienic practices were of a lower level of sanitation (Table A3-4). The presence of helminth and protozoan parasites in leafy greens (Table A3-5), however, likely reflects the ability of these pathogens to resist standard chlorine-based wastewater treatments (Erickson and Ortega, 2006; Graczyk et al., 2007). The relatively low occurrence of pathogen contamination on produce makes it inherently difficult to rank the degree of risk associated with the various sources of contamination by which enteric pathogens are transmitted from animals to plant production environments.

Pathogens in Manures from Domesticated Animal

A large number of zoonotic pathogens reside and grow in the gastrointestinal tract of domesticated animals (poultry, cattle, swine, sheep, and goats) and are shed in their feces asymptotically, often in very large numbers. Those enteric pathogens associated with the largest number of food-borne disease outbreaks and illnesses include *Campylobacter jejuni*, *Salmonella* spp., Shiga toxin-producing

TABLE A3-3 Selected Food-Borne Disease Outbreaks Attributed to Produce During the Period of 2006-2011

Year	Pathogen	Number of cases	Country of origin
2007	<i>Salmonella</i> Weltevreden	45	Italy, seed origin
2009	<i>Salmonella</i> Saintpaul	228	Domestic, seed company
2010	<i>S.</i> Newport	44	Domestic, processor
2010	<i>Salmonella</i> I 4,[5],12:i:-	112	Domestic
2011	<i>E. coli</i> O104:H4	3,911	Egypt
2007	<i>S.</i> Senftenberg	51	Israel
2007	<i>S.</i> Senftenberg	74	Israel
2006	<i>S.</i> Saintpaul	36	Domestic
2008	<i>S.</i> Litchfield	51	Honduras
2011	<i>S.</i> Panama	20	Guatemala
2011	<i>Listeria monocytogenes</i>	146	Domestic
2006	<i>Clostridium botulinum</i>	4	Domestic
2006	<i>Yersinia pseudotuberculosis</i>	427	Domestic, traced to vegetable distributor
2006	Norovirus	43	China
2006	<i>E. coli</i> O157:H7	71	Not known
2006	<i>E. coli</i> O157:H7	81	Domestic
2011	<i>E. coli</i> O157:H7	60	Domestic
2008	<i>Salmonella</i> Newport and Reading	77 (Newport) 30 (Reading)	Domestic
2010	Norovirus and <i>E. coli</i> ETEC	264	France
2010	<i>E. coli</i> O145	33	Domestic, processor
2007	<i>E. coli</i> O157:H-, PT8	50	Netherlands, processing plant
2008	<i>Cryptosporidium parvum</i>	21	Italy
2008	<i>S.</i> Saintpaul	1,442	Mexico
2007	<i>Shigella sonnei</i>	227	Thailand
2006	<i>E. coli</i> O157:H7	204	U.S.
2006	<i>S.</i> Typhimurium	183	Not known

SOURCE: CDC.

enterohemorrhagic *Escherichia coli* (STEC), and *Cryptosporidium parvum*. Many studies have been conducted to determine the prevalence of these pathogens in the feces of domesticated animals. A selection of results of recent studies are shown in Tables A3-6 to A3-9 to illustrate the range of pathogen prevalences and cell numbers that may occur within animal wastes and between and within different groups of animals. For *Cryptosporidium*, not all species are pathogenic for humans. For example, currently there are at least 16 recognized species of *Cryptosporidium*, of which two most affect humans, *C. hominis* and *C. parvum* (Jagai et al., 2010). Therefore, when results do not differentiate species of *Cryptosporidium*, the potential risk of those manures to human health may be overestimated.

Affected regions	Implicated food	Reference
Norway, Denmark, Finland	Alfalfa sprouts	Emberland et al., 2007
U.S., multistate	Alfalfa sprouts	CDC, 2009b
U.S., multistate	Alfalfa sprouts	CDC, 2010a
U.S., multistate	Alfalfa sprouts	CDC, 2011a
Multinational	Fenugreek sprouts	EFSA, 2011
U.K., U.S., Denmark, Netherlands	Basil	Elviss et al., 2009
U.K., Denmark, Netherlands, U.S.	Basil, fresh	Pezzoli et al., 2008
Australia, multijurisdiction	Cantaloupe	Munnoch et al., 2009
U.S., multistate	Cantaloupe	CDC, 2008a
U.S., multistate	Cantaloupe	CDC, 2011b
U.S., multistate	Cantaloupe	CDC, 2011c
U.S., Georgia	Carrot juice	CDC, 2006a
Finland	Carrots, grated	Rimhanen-Finne et al., 2009
Sweden	Frozen raspberries	Hjertqvist et al., 2006
U.S., multistate	Lettuce	FDA, 2006
U.S., multistate	Lettuce	FDA, 2007
U.S., multistate	Lettuce, romaine	CDC, 2011c
Finland	Lettuce	Lienemann et al., 2011
Denmark, Norway	Lettuce, lollo biondo type	Ethelberg et al., 2010
U.S., multistate	Lettuce, shredded romaine	CDC, 2010b
Netherlands, Iceland	Lettuce, shredded, prepacked	Friesema et al., 2007
Sweden	Parsley	Insulander et al., 2008
U.S., Canada	Peppers (jalapeño and Serrano), tomatoes	CDC, 2008b
Denmark, Australia	Raw baby corn	Lewis et al., 2009
U.S., Canada	Spinach	Calvin, 2007
U.S, multistate	Tomatoes	CDC, 2006b

Management of Wastes from Domesticated Animals

Globally, food animal production has increased more than fivefold in the past 50 years due in large part to the adoption of the industrialized concentrated animal production model. With multinational companies expanding their operations overseas, estimates indicate that concentrated animal feeding operations (CAFOs) provide 74 percent of poultry, 50 percent of pork, and 43 percent of beef produced worldwide (Halweil and Nierenberg, 2004). Accompanying this expansion in production has been the challenge of managing the massive quantities of animal wastes that are generated in one location. For example, in China, animal waste was estimated to be 3.2 billion tons, which was three times the amount of

TABLE A3-4 Prevalence of *Salmonella* in Lettuce, Cabbage, and Mixed Salads Throughout the World (2001-2011)

Produce item	Country	Sampling site	Number positive/ number sampled	Prevalence (%)	Reference	
Cabbage	India	Fields	4/33	12.1	Rai and Tripathi, 2007	
	India	Street vendors	2/8	25.0	Viswanathan and Kaur, 2001	
	Ireland	Supermarkets	0/4	0	McMahon and Wilson, 2001	
	Mexico	Supply station	1/100	1.0	Quiroz-Santiago et al., 2009	
	U.S.	Packing sheds, southern U.S.	0/109	0	Johnston et al., 2006	
	U.S.	Farms, organic, conventional, semiorganic	0/291	0	Mukherjee et al., 2004, 2006	
Lettuce	Canada	Retail distribution centers/farmer's markets	1/530	0.2	Arthur et al., 2007	
	Ireland	Supermarkets	0/8	0	McMahon and Wilson, 2001	
	Italy	Producers	2/62	3.2	De Giusti et al., 2010	
	Korea	Department store, supermarket, restaurant	1/30	3.3	Seo et al., 2010	
	Mexico	Markets, supermarkets	10/75	13	Castañeda-Ramírez et al., 2011	
	Nigeria	Fields	0/55	0	Okago et al., 2003	
	Norway	Producers, organic	0/179	0	Loncarevic et al., 2005	
	Spain	Farms, organic, conventional	0/72	0	Oliveira et al., 2010	
	Spain	Retail establishments	1/29	3.4	Abadias et al., 2008	
	U.S.	Farms, organic, conventional, semiorganic	0/261	0	Mukherjee et al., 2004, 2006	
	U.S.	Supermarkets, farmer's markets	0/10	0	Thunberg et al., 2002	
	U.S.	Markets and wholesale distribution centers	2/5,453	0.04	USDA, 2007, 2008, 2009	
	Mixed salads/ vegetables	Brazil	Retailers	1/21	4.8	Fröder et al., 2007
		Cyprus	Production sites, retail outlets	6/294	2.0	Eleftheriadou et al., 2002
Korea		Department store, supermarket, restaurant	1/129	0.8	Seo et al., 2010	
Malaysia		Wet markets	40/112	35.7	Salleh et al., 2003	
U.K.		Catering, retail outlets	5/10,002	0.05	Sagoo et al., 2001, 2003a, 2003b	

SOURCE: CDC.

industrial solid waste produced in that same year (Wang et al., 2005). Within the United States, it has also been reported that confined food animals produce approximately 335 million dry tons of waste per year, which is more than 40 times the amount of human biosolids waste generated from wastewater treatment plants (Graham and Nachman, 2010). The vast majority of this animal waste is applied to land without any required treatment for reduction of pathogens as is required for human biosolids (EPA, 2004).

There are two primary forms of animal wastes generated at CAFOs. In the case of broiler units, solid waste is generated either as single-use, partial reuse, or multiuse litter (Bolan et al., 2010). In confined swine and cattle operations, water is used to flush waste from the floors where the animals are housed, and the liquid slurry is channeled into large ponds for storage (Graham and Nachman, 2010). The application of animal wastes to land is largely based on agronomic requirements, geography, and commodity choices. For example, corn receives more than half of the land-applied manure, of which most of the manure is from dairy and hog stock because of the use of corn as a major feed crop for dairy and hog operations and the high growth nutrient requirement of corn for nitrogen-rich manure. Hay and grasses are the second largest of the crops fertilized by manure, which is mostly from hog, broiler, and dairy producers (MacDonald et al., 2009). Poultry litter, on the other hand, is frequently used as a fertilizer for cotton, peanuts, and fresh produce (Boyhan and Hill, 2008).

Direct Transmission of Enteric Pathogens from Animal Wastes to Produce Fields

Animal manures applied to fields to be used for fruit and vegetable production have the potential to be a direct source of enteric pathogens if there has not been sufficient holding time between planting and harvest. The U.S. Department of Agriculture (USDA) National Organic Program permits the incorporation of raw manure into soil 120 days before harvest if the food crop has direct contact with the soil; however, only 90 days prior to harvest is required if crops have no contact with the soil (7 Code of Federal Regulations [CFR] 205.203). In contrast, more stringent requirements have been set by the Leafy Greens Marketing Agreement in which 1 year between application of raw manure and harvest of the crop is advocated (LGMA, 2012). As part of the Food Safety Modernization Act, it is anticipated that the Food and Drug Administration will include in its produce rule a required time interval between manure application to fields and either the planting or harvest of crops that would be consumed raw.

Transmission via Runoff of Enteric Pathogens from Animal Waste–Applied Lands to Produce Fields

One of the routes by which enteric pathogens may be indirectly transferred to produce fields from domesticated animal waste deposited or stored on land

TABLE A3-5 Prevalence of Helminth and Protozoan Parasites in Leafy Greens from 2005-2010

Produce item	Country	Sampling target	<i>Ascaris</i> spp.		<i>Cryptosporidium</i> spp.	
			Number positive/ number of samples	%	Number positive/ number of samples	%
Cabbage	Ghana	Retail fruit, vegetable markets	33/60	55.0		
	Spain	Fields			2/6	33.3
Lettuce	Turkey	Wholesale markets				
	Ghana	Retail fruit, vegetable markets	36/60	60.0		
	Libya	Wholesale, retail markets	26/27	96.3		
	Spain	Fields			10/13	76.9
	Turkey	Field	6/15	40.0		
	Turkey	Wholesale markets	2/35	5.7		

SOURCE: CDC.

adjacent to produce fields is via storm runoff. Many studies have revealed that enteric pathogens can move both horizontally and vertically to contaminate land, surface waters, and ground waters adjacent to produce fields (Cooley et al., 2007; Forslund et al., 2011). In these situations, the risk of pathogen contamination of produce will be dependent on a number of factors, including the attachment strength of the pathogen to soil particles, the interval between the manure application and the precipitation events, the kinetic energy of the rainfall, the topographical slope that affects the direction and velocity of water flow, and the density of vegetation between the waste source and the destination site (Ferguson et al., 2007; Hodgon et al., 2009; Jamieson et al., 2002; Lewis et al., 2010; Mishra et al., 2008; Saini et al., 2003; Tyrrel and Quinton, 2003). In addition, the physical state of the waste will also affect the direction of movement of the pathogens with greater percolation occurring by a liquid slurry source and greater overland transport for a solid manure source (Forslund et al., 2011; Semenov et al., 2009).

Transmission of Enteric Pathogens from Waste-Contaminated Water Sources to Produce Fields

Storm runoff carrying pathogens from animal wastes does not necessarily have to pass through agricultural produce fields to be a source of contamination. Collection in surface waters and subsequent use of that water to irrigate produce crops is another means to disseminate the pathogens. Surveys of environmental water sources for pathogen contamination have revealed significant contamina-

<i>Giardia</i> spp.		<i>Taenia</i> spp.		<i>Toxocara</i> spp.		Reference
Number positive/ number of samples	%	Number positive/ number of samples	%	Number positive/ number of samples	%	
						Amoah et al., 2006
2/6	33.3	0/14	0			Amorós et al., 2010 Kozan et al., 2005 Amoah et al., 2006
1/27	3.7	9/27	33.3	23/27	85.2	Abougrain et al., 2010
8/13	61.5					Amorós et al., 2010
3/15	20.0			2/35	5.7	Erdoğan and Şener, 2005 Kozan et al., 2005

tion with *Salmonella* spp., STEC, and protozoan parasites (Table A3-10); however, contamination appears to be sporadic and is often associated with recent rain events and seasonality (Gaertner et al., 2009; Haley et al., 2009). Enhanced survival of pathogens in the sediment (Chandran et al., 2011; Garzio-Hadzick et al., 2010) and resuspension of the organisms into the water column may also perpetuate the risk. Contamination of surface waters, moreover, has been associated with the concentration of food animals raised in the area (Cooley et al., 2007; Johnson et al., 2003; Tserendorj et al., 2011; Wilkes et al., 2011). *Salmonella* and *Cryptosporidium* contamination of watersheds not impacted by human or domesticated animal production has been observed (Edge et al., 2012; Patchanee et al., 2010), which suggests that there is a level of natural occurrence of these pathogens from wildlife sources.

Several epidemiological studies lend support to the role that contaminated irrigation water serves as a transmission vehicle of enteric pathogens to fresh produce. In 2002 and 2005, two outbreaks of *S. Newport* infection in the United States were associated with eating tomatoes and the outbreak strain was isolated from the pond water used to irrigate the tomato fields (Greene et al., 2008). Irrigation of fields with contaminated irrigation waters was also indicated as a possible source of contamination of imported cantaloupe associated with an outbreak of *S. Poona* infection in the United States in consecutive years during 2000-2002 (CDC, 2002). Given the often sporadic nature of contamination of irrigation water, these documented cases linking irrigation water to an outbreak may represent only a small fraction of the contamination events that actually occur. World-

TABLE A3-6 Prevalence and Cell Numbers of *Salmonella* spp. in Manures from Domesticated Animals

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>Salmonella</i>	Cattle feces	U.K., England and Wales	7.7% of 810 samples were positive (4.6 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Salmonella</i> spp.	Cattle feces	Britain	7.7-10.0% samples were positive; 3.3-3.4 log CFU/g in positive samples	Hutchison et al., 2005
<i>Salmonella</i>	Cattle, beef and dairy farms, rectal fecal	U.S., TN, NC, AL, WA, CA	0.2% of 480 beef cattle samples were positive; 0.4% of 480 dairy cattle samples were positive; 8.5% of 18 beef farms had at least one positive sample; 17.9% of 18 dairy farms had at least one positive sample	Rodriguez et al., 2006
<i>Salmonella</i> spp.	Cattle, beef, abattoir, feces	Ireland, northern	3% of 200 samples were positive	Madden et al., 2007
<i>Salmonella</i>	Cattle, dairy feces	New Zealand	0% of 155 samples positive	Moriarty et al., 2008
<i>S. enterica</i>	Cattle, feedlot feces	Australia	6% of 32 samples were positive	Klein et al., 2010
<i>S. enterica</i>	Cattle feces	California	0.13% of 795 samples were positive	Gorski et al., 2011
<i>Salmonella</i>	Chicken feces	U.K., England and Wales	17.9% of 67 samples were positive (3.7 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Salmonella</i> spp.	Poultry feces	Britain	11.5-17.9% samples were positive (2.3-3.6 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Salmonella</i>	Turkey feces and litter	U.S., NC	70% of 48 composite fecal samples were positive; 79% of 48 composite litter samples positive ($<1 - 5.3$ log MPN/g in positive samples)	Santos et al., 2005
<i>Salmonella</i>	Chicken litter	Lebanon	0% of 24 samples were positive	Omeira et al., 2006
<i>Salmonella</i>	Chicken/turkey farms, rectal feces	U.S., TN, NC, AL, WA, CA	0.2% of 480 samples were positive; 16.2% of 18 farms had at least one positive sample	Rodriguez et al., 2006
<i>Salmonella</i>	Chickens, laying hens feces	Belgium	0% of fecal samples collected on farm were positive	Van Hoorebeke et al., 2009
<i>Salmonella</i>	Chicken feces	France	8.6% of 370 flocks had at least one positive sample; most prevalent serovar was <i>S. hadar</i> followed by <i>S. anatum</i> and <i>S. mgandaka</i>	Le Bouquin et al., 2010

<i>Salmonella</i>	Pig feces	U.K., England and Wales	7.9% of 126 samples were positive (4.0 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Salmonella</i> spp.	Pig feces	Britain	5.2-7.9% samples were positive (2.8 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Salmonella</i>	Swine farms, rectal feces	U.S., TN, NC, AL, WA, CA	6.0% of 480 samples were positive; 57.3% of 18 farms had at least one positive sample	Rodriguez et al., 2006
<i>Salmonella</i>	Swine, finishing pigs, feces	Italy, Piedmont	9% of 75 fecal samples were positive	Lomonaco et al., 2009
<i>S. enterica</i>	Swine feces	Japan	3.1% of 169 samples were positive 22.0% of farms had positive samples	Kishima et al., 2008
<i>Salmonella</i>	Sheep feces	U.K., England and Wales	8.3% of 24 samples were positive (3.0 log CFU/g in positive samples)	Hutchison et al., 2004
<i>Salmonella</i> spp.	Sheep feces	Britain	8.3-11.1% samples were positive (2.8-3.8 log CFU/g in positive samples)	Hutchison et al., 2005

NOTE: CFU, colony forming unit; MPN, most probable number.

TABLE A3-7 Prevalence and Cell Numbers of *Campylobacter* spp. in Manures from Domesticated Animals

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>Campylobacter</i>	Cattle feces	U.K., England and Wales	12.8% of 810 samples were positive (3.9 log CFU/g in positive samples)	Hutchison et al., 2004
<i>Campylobacter</i> spp.	Cattle feces	Britain	9.8-12.8% samples were positive (2.5-2.7 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Campylobacter</i>	Cattle, dairy feces	New Zealand	64% of 155 samples were positive (5.6 log CFU/g in positive samples)	Moriarty et al., 2008
<i>Campylobacter</i> spp.	Cattle, beef, abattoir, feces	Ireland, northern	24.8% of 220 samples were positive	Madden et al., 2007
<i>C. jejuni</i>	Cattle, feedlot feces	Australia	94% of 32 samples were positive	Klein et al., 2010
<i>Campylobacter</i>	Chicken feces	U.K., England and Wales	19.4% of 67 samples were positive (3.6 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Campylobacter</i> spp.	Poultry feces	Britain	7.7-19.4% samples were positive; (2.4-2.8 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Campylobacter</i>	Broilers ceca	Sweden	47% of 540 ceca samples were positive; proportion of positive samples ranged from 10 to 100% within a flock. (1.7 to 8.6 log CFU/g in positive samples)	Hansson et al., 2010
<i>Campylobacter</i>	Duck, Mallard, feces	U.K.	93.3-100.0% of two groups of 60 farmed ducks tested at 28-56 days of age were positive	Colles et al., 2011
<i>Campylobacter</i>	Pig feces	U.K., England and Wales	13.5% of 126 samples were positive (3.3 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Campylobacter</i> spp.	Pig feces	Britain	10.3-13.5% samples were positive (2.5-3.2 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Campylobacter</i>	Sheep feces	U.K., England and Wales	20.8% of 24 samples positive (2.9 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Campylobacter</i> spp.	Sheep feces	Britain	11.1-20.8% samples were positive (2.0-2.6 log CFU/g in positive samples)	Hutchison et al., 2005

NOTE: CFU, colony forming unit.

TABLE A3-8 Prevalence and Cell Numbers of Shiga Toxin–Producing *E. coli* in Manures from Domesticated Animals

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>E. coli</i> O157:H7	Cattle, beef feedlot, feces	Canada, Alberta	1.9% of 8,682 samples were positive	Berg et al., 2004
<i>E. coli</i> O157	Cattle, dairy beef, farms, rectal feces	Mexico, central	1.2% of 240 samples were positive	Callaway et al., 2004
<i>E. coli</i> O157:H7	Cattle, beef and dairy, rectal feces	U.S., TN, NC, AL, WA, CA	3.9% of 408 dairy cattle samples were positive 4.7% of 408 beef cattle samples positive	Doane et al., 2007
<i>E. coli</i> O157:H7	Cattle, cow and calf farms, rectal feces	U.S.	2.5% of 408 samples were positive; 17.2% of 29 cow-calf farms positive	Dunn et al., 2004
<i>E. coli</i> O157	Cattle feces	U.K., England and Wales	13.2% of 810 samples were positive (6.5 log CFU/g for positive samples)	Hutchison et al., 2004
<i>E. coli</i> O157	Cattle, beef and dairy farms, feces	Korea	1.7% of 864 beef cattle samples were positive; 6.7% of 990 dairy cattle samples were positive	Jo et al., 2004
<i>E. coli</i> O157:H7	Cattle, feedlot	U.S., midwest	10.2% of 10,622 cattle were positive; 52.0% of 711 pens had a positive animal; 95.9% of 73 feedlots sampled had a positive animal	Sargeant et al., 2004
<i>E. coli</i> O157	Cattle feces	Britain	9.1-13.2% samples positive (2.4-3.1 log CFU/g in positive samples)	Hutchison et al., 2005
<i>E. coli</i> STEC	Cattle, beef feces	Japan	23% of 272 samples positive	Kijima-Tanaka et al., 2005
<i>E. coli</i> O157:H7	Cattle, dairy	Switzerland	4.2% of 966 samples were positive	Kuhnert et al., 2005
<i>E. coli</i> O157:H7	Cattle feces	Norway	7.0% of 156 samples were positive	Wasteson et al., 2005
<i>E. coli</i> O157:H7	Cattle, feedlot feces	U.S., KS	9.2% of 891 samples were positive	Alam and Zurek, 2006
<i>E. coli</i> STEC	Cattle, organic and conventional dairy feces	U.S., MN	32.% of 2208 samples were positive; 71.4% of dairy farms had at least one positive sample	Cho et al., 2006

continued

TABLE A3-8 Continued

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>E. coli</i> O26 and O111	Cattle, beef and dairy farms, feces	Korea	6.7%, 4.6%, and 2.0% of 809 samples tested positive for O26, O111, and both O26 and O111, respectively.	Jeon et al., 2006
<i>E. coli</i> O157	Cattle, dairy, mature, rectal feces	U.S., OH and Norway	0.7% of 750 samples were positive in Ohio; 8% of 50 herds had at least one positive sample; 0% of 680 samples positive in Norway	LeJeune et al., 2006
<i>E. coli</i> STEC	Cattle, dairy, mature, rectal fecal samples	U.S., OH and Norway	14% of 750 samples were positive in Ohio; 70% of 50 herds had at least one positive sample; 61% of 680 samples were positive in Norway; 100% of herds had at least one positive sample	LeJeune et al., 2006
<i>E. coli</i> O157	Cattle, feedlot and abattoir, fecal pats and rectal feces	U.S., CO, NE	24.7% of 450 fecal pats were positive; 27.6% of 145 rectal fecal samples were positive	Woerner et al, 2006
<i>E. coli</i> O157:H7	Cattle, rectal, feces	U.S., TX	64.3% of 8 cattle were positive	Edrington et al., 2007
<i>E. coli</i> O157	Cattle, 12-30-month-old beef, feces	Scotland	7.9% of 14,856 samples were positive; 22.8% of 952 farms had at least one positive sample	Gunn et al., 2007
<i>E. coli</i> O157:H7	Cattle feces	U.S., Central California	33.8% of 77 samples tested were positive	Jay et al., 2007
<i>E. coli</i> non-O157	Cattle, feedlot, feces	Canada, Alberta	0.7% of 2099 samples were positive; 57% of 21 feedlots sampled had positive samples	Renter et al., 2007
<i>E. coli</i> , non-O157 STEC	Cattle, beef, rectal fecal	Spain, northern	46.0% of 124 samples were positive	Oporto et al., 2008
<i>E. coli</i> , non-O157 STEC	Cattle, dairy, rectal fecal	Spain, northern	20.7% of 82 samples were positive	Oporto et al., 2008

<i>E. coli</i> O157:H7	Cattle, beef, abattoir, feces	Ireland, northern	0.9% of 220 samples were positive	Madden et al., 2007
<i>E. coli</i> , STEC	Cattle, dairy feces	New Zealand	1.3% of 155 samples were positive	Moriarty et al., 2008
<i>E. coli</i> O157:H7	Cattle, beef, rectal fecal	Spain, northern	1.6% of 124 samples were positive; 6.7% of herds had positive samples	Oporto et al., 2008
<i>E. coli</i> O157:H7	Cattle, dairy, rectal fecal	Spain, northern	7.0% of 82 samples were positive	Oporto et al., 2008
<i>E. coli</i> O157:H7	Cattle feces (perineal swab)	Canada	7.2% of 2,125 cattle were identified as supershedders of <i>E. coli</i> O157:H7 (> 4 log CFU/g) in the spring/summer; 0.5% of 2,000 cattle were identified as supershedders of <i>E. coli</i> O157:H7 (> 4 log CFU/g) in the fall/winter	Stephens et al., 2009
<i>E. coli</i> O157:H7	Cattle, beef GI tract	U.S., KS	20.3% of 815 samples were positive	Walker et al., 2010
<i>E. coli</i> O157	Chickens feces	Korea	0% of 418 samples were positive	Jo et al., 2004
<i>E. coli</i> STEC	Chicken broiler feces	Japan	0% of 158 samples were positive	Kijima-Tanaka et al., 2005
<i>E. coli</i> O157:H7	Chicken feces	Norway	13.6% of 22 samples were positive	Wasteson et al., 2005
<i>E. coli</i> O157:H7	Chicken and turkey, rectal fecal samples	U.S., TN, NC, AL, WA, CA	2.7% of 444 samples were positive	Doane et al., 2007
<i>E. coli</i> O157	Swine, rectal feces	Mexico, central	2.1% of 240 samples were positive	Callaway et al., 2004
<i>E. coli</i> O157	Pig feces	UK, England and Wales	11.9% of 126 samples positive (4.8 log CFU/g for positive samples)	Hutchison et al., 2004
<i>E. coli</i> O157	Swine feces	Korea	0.3% of 345 samples were positive	Jo et al., 2004
<i>E. coli</i> O157	Pig feces	Britain	11.9-15.5% samples were positive (3.1-3.6 log CFU/g in positive samples)	Hutchison et al., 2005
<i>E. coli</i> STEC	Swine feces	Japan	14% of 179 samples were positive	Kijima-Tanaka et al., 2005
<i>E. coli</i> O157:H7	Swine, rectal fecal samples	U.S., TN, NC, AL, WA, CA	8.8% of 426 samples were positive	Doane et al., 2007
<i>E. coli</i> O157:H7	Swine	Spain, northern	0% of 17 samples were positive	Oporto et al., 2008

continued

TABLE A3-8 Continued

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>E. coli</i> , non-O157 STEC	Swine	Spain, Northern	0% of 17 samples were positive	Oporto et al., 2008
<i>E. coli</i> O157	Sheep feces	UK, England and Wales	20.8% of 24 samples positive (4.0 log CFU/g for positive samples)	Hutchison et al., 2004
<i>E. coli</i> O157	Sheep feces	Britain	20.8-22.2% samples were positive (2.4-2.9 log CFU/g in positive samples)	Hutchison et al., 2005
<i>E. coli</i> O157:H7	Sheep feces	Norway	17.1% of 117 samples positive	Wasteson et al., 2005
<i>E. coli</i> O157	Sheep feces	Turkey	9.1% of 175 samples positive; 47% of 15 flocks had at least one positive sample	Turutoglu et al., 2007
<i>E. coli</i> , non-O157 STEC	Sheep, dairy, rectal feces	Spain, northern	50.7% of 122 samples were positive	Oporto et al., 2008
<i>E. coli</i> O157:H7	Sheep, dairy, rectal feces	Spain, northern	8.7% of 122 samples were positive; 7.3% of herds had positive samples	Oporto et al., 2008

NOTE: CFU, colony forming unit; STEC, Shiga toxin-producing *Escherichia coli*.

TABLE A3-9 Prevalence and Cell Numbers of *Cryptosporidium* spp. in Manures from Domesticated Animals

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>C. parvum</i>	Cattle feces	U.K., England and Wales	5.4% of 810 samples were positive (2.4 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Cryptosporidium</i>	Calf feces	Australia, Sydney watersheds	57.1% of 7 samples were positive	Cox et al., 2005
<i>Cryptosporidium</i>	Cattle, adult, feces	Australia, Sydney watersheds	22.2% of 9 samples were positive	Cox et al., 2005.
<i>C. parvum</i>	Cattle feces	Britain	2.8-5.4% samples were positive (1.0-1.3 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Cryptosporidium</i>	Cattle, dairy feces	New Zealand	5.2% of 155 samples were positive	Moriarty et al., 2008
<i>Cryptosporidium</i> spp.	Cattle, feedlot feces	Australia	13% of 32 samples were positive	Klein et al., 2010
<i>Cryptosporidium</i>	Chicken feces	Australia, Sydney watersheds	0% of 7 samples were positive	Cox et al., 2005
<i>C. parvum</i>	Pig feces	U.K., England and Wales	13.5% of 126 samples were positive (2.5 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Cryptosporidium</i>	Pig feces	Australia, Sydney watersheds	77.8% of 9 samples were positive	Cox et al., 2005
<i>C. parvum</i>	Pig feces	Britain	5.2-13.5% samples were positive (1.5-1.8 log CFU/g in positive samples)	Hutchison et al., 2005
<i>Cryptosporidium</i>	Pig slurry	Spain	40% of 5 pig farms were positive	Bornay-Llinares et al., 2006
<i>C. parvum</i>	Swine waste lagoons	U.S., Southeast	1.2% of 407 samples were positive	Jenkins et al., 2010

continued

TABLE A3-9 Continued

Pathogen	Source	Location	Prevalence (% of total samples positive) (average cell numbers in positive samples)	Reference
<i>Cryptosporidium</i>	Swine feces	China, Shanghai	34.4% of 2,323 samples were positive; 82.6% of positive samples were <i>C. suis</i> and 8.7% of positive samples were <i>Cryptosporidium</i> pig genotype II; 100% of 12 pig farms were infected with prevalence ranging from 14.1 to 90.6%	Chen et al., 2011
<i>Cryptosporidium</i>	Swine manure	Canada	55.7% of 122 pooled samples from 10 farms were positive; 55.4% of positive samples were <i>C. parvum</i> and 37.5% of positive samples were <i>Cryptosporidium</i> sp. pig genotype II	Farzan et al., 2011
<i>C. parvum</i>	Sheep feces	U.K., England and Wales	29.2% of 24 samples were positive (1.7 log CFU/g for positive samples)	Hutchison et al., 2004
<i>Cryptosporidium</i>	Sheep feces	Australia, Sydney watersheds	66.6% of 9 samples were positive	Cox et al., 2005
<i>C. parvum</i>	Sheep feces	Britain	29.2% samples were positive (1.0 log CFU/g in positive samples)	Hutchison et al., 2005

NOTE: CFU, colony forming unit.

TABLE A3-10 Prevalence of *Salmonella* spp., STEC, and Protozoan Parasites in Environmental Waters

Pathogen	Water source	Location	Prevalence (% positive of number of samples analyzed)	Reference
<i>Salmonella</i> spp.	Water, pond/creeks	Australia, Brisbane	3% of 32	Ahmed et al., 2009
	Water, river	Canada, Ontario	62% of 32	Droppo et al., 2009
	Water, river	U.S., GA	79.2% of 72	Haley et al., 2009
	Water, surface	Netherlands	14.3% of 49	Heuvelink et al., 2008
	Watersheds, swine	U.S., NC	41.7% of 12	Patchanee et al., 2010
	Watersheds, agriculture crops	U.S., NC	50% of 12	Patchanee et al., 2010
	Watersheds, forestry	U.S., NC	57.1% of 28	Patchanee et al., 2010
	Water, surface	Canada, Alberta	6.2% of 1429	Johnson et al., 2003
<i>E. coli</i> O157	Water, irrigation	Nigeria	8.2% of 196	Okago et al., 2003
	Water, surface	Netherlands	2.0% of 49	Heuvelink et al., 2008
<i>E. coli</i> O157:H7	Water, surface	U.S., Central CA	3.8% of 79	Jay et al., 2007
	Water, well	U.S., Central CA	0% of 19	Jay et al., 2007
	Water, surface	Canada, Alberta	0.9% of 1483	Johnson et al., 2003
STEC	Water, pond and creeks	Australia, Brisbane	9-15% of 32	Ahmed et al., 2009
<i>Cryptosporium</i>	Water, reclaimed	U.S.	70% of 30	Harwood et al., 2005
	Water, irrigation	Mexico	18% of 11	Thurston-Enriquez et al., 2002
	Water, irrigation	U.S.	<1 of 3	Thurston-Enriquez et al., 2002
<i>Giardia</i>	Water, reclaimed	U.S.	80% of 30	Harwood et al., 2005
	Water, irrigation	Mexico	64% of 11	Thurston-Enriquez et al., 2002
	Water, irrigation	U.S.	67% of 3	Thurston-Enriquez et al., 2002
Microsporidia	Water, irrigation	U.S.	67% of 3	Thurston-Enriquez et al., 2002

wide, it is estimated that 17 percent of the world's cropland (1.4 billion hectares) is irrigated and, of that, 20 million hectares are irrigated with untreated wastewater (Jimenez et al., 2010). In the United States and the United Kingdom, extensive irrigation of fresh produce crops occurs and, of the acreage irrigated, 48 percent and 78 percent, respectively, are derived from non-groundwater sources (Knox et al., 2011; USDA NASS, 2009), which are subject to intermittent inputs of pathogens from animal husbandry operations.

Contribution of Bioaerosols to Dissemination of Enteric Pathogens from Animal Production Operations to Produce Fields

Aerosolization of microbial pathogens is an inevitable consequence associated with animal production operations as well as the handling and disposition of animal manure. However, estimating the impact of bioaerosol dispersal on pathogen dissemination has been hampered by the notable absence of standardized and validated methods for enumeration of various types of microorganisms in outdoor bioaerosols. Hence, there has been a wide range of prevalence and cell number values reported across very diverse types of animal operations and landscapes (Millner, 2009).

Studies addressing bioaerosol levels in outdoor air generally address fecal indicator organisms because they are more abundant and easily identified in the aerosols, although it is acknowledged that they may behave differently than the pathogens. The general trend that has been observed is decreasing airborne microorganism concentrations as the distance from the source increases with relative humidity, temperature, and solar irradiance being major factors affecting viability (Dungan, 2010). Other pertinent observations made in studies addressing the levels of the indicator organism, *E. coli*, in aerosols of poultry houses are that the levels of airborne bacteria are intricately linked to the levels of those bacteria in the litter (Chinivasagam et al., 2009; Smith et al., 2012) and the type of ventilation system affects the distance that *E. coli* is disseminated, with *E. coli* traversing 11.1 and 7.5 m downwind from houses using tunnel and conventional fans, respectively (Smith et al., 2012).

Limited studies have been conducted addressing bioaerosol transport following land application of animal manures in contrast to those addressing the application of municipal wastes (Pillai and Ricke, 2002). Although there may be some similar behavior between these two sources, there could be differences given that they vary in their organic matter content that can provide differences in the degree of protection against ultraviolet radiation and drying (Dungan, 2010). In one of the few studies addressing land application of cattle and swine slurry and the method used to disperse the wastes, total bacterial counts in the air were greater at greater distances from spray guns that discharged the slurry upward into the air compared to tank spreading that sprayed the slurries closer to the ground (Boutin et al., 1988). In another study in which swine manure was ap-

plied through a center pivot irrigation system, coliform concentrations decreased to near background concentrations at 23 m downwind (Kim et al., 2008). Wind speed and topography, however, are likely to also factor into the distances traversed by pathogens and, hence, safe distances between produce fields and animal production activities will likely be site specific.

Wildlife as a Vehicle to Transmit Pathogens from Domesticated Animal Waste to Produce Fields

The recent focus on wildlife as a potential source of pathogen contamination of produce fields was driven by the isolation of *E. coli* O157:H7 from feral swine that occupied areas near spinach fields and cattle farms in California following the 2006 spinach outbreak (Jay et al., 2007). More recently, *Campylobacter jejuni* was isolated both from Sandhill crane feces and raw peas and several of the isolates had pulsed-field gel electrophoresis (PFGE) patterns indistinguishable from clinical samples obtained during a *C. jejuni* gastroenteritis outbreak that occurred in Alaska in 2008 (Gardner et al., 2011). Attention was again focused on wildlife as a potential source of contamination when *E. coli* O157:H7 isolated from deer feces was determined to have an identical PFGE pattern as the isolates responsible for 15 people who were ill from eating contaminated fresh strawberries in Oregon in 2011 (IEH Laboratories & Consulting Group, 2011). Given that the same strain was also isolated from soil raises the question as to whether the deer were actually the source of the outbreak or were infected when they ate the contaminated strawberries. Most evidence indicating that wildlife is a potential source of food-borne contamination is from the isolation of clinically relevant pathogens from the animal's feces. In one example, Renter et al. (2006) isolated from deer fecal samples four *Salmonella* serovars (Litchfield, Dessau, Infantis, and Enteritidis) known to be pathogenic to humans and animals. In another example, subtyping of STEC isolates from wildlife meat in Germany identified virulence genes associated with severe clinical outcome (*stx2*, *stx2d*, and *eae*) in 46 of the 140 STEC samples (Miko et al., 2009). More definitive proof that specific types of wildlife could be transmission vectors of pathogens from domesticated animal facilities was obtained with a study of European starlings (Williams et al., 2011). In that study, distinct molecular types of *E. coli* O157:H7 were similar in starlings and cattle on different farms, and these birds were capable of shedding the pathogen in their feces for more than 3 days (Kauffman and LeJeune, 2011). Hence, it is reasonable to assume that European starlings could serve as a vector of pathogens from cattle and dairy farms to produce fields.

In response to the limited studies linking wildlife to produce contamination, processors and buyers have become overreactive in many cases in requiring the absence of many types of wildlife from farms. To illustrate this trend, the percentage of growers that reported being told by their processors or buyers that feral pigs, deer, birds, rodents, and amphibians were a significant risk was 19, 28,

44, 47, and 28 percent, respectively (Lowell et al., 2010). Several studies, however, have revealed that some groups of animals have a very low prevalence of contamination with relevant human enteric pathogens (Table A3-11). It is likely that all animal groups have the potential to be contaminated with a food-borne pathogen, but whether they are significant harbingers of human enteric pathogens is likely dependent on their access to animal husbandry sites as well as on their social behavior (i.e., existence of a social group and its size). This would also be the case with insects. For example, filth flies collected in leafy green fields were believed to have originated from nearby rangelands that contained fresh cattle manure (Talley et al., 2009).

Persistence of Pathogens on Produce in Fields Requires a Systems Approach to Prevent and Monitor Pathogen Introduction

Many field studies have revealed the persistence of human enteric pathogens, albeit typically at low levels, in a number of different vegetables contaminated at various points during their cultivation (Erickson et al., 2010; Gutiérrez-Rodríguez et al., 2011; Islam et al., 2004a, 2004b, 2004c, 2005; Moyne et al., 2011). This is noteworthy because chemical disinfectants typically used during minimal processing of fresh produce are not fully effective in eliminating pathogen contamination (Doyle and Erickson, 2008). Hence, it is paramount to prevent the introduction of these pathogens into produce fields. The primary approach currently used to reduce the risk of pathogen contamination in fields is the application of good agricultural practices (GAPs). To prevent the introduction of pathogens through nontraditional vehicles (storm runoff, intrusions by pathogen-carrying wildlife) will require the development of novel approaches in addition to GAPs. Given that the environment surrounding the produce field would likely be impacted by these pathogen control practices, it is important to implement a systems approach and consider all ramifications to the adoption of any intervention practices. It is also important to be cognizant that storm runoff and fecal deposits from wildlife may only contaminate the plants at discrete locations within a field. The ability to detect this contamination by current sampling plans that rely on uniform contamination is therefore limited and efforts are needed to develop new monitoring systems that can detect contamination when such pathogen introductions occur.

Concluding Comments

Vegetables, fruits, and a variety of plant foods and ingredients are now recognized as major vehicles of food-borne disease outbreaks, and a primary source of pathogen contamination of this commodity group is animal manure. There are several routes by which pathogens can be transmitted from animal production sites to produce fields. The vehicles likely presenting the greatest risk are manure-contaminated soil amendments and irrigation water. Wildlife, insects,

TABLE A3-11 Prevalence of Enteric Food-Borne Pathogens in Wildlife and Insects

Animal	Pathogen	Country	Prevalence (% positive of number of samples analyzed)	Reference
Wild boars/pigs	<i>E. coli</i> O157:H7	U.S.	14.9% of 87	Jay et al., 2007
	<i>E. coli</i> O157:H7	Spain	3.3% of 212	Sánchez et al., 2010
	Non-O157 STEC	Spain	5.2% of 212	Sánchez et al., 2010
	<i>C. parvum</i>	Australia	0% of 5	Cox et al., 2005
	<i>Giardia</i>	Australia	0% of 5	Cox et al., 2005
Coyotes	<i>Salmonella</i>	U.S.	5% of 40	Gorski et al., 2011
Rabbits	<i>E. coli</i> (VTEC)	U.K.	15.5% of 129	Scaife et al., 2006
	<i>Cryptosporidium</i>	Australia	50% of 2	Cox et al., 2005
	<i>Giardia</i>	Australia	0% of 2	Cox et al., 2005
Raccoons	<i>Salmonella</i>	U.S.	0% of 2	Gorski et al., 2011
Skunks	<i>Salmonella</i>	U.S.	30.7% of 13	Gorski et al., 2011
Deer	<i>E. coli</i> O157:H7	U.S.	0% of 4	Jay et al., 2007
	<i>Cryptosporidium</i>	Australia	100% of 1	Cox et al., 2005
	<i>Giardia</i>	Australia	0% of 1	Cox et al., 2005
	<i>Salmonella</i>	U.S.	1% of 500	Renter et al., 2006
	Pigeons/sparrows	<i>E. coli</i> O157	Czech Rep.	0% of 70
Birds (cattle farm)	<i>E. coli</i> O157:H7	U.S.	0.5% of 200 pooled	Hancock et al., 1998
Geese, Canadian	<i>E. coli</i> (EHEC)	U.S.	6.0% of 151	Kullas et al., 2002
Multiple birds (domestic animal farms)	<i>Salmonella</i>	Denmark	1.5% of 1285	Skov et al., 2008
Multiple birds	<i>Salmonella</i>	U.S.	6.6% of 105	Gorski et al., 2011
Reptiles	<i>Salmonella</i>	Spain	41.5% of 94	Briones et al., 2004
Amphibians	<i>Salmonella</i>	Spain	0% of 72	Briones et al., 2004
Rats	<i>E. coli</i> O157	Czech Rep.	40% of 10	Čížek et al., 1999
Mice, wood	<i>E. coli</i> O157	Czech Rep.	0% of 7	Čížek et al., 1999

continued

TABLE A3-11 Continued

Animal	Pathogen	Country	Prevalence (% positive of number of samples analyzed)	Reference
Rodents	<i>E. coli</i> O157:H7	U.S.	0% of 300 pooled	Hancock et al., 1998
Rodents	<i>Toxoplasma gondii</i>	Netherlands	9.1% of 77	Kijlstra et al., 2008
Rodents	<i>Salmonella</i>	Denmark	2% of 135	Skov et al., 2008
Mice/rats	<i>S. Enteritidis</i>	U.S.	16.2% of 715	Henzler and Opitz, 1992
Squirrels	<i>S. enterica</i>	U.S.	0% of 28	Gorski et al., 2011
Mice/rats	<i>C. parvum</i>	U.S.	27.8% of 241	Li et al., 2011
Flies	<i>E. coli</i> O157:H7	U.S.	3.3% of 60	Hancock et al., 1998
Houseflies	<i>E. coli</i> O157:H7	U.S.	2.2% of 3,440	Alam and Zurek, 2004
Flies	<i>Salmonella</i>	Denmark	22.6% of 31	Skov et al., 2008
Houseflies/dump flies	<i>Salmonella</i>	U.S.	18% of 22	Olsen and Hammack, 2000
Houseflies	<i>S. Enteritidis</i>	U.S.	~50% of 120	Holt et al., 2007
Cockroaches	<i>Salmonella</i>	U.S.	14.4% of 90	Kopanic et al., 1994
Slugs	<i>E. coli</i> O157:H7	U.K.	0.2% of 33	Sproston et al., 2006

and vermin, however, may also serve as intermediate vectors of pathogens from animal wastes to plants in the field. The multifaceted routes by which pathogens may be transmitted to produce crops illuminates the value of a One Health approach to minimize pathogen contamination in the production environment while ensuring that adverse effects to the environment be minimized.

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A4

**ONE HEALTH AND FOOD SAFETY—THE CANADIAN EXPERIENCE:
A HOLISTIC APPROACH TOWARD ENTERIC BACTERIAL
PATHOGENS AND ANTIMICROBIAL RESISTANCE SURVEILLANCE**

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Introduction

This paper describes a holistic approach to the prevention and control of human food-borne illness from enteric pathogens, based on implementation of the “One Health” paradigm. Antimicrobial resistance (AMR) has been chosen as a particular illustrative theme for this overview to demonstrate the practical utility of a One Health approach.

The rapid emergence, global spread, morbidity, and mortality associated with emerging infections such as severe acute respiratory syndrome and avian and pandemic influenza is stimulating the global community to develop novel approaches for their prevention and control. Ongoing concerns about food-borne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and various species of *Salmonella*, as well as the arrival and impact of new strains of food-borne pathogens such as *E. coli* O104 as observed during the 2011 outbreak in Germany, add to the need to take into account the complexity of infection from multiple dimensions. These include the following:

1. *Burden of illness.* The World Health Organization estimates that infectious and parasitic diseases are the second leading cause of death in the world (WHO, 2008). Enteric pathogens are the third leading cause of infectious disease worldwide and account for almost 2 million deaths every year (Girard et al., 2006). As in many other countries, these pathogens also cause a significant disease burden in Canada, where there are an estimated 11 million food-borne enteric illnesses per year with an estimated cost of \$3.7 billion dollars (Thomas et al., 2008). Although microbial enteric illness can be caused by bacteria, viruses, parasites, and protozoal organisms, bacteria play a major role in enteric disease (Girard et al., 2006)

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and are the major focus of enteric surveillance programs. Although most enteric bacterial infections result in subclinical or mild illness, their high rate of incidence in the population can be expected to have economic impact on a country simply through loss of short-term individual productivity. In addition, bacterial infections can cause severe disease, particularly in children, the elderly, and immunosuppressed individuals.

2. *Zoonotic and environmental origins.* More than 60 percent of new emerging and reemerging pathogens of humans, including those that are transmitted by food and water, arise from animals and the environment (Jones et al., 2008). The rate of emergence appears to be increasing, most likely related to factors such as human population growth, changing patterns of international trade, globalization, mass population migrations, climate change, and environmental degradation. With regard to food safety pathogens, it is anticipated that the increased industrialization of animal production, as is seen worldwide in both developed and developing countries, creates an environment for increased opportunity for entry of pathogens into the food chain.
3. *Antimicrobial resistance.* The severity of infections and our success in treating the associated clinical diseases are affected by the presence of antimicrobial resistance. Antimicrobial-resistant bacteria are those that are able to replicate in the presence of antimicrobials, here meaning antibiotics and their synthetic derivatives, at levels that normally suppress growth or kill the bacteria. Antimicrobial resistance is a growing concern that threatens animal and human health worldwide, driven mainly by antimicrobial use, both appropriate and inappropriate.

The One Health Paradigm

“One Health” has emerged as a strategic framework for reducing the risks of infectious diseases arising from the animal–human–ecosystems interface. Although a universal definition of One Health has not been achieved, and there are overlaps with integrative approaches used in international research and development, such as “ecosystem approaches to health” (Charron, 2011), there is consensus that One Health is an approach or method of practice that recognizes linkages among human, animal, ecosystem, and economic domains in the context of human health.

The One Health approach focuses on the dynamic interactions at the interfaces between multiple sectors that contribute to the expression of a public health risk. In that interactive context, the approach becomes a tool for disease prevention and control through more informed risk management, encompassing the separate elements of identification, assessment, avoidance, and mitigation of the public health risk. It is worth noting that One Health is bigger than the zoonotic infectious disease issues described below, and incorporates socioeconomic, cultural,

and community conditions (the social determinants of health), as well as individual lifestyle and hereditary health factors.

The economic relevance of early and comprehensive intervention is often overlooked, but can be significant. For instance, the direct economic impacts of individual zoonotic disease events that have occurred over the past 15 years can be in the billions (Figure A4-1).

Canada has been actively engaged in operationalizing the One Health concept through the development of a community of practice by participating and supporting international conferences encompassing the subject. The Public Health Agency of Canada, recognizing the emerging value of the One Health paradigm, hosted an Expert International Consultation on “One World One Health™: from Ideas to Action,” March 16-19, 2009, in Winnipeg, Manitoba (<http://www.phac-aspc.gc.ca/publicat/2009/er-rc/index-eng.php>). Many other major international meetings have helped define One Health, most recently, in November 2011, the High Level Technical Meeting on Health Risks at the Human–Animal–Ecosystems Interfaces in Mexico City. Upcoming is a meeting scheduled for February 2012 in Davos, Switzerland: Global Risk Forum One Health Summit 2012: One Health–One Planet–One Future.

One Health in relation to food safety has multiple dimensions, including science and research, optimizing animal health and ecosystem health, and food inspection and regulatory activities. In Canada work in this area is conducted by several federal government agencies, such as the Public Health Agency of Canada (surveillance, research, and epidemiology of food-borne illness), the Canadian

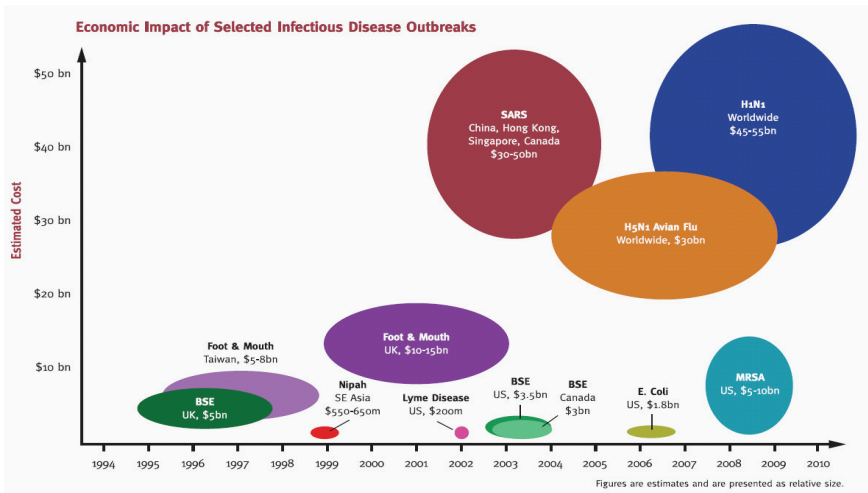


FIGURE A4-1 Economic impact examples.

SOURCE: Newcomb et al. (2011).

Food Inspection Agency (animal health and food inspection), Health Canada (food safety regulations and risk assessment), and Agriculture and AgriFood Canada (food animal production). Canada's provincial and territorial jurisdictions have also started to embrace a One Health approach; for instance, Manitoba has a primer on One Health and food safety and has developed an animal health and food safety strategy for the future ("Protecting Animals, Food and People"), and Québec has an animal health and welfare strategy ("One Health, Health for All"). The Canadian academic sector is a critical contributor to the theme, particularly its five veterinary colleges.

Science and research activities include surveillance, detection, and public health risk assessment of nonhuman bacterial isolates, studies on the population and environmental determinants of food-borne zoonoses, systems modeling of the food chain to identify optimal points of intervention, development of intervention strategies such as vaccines and bacteriophage products, and knowledge translation for uptake by food production and processing workers. The activities also include characterization of impacts of particular practices, such as the use of antibiotics in commercial food animal production and its potential in giving rise to antimicrobial resistance in bacteria pathogenic to humans.

Mechanisms of Antimicrobial Resistance

Resistance can be intrinsic, conferred by naturally occurring characteristics of the bacteria, or acquired. Bacteria can acquire resistance through mutations of preexisting genes or through transfer of resistance determinants from other bacteria (horizontal gene transfer). Horizontal transfer occurs much more commonly than *de novo* development of resistance through mutation (White et al., 2008). It is through horizontal gene transfer that resistance genes, alone or in groups, can spread within bacterial populations and even to other bacterial species.

Resistance genes provide the molecular tools by which bacteria block or oppose the mechanism of action of antimicrobials. Some genes allow bacteria to physically modify their structure to evade drugs, while other genes express enzymes to directly degrade the antimicrobial agent. In addition, resistance mechanisms that are not specific to antimicrobial agents can also be present. For example, cell pumps that allow bacteria to excrete environmental toxins and prevent them from reaching harmful intracellular concentrations can also help bacteria to resist the harmful effects of antimicrobials.

Not all antimicrobial-resistant bacteria are harmful, and resistance genes can be found in nonpathogenic bacteria (Wright, 2007). However, these benign but resistant bacteria may also pose a threat through the transfer of resistance genes to pathogenic bacteria (Figure A4-2).

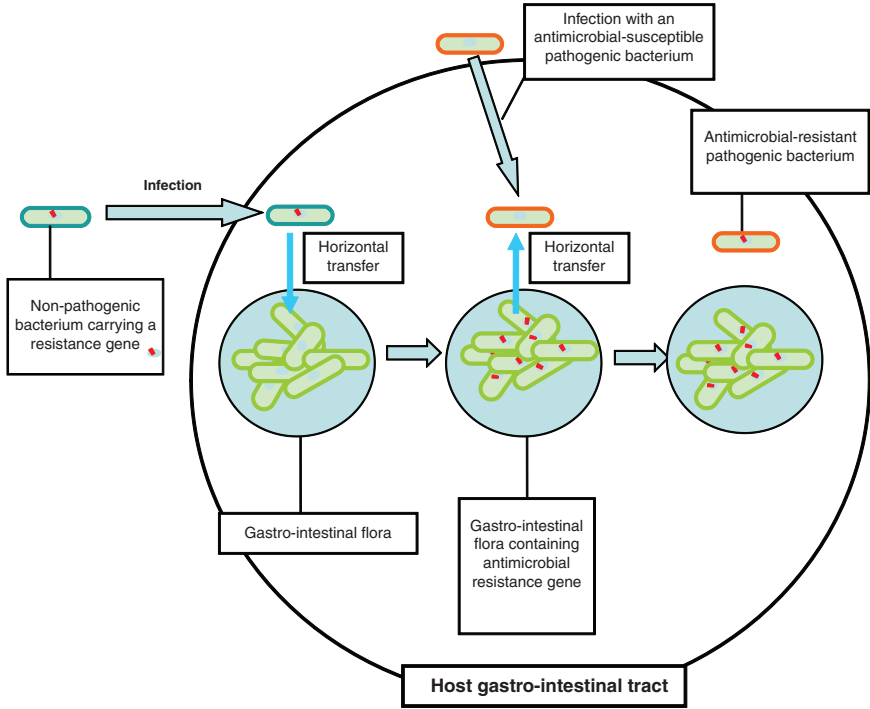


FIGURE A4-2 Transfer model for antimicrobial resistance genes.

Antimicrobial Usage and Resistance

Antimicrobial use (AMU) in animal and human populations is considered to be the major driver of AMR emergence and persistence. Use of antimicrobials exerts a powerful selective influence on bacteria, encouraging the survival and propagation of resistant strains and influencing how quickly AMR develops. Because different resistance genes are often clustered close together on the bacterial genome, especially on transmissible genetic elements such as plasmids and transposons, selection for resistance against one type of antimicrobial may also co-select for resistance against other unrelated antimicrobials. In addition, use of one antimicrobial can select for resistance to closely related antimicrobials (cross-resistance). For example, in Europe, use of avoparcin, an antimicrobial growth promoter used in food animals, has been linked with resistance to vancomycin, an antimicrobial “of last resort” in human medicine (Kruse et al., 1999).

Genetic mechanisms leading to the development and maintenance of AMR are complex. At one time, it was thought that AMR universally negatively im-

pacted the fitness of microorganisms and that, by removing the selective pressure imposed by antimicrobial usage, resistance genes would be selected against in future bacterial generations. However, Wright (2007) identified several genetic mechanisms that may be exceptions to this rule: resistance genes that increase fitness, resistance genes that do not have a fitness cost, and compensatory mutations that restore bacterial fitness. Finally, environmental factors may play a large role in the persistence of “unused” antimicrobial resistance genes. Selection of genes conferring protection against environmental stressors such as heavy metals and biocides may also co-select for resistance genes (Alonso et al., 2001).

The genetic regulation of AMR is complex and not fully understood. Despite our gaps in knowledge, prudent AMU and adherence to the principles of good antimicrobial stewardship are recommended as key elements in a strategy directed at preserving the efficacy of antimicrobials, particularly those that are very important to human and veterinary medicine.

A Holistic Consideration of AMR and Enteric Disease

Figure A4-3 depicts the complex interactions between enteric organisms, animals, and humans, and the many determinants (socioeconomic, environmental, and geopolitical) that affect these relationships. Antimicrobial-resistant bacteria form the central component of our model. A number of different interactions can be described using this model, some of which require greater insight into their mechanisms and importance. For example, certain bacteria that cause disease in animal hosts may not cause disease in people but may exchange genetic material, including resistance genes, with human pathogens, causing community-acquired and nosocomial infections (Guardabassi et al., 2004).

Enteric infections in people generally occur through fecal–oral transmission, of which several risk factors can be identified: increased contact between humans and animals, extended hospitalization, poor hygiene, consumption of improperly handled and improperly cooked foods including meats, and ingestion of contaminated water. Prior treatment with antimicrobials can also increase an individual’s susceptibility to infection by pathogenic bacteria through disruption of the normal bacterial flora and by conferring a competitive advantage to resistant strains of pathogens such as *Salmonella* (Barza and Travers, 2002).

Previous infections with resistant bacteria can also predispose individuals to future resistant infections and disease. As seen in Figure A4-3, an individual may be infected with a commensal bacterium carrying resistance genes. Maintenance of resistance within the individual may occur through colonization of the gastrointestinal tract with this commensal bacterium or via horizontal transfer to gut flora as shown in the diagram. If this same individual is later infected with a pathogenic bacterium, then resistance may be transferred to this pathogen through horizontal transfer from the gut flora.

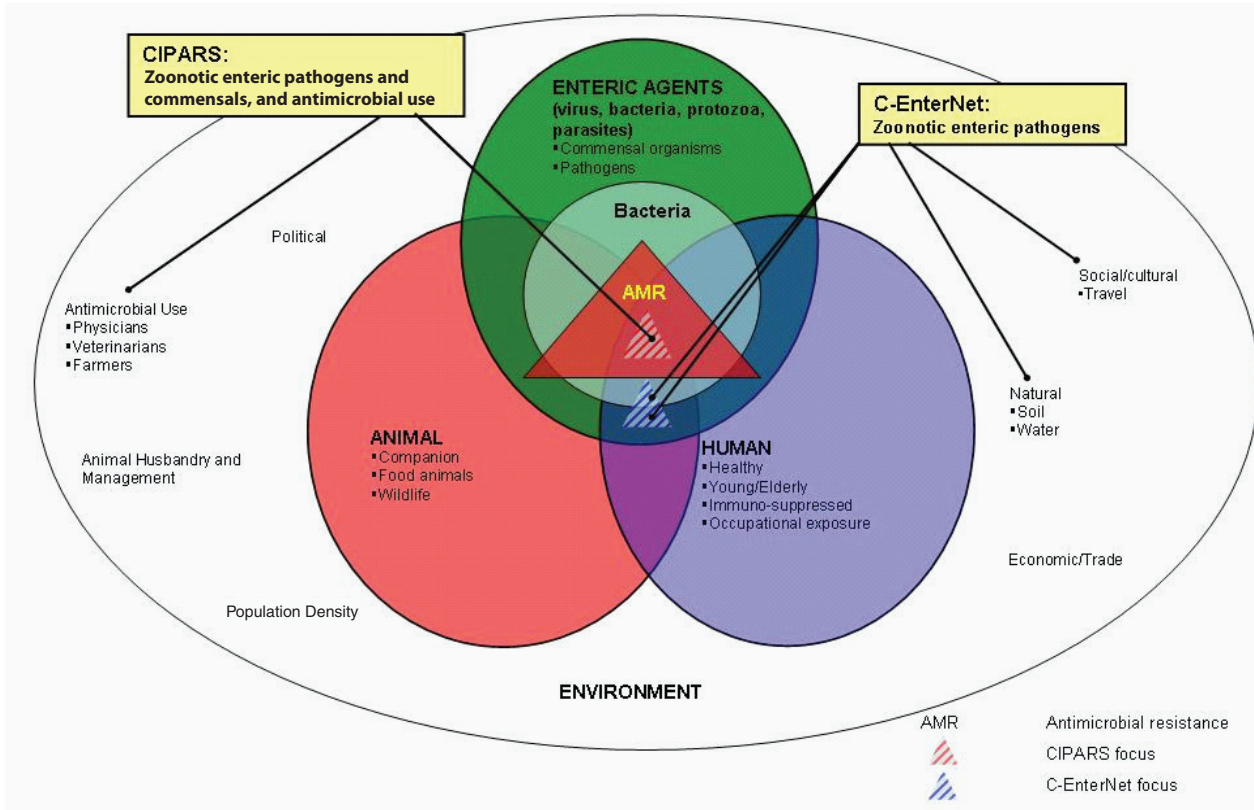


FIGURE A4-3 The intersection of enteric agents, animals, and humans, and the environmental factors that influence the occurrence of zoonotic bacterial infections and the emergence of AMR.

Implications on Global Health

A number of provincial and national reports, including the 2002 Walkerton Commission of Inquiry (Government of Ontario) and the 2004 Renewal of Public Health in Canada report (Government of Canada), have advocated for a holistic approach toward understanding enteric disease. This type of approach is especially useful given the complexity of enteric disease and its importance as a global health issue. AMR also has serious implications for global human and animal health.

AMR impairs our ability to treat infectious diseases and endangers the long-term efficacy of antimicrobial drugs available to human and veterinary medicine. Not only are infections caused by resistant bacteria more difficult and more expensive to treat, but also the longer duration of infection may increase disease shedding and spread. AMR thus has important effects on the pathogenicity and epidemiology of zoonotic bacterial agents.

Along with its global health implications, the emergence of resistant bacteria may have broad economic effects. Weakened public confidence over the safety of agricultural commodities, potential inclusion of AMR bacteria as a product adulterant leading to recalls, and changes to consumer buying patterns are major economic concerns to agricultural industries. At the patient level, AMR may reduce the efficacy of certain antimicrobials and thereby increase the cost of infection (e.g., longer hospital stays and changes in AMU for disease treatment and prevention) in people and animals. As discussed by Foster (2009), the economic burden of AMR may be most dramatic in developing nations because of the higher expense of second- or third-line drugs, and the lack of diagnostic capacity to detect resistance early, which may result in treatment failures and complications in antimicrobial selection.

Developing solutions to AMR and enteric disease requires synthesis of knowledge and analysis of data at the local, national, and global scales. Factors such as agricultural land-use patterns, attitudes toward antimicrobial usage, and the nature and extent of interactions between people and animals can have major effects on the development of AMR at the local and national levels. However, these local influences may also have global significance. Global interactions of people, animals, and animal products mean that AMU and the accompanying regulations in one country can affect the efficacy of a particular antimicrobial in another. Similarly, the global epidemiology of enteric pathogens is important in understanding the local burden of enteric disease. For example, it was estimated that 30 percent of all enteric disease cases at a sentinel site in Ontario, Canada, in 2008 were associated with international travel (Government of Canada, 2009).

Application of a Holistic Approach to Zoonotic Bacterial Infections and AMR in Canada

The Public Health Agency of Canada supports two complementary surveillance programs that together provide a holistic approach to AMR and enteric

disease (Figure A4-3): (1) the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and (2) the National Integrated Enteric Pathogen Surveillance Program (C-EnterNet). Both were modeled after similar programs in other countries: NARMS (United States) and DANMAP (Denmark) for CIPARS and FoodNet (United States) and OzFoodNet (Australia) for C-EnterNet. The two Canadian programs generate and collect data that contribute to our understanding of the transmission of zoonotic bacteria, risk factors for infection, and the drivers of AMR and AMU. As surveillance systems, their ongoing and systematic designs allow for the identification of emerging trends and the ability to identify the impacts of prevention and control measures adopted at the national, provincial, and, occasionally, local levels in Canada.

Both programs also provide a research platform that aims to identify and understand how livestock husbandry and production methods, water-borne routes of exposure, wildlife, companion animals, exotic pets, and socioeconomic factors and high-risk human populations are affected by and contribute to zoonotic bacterial infections and AMR.

While CIPARS performs epidemiological surveillance on AMR and AMU through the generation and collection of nationwide data from farms, abattoirs, retail stores, and both human and animal diagnostic health laboratories, C-EnterNet performs epidemiological surveillance on enteric pathogens at intensively sampled local sentinel sites (currently one site in Ontario and one in British Columbia). Like CIPARS, C-EnterNet collects data at the level of the farm, retail store, and human community (via epidemiological and laboratory data on human cases in partnership with the local public health unit). C-EnterNet also performs environmental surveillance by collecting and testing untreated water samples. This parallel testing is critical to understanding the complex system of food and water-borne disease transmission. Results from both programs are publicly accessible through the Public Health Agency of Canada website as well as through annual reports and newsletters.

The epidemiological strength of CIPARS lies in its breadth of surveillance at major points along the farm-to-fork continuum. These data allow for temporal and spatial analyses of provincial and national trends in bacterial recovery and AMR. This is best demonstrated with the recent study of *Salmonella* Heidelberg and ceftiofur resistance (see the section titled *Success Within CIPARS: A Case Example*). While CIPARS is most effective at studying trends at broad scales, C-EnterNet's value is in its ability to detect subtle epidemiological effects that may only be captured at the local level. In addition, it is one of the only systems that can delineate endemic versus travel-acquired human infections (see the section titled *Success Within C-EnterNet: A Case Example*). The sentinel-site surveillance approach provides rich data that would be cost-prohibitive to collect across all of Canada. But, by understanding sentinel populations, the information can be used to determine the predominant sources of enteric pathogens causing

infection and the risk factors (including individual behaviours) that contribute to the burden of enteric illness.

It is important to recognize the unique operational aspects of both CIPARS and C-EnterNet and their complementary nature. Having two different but linked surveillance models that encompass different scales is essential in providing a comprehensive look at the specific risk factors associated with AMR and enteric disease. When considered together, both programs provide a holistic picture of the complex relationships between enteric pathogens, the environment, and the health of humans and animals.

Success Within CIPARS: A Case Example

Recent analysis of CIPARS data identified a link between ceftiofur (an antimicrobial of high importance to human medicine) usage in poultry and ceftiofur-resistant *Salmonella* Heidelberg isolates obtained from people and chicken meat in Québec (Dutil et al., 2010), as shown in Figure A4-4. Because *S. Heidelberg* is a common serotype that infects and can cause disease in people, this finding had important human health implications.

Communication of this information led to a voluntary ban on the use of ceftiofur in 2005, and the ongoing collection of surveillance data provided the opportunity to follow trends in human and animal infection and in AMR. The findings from this work have provided strong evidence pointing toward changing patterns in AMU affecting clinical bacterial resistance in human and animal isolates. This study has been used to inform policy on the appropriate use of this antimicrobial and is helping to guide physicians and veterinarians in their selection of appropriate antimicrobials and how these drugs are dispensed.

Success Within C-EnterNet: A Case Example

The C-EnterNet program recently looked at 1,773 reported cases of disease caused by enteropathogens such as *Salmonella*, *Campylobacter*, and verotoxigenic *Escherichia coli* in Sentinel Site 1 (Region of Waterloo, Ontario) (Ravel et al., 2011). C-EnterNet and its local public health partners found that more than one in four reported cases of enteric infection were related to travel, including 9 percent involving new immigrants. The most popular destinations of the patients studied were the Caribbean, Latin America, and Asia.

The finding illustrates that travel-related cases of diseases caused by enteric pathogens represent a significant proportion of the burden of total diseases in Canada. These results will help to delineate domestically acquired infections from those acquired abroad. In the One Health framework, this will help target more effective prevention and control measures domestically, considering a broad suite of pathogens and the complex routes of transmission.

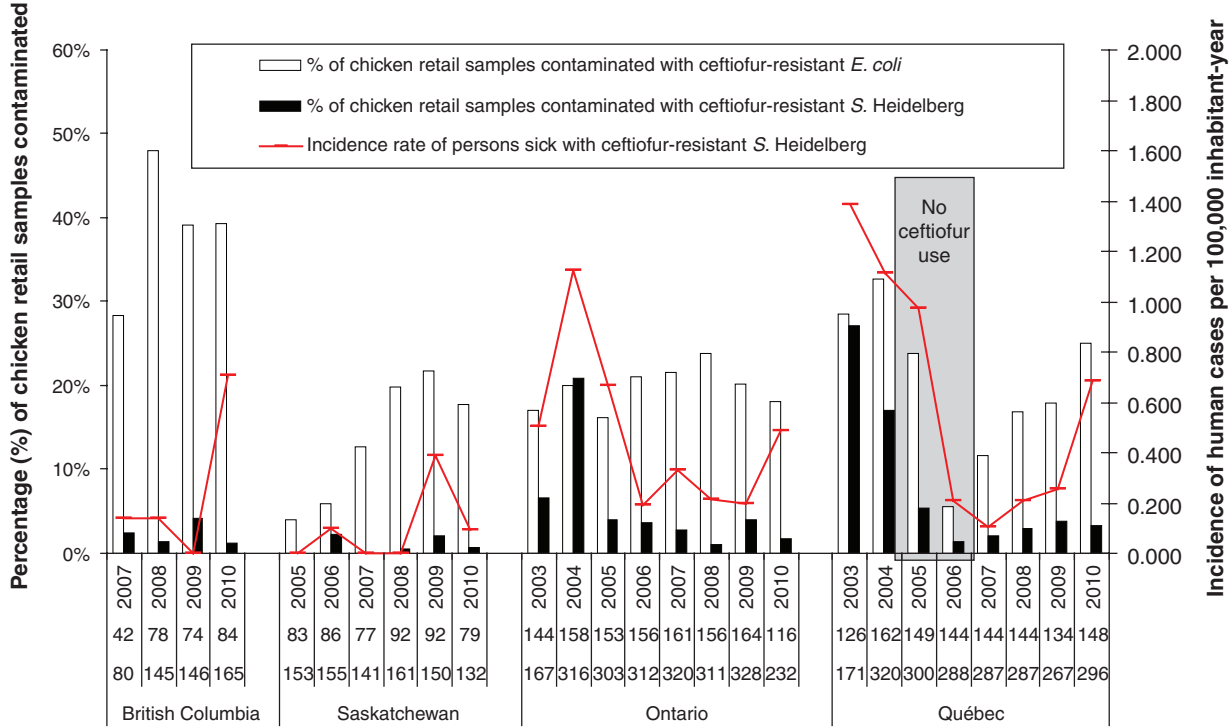


FIGURE A4-4 Ceftiofur resistance in *E. coli* from retail chicken and *S. Heidelberg* from retail chicken and humans, CIPARS 2003-2010. SOURCE: CIPARS (2003, 2004).

Conclusions and Key Policy Implications

The global, transdisciplinary, multiscalar, and multijurisdictional nature of AMR and enteric disease highlights the utility of the One Health approach in framing these health issues. One Health principles encourage public health practitioners to engage and collaborate with stakeholders and to consider the numerous socioeconomic, geopolitical, zoonotic, and environmental factors involved in health issues (Figure A4-2). Veterinarians and physicians as well as other human, animal, and ecosystem health professionals have important roles to play in preserving the efficacy of our antimicrobials through leadership roles in disease surveillance, AMU decision making, and health management decisions to prevent disease. Communication and collaboration with farms, industry, veterinarians, physicians, and other public health practitioners must be strengthened and is emphasized as key to the success of the approach to AMR and enteric disease.

C-EnterNet and CIPARS have successfully operated for 7 and 10 years, respectively. A large part of this success and the sustainability of these programs can be attributed to ongoing collaborations with multiple stakeholders and the flexibility of all the partners to adapt to changing needs and conditions. These programs serve as a model for how government agencies can address, in an integrated fashion, urgent problems and issues that cut across multiple departments and jurisdictions.

Acknowledgments

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A5

OVERVIEW OF THE GLOBAL FOOD SYSTEM: CHANGES OVER TIME/SPACE AND LESSONS FOR FUTURE FOOD SAFETY

Will Hueston^{10,11} and *Anni McLeod*¹⁰

Food systems emerged with the dawn of civilization when agriculture, including the domestication of animals, set the stage for permanent settlements. Inhabitants could grow more crops and raise more animals than necessary to feed those who tended them. This changed human culture; unlike earlier hunter-gatherers, agriculturalists did not need to be in constant motion to find new sources of food. Cultivating grain allowed for drying and storage of some of the harvest for later consumption. Different grain cultures emerged in each of the cradles of civilization: maize in Mexico, rice in China, and wheat and barley in the Middle East. The ability to produce a surplus of grain also set the stage for the development of art, religion, and government.

Since agriculture began, food systems have constantly evolved, each change bringing new advantages and challenges and ever-greater diversity and complexity. This paper looks backward to the drivers of change and forward to the challenges faced by producers, consumers, and policy makers of tomorrow.

Changes Over Time and Space

The emergence of city-states has been a major driver of food system changes, bringing together large populations within defined boundaries and requiring complex governance to deliver sufficient quantities and quality of food. Advances in food storage, with sealed containers and curing methods, the use of animal transport, sailing ships, and trains to move larger volume than can be carried by individuals; trade in ingredients like salt as well as live animals and agricultural products; and increasing political and military conflict for resources all have been developments of the city-state. Extensive trading routes have existed for salt, spices, tea, and pepper for thousands of years.

The Iron Age and the Roman Empire brought expanding empires and the beginning of global food systems, including regional specialization in products traded throughout empires. Food systems began to be organized on a grand scale to feed larger cities and fuel local economies. Trade networks for grain, nuts, oils, fruit, and wine developed using both road systems and sailing routes. Standardized weights and measures were established along with the expansion of money and accounting.

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The Middle Ages saw the emergence of the merchant class and banknotes. Prior to the Middle Ages, selling was considered a task for one of the lower classes of civilization, if not a sin. The *equestrii* in Roman times did the trading, not the citizens of Rome. The Middle Ages also saw banknotes replacing coinage, first with the Song dynasty in China and then later in Europe around 1661. As a wealthy class emerged, they became more sophisticated in their food preferences. The resulting demand of consumers began to affect trade in addition to supply.

Science and technology represent another major driver, changing the way that food is grown, processed, preserved, and transported. The Industrial Age brought a transition from manual labor and draft animal-based economies to machines. Further increases in agricultural productivity brought about by technology such as the seed drill, the iron plow, and the threshing machine freed up labor for the factories in the 1700s. The Industrial Revolution also created per-capita income growth. The emerging middle class had discretionary income to spend on its food preferences. Transportation breakthroughs were ushered in during the industrial age: canal systems, improved roadways, steam engines used for traction, railroads, and steamships. The Erie Canal, as an example, connected the Great Lakes and the northeastern United States with 363 miles of inland waterways by 1825.

Food preservation, important to both storage and transport of food, also changed over time. Drying was one of the early food preservation methods, certainly known in ancient times. Fermentation also was an early method of food preservation, with pasteurization applied to wine in China as early as 1117. Salting of food has been used for at least 500 years, beginning when the fishing fleets from Europe used drying and salting to store fish caught in Newfoundland and the Grand Banks in order to get them back to consumers in Europe.

Two preservation methods, canning and freezing, allowed food to be stored and transported in an almost-fresh state. Canning grew out of military research in 1810. Ice storage was developed in northern climates where ice could be cut from lakes in the winter for use later in the year. Commercial refrigeration followed in the 1800s. The first refrigerated ship, the SS Dunedin in 1882, revolutionized the meat and dairy industries in Australia and New Zealand. Refrigerated and frozen food products now could be traded globally.

The 20th century saw intensification of agricultural production with mechanization of planting and harvesting, selective breeding of animals and plants, and more attention to animal nutrition and feed input costs. Increased scale of production drove down the per-unit cost of products and fostered greater specialization in food systems. Advances in plant and animal disease control also helped, such as the movement of pigs and poultry indoors to decrease disease exposure and to enhance efficiency by controlling the environment.

Colonization and war have been important political influences on food systems, the first creating distributed ownership of food systems and the second highlighting a need for global agreements. Colonialism allowed for population growth of the industrialized countries when there were limited domestic opportunities to create employment or to grow food. Settler colonies captured market opportunities for the colonizing country's exports and provided import sources for raw materials, including food and food ingredients.

Trade underwent dramatic changes in the 20th century as a result of the two world wars. The war-associated food shortages, economic crises, and disease spread set the stage for global trade agreements and organizations designed to address global public good issues. The 1947 General Agreement on Tariffs and Trade was created to reduce tariff-based trade barriers and to prevent the downward spiral of world trade seen in the Great Depression from 1929 to 1933. Monthly trade dropped from \$3.0 billion in January 1929 to \$0.9 billion in March 1933 as protectionist measures reduced trade worldwide (Personal communication, Christiane Wolff, World Trade Organization, March 2012).

Supply-driven to demand-driven Until the 20th century many countries had supply-driven economies, where policies favored increased agricultural production to ensure adequate domestic supplies of basic feedstuffs. Increasing the supply and reducing the costs of food were politically popular national priorities. Food self-sufficiency was a powerful motivation, especially for countries that had experienced food shortages in the past. Countries that exceeded domestic demand used export markets and food aid programs to deal with the excess.

Rising discretionary incomes in Europe and North America in the 20th century impacted food demand and global food trade. Rising consumer demand for chicken drove the development of the broiler industry, but, as marketing moved from whole birds to parts such as leg quarters or breasts, demand disequilibrium resulted. For example, many Americans prefer white meat and do not eat chicken feet, while in other parts of the world people prefer dark meat and consider chicken feet a delicacy. Global food trade provided an opportunity to sell the parts of animals for which there is little or no domestic demand. One reason that the developed world enjoys relatively inexpensive food is the ability to market commodities and specialized products worldwide.

Food systems are dynamic and ever changing in response to natural forces (e.g., weather), demographics (e.g., emergence of megacities), economics (e.g., currency values), technological advances in processing (e.g., high pressure pasteurization), entrepreneurship (e.g., development and marketing of new products), and consumer preferences (e.g., locavores). Every country in the world produces some of its own food and trades food. As a result of these constant changes, food systems are increasingly complex, as adding to the challenge of assuring global food safety.

The Complexity of Current Global Food Systems and Implications for Food Safety

Today's food systems are diverse and complex, involving everything from subsistence farming to multinational food companies. Everyone eats; therefore, everyone relies on food systems, local and global. The movement of food and food ingredients in food systems includes animals and animal products, plants and plant products, minerals, and vitamins. The classic cheeseburger provides an excellent example of the complexity of today's supply chain. Researchers at the University of Minnesota mapped the global supply chain of the cheeseburger working with a large quick-service restaurant chain, Figures A5-1, A5-2, and A5-3 tell the story. Figure A5-1 demonstrates graphically the movement of different commodities from the farm through processing to the restaurant. Figure A5-2 lists all the ingredients found in this company's cheeseburgers and Figure A5-3 provides an idea of the variety of companies supplying key ingredients like vinegar, garlic powder, tomatoes, beef, and wheat gluten. Each cheeseburger includes more than 50 ingredients sourced from countries in every continent of the world except the Arctic.



FIGURE A5-1 Global supply chain complexity. Movement of commodities.
SOURCE: Shaun Kennedy, Director, National Center for Food Protection and Defense, University of Minnesota.

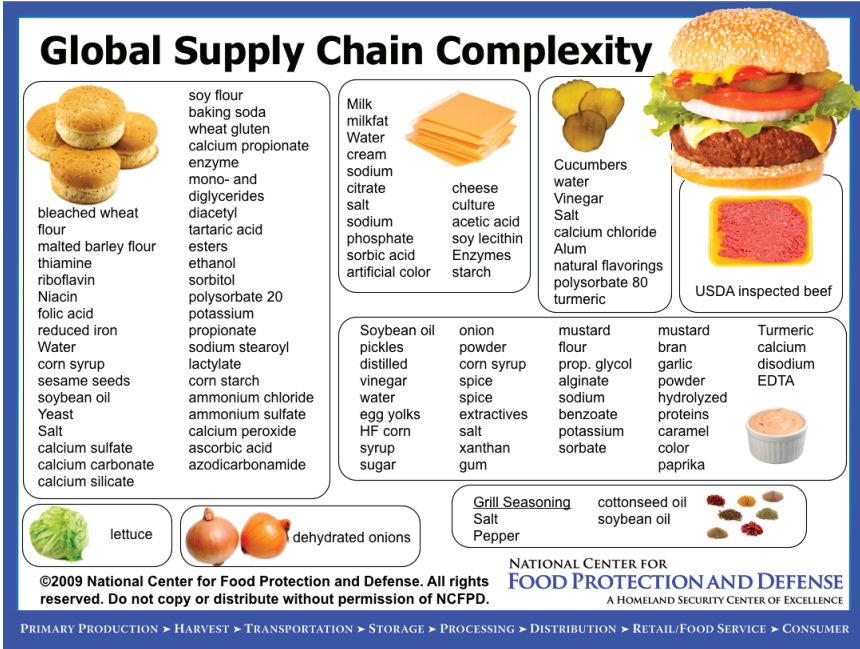


FIGURE A5-2 Global supply chain complexity. Ingredient list.

SOURCE: Shaun Kennedy, Director, National Center for Food Protection and Defense, University of Minnesota.

Food processing supplies also move globally and include processing equipment, packaging, and chemicals such as disinfectants and preservatives. Agricultural inputs move too, from feed to fertilizer, to vaccines and pharmaceuticals, to planting and harvesting equipment. As agricultural commodities are combined with other food ingredients to create processed foods, individual food items commonly include ingredients from multiple countries. The increasing consumer demand for “ready-to-eat” foods has fueled the growth of quick service restaurants and fully cooked, frozen dishes that only require reheating, further expanding supply chains. Government regulatory systems and private-sector initiatives are part of food systems, as are educational efforts and consumer actions.

Food systems are integrally related to food safety. Contamination can occur at any point in the food system, and prevention and control strategies can be implemented at any point. The scale and complexities of today’s food systems contribute to the likelihood and magnitude of food-borne illness (Ercsey-Ravasz et al., 2012). The more complex, the more opportunities for things to go wrong; the larger the scale, the more people are potentially affected.

Complex food systems each involve interconnected subsystems that, taken together, exhibit properties that are not predictable by the properties of the indi-

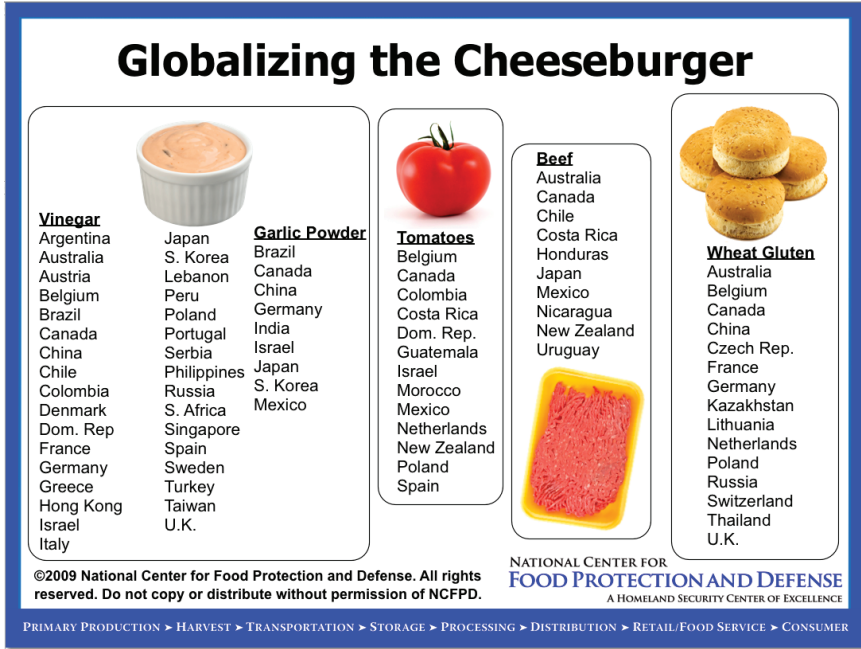


FIGURE A5-3 Globalizing the cheeseburger.

SOURCE: Shaun Kennedy, Director, National Center for Food Protection and Defense, University of Minnesota.

vidual subsystems or their parts. Food systems can be called complex adaptive systems. These have no boundaries; individual actions affect the food systems by what individuals produce and what they purchase. Complex adaptive systems have a memory. While food systems change over time, present behavior is affected by prior behavior. Food systems are nonlinear. A small perturbation in some part of the system may have a large effect, a proportional effect, or no effect. And the relationships of this system of systems have feedback loops. The adaptiveness and nonlinearity of food systems mean that food safety problems are also nonlinear; they can be anticipated but are hard to predict with accuracy or precision.

Feeding the world requires a multitude of systems. Each system is dynamic and the food systems are interdependent; there is no one best system that meets all needs. However, every success in improving the food system perturbs the whole system of systems and changes the nature of the food safety problems.

Lessons for the Future

Looking at existing global food systems and predicated demands for food, we can reasonably speculate the following over the next 10 to 20 years:

1. *Food systems will continue to change, although with additional drivers.* The drivers of urbanization, production and processing technology, transport technology, and political forces that have played a large part in shaping current food systems will continue to be relevant. Newer drivers playing an increasingly important part are a real prospect of a global population of 10 billion, aging populations changing the production and consumption base, climate change leading to constraints on water supplies, severe constraints on nonrenewable energy, and communication technology.
2. *Food systems will continue to shift from being supply driven to being demand driven.* The global quick service restaurant chains like McDonalds and big-box retailers like Walmart have had an enormous impact on food systems. Consumer groups demanding safety, fair trade, “green” production, and animal welfare-related changes in production practices put pressure on policy makers and retailers. The large processors are putting pressure on the primary producers of plants and animals for assurances on source, on identity preservation, on means of production, and on characteristics like animal welfare and labor standards.
3. *Increasing prominence of private standards.* Successful completion of the Uruguay Round of the multinational trade negotiations under the framework of the General Agreement on Tariffs and Trade included approval of the Sanitary and Phytosanitary Agreement (SPS) in 1995 under a new organization, the World Trade Organization (WTO). The SPS established a framework for international standards for trade in animals, plants, and the products derived from them including food. More recently, coalitions of companies are forming to standardize specifications for food products, basically saying, “we can’t wait for the slow process of international standards organizations.” An example is the Global Food Safety Initiative, a nonprofit organization that benchmarks guidelines established by food processors, retail, and food service against the international standards recognized by WTO. Food safety standards used by the large companies who target premium market niches are often above and ahead of the minimum demanded by legislation.
4. *Panarchy.* The term “panarchy” is used in systems theory to describe systems interlinked in continual adaptive cycles of growth, restructuring, and renewal (Gunderson and Holling, 2001). The increased growth in connectedness and efficiency results in a lack of redundancy and at the same time makes individual food systems less resilient, more sensitive to stress, and therefore more susceptible to collapse. If subsystems within complex food systems collapse, the result is systems with greater resiliency that have fewer connections and less efficiency. And the cycle starts again.

Food systems have demonstrated adaptive cycles as they have evolved. Many current food systems have evolved to a point where they are both

complex and sensitive to stress, and the results of a collapse in a subsystem can be wide-reaching. For example, the concentration of production of an ingredient like a vitamin in a single company or country may be the most efficient approach, but if a production problem ensues or a disaster disrupts this supply chain, then all food processors using this vitamin as a food ingredient are affected. They must either remove the vitamin from their recipes or stop production because of lack of supply. Another example is the proliferation of “just-in-time” supply chains. Instead of stockpiling food supplies in warehouses, many large food retailers and food services have worked with food manufacturers to establish these supply chains. Real-time data on usage and inventories are provided directly to the supplier on a regular basis to allow for customized shipments of only those food products needed. If the supply chain is disrupted, there is very little food in reserve. Many cities have less than 2 days’ supply of perishable food like milk and eggs on the shelves at retail outlets. People in countries where systems regularly collapse have coping strategies: they store food, water, and alternative energy at home. Many of those in large modern cities do not. The urban poor have neither the finances nor the storage facilities to store reserves of food.

5. *Culture clash.* Disconnects exist between origination and destination countries because of differences in their cultures and differing levels of economic development. While developed countries have emphasized the importance of food safety and quality, less-developed countries may focus on the opportunity for exports to generate foreign currency reserves. The recent melamine incidents demonstrate economic adulteration in order to achieve greater profit in domestic and international markets.

What Do One Health Approaches Have to Offer Food Safety in the Context of Food Systems?

Food safety is a “wicked problem.” We cannot completely understand the challenge; it is too complex. And yet food safety is compelling: people are getting sick and dying every day as a result of unsafe food and water. We must take action, and we recognize that every action we take perturbs the very food systems we are working to improve. The so-called wicked problem reflects the condition of a complex adaptive system.

If One Health is taken to imply holistic and multidisciplinary approaches to complex challenges (e.g., wicked problems), then a One Health approach offers the possibility of new perspectives on safety in food systems and new ways of working. It implies systems thinking, shared leadership, a holistic view, and a multifaceted approach.

Is this back to the future? The World Health Organization (WHO) definition of health in 1948 was quite broad: “Health is a state of complete physical,

social, and mental well-being, and not merely the absence of disease or infirmity.” However, the public health implementation of food safety focus often is limited to prevention and response to infectious diseases rather than a more holistic approach to food safety as an element of food security (availability, access, and nutrition as well as safety). More recently, the Food and Agriculture Organization and the WHO have developed a much broader definition of food safety: “All the conditions and measures necessary during production, processing, storage, distribution, and preparation of food to ensure that it is safe, sound, wholesome, and fit for human consumption.”

Successfully applying One Health approaches to food safety requires a *sound understanding of the dynamics of food systems*. Food safety must be addressed in a systemic manner rather than an ad hoc approach driven by reaction to crises. These One Health approaches have implications for what we record, measure, and analyze in food systems and how we share information about potential food safety problems as well as existing crises.

One Health approaches also require a *new leadership model* that is adaptive and shared, matching the adaptive nature of food systems and the many ways they are controlled and influenced. Five skill sets for adaptive leaders were identified by a small international working group at a session in Bellagio, Italy, sponsored by the Rockefeller Foundation: communications; getting things done and accomplishing change; working across boundaries, whether disciplinary, sectoral, or political; influence; and vision and strategy.

Applying these skills sets encourages a *move from finger-pointing to shared leadership*. It provides space to accept the fact that food-borne disease happens and will happen. Food safety programs are not always somebody’s fault. After all, “safe food” is an oxymoron. All food has risks and yet “safe” implies the absence of risk. Food systems can either contribute to the risks or be designed to help manage the risks. The very complexity of food systems also means that an infinite number of risk-management strategies are available, if we are only creative enough.

Incremental progress on complex food safety problems may also require a *new model of partnership* that engages producers and the food industry along with government. We do not have an ideal model for partnership or shared leadership, but several initiatives in fisheries and foods are trying to find or build models, and so are others outside of the food sector. A new partnership model would include a *value proposition to engage industry* (examples are beginning to emerge around agriculture and environment, where there is no alternative but for government and the private sector to work together) and a *more flexible and realistic regulatory system*. The idea of zero tolerance makes no scientific sense (zero risk is unachievable) and contributes to the very high levels of waste in U.S. food supply chains (e.g., supermarkets in the United Kingdom are moving to changes in the “use by” label to provide more flexibility in home-freezing, which is anticipated to reduce waste in kitchens with no reduction in food safety).

What Comes Next?

We have proposed a One Health approach that would match the complex, adaptive problems of food safety with shared, adaptive, and holistic problem solving that considers the entire food system. However, an approach is of little use while it remains on paper. The next challenge is to find a complex, subtle, pervasive, and wide-ranging food safety problem that will require adaptive leadership, partnerships, and a wide scope of action—the problem of mycotoxins is excellent example—and put the food systems community to work on it.

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A6

THE AUSTRALIAN PERSPECTIVE, THE BIOSECURITY CONTINUUM FROM PREBORDER, TO BORDER AND POSTBORDER

*Martyn Jeggo*¹²

Executive Summary

Biosecurity is of considerable importance to Australia and managing biosecurity risks through a One Health approach offers many attractive advantages. To date most of the international effort has been focused on adopting a One Health approach from the perspective of infectious diseases and the need to bring together multidisciplinary teams to most effectively understand and mitigate the risks. Central to understanding the skills and knowledge that are required is an appreciation that many recent outbreaks of infectious diseases arise in wildlife, create disease in livestock, and subsequently go on to cause infection in humans. While the drivers for this emergence are still not fully elucidated, a number of key factors play a part, including climate change.

While there are clear differences between the approaches to food safety versus infectious disease management, there is still the basic gain to be made by attacking the risks through reducing likelihood rather than addressing the consequences. This key concept underpins the approach undertaken in Australia,

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where biosecurity activities, preborder, at the border, and postborder, focus on early detection and rapid response. While the approach recognizes the continuum from pre- to postborder, resource allocation is currently being reviewed to ensure an appropriate balance for effective risk mitigation.

Underpinning the Australian biosecurity strategy is the recognition of the value of a One Health, multidisciplinary approach. There is a current awareness that much needs to be done to ensure that the maximum value is achieved from this approach and that a “business-as-usual” mentality does not prevail. Fortunately for Australia, the recent management of Hendra outbreaks in Queensland and New South Wales has provided an excellent example of the gains that can be made through a One Health approach. Similar examples need to be developed in the food safety arena.

Introduction

Biosecurity is the protection of the economy, the environment, social amenity, or human health from the negative impacts associated with the entry, establishment, or spread of animal or plant, pests and diseases, or invasive plant and animal species (Beale et al., 2008). Australia has an enviable biosecurity position having been free of many of the infectious diseases that infect livestock in most other parts of the world. Built on the “island status,” Australia has for many years maintained a stringent import policy around plants, livestock, and agricultural products to ensure the protection of this status. Australia has consistently adopted a precautionary policy, although international trade regulations (OIE, 2011) attempt to ensure that fair trading practices exist in the international agricultural marketplace. Notwithstanding this, the risks continue to increase and disease outbreaks are an unfortunate regular event. Recognizing this, the focus remains on early detection linked to a rapid and effective response. Eradication is the preferred option but not always achievable, particularly in the plant sector. Here a policy of containment is adopted that seeks to limit spread and reduce the impact on both productivity and the environment.

A number of frameworks have been developed to better enable the Australian biosecurity strategy. These include not-for-profit companies providing a framework for industry and government to work in partnership, such as Animal Health Australia (AHA, 2011a) and Plant Health Australia, agreed on plans for how to deal with outbreaks and agreed on processes for who will pay for what in the face of a major disease incursion (AHA, 2011b). Mostly developed for the livestock sector, this approach is now being applied to both the plant and environmental sectors.

There is a growing appreciation that the risks being addressed now encompass environment and human health as well as animals and plants. In order to effectively manage these risks, a One Health approach has much to offer.

The One Health Concept

One Health as a concept emerged some 10 years ago and has gained increasing acceptance as a process for addressing a range of issues involving environmental, animal, and human health (Leboeuf, 2011). Although there are many definitions of One Health, the current focus remains around emerging infectious disease (EID) and recognizes that 75 percent of EID in humans arise from animals, and in large part, from wildlife, often spilling over first into domestic livestock and then infecting humans (Woolhouse and Gowtage-Sequeria, 2005). This also includes the emergence of diseases affecting food safety and food security. A full understanding of these processes and the development of mitigating strategies to reduce the threats from EIDs will require input and engagements from people with a diverse set of skills and a range of disciplines (Vallet, 2009).

The emergence of disease requires an interaction between the pathogen, the host, and the environment. Understanding these interactions and developing effective mitigation strategies requires a complex of One Health disciplines. In the case of pathogen influences these involve such areas as quasispecies variation, genetic recombination, host/vector adaptation, tissue tropism, virulence determinants, and latency or persistence. For host influences it is necessary to understand reservoir host spillover, the range of intermediary hosts, various aspects of vector competence, the susceptible host range, the pathogenesis of the disease in different hosts, and the potential range of immune responses. In looking at the impact of anthropogenic influences it is important to appreciate the broader issues of globalization, urbanization, land-use changes, cultural changes, and regional and global conflicts. Finally in terms of geophysical influences, climate change and variability link to extreme weather events are critical (Cutler et al., 2010; Rushton, 2009; Wolfe et al., 2007).

The One Health approach strives to bring these many sciences and disciplines together to provide the best possible solution to health risk management. Presently much is being done at both the national and international levels to create effective One Health partnerships with the first One Health International Congress being held in 2011 in Melbourne, Australia (*Ecohealth*, 2011). Despite these efforts, few examples exist of real success, and it may require more drastic organizational changes to achieve the cultural changes needed to deliver the anticipated value and impact from a One Health approach.

Infectious Diseases Versus Food Safety

Ensuring the safety and quality of Australian foods within an integrated national biosecurity system is a current challenge for Australia. Although much has been done on characterization of food-borne hazards, on analysis of through-chain risks and the continual development of innovative risk management strategies, the approach is principally post-farm gate. In Australia these differences in infectious disease management versus food safety (Table A6-1) highlight areas

TABLE A6-1 Major Differences to Risk Management of Infectious Diseases Versus Those Associated with Food Safety Issues

Infectious Diseases	Food Safety
Focus on effects of disease on host	Focus on impacts on humans
Includes risk prevention as well as response (e.g., mainly on farm)	Focus on post-farm gate response (but clearly changing)
Looks also at treatment in host (e.g., vaccination)	Looks at treatment of risk product
Driven by government and primary product producers (e.g., farmers)	Driven by product processors and retailers
Historically major outbreaks have driven change	Large events unusual and more about consumer impact

for a rethink and to consider how these two sectors can learn from each other. Central to this will be the application of the One Health principles.

Biosecurity Risk Management and the Biosecurity Continuum

The process of risk management for infectious diseases is concisely documented by the World Animal Health Organization (OIE) (Williberg, 2011) but can be simply considered in terms of the likelihood of a hazard occurring and the consequences if such an event did happen. In considering the likelihood, how the disease spreads and the survival of the pathogen are major components, but for the newer emerging infectious diseases understanding emergence and host switching are critical issues. Indeed, as an appreciation is gained of the emergence of pathogens from a wildlife reservoir into a livestock species and the subsequent potential to cause disease in humans, it becomes crucial to better understand those drivers that lead to a host switch.

On the consequence side of the risk profile, it is important to not only understand the process of disease in the affected host but also to appreciate this in terms of production and trade losses and the potential risks to humans and the environment. This expanded perception of the impact of disease lends even further credence to the concept of a multidisciplinary or One Health approach in managing effectively these consequences.

Australia has studied carefully the most effective approach to managing the risks from infectious disease and has come to the clear conclusion that the greatest return on investment lies in prevention and eradication rather than containment and allowing endemicity (see Figure A6-1).

It thus concludes that it is necessary to understand the risks for emergence and to tackle these directly to reduce or eliminate these risks. This approach, however, will never be 100 percent effective, and thus some resources will need

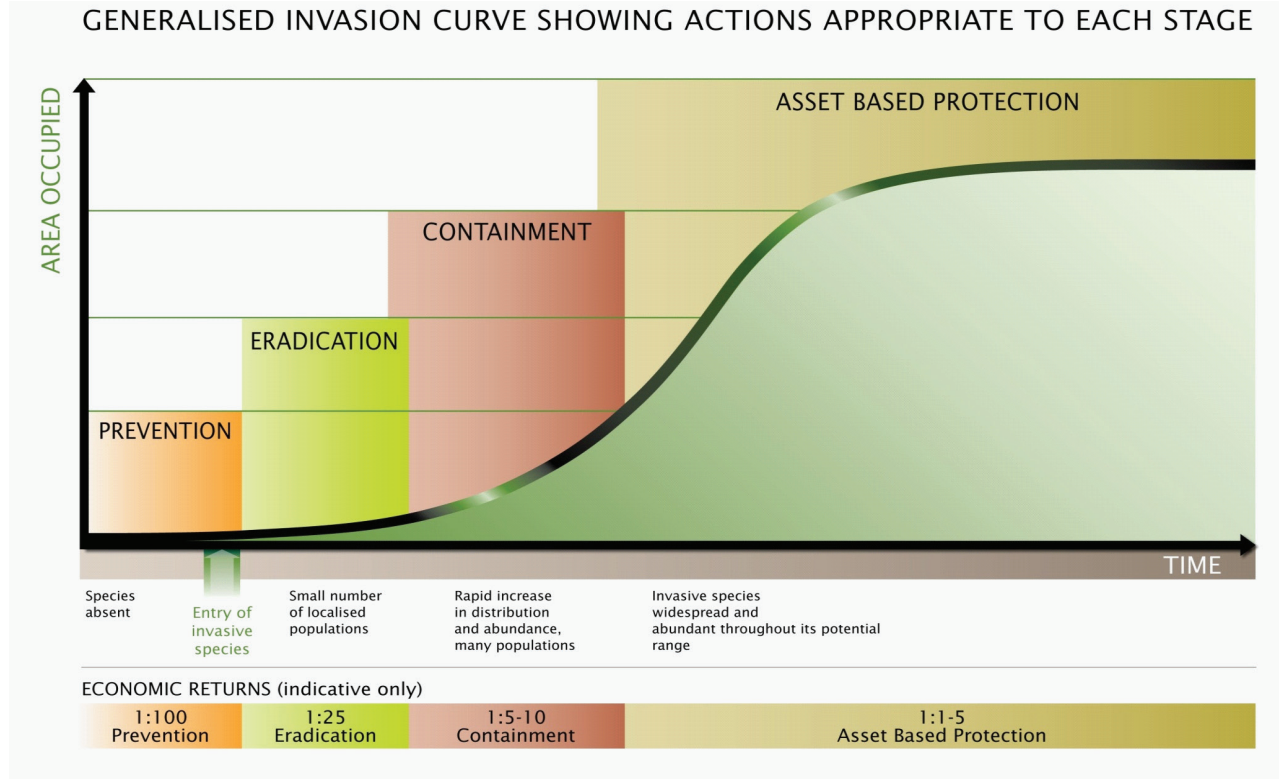


FIGURE A6-1 When to act: Generalized invasion curve showing actions appropriate to each stage.
SOURCE: © State of Victoria Department of Primary Industries, 2009, and reproduced with their kind permission.

to be allocated to consequence management but at a level that appreciates the lower risk if likelihood is significantly reduced.

In considering emerging risks, the movement of people to urbanized areas and the intensification of agriculture, often in close proximity to these urban areas, have clearly changed the risk profile in terms of both opportunities for the pathogen as well as the likely outcome of an infection. This risk is then exacerbated by the significant increased movement of people and products between these urbanized areas both nationally and internationally. There is little doubt that, overlying these issues, climate change has the potential to have a significant impact both directly through a change, for example, in available diseases carrying vectors and survival of the pathogen, but also in terms of change patterns of habitat and feeding by reservoir hosts (Rosenthal, 2009).

Australia has focused efforts for many years at the border and preborder areas in order to best manage the likelihood risks, to ensure detection as early as possible and thus a response that has the best chance to enable eradication. More recently and following a significant review of national biosecurity, an enhancement of postborder activities and the concept of the biosecurity continuum have emerged. To best manage this continuum it has been agreed between the Australian governments (Commonwealth, states, and territories), that the Commonwealth government will focus and take responsibilities for preborder and border activities, with states and territories managing the majority of postborder activities. In recent times, the concept of a significant contribution from industry has emerged and, while government will retain primary responsibility for policy and standard setting, operational activities will in the future likely involve both government and industry working together in implementation of biosecurity activities.

Preborder Activities

It should be recognized from the outset, that although Australia is an island and this has been a significant advantage in maintaining a disease-free status, neighboring countries are in close proximity to Australia (via the Torres Strait) and the huge increase in international travel and trade considerably reduce the “safety factor” of being an island. It is therefore necessary to continually assess the threats from “abroad” and consider these threats in terms of market access and trade. There is a permanent pressure to broaden the trade in agricultural products, with an increase in demand to import from areas with a very different disease status to Australia. Managing these risks requires not only understanding the disease status of trading partners but also influencing the international regulations that govern such trade. Although continual risk analysis is a prerequisite for pre-border activities, threat reduction through a range of activities is also a major component. This starts with building trust and partnerships and has to grow into on-the-ground capacity-building support programs that

assist many countries, particularly in the Asian region, to better manage their own biosecurity programs.

A clear example of the need to reduce risk is that of the support by Australia to countries in the region to control foot and mouth disease (FMD). FMD represents the biggest risk to the Australian livestock industries, and for many years FMD was endemic in most countries in the region. Assistance to initially Indonesia and subsequently to the Southeast Asia FMD control program has considerably reduced this risk through both eradication and effective control in many countries in this region. Building an increased capacity in the region for countries to better manage their own biosecurity leads to a clear reduction of the likelihood risk of disease occurring in Australia. Various programs of the Australian Agency for International Development and the Australian Centre for International Agricultural Research focus in this area.

Border Activities

Australia's Biosecurity Quarantine Operations Division manages those activities at the border that provide quarantine controls to minimize the risks of exotic pests and diseases entering the country. A further activity is the inspection of import and export certification to help retain Australia's highly favorable health status and wide access to overseas markets. These inspections are targeted at activities involving aircraft, ships, and cargo and include the management of the National Australian Quarantine Strategy (NAQS).

Given the nature of the coastline of Australia, a large number of maritime programs are undertaken to ensure the effectiveness of these border operations. These include the management of unauthorized maritime arrivals, marine pollution, illegal activities in protected areas, issue around piracy, robbery or violence at sea, the illegal exploitation of natural marine resources, and maritime terrorism. Linked to this is the availability of a range of response assets including military naval vessels.

NAQS supports the government's broader biosecurity objectives through conducting the monitoring and surveillance for exotic plants and animal disease across the north of Australia from Cairns to Broome and including the Torres Strait. These activities recognize the remote location of this region, the low human population, and the close proximity of neighboring countries and extend to collaborative surveillance and capacity building in Papua New Guinea, Indonesia, and Timor Leste, along with other neighboring countries. The overall strategy is clearly focused on early detection in a high-risk area, linked to an ability to mount an early response.

Postborder Activities

A wide range of activities are conducted postborder, principally through the governments of the states and territories. These aim for the early detection

of an emerging or exotic disease or disease-causing agent; the demonstration of freedom from a disease or disease-causing agent for trade purposes; the detection of changes in the distribution, prevalence, and incidence of a disease or disease-causing agent; and finally the detection of changes in factors or events that influence the risk of disease.

Increasingly a range of sophisticated geographical information systems and genetic-based tools have been used to better understand host and population structures with molecular epidemiology being used to understand the distribution of pathogens.

For the most part these activities have been targeted (or active) in nature with clear resource allocations and deliverables. Background (or passive) surveillance has been a lower priority for some time. The recent formation of a National Animal Health Surveillance System has recognized the critical component of passive surveillance in the overall approach, and increased activities in this area will be part of the future.

Conclusions and Discussions

- A One Health approach is essential to effectively managing the risks associated with both food safety and infectious diseases. Bringing together two necessary disciplines, skills and knowledge, is a real challenge given the current separation of management of environment, human, animal, and plant health. This may require real organizational change at the national level to achieve a genuine multidisciplinary and One Health approach.
- There are many similarities but some important differences between the management of food safety versus infectious disease. Having a whole systems approach (from farm to fork) has much to offer, and there are now real examples of success (e.g., control of salmonellosis in poultry in Denmark).
- Biosecurity (in the Australian concept) looks after both areas but remains somewhat fragmented. Currently biosecurity encompasses agricultural health (plants and animals) and environmental health; human health management remains outside of these activities except in exceptional cases (e.g., influenza management). A true One Health approach is perhaps some way off. The recent management of Hendra virus outbreaks, however, has clearly demonstrated the value of a One Health approach.
- Biosecurity is managed as a continuum from preborder to border and postborder activities.
- In managing biosecurity risks, investments in likelihood considerably outweigh consequence management (but the latter cannot be ignored). Within this framework resource allocation across the preborder, border, and postborder need to be continually reassessed and currently in Australia there is an agreed-upon need to greater invest in postborder activities.

- Much still needs to be done to achieve a genuine One Health approach. Although progress is being made, real gain may require some fundamental changes in thinking and even reorganization at both the national and international levels with the creation of departments and divisions of One Health.
- Training and education in One Health is currently crucial in driving the longer-term cultures necessary to sustain a One Health approach. This needs to be linked to clear examples of success and the added value of a One Health approach.

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A7

FOOD SAFETY: A VIEW FROM THE WILD SIDE

*William B. Karesh, Elizabeth Loh, Catherine Machalaba*¹³

Food-borne illnesses pose a serious threat to public health with growing economic and international trade ramifications. Past outbreaks of food-borne diseases have largely been viewed only through the lens of public health; yet food-borne illnesses are closely associated with the link between human and animal populations, and with the surrounding environment. For example, in 2006, approximately 200 people in 26 states were diagnosed with a particularly virulent strain of *E.coli* O157:H7 found in spinach. Viewed only from a human health perspective, our knowledge of this outbreak would have extended only to morbidity, mortality, outbreak investigation, laboratory diagnosis, and clinical treatment. However, once viewed through the lens of animal health and ecology, the *E. coli* O157:H7 isolates that caused the human deaths and serious illnesses related to spinach were also found in wild pig feces, the feces of several cows, and in a stream on one of the four spinach farms in the area (Warnert, 2007). Thus, a One Health perspective integrating our knowledge of the environment and ecology, in addition to human and animal health, was required to fully investigate and understand this outbreak and has great utility in the food-borne illness discussion.

Food-borne pathogens from wildlife span the taxonomic spectrum from helminthes to viruses (Figure A7-1). While the number of food-borne transmitted emerging disease events due to viruses is fewer than the number due to other groups of pathogens, some such as severe acute respiratory syndrome (SARS) have resulted in devastating consequences. As noted by Tauxe, the vast majority of food-borne illnesses in the United States due to known pathogens have emerged in the past two decades and an even larger percentage are due to yet-to-be-identified pathogens (Tauxe, 2002). Many of the known food-borne pathogens are zoonotic (Figure A7-2), and many may be linked to wildlife. As seen more generally with emerging infectious diseases (Jones et al., 2008), it is rational to assume that future outbreaks of new food-borne illnesses may be linked to wildlife.

From our review of the peer-reviewed literature, the main drivers of wildlife-related zoonotic disease emergence include land-use change and food industry changes. For food-borne disease, food industry changes, human susceptibility (reduced immune function), travel, and antimicrobial resistance were the primary drivers of past emergence events (Figure A7-3).

With more than 70 percent of food-borne EID events being zoonotic in nature (Figure A7-2), contact with or contamination of food or food ingredients

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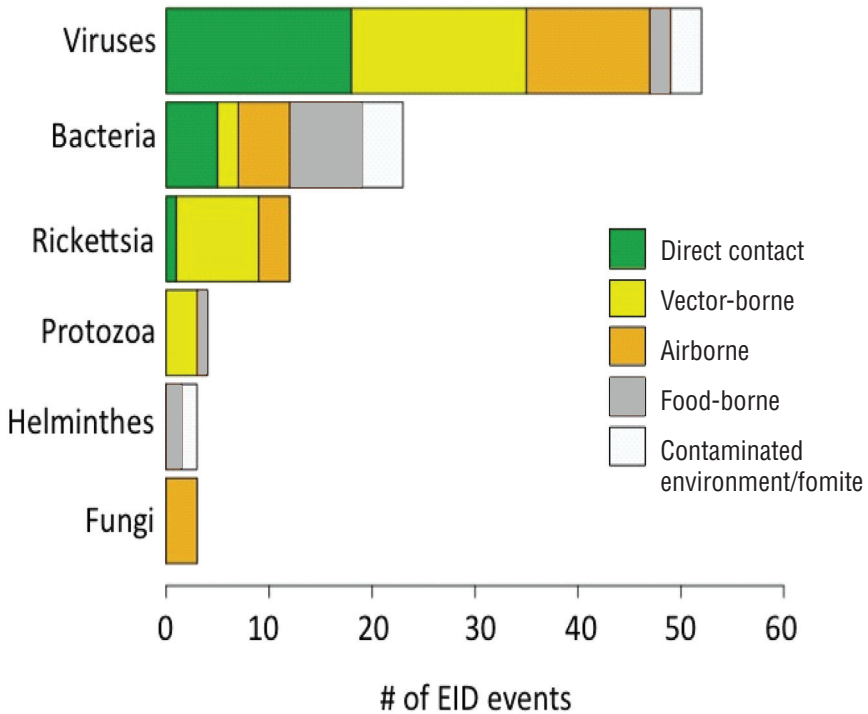


FIGURE A7-1 The number of infectious disease events that emerged from wildlife between 1940 and 2004 as published by Jones et al. (2008). These events ($n = 96$) are broken down by pathogen type, and each event is defined as the first emergence of a given pathogen. The colored areas within each bar depict the different transmission pathways as a percentage of the total number of emerging infectious disease (EID) events. For example, between 1940 and 2004, 52 zoonotic EIDs from wildlife were viruses. Of these events, 34.5 percent of these pathogens are transmitted by direct contact, 32.7 percent are vector-borne, 23.6 percent are air-borne, 5.5 percent are transmitted through contact with a contaminated environment or fomite, and 3.7 percent are food-borne.

by wild animals creates a serious potential for disease transmission. Commonly, both wild and domestic animals are implicated as sources of food contamination (Beuchat and Ryu, 1997; Cima, 2012; Doyle and Erickson, 2008; Gorski et al., 2011; Newell et al., 2010). Yet, definitive identification of a specific source animal or species is rare, particularly with wild species because they are typically no longer present at the time food-borne illness is detected in humans and investigations leading back to farms or processing plants are initiated. Despite the rarity of finding the “smoking gun,” we can operate on the fact that a large number of

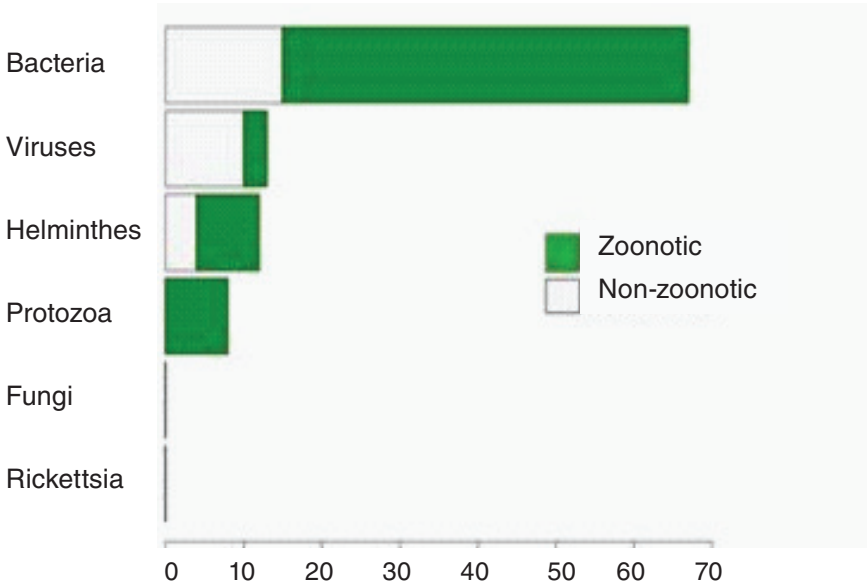


FIGURE A7-2 All food-borne EID events from 1940-2004 (n = 100), broken down by zoonotic versus nonzoonotic origin.

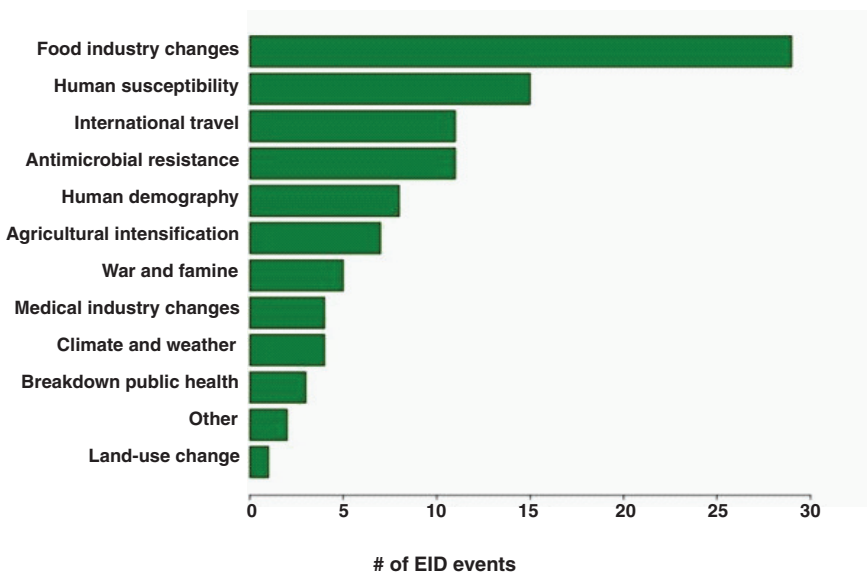


FIGURE A7-3 All food-borne emerging infectious diseases from 1940 to 2004 (n = 100) with their respective “drivers of emergence” as published by Jones et al. (2008).

food-borne illnesses are caused by pathogens frequently associated with wild species such as rodents, deer, feral pigs, reptiles, and birds (Figure A7-4).

Wild animals can provide the original source of pathogen contamination or serve to move pathogens from other infected sources. Similarly, insect reservoirs have been shown to introduce diseases into food processing systems (i.e., Chagas disease; Pereira et al., 2009) and have the potential for serving as mechanical vectors. Research by Wayadande et al. (2011) has identified filth flies as mechanical vectors that acquire and carry bacteria from their development stage environment (i.e., feces, carcasses, or decaying matter) including *Salmonella typhi*, *Mycobacterium tuberculosis*, and *Rotavirus*. The bacteria can also be carried in the excreta of blow flies, presenting risks of spread to external surfaces. Sela et al. (2005) reported similar findings for fruit flies.

In some cases, it may be feasible to limit contact or contamination of food during the production stage from larger wild mammals. Eliminating small-animal access to farm fields is impractical, although reducing exposure could be facilitated by a number of pest management techniques such as waste management and sanitation, eliminating hiding areas, etc. Postharvest contamination of food from small mammals and birds is more easily controlled by good pest management practices.

More directly, wildlife provides a substantial portion of our food globally, with nearly half of all seafood coming from wild sources. In some regions of the world, wild meat from terrestrial animals represents a primary source of protein on which populations are dependent. The volume of wild meat (“bush meat”) harvested from Central Africa alone totals more than 1 billion kg per year (Wilkie and Carpenter, 1999). This volume of meat, almost all of which is processed and distributed to consumers with few if any modern hygiene practices, provides a constant opportunity for human exposure to common food-borne pathogens (Karesh et al., 2005; Smith et al., 2012). Additionally, wild animals are sought after as delicacies based on cultural and consumer preferences. This demand for wildlife, which is both legally and illegally supplied, has global dimensions. Although there is a “not in my backyard” mentality that limits our concern for what diseases are circulating across the world, this is a problem in which the United States is deeply involved, as the United States is the main importer of wildlife (Asmussen et al., 2011). What is present across the world can be in our backyard—and then on our plate—in a matter of days through our importation of tens of millions of legal and illegal animals (Jones et al., 2008; Smith et al., 2009). The United States also contributes to the potential spread of disease to other countries through its export of turtles destined for the food trade in Asia.

The emergence and transmission of food-borne zoonotic diseases from dietary habits and pressures are increasingly being documented. In simplest terms, we are seeing that the consumption of wild animals translates to “you get what you eat.” Hepatitis E (Vasickova et al., 2007), brucellosis (CDC, 2009), and trichinellosis (Roy et al., 2003) are examples of hunter-acquired food-borne illness. The origin

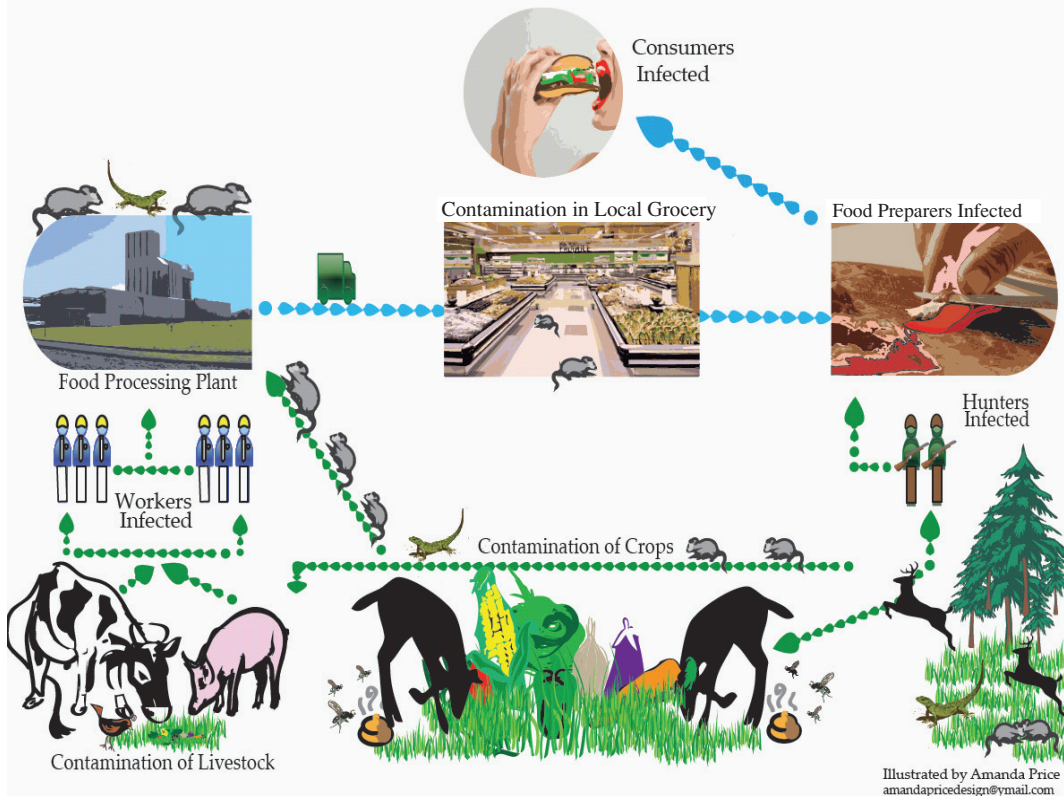


FIGURE A7-4 Routes of contamination resulting in food-borne illness linked to wildlife.
 SOURCE: Illustrated by Amanda Price.

of HIV/AIDS through the transmission of nonhuman primate simian immunodeficiency viruses to humans via bush meat hunting represents a major example of how anthropogenic behaviors can lead to massive and pervasive public health threats, and newer evidence shows that the transmission of nonhuman retroviruses to humans happens on a regular basis (Betsem et al., 2011; Calattini et al., 2011; Peeters et al., 2002). SARS is another well-known example of food-borne illness from animals, spreading to humans after the mixing of live reservoir hosts (e.g., bats) and intermediate hosts (e.g., civets) (Guan et al., 2003; Li et al., 2005).

Food-borne illnesses and the interactions that increase their presence in our food supply are not new. However, we are seeing increased detection of zoonotic food-borne illness as we engage more and more in the practices that drive disease emergence. There are challenges ahead, including climate change and its associated changes in animal migration, water supply demands, and possibly pathogen distribution and abundance. For the latter, Vezzulli et al. reported that, during the past half century, ubiquitous marine bacteria of the *Vibrio* genus, including *V. cholerae*, increased in occurrence within the plankton-associated bacterial community of the North Sea, where an unprecedented increase in bathing infections associated with these bacteria was recently reported. Among environmental variables, increased sea surface temperature explained 45 percent of the variance in *Vibrio* data, supporting the hypothesis that ocean warming is facilitating the spread of vibrios and may be the cause of the globally increasing trend in associated diseases (Vezzulli et al., 2012).

Opportunities for Food-Borne Disease Surveillance

Humans have commonly served as the sentinel species for food-borne illnesses, and as a result early detection and response systems such as PulseNet, FoodNet, the National Electronic Norovirus Outbreak Network (CalciNet), and the National Notifiable Diseases Surveillance System are based on human outbreak (or case) surveillance. There are new approaches in emerging disease surveillance that could possibly be adapted to food-borne disease surveillance to contribute to targeting surveillance efforts, early detection, control, and prevention. Some of these approaches are being developed and tested with the support of the U.S. Agency for International Development's Emerging Pandemic Threats PREDICT program in an attempt to create more upstream focus for early detection of emerging diseases with pandemic potential.

PREDICT's SMART (strategic, measurable adaptive, responsive, and targeted) surveillance method uses continuously refined predictive models, literature reviews and analyses, digital news surveillance, and input from front-line information by field personnel and the public. *Predictive models* can identify areas of greatest risk for outbreaks of food-borne illness (food-borne illness "hotspots") by generating geospatial information on various human–animal interfaces, behaviors, activities, and presence of additional risk factors. These analyses

can be spatially explicit to account for host and pathogen niches and known distributions, as well as differing drivers of disease emergence in different regions of the world. For food-borne illnesses, these interfaces might include areas where food-production farms overlap with habitat used by wildlife (to include species and common pathogens), areas of hunting, wildlife–livestock conflict, natural resource extraction and land-use change, markets, and regions with high levels of global transportation.

Closely linked, and in some ways underpinning the predictive modeling, *analyses of peer-reviewed publications* can be conducted for food-borne diseases related to wildlife to determine species and human activities that present the highest risks. *Databases* on outbreaks or cases of wildlife-related food-borne illnesses could also be queried to identify products with high potential for food-borne diseases, drivers of disease emergence, and risky areas where surveillance and control efforts can be focused. *Analysis of the individual drivers of disease emergence* can help parse out the most likely routes of transmission in a given area, helping to set control measures in place. For the PREDICT program, these analyses are used to target surveillance to key taxonomic groups and key human activities and “interfaces” with wildlife. Similar approaches have been used in the food industry for years in determining the most effective “control points” or monitoring steps for known, common pathogens. Analyses of drivers might contribute to current approaches by revealing additional key points for surveillance and interventions preceding human infection with novel pathogens. Some of this information could be derived from the Foodborne Outbreak Online Database produced by the U.S. National Outbreak Reporting System, which reports annual outbreaks by year, month, state, etiology, location interface, total cases, hospitalizations, deaths, food vehicle, and contaminated ingredient. It currently contains data from 1998–2009. Wildlife-related food-borne illnesses present some additional challenges and opportunities that may require nonconventional approaches and include targeting surveillance outside of the normal farm-to-table production chain. For example, analyses may indicate that sampling by hunters or pest control operators could yield valuable surveillance data (both for pathogen and host species abundance) in a cost-effective manner.

Digital surveillance, the practice of seeking disease news and tracking disease trends via the Internet such as provided by HealthMap, could also help to determine where to target surveillance, control, and capacity-building efforts. Both HealthMap and ProMED-Mail are expanding their coverage of wildlife disease events with the support of the PREDICT program, providing easier access to that information for health workers and the public around the world. While historic, macro-level data can be useful for risk modeling and mapping, it is important to maintain on-the-ground approaches to continually update and target the specific and real-time events and factors driving food-borne disease risk.

Food-borne disease diagnostics. The increase in technological capabilities over the past decade has aided our ability to garner far more information from

surveillance efforts. These technologies can support rapid detection, diagnosis, and control of food-borne illness, as well as preventive measures around potential food-borne illness emergence. Expanded efforts in surveillance for pathogens in wildlife have created a need for simple, inexpensive broad-ranging tests that can be used locally for rapid screening, coupled with networks of labs that can follow up with more detailed, confirmatory testing. For food-borne illness, pathogen-specific and -sensitive tests will always play an important role, but as we expand our concern to wildlife-related and novel pathogens in food, there could be a growing need for some broad-level screening tests for family-, order-, or genus-level testing.

Intervention Strategies

Once a source or a transmission pathway has been established, the use of existing control mechanisms for food-borne illnesses will likely be effective. There is currently no evidence to suggest that wildlife-related food-borne illnesses are inherently different from other sources of contamination; in the case of consumption of wildlife itself, similar risk reduction practices for the safe handling and consumption of poultry, beef, or seafood should suffice. As with traditional food safety surveillance and interventions, stakeholder engagement is crucial for success. For diseases related to wildlife, this concept is the same, but the stakeholders may not be the traditional food safety partners that industry and public health agencies work with. Hunters, conservation organizations, and wildlife management authorities need to be engaged to most effectively develop and implement both surveillance systems and control strategies. As we are learning in most areas of public health, collaboration among multiple disciplines is key in the success of disease risk reduction interventions, and this is especially true as we try to reduce food-borne illnesses linked to wildlife in a variety of ways.

Agency Partnerships and Regulation

There is great potential to learn more about wildlife-related food-borne illnesses through collaboration with surveillance and regulatory agencies. Initial sampling efforts of confiscated wildlife through the Centers for Disease Control and Prevention (CDC) have begun to find pathogens (Smith et al., 2012), but there needs to be dedicated funding and sustained efforts to conduct sufficient inspections and pathogen testing across all agencies that regulate wildlife. This is especially relevant to the Food and Drug Administration, which has regulatory authority for food safety in meat or other animal-derived products from wildlife in interstate commerce not otherwise covered by U.S. Department of Agriculture authority. Greater understanding of wildlife trade risks can inform risk reduction regulations and practices. For example, short-term enforcement of traded animals

in Asian markets following the 2003 SARS outbreak shed light on the scale and composition of wildlife trade in the region (Karesh et al., 2005). In addition to protecting consumers, strong enforcement and surveillance of food-bound wildlife products can help the health, pet, and food industries proactively mitigate risks from potential food-borne threats.

The Convention on the International Trade of Endangered Species (CITES) regulates the trade of endangered wildlife in its 175 member countries. However, illegal wildlife trade is still widespread, with 87 percent of CITES member countries reporting illegal trade activity. Thus, trade regulations must be implemented and enforced at the individual country level as well. There are opportunities to use regulation to directly protect the public from wildlife diseases. The U.S. ban on small turtles resulted in a major decline in *Salmonella* transmission in children, leading to a 77 percent reduction of cases (Cohen et al., 1980). Food-borne disease surveillance can inform targeted regulations to reduce illness spread from high-risk sources.

Collaboration

Food-borne illness is a complex challenge that cannot merely be solved by an “expert solution” and demands diverse stakeholder participation. The public health sector and food industry should not be isolated in addressing food-borne illness concerns involving wildlife. There are ample opportunities for collaboration and mutual benefits among a wide array of stakeholder disciplines. The capture of endangered wildlife and detrimental impacts to wildlife habitats through land-use change—a major driver of emerging infectious diseases (Jones et al., 2008; Patz et al., 2004)—pose serious threats to the sustainability of biodiversity, bringing conservationists into the equation. Hunters and indigenous populations have a vested interest in both the food security and the health of their communities, and their direct involvement and ownership of disease reduction efforts is crucial for creating long-term success.

At the same time, although food-borne illness is a global concern, individual risks can be addressed at a microcosm level. Large-level suppliers can support the health of local communities where their products are sourced. This involves both reducing reliance on wildlife for food (e.g., in logging settlements) and taking measures to educate communities about safe and healthy practices (i.e., in hunting and animal butchering). These approaches have had marked success. For example, Northern Congo has not seen a human case of Ebola since 2005, despite its continuing presence in wildlife. The joint approach of education of village hunters on risk reduction (i.e., high-risk species, hand washing, and cooking techniques) and the assumption of responsibility taken on by hunters for protecting themselves and their families is credited with the prevention of new cases.

Going Forward

Without effective action, the world is slated for an increasing trend of negative health and economic consequences from food-borne illnesses. Fortunately, at present there are opportunities for intervention. Addressing food security issues will decrease reliance on high-risk food sources such as wildlife. Additionally, overall progress around disease surveillance, control, and prevention has allowed us to establish feasible disease monitoring systems and learn important lessons that can be applied to the risk reduction of food-borne illnesses. Integral to these lessons has been the necessity and value of One Health collaborations. The synergies formed by integrating environment, health, and wildlife sectors, in concert with local populations, can provide the perspectives and actions to reduce food-borne illnesses and provide appropriate intervention and prevention strategies before further outbreaks occur.

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A8

ONE HEALTH AND FOOD SAFETY*Lonnie J. King*¹⁴**Introduction**

The concept of One Health is not new but it has reemerged as an important concept to both understand and help address our contemporary challenges and threats to our health.

We live in a world that is rapidly changing, complex, and progressively more interconnected. The convergence of people, animals, and our environment has created a new dynamic—one in which the health of each group is now profoundly and inextricably linked and elaborately connected.

Inherent in this new dynamic is the changing interface between people and animals, including animal products. The human–animal interface is accelerating, expanding, and becoming increasingly more consequential. Over the past three decades, approximately 75 percent of new human infectious diseases have been zoonotic. The global population has now exceeded 7 billion people, and an estimated 30 billion food animals were produced to help feed this population and meet its growing demand for protein from animal sources. The result is a phenomenal global food system that is both a major agricultural and business accomplishment and an unparalleled challenge that is creating major societal issues that, to some extent, threaten human, animal, and environmental health (FAO, 2006).

As a further consequence, the safety of our food is being increasingly scrutinized and questioned by the public, and food-borne illnesses are significant, costly, and a global problem. There continue to be differences of opinion on how to improve food safety, and we lack an integrated and holistic strategy for implementation in the United States and much of the world. While we acknowledge some success in controlling and ameliorating food-borne illnesses and food contamination, these achievements are uneven, often transitory, and especially difficult. Ensuring a safe food supply will likely demand new levels of collaboration, understanding, and thinking. The application of a One Health model where potential solutions are viewed and delivered more holistically and with an emphasis on prevention is a compelling and timely strategy.

One Health Defined

One Health is the collaborative effort of multiple disciplines—working locally, nationally, and globally to attain optimal health for people, animals, and

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our environment (King et al., 2008). The scale and complexity of food safety issues demand that scientists, researchers, and others move beyond the confines of their own disciplines, professions, and mindsets and explore new organizational modes of team science, and the One Health concept embodies this declaration. The scope of One Health is impressive, broad, and growing. Much of the recent focus of One Health has been limited to emerging infectious diseases, yet the concept clearly embraces environmental and ecosystem health, social sciences, ecology, noninfectious diseases and chronic diseases, wildlife, land use, antimicrobial resistance, biodiversity, and much more. While these components are appreciated within our understanding of the broad dimensions of health, they also add to the complexity of One Health and the difficulty in implementing strategies, building effective coalitions, and mobilizing scientific communities who embrace One Health yet who have been trained and think in much narrower scope and scale. Although there may be disagreement on the exact definition of One Health there is broad consensus that a new framework for preventing food-borne diseases is essential rather than the alternative of constantly responding to them reactively.

“Wicked” Problems

We now live in a world that is complex, interconnected, and uncertain, with growing dilemmas and unprecedented societal problems. These problems have been referred to as “wicked problems” and are contrasted with “tame problems,” which can be solved with existing modes of inquiry, technological knowledge, and decision making. Wicked problems are complex, do not have yes-or-no answers, can generate unexpected consequences, may be symptomatic of other problems, and are unique in that past experiences and thinking are not helpful in addressing them. In addition, wicked problems and issues often crop up as organizations face constant change and unparalleled challenges, and they often occur in a social context with diverse opinions from numerous stakeholders who lack consensus in both identifying the total problem and how to resolve them (Brown et al., 2010).

Issues and problems connected with food safety, food security, sustainable production systems that ensure environmental protections, and the capacity to help feed more than 7 billion people collectively qualify as a societal and wicked dilemma. Ensuring safe, accessible, affordable, and nutritious food is increasingly difficult, especially in a global context. Central to this challenge is the development of a One Health strategy and a new level of thinking and acting.

The world population has a growth rate of 1.2 percent per year and the next century will represent a period of exponential growth. There is also a significant demographic fault line between the population growth in developed versus developing countries. Approximately 90 percent of the world’s population growth is occurring in the developing countries of the world. In addition almost 1 billion people live in peri-urban or slum settings in the developing world’s largest cities,

and these sites are where the most rapid growth in our human populations will continue (Smith and Kelly, 2008).

While there is also legitimate concern about the approximately 800 million people who are undernourished, we are concurrently observing a relative increase in wealth in the developing world and as per capita incomes rise; people eat more calories and consume different products, including a demand for meat and protein from animal sources. Today, 3 to 4 billion people consume very little meat but will consume more, should incomes increase. Thus, a new agricultural phenomenon is emerging: the Livestock Revolution. With relative increases in wealth and technological advances in livestock and poultry production, global increases in production and consumption of livestock products are unavoidable. The Food and Agriculture Organization (FAO) estimates that there will be a demand for a 50 percent increase for animal proteins in the next one to two decades. Thus, the entire global food system will adjust into a more intensive, specialized, and integrated system, and production systems will progressively shift to the developing world (Delgado et al., 1999).

As the Livestock Revolution ushers in a rapidly expanding animal agriculture production system in the developing world, there is real concern regarding the animal and public health infrastructures available to support this revolution. The United States now imports approximately 15 percent of its food, but it imports a much higher percentage of seasonable fruits, vegetables, and seafood (Acheson, 2010). The need for inspecting these products is growing much more quickly than the regulatory system now in place to implement such safeguards.

Concurrently, there is unprecedented immigration and movement of people worldwide. Unique diasporas have emerged, and there are large numbers of immunocompromised individuals dispersed throughout the United States and global populations who are especially susceptible to infections including food- and water-borne illnesses. In many countries, the population of seniors is one of the fastest growing cohorts.

There is also a disconnect between global commerce and the remarkable movement of food in trade channels and the commensurate emphasis and assurance of safe food. There is a significant gap between an emphasis on the rapidly growing commerce and business of global food companies and an equal emphasis and investment to address the potential health consequences generated by global food and animal commerce (Kimball, 2010). The 21st century has created a great mixing bowl of people, animals, and animal products and a group of wicked problems, including the protection and safety of our food, that demands a transformation of thought and actions to address these contemporary challenges, threats to our health and well-being, and threats to animal and environmental health that are under increasing pressure. A holistic and integrated approach considering these domains in a One Health strategy is both logical and essential to further success.

Food Safety: Trends and Concerns

The Centers for Disease Control and Prevention (CDC) now estimates that, in the United States, there are 48 million food-borne illnesses, 128,000 hospitalizations, and 3,000 deaths each year (Scallan et al., 2011). Thus, one out of six Americans will have at least one episode of a food-borne illness annually. Although we lack similar global data, a rough extrapolation would suggest that there could be at least 1 billion such illnesses worldwide each year. This would qualify as a global public health epidemic by any definition; however, there are few surveillance systems that can help us track and define the global burden of food-borne illnesses. With these estimates of the burden of illness, the global food system continues to grow increasingly more vulnerable and potentially riskier and progressively connects our global communities daily through our growing imports and exports of food.

Today, microbes can traverse the globe faster than their incubation period; our great convergence offers unique opportunities for them to cross species lines, become resistant to antimicrobial agents, adapt, change, and find new niches, and emerging and reemerging diseases result. Our current era of emerging infections and pace of emergence is accelerated with changing ecosystems, risky human behavior, poverty, travel, trade, globalization, population growth, and our inter-connectiveness. Food as a potential vehicle for disease transmission is embedded in this complex system; food safety has taken on a growing importance and has become a critical public health imperative.

As we learn more about the burden of food-borne illness, we also appreciate and learn about new pathogens transmitted by food and the expansion of the types of food that can transmit potential food-borne pathogens. We are reminded that bacterial contamination of food is a critical issue; however, viruses, parasites, toxins, prions, chemicals, metals, and allergens may also be transmitted by food and water and result in an expanded burden of illness and growing spectrum of threats.

CDC studies have also demonstrated changing patterns of attribution. Plant-derived foods such as leafy greens, tomatoes, and sprouts have been implicated in more and more food-borne disease outbreaks. In the recent past, transmission has been linked to peanut butter, pizza, spinach, ice cream, cookie dough, pet food, melons, peppers, and carrot juice. We are also concerned about the concept of “stealth” vehicles in transmission. There are numerous food ingredients that are often mixed in with foods, such as spices, which can be vehicles for transmission. It is estimated that 75 percent of our food that has been processed has an ingredient from an international source (Doyle and Erickson, 2008).

In addition to the traditional food-borne pathogens such as *Escherichia coli*, *Salmonella*, *Campylobacter*, *Listeria*, and so on, new outbreaks often reveal new agents. The FoodNet system that analyzes outbreaks has revealed adenoviruses, sapoviruses, saffoldviruses, and picobirnaviruses as potential pathogens (Tauxe, 2008). To further complicate our understanding of the safety of our food, trans-

mission vehicles can change when microbes are given new opportunities. For example, the Nipah virus first found in a zoonotic disease outbreak in Malaysia that killed pigs and people associated with them has recently been found as a contaminant in date palm sap, a food source in Bangladesh. *Pteropus* fruit bats are the asymptomatic carriers. *Trypanosoma cruzi* is the parasite that causes Chagas disease and is usually transmitted to people via reduvid insects, yet it has recently been found in sugar cane juice in Brazil. There is a remarkable spectrum of foods and pathogens involved in food-borne illnesses, and this is an ever-changing dynamic. There is a growing importance of produce as a vehicle for food-borne pathogens, yet animal reservoirs are often the origin of these infections. One Health gives us the proper lens to view and better understand this linkage and, more importantly, to develop new insights for changing our interventions and prevention strategies. In many instances, ill people are the end point of a complicated epidemiological cycle and serve as indicator hosts; however, if we continue to focus exclusively on food-borne illness by responding to human outbreaks and just conducting retrospective analyses, then we will miss the true sites and origins of these diseases and we will forgo critical prevention strategies. To a certain extent, ill people serve as sentinels of a larger ecological problem and, as such, may not be the best focal point for our interventions. One Health is a mindset that is proactive and preventive and helps to shift our attention “upstream” to the ecological, animal, and environmental sources and influences responsible for these illnesses and helps us to identify the most effective points for the initiation of food safety actions.

According to Jared Diamond, in his book *Guns, Germs, and Steel*, diseases such as measles, smallpox, influenza, and tuberculosis likely evolved from animal diseases as the first group of zoonotic diseases (Diamond, 1999). The advent of agriculture and the domestication of animals approximately 8,000 to 10,000 years ago were drivers of a new human–animal interface and the first era of emerging zoonotic diseases. Although animal agriculture is much more sophisticated today, it is also growing more intensified and complex. Domestication has resulted in the development of new and more efficient food-animal species, and the human–animal interface has accelerated and multiplied through the globalization of our food system and has created the potential exposure of billions of people to potential pathogens. As our food-animal production and ecosystems continue to change to produce more and more, microbes are given further opportunities to adapt and find new niches. Transboundary diseases have again emerged at an alarming rate, suggesting that our new era of disease emergence has a troubling similarity to the past era that was created 8,000 to 10,000 years ago.

One Health Lens to View and Improve Food Safety

Dr. Gro Brundtland, former Director of the World Health Organization, stated, “In the modern world, bacteria and viruses travel almost as fast as money.

With globalization, a single microbial sea washes all of humankind. There are no health sanctuaries.” In actuality, that microbial sea washes not only over all humankind, but also across our animal and environmental domains. This dynamic exposes and connects the human, animal, and environmental domains in ways never previously experienced. Positive and negative actions and impacts in one domain now may significantly impact the others, and solutions to address threats in any single domain may have multiplier effects in the others. This is the essence of One Health, and the safety of our food must also be considered using this mindset.

Yet as our food systems grow ever more complex, larger, and more vulnerable, our scientific, medical, agricultural, environmental, and health systems and studies remain too isolated and entrenched. Perhaps our greatest challenge today may be our ability to reconcile the changes and challenges of our global convergence with our traditional thinking and habitual ways of working. For many zoonotic diseases, including certain food-borne illnesses, we focus on the risk to human health while the most effective control strategies are in animals, animal products, and the environment. There are divided constituents and responsibilities for animal and human health that must be integrated in order to make significant progress in the reduction of many food-borne illnesses. The microbes seldom distinguish among species as they just seek opportunities to survive and multiply. Our own bias and artificial separation between veterinary and animal health and public health is a critical barrier to the acceptance of One Health.

I have discussed the concept of wicked problems and the need to view many contemporary problems as interconnected issues that have created larger societal dilemmas. Patterns of thought of a previous era may not be useful to address current problems. Because wicked problems are part of the society that creates them, future solutions and actions must be based in that society. We can no longer focus on a single domain of health or any singular inquiry; we now must be open to new ways of thinking and be receptive to new ideas and directions that match our challenging times. The status quo in food safety must be replaced by a new transdisciplinarity and a new collective understanding of food safety characterized by a One Health mindset and approach. A One Health emergent community of practice now exists where new views, approaches, and knowledge can inform each other synergistically and more productively.

In Thomas Kuhn’s seminal book *The Structure of Scientific Revolution* (Kuhn, 1962), he discussed new paradigms and the conditions and factors that create them. A paradigm shift is often manifested because old models to solve problems do not work as well and new models have yet to be created or substantiated. Basic assumptions are questioned, and the evidence to change is not uniformly accepted. While we acknowledge that progress has been made in making our food safer, especially in the United States, breakthrough thinking is minimal. Kuhn suggested that changing mindsets can be difficult and protracted and that new paradigms are not necessarily led by a scientific community. In

the case of food safety, much of the force for change is being led by consumers and more recently retailers. Also new paradigms often lead to new fields of study, inquiry, and work. One Health, although not new, is certainly a renewed field of inquiry and transdisciplinary thinking.

The convergence of people, animals, and our environment has created a new dynamic in which the health of each domain is inextricably interconnected. The challenges associated with this new reality are demanding, profound, and unprecedented. This remarkable convergence is a critical factor in disease emergence, and there is nothing on the horizon to suggest that this dynamic will be altered or abated. The safety of our food supply is a microcosm of this larger dynamic, and our food is increasingly vulnerable to both intentional and unintentional contaminations and changing microbial communities. Working successfully to address these threats will require new thinking, changing partnerships, and shifting our emphasis “upstream,” closer to the origin of pathogens in other domains. A One Health paradigm shift holds great promise but is also a new mindset that will be disruptive to the status quo; thus, old systems, habitual thinking, and working with old modes of inquiry that are sharply divided among diverse cultures and interests and that compete for resources and are part of strongly embedded belief systems remain as challenges.

Dr. Josh Lederberg, a Nobel Laureate and founder of this Forum, published an article in *Science* in 2000 titled “Infectious History.” He stated, “An axiomatic starting point for progress is the simple recognition that humans, animals, plants and microbes are cohabitants of this planet. That leads to refined questions that focus on the origin and dynamics of instabilities within this context of cohabitation. These instabilities rise from two main sources loosely definable as ecological and evolutionary” (Lederberg, 2000). I suggest that our dynamic, complex food system, and the challenge of its safety, is about controlling and preventing instabilities and using One Health as the construct to understand this ecological dilemma and as the foundation to devise new solutions and interventions. Dr. Lederberg further remarked that the future of humanity and microbes will be based on “our wits versus their genes.” This is a prophetic statement underpinning One Health’s application to food safety.

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A9

FOOD-BORNE VIRUSES FROM A GLOBAL PERSPECTIVE

*Marion Koopmans*¹⁵

Abstract

Food-borne transmission has been described as one of the modes of transmission for many different viruses, associated with diseases ranging from mild diarrhea to severe neurological symptoms. The potential for such transmission can be studied by using common human pathogens as a model. By genomic epidemiology approaches, this has revealed significant food-related disease for noroviruses and hepatitis A viruses associated with food-handler transmission and sewage-contaminated foods. In the latter category, complex mixtures of viruses and other pathogens may be present in a single food item, creating potential for genetic recombination or reassortment and thus further expansion of the diversity of these pathogens. Therefore, bringing expertise and data together from veterinary, food, and clinical microbiology may help unravel these complexities and identify areas amenable to intervention and prevention.

Introduction

When it comes to food safety, most people would agree that food has become safer than ever. The potential for contamination with pathogenic bacteria, viruses, and parasites has been recognized and translated into control programs aimed at

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reducing the burden of food-borne diseases in many parts of the world (Newell et al., 2010). Legislation exists to support countries in these control activities and to advise industries by developing guidelines targeting specific pathogens, commodities, or processes (Havelaar et al., 2004). Nevertheless, food-borne disease remains a significant cause of illness, of which the true burden is difficult to estimate (Scallan et al., 2011). The growing population density impacts upon the environment, for instance through sewage discharges, making it challenging to find clean waters for shellfish production in some parts of the world. Such environmental sources of contamination also may contain a mixture of human and animal pathogens, emphasizing the potential for introduction of animal pathogens into the food chain through routes that are not controlled (Figure A9-1). The increasing demand for seasonal produce year-round has globalised the food market, with the ensuing challenge to work with the same high hygienic standards across the world. While these production programs are largely successful, they also illustrate the vulnerability of the global food supply: *if* there is a flaw in the process, then contamination may occur with pathogens from across the globe, including those that have recently emerged (Newell et al., 2010). Therefore, thinking in terms of the future of food safety from a public health perspective does require a

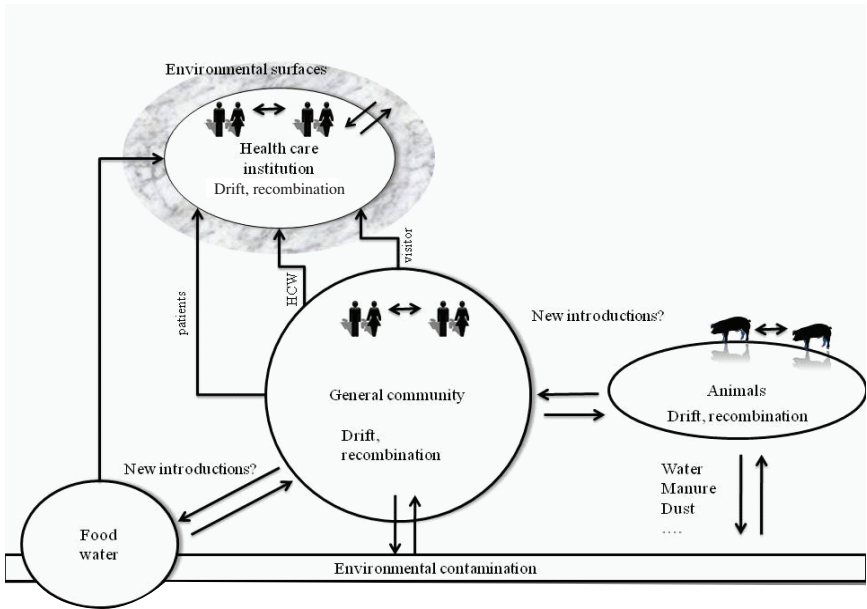


FIGURE A9-1 Epidemiology of food- and water-borne viruses, showing complexity of transmission and possible sources of infection. Which of the factors shown here apply may differ for different food-borne viruses.

holistic view, including the careful review of possible scenarios that may require our attention. Here, we focus on viral food-borne disease, reviewing the current state of knowledge with this forward-looking perspective. For detailed reviews of the state of the art, we refer to other recent publications (Baert et al., 2011; Iwamoto et al., 2010; Khuroo and Khuroo, 2008; Koopmans and Duizer, 2004; Strawn et al., 2011).

Most Common Viral Food Safety Concerns

Currently known viruses that can infect humans are grouped into 24 families. Food-borne transmission has been documented for viruses belonging to at least 10 of these, and the diseases associated with these infections range from mild diarrheal illness to severe encephalitis. The burden of food-borne illness is thought to be greatest for human viruses that are transmitted through poor hygienic practices, either by food handlers or during food production (Scallan et al., 2011). This applies to viruses that are transmitted by the faecal-oral route, hence infecting their host after ingestion, followed by invasion of cells in the epithelial lining of the gut, and subsequent replication in the same site or elsewhere in the body (Koopmans and Duizer, 2004).

Food-borne transmission can occur by contamination of food by infected food handlers, by contamination of food during the production process (e.g., in shellfish production), or more rarely by consumption of products of animal origin harboring a zoonotic virus. While intuitive, understanding these different potential sources is important because the disease ecology differs for these different sources of contamination. These differences are qualitative but, nevertheless, can help direct outbreak investigations.

Food Handler–Associated Illness

Food handler–associated food-borne illness results from the manual preparation of food by a food handler shedding viruses. The potential impact of such contamination events depends on the product type and preparation. There are numerous reports of food handler–associated viral outbreaks, usually resulting in limited outbreaks (Greig et al., 2007). Understandably, the most frequently identified viruses through this transmission route are highly prevalent. Priority concern in this category are noroviruses (NoVs) as the most common cause of gastroenteritis in all age groups, but outbreaks with several other enteric viruses are possible, particularly with hepatitis A (WHO, 2008). Contamination events are not limited to symptomatic persons, although there is no quantitative information about the relative contribution of symptomatic versus asymptomatic food handlers (Okabayashi et al., 2008; Todd et al., 2008). Food handling may occur throughout the food chain, but reported food handler–associated outbreaks often reflect contamination during the final food preparation or serving. This may be a

bias in surveillance, as end-of-the-chain food handler–associated outbreaks are easier to identify through regular outbreak investigations. Risk foods, therefore, are all foods that are handled manually and not further processed before consumption. Freezing is not sufficient to inactivate viral pathogens (Koopmans and Duizer, 2004).

Source Contamination

Food contamination at source occurs when food is contaminated during the primary production, as has been observed in particular in fresh produce such as berries and green onions, or bivalve filter-feeding shellfish. Here the nature of contamination may vary greatly, depending on location of the production area and nature of sewage contamination, but NoV and hepatitis A virus (HAV) were considered to be priority concerns in a coordinated expert meeting of the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Animal Health Organization (OIE) (WHO, 2008). In contrast with food handler–associated contamination, source contamination events may involve multiple pathogens that may be present in sewage, including animal viruses (Myrmel et al., 2006; Pommepuy et al., 2004; Costantini et al., 2006). This simultaneous exposure to mixtures of viruses theoretically increases the probability of recombination or reassortment of viral genomes when a person is simultaneously infected with multiple related viruses (Gallimore et al., 2005; Koopmans and Duizer, 2004; Le Guyader et al., 2006a; Symes et al., 2007). As with food handler–associated outbreaks, this mode of transmission typically involves the most common human viruses that are present in abundance in sewage (Iwai et al., 2009; Myrmel et al., 2006; Pommepuy et al., 2004; Shieh et al., 2003; Victoria et al., 2010; Wolf et al., 2010). However, treatment of sewage appears to selectively reduce levels of contamination with genogroup II NoV, possibly explaining the relatively high frequency of genogroup I viruses in sewage-related food-contamination events (van den Berg et al., 2005).

Zoonotic Food-Borne Viruses

Zoonotic food-borne infection occurs when meat, organs, or other products from an infected animal are consumed. For viruses, this is the least common mode of transmission, although the potential for such transmission is a cause for concern with every emerging disease outbreak. There is evidence that severe acute respiratory syndrome (SARS), monkeypox, and Nipah virus have been transmitted through food-related incidents (Leung et al., 2006; Luby et al., 2006; Rimoin et al., 2010; Wang et al., 2005a). However, more detailed review of these events suggests that it is more likely the process of food preparation (slaughter of the animal) that constitutes the greatest risk. For hepatitis E, there is documentation of food-borne infection through meat consumption.

In the WHO/FAO/OIE expert meeting, conclusions about priority food-commodity combinations of concern were based on available evidence from the literature, but it was also noted that large data gaps exist: trends in disease reporting are available in many parts of the world for hepatitis A, but not for the other viruses. Estimates of the proportion of illness caused by these pathogens that can be attributed to consumption of contaminated food are based on very few studies and would require the addition of systematic strain typing to routine surveillance, and more systematic studies to provide the data for burden estimates (Scallan et al., 2011; WHO, 2008). Finally, testing for viruses in commodities is difficult, and there is considerable debate over interpretation of findings from molecular assays, because these do not provide information on the viability of the pathogens detected (Baert et al., 2011). As a consequence, data from product monitoring are patchy at best.

Short Description of Common Food-borne Viruses

Norovirus

Virological aspects NoVs belong to the Family Caliciviridae, which is divided into genera. *Norovirus* and *Sapovirus* are the two out of five genera of the family Caliciviridae that contain viruses that cause infections in humans. NoVs have also been detected in pigs, cattle, mice, cats, dogs, and sheep, and sapoviruses in pigs (Han et al., 2004; Martella et al., 2007, 2008, 2011; Ntafis et al., 2010; Oliver et al., 2006; Smiley et al., 2003; Wang et al., 2005b, 2006; Wobus et al., 2006; Wolf et al., 2009). In humans, NoVs cause gastroenteritis, while the animal viruses can cause a range of different clinical syndromes, including oral lesions, systemic disease with hemorrhagic syndromes, upper respiratory tract infections, and others. Furthermore, one other potential genus comprising viruses detected in rhesus macaques has been described (Farkas et al., 2008). So far, the NoVs and sapoviruses are the only caliciviruses known to cause disease in humans, with the exception of anecdotal zoonotic infection with vesiviruses. NoVs can be divided into distinct genogroups, based on phylogenetic analyses of the capsid protein. To date, five norovirus genogroups (G) have been recognized (GI-GV) (Kroneman et al., 2011; Zheng et al., 2005). Viruses of GI, GII, and GIV are known to infect humans. GII viruses have additionally been detected in pigs, and GIV viruses have been detected in carnivores (a lion cub and a dog). GIII viruses infect cattle and sheep, and GV viruses infect mice. The host barrier is not absolute—a suggestion that there may be opportunity for genetic mixing if circumstances are favourable (Souza et al., 2007). Recombination between viruses from different genogroups is rare, suggesting that this constitutes a species level in taxonomy. Within each genogroup, viruses are further segregated into lineages, termed genotypes (Kroneman et al., 2011; Phan et al., 2007). Where known, these seem to have a global distribution, with little evidence for geographic clustering. Direct

comparison of data across countries is challenging because of differences in study design and laboratory diagnostics, resulting in poorly defined biases (Kroneman et al., 2008a, 2008b). This is particularly the case when trying to establish causes of food-borne illness. Here, the less common genotypes of norovirus are likely to play a bigger role, and it is these viruses that are less available for assay test validation studies (Duizer et al., 2007; Fisman et al., 2009; Gray et al., 2007). The development of quality assurance schemes for molecular diagnostics, therefore, is particularly important for detection of such highly diverse viruses.

Epidemiology The etiological importance of NoVs as causes of diarrheal illness has been documented worldwide, but few studies have been performed in a standardized way that allows international comparison and true burden of disease estimates (Hall et al., 2005; Scallan et al., 2011). Community studies have provided evidence for the abundance of NoVs and established that these viruses are the number one cause of community-acquired gastroenteritis, with one out of four or five persons infected per year (de Wit et al., 2001, 2003; Jansen et al., 2008; Kirkwood et al., 2005; Olesen et al., 2005; Patel et al., 2008; Tam et al., 2012; Tompkins et al., 1999; Wheeler et al., 1999). The burden of illness is highest in young children and the elderly (de Wit et al., 2001; Tompkins et al., 1999). The best described feature of NoVs is their propensity to cause outbreaks, resulting from some basic properties: the dose required for productive infection is very low (1-10 particles), and infected persons shed huge amounts of viruses (up to 10^{10} million per gram of stool) (Atmar et al., 2008; Teunis et al., 2008). In addition to this, the most common NoVs evolve through accumulation of mutations and selection of fitter variants that escape the receptor-blocking activities from antibodies triggered by prior infections (Lindesmith et al., 2008; Lochridge et al., 2005; Siebenga et al., 2007, 2010). In addition, the interaction of NoVs with histo-bloodgroup antigens determines the outcome of exposure, and strain-dependent differences in host susceptibility have been observed (Donaldson et al., 2008; Marionneau et al., 2005; Rydell et al., 2011; Tan and Jiang, 2011). Although there is insufficient literature to substantiate this, the transmissibility is likely to differ between genotypes, and such differences may explain why relatively little diversity is seen in outbreak reporting, particularly when outbreaks notified include those in health care institutions: here, genogroup II.4 viruses are by far the most commonly identified outbreak strains (Kroneman et al., 2008; Sukhrie et al., 2011). In a study in hospitalized patients, the probability of secondary transmission of NoVs differed by age and genotype (Sukhrie et al., 2011). In recent years, the incidence of norovirus outbreaks has increased with the emergence of a particular variant (Lopman et al., 2004; Siebenga et al., 2010). More severe complications are seen in immunocompromised patients, and mortality in the elderly (Siebenga et al., 2008; van Asten et al., 2011; Westhoff et al., 2009).

Estimation of the burden of food-borne infection A challenging question, therefore, is how much disease caused by NoVs can be attributed to the different modes of transmission, in particular food-borne spread (Figure A9-1). One source of information comes from outbreak reporting, for instance the European Union (EU) Community Summary Report, the Centers for Disease Control and Prevention's (CDC's) FoodNet overviews, and the Australian FoodNet reports. These list NoVs as frequent causes of outbreaks (CDC, 2011; EFSA, 2010; Hall et al., 2005; OzFoodNet Working Group, 2009). In the EU, in 2008, crustaceans, shellfish, mollusks, and products thereof were the most frequently implicated food items in NoV and HAV outbreaks, but this may also reflect an ascertainment bias, because testing for the presence of viruses in shellfish is well established across Europe. The use of epidemiological criteria in the United States concluded that an estimated 28 percent of all reported outbreaks with unknown etiology were likely caused by NoVs (Turcios et al., 2006). An important caveat in using these data is that testing of patients with gastroenteritis for NoV is not yet an established routine, although this is rapidly changing (Tam et al., 2012). With that, numbers and proportion of reported viral outbreaks will most likely increase in the near future. In addition to the recognized food-borne outbreaks, the high rate of secondary infections in NoV outbreaks can rapidly mask an initial food-borne introduction. Therefore, a relevant question is what proportion of such outbreaks in fact were triggered by a food contamination event (Verhoef et al., 2010). What remains anecdotal is the geographic spread of most food-borne outbreaks, because this requires systematic incorporation of molecular typing into outbreak investigations and international data sharing to identify clusters (Koopmans et al., 2003). Therefore, the current reporting is likely to reflect the tip of the iceberg of true food-borne incidents. The available data also illustrates current challenges in using the notified outbreaks for action: only 5 percent of all reported NoV outbreaks are fully confirmed, reflecting the challenges of virus detection in or on food items (Kroneman et al., 2008a).

Given the paucity of evidence, few studies have attempted to quantify burden of food-borne illness attributable to viruses. In the Netherlands, approximately 12 to 15 percent of community cases of NoV gastroenteritis were attributed to food-borne transmission, based on risk factor analysis using questionnaire data. This makes NoV as common a cause of food-borne gastroenteritis as *Campylobacter* and more common than *Salmonella* (de Wit et al., 2003). A recent analysis of available data estimates that almost 60 percent of illness cases, 26 percent of hospitalizations, and 11 percent of deaths from food-borne illness are caused by NoV (Scallan et al., 2011). Similarly, an estimate based on data from Australia suggests that NoVs are important causes of food-borne illness (Hall et al., 2005).

In studies of outbreak reports, the term "food-borne" has been used loosely and has not been standardised. Also, the ultimate number of persons affected by a food-borne outbreak is rarely known, and reported outbreaks are likely to be biased (Kroneman et al., 2008b; Todd et al., 2008). The average size of reported

outbreaks is limited, but there are examples of widespread dissemination, for instance following consumption of wedding cake, sandwiches from an ill baker, deli meat during rafting trips down the Grand Canyon, frozen shellfish, or a manually prepared salad (de Wit et al., 2007; Friedman et al., 2005; Malek et al., 2009; Schmid et al., 2007; Webby et al., 2007). An interesting example was the simultaneous emergence of a new recombinant NoV in nine countries across Europe in 2001 (Ambert-Balay et al., 2005; Koopmans et al., 2003; Reuter et al., 2006). This variant was found in association with four different capsids until equilibrium was reached and the virus continued to circulate in combination with GI3 capsid. These viruses currently are the second most common cause of infection in children hospitalized with NoV (Beersma et al., 2009).

This example also raises the question of where to draw the line in terms of estimation burden of food-borne disease: could the widespread circulation of the GIb strains have been prevented? Or is it only the first round of infections that should be attributed to food? While difficult to prove with certainty, these examples illustrate the contribution of food-borne introduction to the diversity of viruses circulating in the population, a situation that is not desirable from a virological standpoint: novel combinations of genes may have unpredictable effects on viral behavior and virulence and should be avoided when possible.

Hepatitis A (HAV)

Virology The hepatitis A virus belongs to the family Picornaviridae, genus *Hepatovirus*. Hepatoviruses have only been found in humans and primates, suggesting there is no risk of introduction from a reservoir. Based on genetic diversity, hepatitis A viruses are divided into six lineages or genotypes, of which genotypes I-III infect humans (Robertson et al., 1992). Genotypes I and II contain subgenotypes (Ia, Ib, IIa, and IIb). In regions with endemic HAV circulation, further segregation into geographically defined clusters is observed, a property that can be used to support source tracing activities in food-borne outbreaks (Costa-Mattioli et al., 2003; Robertson et al., 1992).

Epidemiology HAV is less transmissible than NoVs, and its incidence is greatly reduced in regions with proper sanitation and good hygienic conditions. As a consequence, great differences can be observed in the incidence of HAV in communities across the globe, related to socioeconomic status (Jacobsen and Wiersma, 2010; Mohd Hanafiah et al., 2011). These differences also affect the level of population immunity and, thus, the susceptibility to food-contamination events. In highly endemic regions, HAV is one of the childhood infections that, in the majority of cases, runs an asymptomatic course, while triggering a protective immune response that lasts long, possibly even lifelong (Hollinger and Emerson, 2007). In such regions, sustained circulation of HAV strains is found, resulting in geographically distinct genetic fingerprints (Baramuechai et al., 2008;

Broman et al., 2010; Cao et al., 2011; Davidkin et al., 2007; Faber et al., 2009; Gharbi-Khelifi et al., 2006; Klevens et al., 2010; Kokkinos et al., 2010; Munné et al., 2007; Nejati et al., 2012; Pérez-Sautu et al., 2011; Sulbaran et al., 2010; Yun et al., 2008). Although this geographical diversity is not robustly defined, this information is used to support investigations into the possible source of an outbreak, or in defining where a patient most likely contracted the disease (Bialek et al., 2007; Pettrignani et al., 2010; Shieh et al., 2007).

In regions with high socioeconomic status, HAV circulation is very limited and mostly restricted to risk groups such as men who have sex with men, to immigrant populations from regions with higher endemicity that may reintroduce viruses when infected during family visits in their country of origin, to travelers who contracted infection while visiting an endemic country and may transmit infection to nonimmune contacts, and to food- and water-borne infection. In such regions, population immunity builds up much slower, leading to an increase in the size of the susceptible population, and a right shift of first-time infections to higher age groups (Jacobsen and Wiersma, 2010). With increasing age, the probability of having symptomatic illness increases, and complications such as fulminant hepatitis are more common. This leads to the somewhat contrasting situation that food-contamination events may have a greater impact in regions with low endemicity of hepatitis A than in highly endemic regions (Greig et al., 2007; Koopmans and Duizer, 2004). This different epidemiological pattern also has consequences for the use of molecular typing in HAV source tracing; in low endemic regions, most people with HAV will have contracted the infection in a different region, and, as a consequence, a great diversity of HAV strains may be seen, reflecting the geographic fingerprints from the regions where they contracted the illness. This basic pattern can be greatly influenced by changing the population immune status through vaccination. Vaccination confers clinical protection that is thought to be long lasting (Van Damme et al., 2011). Whether vaccinated individuals contribute to shedding also is not well known.

Evidence for food-borne infection HAV is quite stable outside a host and, therefore, can persist on contaminated environments, food, and water. Food- and water-borne outbreaks have been documented, although again, as for NoVs, the most common mode of transmission occurs between persons (Bosch et al., 2001; Dentinger et al., 2001; Pinto et al., 2009; Sanchez et al., 2002). Because of the risk pattern described above, the biggest risk of food-borne HAV currently is introduction through food into regions where population immunity is relatively limited. Foods of primary importance, therefore, are those susceptible to contamination during the production phase, such as bivalve filter-feeding mollusks (oysters, clams, mussels) or produce that is irrigated with water that may be contaminated (e.g., lettuce, green onions, and soft fruits, such as raspberries and strawberries). An extreme example of the potential impact dates from 1988, when almost 300,000 cases were caused by consumption of clams harvested from

a sewage-polluted area (Halliday et al., 1991). A specific problem with shellfish is that the current microbiological quality control criteria are based on testing for bacterial contamination, which does not reliably predict the presence or absence of viruses. Also, mildly polluted products can be put on the market after “rinsing” the shellfish by storing them for a period of time in clean water in a process called depuration. Depurated shellfish have been associated with outbreaks of norovirus, hepatitis A, gastroenteritis, and other viral diseases (Ueki et al., 2007). For NoV, specific binding to histo-bloodgroup antigens in oyster tissues has been demonstrated, possibly further explaining the retention of viruses in these animals (LeGuyader et al., 2006b).

Estimation of the food-borne burden of illness In the CDC assessment of food-borne pathogens, hepatitis A is the second virus listed and is considered a significant cause of severe disease (Scallan et al., 2011). This may be related to the increased severity when HAV infection is first acquired during adulthood, although there also are differences in virulence between genotypes (Yoon et al., 2011).

Hepatitis E Virus (HEV)

Virology Hepatitis E viruses have been listed as genus *Hepevirus* in the family Hepeviridae in the database of the International Committee for Taxonomy of Viruses, along with the more distantly related avian hepatitis E viruses. The hepatitis E viruses can be grouped into four genotypes, with different geographical distribution and host range. Genotype 1 is endemic in Asia and Africa, and genotype 2 is endemic in Mexico and western Africa. Whereas these genotypes have been found exclusively in humans, genotypes 3 and 4 have also been detected in pigs and other animal species (e.g., wild boar and deer) (Lu et al., 2006; Teo, 2009). Genotype 3 is distributed worldwide, and genotype 4 is found commonly in Southeast Asia, although recent findings suggest these lineages also may be more widespread (Tessé et al., 2012). Nevertheless, current information suggests that the endemic strains found in pigs in Europe, Japan, and the United States are usually of genotype 3. In addition to the HEV genotypes 1 to 4, distinct HEV-like viruses with lower sequence identity to the strains found in humans have been detected in chicken, rats, and farmed rabbits in China (Huang et al., 2004; Johne et al., 2010; Zhao et al., 2009). In addition, serological data suggest the presence of HEV-related agents in cattle, horses, and some pet animals, but these remain to be confirmed by virological methods (Teo, 2009).

Epidemiology Historically, HEV has been considered to be endemic in developing countries, where genotype 1 and 2 HEV strains have been associated with large outbreaks of hepatitis, primarily in Asia and Africa. The most commonly

recognized mode of transmission in these outbreaks is water-borne, associated with poor-quality drinking water (Purcell and Emerson, 2001). Although HEV outbreaks are only observed in developing countries, antibodies have been found at lower prevalence levels globally, with estimates ranging from very low (around 1 percent) up to 33 percent. Some of these antibodies reflect exposures to genotypes 1 and 2 HEV in the recognized endemic regions through travel, but an increasing number of non-travel-related cases have been reported (Lewis et al., 2010). This follows the discovery of the presence of other lineages (genotypes 3 and 4) in farmed pigs across the world, with evidence for human infections with genotype 3 viruses in a wide geographic region 3 and for genotype 4 viruses in China, and recently in France (one case) (Liu et al., 2012; Tessé et al., 2012). The broader genetic diversity influences the use of existing commercial antibody tests that show large differences in baseline seroprevalence in populations where HEV genotype 3 strains are endemic in pigs, depending on the test used (Herremans et al., 2007). Therefore, type-specific validated methods are needed before robust conclusions can be drawn about the differences in population immunity across countries (Lewis et al., 2010). However, targeted studies suggest that HEV infections may be as common as HAV in some industrialized countries, although the risk profile of patients suggests that genotype 3 HEV is less virulent for humans because illness is mostly observed in persons with comorbidities (Borgen et al., 2008; Dalton et al., 2007; Fogeda et al., 2009; Wichmann et al., 2008). Men over 50 with comorbidities such as underlying chronic liver disease, liver cirrhosis, or a history of high alcohol consumption are at increased risk for symptomatic HEV. Chronic infections have been found in immunocompromised persons (Haagsma et al., 2008; Kamar et al., 2011).

Person-to-person transmission appears to be rare, but the exact mode of transmission of most HEV cases outside the previously recognized risk areas remains to be established. In addition to water-borne transmission, there is evidence for food-borne transmission, transmission by transfusion of blood products or organs, and maternofetal transmission (Aggarwal and Jameel, 2011).

Evidence for food-borne transmission As indicated above, the sources of most HEV infections remain unknown, but there is some evidence for food-borne transmission of genotype 3 HEV from undercooked wild boar and deer (Li et al., 2005; Tei et al., 2003). Epidemiological studies have provided evidence for consumption of undercooked or raw (wild) pork meat as risk factors for acquisition of HEV infection, but only very few systematic studies have been performed so far (Colson et al., 2010; Lewis et al., 2010; Wichmann et al., 2008).

Estimation of the food-borne burden of illness Currently, there is insufficient information to allow burden-of-illness estimates for food-borne HEV infection.

Detection of Food-borne Viral Disease: Specific Challenges

The detection of food-borne illness relies on a combination of laboratory diagnosis, epidemiological investigation, pathogen typing, and food traceback investigations. All of these activities need to be aligned for optimal detection, and the specific challenges differ for the different viruses discussed above (Figure A9-2).

Diagnosis and Genotyping of NoV, HAV, and HEV in Humans

For NoVs, the incidence in the community and the contribution of person-to-person spread dominate the picture (Figure A9-1). Testing of patients with diarrhea and vomiting for NoVs is not always routine because of the lack of low-cost rapid tests with adequate sensitivity and specificity, and in particular because it usually does not inform the decision making of the treating physician. For diarrheal disease outbreaks, norovirus testing is more common, and this has formed the basis of surveillance in most countries that have surveillance of food-borne viral disease in place. Again, however, the rapid secondary spread of NoVs leads to a bias for outbreaks with person-to-person transmission. More in-depth outbreak investigations that involve taking a detailed food consumption history are needed to identify those outbreaks related to food-borne introduction (Figure A9-2). Here, the use of genetic typing has shown to be informative: NoVs are a diverse genus, infecting humans and animals, and divided in lineages termed genotypes. Analysis of the aggregated data from outbreak reporting across Europe

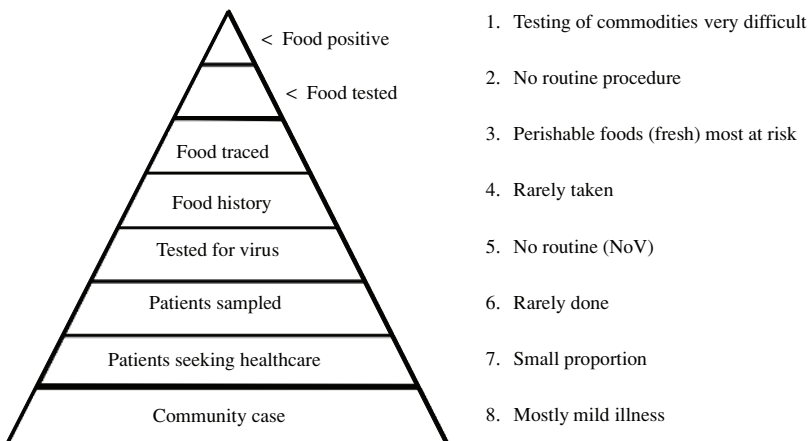


FIGURE A9-2 Steps required (left) and common challenges (right) for establishing proof of food-borne (viral) infection.

SOURCE: Modified from http://www.cdc.gov/foodnet/surveillance_pages/burden_pyramid.htm.

has shown that the probability of a food- or water-borne source differs greatly between genotypes. Therefore, if outbreak investigations need to be triaged for lack of resources, genetic typing may be used to guide this decision making. Clearly, this is not ideal because food-related outbreaks also have been documented for the genotypes that spread most efficiently, hence dominating the reporting when outbreaks in health care settings are included.

For hepatitis A, diagnostic tests are part of the standard diagnostic repertoire; thus, underascertainment of the number of cases in vaccinated individuals is less of a problem than for hepatitis A. The challenge here, however, is the long incubation period, which may be between 15 and 50 days (CDC, 2008). Getting a reliable food consumption history this long after exposure is virtually impossible, unless an incident relates back to a specific event. Analysis of viral sequences may help identify the source of an outbreak (Bosch et al., 2001; Dentinger et al., 2001; Hutin et al., 1999; Sanchez et al., 2002; Shieh et al., 2007; Wheeler et al., 2005); systematic typing of outbreak strains has helped to identify clusters of patients related to food consumption that had not been recognized as such from the notifications, but this is done rarely (Petrignani et al., 2010).

For HEV, routine diagnostic evaluation of patients with acute hepatitis in regions with no known circulation of the human HEV genotypes (1 and 2) is rare, although the recent finding that genotype 3 HEV may cause chronic illness in immunocompromised individuals may change this practice. Therefore, HEV is likely to be largely underdiagnosed. Again, strain typing may be used to identify patient clusters, but this practice currently is limited to specific outbreak investigations and done in research settings.

Detection and Genotyping of NoV, HAV, and HEV in Food (Animals)

For all of the above viruses, there are great challenges in reliable detection in food products, a practice that is seen as an essential part of outbreak investigations (Gentry et al., 2009b; Le Guyader et al., 2008a, 2008b; Li et al., 2011; Rutjes et al., 2006). Recent publications have shown a high prevalence of viral genes on fresh produce, questioning the relevance of such findings as they do not reflect infectious articles (Baert et al., 2011; Stals et al., 2011). A practical problem is that there are no cell culture methods available for noroviruses (Duizer et al., 2004). An elegant study in Europe suggests a correlation between quantities of viral RNA in shellfish and illness in consumers, providing a possible basis for regulatory action (Lowther et al., 2010). Levels of virus contamination, however, vary greatly across production sites, typically reflecting population densities and the ensuing environmental impact from sewage contamination, particularly following heavy rainfall (Boxman et al., 2006; Elamri et al., 2006; Gentry et al., 2009a; Groci et al., 2007; Le Guyader et al., 2008b; Lowther et al., 2010; Myrmel et al., 2004; 2006; Nishida et al., 2007; Nordgren et al., 2009; Pommepuy et al., 2004; Shieh et al., 2003; Suffredini et al., 2008).

Linking Epidemiological and Virological Data for Source Tracing and Attribution

In order to gain a better understanding of the trends in enteric viruses and the possible role of food-borne transmission, the Foodborne Viruses in Europe network was launched in 1999. Participating epidemiologists and virologists from academia, and clinical and public health laboratories, covering medical and food virology agreed to compile data related to outbreaks into a joint database. Since the launch of this network, data have been compiled for more than 8,000 outbreaks involving 13 countries, and some important importations were made. First of all, it became clear that the proportion of food-borne outbreaks reported differed greatly, reflecting differences in the surveillance setup of each country (Koopmans et al., 2003). This background also influenced the diversity of outbreak strains, with limited diversity and strong seasonal effect seen in healthcare-associated outbreaks and greater diversity with limited seasonality in outbreaks reported as food-related (Kroneman et al., 2008). For the common strains for which this was investigated, the strain diversity observed was very similar in different countries, showing that the epidemiology of these viruses is shaped by the global interlinked circulation of pathogens, with little evidence for geographic differences (Lopman et al., 2004; Siebenga et al., 2007, 2009, 2010; Verhoef et al., 2008). Food-borne outbreaks were rarely reported, but their number increased by almost 20-fold when genome sequencing was used to identify linked outbreaks (Verhoef et al., 2010, 2011). The analysis required the availability of both epidemiological and laboratory data, and it included approaches aiming to determine robustness of conclusions drawn, based on choice of target genes and fragment lengths. This was done because international standardization of molecular detection and genetic typing methods across clinical, public health, and food laboratories is very difficult because of the differences in focus and required levels of resolution at each level. In particular the virus detection in food requires such low detection limits that optimal target choice is a luxury that cannot be afforded. By using multiple genome targets to study food-borne NoV outbreaks, multiple recombinant genomes have been identified (Ambert-Balay et al., 2005; Bon et al., 2005; Reuter et al., 2006; Le Guyader et al., 2006). In food-related outbreaks where sewage contamination was the most likely cause, multiple viruses can be found within the same batch, thus favoring conditions for generation of recombinant genomes (Symes et al., 2007).

Emerging Viruses and Food-borne Transmission*Globalization and Risk of Introduction of New Diseases*

With changing consumer behavior and the growing preference for consumption of fresh produce with year-round availability, food has become a commodity in the global market, dictated by availability and (low) cost. Seemingly unrelated events can lead to market shifts and, with that, to potential introduction of new

risks into the food chain. A recent example is the emergence of a highly lethal infection affecting a high proportion of oysters in European banks (Peeler et al., 2012). Although not documented, the lack of locally grown oysters may move the market to Southeast Asia, which has the fastest growth in the market of aquaculture products. Assuming that failures in the production system may occur, as evidenced from the NoV studies, such incidents would potentially lead to contamination of products with locally circulating strains, such as the distinct lineages of enterovirus 71 viruses causing large outbreaks of hand, foot, and mouth disease in that region only (van der Sanden et al., 2009). Even if this is not the prevailing way of spreading, dissemination of viruses via international food trade could disperse an otherwise localized outbreak. This concern has led to in-depth investigations during the emergence of SARS, highly pathogenic avian influenza, filoviruses in pigs in the Philippines, and Nipah virus outbreaks in Malaysia and Bangladesh (Leung et al., 2003; McKinney et al., 2006; Miranda and Miranda, 2011; Parashar et al., 2000). For all of these viruses, there is evidence of introduction of the viruses into the human population through the harvesting, preparation, and/or consumption of food. For all of these examples, the biggest concern is not widespread food-borne transmission, but the fact that this mode of transmission may favor cross-species infections that are not evident otherwise, with the potential for adaptation of these viruses to humans. A systematic review of emerging infectious disease outbreaks suggested that 76 percent of these resulted from zoonotic introductions, and the pressure on the environment from population growth is increasing the contact rates between humans and animals in biotopes that were previously untouched, attesting to the opportunity for cross-species transmissions (Jones et al., 2008). Consumption of virus-containing food, either through bush meat or food contaminated with excreta from animals, is one of the potential routes (Costantini et al., 2006).

Food Safety and the Era of Virus Discovery

The classical toolbox for virology was greatly expanded when sequencing-based technologies entered the playing field, and with this it also became clear that viruses are among the most prevalent entities in the world. Unbiased sequencing has established that a large proportion of ocean waters contain viral sequences, many of them unknown (Breitbart et al., 2004; Rosario et al., 2009). Based on these studies, an estimated 10^4 genotypes per kilogram of sediment have been identified, and the current view is that viral communities are powerful manipulators of microbial diversity, biochemistry, and evolution in the marine environment. Similarly, samples collected from humans when subjected to unbiased analysis of the gene content contain high quantities of viral information, with a dominance of plant and bacterial viruses, but also typically multiple human viruses (Breitbart et al., 2003). These findings are opening an entirely new field of research in host/microbiome and pathogen interaction that is likely to fundamen-

tally change how we view infectious diseases. Sequence-based virus discovery programs identify new viruses in humans and (wild) animals with high frequency (Allander et al., 2005). While most of these newly discovered viruses likely have been present for a long time, these observations do underscore the notion that there is ample potential for new human pathogens. There is consensus among virologists that the probability of the emergence of new viruses or the evolution of old viruses into new forms is inevitable, given the demographic, economical, and sociological changes that we are now facing. Therefore, having mechanisms in place to rapidly address the probability and possible consequences of food-borne transmission of a new infectious disease when it emerges should be a priority.

Another consequence is a revision of how we view the detection of viruses in food or clinical samples (Nakamura et al., 2009; Svraaka et al., 2010). As the methods develop further, more diversity of viruses (and microbes) are found in any of the samples that have been tested, calling for the challenging task to answer what these findings signify. This is no different in clinical virology, where applications of multiplex polymerase chain reaction–based methods or deep sequencing increasingly find complex mixtures of potential pathogens in patients that are tested. This makes it difficult to decide which one or which combination of these was the cause of the symptoms. Methods will be needed to filter the data for relevance for the question addressed.

Conclusion

Food-borne transmission is common but largely underdiagnosed. While viruses from at least 10 families have been associated with food-borne transmission, NoV and HAV have been listed as priority concerns. By genomic epidemiology approaches, significant food-related disease associated with food handler transmission and sewage-contaminated foods has been identified for these viruses. In the latter category, complex mixtures of human and animal viruses and other pathogens may be present in a single food item, creating the potential for genetic recombination or reassortment and, thus, further expansion of the diversity of these pathogens. Bringing expertise together from veterinary, food, and clinical microbiology may help unravel these complexities and identify areas amenable to intervention and prevention.

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A10

MICROBE HUNTING AND PATHOGEN DISCOVERY¹⁶

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Introduction to Pathogen Discovery

Over the past few decades, numerous factors have contributed to a dramatic increase in the rate of microbial and pathogen discovery. The globalization of travel and trade, changes in demographics and land use, susceptibility to opportunistic organisms associated with immunosuppression, and climate change have all contributed to the physical emergence and reemergence of novel and known microbial pathogens (Morse, 1995). Advanced molecular technologies, such as MassTag polymerase chain reaction (PCR) (Briese et al., 2005), microbial microarrays (Cox-Foster et al., 2007; Palacios et al., 2007; Wang et al., 2002), and unbiased high-throughput sequencing (Cox-Foster et al., 2007), have enabled efficient microbial surveillance and detection. As a result of technological advancements, our understanding of sample quality has increased, and specimen collection has become more sophisticated and comprehensive. Dramatic improvements in bioinformatics expertise and computing power have enabled the creation and management of databases needed to compare and distinguish genetic sequences between host and microbe. Finally, our models for pathogenesis embrace increas-

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ingly complex mechanisms that consider host–microbe–timing interactions in both acute and chronic diseases.

This paper summarizes and compares methods now in use and suggests possible directions in which we might travel over the next few years, providing examples of discoveries from our research group that stress the importance of the One Health approach to the topic of food supply and safety.

Diversity in the Microbial Universe

The introduction of cultivation-independent methods for microbial discovery and surveillance has dramatically altered our view of the breadth and diversity of the microbial world. Not only can we now detect and characterize disease agents for which we have no culture system, we can also more rapidly survey ourselves and the larger biosphere. These advances have enabled extraordinary revelations, including the extent to which humans represent microbial vessels. While the number of cells of the human body has been estimated to be 10^{13} , our bacterial passengers on internal and external surfaces are estimated to number at least 10^{14} , or ten times the number of microbial cells to host cells (Savage, 1977). 16S rRNA gene analyses of the oropharynx (Aas et al., 2005), esophagus (Pei et al., 2004), stomach (Bik et al., 2006), intestine and colon (Eckburg et al., 2005), vagina (Oakley et al., 2008), and skin (Gao et al., 2007) indicate differences in human bacterial microflora by anatomical location, individual, and area of residence. This dynamic bacterial composition can also vary over time and can be modified as a function of diet (including the use of probiotics), antibiotics (Hoban, 2003), hygiene, and, in the instance of intestinal microflora, surgical interventions such as bypass procedures (Zhang et al., 2009). The mouth alone has been shown to harbor more than 600 species of bacteria (Paster et al., 2001), and recent improvements in throughput, reductions in costs, and investments in metagenomic sequencing will predictably drive this figure much higher. Environmental sampling has also revealed bacteria and fungi that thrive in extreme temperatures and in the presence of radioactivity, organic compounds, and heavy metals not tolerated by higher organisms (Degryse et al., 1978; Nicholson et al., 2000; Rothschild and Mancinelli, 2001).

Unlike bacteria, viruses do not comprise regions of sequence conservation that enable surveillance and discovery by a method analogous to 16S rRNA gene PCR. Thus, with a few notable exceptions in which agents have been shown to be present because investigators invested in more complex analyses (e.g., subtractive cloning [Lipkin et al., 1990] or consensus PCR using sequences of related agents) based on clues from immunohistochemistry (Nichol et al., 1993), studies of viral diversity have come into their own only more recently with the introduction of high-throughput sequencing. However, even with the use of this technology, we are limited by our capacity to recognize similarities between what we observe for a sample and what is present in a database. The number of vertebrate species

is estimated to be greater than 50,000 (Personal communication, P. Daszak), and if each is associated with only 20 endemic viruses, the vertebrate virome would exceed 1 million. Furthermore, up to 10 percent of the human genome comprises retroviral sequences (Griffiths, 2001).

Virus abundance in aquatic environments is also extremely high, with concentrations estimated at 10^6 per 1 ml in the deep sea and 10^8 per 1 ml in coastal waters, for a total of approximately 10^{30} viruses throughout the world's oceans (Suttle, 2005). The extent to which these viruses pose threats to human health remains to be determined. Nonetheless, their sheer mass and diversity are staggering, and it is clear that we have only begun to scratch the surface of virus discovery. Figure A10-1 illustrates this point by tracking the annual growth of the viral sequence database vis-à-vis selected seminal discoveries and improvements in sequencing technology since 1982.

Links to Causation

Detecting an organism in a sample is only one step in establishing a causal relationship or understanding how it may be associated with disease, and many have wrestled with the challenge of codifying the process of proving causation. Based on the germ theory of disease of Pasteur, Koch and Loeffler proposed criteria that define a causative relationship between agent and disease: the agent is present in every case of a disease; it is specific for that disease; and it can be propagated in culture and inoculated into a naïve host to cause the same disease. Known as Koch's postulates, these criteria were modified by Rivers for viruses (Rivers, 1937) and by Fredericks and Relman to reflect the introduction of molecular methods (Fredericks and Relman, 1996). Although fulfillment of Koch's postulates remains the most persuasive evidence of causation, there are numerous challenges with holding to this standard. Overlap in signs and symptoms due to infection with different agents is common, and results of infection may vary with genetic background, age, nutrition, and previous exposure to similar agents. Furthermore, many agents cannot be cultured and/or there may be no animal model for experimentation. Proving causation is particularly difficult where agents have remote effects or require cofactors for expression. Here, one may resort to a statistical assessment of the strength of epidemiological association based on the presence of the agent or its footprints (nucleic acid, antigen, and, preferably, an immune response) and biological plausibility as indicated by analogy to diseases with other organisms where linkage is persuasive.

Complex Pathogenesis as a Confounder of Microbial Implication

In the most straightforward pathogen discovery expeditions, an agent is present in high concentrations at a site where pathology is readily apparent and organ dysfunction is dramatic. Classical examples include infections with polioviruses

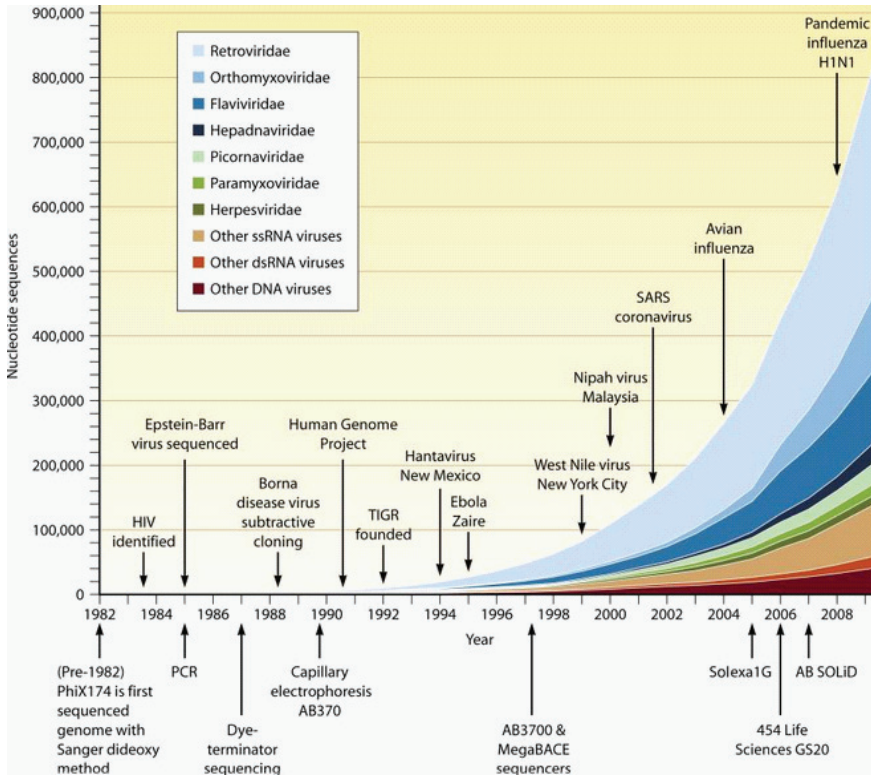


FIGURE A10-1 Growth of the viral sequence database mapped to seminal discoveries and improvements in sequencing technology. EM, electron microscopy. (Image courtesy of Omar Jabado, reproduced with permission.)

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and motor neuron disease, an influenza virus or *Streptococcus pneumoniae* and acute respiratory disease, and a rotavirus or *Shigella* sp. and diarrhea. Viruses may kill cells directly through intracellular replication and lysis, the induction of apoptosis, or autophagy. They may also do so indirectly by presenting antigens that are recognized by cytotoxic T lymphocytes or that become bound to antibodies and trigger the activation of the classical complement cascade. Causal links may be more difficult to establish when damage is indirect, particularly when effects are manifest at sites other than the replication site. *Clostridium botulinum* and *C. tetani* bacteria, for example, grow in the skin or the gastro-

intestinal tract and release zinc metalloproteases that have distal effects on motor function by modulating neurotransmitter release (botulism [Segelke et al., 2004] and tetanus [Bruggemann et al., 2003]).

Another example of indirect pathogenesis is Sin Nombre virus infection, a hantavirus that induces the expression of cytokines that in turn promote pulmonary capillary leakage, culminating in an acute respiratory distress syndrome (Mori et al., 1999). Microbes can elicit immune responses that break tolerance to self, resulting in autoimmune disease. A well-known example is group A beta-hemolytic streptococcus (GABHS). GABHS infection of the oropharynx may cause local inflammation or be asymptomatic. In either case, infection in susceptible individuals elicits a humoral immune response that can cause both cardiac valvular damage (rheumatic heart disease) and abnormalities in movement and behavior (Sydenham's chorea) (Pichichero, 1998). *Campylobacter jejuni* has been linked to Guillain-Barré syndrome (GBS), an acute demyelinating neuropathy treated by plasmapheresis or the administration of intravenous immunoglobulin (Buchwald et al., 2002). *C. jejuni* elicits an immune response that cross-reacts with the ganglioside GM1 in host neural tissue (Yuki et al., 2004), and more than 25 percent of individuals with GBS are infected with *C. jejuni*.

Infection with one organism may increase vulnerability to others. HIV/AIDS is an extreme example of this occurrence, in which immunosuppression sets the stage for opportunistic infection with *Toxoplasma gondii*, *Pneumocystis jirovecii*, human herpesvirus 8, or *Cryptococcus neoformans*. This phenomenon was previously described in 1908 when von Pirquet reported that measles was associated with a loss of delayed-type hypersensitivity to tuberculin antigen and suggested that impaired immunity might explain the dissemination of tuberculosis in individuals with measles (von Pirquet, 1908). Infection with one microbe may also directly facilitate local invasion with another. *S. pneumoniae* invasion in influenza, for example, is linked to damage to respiratory tract epithelium and is correlated with the sialidase activity of influenza virus neuraminidase (McCullers and Bartmess, 2003).

The duration of pathogenesis is also a complicating factor; some have both acute and long-term effects. Viruses can express gene products (oncoproteins) that impair cell cycle regulation (Weinberg, 1995) or integrate into the host genome (zur Hausen, 2002) to promote neoplasia. Inflammation associated with persistent bacterial, parasitic, or viral infection has also been implicated in cancer (Mantovani et al., 2008). During vulnerable periods of embryogenesis, any of a variety of agents may cause similar types of structural damage to the central nervous or cardiovascular system, damage that continues long after the infection has cleared. In TORCH syndrome, for example, neurological effects of prenatal infection with *T. gondii*, rubella virus, cytomegalovirus, or herpes simplex virus cannot be distinguished by clinical criteria. In some animal models of autism, schizophrenia, and attention deficit hyperactivity disorder, the neurological effects of prenatal infection with RNA viruses and Gram-negative bacteria

can be recreated by using the double-stranded RNA virus mimic, polyinosine/cytosine (De Miranda et al., 2009), and lipopolysaccharide (Cai et al., 2000), respectively. It is unknown whether sequelae in these examples are mediated by the loss of somatic or stem cells (or both), by altered signaling that impedes the trafficking of cells to their appropriate destinations, or by another mechanism. As these examples demonstrate, it may be that any heuristic is perhaps too stringent if it requires an exclusive relationship between a pathogen and a specific outcome.

Strategies for Pathogen Discovery

Although reviews on pathogen surveillance and discovery typically focus on the latest molecular technologies, it is important to emphasize the pivotal roles of clinical acumen, pathology, serology, and classical culture techniques. Clinicians, veterinarians, and epidemiologists are the prime movers in pathogen discovery. They recognize the appearance of new syndromes, collect materials for investigation, and persuade their colleagues to take up the search. When possible, a comprehensive collection of biological specimens allows for the complementation of various diagnostic and discovery techniques. For example, the use of anatomic pathology can be instrumental in directing downstream molecular work. The discoveries of Nipah virus (Paton et al., 1999) and West Nile virus (Briese et al., 1999; Shieh et al., 2000) were facilitated by demonstration of henipavirus and flavivirus proteins in tissues, which allowed focused genetic analyses. In addition to serology, classical virological methods, such as tissue culture, proved pivotal in the 2003 severe acute respiratory syndrome (SARS) outbreak (Challoner et al., 1995). Propagation of the virus in tissue culture enabled its rapid characterization by a variety of molecular and morphological techniques, including consensus and random PCR, cloning, microarray, and electron microscopy.

Isolation and Visualization of Infectious Agents

Microbe hunters employ a wide range of media and tissue culture systems, including complex organotypic cultures (Braun et al., 2006; Honer zu Bentrup et al., 2006), to isolate and grow prokaryotic and eukaryotic organisms. When these efforts fail, alternative strategies may include inoculation of immature or genetically modified higher organisms that possess innate immune responses that are inefficient or disabled (e.g., newborn [Bowen et al., 1977] and knockout [Glaser et al., 2007] mice) or transgenes that are introduced to express products essential to the entry or replication of viruses (Martina et al., 2006; Ren et al., 1990) or prions (Scott et al., 1989). The choice of an *in vitro* versus an *in vivo* strategy for the isolation of infectious agents can have a profound impact on what one can find. For example, whereas the surveillance of human stool for enteroviruses by the inoculation of suckling mice favors detection of human enterovirus type A, tissue culture favors the detection of human enterovirus type B (Witso et al., 2007).

Although isolation of the agent is highly encouraged (Arrigo et al., 2012), it is not always possible. If the sequence of the pathogen candidate is known, genomic reconstruction can circumvent the need for a viable isolate (Handelsman, 2004). This approach has enabled a new field of archaeovirology wherein infectious retroviruses have been built from endogenous retroviral sequences (Lower et al., 1996), and the 1918 pandemic influenza strain was rebuilt and analyzed for pathogenetic properties (Tumpey et al., 2005).

When an agent cannot be isolated, propagated, or studied through reconstruction, one may nonetheless find evidence of its presence by imaging it morphologically via light or electron microscopy or imaging its proteins or nucleic acids through immunohistochemistry or in situ hybridization, respectively. In some instances, a candidate agent is sufficiently similar to known ones such that available antibodies to the latter are cross-reactive with the former. Indeed, immunohistochemistry has been used not only to confirm the presence of an agent or determine its anatomic distribution, but also as a clue to its identity. Prominent examples include the identification of Sin Nombre virus (Chizhikov et al., 1995), Nipah virus (Paton et al., 1999), and West Nile virus (Briese et al., 1999), for which the screening of tissues from victims of unrecognized infectious diseases with broadly reactive sera led investigators to focus on candidate viral families by consensus PCR.

Molecular Methods

The advent of methods of detecting and cloning nucleic acids of microbial pathogens directly from clinical specimens ushered in a new era in pathogen discovery. Over the past two decades, subtractive cloning, expression cloning, consensus PCR, and high-throughput sequencing resulted in identification of novel agents associated with both acute and chronic diseases, including Borna disease virus, hepatitis C virus, Sin Nombre virus, HHV-6, HHV-8, *Bartonella henselae*, *Tropheryma whippelii*, Nipah virus, SARS coronavirus, and Israel Acute Paralysis virus (Challoner et al., 1995; Chang et al., 1994; Cox-Foster et al., 2007; Lipkin et al., 1990; Nichol et al., 1993; Paton et al., 1999; Peiris et al., 2003; Relman et al., 1990, 1992; VandeWoude et al., 1990).

Singleplex Assays The most common singleplex assays employed in clinical microbiology and microbial surveillance are conventional or quantitative PCR assays. The DNA products of conventional PCR are visualized via ethidium bromide-stained agarose gels, while DNA strand replication in quantitative PCR results in cleavage of a fluorescence-labeled oligonucleotide probe bound to a sequence between the forward and reverse nucleotide primers. Equipment needs are simple (thermal cycler, fluorescent reader, and laptop computer), and rugged instruments have been implemented for field use with battery power. Loop-mediated isothermal amplification (LAMP) does not require programmable

thermal cyclers (Hagiwara et al., 2007; Notomi et al., 2000; Shirato et al., 2007). In laboratory settings, LAMP products are also detected via ethidium bromide–stained agarose gels. However, in the field, changes in the turbidity of the amplification solution may be sufficient, and assays in which the accumulation of product can be detected by eye have been described (Jayawardena et al., 2007).

The most sensitive assays are those for which primers and/or probes perfectly match a single genetic target. Fluorescence reporter–based TaqMan or molecular beacon singleplex PCR assays, for example, typically have detection thresholds of <10 RNA molecules. Although ideal for detecting the presence of a specific agent and for quantitating burden (Heid et al., 1996; Tyagi and Kramer, 1996), these assays may nonetheless fail with RNA viruses characterized by high mutation rates and genetic variability. Consensus PCR assays that use degenerate primers are less likely to be confounded by sequence divergence, but they are less sensitive than specific PCR assays. Furthermore, given that many potential pathogens can overlap in clinical presentation, unless one has the sample mass, resources, and time to invest in many singleplex assays for different agents, there is the risk that a spurious candidate or candidates will be selected. Bacterial 16S rRNA gene assays are increasingly particularly powerful tools, with such seminal contributions as the discovery of *Tropheryma whippeli* (Relman et al., 1992), and are becoming more powerful with the introduction of sequencing technologies that enable the description of microbial communities.

Nested PCR, in which two amplification reactions are pursued sequentially with either one (heminested) or two (fully nested) primers located 3' with respect to the original primer set, may be more sensitive than fluorescence reporter–based singleplex assays. However, because the original reaction vessels must be opened to add reagents for the second nested reaction, the risk for contamination is high, even in laboratories with scrupulous experimental hygiene.

Multiplex Assays Signs and symptoms of disease are rarely pathognomonic of a single agent, particularly early in the course of an illness. Multiplex assays may be helpful in such situations because they may be used to entertain many hypotheses simultaneously. The number of candidates considered ranges from 10 to 100 with multiplex PCR, to thousands with microarrays, and to the entire tree of life with unbiased high-throughput sequencing. In multiplex assays many genetic targets compete for assay components (e.g., nucleotides, polymerases, and dyes), in some instances with variable efficiencies. Thus, multiplex assays tend to be less sensitive than singleplex assays.

Multiplex PCR Assays Multiplex PCR assays are more difficult to establish because primer sets may differ in optimal reaction conditions (e.g., annealing temperature and magnesium concentration). Furthermore, complex primer mixtures are more likely to result in primer–primer interactions that reduce the assay sensitivity and/or specificity. To enable multiplex primer design, we developed

Greene SCPrimer, a software program that automates consensus primer design over a multiple-sequence alignment and allows users to specify the primer length, melting temperature, and degree of degeneracy (Jabado et al., 2006).

Gel-based multiplex PCR assays, wherein products are distinguished by mass, can detect as many as 10 distinct targets (Casas et al., 1997; Templeton et al., 2004). Fluorescence reporter-based multiplex assays are more sensitive but are limited by the number of fluorescent emission peaks that can be unequivocally separated. At present, up to four fluorescent reporter dyes are detected simultaneously. "Sloppy molecular beacons" can address this limitation in part by binding to related targets at different melting temperatures; however, they cannot detect targets that differ by more than a few nucleotides and, thus, their applications are limited.

Two platforms that combine PCR and mass spectroscopy (MS) for the sensitive, simultaneous detection of several targets have been established. The Ibis T5000 biosensor system uses matrix-assisted laser desorption-ionization (MALDI) MS to directly measure the molecular weights of PCR products obtained in an experimental sample and to compare them with a database of known or predicted product weights (Hofstadler et al., 2005; Sampath et al., 2007; Van Ert et al., 2004). MassTag PCR uses atmospheric pressure chemical ionization (APCI) MS to detect molecular weight reporter tags attached to PCR primers. Whereas the Ibis system is confined to specialized laboratories, MassTag PCR can be performed by using smaller, less expensive instruments and does not require sophisticated operators. The Ibis system has an advantage in that it can detect novel variants of known organisms via a divergent product weight; nonetheless, like MassTag PCR, it too requires sequencing for a detailed characterization. Syndrome-specific MassTag PCR panels have been established for the detection of viruses, bacteria, fungi, and parasites associated with acute respiratory diseases, diarrheas, encephalitides/meningitides, and hemorrhagic fevers (Briese et al., 2005; Lamson et al., 2006; Palacios et al., 2006).

The Bio-Plex (also known as Luminex) is a multiplex platform that employs flow cytometry to detect PCR amplification products bound to matching oligonucleotides on fluorescent beads (Brunstein and Thomas, 2006; Han et al., 2006; Li et al., 2007). Assay panels that allow the detection of up to 50 genetic targets simultaneously have been developed.

Although multiplex PCR methods are designed to detect known agents, they can nonetheless facilitate pathogen discovery. For example, the use of MassTag PCR to investigate influenza-like illness in New York State revealed the presence of a novel rhinovirus clade. This discovery enabled follow-up studies across the globe wherein this novel genetic clade was implicated not only in influenza-like illnesses but also in asthma, pediatric pneumonia, and otitis media (Blomqvist et al., 2009; Briese et al., 2008; Dominguez et al., 2008; Khetsuriani et al., 2008; Kistler et al., 2007; Lau et al., 2007; Lee et al., 2007; Renwick et al., 2007; Savolainen-Kopra et al., 2009).

Microarrays Microarray technology has been used to develop assays that comprise hundreds to millions of genetic probes for applications in diagnostics, screening, pathogen identification, and discovery. Probes can be designed to discriminate differences in related sequences of known agents with the purpose of speciation. An example of this application is respiratory virus resequencing arrays, where specific genetic targets are amplified by multiplex consensus PCR and the resultant products are hybridized to oligonucleotide probes less than 25 nucleotides in length (Chiu et al., 2006; Lin et al., 2007; Wong et al., 2004). These arrays are easily implemented when one considers only a limited number of known agents. However, because the signal is dependent on precise complementarity between probes and their genetic targets, these arrays are not ideal for pathogen discovery. In contrast, arrays comprising longer probes (e.g., >60 nucleotides) are more tolerant of sequence mismatches and may detect agents more divergent than those presently known.

Two longer probe array platforms that are in common use are the GreeneChip and the Virochip (Palacios et al., 2007; Wang et al., 2002). Although they differ in design, both employ random amplification strategies to allow an unbiased detection of microbial targets, which is critical to exploiting the broad probe repertoire of these arrays. Recently, Lawrence Livermore National Laboratory developed the Lawrence Livermore Microbial Detection Array, which incorporated into its first design all available viral and bacterial sequences to detect 2,000 viral and 900 bacterial species. A newer version will expand this capability with the goal of detecting nearly 6,000 viruses, 15,000 bacteria, and fungi and protozoa (Gardner et al., 2010). A challenge with each of these platforms is that host and microbe sequences are amplified with similar efficiencies, reducing sensitivity for microbial detection in tissues rich in host genetic material. Host DNA can be eliminated by enzymatic digestion; however, host rRNA remains a major confounder, making these platforms most successful with acellular template sources, such as virus cell culture supernatant, serum, plasma, cerebrospinal fluid, or urine. Methods for depleting host rRNA prior to amplification through subtraction or the use of random primers selected for the lack of complementarity to rRNA have been described (Armour et al., 2009). Whether these interventions will sufficiently enhance sensitivity to enable pathogen discovery in tissues remains to be determined.

At present, hybridization to probes representing pathogen targets is detected by binding of a fluorescent label. However, platforms that will detect hybridization as changes in electrical conductance are in development, which may enhance both ease of use and sensitivity. During a Marburg virus outbreak, the GreeneChip panmicrobial array implicated *Plasmodium falciparum* in a fatal case of hemorrhagic fever that was not resolved using standard diagnostic methods (Palacios et al., 2007), and a variant of the GreeneChip array facilitated the discovery of Ebola virus Reston in a porcine respiratory illness outbreak in the Philippines (Barrette et al., 2009). The Virochip was also successfully employed in the characterization of the SARS coronavirus in 2003 (Wang et al., 2002).

Unbiased High-Throughput Sequencing The power of unbiased high-throughput sequencing has enabled unique advances in microbial surveillance and discovery. Applications include metagenomic characterization of environmental and clinical samples, rapid and comprehensive sequence analysis of microbial strains and isolates, and pathogen discovery. Unlike consensus PCR or microarray methods, whereby investigators are limited by known sequence information and must choose the pathogens to be considered in an experiment, high-throughput sequencing can be unbiased, providing an opportunity to inventory the entire tree of life. Research in our laboratory has chiefly used the 454 Life Sciences pyrosequencing system; however, applications and principles are similar across platforms, including Illumina sequencing by synthesis (SBS) technology and Life Technologies' Ion Torrent semiconductor sequencing technology. The Ion Torrent Personal Genome Machine™ was recently used to sequence the novel Shiga toxin-producing *Escherichia coli* variant responsible for an outbreak with unusually high mortality in Germany in June 2011 (Mellmann et al., 2011). With whole-genome results in only 2 hours, this situation demonstrates the powerful potential of advancements in this technology not only for basic research, but also as a valuable public health tool.

While our laboratory primarily focuses use of unbiased high-throughput sequencing on pathogen discovery, we have also employed primers designed to amplify phyla (e.g., 16S rRNA gene analyses of gastrointestinal flora) or specific viruses (e.g., characterizations of influenza or dengue virus isolates). Similar to obstacles faced with applying microarray technology to unbiased PCR amplification strategies, host nucleic acid can be a critical impediment to the sensitivity of unbiased high-throughput sequencing. The same caveats and potential solutions also apply. After amplification and sequencing, raw sequence reads are clustered into nonredundant sequence sets. Unique sequence reads are assembled into contiguous sequences, which are then compared to databases using programs that examine homology at the nucleotide and amino acid levels considering all six potential reading frames. A truly novel pathogen might elude this level of analysis, thus our laboratory and others are exploring the implementation of additional characteristics to aid in pathogen discovery, including relative nucleotide composition or predicted secondary or tertiary structures.

A Staged Strategy for Pathogen Detection and Discovery

A staged investment strategy for pathogen discovery is important to contain costs, reduce extraneous efforts, and conserve valuable sample materials. When epidemiology, serology, and/or pathology suggest one or a few candidates, singleplex PCR is an ideal approach. Where no such clues pertain or singleplex assays are negative, syndromic multiplex PCR assays allow rapid examination of up to 30 candidates with only a modest increase in time and expense. Microarrays are the next step indicated if multiplex PCR fails to provide a result. Because

each of these methods requires that an agent be related to those already known, novel or distantly related agents may require subtractive cloning or unbiased high-throughput sequencing. Irrespective of the route that results in identification of a candidate, subsequent steps include quantitation of pathogen burden in affected hosts and unaffected controls, detailed characterization of the pathogen for features that may contribute to virulence or provide clues to provenance, and serology to indicate acute versus convalescent infection and examine the prevalence of infection over time and geography. Figure A10-2 depicts the complementary and parallel or progressive use of multiple techniques in a typical strategy for pathogen discovery.

Examples of Pathogen Discovery Related to Food Safety and One Health

As population increases and globalization and trade expand, the modern food supply chain becomes more diverse and complex. In the past few years, our research group has detected a number of novel and known pathogens in animal species that serve as food products, including salmon (Palacios et al., 2010), turkey (Honkavuori et al., 2011), and imported bush meat (Smith et al., 2012). While a direct threat to human health through consumption is not clear, the following examples demonstrate the application of pathogen discovery to monitoring food safety and emphasize the importance of pursuing esoteric events in consumable animal species or those that could potentially threaten independent or commercial food sources.

Salmon: Heart and Skeletal Muscle Inflammation of Farmed Salmon Is Associated with Infection with a Novel Reovirus

Abstract¹⁸

Atlantic salmon (*Salmo salar L.*) mariculture has been associated with epidemics of infectious diseases that threaten not only local production, but also wild fish coming into close proximity to marine pens and fish escaping from them. Heart and skeletal muscle inflammation (HSMI) is a frequently fatal disease of farmed Atlantic salmon. First recognized in one farm in Norway in 1999, HSMI was subsequently implicated in outbreaks in other farms in Norway and the United Kingdom. Although pathology and disease transmission studies indicated an infectious basis, efforts to identify an agent were unsuccessful. Here we provide evidence that HSMI is associated with infection with piscine reovirus (PRV). PRV is a novel reovirus identified by unbiased high-throughput DNA sequencing and a bioinformatics program focused on nucleotide frequency as well as sequence alignment and motif analyses. Formal implication of PRV in HSMI will

¹⁸ Abstract reprinted with proper citation and open access to Palacios et al. (2010).

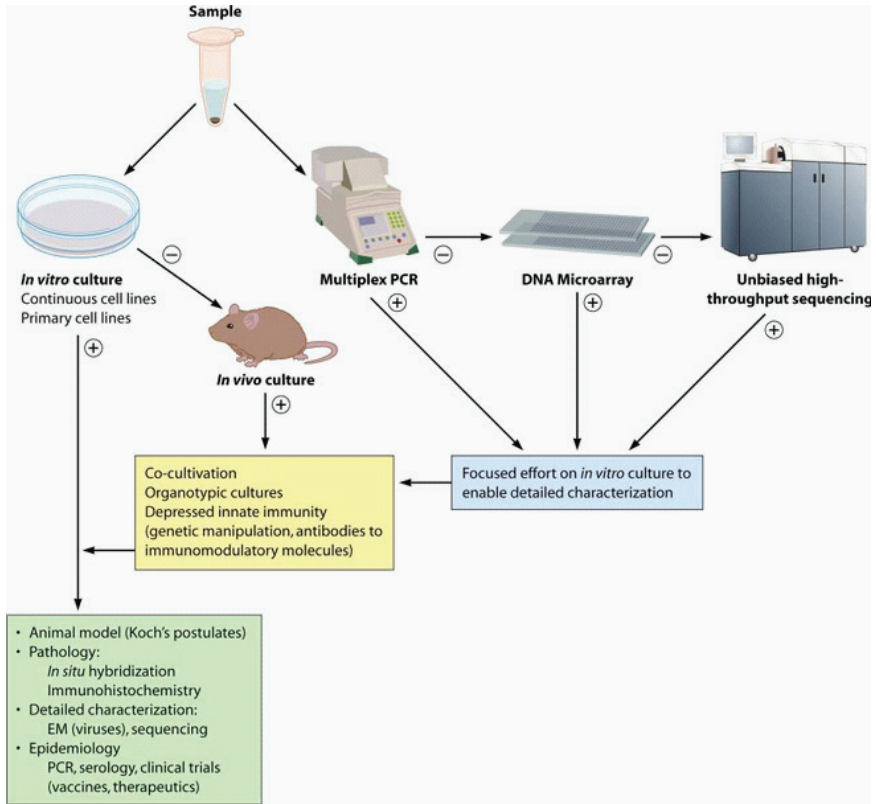


FIGURE A10-2 Staged strategy for pathogen discovery and link to causation. In the molecular era of pathogen discovery, culture and molecular methods are pursued in parallel until an agent is detected, isolated, and characterized. +, positive result; -, negative result. ssRNA, single-stranded RNA; dsRNA, double-stranded RNA.

SOURCE: Lipkin (2010) Copyright © American Society for Microbiology, Lipkin, W. I. (2010). Microbe Hunting. *Microbiology and Molecular Biology Reviews*. 74 (3):363-377; doi:10.1128/MMBR.00007-10. Reproduced with permission from American Society for Microbiology.

require isolation in cell culture and fulfillment of Koch’s postulates, or prevention or modification of disease through use of specific drugs or vaccines. Nonetheless, as our data indicate that a causal relationship is plausible, measures must be taken to control PRV not only because it threatens domestic salmon production but also due to the potential for transmission to wild salmon populations.

Poultry: The Discovery of a Novel Picornavirus in Turkey Poults with Hepatitis in California, U.S.A

Abstract¹⁹

To identify a candidate etiologic agent for turkey viral hepatitis, we analyzed samples from diseased turkey poults from 8 commercial flocks in California, USA, that were collected during 2008-2010. High-throughput pyrosequencing of RNA from livers of poults with turkey viral hepatitis (TVH) revealed picornavirus sequences. Subsequent cloning of the ≈9-kb genome showed an organization similar to that of picornaviruses with conservation of motifs within the P1, P2, and P3 genome regions, but also unique features, including a 1.2-kb sequence of unknown function at the junction of P1 and P2 regions. Real-time PCR confirmed viral RNA in liver, bile, intestine, serum, and cloacal swab specimens from diseased poults. Analysis of liver by in situ hybridization with viral probes and immunohistochemical testing of serum demonstrated viral nucleic acid and protein in livers of diseased poults. Molecular, anatomic, and immunologic evidence suggests that TVH is caused by a novel picornavirus, tentatively named turkey hepatitis virus.

Bushmeat: The Identification of Zoonotic Viruses Associated with Illegally Imported Wildlife Products

Abstract²⁰

The global trade in wildlife has historically contributed to the emergence and spread of infectious diseases. The United States is the world's largest importer of wildlife and wildlife products, yet minimal pathogen surveillance has precluded assessment of the health risks posed by this practice. This report details the findings of a pilot project to establish surveillance methodology for zoonotic agents in confiscated wildlife products. Initial findings from samples collected at several international airports identified parts originating from nonhuman primate (NHP) and rodent species, including baboon, chimpanzee, mangabey, guenon, green monkey, cane rat and rat. Pathogen screening identified retroviruses (simian foamy virus) and/or herpesviruses (cytomegalovirus and lymphocryptovirus) in the NHP samples. These results are the first demonstration that illegal bushmeat importation into the United States could act as a conduit for pathogen spread, and suggest that implementation of disease surveillance of the wildlife trade will help facilitate prevention of disease emergence.

¹⁹ Abstract reprinted with proper citation and open access to Honkavuori et al. (2011).

²⁰ Abstract reprinted with proper citation and open access to Smith et al. (2012).

Future Perspectives

Molecular platforms are rapidly evolving, with enhancements in sensitivity and throughput at a lower cost. Such improvements are facilitating the decentralization of technology such that studies now restricted to a few specialized laboratories will soon be feasible on a global scale and to a broader industry base. This technology transfer will, in turn, circumvent logistical and political issues relating to specimen transfer that can delay informed responses to outbreaks of acute disease, which is a particularly important issue when considering food safety.

With some mature technology, such as multiplex PCR, advances are likely to be incremental. In contrast, microarray technology is less advanced, and predictable, near-term improvements include higher-density arrays, automation, microfluidic sample processing, and alternatives to imaging of results, such as the direct measurement of conductance changes associated with hybridization. Unbiased high-throughput sequencing technology is expected and currently progressing rapidly, and a corresponding need for advancements in data management and bioinformatics are becoming increasingly important with the growing complexity of each of these platforms.

Although significant, this article did not address the emerging fields of proteomics and host response profiling, nor did it discuss new platforms for serology. It is conceivable that biomarkers will be found that are specific for classes of infectious agents and/or provide insights that can guide clinical management. Although less advanced, there are also efforts to develop high-density serological arrays capable of depicting previous microbial exposures to a wide range of pathogens. There is also an increasing appreciation for individualistic responses to infectious agents based on differences in genetic and epigenetic factors, nutritional status, age, exposure history, and simultaneous infections with other microbes. Thus, it is anticipated that many substantive advances may come not from technical improvements but from investments in prospective serial sampling and a shifting perspective that many diseases reflect a more complex and temporal intersection of genes and the environment.

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A11

TRANSMISSION OF HUMAN INFECTION WITH NIPAH VIRUS²¹

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Nipah virus (NiV) is a paramyxovirus whose reservoir host is fruit bats of the genus *Pteropus*. Occasionally the virus is introduced into human populations and causes severe illness characterized by encephalitis or respiratory disease. The first outbreak of NiV was recognized in Malaysia, but 8 outbreaks have been reported from Bangladesh since 2001. The primary pathways of transmission from bats to people in Bangladesh are through

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contamination of raw date palm sap by bats with subsequent consumption by humans and through infection of domestic animals (cattle, pigs, and goats), presumably from consumption of food contaminated with bat saliva or urine with subsequent transmission to people. Approximately one-half of recognized Nipah case patients in Bangladesh developed their disease following person-to-person transmission of the virus. Efforts to prevent transmission should focus on decreasing bat access to date palm sap and reducing family members' and friends' exposure to infected patients' saliva.

Human Nipah virus (NiV) infection was first recognized in a large outbreak of 276 reported cases in peninsular Malaysia and Singapore from September 1998 through May 1999 (Chua, 2003; Chua et al., 2000; Paton et al., 1999). Most patients had contact with sick pigs (Parashar et al., 2000). Patients presented primarily with encephalitis; 39% died (Chua, 2003; Goh et al., 2000). Autopsy studies noted diffuse vasculitis most prominently involving the central nervous system with intense immunostaining of endothelial cells with anti-Nipah virus hyperimmune serum (Chua et al., 2000). The virus, a member of the recently designated genus *Henipavirus*, within the family Paramyxoviridae, was first isolated from a patient from Sungai Nipah village (Chua, 2003; Chua et al., 2000). The human outbreak of Nipah infection ceased after widespread deployment of personal protective equipment to people contacting sick pigs, restriction on livestock movements, and culling over 900,000 pigs (Uppal, 2000).

Large fruit bats of the genus *Pteropus* appear to be the natural reservoir of NiV. In Malaysia the seroprevalence of neutralizing antibodies to NiV in colonies of *Pteropus vampyrus* and *Pteropus hypomelanus* ranged from 7% to 58% (Yob et al., 2001; Daszak et al., 2006). Antibodies against henipaviruses have been identified in *Pteropus* bats wherever they have been tested including Cambodia, Thailand, India, Bangladesh, and Madagascar (Epstein et al., 2004; Hsu et al., 2004; Ihle et al., 2007; Reynes et al., 2005; Wacharapluesadee et al., 2005). NiV was isolated from urine specimens collected underneath a *P. hypomelanus* roost and from partially eaten fruit dropped during feeding activity in Malaysia (Chua et al., 2002), from urine collected underneath a *Pteropus lylei* roost in Cambodia (Reynes et al., 2005), and from saliva and urine of *P. lylei* in Thailand (Wacharapluesadee, 2005). Experimental infection of *Pteropus* bats with NiV does not cause illness in the bats (Middleton et al., 2007). Surveys of rodents and other animals have not identified other wildlife reservoirs for NiV (Hsu et al., 2004; Yob et al., 2001). Over 50 species of *Pteropus* bats live in South and South East Asia (Figure A11-1) (Nowak, 1994). *Pteropus giganteus*, the only *Pteropus* species found in Bangladesh, is widely distributed across the country and frequently has antibody to NiV (Bates and Harrison, 1997; Hsu et al., 2004).

The growth of large intensively managed commercial pig farms in Malaysia with fruit trees on the farm created an environment where bats could drop partially eaten fruit contaminated with NiV laden bat saliva into pig stalls. The pigs

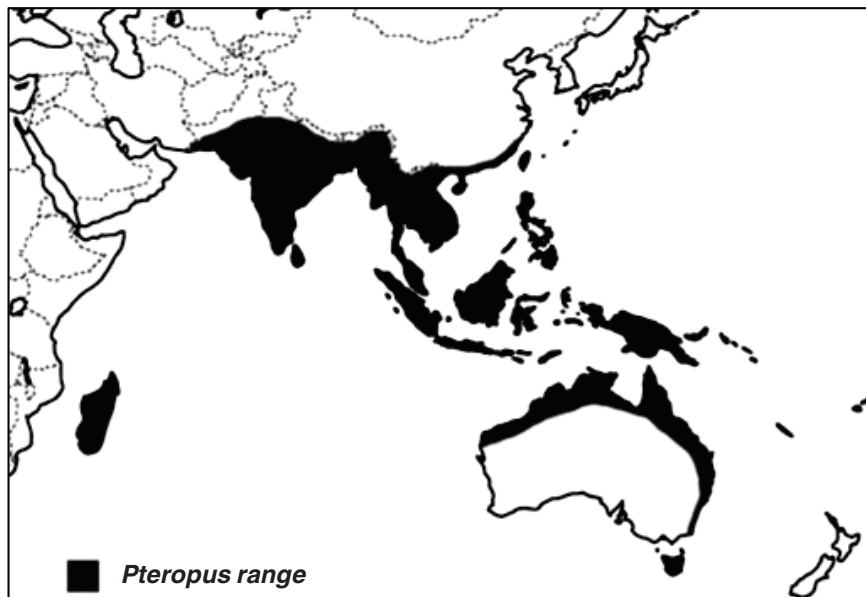


FIGURE A11-1 Range of Pteropus bats based on RM Nowak (Nowak, 1994).

could eat the fruit, become infected with NiV, and efficiently transmit virus to other pigs because of the dense pig population on the farms, frequent respiratory shedding of the virus among infected pigs (Middleton et al., 2002), and the pigs' high birth rate that regularly brought newly susceptible young pigs into the population at risk (Epstein et al., 2006).

Recurrent Outbreaks of NiV Infection in Bangladesh

In the 10 years following the Nipah outbreak in Malaysia, no further human cases of NiV infection have been reported from Malaysia, but 8 human outbreaks of NiV infection in Bangladesh were reported from 2001 through 2008, all occurring between December and May (Gurley et al., 2007; Hsu et al., 2004; ICDDR, 2007; ICDDR, 2008; Luby et al., 2006; Montgomery et al., 2008). A total of 135 human cases of Nipah infection in Bangladesh were recognized; 98 (73%) died. One outbreak of NiV occurred in Siliguri, India, 15 kilometers north of the Bangladesh border in January and February 2001 (Chadha et al., 2006) and a second NiV outbreak was reported by newspapers in Nadia District, India also close to the border with Bangladesh, in 2007 (Mandal and Banerjee, 2007). In addition to the outbreaks, between 2001 and 2007, 17 other NiV transmission events, ranging from single sporadic human cases to clusters of 2–4 human

cases were recognized in Bangladesh (Luby et al., 2009). Thus, in contrast to the Malaysia-Singapore outbreak, which could be coherently explained by a single or perhaps a few transmissions of NiV from an infected bat to pigs, leading to a porcine epidemic which in turn led to a human epidemic (Epstein et al., 2006), in Bangladesh NiV transmission from bats to human is repeated and ongoing.

The diversity of NiV strains recovered from Bangladesh also supports multiple introductions of the virus from bats into human populations even within a single year. Among 4 NiV isolates from human NiV cases in 2004, the sequences of the nucleoprotein open reading frames of the isolates differed by 0.9% in nucleotide homology, in contrast to the sequences obtained from all of the human cases in Malaysia which were nearly identical to each other (AbuBakar et al., 2004; Chan et al., 2001; Harcourt et al., 2005).

The clinical presentation of NiV infection in Bangladesh differed from Malaysia. In Bangladesh, severe respiratory disease is more common, with 62% of cases having cough, 69% developing respiratory difficulty, and available chest radiographs showing diffuse bilateral opacities covering the majority of the lung fields (Hossain et al., 2008). By contrast, in Malaysia, 14% of patients had a non-productive cough on presentation; only 6% of chest radiographs were abnormal and these abnormalities were mild and focal (Goh et al., 2000). The case fatality rate was higher in Bangladesh at 73%, compared with 39% from Malaysia (Goh et al., 2000; Hossain et al., 2008), but much of this difference results from the more sophisticated clinical care provided in Malaysia. One-half of Malaysian Nipah patients received mechanical ventilatory support compared to a single patient (1%) in Bangladesh (Goh et al., 2000) (unpublished data). One third of Nipah survivors in Bangladesh have moderate to severe objective neurological dysfunction 7–30 months after infection (Sejvar et al., 2007).

NiV Transmission from Bats to People

Epidemiological investigations in Bangladesh have identified three pathways of transmission of NiV from bats to people. The most frequently implicated route is ingestion of fresh date palm sap. Date palm sap is harvested from December through March, particularly in west central Bangladesh. A tap is cut into the tree trunk and sap flows slowly overnight into an open clay pot. Infrared camera studies confirm that *P. giganteus* bats frequently visit date palm sap trees and lick the sap during collection (Khan et al., 2008). NiV can survive for days on sugar-rich solutions such as fruit pulp (Fogarty et al., 2008). Most date palm sap is processed at high temperature to make molasses, but some is enjoyed as a fresh juice, drunk raw within a few hours of collection. In the 2005 Nipah outbreak in Tangail District, Bangladesh, the only exposure significantly associated with illness was drinking raw date palm sap (64% of case patients vs 18% of control patients; odds ratio [OR], 7.9; 95% confidence interval [CI], 1.6–38; $P = .01$) (Luby et al., 2006). Twenty-one of the 23 index NiV case patients recognized

in Bangladesh developed their initial symptoms during the December through March date palm sap collection season (Luby et al., 2009).

A second route of transmission for NiV from bats to people in Bangladesh is via domestic animals. Fruit bats commonly drop partially eaten saliva-laden fruit. Domestic animals in Bangladesh forage for such food. Date palm sap that is contaminated with bat feces and so is unfit for human consumption is also occasionally fed to domestic animals. The domestic animals may become infected with NiV, and shed the virus to other animals, including humans. Contact with a sick cow in Meherpur, Bangladesh in 2001 was strongly associated with Nipah infection (OR, 7.9; 95% CI, 2.2–27.7; $P = .001$) (Hsu et al., 2004). A pig herd visited the community two weeks before the 2003 Nipah outbreak in Naogaon and contact with the pigs was associated with illness (OR, 6.1; 95% CI, 1.3–27.8; $P = .007$) (Khan et al., 2008). In 2004, one family explained that they owned 2 goats that their son frequently played with. The goats became ill with fever, difficulty walking, walking in circles, and frothing at the mouth. The parents believe their son had contact with goat saliva while the goats were ill. Both goats died. Within 2 weeks of the goats' death, the child developed encephalitis that was confirmed to be Nipah by antibody testing (unpublished data). Third, some people may come into direct contact with NiV-infected bat secretions. In the Goalando outbreak in 2004, persons who climbed trees were more likely to develop NiV infection than were control patients (OR, 8.2; 95% CI, 1.3– ∞) (Montgomery et al., 2008).

Person-to-Person Transmission

Several Bangladesh Nipah outbreaks resulted from person-to-person transmission. The clearest illustration of person-to-person NiV transmission occurred during the Faridpur outbreak in 2004 (Gurley et al., 2007a). Four persons who cared for the index patient—his mother, his son, his aunt, and a neighbor—became ill 15–27 days after the index patient first developed illness (Figure A11-2). During her hospitalization, the index patient's aunt was cared for by a popular religious leader who lived in a nearby village and who became ill 13 days later. When the religious leader became seriously ill, many of his relatives and members of his religious community visited at his home. Twenty-two persons developed Nipah infection after contact with the religious leader. One of these followers moved to his family's house in an adjacent village to receive care after becoming ill where he was cared for by a friend and 2 family members. These 3 caregivers and a rickshaw driver, who helped carry him to the hospital as his condition deteriorated, became ill. Ultimately, the chain of transmission involved 5 generations and affected 34 people (Gurley et al., 2007a) (Figure A11-2). Physical contact with an NiV-infected patient who later died (OR, 13.4; 95% CI, 2.0–89) was the strongest risk factor for developing NiV infection in the outbreak.

The transmission pattern in Faridpur is not unique. For example, in 2007 in Thakurgaon, 6 family members and friends who cared for an NiV-infected patient

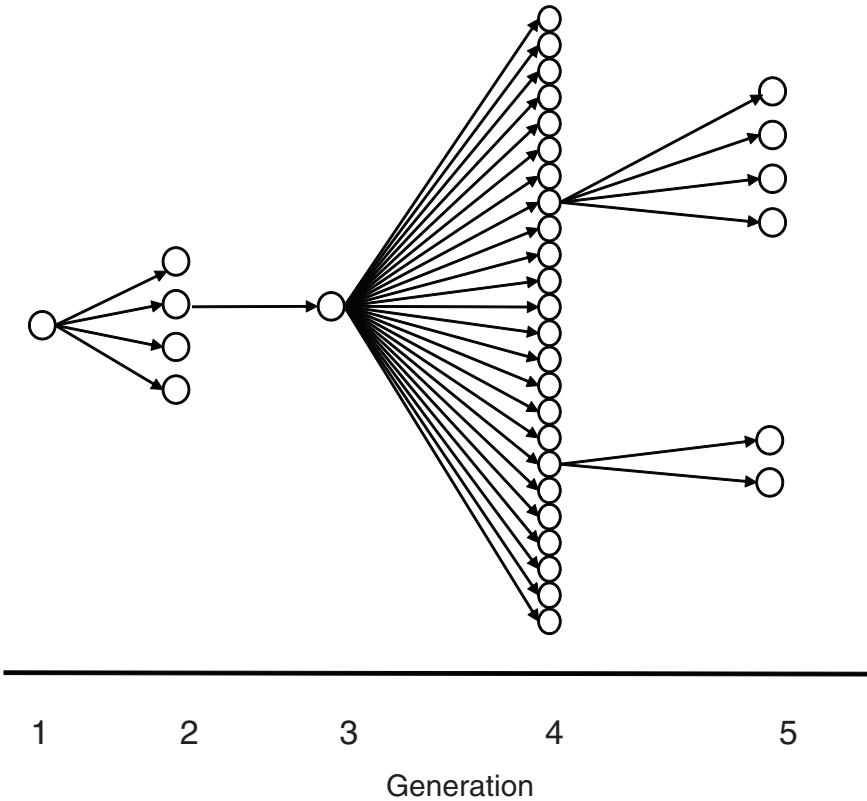


FIGURE A11-2 Chain of person to person transmission in Nipah outbreak, Faridpur, Bangladesh, 2004.

developed Nipah infection. Case patients were more likely than control patients to have been in the same room when the index case was coughing (ICDDR, 2007). In a review of the 122 Nipah case patients identified in Bangladesh from 2001 through 2007, 62 (51%) developed illness after close contact with another Nipah patient (Luby et al., 2009). A small minority of patients infected with NiV (ie, 9 [7%] of 122 recognized cases) transmitted NiV to 62 other persons.

Respiratory secretions appear to be particularly important for person-to-person transmission of NiV. NiV RNA is readily identified in the saliva of infected patients (Chua et al., 2001; Harcourt et al., 2005). Anthropological investigations during the Faridpur outbreak highlighted multiple opportunities for the transfer of NiV contaminated saliva from a sick patient to care providers (Blum et al., 2009). Social norms in Bangladesh require family members to maintain close physical contact during illness. The more severe the illness, the more hands-

on care is expected. Family members and friends without formal health care or infection control training provided nearly all the hands on care to Nipah patients both at home and in the hospital (Hadley et al., 2007). Care providers during the Faridpur outbreak continued to share eating utensils and drinking glasses with sick patients. Leftovers of food offered to ill Nipah patients were commonly distributed to other family members. Family members maintained their regular sleeping arrangements, which often involved sleeping in the same bed with a sick, coughing Nipah patient. There was a particularly strong desire to have close physical contact near the time of death, demonstrated by such behaviors as cradling the patients head on the family member's lap, attempting to give liquids to the patient with a spoon or glass between bouts of coughing, or hugging and kissing the sick patient (Blum et al., 2009). In both the Faridpur outbreak in 2004 and the Thakurgaon outbreak in 2007, persons who were in a room when a Nipah patient was coughing or sneezing were at increased risk of Nipah virus infection (Gurley et al., 2007a; ICDDR, 2007). Across all recognized outbreaks in Bangladesh from 2001 through 2007, Nipah patients with respiratory symptoms were more likely to transmit Nipah (Luby et al., 2009).

The capacity for NiV to spread in hospital settings to both staff and patients was clearly illustrated in a large outbreak affecting 66 people in Siliguri, India in 2001. The outbreak apparently originated from an unidentified patient admitted to Siliguri District Hospital who transmitted infection to 11 additional patients, all of whom were transferred to other facilities. In 2 of the facilities, subsequent transmission infected 25 staff and 8 visitors (Chadha et al., 2006). However, transmission to health care workers is rarely recognized. Among a cohort of 338 health care workers who cared for Nipah patients at 3 Malaysian hospitals and reported a combined 89 episodes of Nipah patient blood or body fluid directly contacting their bare skin, 39 splash exposures of blood or body fluid into their eyes, nose or mouth, and 12 needle stick injuries, none developed clinical illness associated with Nipah infection (Mounts et al., 2001). Health care workers in Bangladesh have much less direct physical contact with patients than in western hospitals (Hadley et al., 2007). Hands-on care is generally provided by family members and friends. No health care workers in Bangladesh who cared for identified Nipah patients have been identified with illness, although confirmed cases include 1 physician whose source of infection is unknown. A serosurvey among 105 health care workers who cared for at least 1 of 7 patients admitted with Nipah infection at one hospital in Bangladesh identified 2 health care workers with serological evidence of NiV infection; however, their antibody responses (IgG only, no IgM) and lack of symptoms suggest a previous infection, not recent nosocomial transmission (Gurley et al., 2007b).

Might person-to-person transmission be associated with particular strains of NiV that have genetic characteristics that lead to person-to-person transmission? The closely related strains in Malaysia resulted in less frequent and less severe respiratory disease than observed in Bangladesh and were not associated

with frequent person-to-person transmission. However, the pattern of the outbreaks in Bangladesh and India suggests that person-to-person transmission is more dependent on the characteristics of the occasional Nipah transmitter than a specific strain. If the NiV strain was central to person-to-person transmission, then secondary cases of NiV would be more likely to become NiV transmitters, than primary cases (because secondary cases would already have selected for strains predisposed to person-to-person transmission). However, in the review of 7 years of human Nipah infection in Bangladesh, secondary cases were no more or less likely to become Nipah transmitters than were primary cases (Luby et al., 2009). All persons who transmitted Nipah died, suggesting that late stages of infection, presumably with high virus titers, increases the risk of transmission. Even the pattern in Siliguri, the largest recognized Nipah outbreak from apparent person-to-person transmission, is consistent with the review of 7 years of human Nipah infection in Bangladesh. The unidentified index case in Siliguri District Hospital infected 11 patients, 2 of whom infected an additional 33 patients. The 13 day duration of the outbreak at Medinova Hospital suggests 2 generations of transmission likely occurred there. Taken together, this pattern suggests 4 NiV transmitters propagated human infection across 4 generations. There were 67 cases (66 recognized plus the unidentified index case), 4 (5.9%) of whom became Nipah transmitters, a proportion very close to the 7% recognized in Bangladesh. This suggests that the virus strain responsible for this largest recognized person-to-person outbreak was not exceptional. Its rate of secondary transmission was similar to other strains circulating in South Asia.

Exposures Not Associated with NiV Transmission

Outbreak investigations have both identified important routes of transmission of human NiV infection, and identified exposures not associated with transmission. NiV was recovered from the urine of *Pteropus* bats in Malaysia, Cambodia, and Thailand (Iehle et al., 2007; Reynes et al., 2005; Wacharapluesadee et al., 2005). In Bangladesh, *P. giganteus* bats live in close proximity to human populations, often roosting in trees located in rural Bangladeshi villages. Thus, bat urine, intermittently laced with NiV, contaminates the immediate physical environment in many villages in Bangladesh. Yet in each of the 8 Nipah outbreaks investigated in Bangladesh, an association between living near a bat roost and infection with Nipah was looked for but was never found. This suggests that the quantity of viable virus shed in bat urine is too low to initiate clinically apparent infection in humans.

Eating bat-bitten fruit is often suggested as a pathway of transmission for human Nipah infection. NiV was recovered from fruit dropped by *Pteropus* bats in Malaysia (Chua et al., 2002). It is the most commonly suggested pathway for NiV transmission from bats to domestic animals. In contrast to general environmental contamination with urine, punctured fruit contaminated with bat saliva

may favor virus survival. In Bangladesh where 43% of children under the age of 5 years meet the World Health Organization standards for chronic malnutrition (NIPORT, 2007), little food is wasted. In outbreak investigations, villagers, especially children, commonly report consuming fruit which was partially eaten by bats. However, in the 6 NiV outbreak investigations where the question was asked, case patients never reported consuming partially eaten fruit significantly more than did controls.

Unanswered Questions

Did outbreaks of human NiV infection occur in Bangladesh before the first outbreak was recognized in 2001? Almost certainly. *P. giganteus* are widely distributed across Bangladesh (Nowak, 1994), and wherever *Pteropus* bats have been tested they have antibody to henipavirus (Epstein et al., 2008; Hsu et al., 2004; Ihle et al., 2007; Reynes et al., 2005; Wacharapluesadee et al., 2005). When *Pteropus* bats are experimentally infected with NiV they do not become clinically ill (Middleton et al., 2007), which suggests that NiV likely coevolved with its *Pteropus* hosts over millennia. Bangladesh has long been densely populated, and date palm sap harvesting is an old profession using techniques and simple tools that are passed on from father to son. Moreover, people frequently die in Bangladesh of unknown causes, often outside of hospitals. Three factors that have contributed to recognition of Nipah outbreaks recently include development of diagnostic tests for Nipah infection following the Malaysian outbreak, expansion of surveillance for a range of communicable disease by the government of Bangladesh, and expansion of news media coverage in rural Bangladesh.

Unanswered questions regarding Nipah transmission include the following: (1) Why is respiratory disease and person-to-person transmission more common among human NiV infection in Bangladesh compared to Malaysia? Are certain strains of virus more likely to cause respiratory tract disease in humans, or might the different clinical syndromes in Bangladesh and Malaysia reflect differences in host susceptibility from malnutrition or other causes? (2) How stable is the genome of Nipah? The overall nucleotide homology between a prototypical Malaysian strain of NiV and a strain of NiV from Bangladesh was 91.8% (Harcourt et al., 2005). Is there a substantial risk of mutation that would improve the efficiency of person-to-person transmission of the virus? (3) How common is unrecognized, including subclinical, infection with NiV?

Prevention Strategies

The epidemiology of NiV transmission in Bangladesh suggests two avenues to prevent human disease. The first is limiting exposure of Bangladeshi villagers to NiV contaminated fresh date palm sap. Date palm sap collection provides critical income to low-income collectors and is a seasonal national delicacy enjoyed

by millions every year. Steps to make the date palm sap consumption safer include diverting more of the production to molasses where the sap is cooked at temperatures above the level that NiV can survive and limiting bat access to date palm trees where the sap will be consumed fresh. A number of methods have been occasionally employed by date palm sap collectors to restrict bat access to date palm trees (Nahar et al., 2008). We are currently evaluating the effectiveness and scalability of these methods.

A second area for targeted intervention is reducing the exposure of caretakers to the saliva of seriously ill persons. When a Nipah outbreak is recognized, it is appropriate to implement standard precautions (Siegel et al., 2007), but recommendations to improve infection control practices more broadly in Bangladesh must consider the social and health care context in the country, where (1) the annual total per capita spending on health is \$12 per person per year (Health Economics Unit MoHaFW, 2007); (2) over 99% of respiratory disease and over 99% of acute meningoencephalitis in Bangladesh is not caused by Nipah; (3) most of the people who contract Nipah are dead by the time the diagnosis is considered by local practitioners; and (4) even in the hospital setting most hands-on care is provided by family members, not health care professionals. If we recommend an unachievable level of infection control practices for persons caring for pneumonia and acute meningoencephalitis patients from rural communities in Bangladesh, we will not reduce the risk of person-to-person transmission of NiV in Bangladesh. An important research priority is to identify approaches that can be consistently implemented in these low income settings where family members are caring for patients with severe respiratory and neurological disease. For example, family members who washed their hands with soap after caring for Nipah patients were significantly less likely to become infected (Gurley et al., 2007b). If such practices were widely adopted, they would lessen the risk of person-to-person transmission of NiV and other pathogens.

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A12

DATE PALM SAP LINKED TO NIPAH VIRUS OUTBREAK IN BANGLADESH, 2008²⁴

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Abstract

Introduction: We investigated a cluster of patients with encephalitis in the Manikgonj and Rajbari Districts of Bangladesh in February 2008 to determine the etiology and risk factors for disease.

Methods: We classified persons as confirmed Nipah cases by the presence of immunoglobulin M antibodies against Nipah virus (NiV), or by the presence of NiV RNA or by isolation of NiV from cerebrospinal fluid or throat swabs who had onset of symptoms between February 6 and March 10, 2008. We classified persons as probable cases if they reported fever with convulsions or altered mental status, who resided in the outbreak areas during that period, and who died before serum samples were collected. For the case–control study, we compared both confirmed and probable Nipah case-patients to controls, who were free from illness during the reference period. We used motion-sensor-infrared cameras to observe bat’s contact of date palm sap.

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Results: We identified four confirmed and six probable case-patients, nine (90%) of whom died. The median age of the cases was 10 years; eight were males. The outbreak occurred simultaneously in two communities that were 44km apart and separated by a river. Drinking raw date palm sap 2–12 days before illness onset was the only risk factor most strongly associated with the illness (adjusted odds ratio 25, 95% confidence intervals 3.3–N, $p < 0.001$). Case-patients reported no history of physical contact with bats, though community members often reported seeing bats. Infrared camera photographs showed that *Pteropus* bats frequently visited date palm trees in those communities where sap was collected for human consumption.

Conclusion: This is the second Nipah outbreak in Bangladesh where date palm sap has been implicated as the vehicle of transmission. Fresh date palm sap should not be drunk, unless effective steps have been taken to prevent bat access to the sap during collection.

Introduction

Nipah virus (NiV) causes encephalitis in humans and has a high fatality rate (Hossain et al., 2008; Luby et al., 2009). Species of fruit-bats in the *Pteropus* genus are the presumed natural reservoirs of NiV. NiV has been isolated and/or NiV RNA has been identified in bats in Malaysia (Chua et al., 2002; Rahman et al., 2010), Cambodia (Reynes et al., 2005), and Thailand (Wacharapluesadee et al., 2005). Researchers identified antibodies against NiV in *Pteropus* fruit-bats in Malaysia, India, and Bangladesh (Yob et al., 2001; Hsu et al., 2004; Epstein et al., 2008). There were seven recognized Nipah outbreaks in Bangladesh from 2001 to 2007 (Luby et al., 2009) (Figure A12-1). Investigators implicated various routes of transmission in those outbreaks, including person-to-person transmission, drinking raw date palm sap, and contact with sick animals (Luby et al., 2006, 2009; Gurley et al., 2007).

Surveillance for human Nipah infection has been ongoing since 2006 in six hospitals serving communities in the northwestern districts of Bangladesh where previous Nipah outbreaks have been reported (Figure A12-1). The surveillance has been conducted by the Institute of Epidemiology, Disease Control and Research (IEDCR), in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

On February 26, 2008, government health workers reported that one child from Manikgonj District died with illness characterized by fever, generalized body ache, cough, difficulty breathing, and mental status changes. Another two siblings from the same household were admitted to a subdistrict healthcare facility in Manikgonj District with similar symptoms. Both of them were referred to the District Hospital for more advanced medical care; one of them died on the way to the referral hospital and the other child was taken to a private hospital in Dhaka District, where the child died on the following day. On February 27,

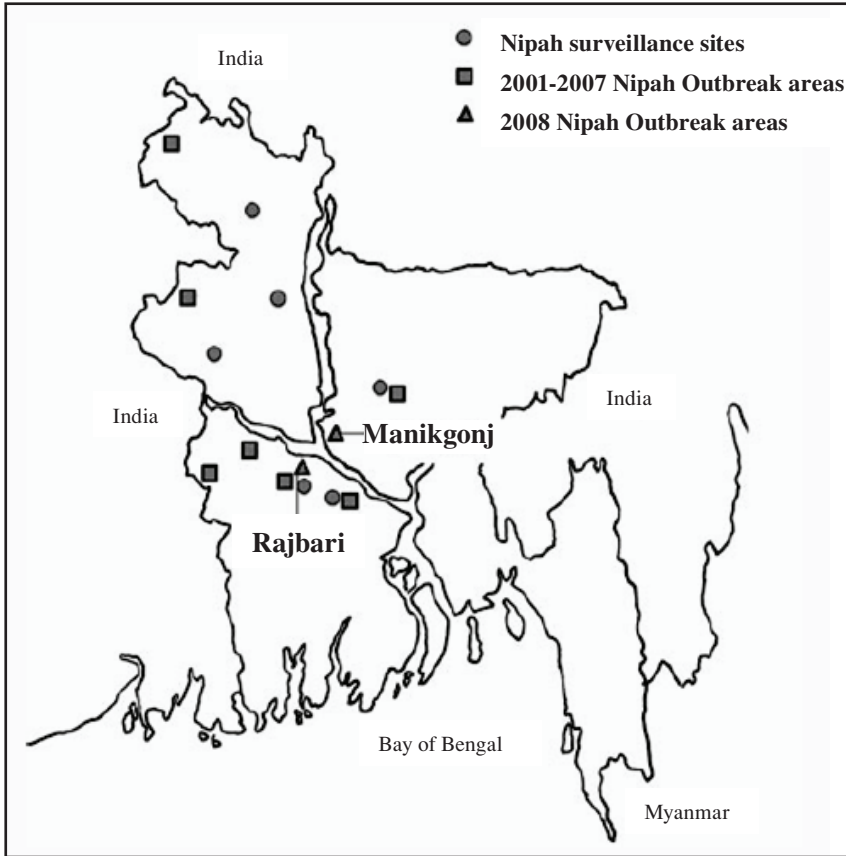


FIGURE A12-1 Bangladesh map showing location of Nipah surveillance sites, previous Nipah outbreak areas, and February 2008 outbreak areas of Bangladesh.

2008, a Nipah Surveillance physician from the Rajbari District reported another cluster of encephalitis cases. Four patients from the same village were admitted to the Rajbari District Hospital; another patient from the same area died before reaching the hospital and another patient from the neighboring village died upon reaching the hospital. All of them presented with fever, generalized weakness, cough or respiratory distress, progressive mental status changes, and unconsciousness.

IEDCR initiated an investigation of both clusters in collaboration with ICDDR,B on the day they received the reports. The objectives of the investigation were to determine the cause of the outbreak, identify risk factors for illness, and develop strategies for prevention.

Materials and Methods

Study Site and Study Population

The communities affected by the outbreak were located in Doulatpur Upazila (subdistrict) (population 155,674) of Manikgonj District and Baliakandi Upazila (population 186,562) of Rajbari District in central-western region of Bangladesh (Bangladesh Bureau of Statistics, 2010). These sites are located 44km apart and are separated by a river (Figure A12-1). Date palm sap is widely harvested, sold, and consumed in both areas.

Case Identification and Sample Collection

The investigation team sought for suspect cases who had fever and convulsions or altered mental status in the outbreak areas between February 6 and March 10, 2008 (Figure A12-2). First, we visited the hospitals where the patients were treated. We identified the suspect case-patients and interviewed them or their proxy respondents. Then we searched for additional case-patients in the affected villages. We collected blood, cerebrospinal fluid (CSF), and throat swab samples from all the living suspect case-patients. The serum and CSF samples were aliquoted locally. The samples were transported to ICDDR, B in cold pack or in liquid nitrogen for storage in -70°C freezer.

We classified persons as confirmed Nipah case-patients by the presence of immunoglobulin M (IgM) antibodies against NiV or by the presence of NiV RNA or by isolation of NiV from CSF or throat swabs. The probable cases were defined as suspect cases who died before sample collection or who had no IgM against NiV in serum collected within 8 days of onset of illness and who died before a follow-up serum sample could be obtained.

Case–Control Study

The investigation team returned to the outbreak communities to conduct a case–control study from March 5 to 10, 2008, to determine the risk factors for illness. We enrolled all confirmed and probable case-patients as cases. Individuals who lived in the same communities as the cases, and who were close in age and were free from any febrile illness with convulsions or altered mental status between February 6 and March 10, 2008, were eligible to be enrolled as controls. We identified controls by visiting the fourth closest house to the case-patient's, confirming that no one in the house met the case definition, and identifying the household resident closest in age to the case-patient. We enrolled only one control per household. If the household resident closest in age to the case-patient declined to participate in the study; no other person in the household was sought as a control. This process was repeated at the next closest household until four controls were enrolled for each case-patient.

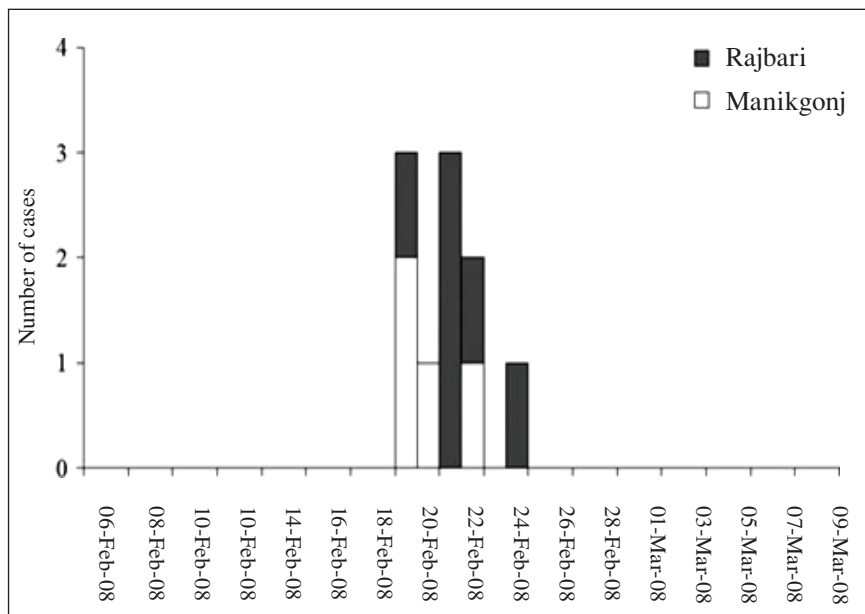


FIGURE A12-2 Date of illness onset from both clusters occurred over 6 days during February 2008, Manikgonj and Rajbari Districts, Bangladesh.

Data Collection

Trained interviewers collected information from cases and controls using a standardized structured questionnaire in Bengali language, based upon the questionnaires used in previous Nipah outbreak investigations in Bangladesh. We collected a detailed exposure history to previously identified risk factors for cases and respective controls for 1 month preceding the onset of illness of cases. For each case-patient who had died or was unable to respond and for each of the controls who were < 10 years of age, we identified proxy respondents. Proxy respondents included spouses, family members, friends, and neighbors who were knowledgeable about the illness or the exposures of the case-patients and controls. We also conducted informal interviews with several date palm sap collectors and local community residents about the date palm sap collection procedure, recognition of bats in the areas, and possible contamination of date palm sap by bats. We used global positioning system to determine the location of the outbreak areas.

Laboratory Analysis

Serum and CSF samples were tested for IgM and IgG antibodies against NiV using IgM-capture and indirect IgG enzyme immunoassay (Daniels et al., 2001).

CSF and throat swab specimens from five patients were tested at the U.S. Centers for Disease Control and Prevention (CDC) laboratory for molecular detection and virus isolation of NiV. Real-time RT-polymerase chain reaction (rRT-PCR) was performed using the following primers that amplified a 112-nucleotide (nt) fragment spanning from position 538 to 660 in the NiV N gene: forward primer NVBNF2B-5'-CTGGTCTCTGCAGTTAT CACCATCGA-3', reverse primer NVBN593R 5'-ACGTA CT TAGCCCATCTTCTAGTTTCA-3', and probe NVBN542P 5'-CAGCTCCCGACACTGCCGAGGAT-3', with the FAM dye incorporated at the 5' end and a BHQ1 molecule at the 3' end. PCR products were sequenced as previously described (Chadha et al., 2006), and were analyzed using Sequencher 4.10.1 software (Gene Codes).

Date Palm Sap Evaluation

The field team also collected date palm sap early in the morning from both the outbreak areas from February 27 to March 5, 2008. Two separate aliquots for a sample were collected from a tree: one in viral transport medium and another in trizol. The sap specimens were stored in a cold box maintaining temperature around 2°C–8°C and transferred to liquid nitrogen within several hours and later stored in –70°C. The sap was tested at CDC for the presence of NiV RNA by rRT-PCR; the sap was also cultured for NiV.

Infrared Camera Observation

We identified seven date palm trees where sap was collected for the cases' consumption in Manikgonj and Rajbari District outbreak sites. To identify the possible contamination of date palm sap by bats' secretions and to understand bat sap contamination behavior, we mounted one motion-sensor-infrared camera focusing on date palm trees' shaved surface, sap stream, tap, and collection pot in each of the seven trees for a full night (5:00 PM to 6:00 AM).

Data Analysis

We used exact logistic regression to estimate the univariate odds ratios (ORs) with 95% confidence intervals (CIs) between exposures and case status. We stratified on the case–control pairs to account for the matched design. We assessed for confounding by constructing a multivariate exact logistic regression model. We included all exposures during multivariate analysis that had ($p < 0.20$) in the initial model and removed those exposures one at a time that were not significantly associated with case status. We performed all statistical analyses with STATA version 10.0.

Ethics

Interviewers obtained voluntary informed consent from all participants or proxies; for those <18 years of age, the team obtained individual assent as well as parental consent. This investigation was part of an emergency response to the outbreak, and so a complete human subjects review of all activities was not possible, but the Ethical Review Committee at ICDDR,B had previously reviewed and approved a general protocol for Nipah surveillance and response to outbreaks.

Results

The outbreak occurred in two adjoining Districts of Manikgonj and Rajbari over the same 6-day period in February 2008 (Figure A12-2). We identified a total of 10 case-patients: 4 from Manikgonj and 6 from Rajbari Districts. Nine of them died (90%); one 12-year-old child from Rajbari survived. The median age of all case-patients was 10 years, and eight (80%) were males. All of the cases presented with fever, progressive altered mental status, and loss of consciousness. The mean duration from illness onset to death was 6 days (Table A12-1).

CSF specimens were available from five (50%) case-patients and serum specimens were available from six (60%). Four patients died before the investigation team could collect any specimen. The field team was able to collect a second set of serum specimens from three case-patients (30%) within 1–6 days of first sample collection. There were four (40%) confirmed and six (60%) probable case-patients. Table A12-2 shows the laboratory results of each case-patient.

Sequencing of the complete NiV nucleoprotein (N) ORF amplified from these two isolated viruses from conventional two-step RT-PCR indicated an identical match. Complete genome sequence analysis of the two isolates confirmed that the two viruses were identical (Lo et al., 2011). The N ORF sequence shared nt sequence identity at all but seven positions with NiV isolated from India in 2007 (accession FJ513078), and at all but 10 positions with NiV isolated from Bangladesh in 2004 (accession AY988601). The amino acid sequence of N ORF differs at only one position from the 2007 Indian (R211/Q) and the 2004 Bangladesh (D188/E) isolates (Table A12-3).

We enrolled 40 controls for the 10 case-patients in the case-control study. All case-patient interviews were conducted by proxies as nine patients had died before the investigation began, and the final child was still recovering from the illness and was unable to communicate with us. We also identified proxies for 19 (48%) of the controls who were <10 years old. None of the selected cases, controls, or proxies refused to participate in the study.

In both outbreak areas, all of the case-patients drank fresh raw date palm sap 2–12 days before onset of their illness compared with 10 (25%) controls who consumed fresh date palm sap during the period of investigation ($p < 0.001$). Household members of case-patients were more frequently involved in date palm sap harvesting than household members of controls (30% vs. 3%, $p < 0.05$).

TABLE A12-1 Profiles of the Case-Patients in February 2008 Nipah Outbreaks, Manikgonj and Rajbari Districts, Bangladesh

Characteristics	Total, n=10; no. (%)
Age	
Mean (SD) in years	19 (17)
Median (range) in years	10 (7-55)
Male	8 (80)
Clinical features	
Fever	10 (100)
Altered mental status	10 (100)
Loss of consciousness	10 (100)
Convulsions	0 (0)
Severe weakness	9 (90)
Muscle pain	6 (60)
Headache	5 (50)
Cough	5 (50)
Difficulty in breathing	4 (40)
Diarrhea	3 (30)
Joint pain	3 (30)
Vomiting	2 (20)
New onset of seizures	1 (10)
Died	9 (90)
Onset of illness to death (n=9)	
Mean (range) in days	6 (1-10)
Category of cases	
Confirmed Nipah	4 (40)
Probable Nipah	6 (60)

TABLE A12-2 Laboratory Results of Case-Patients in February 2008, Manikgonj and Rajbari Districts, Bangladesh

Case-patients			Sampling days after illness onset	CSF				Sampling days after illness onset	Serum-1		
No	Site	Category		IgM	IgG	Isolation	PCR		IgM	IgG	Isolation
1	R	Confirmed	9	Neg	Neg	Neg	Neg	5	Pos	Neg	ND
2	R	Confirmed	6	Neg	Neg	Neg	Neg	6	Neg	Neg	ND
3	R	Confirmed	3	Neg	Neg	Neg	Neg	3	Neg	Neg	ND
4	M	Confirmed	6	Neg	Neg	Neg	Neg	6	Pos	Neg	NA
5	R	Probable	8	Neg	Neg	Neg	Neg	8	Neg	Neg	ND
6	R	Probable	NA	NA	NA	NA	NA	4	Neg	Neg	ND
7	R	Probable	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	M	Probable	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	M	Probable	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	M	Probable	NA	NA	NA	NA	NA	NA	NA	NA	NA

R, Rajbari; M, Manikgonj; IgM, immunoglobulin M; IgG, immunoglobulin G; PCR, polymerase chain reaction; Neg, negative; Pos, positive; NA, not available; ND, not done; CSF, cerebrospinal fluid.

TABLE A12-3 Nucleotide Differences Between Nipah Virus Isolated from Bangladesh (2004, 2008) and India (2007)

Nipah virus isolate ID	Nucleotide position													
	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	5	5	6	6	6	0	1	2	3	4	5	5	
	7	4	6	3	3	5	7	1	6	1	9	0	3	
	0	9	4	2	3	4	7	9	9	1	7	0	3	
NIVBGD2004RAJBARI1 AY988601	T	C	T	A	A	A	T	G	A	A	C	A	A	
NIVBGD2008MANIKGONJ	C	A	G	.	G	G	.	A	G	G	T	.	G	
NIVBGD2008RAJBARI	C	A	G	.	G	G	.	A	G	G	T	.	G	
NIVIND2007NADIA FJ513078	C	A	G	G	.	.	C	A	G	.	T	G	.	

NIVBGD2004RAJBARI1 AY988601 serves as consensus sequence by which to compare the others.

“.” Indicates nucleotide identity with consensus sequence.

TABLE A12-4 Bivariate Analysis of Exposures for Nipah Virus Infection in February 2008, Manikgonj and Rahbari Districts, Bangladesh

Exposures	Case patients with this exposure, n = 10; no. (%)	Control patients with this exposure, n = 40; no. (%)	p-Value	Odds ratio	95% confidence intervals
Drank raw date palm sap	10 (100)	10 (25)	0.000	38	5.4-∞
Date sap					
Given	6 (60)	4 (10)	0.656	2.2	0.3-19
Purchased					
From local vendor	0 (0)	1 (3)	0.429	Undefined	Undefined
From any house	1 (10)	2 (5)	0.486	1.0	0-39
From any other sources	3 (30)	0 (0)	0.143	1.0	0-∞
Collected from tree	0 (0)	3 (8)	0.211	0.2	0-2.1
Date sap harvesting					
Self	0 (0)	3 (8)	1.000	1.1	0-10
Household members	3 (30)	1 (3)	0.022	15	1.0-860
Climbed trees					
Fruit trees	5 (50)	9 (23)	0.099	3.2	0.5-351
Date palm trees	0 (0)	4 (10)	0.549	0.4	0-4.3
Physical contact with living animals					
Cow	4 (40)	17 (43)	1.000	0.9	0.2-4.5
Cat	6 (60)	4 (10)	0.002	11	1.9-84
Physical contact with sick animals					
Cow	0 (0)	1 (3)	1.000	3.9	0-152
Chicken	0 (0)	1 (3)	1.000	4.1	0-159
Physical contact with dead animals (chicken)	1 (10)	0 (0)	0.200	4.2	0.1-∞
Ate sick animals	0 (0)	3 (8)	1.000	1.0	0-11
Ate dropped fruit					
Banana	0 (0)	1 (3)	1.000	11	0-429
Local plum (Boroi)	5 (50)	24 (60)	0.389	0.5	0.1-3.9
Papaya	0 (0)	4 (10)	1.000	Undefined	Undefined
Carambola (Kamranga)	1 (10)	1 (3)	0.250	2.0	0.1-∞
Guava	0 (0)	13 (33)	0.435	0.3	0-13
Tamarind	4 (40)	11 (28)	1.000	1.0	0-66
Custard apple	1 (10)	0 (0)	0.333	Undefined	Undefined
Traveled outside subdistrict	1 (10)	6 (15)	1.000	0.6	0-6.3
Touched persons with fever and altered mental status who died later	1 (10)	0 (0)	0.184	4.1	0.1-∞
Been in the same room with persons with fever and altered mental status who died later	2 (20)	3 (8)	0.258	2.9	0.2-30

Bolded type indicates significant results.

The median incubation period from intake of raw date palm sap to the onset of illness was 10 days (range: 9–12 days) in Manikgonj and 4 days (range: 2–7 days) in Rajbari District. All of the cases had consumed about 100 mL of date palm sap. All of the cases consumed the sap before 9 AM.

Cases were more likely to be exposed to three risk factors than controls in the initial bivariate analysis. However, in the multivariate analysis, only a single risk factor, drinking raw date palm sap, was significantly and independently associated with the illness. Nipah cases were 25 times more likely than controls to have consumed raw date palm sap (adjusted ORs 25, 95% CIs 3.3–∞, $p < 0.001$) in the preceding month. Physical contact with a living cat was also associated with illness in univariate analysis (ORs 11, 95% CIs 1.9–84, $p = 0.002$), but was not statistically significant in the multivariate analysis (adjusted ORs 9.2, 95% CIs 0.6–675).

Infrared photographs showed that bats frequently visited date palm trees during sap collection. During seven nights of camera observation, 104 bats visits were photographed around the date palm tree's sap producing area (mean: 14.9 visits per tree per night standard deviation [SD] 30.1) with 47 visits to the shaved surface (mean: 6.7 visits per tree per night SD 13.1). Bats were seen to lick date palm sap 59 times during the observation, and almost half of them (49%) were *Pteropus* bats. The fresh date palm sap samples (15 samples collected from 7 trees for 8 consecutive days), collected at least 9 days after consumption by the last case of this outbreak, were negative for NiV RNA by PCR and virus isolation.

Discussion

This outbreak, involving the death of nine people in two communities separated by a river in Manikgonj and Rajbari Districts over a 6-day period in February 2008, was almost certainly caused by NiV infection. The presenting clinical signs and symptoms of the case-patients were fever, central nervous system involvement, and rapid progression to death, which are consistent with other Nipah outbreaks in Bangladesh (Hossain et al., 2008). Four of the case-patients from both communities had laboratory evidence of Nipah infection; tight clustering in space and time of all case-patients, including those who were not laboratory confirmed and drinking of raw date palm sap on the same day from the same pot, which is a known risk factor for NiV infection, supports the hypothesis that the probable cases also had Nipah infection. This is the second Nipah outbreak where date palm sap has been implicated as the exposure most strongly associated with the illness.

Pteropus bats are the presumed reservoir of NiV (Chua et al., 2002; Rahman et al., 2010). They shed the virus in both saliva and urine (Reynes et al., 2005; Middleton et al., 2007); *Pteropus* bats were observed to be licking the raw date palm sap collected in the outbreak areas. Indeed, in contrast to an earlier infrared camera study in Bangladesh, where only 5% of the bats that contacted date palm

sap were *Pteropus* bats (Khan et al., 2011), when we evaluated the trees those were the sources of date palm sap consumed by cases in this outbreak, 49% of the bats that visited the trees and contacted date palm sap were *Pteropus* bats. There is evidence of survival of NiV in mango flesh, mango juice, pawpaw juice, and lychee juice for up to 3 days, depending upon the pH of the juice (Fogarty et al., 2008). Moreover, the half-life of NiV in bats' urine, with pH adjusted to 7 at a temperature up to 22°C, is 18 h (Fogarty et al., 2008). The pH of date palm sap is 7.2 (Aidoo et al., 2006), suggesting that survival of NiV in date palm sap may be similar. In Bangladesh, winter is the peak collection period of date palm sap and this Nipah outbreak corresponds with the seasonality of all previously reported Nipah outbreaks in Bangladesh. In winter, the temperature remains between 15°C and 28°C (Bangladesh Meteorological Department, 2008) and this low temperature might extend the survival of NiV in bat secretions or in sap (Fogarty et al., 2008). As date palm sap is usually collected early in the morning (5 AM–7 AM) and all of the cases in this outbreak drank the raw date palm sap before 9 AM, NiV apparently survived until that time.

The date palm sap samples that were evaluated for the presence of NiV were collected 24 days after the onset of illness of the last identified case in the outbreak. The median incubation period of NiV is 9 days (Hossain et al., 2008). The absence of NiV in the sap 2 weeks after the putative transmission event suggests that date palm sap is only intermittently contaminated, a pattern of contamination that is consistent with the observed intermittent outbreaks in Bangladesh.

The distance between the two areas (44 km) was within the 50 km foraging ranges for the *Pteropus* bat (Kunz and Jones, 2000). The genetic sequences of the isolated viruses from the two sites were identical in contrast to substantial diversity in NiV isolates noted previously from different outbreaks in Bangladesh (Harcourt et al., 2005). While we do not know if the same bat or the same colony of bats contaminated date palm sap at these two sites, the near simultaneous occurrence of these uncommon outbreaks by an identical strain of NiV and the similar pattern of transmission suggests that they resulted from the same underlying process.

Although the association between cats and Nipah infection was not statistically significant in the multivariate model, cats are susceptible to infection with NiV and when infected can shed virus in their saliva (Middleton et al., 2002). Other domestic animals, including pigs and cattle, were associated with Nipah illness in earlier outbreaks in Bangladesh (Luby et al., 2009). The role of domestic animals in transmission of NiV is an important area for continued research.

Limitations include that we could not test samples from four cases as they died before specimens could be collected, which might be misclassified as cases in our study, but all of these cases were previously healthy people whose symptoms were consistent with confirmed Nipah cases. Moreover, we could not collect follow-up samples from the patients, who were negative in the samples collected 3, 4, and 8 days of illness onset. However, in previously investigated outbreaks, IgM against NiV was present in the follow-up samples collected 2 or more weeks

after illness onset among 56% of the Nipah cases, who did not have detectable IgM against NiV detected from earlier specimens (Hossain et al., 2008). So, it is likely that the probable cases in this outbreak also had Nipah illness. During the outbreak periods, the Government of Bangladesh conducted local awareness raising activities to notify the community about Nipah-like symptoms and to avoid drinking raw date palm sap, eating partially eaten fruits, and having contact with bats. This might have sensitized our study population regarding their response, but awareness regarding the risk factors possibly motivated study subjects and their proxies to recall their exposure history more elaborately, rather than encouraging them to hide those exposures. So, it is unlikely that the awareness raising activities affected the results of our study.

There is evidence of recurrent fatal outbreaks from 2001 through 2007 by the transmission of NiV from its fruit-bat reservoirs to humans in Bangladesh (Luby et al., 2009). The present investigation also suggests that date palm sap is an important pathway for this transmission. Drinking fresh date palm sap was the most strongly associated risk factor among the exposures investigated for this outbreak of human NiV infection in this study. The outbreak ended following local warning against drinking fresh date palm sap from the Government of Bangladesh. To prevent this illness, date palm sap should not be drunk fresh unless effective steps have been taken to prevent bat access to the sap during collection. We are working with local date palm sap collectors to develop socially acceptable low cost technologies to prevent bats' access to the date palm sap producing parts of the tree. Studies in Bangladesh involving local date palm sap collectors suggest that using a bamboo-skirt to cover the shaved part of the date palm tree and sap collection pot might be a practical, affordable method to prevent bats' access to the date palm sap (Nahar et al., 2008; Khan et al., 2011). Drinking raw date palm sap is a long-practiced tradition in Bangladesh, so public health recommendations to avoid drinking fresh date palm sap are unlikely to be universally followed. Research to identify culturally acceptable approaches to produce safe date palm sap may provide an additional lifesaving prevention strategy.

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Disclosure Statement

No competing financial interests exist for any of the authors.

Disclaimer Statement

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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A13

FOOD-BORNE PATHOGEN CONTROL PROGRAMS

*Mike Robach*³⁰

Cargill is an international producer and marketer of food, agricultural, financial, and industrial products and services. Founded in 1865, our privately held company employs 139,000 people in 65 countries. We help customers succeed through collaboration and innovation and are committed to sharing our global knowledge and experience to help meet economic, environmental, and social challenges.

In fiscal year 2011, Cargill had US\$119.5 billion in sales and other revenues. Earnings from continuing operations were US\$2.69 billion. The company also realized US\$1.55 billion in income from discontinued operations.

Cargill has a very focused purpose to be the global leader in nourishing people. That purpose takes into account health and nutrition, as well as food safety and food security. We have a mission to create distinctive value, and our approach is to be trustworthy, creative, and enterprising.

Thousands of customers turn to Cargill for innovative solutions across our four major market segments.

Agriculture: We buy, process, and distribute grain, oilseeds, and other commodities to makers of food and animal nutrition products. We also provide crop and livestock producers with products and services.

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Financial: We provide our agricultural, food, financial, and energy customers around the world with risk management and financial solutions.

Industrial: Cargill serves industrial users of energy, salt, starch, and steel products. We also develop and market sustainable products made from agricultural feedstocks.

As an agricultural and food company, food safety is fundamental to Cargill's ongoing business. Our goal is to provide high-quality, safe food every time, everywhere. We recognize that our work in this important area is never done. Every day we work to earn the trust of our customers and consumers, beginning with the safety of the products we produce and extending to improving food safety around the world.

Our definition of food safety is simple—protecting people and animals from illness or injury from handling or consuming our food products. Our efforts to ensure this—all along the vast supply chain, from production to consumption—are much more complex. Because we touch the global food supply chain in so many ways and in so many places, we take a broad, comprehensive science- and risk-based approach to ensure the safety and integrity of all of our products. This comprehensive approach is designed to address biological, chemical, and physical hazards.

Because we recognize that food safety practices, legislation, and regulatory oversight vary between and even within nations, we have adopted one global systems approach to which we hold ourselves accountable across all of our business, in all of our geographies.

It's everyone's responsibility, and we take a very holistic approach from the farm all the way to the plate. We embrace the concept of One Health.

I want to share this as a roadmap. I'm going to break this down as we go through here, but I think this is a very good example of what One Health is all about. We've worked on this with a number of other colleagues in the food industry and through Michigan State University to create this road map for the components around global food safety (Figure A13-1).

The journey starts out with international governance up in the upper left-hand corner. Then there's a track that goes across the top around how governments can adopt the principles, guidelines, and recommendations coming out of Codex Alimentarius (CODEX), the OIE (World Organization for Animal Health), and the International Plant Protection Commission (IPPC) as a basis for the regulatory oversight program. These organizations are the international standard setting bodies prescribed by the World Trade Organization's (WTO's) Sanitary and Phytosanitary Agreement (SPS).

The bottom track outlines how industry has taken those same principles, guidelines, and recommendations and, through an ISO framework, transformed them into food safety systems that can be implemented and then audited against to ensure that the systems have been appropriately deployed. These systems can

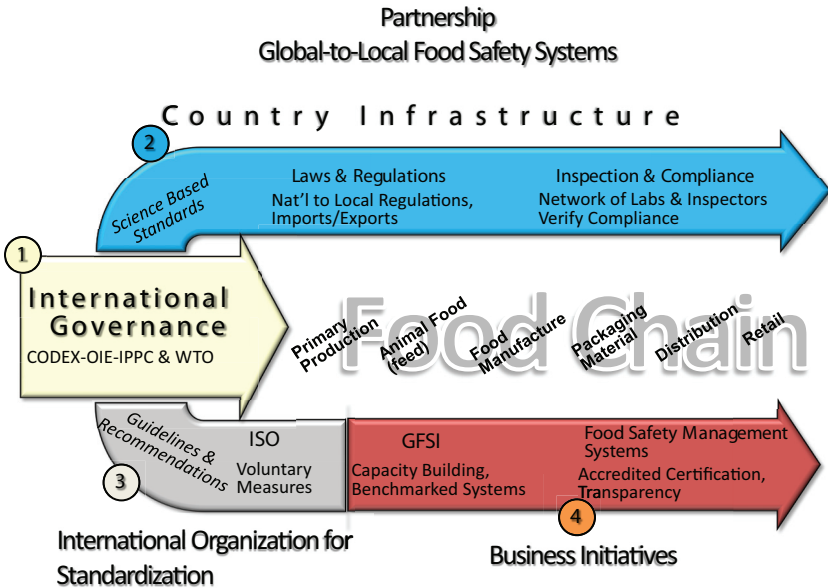


FIGURE A13-1 Roadmap for the components of global food safety.
SOURCE: Cargill.

cover the entire food network going from the farm on the left all the way through to the consumer on the right. It’s a shared responsibility, shared accountability thought process through the whole thing.

We will discuss public–private partnerships as we go through the map.

Countries are dependent on each other for food. We know that we don’t produce enough food in the areas where a lot of people live; therefore, there is going to be movement of food. This isn’t anything new. Food historically has been a basis of trade, and it used to be traded for other goods and services over time. This continues on today.

National governments established the WTO and the SPS agreements, and use CODEX, OIE, and IPPC for the process for setting international food safety standards. Out of these organizations you have science-based standards that have been internationally vetted, discussed, and adopted. Out of this comes guidelines and recommendations that can be utilized by both the public and the private sector in global food safety.

Going across the top of the road map, we actually started to create these slides as we got into discussions with various governments on regulatory reform. We’ve used this with the Chinese government (Administration of Quality Supervision, Inspection and Quarantine, Ministry of Health) in discussions about how

they can effectively implement food safety systems from a regulatory oversight standpoint.

We've also used it over the past year with the Food and Drug Administration (FDA) as it has looked at implementing the Food Safety Modernization Act to provide an idea of what the industry's thinking and how we're taking these international standards and adopting them in our food safety systems across the global food supply network.

From a government standpoint we all know that strong systems will protect customers and consumers, and also facilitate trade. A number of countries already used CODEX as a basis for a number of their regulations. Many of them reference ISO as voluntary measures, and as suggestions for the industry in terms of adoption. Regarding government inspections and compliance, if a regulatory agency is verifying compliance and evaluating a firm's preventative measures, and the focus is on the elements that come out of international governance, then you will have industry and government looking at the same criteria and thus aligned as to what it is that is important as it relates to the safety of our food system.

On the private-sector side, the rationale is to build on science-based standards coming out of CODEX, OIE, and IPPC. A strategic partnership exists between ISO and WTO to facilitate market requirements. They're working together to make sure that there's a framework available for the private sector to adopt these principles.

ISO does not regulate, legislate, certify, or accredit. We have 163 countries and national standards that collaborate with ISO on the development of these voluntary measures. There is a lot of input and a lot of ownership through this process. They are voluntary measures, but there are measures for the accreditation process, for the certification process, for auditing, for auditor competency, and then also for food safety management systems. All together it gives industry the basis for consistent, harmonized food safety management systems.

The process standardizes implementation; it gives us harmonization, alignment, and consistency across the food chain from origination through consumption. In some cases there may be a market requirement, or it may be referred to in regulations and legislation. For the industry it's a good framework—using the guidelines, recommendations, and principles out of CODEX, OIE, and IPPC and putting them into a framework that can be adopted by facilities in their food safety systems.

How do we do that? Within the industry there has been a lot of discussion about food safety being a competitive issue. Let's go back to the mid-1990s when the beef industry got together as it was struggling with *E. coli* O157:H7 and how to deal with the situation. The industry got together and made a decision that food safety would not be a competitive issue in the ground beef business.

So all of a sudden companies were coming together and sharing insight, best practices, and data. Together we've driven the O157 presence down with the help of the ground beef industry and other stakeholders focusing in on what were the

important elements of a food safety system and aligning on how to address the challenge.

The work that we've done as industry is readily available. Everybody has access to that information and those processes. We all know the tools that are out there for the entire industry to achieve the goals of improved ground beef safety.

We've also been working through an organization called the Global Food Safety Initiative (GFSI). GFSI is a multistakeholder group that benchmarks food safety systems. We just came out with guidance document 6 earlier this year. It is based on the principles of good hygiene and hazard analysis and critical control point (HACCP) from CODEX.

The guidance document has requirements for food safety systems and their delivery. It also has a component around capacity building that allows these principles to be implemented in emerging markets where the capacity might not be there. There's a process that takes countries or individual facilities in countries through a stepwise progression so that they can achieve this certification process.

We think food safety management systems are really the way to go in terms of having a robust program, and an accredited certification gives us third-party assurances that we're doing the right thing. We strive to create transparency and confidence in the supply chain. This has to be done through a partnership. We believe it's effective and efficient. We believe that it protects consumers around the globe. This has been implemented within Cargill (Figure A13-2).

This document is in every one of our facilities around the world. Everybody is aware of it. Everybody knows it and understands it. In most places around the world you're going to have both the business unit leader and the plant manager also signing this document. It's a true reflection of both top-down and bottom-up commitment to the policy.

We have based our policy and procedures on CODEX. It's a focus on food safety management systems. We have general requirements that are required to be documented. The next section describes management responsibility. Every business unit leader and every plant manager has a responsibility that they must achieve in order to be compliant with the policy.

We also have a resource management section. We have a section describing planning and realization of safe products. These may sound like strange section titles to you, but they're taken from ISO, coming right out of CODEX. These are very consistent all the way through. In fact, we just this past year renumbered our policy and procedures manual to be in line with CODEX, so it's quite clear.

In looking at planning and realization of safe products, the key is prerequisite programs, steps you must take to enable a hazard analysis, doing the hazard analysis, and then putting in your operational prerequisite programs and establishing your HACCP plan.

You must update your plan on a regular basis; we require an annual reassessment of the plan. We also have a verification component. Traceability is included in this, and then a control of any nonconforming products. It's very consistent in



Cargill Food Safety Policy

Cargill is committed to providing safe food and animal feed products and services. We will use only those product development, procurement, supply chain, transportation, storage, production, manufacturing, and distribution systems and suppliers that ensure the safety and regulatory compliance of our products. We will communicate relevant food safety matters both internally and externally. We will adhere to the food safety requirements we have mutually agreed to with our customers. Every Cargill Business Unit, Function, and employee has a responsibility to ensure the production of safe products that comply with the applicable laws, regulations and Corporate Food Safety and Regulatory Affairs requirements. Cargill management will provide the resources and support necessary to enable our employees to fulfill this responsibility and continually improve our programs and processes.

A handwritten signature in black ink, appearing to read "Greg Page".

Greg Page
Chairman and Chief Executive Officer

A handwritten signature in black ink, appearing to read "Mike Robach".

Mike Robach
Vice President
Corporate Food Safety & Regulatory Affairs

FIGURE A13-2 Cargill food safety policy.
SOURCE: Cargill.

the approach. The next essential section includes validation and verification as well as continuous improvement of the management system.

You've got to be able to validate that what you've put in place is effective. Then you've got to verify that you're doing what you said you were going to do over time. These all become important components. It's important for us to remain outcome based so that we can drive continuous improvement.

When new technology becomes available, new interventions become available; we want to be able to take advantage of those and not be constrained by a regulatory construct that is prescriptive and telling us how to do it. Let's focus on outcomes. Let's agree on what those performance standards need to be. Let's agree what the outcome needs to be, and then let industry move forward and innovate and continuously improve and share that information across the supply chain.

There are a lot of prerequisite programs. I'm going to discuss management of purchased materials and measures for the prevention of cross-contamination as we go through here. These are prerequisite programs coming out of CODEX and some Cargill-specific prerequisite programs included. We have a lot of them. We spent a lot of time on this. This is really the soul of what we do each and every day in our food-producing plants.

I'll outline management of purchased materials. This really gets into our supplier and external manufacturer program. We have more than 1,000 plants. We have hundreds of thousands of suppliers and at least 400-500 external manufacturers who produce product on Cargill's behalf. They're expected to have the same systems in place that we do.

We have a program around the selection and management of those suppliers, incoming raw materials, and then our supplier and external manufacturer qualification and management scheme. This is a very simple diagram in the way we approach this with our suppliers (Figure A13-3). We believe this will mitigate food safety and quality risks, keeping people and animals safe. That's the core of what we do each and every day.

We do a risk assessment on the product and on the plant. We look at their competency development. We look at the relationship management. One of the most important components of this is know your supplier. We follow the "trust but verify" model. You must know who you're working with as a supplier. We have accountability; they have accountability. We're going to collaborate to make sure that we're assessing food safety appropriately and that there's



FIGURE A13-3 Corporate food safety and regulatory affairs.

SOURCE: Cargill.

transparency in the information that's being generated. This is at the core of what we do.

We've put together a very simple risk assessment model that we use with all of our suppliers. There's a material risk that you can see that goes from low to high, and we have a quantitative score that we go through in looking at what that risk is associated with that product. But then we also assess the capability of the supplier to manage that risk.

If you've got a high-risk material, but you've got a company that's controlling that risk, they're going to go into that medium category. If I've got a high-risk material and a high-risk supplier, they're in the priority list. About 6 percent of our suppliers fall into that range. We're working intensely with them, and they're under increased scrutiny, obviously, as we go through the process. We're rolling this out across the entire company over the course of the next 3 years, and it's quite an undertaking.

Microbiological cross-contamination is extremely important for us. We focus on environmental monitoring for most of our facilities. We also have a specific *Salmonella* control program and a *Listeria monocytogenes* control program in those facilities where those hazards are identified as being reasonably likely to occur.

The environmental monitoring program goes into play where you have a risk that's reasonably likely to occur and could find its way into product (Figure A13-4). We've put together a decision tree that we've taken through every one of our facilities so that everybody is operating against the same criteria.

We look at the difference between shelf-stable and not-shelf-stable products. But then there are very specific processes that everybody goes through to assess the risk associated with their product and their facility. That will take you down to whether or not you need an environmental monitoring program in place.

We've found this to be very useful in really driving people to think critically about the products that they produce, the facilities that they manage, and the products—they have to understand where they're going and who's consuming them. This has been a really valuable tool for us to get people really thinking critically about the business that they're managing.

In summary, I believe that we do have a path forward here. I think the One Health approach makes a lot of sense. We live it every day. We're originating grain and producing ready-to-eat food. To me, One Health is what we do every day.

We believe that we have a structure and a mechanism for effective global partnerships in place. We work closely not only with our supply chain and our competitors in the industry, but also with our customers and with the regulatory agencies. And we do a lot of work with the Centers for Disease Control and Prevention (CDC)—sharing information with CDC, talking with them, understanding epidemiology and microbiology.

Working with academia, consumer groups, government, and industry is the way forward. We've all tried to do it alone. The private sector has tried to do it alone. Government has tried to do it alone. It doesn't work. We've got to work

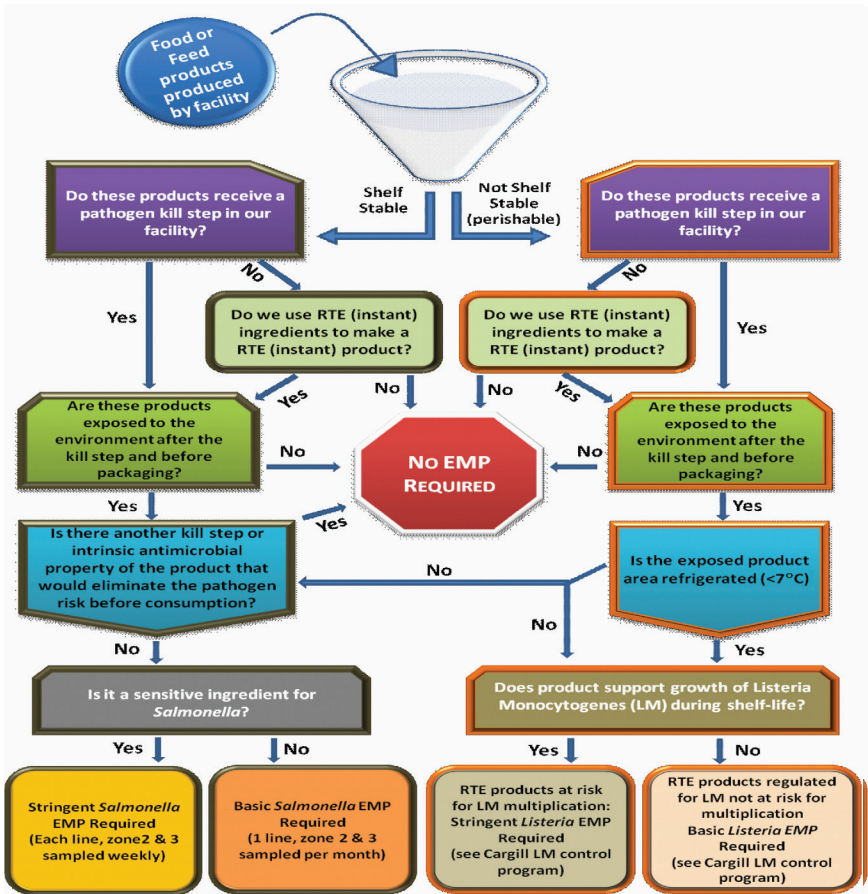


FIGURE A13-4 Cargill environmental monitoring decision tree. SOURCE: Cargill.

together. We’ve got to get on the same page. We’ve got to get aligned around some of these issues.

We believe that resources must be deployed based on risk. You must have a science base and a risk base to apply resources. We’re all operating with reduced resources.

We’re trying to do more with less, so it becomes even more important that we’re focused on the science, we’re focused on the risk, and we’re applying resources against the areas of greatest need. Focus has to be on prevention. It has to be on preventing issues from happening in order to maintain confidence in the food supply and to have a shared goal of safe, affordable food.

Food security plays into this in a major way, and the more preventative measures we can have in place around the world, the more assurance we're going to have of an abundant, safe food supply. It builds confidence in food safety, enhances global trade. It enhances food security. It enhances people's enjoyment of their nutrition.

Lastly, I have to finish with this statement. Business shoulders the responsibility for safe food. I know a lot of times government thinks it has the responsibility. It doesn't. We do. It's our product. It's our brand. They're our customers. We want to work together, and we want to work collaboratively. But at the end of the day, we're the ones who have the responsibility, and we accept that.

A14

EMERGING FOODBORNE PATHOGENS AND PROBLEMS: EXPANDING PREVENTION EFFORTS BEFORE SLAUGHTER OR HARVEST

*Casey Barton Behravesh, Ian T. Williams, Robert V. Tauxe*³¹

Introduction

Infections caused by microbes that contaminate the food supply are a frequent reminder of the complex food web that links us with animal, plant, and microbial populations around the world. In the United States, an estimated 46 million foodborne infections occur each year, along with 250,000 hospitalizations and 3,000 deaths (Scallan et al., 2011a, 2011b). While all are at risk, the consequences are the most severe in the vulnerable populations of the very young, the elderly, and those with compromised immune systems. Of the many pathogens that can contaminate food, some, like norovirus and *Salmonella* serotype Typhi, are sustained in human reservoirs and contaminate the food supply via the excreta of infected humans. Many others are sustained in animal reservoirs and contaminate our food supply because they are present in the flesh, milk, or eggs in the living animal, or because they are in the excreta of infected animals that subsequently contaminate the foods we eat. Some pathogens persist in the environment, or in multiple hosts, and can contaminate the foods we eat via pathways that reflect the variety of ecosystems that make up our food supply.

Food safety depends on understanding these pathways well enough to prevent them. In the United States, substantial progress during the 20th century in animal disease control efforts has greatly reduced the foodborne infections re-

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lated to zoonotic diseases such as brucellosis and bovine tuberculosis (Tauxe and Esteban, 2006). At the same time, an increasing number of microbes have been recognized that can cause serious illness in humans, but rarely cause illness in the animals that carry them. The presence of these microbes is thus not apparent to the rancher or farmer, and the animal appears entirely healthy on inspection at slaughter; addressing these microbes requires a different prevention paradigm based on reducing levels of microbial contamination throughout the food chain. This effort starts on the farm or ranch where animals are raised, with attention to fodder, water, and biosecurity there. An early success was the virtual elimination of the parasite *Trichinella* from the nation's swineherds, and the prevention of pork-related trichinosis in people, through attention to the fodder fed to pigs (Schantz, 1983). Recent outbreaks show that plants can also be contaminated with human pathogens on the farm, through manures, water, and wild animal incursions (Lynch et al., 2009). The need to reduce and prevent contamination continues through harvest and slaughter, subsequent processing, and the food preparation steps in the final kitchen (Figure A14-1). Indeed, reducing the num-

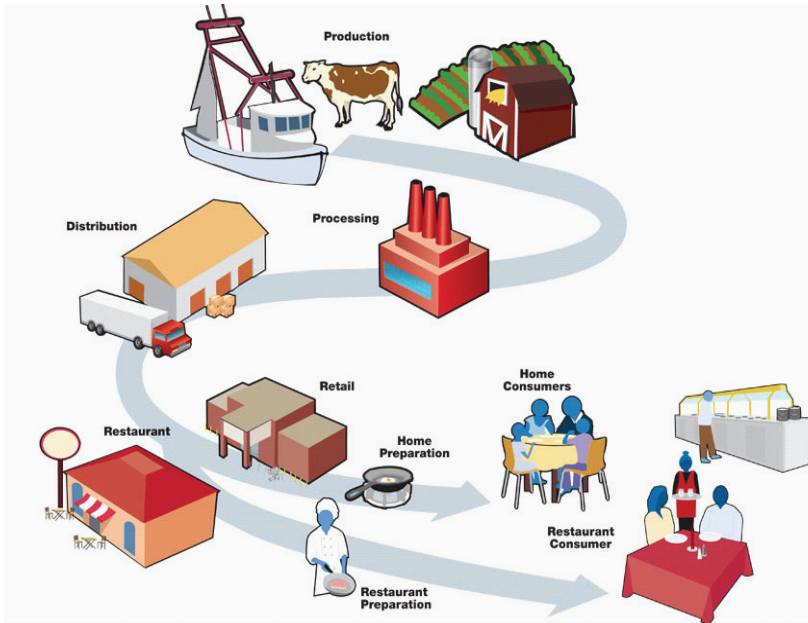


FIGURE A14-1 The food production chain from the farm to the table.

SOURCE: Adapted from Tauxe (2006) Table 3-1, page 73. http://www.cdc.gov/outbreaknet/investigations/figure_food_production.html (accessed April 10, 2012).

ber of foodborne infections by making food safer is the result of efforts by many partners in the food safety system.

Concentrated animal production has parallels with human urbanization, like the challenge of providing water, food, and fecal disposal for thousands of individuals every day. Just as the spread of many infections in human cities depends critically on treating the drinking water, and collecting sewage and keeping it out of the food and water supplies, and immunizing ourselves against many infections, so will the health of animals raised in virtual cities depend on attention to the safety of their water and food supplies, coupled with selective immunization.

New Pathogens and Problems

New food-borne pathogens emerge when previously unrecognized pathogens are identified and are linked to foodborne transmission from the beginning, or when foodborne transmission is documented for pathogens that are already well known. The list of foodborne agents that have emerged in the past three decades includes bacteria, viruses, parasites, biotoxins, and a prion (Table A14-1). They often emerge from animal reservoirs; 70 percent are sustained in animal populations and affect humans only incidentally. Many were identified in the course of outbreak investigations, when both their pathogenicity and association with food could be determined. Some common bacterial foodborne pathogens are adapted to particular reservoirs, making targeted control strategies feasible (Table A14-2).

TABLE A14-1 Major Pathogens Identified as Foodborne Since 1970

Bacterial	Parasitic
<i>Arcobacter butzleri</i> *	<i>Cryptosporidium</i> *
<i>Campylobacter jejuni</i> *	<i>Cyclospora cayetanensis</i>
<i>Campylobacter fetus</i> *	<i>Sarcocystis</i> *
<i>Cronobacter sakazakii</i>	<i>Trypanosoma cruzi</i> *
<i>E. coli</i> O157:H7*	
<i>E. coli</i> , non-O157 STEC*	Viral
<i>E. coli</i> , enteroaggregative/STEC	Astrovirus
<i>E. coli</i> , other diarrheagenic	Caliciviridae (norovirus and sapovirus)
<i>Listeria monocytogenes</i> *	Hepatitis E*
<i>Vibrio cholerae</i> O139, toxigenic*	Nipah virus*
<i>Vibrio vulnificus</i> *	Rotavirus
<i>Vibrio parahaemolyticus</i> *	
<i>Yersinia enterocolitica</i> *	Fungal
<i>Yersinia pseudotuberculosis</i> *	<i>Aspergillus flavus</i> aflatoxin
Algal	Prion Agent
<i>Pseudo-nitzschia pungens</i> *	new Variant Creutzfeldt Jacob Disease prion*
(domoic acid-producing)	

* Reservoir or major transmission pathway through animals.

SOURCE: Adapted from Tauxe (2006), Table 3-1, p. 73.

TABLE A14-2 Major Food-Animal Reservoirs for Human Foodborne Bacterial Pathogens

-
- *Campylobacter*—poultry, other birds, cattle
 - Shiga toxin–producing *E. coli* O157:H7—cattle and other ruminants
 - *Salmonella*—poultry, cattle, swine, reptiles, and others
 - *Salmonella* serotype Enteritidis—poultry
 - MDR *Salmonella* serotype Newport—dairy and beef cattle
 - *Salmonella* serotype Choleraesuis—swine
 - *Vibrio*—molluscan shellfish
 - *Yersinia enterocolitica*—swine
-

For example, *Campylobacter* are adapted to birds and particularly to poultry, among which they are commensal intestinal flora, and contaminate poultry meat by cross-contamination at slaughter. Some strains can also colonize cattle and are transmitted via raw cows' milk. Shiga toxin–producing *E. coli* O157:H7 can colonize the peri-rectal glands of ruminants and transfer from hides and feces to meat during the slaughter process. Strains of *Salmonella* serotype Enteritidis that spread around the world in the 1980s colonize the peri-ovarian tissues of the hen's reproductive tract, where they come in contact with the egg yolk as it forms, and contaminates the internal contents of normal-appearing eggs. If the egg is fertilized, these *Salmonella* then colonize the reproductive tissues of the chick embryo and reach the next generation, while in the unfertilized table egg, *Salmonella* can multiply in the yolk and infect the eater of a less than fully cooked egg.

The reservoirs where these pathogens persist, and the pathways by which they reach humans, are revealed in outbreak investigations. By epidemiological methods, the illnesses in an outbreak can often be associated with consuming a particular food, the food vehicle of infection. Between 2003-2008, the food vehicles identified in 1,565 outbreaks reported to the Centers for Disease Control and Prevention (CDC) with specific food vehicles are a broad spectrum of animal- and plant-derived foods (Figure A14-2). The list of implicated foods is regularly expanded as new ones are identified in outbreak investigations. Between 2006 and early 2012, 15 new specific food types were identified as food vehicles in outbreaks affecting the United States (Table A14-3). It is curious that while many of the pathogens have animal reservoirs, many new food vehicles are plant derived. This includes plant-derived processed foods, like peanut butter, peanut paste, and spinach powder; spices such as black and white pepper; tree nuts; and fresh produce items.

The identification of new food safety problems has been accelerated by important improvements in surveillance and response. These new surveillance tools capture information about infections in humans as well as in animals and contamination of foods, providing important information that is integrated across sectors.

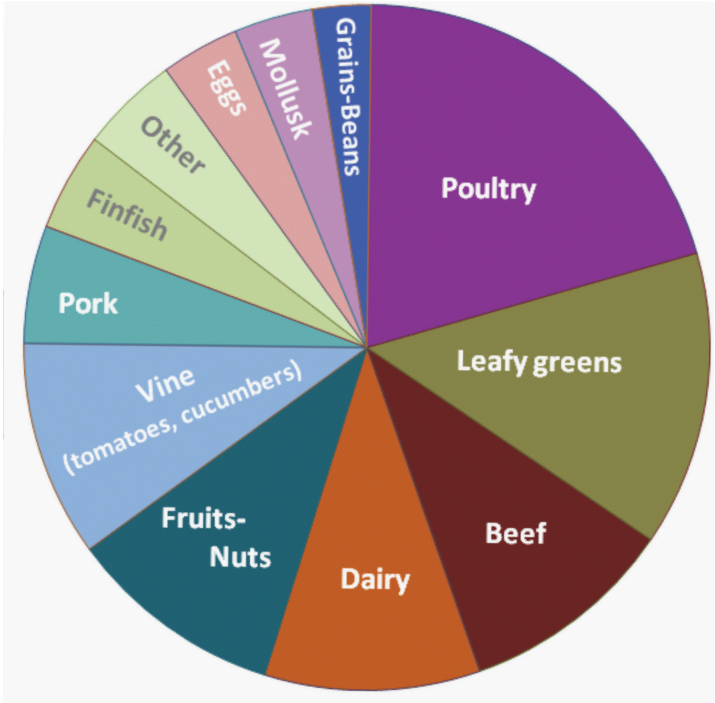


FIGURE A14-2 Distribution of illnesses by food type in 1,565 foodborne outbreaks caused by a single food type and reported to CDC’s National Foodborne Disease Outbreak Surveillance System, 2003-2008.
 SOURCE: CDC, unpublished data.

TABLE A14-3 Fifteen New Food Vehicles Identified from 2006 Through March 2012 in Foodborne Outbreaks Affecting the United States

-
- Bagged spinach
 - Pasteurized carrot juice
 - Peanut butter
 - Broccoli powder on a children’s snack food
 - Dry dog food
 - Frozen pot pies
 - Canned chili sauce
 - Hot peppers
 - White and black pepper
 - Raw cookie dough (likely flour)
 - Hazelnuts
 - Fenugreek sprouts
 - Papayas
 - Pine nuts
 - Raw frozen scraped ground tuna
-

SOURCE: CDC, unpublished data.

In the United States, public health surveillance that tracks the frequency of human infections with specific pathogens has expanded greatly since 1996 to capture different kinds of information that is needed for making public health decisions. FoodNet, the network for active surveillance of infections often transmitted through foods in 10 sentinel sites around the country, led by the CDC and supported by the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA), provides accurate tracking of what is diagnosed in human clinical laboratories, overcoming local variation in reporting requirements (Scallan, 2007). FoodNet data are fundamental to estimating the total number of illnesses that occur and to tracking trends over time. By 2010, FoodNet data showed that the incidence of *E. coli* O157 infections had declined by 44 percent since the baseline period of 1996-1998, that of *Campylobacter* by 27 percent, and that of *Listeria* infections by 38 percent, while those caused by *Salmonella* had not decreased at all (Figure A14-3) (CDC, 2011e). The substantial progress made in reducing *E. coli* O157, *Campylobacter*, and *Listeria* infections between 1996 and 2003 was largely the result of improvements in sanitation at slaughter and meat processing for meat and poultry. There has been little progress in more recent years.

A second surveillance enhancement is PulseNet, the national molecular subtyping network for bacterial foodborne pathogens, which has enhanced detection of outbreaks (Swaminathan et al., 2001). In each state, clinical laboratories send strains of Shiga toxin-producing *E. coli* (STEC), *Salmonella*, and *Listeria*

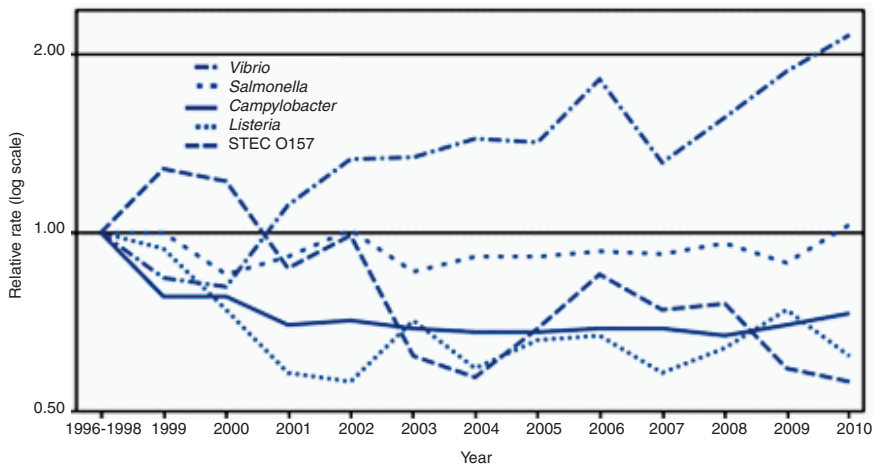


FIGURE A14-3 Relative rates of laboratory-confirmed infections with *Campylobacter*, Shiga toxin-producing *Escherichia coli* O157, *Listeria*, *Salmonella*, and *Vibrio*, compared with 1996-1998 rates, by year, in the United States during 1996-2010, based on data from the Foodborne Diseases Active Surveillance Network (FoodNet).

SOURCE: CDC (2011e).

monocytogenes isolated from ill persons to the public health laboratories where they are subtyped by molecular methods and added to a national database. To find clusters of infections that may be related, the database is scanned looking for surges in particular subtypes. Each state can review its own data and national data, and the CDC looks for multistate increases. The same laboratory methods are applied by the USDA to *Salmonella* and *E. coli* isolated from animals and meats, and by FDA to isolates from other foods, so that PulseNet can explore possible connections between animal reservoirs and foods, in addition to clusters of human infections. Epidemiological investigation of such clusters may reveal an exposure that the cases all have in common, such as eating a particular food, contact with an unusual pet, or travel to a particular place. The growth of this surveillance system, which now adds patterns of 50,000 isolates a year to the national database, and the increasingly sophisticated epidemiological approach to the clusters identified have led to a dramatic increase in the number of multistate outbreaks detected and investigated.

A third surveillance enhancement tracks the frequency of antimicrobial resistance in enteric bacterial pathogens (CDC, 2011c; Holmes and Chiller, 2004). The National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria also depends on the submission of isolates to public health laboratories. For NARMS, a systematic subset of those isolates is tested at the CDC to determine their resistance to a panel of antimicrobial agents. In parallel, isolates from retail meats are tested at FDA, and isolates from animal carcasses are tested in USDA laboratories. These three arms of NARMS provide ongoing monitoring of the progression of resistance to specific agents that are used in agriculture and human health and have been important to promoting prudent use and regulation.

Finally, the National Outbreak Reporting System (NORS) collects reports of outbreaks of foodborne and waterborne illnesses that are investigated by public health departments, providing the summary information about frequency by pathogen and vehicle type (CDC, 2011d). In 2009, this system was expanded to include outbreaks of gastroenteritis caused by contact with animals and by person-to-person transmission. NORS thus captures information about a range of transmission events likely to lead to gastrointestinal disease.

Improving Preharvest Prevention: The Animal Sector

Long-term prevention of foodborne disease depends on actions of many partners in the food production chain, stretching from farm to table. Some critical prevention measures include quality assurance programs at egg farms; safe agricultural practices for produce farmers; inspection systems at meat processing plants; use of pasteurization, canning, cooking, irradiation, and other steps to kill pathogens in food processing; and food safety education for consumers and staff in the food industry. Much of the progress has been focused on safer processing of animals and plants after they are harvested, with less emphasis on the preven-

tion that can be achieved before harvest or slaughter. Making food safer in the future will depend on reducing preharvest contamination.

Outbreak investigations show that contamination events often start with problems in production, that is, while growing the plants we harvest or raising food animals. Many factors throughout all stages of the food production and distribution system can affect food safety. For meat products, what happens on farms, in feedlots, during transport and lairage before slaughter, as well as during slaughter and further processing can have a major effect on human health (Miller and Griffin, 2012). Domesticated food animals can also serve as a source of contamination of nearby produce-growing fields and can lead to human infection through direct contact at petting farms and mail order hatcheries. Preventing such infections also means reducing the carriage and spread of human pathogens among live animals.

Bacterial and other microbial pathogens in animal feces can contaminate the environment in which animals are raised, where they roam, and where they are kept while awaiting slaughter. Because animal hides and intestinal contents may have pathogens, efforts at slaughter are focused on cleaning the hides, removing them with care, and preventing the contamination of meat with intestinal contents. Poultry farms with large populations of birds are a setting where infectious agents can spread rapidly. When birds are slaughtered, hot water dips help remove feathers but can spread intestinal contents to subsequent carcasses. *Campylobacter jejuni/coli*, a common cause of illness in the United States, contaminates at least 40 percent of chicken breasts at retail (FDA, 2011). As a result, poultry is a major source of *Campylobacter* infections in humans. People become infected by consuming inadequately cooked poultry or other foods that become cross-contaminated via contact with poultry. Even infants riding in shopping carts containing raw poultry are at increased risk (Patrick et al., 2010).

Control measures for *Campylobacter* focused on slaughter sanitation; chlorinating water baths and chiller tanks have been associated with a decrease in *Campylobacter* infections in the United States in the late 1990s, although there has been no progress since 2002 (Figure A14-3). In New Zealand, similar control measures implemented at slaughter led to a dramatic 50 percent decline in campylobacteriosis in 2008 and a parallel decline in Guillain-Barre syndrome cases (Baker et al., 2012; Sears et al., 2011). In Scandinavia, a new control strategy is “test and freeze,” developed first in Iceland and then adopted in Norway and Denmark, in which flocks are tested preslaughter for the presence of *Campylobacter* (Tustin et al., 2011). Birds from flocks that have *Campylobacter* must be frozen after slaughter, which reduces the level of *Campylobacter* contamination, while birds from poultry farms without *Campylobacter* can be sold fresh at a premium price. This provides farmers with an economic incentive to reduce *Campylobacter* contamination and may be a model for how to incentivize preharvest food safety measures. To make further progress, short of irradiating poultry, control measures will need to include a preharvest focus (Wagenaar et

al., 2008). Such measures may include chlorinating the drinking water, probiotics, and vaccination (Wagenaar et al., 2008). In Denmark, field evaluations show that putting insect screens on henhouses can lead to a 70 percent decrease in *Campylobacter* flock prevalence (Hald et al., 2007).

Escherichia coli O157:H7 infection has emerged as an important cause of human illness ranging from simple diarrhea, to hemorrhagic colitis, to hemolytic-uremic syndrome, characterized by hemolytic anemia, thrombocytopenia, and renal injury (Griffin and Tauxe, 1991; Rangel et al., 2005). It was first recognized as a human pathogen in foodborne outbreaks associated with ground beef in 1982 (Riley et al., 1983). In 1993, after a large multistate *E. coli* O157 outbreak was linked to undercooked ground beef patties sold from a fast-food restaurant chain, *E. coli* O157 became broadly recognized as an important human pathogen (Bell et al., 1994). In 1994, officials at the USDA declared *E. coli* O157:H7 an adulterant of ground beef, so that finding these bacteria in ground beef resulted in its mandatory recall, and then implemented a new inspection procedure for beef carcasses based on hazard analysis critical control point strategies. In 2002, after a large multistate outbreak and recall of ground beef, regulators and slaughter and beef grinding companies focused more intensive effort on preventing the contamination of ground beef itself, including increased focus on hide removal, testing beef trim before it reached the grinder, and holding ground beef lots until they were found not to be contaminated. These efforts helped to reduce the contamination of ground beef and in turn may have led to the decrease in laboratory-confirmed *E. coli* O157:H7 cases measured in the U.S. FoodNet active surveillance system (See Figure A-14-3) (CDC, 2011e). However, beef remains the most frequently identified vehicle for *E. coli* O157:H7 infections, followed by produce-associated outbreaks (CDC, 2010a). Reducing these infections further will depend on pre-harvest interventions to decrease the shedding of *E. coli* O157 by cattle before they come to slaughter. *E. coli* O157 is common among cattle, particularly in the summertime, and reducing carriage may be achieved using a suite of interventions, including vaccines (two are currently available for evaluation), probiotics, and bacteriophage treatments, and microbicidal agents such as sodium chlorate (Loneragan and Brashears, 2005). Leveraging the need for safer meat with economic incentives for lower contamination rates in cows has yet to be achieved.

Better prevention can occur with multifaceted on-farm preharvest control measures and with test and diversion strategies. One example is egg safety in the United States. Shell eggs and poultry are common sources of human *Salmonella* serotype Enteritidis (SE) infections, which cause 6,000 to 7,000 laboratory-confirmed illnesses annually in the United States. These strains of SE increased dramatically in the 1980s in the United States and many other countries, causing egg-associated infections because they can silently infect the ovaries of healthy hens, resulting in internally contaminated eggs (St. Louis et al., 1988). In the United States, initial control measures included use of pasteurized liquid egg product for high-risk foods and institutions, refrigeration during transporta-

tion and sale, and voluntary programs of flock-based interventions. These egg safety programs typically included obtaining SE-free chicks from hatcheries, preventing spread among flocks by biosecurity, cleaning and disinfection, and testing henhouse environments with diversion of eggs to pasteurization if SE was found; these programs were associated with significant decreases in SE infections (Mumma et al., 2004). However, by themselves they are not enough. In July 2010, a nationwide increase in SE infections was identified in the most common pulsed-field gel electrophoresis (PFGE) pattern (CDC, 2010b). In PulseNet, this common PFGE pattern accounted for 30 to 40 percent of all SE isolates. In 2010, approximately 1,900 more laboratory-confirmed illnesses with this outbreak strain were reported than were expected; adjusting for underreporting approximately 55,000 illnesses likely occurred. Epidemiological investigations focused on 29 restaurant and event clusters in 11 states; egg suppliers were identified for 15 of these clusters. A single producer in Iowa was identified as a supplier of shell eggs in 92 percent of clusters with completed tracebacks, and a second Iowa producer supplied eggs to at least one cluster. The first producer was found to sell feed to the second; both producers shared the same source of pullets. Inspection of these producers identified 13 environmental samples matching the outbreak strain and found substantial potential for egg contamination. These producers recalled more than 550 million shell eggs in August 2010. Also in 2010, a year after publication, a new regulation titled “Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Transportation and Storage” was implemented for producers with 50,000 or more laying hens (FDA, 2009). The shell egg rule mandates what had previously been voluntary in egg safety programs to prevent SE contamination of eggs during production; to prevent further growth of SE during transportation and storage of shell eggs; and to require recordkeeping of testing results for SE. Shell eggs remain an important vehicle for SE infection, and this new rule is an important step toward enhancing egg safety.

A similar program was launched in the United Kingdom in 1998 to reduce SE infections. In the “British Lion” program, egg producers implemented measures voluntarily; including on-farm biosecurity, cleaning and disinfecting henhouses between flocks, vaccinating hens against SE, and monitoring them for the presence of infection (see <http://www.britegg.co.uk/>). Eggs from those producers were stamped with a red lion rampant and were thus differentiated from other eggs, including those imported from continental Europe. As with the Scandinavian *Campylobacter* program, this aligned the consumers’ desire for local and safer eggs to support the cost of the flock-based control programs. Following this launch of this control program, the incidence of SE infections in the United Kingdom fell substantially (Figure A14-4).

SE infections are transmitted through chicken meat as well as through eggs. Recent sampling of retail chicken breasts as part of NARMS indicated that 2 percent are contaminated with SE (FDA, 2011). This is occurring despite efforts to improve slaughter hygiene and inspection processes. As with *Campylobacter*, it

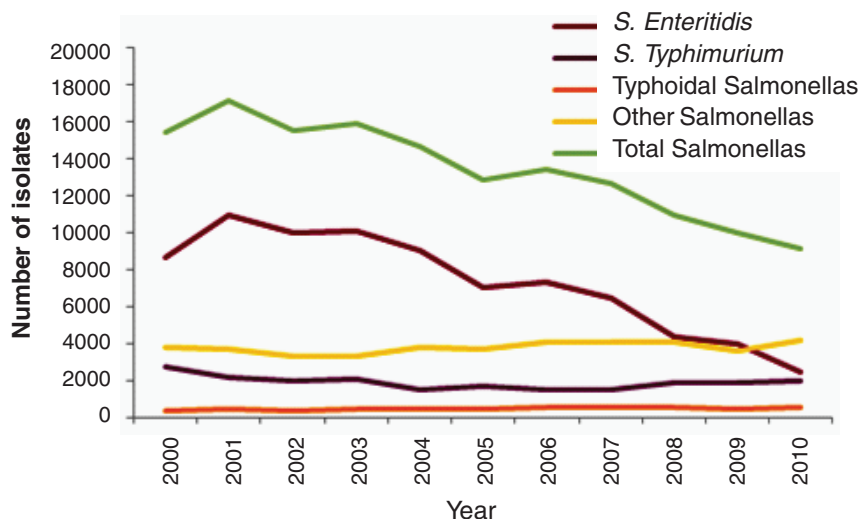


FIGURE A14-4 Number of reported cases of salmonellosis, by serotype, England and Wales, 2000-2010.

SOURCE: Health Protection Agency, <http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Salmonella/EpidemiologicalData/salmDataHuman> (accessed April 8, 2012).

is likely that making further progress with poultry-associated SE and other types of *Salmonella* will require on-farm preharvest interventions. Indeed, it is notable that, despite the extensive efforts to reduce flock contamination of egg-laying flocks, virtually nothing similar has been done with broiler flocks. And as with *Campylobacter*, there is evidence that on-farm control measures for *Salmonella* may be effective. For example, parent flock vaccination, combined with hatchery sanitation and on-farm biosecurity in production flocks was recently documented to greatly reduce the *Salmonella* contamination of poultry carcasses at slaughter (Berghaus et al., 2011; Dorea et al., 2010). This suggests that *Salmonella* in poultry flocks may often be vertically acquired and that a focused program to reduce contamination in parent flocks may have value, although questions remain about the impact of concurrent immunocompromising infections on immune response to vaccines in chicken flocks (Hoerr, 2010). Aligning the benefit of less contaminated poultry meat with the cost to the producer of taking such measures could lead to their adoption.

Another emerging challenge for food safety officials is multidrug-resistant *Salmonella* infections. In the United States, many outbreaks of multidrug-resistant *Salmonella* infections have been investigated in recent years. Because infections with multidrug-resistant *Salmonella* may be associated with an increased risk of hospitalization and antibiotic treatment failure, these illnesses

are especially concerning (Anderson et al., 2005). In 2011, an outbreak of 136 laboratory-confirmed infections with *Salmonella* serotype Heidelberg infections were identified in 32 states (CDC, 2011b). Epidemiological and traceback investigations linked these illnesses with consumption of ground turkey from a common production facility, resulting in a recall of 36 million pounds of ground turkey, the largest USDA Class I recall in U.S. history. Antibiotic resistance profiles of patient and environmental samples matched an identical multidrug-resistant pattern, which included several clinically relevant antibiotics. These outbreaks of multidrug-resistant salmonellosis highlight the importance of preharvest food safety programs to reduce the need for antibiotic usage in animals, and for considering further measures to reduce contamination with multidrug-resistant *Salmonella*.

Traditional animal disease control measures, such as those for trichinosis and brucellosis, have had important impacts on the reduction of infections. Presently, concern exists for agents that may be less pathogenic in animals but can cause serious human disease. Often these animals appear asymptomatic, but they can be shedding bacteria and other agents that cause disease in humans. One example of a recurring public health issue involving human illness linked to contact with asymptomatic animals is the problem of human *Salmonella* infections linked with live poultry (chicks, ducklings, chickens, ducks, turkeys, and geese) (Loharikar et al., 2012). Since 1990, at least 35 such outbreaks of human *Salmonella* infections have been reported in the United States (CDC, 2011a). Various *Salmonella* serotypes have been associated with these outbreaks, and specific outbreak strains have been linked to single mail-order hatcheries over several years (CDC, 2006a). These illnesses are especially severe among young children who account for the majority of infections. Chicks and ducklings appear healthy and clean, but their bodies and areas where they live and roam can be contaminated with *Salmonella*, leading to human illness. For example, since 2004, *Salmonella* serotype Montevideo infections having the same genetic fingerprint profile have been reported annually and linked to a single mail-order hatchery. Starting in late 2007, this hatchery implemented numerous interventions to reduce *Salmonella* transmission in its birds, including biosecurity measures and introduction of an autogenous vaccine specific to the outbreak strain. As of 2011, these measures reduced, but did not eliminate, corresponding human *Salmonella* infections associated with live poultry from the implicated mail-order hatchery.

Human contact with animals of many types and in various settings carries a risk of infectious illnesses. Petting zoos and similar venues that feature animals like goats, sheep, cattle, pigs, and poultry can be particularly risky for high-risk individuals including children, elderly, and those with weakened immune systems, especially when handwashing facilities are also inadequate. Since the 1990s, more than 150 outbreaks linked to animals displayed in public settings have been reported in the United States (CDC, 2011a). Cultures of specimens from patients, animals, and animal environments have yielded the outbreak strain

in numerous investigations. These animals usually appear healthy but can be shedding zoonotic pathogens that also contaminate the areas where the animals are displayed, leading to infections in people who do not directly contact an animal (Friedman et al., 1998). Strategies that reduce contamination of live animals used for food could also help prevent transmission by direct contact.

Improving Preharvest Prevention: The Plant Sector

Large outbreaks of human infections linked to fresh produce consumed after minimal processing have been more frequently identified in recent decades (Lynch et al., 2009; Sivapalasingam et al., 2004). There is little that consumers can do to protect themselves because these foods are not cooked, washing them has little effect on contamination, and the contamination itself is undetectable, so it is particularly important to prevent such contamination from happening in the first place.

Outbreak investigations have revealed direct links between produce and animal reservoirs. Several recent produce-associated outbreaks have followed wildlife intrusion into growing fields or fecal contamination from nearby animal production facilities that likely led to produce contamination. In 2006, a multi-state outbreak of approximately 200 illnesses with *E. coli* O157:H7 infection from 26 states was linked to the consumption of fresh spinach (CDC, 2006b). An environmental investigation identified *E. coli* O157:H7 isolates with a PFGE pattern indistinguishable from the outbreak strain in samples obtained from river water, cattle manure, and wild pig feces in and around a field used to grow one brand of spinach from the implicated lot (Wendel et al., 2009). The investigation team also found evidence that wild pigs had been in the spinach fields (FDA, 2006). In August 2006, FDA launched a lettuce safety initiative to address recurring outbreaks of *E. coli* O157 infections. After this outbreak, the initiative was expanded to include all leafy greens.

An instructive outbreak of produce-related illness linked to wildlife intrusion was identified in Alaska in 2008, when there was a sharp increase in the incidence of *Campylobacter* infections around Anchorage (Gardner et al., 2011). Raw peas had been suspected as the source of a small cluster in 2005, and a larger increase in 2008 was rapidly shown to be associated with eating raw peas, from one local farm, which was adjacent to a nature preserve for the Sandhill Crane, *Grus canadensis*. Peas were harvested mechanically and washed in a tank without added chlorine. After harvest, shelled peas were bagged and labeled with directions for blanching, though they were often repacked in bags without this advice, and eaten without blanching. Cranes were observed feeding on peas in the growing fields at the time of harvest, and molecular subtyping studies confirmed that some *Campylobacter* bacteria isolated from patients were indistinguishable from strains isolated from peas, and from crane feces. Harvest was halted that year, and resumed the following year with scarecranes in the fields, chlorination

in the wash water, and clearly visible advice on the packages to blanch the peas before eating. To date, the outbreak has not recurred. This investigation shows that wild birds may be an underrecognized source of produce contamination, and that some basic prevention measures may make it safer. Animal intrusions have also been suspected as the likely source of contamination of apples in cider orchards by cattle or deer with *E. coli* O157 and *Cryptosporidium* (Besser et al., 1993; CDC, 1997), strawberries by deer with *E. coli* O157 (Laidler and Keene, 2012), and lettuces by wild animals with *Yersinia pseudotuberculosis* in Finland (Nuorti et al., 2004).

Water contaminated with animal feces and then used to irrigate plants has also been a route connecting plant production with animal reservoirs. In 2006, an outbreak of approximately 80 persons with *E. coli* O157:H7 infection was linked to lettuce served at locations of a Mexican-style fast food restaurant chain in Iowa and Minnesota (CDPH, 2006). An investigation identified dairy farms near lettuce fields in California that provided lettuce to the restaurants where ill persons had eaten. Environmental samples from the dairy farm and water in soil samples in close proximity to the growing fields identified *E. coli* O157 indistinguishable from the outbreak strain. The irrigation system was connected to the dairy wastewater blending and distribution system, with inadequate backflow protection devices, presenting a possible route for contaminated water to be used on fields adjacent to the lettuce-growing fields associated with this outbreak. These findings indicated that the nearby dairy farm was the likely source of this outbreak. Contaminated water used for irrigation or for processing has been suspected as the likely source of contamination of outbreaks of *E. coli* O157 infections traced to lettuce (Hilborn et al., 1999), and tomatoes, mangoes, and cantaloupes with *Salmonella* (Bowen et al., 2006; Greene et al., 2008; Hedberg et al., 1999; Sivapalasingam et al., 2003).

Sprout-associated outbreaks represent a special scenario, in which the presence of even a few bacterial cells on seeds can be amplified to a large number as a result of the sprouting process itself (Taormina et al., 1999). As seeds are a raw agricultural commodity rather than a food, they may not be expected to be free of pathogens, and their transformation into a food (the sprouts themselves) actually increases the risk, unless special measures are taken to decontaminate the seeds before sprouting and to regularly test the sprouting environment for contamination.

Observations of the biology of human pathogens on plants suggest that interactions between pathogens and produce may sometimes lead to internalization of the pathogen into edible parts of the plant, where it cannot be washed off or eliminated by surface treatments (Berger et al., 2010; Erickson, 2012). This internalization can occur via different mechanisms. Human bacterial enteric pathogens can enter cut or bruised surfaces of leaves and fruit and then multiply in the interior. Air spaces in many fruits contract with a sudden decrease in temperature, and this contraction can draw in water and pathogens from the outside of a fruit,

as shown for apples, mangoes, and tomatoes (Burnett et al., 2000; Penteadó et al., 2004; Rushing et al., 1996). The interactions may be active as well as passive. In the case of sprouts, grown hydroponically without accompanying soil flora, *E. coli* O157 and *Salmonella* present in seeds can enter via the young sprouts' root hairs and are rapidly found throughout the entire plant (Itoh et al., 1998; Jaquette et al., 1996). The interactions may be complex. For example, in the dark, *Salmonella* distribute randomly over the surface of fresh lettuce leaves, but when light stimulates photosynthesis, they concentrate at the stomatal openings that are the respiratory pores on the leaf, as though drawn to products of photosynthetic metabolism (Kroupitski et al., 2009). Some pathogens may be able to manipulate the stomata directly, with a type 3 secretion system that targets the guard cells that ordinarily hold stomata closed in the presence of Gram-negative flagellated bacteria (Saldaña et al., 2011).

These observations raise the question of whether some enteric bacterial pathogens have a life cycle with plant as well as animal hosts. An enteric organism that colonizes herbivores and that also can enter and persist in the plants the herbivores eat gains ready access to the next generation of herbivores. Transfer events from herbivore to plant and plant to herbivore are frequent in prairie or pasture. If enteric pathogens cycle between animal and plant hosts, then the omnivorous human can encounter them on both sides of the cycle. This suggests that, just as food animals may need safer water, fodder, and environments, so food plants may be safer with further attention to the water, soil amendments, and environments used to grow them.

Emerging Foodborne Infections Around the World

Major changes in the global food trade in the past several decades have led to a transformation in the patterns of food production (Florkowski, 2008). The food we eat is sourced from around the globe and distributed over larger distances than ever before. This global trade provides opportunities for exporting countries to earn foreign exchange and drives increases in the standard of living in developing countries. Not only have supply chains become longer, but also the global trade in food has become more specialized. Higher-income countries export grains and processed food to low- and middle-income countries, which in turn export labor-intensive horticultural and fishery products to higher-income countries. Finally, there has been a move toward integration and consolidation of agriculture and food industries, and large corporations have ownership and control across all stages of food production and distribution.

Changes in the globalization of the food trade have important implications for food safety (Tauxe et al., 2010). More imported foods and food ingredients means we depend on food safety systems in other countries. Centralized production of foods means when a problem occurs, it can lead to a widespread outbreak. In this setting, a contaminated food can rapidly cause a geographically

widespread or “dispersed scenario” type of foodborne disease outbreak. In these outbreaks, there are a small numbers of cases in many jurisdictions, typically detected by lab-based subtype surveillance, leading to a multistate or country investigation, and they are usually a result of an industrial contamination event with broad implications. Effective investigations of these types of outbreaks are key to reducing the burden of foodborne disease as we identify food vehicles and factors that lead to outbreaks.

A recent example of a dispersed scenario outbreak was an outbreak of *Salmonella* serotype Montevideo infections in the United States associated with salami products from one company made with contaminated imported black and red pepper (Julian et al., 2010). A total of 272 cases were identified from 44 states and the District of Columbia during 2009 and 2010. In a multistate case-control study, consumption of salami was associated with illness. The outbreak strain was identified in salami products, one company facility environmental sample, and sealed containers of black and red pepper used to produce the salami products. Pepper tracebacks revealed that the pepper originated from Asian countries, although the locus of contamination was not determined.

Multicontinental outbreaks have been recognized when the same subtype of a pathogen is recognized as a simultaneous source of infection in widely separated populations as a result of global trade in foods and feeds. Detecting such events depends on using the same subtyping strategies in many countries, and on collaboration and information sharing when possible links are recognized, and it is likely that many are missed. For example, a global pandemic of *Salmonella* serotype Agona in the early 1970s was the result of contaminated anchovy meal shipped from Peru and used in chicken feed around the world. This was first recognized as a restaurant-associated outbreak in Arkansas traced back to one poultry farm and was subsequently linked to sudden increases in *Salmonella* Agona infections in many countries (Clark et al., 1973). In 1995, simultaneous outbreaks of sprout-associated *Salmonella* serotype Stanley infections in Arizona, Michigan, and Finland were all linked to seeds from one shipper in the Netherlands, who obtained and blended seeds from many other countries (Mahon et al., 1997). In 1998, a savory snack produced in Israel caused *Salmonella* serotype Typhimurium infections in the United Kingdom and North America and was subsequently shown to be a common source of infections in Israel itself; contamination had apparently occurred at the factory where the snack was made (Killalea et al., 1996; Shohat et al., 1996). In 2001, contaminated peanuts from China caused outbreaks of *Salmonella* serotype Stanley infections in Canada, the British Isles, and Australia (Kirk et al., 2004). In 2010, semi-dried tomatoes from Turkey were recognized as the source of similar hepatitis A virus infections in Australia, France, and the Netherlands (Donnan et al., 2012; Petriagnani et al., 2010). These events are surely the tip of the proverbial iceberg and are likely to become more frequently recognized as subtype-based surveillance networks increase worldwide (Swaminathan et al., 2006). They illustrate how a foodborne

pathogen arising in one part of the world can be rapidly disseminated if it is introduced into the global trade networks.

New and unusual foodborne infections continue to arise around the world, as the expanding ecologies of human food production provide niches for the emergence of foodborne pathogens. Two striking examples are described elsewhere in this symposium: Nipah virus encephalitis in Bangladesh, and Shiga toxin–producing *E. coli* O104 infections in Germany (See Burger, 2011, and Luby et al., 2011, in this volume). In Brazil, Chagas disease, a parasitic infection caused by *Trypanosoma cruzi*, transmitted by the feces of the triatomid bug, and carried by the opossum, is a classic vectorborne infection, long associated with primitive rural thatched housing. An urban foodborne outbreak occurred in 1986, linked to consumption of fresh sugar cane juice, apparently contaminated by triatomids or opossum feces present in the cane as it was crushed to extract the juice (Shikanai-Yasuda et al., 1991). Since then, such outbreaks have been more frequently recognized, particularly with production of fresh juice of the açai berry, a jungle fruit that is now being grown in orchards to satisfy consumer demand (Nobrega et al., 2009). The transmission may depend on the intersection of this production with Amazonian ecology (Valente et al., 2009). Such outbreaks may be due to the direct contamination of freshly processed juice with bugs; illness has not been reported from commercial pasteurized product.

In western China, between 2006 and 2009, public health officials investigated several foodborne outbreaks of infection with toxigenic *Vibrio cholerae* O139, a variant strain of the dominant *V. cholerae* O1 that first appeared in India in 1995 and spread through Southeast Asia (Li et al., 2008; Tang et al., 2010; Xia et al., 2010). In these outbreaks, the food vehicle has been the soft-shelled turtle, steamed and served at banquet celebrations. These turtles are brought from sources in other parts of China or Asia, where they are produced commercially. A survey of such turtles in Hunan markets found that 7 of 437 had toxigenic *V. cholerae* O1 or O139 (Xie et al., 2009). The traditional virtues of the long-lived turtle may make it an attractive delicacy for banquets. As with the crab-associated cholera in the United States, future control for this problem may depend on better understanding of circumstances where the turtles are harvested, as well as on better cooking practices (Lowry et al., 1989).

In Israel, where *Tilapia* species have been raised in fish farms for more than 30 years, a new pathogen emerged in the 1990s, after marketing practices changed. The previous practice had been to market the fish frozen, but in 1996, some began to sell them alive. In 1996–1997, 62 cases of severe *Vibrio vulnificus* biotype 3 infections were reported, among persons handling the live fish (Bisharat et al., 1999). These infections were food-associated, although not caused by eating the fish itself. They typically started as local wound infection in the person buying the fish, after a penetrating injury from the many spines on the dorsal fin, and rapidly progressed to bacteremia; 41 required surgical wound debridement. Unlike infections with *Vibrio vulnificus* biotype 1, which cause severe wound

infections in persons who have poor immune systems, and primary bacteremia in oyster eaters with serious liver disease, the patients in Israel were previously healthy. The implicated fish were also healthy and came from a variety of farms. Biotype 3 is a novel recombination of biotype 1 and biotype 2, a pathogen of eels (Bisharat et al., 2005). In 1998, the marketing policy was changed back to selling fish frozen, although cases still occur among those who handle the live fish (Zaidenstein et al., 2008). While it remains unclear where the recombination first occurred, the event is an example of how a pathogen can expand in an agricultural niche and reach the consumer if the circumstances are right.

In Taiwan, since 2000, extremely resistant strains of *Salmonella* serotype Choleraesuis have caused serious infections in humans and have also been detected in local swineherds (Chiu et al., 2002). This highly invasive serotype has a predilection in humans for endothelial tissues. Most hospitalized patients were admitted with primary bacteremia, sepsis, aortitis, and aortic valve infections (Jean et al., 2006). Human strains have high levels of resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and ciprofloxacin (Chang et al., 2005). More recently, some have been resistant to extended-spectrum cephalosporins as well, due to a transmissible plasmid with *bla*CMY2 that has recently been found in four other common *Salmonella* serotypes in addition to Choleraesuis (Su et al., 2011). Indistinguishable strains have been reported from pigs, for which Choleraesuis is a host-adapted pathogen, and it was noted that 40 percent of pig farmers used fluoroquinolones to treat their herds (Hsueh et al., 2004). It seems likely that many of the human infections come from the porcine source, and full control is likely to require changes in agriculture practices to control the infection in pigs.

The challenge of global emerging foodborne infections underlines our interdependence on the public health and food safety systems around the world. Several collaborative programs are actively improving basic public health capacity, promoting standard laboratory identification and subtyping methods, and providing rapid communication. For example, the Training Programs in Epidemiology and Public Health Interventions Network, and the European Program for Intervention Epidemiology Training provide long-term training and practical experience in more than 56 countries (Anonymous, 2006; Moren et al., 1996). The World Health Organization (WHO) Global Foodborne Infections Network (formerly known as WHO Global Salm-Surv) has held 73 short-term multinational multidisciplinary courses to train microbiologists and epidemiologists together from public health, food, and animal medicine sectors (WHO, 2010). PulseNet International has training laboratories in all regions of the world, members in more than 75 countries who have been trained in standardized methods for molecular subtyping, and a global platform for evaluating and introducing new standard methods as they are developed (CDC, 2010c; Swaminathan et al., 2006). The WHO INFOSAN communication channel can link food safety authorities in all countries, so that information about newly identified hazards can be rapidly

disseminated (WHO, 2007). Managing international foodborne outbreaks relies on robust investigations in the countries where disease occurs, and in the countries where the implicated food is grown or manufactured (Tauxe et al., 2008). In many of these investigations, close collaboration with the exporting country authorities and with the food industry can lead to better prevention strategies for the long term.

Conclusion

The complex and changing biological web of the human food supply means that we can expect new pathogens to emerge and novel food vehicles to be identified. Many of these will start in animal reservoirs and may reach us through both animal- and plant-derived foods. Much of the recent progress that has occurred in food safety has been the result of focused efforts to reduce contamination after harvest, for example with better sanitation and process control for meat and poultry at slaughter and in subsequent processing, and better control of processed foods to reduce contamination with *Listeria*. Contamination can start well before harvest or slaughter, and interventions that focus on the live animal or plant are needed to make further progress in making food safer. Such intervention will depend on understanding the biology of pathogens in the field, their life cycles, and the points at which contamination can be prevented or interrupted. Detecting the new problems will depend on robust capacity for public health surveillance and investigation and on multidisciplinary understanding of the ecologies that sustain them. With our global food supply, problems that arise in one part of the world can spread rapidly, if they enter the global food trade. Improving the safety of the food supply thus depends on stronger public health capacity around the world, better understanding of new challenges wherever they are identified, and translating that understanding into effective prevention from farm to table.

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A15

ANTIBIOTIC RESISTANCE—LINKING HUMAN AND ANIMAL HEALTH

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This paper will address the transmission of antibiotic-resistant microorganisms between animals and humans in a One Health perspective. It will give a general introduction to the epidemiology of antibiotic resistance in zoonotic pathogens and then focus on some national and international programs for integrated surveillance and control of antimicrobial resistance at the human–animal interface, with particular emphasis on programs implemented in the authors’ home country, Denmark.

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Overview

The epidemiology of antimicrobial-resistant microorganisms at the human–animal interface involves complex and largely unpredictable systems that include transmission routes of resistant bacteria as well as resistance genes and the impact of antimicrobial selective pressures in several reservoirs (animals, humans, and the environment) (Figure A15-1).

Thus the One Health approach is useful when it comes to addressing zoonotic transmission of pathogens that are resistant to antimicrobials, because we need to engage a wide range of stakeholders including farmers, veterinarians, food safety professionals, medical doctors, as well as environment and wildlife experts in monitoring and control activities.

The feature that particularly differentiates antimicrobial resistance from other food safety–related problems is the role of the chemical driver, the antimicrobials, which selects for the resistant bacteria that subsequently can spread between animals and humans.

Antimicrobials are used widely to prevent or treat disease in food animals. The major part of the usage is for prevention of disease, and their use has become an integral part of modern industrialized food-animal production, to the extent where nearly all feed for growing animals is supplemented with antimicrobials in various doses, ranging from so-called “subtherapeutic concentrations” to full therapeutic doses. It is estimated that the volumes of antimicrobials used in food animals exceeds the use in humans worldwide, and nearly all the classes of antimicrobials that are used for humans are also being used in food animals, including the newest classes of drugs such as third- and fourth-generation cephalosporins, fluoroquinolones, glycopeptides, and streptogramins (Aarestrup et al., 2008).

The massive use of antimicrobial agents in agriculture has supported the intensification of modern food-animal production since the early 1960s by facilitating earlier weaning, higher animal densities, and the use of cheaper feed sources, among others, and has most likely contributed to increased outputs and lower prices of meat. However, the gains have come at a cost, which is being borne, in part, by other stakeholders, in particular public health. Furthermore, the production gains achieved by indiscriminate antimicrobial usage in the 1960s production systems may to a large extent be achievable by other means in modern and more environmentally sustainable food-animal production systems, where higher emphasis is placed on animal welfare, a smaller environmental footprint, and disease prevention through hygiene and intelligent herd management.

The amounts and patterns of antimicrobials used in food animals is the major determinant for the propagation of resistant bacteria in the animal reservoir. Thus, the levels and patterns of resistance observed in food animals to a wide extent reflect the patterns of drug usage; however, other determinants also play a part, such as spread of bacterial clones between animals, in particular vertical spread between the generations (e.g., the spread of resistant *Salmonella* in the poultry

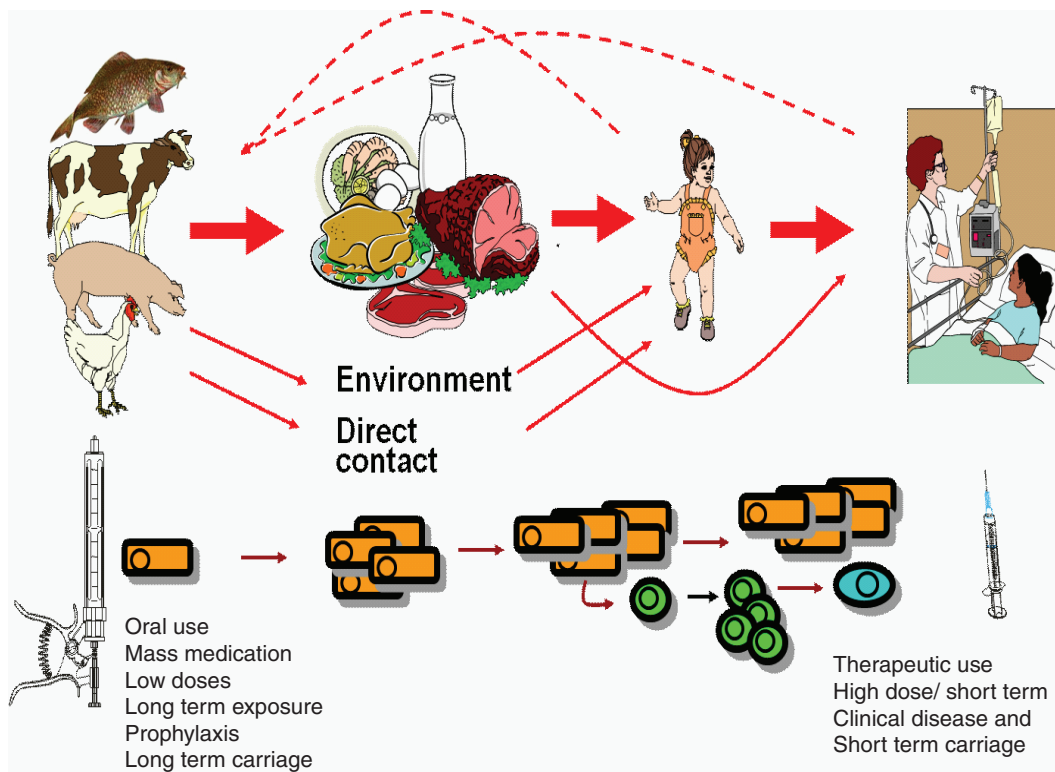


FIGURE A15-1 The epidemiology of antimicrobial resistance at the human–animal interface is invariably complex. It involves a multitude of potential transmission routes and vehicles, antimicrobial selective pressures and other ecological drivers, as well as horizontal transmission of resistance genes between bacterial species and genera.

and swine breeding pyramids), and successful adaptation of clones resistant to the animal reservoir (e.g., MRSA CC398) (Aarestrup et al., 2008).

Transmission of resistance from animals to humans can take place through a variety of routes (Figure A15-1), where the food-borne route probably is the most important (most infections with enteric bacterial pathogens, such as *Salmonella enterica*, *Campylobacter coli/jejuni*, and *Yersinia enterocolitica*, probably occur through this route in industrialized countries), whereas, for other resistant pathogens, direct contact between animal and humans may be the major route of transmission (e.g., MRSA CC398). Bacteria as well as antibiotic residues from food-animal production are spread widely in the environment, mainly with manure, where it affects bacteria in the environment as well as in wild fauna. Thus, the environment and wild fauna can become reservoirs of resistance and a source of reintroduction of resistant bacteria into the food-animal and human reservoirs.

The public health consequences of zoonotic antibiotic resistance are invariably difficult to assess for a number of reasons: the epidemiology is highly complex because it involves complex production and distribution systems of animals and food, it involves the spread of bacterial clones as well as resistance genes, and, finally, the impact on public health includes several end points that are difficult to determine, such as infections that would otherwise not have occurred, increased morbidity and mortality, and higher costs of treatment of disease. In the most comprehensive assessment of the problem to date, an expert group gathered by the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Animal Health Organization (OIE) in 2003 concluded

there is clear evidence of adverse human health consequences due to resistant organisms resulting from non-human usage of antimicrobials. These consequences include infections that would not have otherwise occurred, increased frequency of treatment failures (in some cases death) and increased severity of infections, as documented for instance by fluoroquinolone resistant human *Salmonella* infections. Evidence shows that the amount and pattern of non-human usage of antimicrobials impact on the occurrence of resistant bacteria in animals and on food commodities and thereby human exposure to these resistant bacteria. The foodborne route is the major transmission pathway for resistant bacteria and resistance genes from food animals to humans, but other routes of transmission exist. There is much less data available on the public health impact of antimicrobial usage in aquaculture, horticulture and companion animals.” (FAO et al., 2003)

Investigating the zoonotic antimicrobial resistance problem in its full complexity requires monitoring of antimicrobial usage and resistance in all relevant reservoirs and stages in the transmission route, and coherent analysis of the data (i.e., “integrated monitoring”). For the purpose of intervention, there are multiple potential points of control that may be used, depending on the specific nature of

the problem. Identifying and intervening at the most efficient points of control requires a comprehensive assessment of the risk based on integrated monitoring, as well as good collaboration between all the stakeholders involved.

Already in the early 1960s, findings of resistant *Salmonella* in food animals and humans, and studies that showed that they could pass their resistance traits on to other enteric bacteria, gave rise to major concern in the United Kingdom. This led to the formation of the “Swann Committee,” which recommended

that only antibiotics which “have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic drug or drugs through the development of resistant strains of organisms” should be usable for growth promotion. (Swann et al., 1969)

This was put into the UK legislation and subsequently the European Union legislation. The United States and the rest of the world, however, did not follow the same path.

In the mid-1990s the detection of vancomycin-resistant *Enterococcus faecium* as well as quinolone-resistant *Salmonella* and *Campylobacter* in food animals and evidence of their spread to humans elevated the scientific and public concerns to new levels. This prompted a series of international expert consultations and meetings under the auspices of the WHO and/or the OIE, and it also led to implementation of specific interventions to contain antimicrobial resistance in the food-production chain in many countries, most importantly the complete termination of the use of antimicrobial growth promoters in Europe (FAO et al., 2004; WHO, 1997).

Recently a number of antimicrobial-resistant pathogens have emerged in the food-production chain: extended beta-lactamase producing *Salmonella* and *Escherichia coli*, transmissible quinolone resistance (*qnr*) in *Salmonella* and *E. coli* and animal-associated methicillin-resistant *Staphylococcus aureus* (MRSA), which can transmit to, and cause infections in, humans. These emergences can all be associated with the use of antimicrobial agents in food animals, and they have led to renewed attention to the use of certain types of antimicrobials in food animals that are considered critically important for human health (Aarestrup et al., 2008; Xia et al., 2010).

Residues of antimicrobial agents that may occur in animal-derived products appear to be of a lesser concern for public health than the resistant bacteria. A WHO expert committee concluded in 2003 that residues of antimicrobials in foods, under present regulatory regimes, represent a significantly less important human health risk than the risk related to antimicrobial-resistant bacteria in food (FAO et al., 2003).

Use of antibiotic resistance genes as marker genes in genetically modified plants, which may serve as feed for animals or food for humans, has also raised concerns in this context. Recently, the European Food Safety Authority (EFSA) conducted a risk assessment based on the current state of the science and con-

cluded the following: “Notwithstanding these uncertainties, the current state of knowledge indicates that adverse effects on human health and the environment resulting from the transfer of these two antibiotic resistance genes from GM plants to bacteria, associated with use of GM plants, are unlikely” (EFSA, 2009).

Increased overlap between humans and wildlife populations may increase the risk for novel disease emergence in wildlife in a recent study by Wheeler et al. (2012). Antibiotic resistance was used as a molecular marker for the intensity of human–wildlife microbial connectivity in the Galápagos Islands. Antibiotic-resistant bacteria were found in reptile feces from tourism sites, whereas no resistance was detected at protected beaches on more isolated islands, indicating that human contact may be the source of resistant enteric bacteria (*E. coli* and *Salmonella*) in Galápagos wildlife (Wheeler et al., 2012).

Recognizing the continued emergence of new bacterial pathogens, in animals, that are resistant to antimicrobials considered critically important for human therapy, there is good reason to further strengthen global efforts to prevent and control the emergence and spread of resistance from animals to humans. The One Health concept and its focus on the interdependencies and links between the three health systems of animals, humans, and the environment, respectively, are extremely well suited for this purpose.

The remaining part of this article describes some examples of national and international interventions to contain antimicrobial resistance in the food-production chain, with main emphasis on interventions employed in the authors’ home country, Denmark, which happen to be some of the most advanced and also best documented interventions in this regard.

National and International Attempts to Monitor and Control Transmission of Antimicrobial Resistance Between Animals and Humans

A large number of national and international rules and regulations are involved in the regulation and control of food-borne antimicrobial resistance.

Legal Framework at the International Level

The Codex Alimentarius Commission (CAC), under the WHO and the FAO, has issued recommendations that should be implemented by all countries as a code of practice to minimize and contain antimicrobial resistance (CAC, 2005). This code of practice gives recommendation for the responsibilities of regulatory authorities, the veterinary pharmaceutical industry, veterinarians, and wholesale and retail distributors and producers.

As examples, regulatory authorities should ensure that antimicrobial agents are prescription only (thus, not used for growth promotion), only drugs that are efficacious and with well-established dosages should be approved, surveil-

lance programmes for monitoring drug use and resistance should be established, research should be encouraged, and all unused drug should be collected and destroyed. It is stated that veterinarians should only prescribe drugs for animals under their care and ensure that the drugs used are aimed at clinical disease. In addition, the professional organization should develop clinical practice guidelines on responsible use. In addition to these international recommendations a large number of different national legislation regulates the use of antimicrobial agents and the control of antimicrobial-resistant bacteria.

Below are some examples of control options and their effect on resistance.

Possible Risk Management Options

Currently from a practical and legal point of view, control options are usually divided into pre-harvest (e.g., on farm) and post-harvest (e.g., slaughterhouse and food). However, a more logical way to look at this problem would be to either avoid selection and/or stop the spread of resistant bacteria. Thus, the control of antimicrobial resistance can be controlled either through management of the selective pressure leading to resistance or through interventions aimed at limiting the spread of the selected resistance.

Continuous and updated information is essential to guide risk management and to determine the effect of possible interventions. Thus, continuous monitoring of the occurrence of food-borne pathogens, antimicrobial resistance, and drug use as well as research studies determining the effects of interventions and the associations between different reservoirs, the spread of bacterial clones and genes, and risk factors for the development and spread of resistance are essential for efficient risk management.

Monitoring of Antimicrobial Resistance Information on the occurrence of antimicrobial resistance is needed at the local, national, and international levels to guide policy and detect changes that require intervention strategies. Such monitoring programmes should be continuous and standardized, enabling comparison between countries as well as over time. The main aspects to be considered in establishing a monitoring system include animal or food groups to be sampled, the number of samples to take and the strategy for collection, bacterial species to be included, methods for susceptibility testing, antimicrobials to test, break points to use, quality control, data to be reported, analysis and interpretation of data, and reporting (Bager et al., 1999). The Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP) established in 1995 was the first integrated program in Europe (Figure A15-2). Recently a proposal for a common protocol for antimicrobial resistance monitoring was proposed for Europe (EFSA, 2008). It can be hoped that this can form the basis for a future establishment of a common global standard.

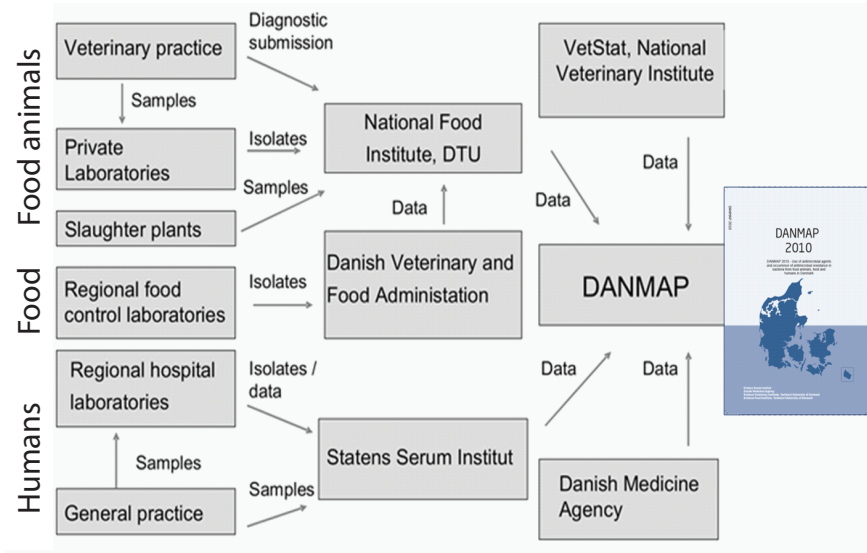


FIGURE A15-2 Flow of samples, isolates, and data in the Danish Integrated Antimicrobial Resistance and Antimicrobial Usage Monitoring Programme—DANMAP. SOURCE: DANMAP (2010).

Monitoring of Antimicrobial Drug Use Data on drug usage is essential for the development of national and international policies for containment of antimicrobial resistance. In Denmark, a programme called Vetstat was implemented in August 2000 and has since collected data from veterinarians, pharmacies, and feed mills. The programme monitors the use of all prescription medicine in production animals, including sera and vaccines, as well as the use of coccidiostatics (Stege et al., 2003). Data are collected at the farm level and include information concerning animal species, age of animal, disease, farm identification number, veterinarians' number, drug identification number, amount of medicine, and date for use of medicine. Today Vetstat enables the authorities to assess usage patterns at the level of the individual herd and individual prescriber. Furthermore, many veterinarians use Vetstat daily as a tool in relation to their service for their clients (farmers). Because all data are converted to defined animal daily doses (ADDs) it is possible to compare the use of antibiotics on one farm with a similar average for the whole country.

In 2010 the Danish Veterinary and Food Authority (DVFA) introduced the “Yellow Card Initiative” based on Vetstat (DVFA, 2012). Each year, DVFA will issue threshold limits for antimicrobial consumption in pigs (other animal species may follow later). The limits for pigs in 2010 were as follows:

1. Weaners (7-30 kg): 28 ADD per 100 weaners per day.
2. Young pigs, including young females (over 30 kg), excluding sows, gilts, and boars: 8 ADD per 100 pigs per day.
3. Sows, gilts, and boars: 5.2 ADD per 100 pigs per day.

If the average antimicrobial consumption in a holding within a 9-month period exceeds one or more of the threshold limits, DVFA may issue an order or injunction (the yellow card) compelling the owner of the holding, in collaboration with the veterinary practitioner, to reduce the antimicrobial consumption in the holding below the threshold limits within 9 months.

The total use of antimicrobials in swine has been reduced by 21 percent in Denmark, following the introduction of the Yellow Card Initiative, when comparing national data on usage for the years 2009 and 2011, respectively (DVFA, 2012).

Recently a first attempt to collect comparable veterinary antibiotic usage data for the European countries was carried out (Figure A15-3) (Grave et al., 2010). The rather large differences between the different countries can be explained by differences in types of animal production systems, different veterinary antibiotic policies and practices, or differences in disease occurrence. A recent study reported that a conservative estimate of the comparable figure for the United States was considerably higher, approximately 300 mg/kg (Aarestrup et al., 2010).

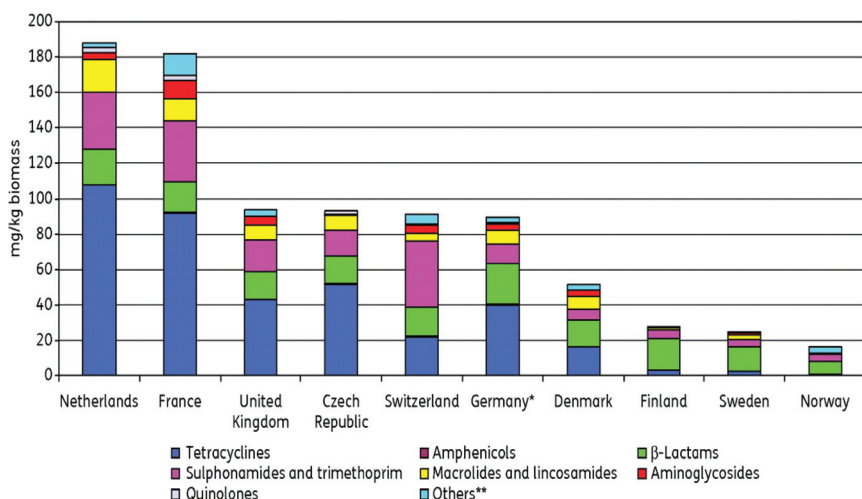


FIGURE A15-3 Comparison of the sales of veterinary antibacterial agents between 10 European countries (mg per kg meat produced).

SOURCE: Grave et al. (2010). The comparable figure for the United States is estimated to be approximately 300 mg/kg according to Aarestrup et al., 2010.

Limiting the Selective Pressure

Prescription One of the basic principles in the Codex Alimentarius codes of practice to minimize and contain antimicrobial resistance is that all antimicrobial agents should be on prescription, and the right to prescribe drugs should rest with the veterinarians or other animal health professionals with an appropriate education. Prescribing and dispensing should be separated to avoid conflicts of interest.

Drug Approval All drugs intended for human or animal use undergo an approval process before licensing, which differs somewhat between countries even though some general guidelines are used. The traditional risks that are considered in the approval process include proof of efficacy against the target pathogen, target animal safety, environmental safety, and human health safety with a focus on toxicological effects (residues). Human hazards related to the transfer of antimicrobial resistance are of more recent concern and have so far only had limited focus in the approval process. In 2003 the U.S. Food and Drug Administration (FDA) published a guidance document for a qualitative risk assessment to be performed prior to the approval of an antimicrobial agent for animal use (FDA, 2003). This guideline outlines an evidence-based approach to preventing antimicrobial resistance from emerging in humans as a consequence of using antimicrobial agents in animals. This guidance requires a ranking into high, medium, and low of the following factors: (1) probability that resistant bacteria are present in target animals as a consequence of drug use, (2) probability for humans to ingest the bacteria in question, and (3) probability that human exposure results in an adverse effect. These three factors are then joined together in an overall risk estimate ranked as high, medium, or low. In combining the three factors, the most value is put on the consequence estimate. Thus, in essence, antimicrobial agents considered “critically important” will be ranked as having a high risk no matter what the probability for selection or transfer. Thus, already in the approval process consideration as to whether antimicrobials are of critical importance for human health can be taken into account. As an example, in Australia fluoroquinolone use was never approved for use in food animals. Fluoroquinolone-resistant strains are either at very low levels or nonexistent in food animals. The rates of resistance are also very low in human isolates in comparison to other countries (e.g., community onset bloodstream infection resistance rate in *E. coli* of 2 percent) (Kennedy et al., 2008).

It is also possible in the approval process to implement certain restrictions. Thus, it could be possible to approve drugs for a limited number of indications, without accepted extralabel or off-label usage or for some modes of administration only (e.g., only parenteral use). This has recently been done by FDA in the United States, which in July 2008 issued an order coming into effect by October 2008 prohibiting the extralabel use of cephalosporin antimicrobial drugs in food-producing animals (FDA, 2008).

Treatment formularies and prescriber guidelines In Denmark a veterinary treatment formulary was published by the National Food Institute in 1997 (Pedersen et al., 1999). This formulary was mainly targeted toward concerns for human health, but it also took into account the prevalence of antimicrobial resistance among bacteria causing infections in animals. In the formulary, antimicrobials for every disease and associated pathogen(s) are listed and scored (1-3) within the following four categories: efficacy, resistance among the pathogen causing infection in animals, national criteria for human importance in Denmark, and WHO criteria for Critically Important Antimicrobials (WHO, 2005).

It is difficult to evaluate the specific effects of the guidelines. However, considering that one of the main recommendations in Denmark has been to limit the use of macrolides and cephalosporins and that the use of these classes of antimicrobials for pigs, which constitutes 80 percent of the usage for animals in Denmark, has increased by 30 and 33 percent, respectively, between 2005 and 2006 (DANMAP, 2006), the effect seems to be minor.

Restrictions on the use of certain antimicrobial classes It is also possible to implement national or international restrictions on certain antimicrobial classes. As mentioned, in Australia fluoroquinolones are not registered for use in food animals. In Denmark fluoroquinolones were approved for use in production animals in 1993, and in the following years an emergence of resistance was observed. In the year 1999 the farmers voluntarily stopped the use of in-feed fluoroquinolones, and in 2002 the veterinarians' use and prescription of fluoroquinolones to food-producing animals were further restricted by the authorities. Thus, fluoroquinolones can only be used in food-producing animals if a current laboratory test of resistance patterns shows that no other antimicrobial will be effective in treatment of the disease in question and this has been reported to the regional veterinary officer. Furthermore, fluoroquinolones can only be administered by injection and by the veterinarian only. This reduced the total usage of fluoroquinolones in animals in Denmark from 183 kg in 2001 to 49 kg in 2006 (Figure A15-4).

Limiting the prescribers' profit on the sale of antimicrobial agents In many countries a considerable part of the veterinarians' income comes from the direct sale of antibiotics to the farmers. This could tempt some veterinarians to overprescribe antibiotics because of the financial benefit. An example from Denmark showed that limiting the possibility of veterinarians to profit from the sale of drugs led to a reduction in total usage. In 1995 the Danish government issued a new legislation reducing and fixing the veterinarians' profit from the direct sale of antibiotics to a maximum of 5 percent. Furthermore, a veterinarian can only sell antibiotics to a farmer during a visit and for a maximum of 5 days of use. The rest has to be bought at a pharmacy. This resulted in a reduction of 40 percent in total use of

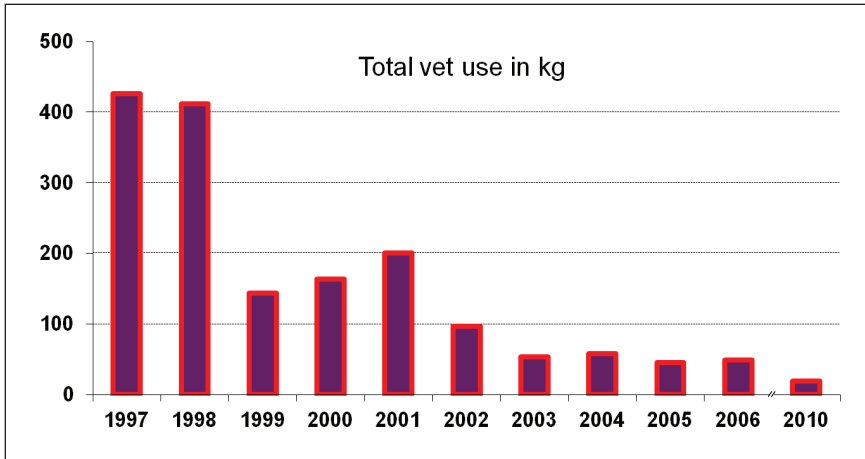


FIGURE A15-4 The total consumption of fluoroquinolones in Danish food-animal production, following voluntary and regulatory efforts to reduce the amounts used in 1999 and 2002, respectively.

therapeutic agents and a reduction in tetracycline use from almost 37 tonnes in 1994 to 9 tonnes in 1995 (Grave and Wegener, 2003).

Price and taxation In human medicine several studies have shown an association between expenses and the prescription of a specific drug. It is a reasonable assumption that the cost of the drug is a considerable factor for the farmer's decision on when and how to use antimicrobials over other disease control and prevention options. In Denmark, a tax was imposed on antimicrobial growth promoters in 1998. The purpose of the tax was to remove the postulated financial benefit from using the antimicrobial growth promoters. From 1998, and until the ban in 2000, a sharp reduction on overall use of antimicrobial growth promoters occurred, but this could also be explained by other factors such as public and media attention, implementation of industry codes of practice, etc. More scientific studies addressing the effects of taxation as a risk management tool are needed.

Voluntary withdrawals or banning of drugs In the United Kingdom the use of tetracyclines and penicillin as growth promoters was banned following the recommendation the Swann report.

In 1995 the Danish Ministry of Agriculture, Fisheries and Food decided to ban the use of the growth promoter avoparcin because of its cross-resistance to vancomycin, a critically important antimicrobial for human use. In 1997, the European Union (EU) banned the use of avoparcin. In 1998 Denmark banned

the use of virginiamycin because of cross-resistance to the critical important Quinupristin-Dalfopristin used in humans. In 1998, the Danish animal production industry voluntarily stopped the use of growth promoters; only swine up to 35 kg bodyweight were still treated with growth promoters until January 2000. In 1999 the EU banned tylosin, spiramycin, virginiamycin, and bacitracin, and the remaining growth promoters were banned in the EU from January 2006. The gradual banning of growth promoters in Denmark resulted in a 50 percent reduction of the usage of antimicrobial agents in animal production from 1997 to 1998, and consequential reductions in the levels of antimicrobial resistance in a range of different bacterial species in food animals (Figures A15-5 and A15-6) (Aarestrup et al., 2008).

In the first quarter of 2005 there was a voluntary withdrawal in Québec chicken hatcheries of the extralabel use of ceftiofur. After the withdrawal, a significant decrease in ceftiofur resistance was seen in *Salmonella* Heidelberg isolates from retail chicken and humans, as well as in *E. coli* from retail chickens (Figure A15-7) (Dutil et al., 2010).

The examples above show that reduction in the use of antimicrobial agents can have a positive effect on the occurrence of antimicrobial resistance. The

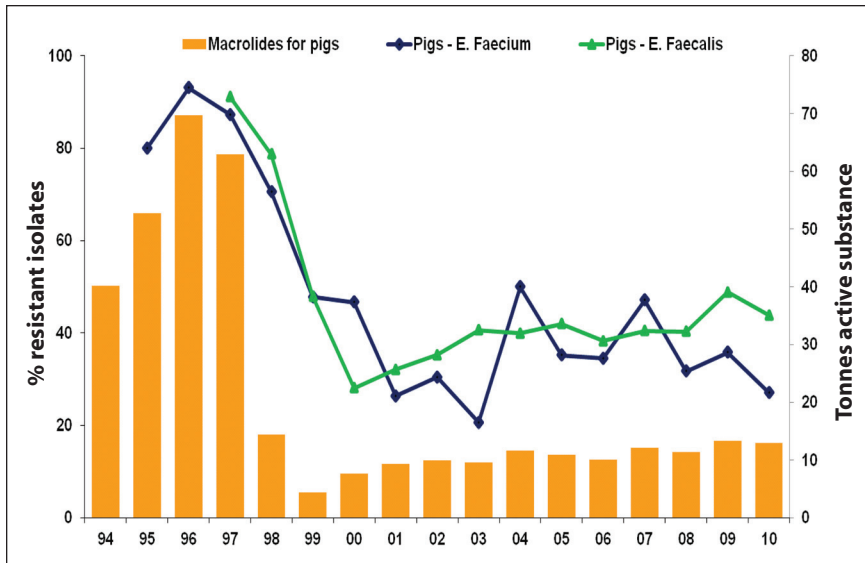


FIGURE A15-5 Resistance (%) to erythromycin among *Enterococcus faecium* and *Enterococcus faecalis* from swine (left Y axis) and the consumption of macrolides in swine, Denmark (right Y axis).
 SOURCE: DANMAP (2010).

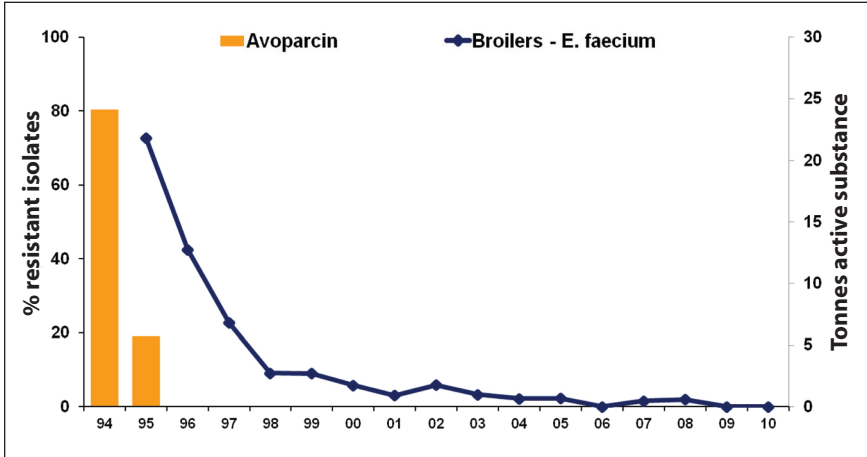


FIGURE A15-6 Resistance (%) to vancomycin in *Enterococcus faecium* from broilers and the consumption of avoparcin, Denmark.
SOURCE: DANMAP (2010).

disadvantage of relying on voluntary withdrawals is that there are no controls that prevent the same groups from later reintroducing these antibiotics and the consequential rapid rise in resistance rates that will result. In fact the chicken hatcheries in Québec have already begun using ceftiofur again.

Preventive veterinary medicinal strategies Disease prevention is an integrated part of food-animal production, and Specific Pathogen Free swine and poultry production systems use this option actively. Preventing disease is considered an essential factor in reducing antimicrobial usage. Strangely only few published scientific studies seem to have addressed this specific point. The most likely explanation for the lack of scientific confirmation thereof is the lack of combined data on management systems, drug use, disease incidence, and antimicrobial resistance.

In a study from Norway the effect of introducing vaccines for prevention of disease in farmed salmon was investigated. The introduction of vaccines led to a substantial reduction in the use of antimicrobials in Norwegian aquaculture (Figure A15-8) (Markestad and Grave, 1997).

It is important to note that whenever antibiotics have been removed as routine food additives for growth promotion and disease prevention purposes there has been no or little evidence that this has resulted in any decrease in animal health or food production.

Controlling Spread of Resistant Bacteria Improved hygiene and infection control is a well-established and essential part of controlling infectious diseases.

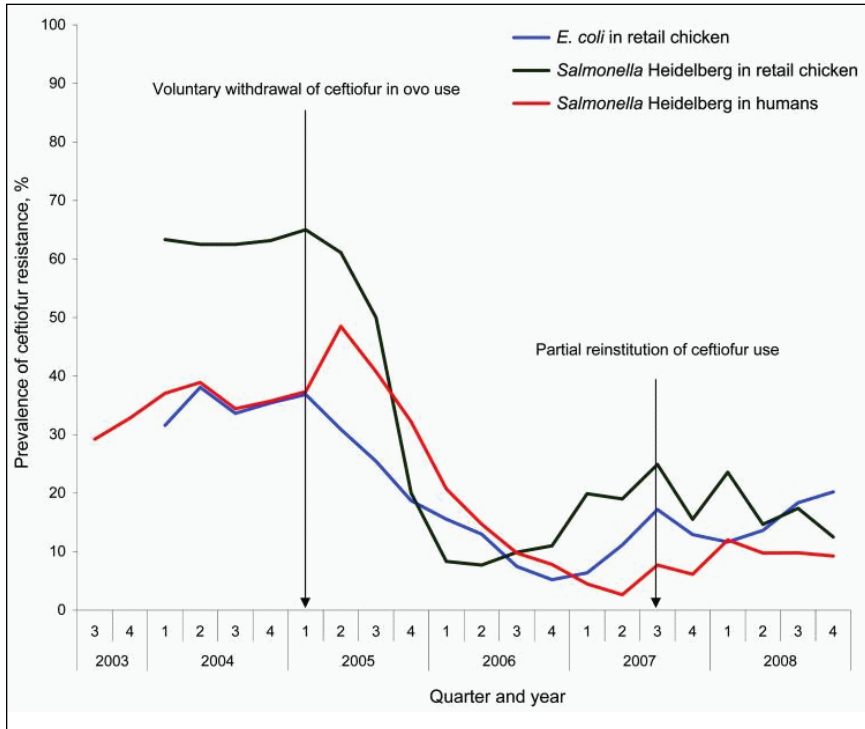


FIGURE A15-7 Prevalence of ceftiofur resistance (moving average of the current quarter and the previous two quarters) among retail chicken *Escherichia coli*, and retail chicken and human clinical *Salmonella enterica* serovar Heidelberg isolates during 2003-2008 in Québec.

Improving the general hygiene in all stages of production and thereby reducing the microbial load on food products will also reduce the antimicrobial resistance load. However, a number of additional options aimed directly at reducing antimicrobial resistance are available for authorities and other stakeholders.

Setting thresholds for the acceptable level of pathogenic bacteria in foodstuffs is a well-established risk management praxis. Thresholds exist for a wide range of bacterial species or subtypes in foodstuffs (e.g., *Listeria monocytogenes* and *E. coli* O157:H7). Thus, establishing thresholds for bacteria resistant to certain antimicrobials is a valid, however rarely used, option to be considered. In Denmark, a specific control programme aimed at *S. Typhimurium* DT104 was implemented in 1998 (Wegener, 2006). As a part of this programme a zero tolerance for DT104 in food was established. The programme has led to a reduction of DT104 in domestically produced and imported foodstuffs.

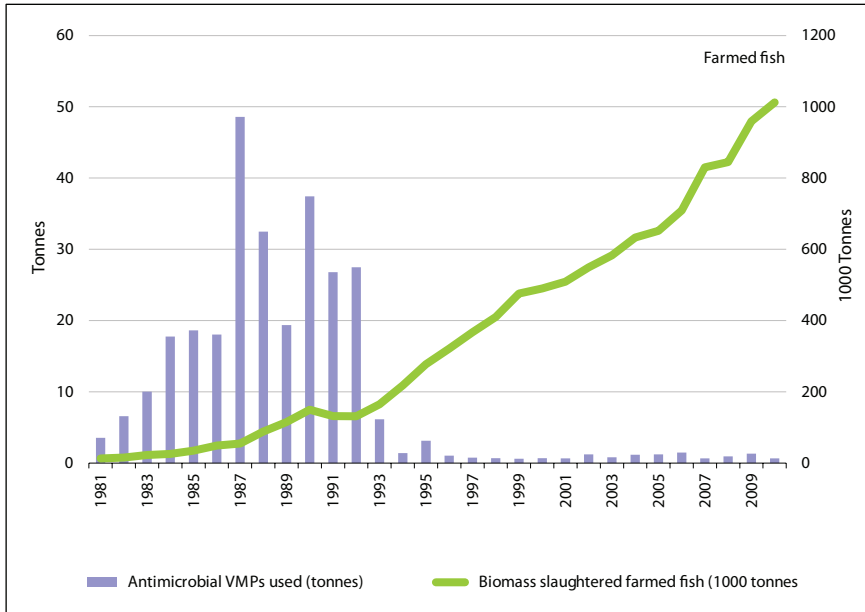


FIGURE A15-8 Sales of antimicrobials for therapeutic use in farmed fish in Norway versus produced biomass.

SOURCE: NORM/NORM-VET (2010).

While several food safety standards for traded food products exist, there seems to be a bigger problem in relation to the trade of live animals. Requirements in relation to epizootic diseases do exist, but none seem to be in place in relation to zoonotic bacteria or in particular antimicrobial resistance. Thus, today breeding animals with resistant *Salmonella* and other pathogens can be traded freely between countries, constituting an efficient route of global dissemination of resistant bacteria.

Conclusions

Integrated surveillance systems are essential to monitor the emergence and spread of antimicrobial resistance along the food production chain. Such systems require

- systematic sampling, harmonized laboratory methods, and good data management;
- detailed denominator data about the origin of the samples;

- subtyping of bacterial isolates, and molecular characterization of resistance genes;
- detailed antimicrobial usage data; and,
- flawless collaboration and coordination, including sharing and comparing data.

Based on existing surveillance systems it is fair to conclude the following:

- There is a close relationship between the patterns of antimicrobial usage and the observed patterns of antimicrobial resistance in food animals; however, other factors such as co-selection and clonal spread also play a part.
- There is a close relationship between levels and patterns of antimicrobial resistance in the food supply and antimicrobial resistance in human food-borne infections, bearing in mind that some food is imported and other foods are consumed while travelling abroad, and that all sources need to be accounted for.

There is a great need to reduce the overall use of antimicrobials in agri- and aquaculture worldwide, and the experiences from different countries suggest that major reductions can be achieved without significant negative effects on animal health or productivity, and for the long-term benefit of public, environmental, and animal health.

A number of effective upstream interventions to reduce resistance have been documented, including banning nontherapeutic uses in food animals, enforcing prescription-only policies, removing financial incentives for prescribing therapeutic drugs, restricting the use of drugs considered critically important for human health, monitoring usage at the farm level and providing advice to high-end users, and establishing thresholds for resistant pathogens in food.

Reducing antimicrobial usage requires collaboration between experts, regulatory authorities, and producers, and integrated monitoring of the effects of interventions is essential. This may be facilitated by establishing a coordinating body, for example, an antibiotic council, including all relevant stakeholders.

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A16

ORIGINS OF MAJOR HUMAN INFECTIOUS DISEASES³³

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Many of the major human infectious diseases, including some now confined to humans and absent from animals, are ‘new’ ones that arose only after the origins of agriculture. Where did they come from? Why are they overwhelmingly of Old World origins? Here we show that answers to these questions are different for tropical and temperate diseases; for instance, in the relative importance of domestic animals and wild primates as sources. We identify five intermediate stages through which a pathogen exclusively infecting animals may become transformed into a pathogen exclusively infecting humans. We propose an initiative to resolve disputed origins of major diseases, and a global early warning system to monitor pathogens infecting individuals exposed to wild animals.

Human hunter/gatherer populations currently suffer, and presumably have suffered for millions of years, from infectious diseases similar or identical to diseases of other wild primate populations. However, the most important infectious diseases of modern food-producing human populations also include diseases that could have emerged only within the past 11,000 years, following the rise of agriculture (Diamond, 1997; Dobson and Carper, 1996). We infer this because, as discussed below, these diseases can only be sustained in large dense human populations that did not exist anywhere in the world before agriculture. What were the sources of our major infectious diseases, including these ‘new’ ones?

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Why do so many animal pathogens, including virulent viruses like Ebola and Marburg, periodically infect human hosts but then fail to establish themselves in human populations?

A tentative earlier formulation noted that major infectious diseases of temperate zones seem to have arisen overwhelmingly in the Old World (Africa, Asia and Europe), often from diseases of Old World domestic animals. Hence one goal of this article is to reappraise that conclusion in the light of studies of the past decade. Another goal is to extend the analysis to origins of tropical diseases (Diamond and Panosian, 2006). We shall show that they also arose mainly in the Old World, but for different reasons, and mostly not from diseases of domestic animals. These results provide a framework for addressing unanswered questions about the evolution of human infectious diseases—questions not only of practical importance to physicians, and to all the rest of us as potential victims, but also of intellectual interest to historians and evolutionary biologists. Historians increasingly recognize that infectious diseases have had major effects on the course of history; for example, on the European conquest of Native Americans and Pacific Islanders, the inability of Europeans to conquer the Old World tropics for many centuries, the failure of Napoleon's invasion of Russia, and the failure of the French attempt to complete construction of a Panama Canal (Crosby, 1986; McNeill, 1976; Ramenofsky, 1987). Evolutionary biologists realize that infectious diseases, as a leading cause of human morbidity and mortality, have exerted important selective forces on our genomes (Anderson and May, 1991; Dobson and Carper, 1996).

We begin by defining five stages in the evolutionary transformation of an animal pathogen into a specialized pathogen of humans, and by considering why so many pathogens fail to make the transition from one stage to the next. We then assemble a database of 15 temperate and 10 tropical diseases of high evolutionary and/or historical impact, and we compare their characteristics and origins. Our concluding section lays out some unresolved questions and suggests two expanded research priorities. We restrict our discussion to unicellular microbial pathogens. We exclude macroparasites (in the sense of Anderson and May, 1991), as well as normally benign commensals that cause serious illness only in weakened hosts. The extensive Supplementary Information provides details and references on our 25 diseases, robustness tests of our conclusions, factors affecting transitions between disease stages, and modern practices altering the risk of emergence of new diseases.

Evolutionary Stages

Box A16-1 delineates five intergrading stages (Figure A16-1) through which a pathogen exclusively infecting animals (Stage 1) may become transformed into a pathogen exclusively infecting humans (Stage 5). Supplementary Table S1 assigns each of the 25 major diseases discussed (Supplementary Note S1) to one of these five stages.

BOX A16-1 Five Stages Leading to Endemic Human Diseases

We delineate five stages in the transformation of an animal pathogen into a specialized pathogen of humans (Figure A16-1). There is no inevitable progression of microbes from Stage 1 to Stage 5: at each stage many microbes remain stuck, and the agents of nearly half of the 25 important diseases we selected for analysis (Supplementary Table S1) have not reached Stage 5.

- **Stage 1.** A microbe that is present in animals but that has not been detected in humans under natural conditions (that is, excluding modern technologies that can inadvertently transfer microbes, such as blood transfusion, organ transplants, or hypodermic needles). Examples: most malarial plasmodia, which tend to be specific to one host species or to a closely related group of host species.
- **Stage 2.** A pathogen of animals that, under natural conditions, has been transmitted from animals to humans ('primary infection') but has not been transmitted between humans ('secondary infection'). Examples: anthrax and tularemia bacilli, and Nipah, rabies and West Nile viruses.
- **Stage 3.** Animal pathogens that can undergo only a few cycles of secondary transmission between humans, so that occasional human outbreaks triggered by a primary infection soon die out. Examples: Ebola, Marburg and monkeypox viruses.
- **Stage 4.** A disease that exists in animals, and that has a natural (sylvatic) cycle of infecting humans by primary transmission from the animal host, but that also undergoes long sequences of secondary transmission between humans without the involvement of animal hosts. We arbitrarily divide Stage 4 into three substages distinguished by the relative importance of primary and secondary transmission:
 - Stage 4a.** Sylvatic cycle much more important than direct human-to-human spread. Examples: Chagas' disease and (more frequent secondary transmission approaching Stage 4b) yellow fever.
 - Stage 4b.** Both sylvatic and direct transmission are important. Example: dengue fever in forested areas of West Africa and Southeast Asia.
 - Stage 4c.** The greatest spread is between humans. Examples: influenza A, cholera, typhus and West African sleeping sickness.
- **Stage 5.** A pathogen exclusive to humans. Examples: the agents causing *falciparum* malaria, measles, mumps, rubella, smallpox and syphilis. In principle, these pathogens could have become confined to humans in either of two ways: an ancestral pathogen already present in the common ancestor of chimpanzees and humans could have co-speciated long ago, when the chimpanzee and human lineages diverged around five million years ago; or else an animal pathogen could have colonized humans more recently and evolved into a specialized human pathogen. Co-speciation accounts well for the distribution of simian foamy viruses of non-human primates, which are lacking and presumably lost in humans: each virus is restricted to one primate species, but related viruses occur in related primate species (Switzer et al., 2005). While both interpretations are still debated for *falciparum* malaria, the latter interpretation of recent origins is widely preferred for most other human Stage 5 diseases of Supplementary Table S1.

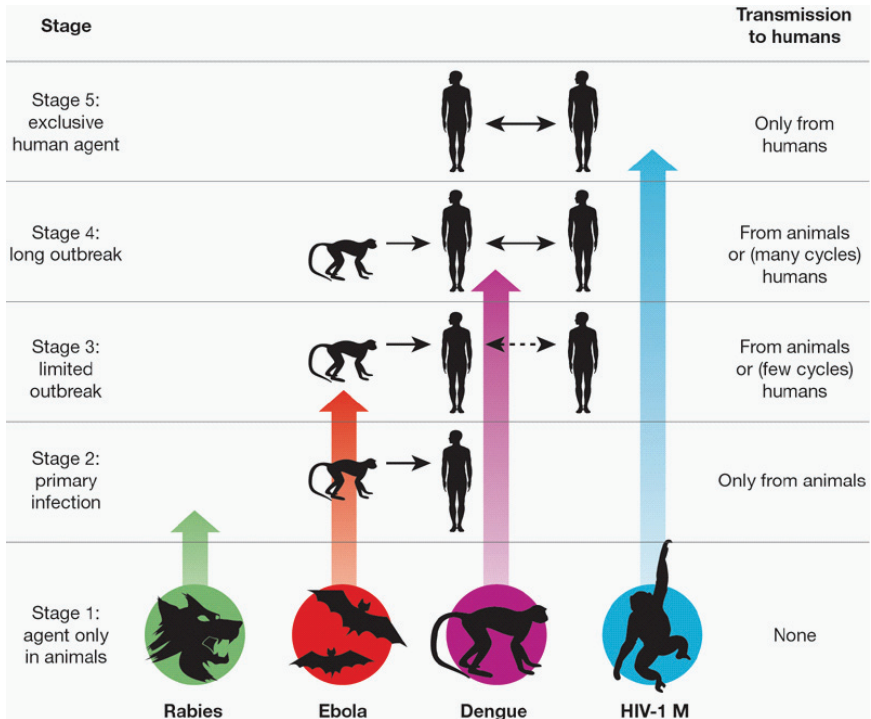


FIGURE A16-1 Illustration of the five stages through which pathogens of animals evolve to cause diseases confined to humans. (See Box A16-1 for details.) The four agents depicted have reached different stages in the process, ranging from rabies (still acquired only from animals) to HIV-1 (now acquired only from humans).

A large literature discusses the conditions required for a Stage 5 epidemic to persist (Anderson and May, 1991; Dobson and Carper, 1996). Briefly, if the disease infects only humans and lacks an animal or environmental reservoir, each infected human introduced into a large population of susceptible individuals must on average give rise during his/her contagious lifespan to an infection in at least one other individual. Persistence depends on factors such as the duration of a host's infectivity; the rate of infection of new hosts; rate of development of host protective immunity; and host population density, size and structure permitting the pathogen's regional persistence despite temporary local extinctions.

Less well understood are two of the critical transitions between stages, discussed in Box A16-2. One is the transition from Stage 1 to Stage 2, when a pathogen initially confined to animals first infects humans. The other is the tran-

sition from Stage 2 to Stages 3 and 4 (see also Supplementary Note S2), when a pathogen of animal origin that is nevertheless transmissible to humans evolves the ability to sustain many cycles of human-to-human transmission, rather than just a few cycles before the outbreak dies out (as seen in modern Ebola outbreaks).

BOX A16-2 Transitions Between Stages

Transition from Stage 1 to Stage 2. Most animal pathogens are not transmitted to humans, that is, they do not even pass from Stage 1 to Stage 2. This problem of cross-species infection has been discussed previously (Antia et al., 2003; May et al., 2001; Moya et al., 2004; Taylor et al., 2001). Briefly, the probability-per-unit-time (p) of infection of an individual of a new (that is, new recipient) host species increases with the abundance of the existing (that is, existing donor) host, with the fraction of the existing host population infected, with the frequency of 'encounters' (opportunities for transmission, including indirect 'encounters' via vectors) between an individual of the existing host and of the new host, and with the probability of transmission per encounter. p decreases with increasing phylogenetic distance between the existing host and new host. p also varies among microbes (for example, trypanosomes and flaviviruses infect a wide taxonomic range of hosts, while plasmodia and simian foamy viruses infect only a narrow range), and this variation is related to a microbe's characteristics, such as its ability to generate genetic variability, or its ability to overcome host molecular barriers of potential new hosts (such as humoral and cellular defenses or lack of cell membrane receptors essential for microbe entry into host cells).

These considerations illuminate different reasons why a given animal host species may or may not become a source of many infections in humans. For instance, despite chimpanzees' very low abundance and infrequent encounters with humans, they have donated to us numerous zoonoses (diseases that still mainly afflict animals) and one or two established human diseases (AIDS and possibly hepatitis B) because of their close phylogenetic relationship to humans. Despite their large phylogenetic distance from humans, many of our zoonoses and probably two of our established diseases (plague and typhus) have been acquired from rodents, because of their high abundance and frequent encounters with humans in dwellings. Similarly, about half of our established temperate diseases have been acquired from domestic livestock, because of high local abundance and very frequent contact. Conversely, elephants and bats are not known to have donated directly to us any established diseases and rarely donate zoonoses, because they are heavily penalized on two or three counts: large phylogenetic distance, infrequent encounters with humans, and (in the case of elephants) low abundance. One might object that Nipah, severe acute respiratory syndrome (SARS) and rabies viruses do infect humans from bats, but these apparent exceptions actually support our conclusion. While bats may indeed be the primary reservoir for Nipah and SARS, human infections by these viruses are acquired mainly from intermediate animal hosts that frequently encounter humans (respectively, domestic pigs,

continued

BOX A16-2 Continued

and wild animals sold for food). The rare cases of rabies transmission directly to humans from bats arise because rabies changes a bat's behaviour so that it does encounter and bite humans, which a healthy bat (other than a vampire bat) would never do.

Transition from Stage 2 to Stage 3 or 4. Although some Stage 2 and 3 pathogens, such as the anthrax and Marburg agents, are virulent and feared, they claim few victims at present. Yet if they made the transition to Stage 4 or 5, their global impact would be devastating. Why do animal pathogens that have survived the initial jump across species lines into a human host (Stages 1 to 2) usually reach a dead end there, and not evolve past Stages 3 and 4 into major diseases confined to humans (Stage 5)? Barriers between Stages 2 and 3 (consider the rabies virus) include differences between human and animal behaviour affecting transmission (for example, animals often bite humans but humans rarely bite other humans); a pathogen's need to evolve adaptations to the new human host and possibly also to a new vector; and obstacles to a pathogen's spread between human tissues (for example, BSE is restricted to the central nervous system and lymphoid tissue). Barriers between Stages 3 and 4 (consider Ebola virus) include those related to human population size and to transmission efficiency between humans. The emergence of novel pathogens is now being facilitated by modern developments exposing more potential human victims and/or making transmission between humans more efficient than before (Morens et al., 2004; Morse, 1995; Weiss and Michael, 2004; Wilson, 1995). These developments include blood transfusion (hepatitis C), the commercial bushmeat trade (retroviruses), industrial food production (bovine spongiform encephalitis, BSE), international travel (cholera), intravenous drug use (HIV), vaccine production (simian virus 40, SV40), and susceptible pools of elderly, antibiotic-treated, immunosuppressed patients (see Supplementary Note S2 for details).

Database and Conclusions

Database. Supplementary Table S1 lists 10 characteristics for each of 25 important 'temperate' (15) and 'tropical' (10) diseases (see Supplementary Note S3 for details of this distinction). Our aim was to select well-defined diseases causing the highest mortality and/or morbidity and hence of the highest historical and evolutionary significance (see Supplementary Note S1 for details of our selection criteria). Of the 25 diseases, we selected 17 because they are the ones assessed by Lopez et al. (2005) as imposing the heaviest world burdens today (they have the highest disability-adjusted life years (DALY) scores). Of the 17 diseases, 8 are temperate (hepatitis B, influenza A, measles, pertussis, rotavirus A, syphilis, tetanus and tuberculosis), and 9 are tropical (acquired immune deficiency syn-

drome (AIDS), Chagas' disease, cholera, dengue haemorrhagic fever, East and West African sleeping sicknesses, *falciparum* and *vivax* *malarias*, and *visceral leishmaniasis*).

We selected eight others (temperate diphtheria, mumps, plague, rubella, smallpox, typhoid and typhus, plus tropical yellow fever) because they imposed heavy burdens in the past, although modern medicine and public health have either eradicated them (smallpox) or reduced their burden. Except for AIDS, dengue fever, and cholera, which have spread and attained global impact in modern times, most of these 25 diseases have been important for more than two centuries.

Are our conclusions robust to variations in these selection criteria? For about a dozen diseases with the highest modern or historical burdens (for example, AIDS, malaria, plague, smallpox), there can be little doubt that they must be included, but one could debate some of the next choices. Hence we drew up three alternative sets of diseases sharing a first list of 16 indisputable major diseases but differing in the next choices, and we performed all 10 analyses described below on all three sets. It turned out that, with one minor exception, the three sets yielded qualitatively the same conclusions for all 10 analyses, although differing in their levels of statistical significance (see Supplementary Note S4). Thus, our conclusions do seem to be robust.

Temperate/tropical differences. Comparisons of these temperate and tropical diseases yield the following conclusions:

- A higher proportion of the diseases is transmitted by insect vectors in the tropics (8/10) than in the temperate zones (2/15) ($P < 0.005$, χ^2 -test, degrees of freedom, d.f. = 1). This difference may be partly related to the seasonal cessations or declines of temperate insect activity.
- A higher proportion ($P = 0.009$) of the diseases conveys long-lasting immunity (11/15) in the temperate zones than in the tropics (2/10).
- Animal reservoirs are more frequent ($P < 0.005$) in the tropics (8/10) than in the temperate zones (3/15). The difference is in the reverse direction ($P = 0.1$, NS, not significant) for environmental reservoirs (1/10 versus 6/15), but those environmental reservoirs that do exist are generally not of major significance except for soil bearing tetanus spores.
- Most of the temperate diseases (12/15) are acute rather than slow, chronic, or latent: the patient either dies or recovers within one to several weeks. Fewer ($P = 0.01$) of the tropical diseases are acute: 3/10 last for one or two weeks, 3/10 last for weeks to months or years, and 4/10 last for many months to decades.
- A somewhat higher proportion of the diseases ($P = 0.08$, NS) belongs to Stage 5 (strictly confined to humans) in the temperate zones (10/15 or 11/15) than in the tropics (3/10). The paucity of Stage 2 and Stage 3

diseases (a total of only 5 such diseases) on our list of 25 major human diseases is noteworthy, because some Stage 2 and Stage 3 pathogens (such as anthrax and Ebola) are notoriously virulent, and because theoretical reasons are often advanced (but also denied) as to why Stage 5 microbes with long histories of adaptation to humans should tend to evolve low morbidity and mortality and not cause major diseases. We discuss explanations for this outcome in Supplementary Note S5.

Most (10/15) of the temperate diseases, but none of the tropical diseases ($P < 0.005$), are so-called ‘crowd epidemic diseases’ (asterisked in Supplementary Table S1), defined as ones occurring locally as a brief epidemic and capable of persisting regionally only in large human populations. This difference is an immediate consequence of the differences enumerated in the preceding five paragraphs. If a disease is acute, efficiently transmitted, and quickly leaves its victim either dead or else recovering and immune to re-infection, the epidemic soon exhausts the local pool of susceptible potential victims. If in addition the disease is confined to humans and lacks significant animal and environmental reservoirs, depletion of the local pool of potential victims in a small, sparse human population results in local termination of the epidemic. If, however, the human population is large and dense, the disease can persist by spreading to infect people in adjacent areas, and then returning to the original area in a later year, when births and growth have regenerated a new crop of previously unexposed non-immune potential victims. Empirical epidemiological studies of disease persistence or disappearance in isolated human populations of various sizes have yielded estimates of the population required to sustain a crowd disease: at least several hundred thousand people in the cases of measles, rubella and pertussis (Anderson and May, 1991; Dobson and Carper, 1996). But human populations of that size did not exist anywhere in the world until the steep rise in human numbers that began around 11,000 years ago with the development of agriculture (Bellwood, 2005; Diamond, 1997). Hence the crowd epidemic diseases of the temperate zones must have evolved since then.

Of course, this does not mean that human hunter/gatherer communities lacked infectious diseases. Instead, like the sparse populations of our primate relatives, they suffered from infectious diseases with characteristics permitting them to persist in small populations, unlike crowd epidemic diseases. Those characteristics include: occurrence in animal reservoirs as well as in humans (such as yellow fever); incomplete and/or non-lasting immunity, enabling recovered patients to remain in the pool of potential victims (such as malaria); and a slow or chronic course, enabling individual patients to continue to infect new victims over years, rather than for just a week or two (such as Chagas’ disease).

Pathogen origins. (See details for each disease in Supplementary Note S10). Current information suggests that 8 of the 15 temperate diseases probably or possibly reached humans from domestic animals (diphtheria, influenza A, measles,

mumps, pertussis, rotavirus, smallpox, tuberculosis); three more probably reached us from apes (hepatitis B) or rodents (plague, typhus); and the other four (rubella, syphilis, tetanus, typhoid) came from still-unknown sources (see Supplementary Note S6). Thus, the rise of agriculture starting 11,000 years ago played multiple roles in the evolution of animal pathogens into human pathogens (Diamond, 1997; Diamond, 2002; McNeill, 1976). Those roles included both generation of the large human populations necessary for the evolution and persistence of human crowd diseases, and generation of large populations of domestic animals, with which farmers came into much closer and more frequent contact than hunter/gatherers had with wild animals. Moreover, as illustrated by influenza A, these domestic animal herds served as efficient conduits for pathogen transfers from wild animals to humans, and in the process may have evolved specialized crowd diseases of their own.

It is interesting that fewer tropical than temperate pathogens originated from domestic animals: not more than three of the ten tropical diseases of Supplementary Table S1, and possibly none (see Supplementary Note S7). Why do temperate and tropical human diseases differ so markedly in their animal origins? Many (4/10) tropical diseases (AIDS, dengue fever, *vivax* malaria, yellow fever) but only 1/15 temperate diseases (hepatitis B) have wild non-human primate origins ($P < 0.04$). This is because although non-human primates are the animals most closely related to humans and hence pose the weakest species barriers to pathogen transfer, the vast majority of primate species is tropical rather than temperate. Conversely, few tropical but many temperate diseases arose from domestic animals, and this is because domestic animals live mainly in the temperate zones, and their concentration there was formerly even more lop-sided (see Supplementary Note S8).

A final noteworthy point about animal-derived human pathogens is that virtually all arose from pathogens of other warm-blooded vertebrates, primarily mammals plus in two cases (influenza A and ultimately *falciparum* malaria) birds. This comes as no surprise, considering the species barrier to pathogen transfer posed by phylogenetic distance (Box A16-2). An expression of this barrier is that primates constitute only 0.5% of all vertebrate species but have contributed about 20% of our major human diseases. Expressed in another way, the number of major human diseases contributed, divided by the number of animal species in the taxonomic group contributing those diseases, is approximately 0.2 for apes, 0.017 for non-human primates other than apes, 0.003 for mammals other than primates, 0.00006 for vertebrates other than mammals, and either 0 or else 0.000003 (if cholera really came from aquatic invertebrates) for animals other than vertebrates (see Supplementary Note S9).

Geographic origins. To an overwhelming degree, the 25 major human pathogens analysed here originated in the Old World. That proved to be of great historical importance, because it facilitated the European conquest of the New World

(the Americas). Far more Native Americans resisting European colonists died of newly introduced Old World diseases than of sword and bullet wounds. Those invisible agents of New World conquest were Old World microbes to which Europeans had both some acquired immunity based on individual exposure and some genetic resistance based on population exposure over time, but to which previously unexposed Native American populations had no immunity or resistance (Crosby, 1986; Diamond, 1997; McNeill, 1976; Ramenofsky, 1987). In contrast, no comparably devastating diseases awaited Europeans in the New World, which proved to be a relatively healthy environment for Europeans until yellow fever and malaria of Old World origins arrived (McNeill, 2006).

Why was pathogen exchange between Old and New Worlds so unequal? Of the 25 major human diseases analysed, Chagas' disease is the only one that clearly originated in the New World. For two others, syphilis and tuberculosis, the debate is unresolved: it remains uncertain in which hemisphere syphilis originated, and whether tuberculosis originated independently in both hemispheres or was brought to the Americas by Europeans. Nothing is known about the geographic origins of rotavirus, rubella, tetanus and typhus. For all of the other 18 major pathogens, Old World origins are certain or probable.

Our preceding discussion of the animal origins of human pathogens may help explain this asymmetry. More temperate diseases arose in the Old World than New World because far more animals that could furnish ancestral pathogens were domesticated in the Old World. Of the world's 14 major species of domestic mammalian livestock, 13, including the five most abundant species with which we come into closest contact (cow, sheep, goat, pig and horse), originated in the Old World (Diamond, 1997). The sole livestock species domesticated in the New World was the llama, but it is not known to have infected us with any pathogens (Diamond, 1997; Dobson, 1996)—perhaps because its traditional geographic range was confined to the Andes, it was not milked or ridden or hitched to ploughs, and it was not cuddled or kept indoors (as are some calves, lambs and piglets). Among the reasons why far more tropical diseases (nine versus one) arose in the Old World than the New World are that the genetic distance between humans and New World monkeys is almost double that between humans and Old World monkeys, and is many times that between humans and Old World apes; and that much more evolutionary time was available for transfers from animals to humans in the Old World (about 5 million years) than in the New World (about 14,000 years).

Outlook and Future Research Directions

Many research directions on infectious disease origins merit more effort. We conclude by calling attention to two such directions: clarifying the origins of existing major diseases, and surveillance for early detection of new potentially major diseases.

Origins of established diseases. This review illustrates big gaps in our understanding of the origins of even the established major infectious diseases. Almost all the studies that we have reviewed were based on specimens collected opportunistically from domestic animals and a few easily sampled wild animal species, rather than on systematic surveys for particular classes of agents over the spectrum of domestic and wild animals. A case in point is our ignorance even about smallpox virus, the virus that has had perhaps the greatest impact on human history in the past 4,000 years. Despite some knowledge of poxviruses infecting our domestic mammals, we know little about poxvirus diversity among African rodents, from which those poxviruses of domestic mammals are thought to have evolved. We do not even know whether ‘camelpox’, the closest known relative of smallpox virus, is truly confined to camels as its name implies or is instead a rodent virus with a broad host range. There could be still-unknown poxviruses more similar to smallpox virus in yet unstudied animal reservoirs, and those unknown poxviruses could be important not only as disease threats but also as reagents for drug and vaccine development.

Equally basic questions arise for other major pathogens. While *falciparum* malaria, an infection imposing one of the heaviest global burdens today, seems to have originated from a bird parasite whose descendants include both the *Plasmodium falciparum* infecting humans and the *P. reichenowii* infecting chimpanzees, malaria researchers still debate whether the bird parasite was introduced to both humans and chimpanzees (Waters et al., 1991) a few thousand years ago in association with human agriculture, or instead more than five million years ago before the split of humans and chimpanzees from each other (Ayala et al., 1999). Although resolving this debate will not help us eradicate malaria, it is fascinating in its own right and could contribute to our broader understanding of disease emergence. In the case of rubella, a human crowd disease that must have emerged only in the past 11,000 years and for which some close relative may thus still exist among animals, no even remotely related virus is known; one or more may be lurking undiscovered somewhere. Does the recent identification of porcine rubulavirus and the Mapuera virus in bats as the closest known relatives of mumps virus mean that pigs infected humans, or that human mumps infected pigs, or that bats independently infected both humans and pigs? Is human tuberculosis descended from a ruminant mycobacterium that recently infected humans from domestic animals (a formerly prevalent view), or from an ancient human mycobacterium that has come to infect domestic and wild ruminants (a currently popular view)?

To fill these and other yawning gaps in our understanding of disease origins, we propose an ‘origins initiative’ aimed at identifying the origins of a dozen of the most important human infectious diseases: for example, AIDS, cholera, dengue fever, *falciparum* malaria, hepatitis B, influenza A, measles, plague, rotavirus, smallpox, tuberculosis and typhoid. Although more is already known about the origins of some of these agents (AIDS, influenza A and measles) than about

others (rotavirus, smallpox and tuberculosis), more comprehensive screening is still likely to yield significant new information about even the most studied agents, as illustrated by the recent demonstration that gorillas rather than chimpanzees were probably the donor species for the O-group of human immunodeficiency virus (HIV)-1 (Van Heuverswyn et al., 2006). The proposed effort would involve systematic sampling and phylogeographic analysis of related pathogens in diverse animal species: not just pigs and other species chosen for their ready availability, but a wider range of wild and domestic species whose direct contact (for example, as bushmeat) or indirect contact (for example, vector-mediated) with humans could plausibly have led to human infections. In addition to the historical and evolutionary significance of knowledge gained through such an origins initiative, it could yield other benefits such as: identifying the closest relatives of human pathogens; a better understanding of how diseases have emerged; new laboratory models for studying public health threats; and perhaps clues that could aid in predictions of future disease threats.

A global early warning system. Most major human infectious diseases have animal origins, and we continue to be bombarded by novel animal pathogens. Yet there is no ongoing systematic global effort to monitor for pathogens emerging from animals to humans. Such an effort could help us to describe the diversity of microbial agents to which our species is exposed; to characterize animal pathogens that might threaten us in the future; and perhaps to detect and control a local human emergence before it has a chance to spread globally.

In our view, monitoring should focus on people with high levels of exposure to wild animals, such as hunters, butchers of wild game, wildlife veterinarians, workers in the wildlife trade, and zoo workers. Such people regularly become infected with animal viruses, and their infections can be monitored over time and traced to other people in contact with them. One of us (N.D.W.) has been working in Cameroon to monitor microbes in people who hunt wild game, in other people in their community, and in their animal prey (Wolfe et al., 2004). The study is now expanding to other continents and to monitor domestic animals (such as dogs) that live in close proximity to humans but are exposed to wild animals through hunting and scavenging. Monitoring of people, animals, and animal die-offs (Kuiken et al., 2003) will serve as an early warning system for disease emergence, while also providing a unique archive of pathogens infecting humans and the animals to which we are exposed. Specimens from such highly exposed human populations could be screened specifically for agents known to be present in the animals they hunt (for example, retroviruses among hunters of non-human primates), as well as generically using broad screening tools such as viral microarrays (Wang et al., 2003) and random amplification polymerase chain reaction (PCR) (Jones et al., 2005). Such monitoring efforts also provide potentially invaluable repositories, which would be available for study after future outbreaks in order to reconstruct an outbreak's origin, and as a source of relevant reagents.

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A17

THE OUTLOOK FOR PUBLIC FOOD SAFETY RESEARCH AND USDA SCIENCE

Catherine Woteki^{37, 38}

Thank you and good afternoon. I'd like to take this opportunity to speak with you today to talk about food safety research at the U.S. Department of Agriculture (USDA) and One Health.

As some of you might know, I served as the first Under Secretary for Food Safety at USDA from 1997 to 2001, where I oversaw U.S. government food safety policy development and USDA's continuity of operations planning.

I'm now USDA's Chief Scientist and Under Secretary for Research, Education and Economics, where I play a role in managing USDA's food and agriculture research portfolio.

My own academic background is in human nutrition—so I've been lucky to have been able to focus on different aspects of our food and agricultural system.

But in reality, all three of these fields—food safety, nutrition, and production agriculture research—are intimately connected.

At USDA these three fields converge, and in order to *ensure* our food and feed systems are safe, health promoting, productive, and sustainable, we need to

³⁷ USDA Chief Scientist and Under Secretary, Research, Education, and Economics.

³⁸ Remarks delivered at "Improving Food Safety Through One Health—Institute of Medicine/Forum on Microbial Threats," December 14, 2011.

make sure that our research programs are planned with these goals in mind. As we used to say in Food Safety—no food is nutritious unless it is safe.

As you are well aware, 65 percent of the emerging infectious diseases of the past 60 years have come from pathogens that have jumped from animals to humans. Many of the pathogens that cause food-borne illness for thousands of Americans every year reside in animals without causing severe illness but can cause life-threatening illness in people.

Greater coordination between human and animal health professionals is becoming a prominent theme among many infectious disease professionals and is becoming the new norm for addressing emerging pathogens.

USDA is the premier organization with veterinary, food safety, nutrition, wildlife, plant, economics, and biotechnology expertise to meet the challenges of this growing coordination and communication among human, animal, and environmental infectious disease professionals.

Given this diversity of expertise, USDA identified a need for a comprehensive national strategy for One Health based upon pandemic planning for both highly pathogenic H5N1 avian influenza and 2009 pandemic H1N1 influenza viruses.

USDA formed two new department-wide interdisciplinary groups to support interdepartmental initiatives at both the policy and technical levels that enhance human, animal, and environmental health.

In a world where disease knows no boundaries, the One Health concept has evolved as the most practical and common-sense means for coordinating between the public health and animal health sectors as well as acknowledging the impact of the environment in the incubation and transfer of infectious diseases.

This comprehensive approach will improve global capabilities to detect, prevent, prepare for, and respond to emerging diseases, pandemic threats, and other issues at the human animal and ecosystem interface.

USDA is developing a greater clarity, understanding, and definition of its One Health approach.

The many cross-cutting organizational structures being created around One Health will help USDA meet the complex challenges of world hunger, food security, environmental stewardship, climate change, and emerging diseases in an ever-changing world. USDA is using the One Health working group to coordinate efforts addressing the use of antibiotics in farm animals and its impact on antibiotic resistance. USDA is also working with the Food and Drug Administration (FDA) in addressing this important societal issue.

By applying One Health principles, it is USDA's hope to encourage a synergy of ideas, reduce program redundancy, and apply this holistic approach—ultimately—to improving global (human, animal, and environmental) health.

As many of you know, USDA, through its Food Safety and Inspection Service (FSIS), ensures the safety of meat, poultry, and processed egg products both domestically and from countries approved to export to the United States.

Prevention is the guiding principle of USDA's food safety efforts.

USDA, FDA, and Centers for Disease Control and Prevention (CDC), along with help from consumers and the industry, have made great strides in reducing *E. coli* O157 illnesses. O157 illnesses have declined by nearly half over the past 14 years, and in the past 2 years, the nation's public health objective in this area has been met. This success is due, in large part, to the Pathogen Reduction and Hazard Analysis Critical Control Point (PR_HACCP) introduced to enhance meat safety practices and is followed up with other preventive measures within USDA and the industry. HACCP by its nature is a holistic approach to an environment where food is processed.

But prevention and inspections are most effective when they are based on good scientific principles. USDA's primary research agencies—through intramural and extramural programs—provide the science-based knowledge to inform food safety policies and regulatory decisions.

As USDA's Chief Scientist, I believe we need to reinforce the role that science is playing in our food safety efforts. Food safety research really *is* a prerequisite for food safety intelligence, and our ability to keep our food system safe is circumscribed by our knowledge of threats to the health of that system.

Research is often a silent partner in food safety, working behind the scenes, before the inspections. We often speak of taking a “farm-to-table” approach to food safety—food and agricultural research is a vital third factor in this equation.

Only occasionally is the value of food and agricultural science brought into the limelight—an unfortunate outbreak of food-borne illness, for example, or recent movies like *Contagion*, raise awareness of ongoing research—but are quickly forgotten when the crisis passes or movie ends.

Outbreaks draw the public into the conversation. But we need to raise awareness of the food safety research that outlasts the news cycle surrounding outbreaks. For food safety, we can't afford to take our eyes off the ball.

Our research programs are our best weapon for identifying new threats. We monitor the latest food-borne illness epidemiological data to identify emerging threats. We work closely with our research partners to develop tests and new technological approaches that work in a regulatory setting, as well as to develop intervention strategies to reduce risk throughout the food chain.

USDA's Agricultural Research Service (ARS) conducts research on the highest priority national and international food-borne pathogens and contaminants. ARS also conducts research for its stakeholders, including its regulatory clients, FSIS and FDA.

Because of its infrastructure, ARS is able to conduct long-term research as well as to quickly respond to newly identified threats. ARS remains flexible to emerging needs, and can and does redirect programs to respond to requests from FSIS, FDA, and CDC.

The ARS food safety program has several centers across the United States dedicated to research covering important food-borne pathogens and contaminants. ARS research focuses on identifying ways to assess, control, or eliminate potentially harmful food contaminants, including those that are accidentally or

intentionally introduced and naturally occurring pathogenic bacteria, viruses, parasites, and chemical contaminants to ensure the food supply is safe and secure and that foreign and domestic regulatory requirements are met.

Based on stakeholders' needs, ARS research focuses on several major areas, including pathogen sources and reservoirs, detection methods, and postharvest processing.

Scientists around the world recognize that the emerging human diseases of this century will continue to arise from the animal kingdom. This past decade has seen an unprecedented epizootic, or animal epidemic, of highly pathogenic avian influenza viruses, mainly affecting poultry, but also infecting several other animal species and humans. Human infections have been associated with direct or indirect contact with live or dead poultry, and animals have been infected through the consumption of infected birds or their products, so there continues to be great concern with these viruses.

USDA-funded scientists have been researching this disease since 1963 and have developed and evaluated avian influenza vaccines, helped assess public health threats, evaluated virus virulence, and helped develop protocols for inactivating flu viruses in food.

When the 2009 pandemic H1N1 influenza A virus emerged in April that year, scientists at first concluded that the virus came from pigs. Pigs can serve as one of the vessels for the "mixing" of avian influenza and human influenza that could set the stage for pandemic avian influenza. We now know that H1N1 is a "triple reassortant virus," which means it contains genetic material from swine, avian, and human influenza viruses—a mix that may help the virus spread quickly and pass between humans and pigs and, importantly, become more virulent.

As early as 2007, USDA-funded scientists had been monitoring for strains of influenza that could spread between pig and human populations. When, in August 2007, several people exhibiting their pigs at a county fair in Ohio developed flu-like symptoms, ARS scientists quickly characterized the virus and found that in pigs it was more virulent than average, instigating immediate close monitoring of the virus in swine, birds, and other species.

The following year USDA and CDC launched a collaborative effort to develop a national swine influenza virus (SIV) surveillance pilot program to better understand the epidemiology of SIV infections and to improve diagnostic tests, preventive management, and vaccines for swine and humans. This program was instrumental in implementing surveillance for the 2009 outbreak.

USDA-funded research contributes to public health by identifying emerging disease strains, assessing current vaccines against emerging strains, and developing standards for inactivating food-borne elements. These research programs are vital as emerging strains continue to evolve, including the nonseasonal H3N2 virus that has just been reported in children in the United States in 2011.

Recognizing that food safety and food security are global issues, the ARS

Food Safety and Animal Health national programs participate in both national and international collaborations through formal and informal partnerships.

Currently, ARS has numerous federal research relationships, including with FSIS, APHIS, FDA, CDC, EPA, DHS, and NIFA. ARS, APHIS, NIFA, FSIS, and FDA have annual and quarterly meetings with leadership to discuss ongoing and upcoming research needs and set priorities.

ARS has many research relationships with academia, especially where the food safety program is collocated with or near a university. ARS is also a member of the National Alliance for Food Safety and Security (NAFSS), a consortium of more than 20 State Agricultural Universities. ARS is co-chair of the Joint Committee on Research (JCR) to address food security research (under Homeland Security Presidential Directive-9) with industry and government.

Our extramural program is the National Institute of Food and Agriculture (NIFA). NIFA provides grants that support important research, education, and extension needs and can be used to conduct large population-based studies and other types of basic and applied food safety research.

USDA's extramural grant programs at NIFA reach out annually to FDA and FSIS to share research priorities and to identify areas where joint research can benefit each agency, as well as our shared publics. In 2011, a series of joint meetings were held to determine priorities, to identify areas of potential collaboration, and to identify gaps in the current research.

In 2009, FDA and NIFA collaborated to solicit research focused on integrating food system signals with geospatial or other innovative technologies used to detect produce contamination. Food system signals are clusters of illnesses reported by government authorities, or problems identified through routine testing. Geospatial technologies include a range of tools for mapping and analyzing data derived from natural resource information, such as climate and environmental monitoring, to predict a future event.

Recently, NIFA awarded two Coordinated Agricultural Project (CAP) grants. One of the CAP grants will help to facilitate research on norovirus, which is a little-understood but difficult virus that causes food-borne and environmentally transferred illness. The other CAP grant will focus on developing intervention and risk management strategies for reducing Shiga toxin-producing *E. coli* contamination in pre- and postharvest environments for beef and beef products. Both CAP grants were awarded through the Agriculture and Food Research Initiative (AFRI), the flagship competitive grant program administered by NIFA.

The National Integrated Food Safety Initiative (NIFSI), another competitive grant program administered by NIFA, has awarded more than \$15 million annually to support a variety of food safety priorities in applied research, education, and extension. For the past several years, the NIFSI program identified produce safety as a special emphasis area in its annual Request for Applications. Special emphasis areas are selected based on current food safety trends (illness

outbreaks), stakeholder input, and collaboration with other federal food safety agencies.

Other NIFA competitive grant programs that provide extramural grant funding for food safety include the Expanded Food and Nutrition Education Program, the Specialty Crop Research Initiative, and the Water Quality Program. Food safety program priorities for all NIFA grant programs are developed with stakeholder input from USDA's sister federal food safety agencies, university, and industry partners and stakeholders.

The major advantage here is that when it comes to food safety there is great deal of consensus that cuts across institutional and international borders as well as public-private interests.

Collectively, USDA agencies, such as FSIS, APHIS Veterinary Services, ARS, and NIFA, are working with industry partners to ensure that hazards are identified and controlled throughout various stages of food production.

But despite the value our research brings to food safety, the continued success and growth of that system is currently being challenged on two fronts.

In 2006, the total domestic food and agriculture R&D performed was just over \$11 billion, with \$5 billion from the public sector and \$6 billion from the private sector. The public sector tends to do the more fundamental, precompetitive, public good research that does not provide an immediate "return on investment," while the private sector picks up the public-sector research and does the development that leads to new products and new technologies.

We know that other countries, most notably China, are ramping up their investments in agriculture research just as the United States is cutting back. Historically, over much of the life of our 150-year-old public research system, the United States has been the leader in agriculture research, which has driven the evolution of science and technology. Recently that dedication has fallen off.

This trend doesn't bode well for our country, its health, the health of our economy, or our food safety research leadership. There is no country other than ours that holds the leadership position or the trust of the rest of the world to do this crucial research.

Our research has a proven track record of success—now more than ever, policy needs to be as scientific as our science: evidence and performance based.

Part of the issue here is that the USDA science agencies are suffering from a funding gap when compared to other U.S. government science agencies.

As many of you know, much of USDA's capacity for doing cutting-edge research depends on both the authorizing and appropriating cycles, and in the past several weeks we've seen a lot of activity on both fronts. On November 17, Congress approved the annual spending bill for USDA and it was signed into law the next day. While the final version emerging from Congress was not as damaging as the House Agriculture Appropriations Committee's proposal earlier this year, this 2012 agriculture appropriations legislation continues the steady stream of cuts to agricultural science that started with the 2011 spending bill.

On the authorizing front, with the demise of the “Supercommittee” process, it is expected now that the House and Senate Agriculture Committees will consider a reauthorization of the 2008 Farm Bill next year. As many of you may know, the Farm Bill process comes around every 5 years when existing authorities for the Department expire. Not every Farm Bill is expected to be as transformational for agricultural science as the 2008 Farm Bill, which created NIFA to be the foremost extramural agricultural research granting agency in the nation, as well as NIFA’s flagship granting program, the Agriculture and Food Research Initiative (AFRI). But every Farm Bill has a significant impact on research.

More fundamentally than funding (and this is the second primary challenge), our country simply isn’t doing enough to educate a sufficient number of students in the STEM (science, technology, engineering, and mathematics) disciplines, and particularly in the food, agriculture, and natural resource sciences, to meet future demand.

Over the past 30 years, the total number of Ph.D. recipients in agricultural fields has only remained constant, while the numbers of Ph.D.s awarded in other life science fields has grown. Because of the tight correspondence of grant funding to graduate student training, it’s not surprising that flat funding of research leads to flat education of graduate students.

Within agricultural disciplines, there has actually been a decline in the number of Ph.D.s awarded in plant, animal, and forestry sciences while the number in environmental science has risen. So our education isn’t keeping up with our scientific needs. The private sector often highlights that it does not have the workforce needed for agricultural research—meaning that, at a time where jobs are in short supply for most of the population, there are jobs going unfilled in these crucial sciences.

Training the scientists today to solve the food and agricultural challenges of tomorrow is one of the smartest investments we can make—*must* make—if we are serious about leading the world to a food secure future.

At every turn, in every partnership, USDA science agencies are delivering on their mission to help ensure a healthy, productive, safe, and sustainable food and agricultural system, while protecting our precious natural and human resources.

Now more than ever we cannot relent in our support for food and agricultural science, nor neglect to educate and train the future scientists who will take the advances made today to new heights.

So we need to continue to stress the vital importance of this research and emphasize the benefits it brings to society.

As awareness grows, sustained support for food and agricultural research will follow. I look forward to continue working with many of you here today to strengthen the ability of food and agricultural science to keep our food system safe and secure. Thank you.

Appendix B

Agenda

Improving Food Safety Through One Health

December 13-14, 2011
500 Fifth St., NW
Washington, DC

DAY 1: TUESDAY, DECEMBER 13

- 8:00 – 8:30: Registration & Continental Breakfast
- 8:30 – 8:45: Welcoming Remarks – Relman/Hughes/King
- 8:45 – 9:30: KEYNOTE: Overview “What Is One Health and Why Is It Relevant to Food Safety?”
Lonnie King & Peter Daszak
Jim Hughes, Moderator
- 9:30 – 10:00: Discussion
- 10:00 – 10:30: BREAK**

SESSION I: Patterns of Emergence and Lessons Learned from Recent Food-borne Disease Outbreaks

Lonnie King, Moderator

- 10:30 – 11:00: Overview of the Global Food System: Changes over Time/Space and Lessons for the Future
Will Hueston, University of Minnesota
- 11:00 – 11:30: Emerging Pathogens in Food—Trends and Changes over the Past 20 Years
Rob Tauxe, Centers for Disease Control and Prevention
- 11:30 – 12:00: EHEC O104:H4 in Germany 2011: Large Outbreak of Bloody Diarrhea and Haemolytic Uraemic Syndrome by Shiga Toxin–Producing *E. coli* via Contaminated Food
Reinhard Burger, Robert Koch Institute
- 12:00 – 12:30: Nipah Virus in Bangladesh
Steve Luby, Centers for Disease Control and Prevention
- 12:30 – 1:00: Discussion
- 1:00 – 1:45: LUNCH**

SESSION II: Surveillance, Detection, and Response

David Relman, Moderator

- 1:45 – 2:15: Norovirus (Viruses as an Emerging Threat to Food Safety & Public Health)
Marion Koopmans, Erasmus University
- 2:15 – 2:45: Global Food Systems: Potential Hot Spots for EID
Peter Daszak, EcoHealth Alliance
- 2:45 – 3:15: Novel Approaches for Detecting Food-borne Outbreaks
Nathan Wolfe, Global Virus Forecasting Initiative, and Stanford University
- 3:15 – 3:45: BREAK
- 3:45 – 4:15: Antibiotic Resistance: Linking Human and Animal Health
Henrik Wegener, Danish National Food Institute

4:15 – 4:45: Plant Food Safety Issues: Linking Production Agriculture with One Health

Michael Doyle, University of Georgia

4:45 – 5:15: Costco’s Food-borne Pathogen QA/QC Program

Craig Wilson, Costco Wholesale

5:15 – 6:00: Discussion and Concluding Remarks

6:00 – 6:15: ADJOURN DAY ONE

DAY 2: WEDNESDAY, DECEMBER 14

8:00 – 8:30: Registration and Continental Breakfast

8:30 – 8:45: Summary of Day One—David Relman

8:45 – 9:30: KEYNOTE: Michael Taylor, Food and Drug Administration
Rima Khabbaz, Moderator

9:30 – 10:00: Discussion

10:00 – 10:15: BREAK

**SESSION III: Views on the “One Health” Paradigm—
Relevance to Stronger Food Safety**
Rima Khabbaz, Moderator

10:15 – 10:45: Food Safety: A View from the Wild Side
William Karesh, EcoHealth Alliance

10:45 – 11:15: One Health: The Australian Perspective on the Biosecurity Continuum from Preborder to Border and Postborder Review
Martyn Jeggo, CSIRO, Australian Animal Health Laboratory

11:15 – 11:45: One Health and Food Safety—The Canadian Experience
Rainer Engelhardt, Public Health Agency of Canada

11:45 – 12:15: Microbe Hunting
Ian Lipkin, Columbia University

12:15 – 12:45: Discussion

12:45 – 1:30: LUNCH

**SESSION IV: One Health and Food Safety—
Perspectives and Opportunities for Global Engagement**

Roger Breeze, Moderator

1:30 – 2:00: Microbiological Food Protection Activities at the USDA
Cathie Woteki, USDA

2:00 – 2:30: Food-Borne Pathogen Control Programs
Mike Robach, Cargill, Inc.

2:30 – 3:00: BREAK

3:00 – 3:30: Complexity and Vulnerabilities of the Fresh Produce Food Sector
David Gombas, United Fresh Produce Association

3:30 – 4:00: How Well Are Food Companies Addressing Microbiological Safety Issues?
David Acheson, Leavitt Partners, LLC

4:00 – 4:45: Discussion

4:45 – 5:00: Concluding Remarks

5:00: ADJOURN

Appendix C

Acronyms

ADD	animal daily dose
A/EEC	attaching and effacing <i>Escherichia coli</i>
AIDS	acquired immune deficiency syndrome
AMR	antimicrobial resistance
AMU	antimicrobial use
APCI	atmospheric pressure chemical ionization
AVMA	American Veterinary Medicine Association
CAC	Codex Alimentarius Commission
CAFO	concentrated animal feeding operation
CDC	Centers for Disease Control and Prevention
CFU	colony forming unit
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CITES	Convention on the International Trade of Endangered Species
CRS	Congressional Research Service
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVM	Center for Veterinary Medicine
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
DEFRA	Department for Environment, Food and Rural Affairs
DNA	deoxyribonucleic acid
DRC	Democratic Republic of the Congo
DVFA	Danish Veterinary and Food Authority

EAggEC	enteroaggregative <i>Escherichia coli</i>
EFSA	European Food Safety Authority
EID	emerging infectious disease
EIEC	enteroinvasive <i>Escherichia coli</i>
EHEC	enterohemorrhagic <i>Escherichia coli</i>
EPEC	enteropathogenic <i>Escherichia coli</i>
ERS	Economic Research Service
ESBL	extended-spectrum β -lactamase
ETEC	enterotoxigenic <i>Escherichia coli</i>
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FMD	foot and mouth disease
FMI	Food Marketing Institute
FSIS	Food Safety and Inspection Service
FSMA	Food Safety Modernization Act
GABHS	Group A beta-hemolytic streptococcus
GAO	government accountability office
GAP	good agricultural practice
GBS	Guillain-Barré syndrome
GVFI	global virus forecasting initiative
HACCP	hazard analysis and critical control point
HAV	hepatitis A virus
HEV	hepatitis E virus
HIV	human immunodeficiency virus
HPAI	highly pathogenic avian influenza
HTLV	human T-lymphotropic viruses
HUS	hemolytic uremic syndrome
IHR	International Health Regulations
IOM	Institute of Medicine
LAMP	loop-mediated isothermal amplification
MALDI	matrix-assisted laser desorption–ionization
MMR	measles, mumps, and rubella
MS	mass spectroscopy
NAQS	National Australian Quarantine Strategy
NARMS	National Antimicrobial Resistance Monitoring System
NIFA	National Institute of Food and Agriculture

NiV	Nipah virus
NOAA	National Oceanic and Atmospheric Administration
NORS	National Outbreak Reporting System
NoV	norovirus
OIE	World Animal Health Organization (Office International des Epizooties)
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
QA/QC	quality assurance/quality control
RNA	ribonucleic acid
SARS	severe acute respiratory syndrome
SE	<i>Salmonella</i> serotype Enteritidis
SFV	simian foamy virus
SIV	swine influenza virus
SRSV	small round structured virus
STEC	Shiga toxin-producing <i>Escherichia coli</i>
STLV	simian T-lymphotropic viruses
TEM	transmission electron microscope
UN	United Nations
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
VTEC	verotoxin-producing <i>Escherichia coli</i>
WHO	World Health Organization

Appendix D

Glossary

Adulterant: An additive causing an undesirable effect; impurity.

Aflatoxin: Any of several carcinogenic mycotoxins that are produced especially in stored agricultural crops (such as peanuts) by molds.

Antibiotic: Class of substances that can kill or inhibit the growth of some groups of microorganisms. Originally antibiotics were derived from natural sources (e.g., penicillin from molds), but many currently used antibiotics are semisynthetic and modified with additions of man-made chemical components. See *Antimicrobials*.

Antibiotic resistance: Property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics.

Antimicrobials: Class of substances that can destroy or inhibit the growth of pathogenic groups of microorganisms, including bacteria, viruses, parasites, and fungi.

Asymptomatic infection: An infection where the patient does not have any apparent symptoms (also known as a subclinical infection).

Avian influenza: Highly variable influenza A of birds caused by any of the subtypes of the causative orthomyxovirus of which some strains have been or may be transmitted to other vertebrates, including humans, especially after undergoing mutation.

Bacteria: Microscopic, single-celled organisms that have some biochemical and structural features different from those of animal and plant cells.

Bush meat: Wildlife species that are hunted in the “bush” or forests.

Campylobacter: A genus of slender spirally curved rod bacteria of the family Spirillaceae that are Gram-negative, microaerophilic, and motile with a characteristic motion resembling a corkscrew, that do not form spores, and that include forms formerly included in the genera *Spirillum* or *Vibrio* of which some are pathogenic for domestic animals or humans.

Clostridium: A genus of saprophytic rod-shaped or spindle-shaped usually Gram-positive bacteria of the family Bacillaceae that are anaerobic or require very little free oxygen and are nearly cosmopolitan in soil, water, sewage, and animal and human intestines, that are very active biochemically comprising numerous fermenters of carbohydrates with vigorous production of acid and gas, many nitrogen fixers, and others that rapidly putrefy proteins, and that include important pathogens.

Codex Alimentarius: A document titled “Recommended International Codes of Hygienic Practice for Fresh Meat, for Ante-Mortem and Post-Mortem Inspection of Slaughter Animals and for Processed Meat Products” published by FAO/WHO in 1976. It serves as an international code of voluntary standards for food additives, pesticide residue, veterinary drugs, and other issues that affect consumer food safety. The CODEX also contains rules and guidelines to promote fair practices in food trade, and recommends an international code of hygiene and technological practices. Published by the Codex Alimentarius Commission. Also called CODEX.

Contaminant: An impurity, any substance or material that enters a system—the environment, human body, food, etc.—where it is not normally found.

Cryptosporidium: A genus of protozoans of the order Coccidia that are parasitic in the gut of many vertebrates including humans and that sometimes cause diarrhea, especially in individuals who are immunocompromised.

Cyclospora: A genus of coccidian protozoans that produce an oocyst containing two sporocysts with each sporocyst containing two sporozoites and that include one (*C. cayetanensis*) causing diarrhea in humans.

Disease burden: The impact of a health problem in a population measured by financial cost, mortality, morbidity, or other indicators.

DNA (deoxyribonucleic acid): Any of various nucleic acids that are usually the molecular basis of heredity, are constructed of a double helix held together by hydrogen bonds between purine and pyrimidine bases that project inward from two chains containing alternate links of deoxyribose and phosphate, and that in eukaryotes are localized chiefly in cell nuclei.

Emerging infectious diseases: Infections that are rapidly increasing in incidence or geographic range.

Enterohemorrhagic *Escherichia coli* (EHEC): A strain of *E. coli* that causes hemorrhage in the intestines. The organism produces Shiga toxin, which damages bowel tissue, causing intestinal ischemia and colonic necrosis. Symptoms are stomach cramping and bloody diarrhea. An infectious dose may be as low as 10 organisms. Spread by contaminated beef, unpasteurized milk and juice, sprouts, lettuce, and salami, as well as contaminated water, the infection can be serious although there may be no fever. Treatment consists of antibiotics and maintenance of fluid and electrolyte balance. In advanced cases, surgical removal of portions of the bowel may be required.

Epidemic: Affecting or tending to affect an atypically large number of individuals within a population, community, or region at the same time.

***Escherichia coli*:** A straight rod-shaped Gram-negative bacterium that is used in public health as an indicator of fecal pollution (as of water or food) and in medicine and genetics as a research organism and that occurs in various strains that may live as harmless inhabitants of the human lower intestine or may produce a toxin causing intestinal illness.

Food-borne diseases: Disease caused by consuming contaminated foods or beverages. Many different disease-causing microbes, or pathogens, can contaminate foods, so there are many different food-borne infections. In addition, poisonous chemicals or other harmful substances can cause food-borne diseases if they are present in food. (<http://www.cdc.gov/foodsafety/facts.html#what>)

Genomics: The study of genes and their associated functions.

***Giardia*:** A genus of flagellate protozoans inhabiting the intestines of various mammals and including one (*G. lamblia* synonym *G. intestinalis*) that is associated with diarrhea in humans.

Globalization: The increased interconnectedness and interdependence of peoples and countries, generally understood to include two interrelated elements: the opening of borders to increasingly fast flows of goods, services, finance, people,

and ideas across international borders; and the changes in institutional and policy regimes at the international and national levels that facilitate or promote such flows. (<http://www.who.int/trade/glossary/story043/en/index.html>)

Hazard Analysis and Critical Control Points (HACCP): A management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement, and handling, to manufacturing, distribution, and consumption of the finished product.

Hemolytic-uremic syndrome (HUS): A rare disease that is marked by the formation of thrombi in the capillaries and arterioles especially of the kidney, that is characterized clinically by hemolytic anemia, thrombocytopenia, and varying degrees of kidney failure, that is precipitated by a variety of etiologic factors (as infection with *Escherichia coli* or *Shigella dysenteriae*), and that primarily affects infants and young children.

Hemorrhagic fever: Any of a diverse group of virus diseases (as Korean hemorrhagic fever, Lassa fever, and Ebola) that are usually transmitted to humans by arthropods or rodents and are characterized by a sudden onset, fever, aching, bleeding in the internal organs (as of the gastrointestinal tract), petechiae, and shock.

Hendra virus: A paramyxovirus that causes encephalitis in humans and is transmitted from animals.

Hepatitis A: An acute usually benign hepatitis caused by a single-stranded RNA virus of the family *Picornaviridae* that does not persist in the blood serum and is transmitted especially in food and water contaminated with infected fecal matter.

Host: Animal or plant that harbors or nourishes another organism.

Hotspot: As used in this report, regions predisposed to disease emergence.

Human immunodeficiency virus (HIV): Any of several retroviruses and especially HIV-1 that infect and destroy helper T cells of the immune system, causing the marked reduction in their numbers that is diagnostic of AIDS.

Human T-lymphotropic virus: A strain of primate T-lymphotropic virus 1 isolated from mature T4 cells in patients with T-lymphoproliferation malignancies. It causes adult T-cell leukemia and T-cell lymphoma, and is involved in mycosis fungoides, and tropical spastic paraparesis.

Infection: The invasion of the body or a part of the body by a pathogenic agent, such as a microorganism or virus. Under favorable conditions the agent develops or multiplies, the results of which may produce injurious effects. Infection should not be confused with disease.

International Health Regulations (IHR): An international legal instrument that is binding on 194 countries across the globe, including all the Member States of the WHO. Their aim is to help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide.

The IHR, which entered into force on June 15, 2007, require countries to report certain disease outbreaks and public health events to the WHO. Building on the unique experience of the WHO in global disease surveillance, alert and response, the IHR define the rights and obligations of countries to report public health events, and establish a number of procedures that the WHO must follow in its work to uphold global public health security (http://www.who.int/topics/international_health_regulations/en/).

Listeria monocytogenes: A common species of Gram-positive motile bacilla that cause listeriosis and a noninvasive food-borne diarrheal disease.

Melamine: An industrial chemical that can cause health problems such as kidney disease.

Microbe: A microorganism or biologic agent that can replicate in humans (including bacteria, viruses, protozoa, fungi, and prions).

Microbial threat: Microbes that lead to disease in humans.

Morbidity: Disease, illness; any departure, subjective or objective, from a state of physiological or psychologic well-being.

Mycotoxin: A poisonous substance produced by a fungus and especially a mold.

Nipah virus: A single-stranded RNA virus of the family *Paramyxoviridae* that has caused epidemics of respiratory disease in pigs and often fatal encephalitis in humans in Malaysia, Singapore, and Bangladesh.

Norovirus: A group of related, single-stranded RNA (ribonucleic acid) viruses of the family *Caliciviridae* that cause acute gastroenteritis in humans.

One Health: One Health is the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals, and our environment (<http://www.avma.org/onehealth/charge.pdf>).

Pandemic: Occurring over a wide geographic area and affecting an exceptionally high proportion of the population.

Paramyxovirus: Any of the family *Paramyxoviridae* of single-stranded RNA viruses.

Parasite: An organism that lives in or on and takes its nourishment from another organism. A parasite cannot live independently. Parasitic diseases include infections by protozoa, helminths, and arthropods (<http://www.medterms.com/script/main/art.asp?articlekey=4769>).

Pathogen: Organism capable of causing disease.

Pathogenic: Capable of causing disease.

Polymerase chain reaction (PCR): A scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Prions: A newly discovered type of disease-causing agent, neither bacterial nor fungal nor viral, and containing no genetic material. A prion is a protein that occurs normally in a harmless form. By folding into an aberrant shape, the normal prion turns into a rogue agent. It then co-opts other normal prions to become rogue prions. They have been held responsible for a number of degenerative brain diseases, including mad cow disease, Creutzfeldt-Jakob disease, and possibly some cases of Alzheimer's disease.

Public health: The art and science of dealing with the protection and improvement of community health by organized community effort and including preventive medicine and sanitary and social health.

Quinolones: Class of purely synthetic antibiotics that inhibit the replication of bacterial DNA; includes ciprofloxacin and fluoroquinolone.

Reservoir: Any person, animal, arthropod, plant, soil, or substance (or combination of these) in which an infectious agent normally lives and multiplies, on which it depends primarily for survival, and in which it reproduces itself in such manner that it can be transmitted to a susceptible vector.

Resistance: see *Antibiotic resistance*.

RNA (ribonucleic acid): Any of various nucleic acids that contain ribose and uracil as structural components and are associated with the control of cellular chemical activities.

***Salmonella*:** A group of bacteria that cause typhoid fever, food poisoning, and enteric fever from contaminated food products.

Salmonellosis: An infection with bacteria called *Salmonella*. Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment.

Severe acute respiratory syndrome (SARS): A viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). SARS was first reported in Asia in February 2003.

Shiga toxin–producing *Escherichia coli* (STEC): A type of enterohemorrhagic *E. coli* (EHEC) bacteria that can cause illness ranging from mild intestinal disease to severe kidney complications. Other types of enterohemorrhagic *E. coli* include the relatively important serotype *E. coli* O157:H7, and more than 100 other non-O157 strains.

***Shigella*:** A genus of nonmotile aerobic bacteria of the family Enterobacteriaceae that form acid but no gas on many carbohydrates and that cause dysenteries in animals and especially humans.

Simian T-lymphotropic virus: A strain of primate T-lymphotropic virus 2, closely related to the human HTLV-1 virus. The clinical, hematological, and histopathological characteristics of the disease in STLV-infected monkeys are very similar to those of human adult T-cell leukemia.

Surveillance: The continuing scrutiny of all aspects of occurrence and spread of a disease that is pertinent to effective control.

***Toxoplasma*:** A genus of sporozoans that are typically serious pathogens of vertebrates.

Vector: A carrier—especially an arthropod—that transfers an infective agent from one host (which can include itself) to another.

Vibrio: A genus of short rigid motile bacteria of the family Vibrionaceae that are straight or curved rods, have one or sometimes two or three polar flagella enclosed in a sheath, and include various saprophytes and a few pathogens (as *V. cholerae*, the cause of cholera in humans).

Virus: A small infectious agent that can only replicate inside the cells of another organism. Viruses are too small to be seen directly with a light microscope. Viruses infect all types of organisms, from animals and plants to bacteria and archaea.

Wet market: A market where most of the commodities for sale are live animals or readily perishable foods.

***Yersinia enterocolitica*:** A pleomorphic Gram-negative bacillus that belongs to the family Enterobacteriaceae. As a human pathogen, *Y. enterocolitica* is most frequently associated with acute diarrhea, terminal ileitis, mesenteric lymphadenitis, and pseudoappendicitis.

Zoonoses: Microbes that are naturally transmitted between animals and humans that cause disease in human populations but can be perpetuated solely in non-human host animals (e.g., influenza, rabies).

Zoonotic infection: Infection that causes disease in human populations but can be perpetuated solely in nonhuman host animals (e.g., bubonic plague); may be enzootic or epizootic.

Appendix E

Speaker Biographies

David W. K. Acheson, M.D., FRCP, graduated from the University of London Medical School in 1980, and following training in internal medicine and infectious diseases in the United Kingdom, moved to the New England Medical Center and Tufts University in Boston in 1987. As an associate professor at Tufts University, he undertook basic molecular pathogenesis research on food-borne pathogens, especially Shiga toxin-producing *E. coli*.

In September 2002, Dr. Acheson became the Chief Medical Officer at the U.S. Food and Drug Administration's (FDA's) Center for Food Safety and Applied Nutrition. Following several other positions at FDA he was appointed as Associate Commissioner for Foods, which gave him an agency-wide leadership role for all food and feed issues, including health promotion and nutrition.

In August 2009, Dr. Acheson took up a new position as the Managing Director for Food and Import Safety at Leavitt Partners LLC, a consulting firm with offices in Salt Lake City and Washington, DC. Leavitt Partners is working with a variety of multinational clients from the farm to retail to address food safety and food defense challenges.

Reinhard Burger, Ph.D., received his doctorate in 1976 and habilitation in 1982 at the Institute for Medical Microbiology at the University of Mainz. From 1983 to 1987 he served as professor for immunology, faculty for theoretical medicine at the University of Heidelberg. Since 1989 he has held the position of professor of immunology at the Free University of Berlin. Professor Burger has also been a visiting scientist at the National Institutes of Health in Bethesda, Harvard Medical School, and the Medical University in Wuhan, China. From 1987 to 2011 he was the head of the Department of Infectious Diseases and Immunology at the

Robert Koch Institute, where he also served as Vice President from 2001 to 2010 and President since 2010.

Professor Burger is a member of numerous scientific societies and numerous national and international expert committees. In 1993 he was appointed Chairman of the National Advisory Committee on Blood of the German Federal Ministry for Health, advising the federal government on all aspects of the safe and efficient use of blood components and plasma derivatives. He has many publications related to immunology and has also authored many recommendations, reports, and guidelines in the field of infectious diseases, immunology, and transfusion medicine, particularly concerning the safety of blood and blood products against transmission of infections and the detection of infectious agents.

Peter Daszak, Ph.D., is President of EcoHealth Alliance, a United States–based organization that conducts research and field programs on global health and conservation. At EcoHealth Alliance, Dr. Daszak manages a headquarters staff of 35 and a global staff of more than 700 that conducts research and outreach initiatives to prevent emerging pandemics and conserve wildlife biodiversity. This includes research on zoonoses that spill over from wildlife in emerging disease “hotspots,” including influenza, Nipah virus, severe acute respiratory syndrome (SARS), West Nile virus, and others. Dr. Daszak’s work includes identifying the first case of a species extinction due to disease, the discovery of chytridiomycosis, the major cause of global amphibian declines, publishing the first paper to highlight emerging diseases of wildlife, coining the term “pathogen pollution,” discovery of the bat origin of SARS-like coronaviruses, identifying the drivers of Nipah and Hendra virus emergence, and producing the first ever emerging disease “hotspots” map.

Dr. Daszak is a member of the Institute of Medicine’s Forum on Microbial Threats and served on the IOM Committee on global surveillance for emerging zoonoses, the National Research Council (NRC) committee on the future of veterinary research, and the International Standing Advisory Board of the Australian Biosecurity CRC. In 2010, he advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak is a member of the Council of Advisors of the One Health Commission, Treasurer of DIVERSITAS (ICSU), Editor-in-Chief of the Springer journal *Ecohealth*, and former Treasurer and a Founding Director of the International Ecohealth Association. In 2000, he won the Commonwealth Scientific and Industrial Research Organisation (CSIRO) medal for collaborative research in the discovery of amphibian chytridiomycosis. He has published more than 150 scientific papers, including in *Science*, *Nature*, *PNAS*, *The Lancet*, *PLoS Biology*, and other leading journals. Dr. Daszak was invited to present his work at TEDMED in 2010, and has been the focus of articles in the *New York Times*, *The Wall Street Journal*, *The Economist*, *The Washington Post*, *US News & World Report*, *CBS 60 Minutes*, *CNN*, *ABC*, *NPR’s Talk of the Nation*, and *Morning Edition & Fresh Air with Terri Gross*. He

is a former guest worker at the CDC, where he assisted in the pathology activity during the 1999 Nipah virus outbreak. His work is funded by the National Institutes of Health (NIH) Fogarty International Center, the National Institute of Allergy and Infectious Diseases (NIAID), the National Science Foundation (NSF), the U.S. Agency for International Development (USAID), Google.org, Rockefeller, and other foundations. To date, his organization is one of the few to have been awarded three prestigious NIH/NSF “Ecology of Infectious Disease” awards and is one of four partners to share a recent \$75 million dollar award from USAID (“PREDICT”) with the goal of predicting and preventing the next emerging zoonotic disease.

Michael P. Doyle, Ph.D., is a Regents Professor and Director of the Center for Food Safety at the University of Georgia, Griffin. A native of Madison, Wisconsin, he received his B.S., M.S., and Ph.D. degrees from the University of Wisconsin in bacteriology/food microbiology. From 1977 to 1980 he was senior project leader of corporate microbiology at Ralston Purina Company, and from 1980 to 1991 he advanced from assistant professor to Wisconsin Distinguished Professor of Food Microbiology, Food Research Institute, University of Wisconsin-Madison. He serves on food safety committees of many organizations and has served as a scientific advisor to many groups, including the World Health Organization, the IOM, the National Academy of Sciences (NAS)-NRC, the International Life Sciences Institute-North America, the FDA, the U.S. Department of Agriculture (USDA), the U.S. Department of Defense (DOD), and the U.S. Environmental Protection Agency. He has published more than 500 scientific papers on food microbiology and food safety topics and has received several awards for his research accomplishments, including the Nicholas Appert Award of the Institute of Food Technologists. He is a fellow of the American Academy of Microbiology, the American Association for the Advancement of Science, the Institute of Food Technologists, and the International Association for Food Protection, and is a member of the IOM, for which he chairs the Food Forum.

Rainer Engelhardt, Ph.D., is the Assistant Deputy Minister of the Infectious Disease Prevention and Control Branch of the Public Health Agency of Canada. Dr. Engelhardt has extensive senior executive management experience, as Chief Executive Officer of companies in the health sector, and in federal government positions. His corporate responsibilities have included serving as director and chairman of numerous boards of institutes and of private companies. Dr. Engelhardt’s broad experience includes development of federal legislation and environmental regulation, as well as management of large-scale national and international multisector science and technology research programs in Canada and the United States. Dr. Engelhardt has an extensive academic and applied research and publications record. He received his Ph.D. in biomedical physiology from the University of Guelph and began his career in academia, as a professor at Southeastern Massachusetts University and at the University of Ottawa.

David Gombas, Ph.D., is Senior Vice President, Food Safety and Technology, for United Fresh Produce Association. In that position, he provides food safety, microbiology, and regulatory and public policy assistance for the fresh and fresh-cut produce industry. He has numerous publications on food safety, and recently served as co-editor of the *Food Safety Programs and Auditing Protocol for the Fresh Tomato Supply Chain*. Dr. Gombas is currently serving as coordinator of the Produce GAPs Harmonization Initiative and the U.S. National Technical Working Group for GlobalGAP. Dr. Gombas received his bachelor's and master's degrees in food science from Rutgers University and Massachusetts Institute of Technology, respectively, and his Ph.D. in food microbiology from University of Massachusetts. Previously, Dr. Gombas has held food safety and microbiology positions with the National Food Processors Association, Campbell Soup Company, Kraft Foods, and the National Center for Food Safety and Technology, where he worked with FDA to develop hazard analysis and critical control point (HACCP) training courses for FDA investigators.

Will Hueston, D.V.M., Ph.D., is an internationally recognized veterinary public health leader known for his contributions to epidemiology tools such as surveillance systems and risk analysis and his expertise on the epidemiology and control of bovine spongiform encephalopathy (mad cow disease). Dr. Hueston is Executive Director and Secretariat of the Global Initiative for Food Systems Leadership, a unique international educational consortium committed to enhancing teamwork and leadership in support of global food security. Well known for his expertise in risk communications, Dr. Hueston remains active in facilitating innovative approaches for tackling the increasingly complex dilemmas facing local, regional, and global food systems. Dr. Hueston's leadership experience began as a community organizer and he has worked as a private veterinary practitioner, a resident veterinarian for a large agricultural cooperative, a field epidemiologist and professor, as well as in government service. He has demonstrated a lifelong commitment to the development of robust food systems that promote public health and sustainable development and livelihoods. Much of his work has focused on building successful public-private partnerships around shared interests such as global food security. Dr. Hueston has extensive experience working with livestock producers, animal health officials, national government agencies, and intergovernmental organizations as well as consumer groups and multinational food companies.

Martyn Jeggo, BVetMed, Ph.D., is the Director of CSIRO's Australian Animal Health Laboratory (AAHL) in Geelong and has held this position since 2002. Professor Jeggo brings a wealth of experience in controlling and detecting exotic and emerging animal disease to this vital role. AAHL is a global leader in research into and diagnosis of major diseases affecting livestock throughout the world. The Laboratory is a frontline defense, helping to protect Australia from

the threat of exotic and emerging animal diseases. In 2004, AAHL was designated as an international collaborating center for new and emerging diseases by the World Organization for Animal Health (OIE). This designation places AAHL at the forefront of international efforts to manage the growing number of disease agents that pose a risk to animal and human health. From 1996 to 2002, Professor Jeggo was the Head of the Animal Production and Health Science Section of the Joint Food and Agricultural Organization (FAO)/International Atomic Energy Agency (IAEA) Division of Agriculture in Vienna, Austria. In that role, he managed a range of FAO/IAEA Coordinated Research Programs involving more than 200 research contracts relating to animal production and health. These were operational in some 130 countries.

William Karesh, D.V.M., is Executive Vice President for Health and Policy at EcoHealth Alliance. He serves as President of the World Animal Health Organization (OIE) Working Group on Wildlife Diseases and chairs the International Union for the Conservation of Nature (IUCN) Wildlife Health Specialist Group, a global network of hundreds of wildlife and health experts. Currently, Dr. Karesh is the Technical Director for the USAID Emerging Pandemic Threats PREDICT program, a \$75 million effort focused on predicting and preventing pandemic diseases. Dr. Karesh has pioneered initiatives focusing attention and resources on solving problems created by the interactions among wildlife, people, and their animals and created the “One World—One Health” initiative linking public health, agriculture, and environmental health agencies and organizations around the world. International programs under his direction have covered terrain from Argentina to Zambia and include efforts to reduce the impact of diseases on humans and endangered species to global surveillance systems for emerging diseases. In addition to his work in the private sector, Dr. Karesh has worked for the USDA, DOD, the Department of the Interior (DOI), and the FAO of the United Nations and has published more than 150 scientific papers and numerous book chapters.

Lonnie J. King, D.V.M., is the 10th dean of the College of Veterinary Medicine at The Ohio State University (OSU). In addition to leading this college, Dr. King is also a professor of preventive medicine and holds the Ruth Stanton Endowed Chair in Veterinary Medicine. Before becoming dean at OSU, he was the Director of the CDC’s new National Center for Zoonotic, Vector-Borne, and Enteric Diseases (NCZVED). In this new position, Dr. King leads the Center’s activities for surveillance, diagnostics, disease investigations, epidemiology, research, public education, policy development, and disease prevention and control programs. NCZVED also focuses on water-borne, food-borne, vector-borne, and zoonotic diseases of public health concern, which also include most of the CDC’s select and bioterrorism agents, neglected tropical diseases, and emerging zoonoses. Before serving as director, he was the first chief of the agency’s Office of Strategy and Innovation.

Dr. King served as dean of the College of Veterinary Medicine, Michigan State University, from 1996 to 2006. As at OSU, he served as the CEO for academic programs, research, the teaching hospital, the diagnostic center for population and animal health, basic and clinical science departments, and the outreach and continuing education programs. As dean and professor of large-animal clinical sciences, Dr. King was instrumental in obtaining funds for the construction of a \$60 million Diagnostic Center for Population and Animal Health; he initiated the Center for Emerging Infectious Diseases in the college, he served as the campus leader in food safety, and he had oversight for the National Food Safety and Toxicology Center.

In 1992, Dr. King was appointed administrator for the Animal and Plant Health Inspection Service (APHIS), USDA, in Washington, DC. In this role, he provided executive leadership and direction for ensuring the health and care of animals and plants, to improve agricultural productivity and competitiveness, and to contribute to the national economy and public health. Dr. King also served as the country's chief veterinary officer for 5 years, worked extensively in global trade agreements within the North American Free Trade Agreement and the World Trade Organization, and worked extensively with the World Animal Health Association. During this time he was the Deputy Administrator for Veterinary Services of APHIS, USDA, where he led national efforts in disease eradication, imports and exports, and diagnostics in both Ames, Iowa, and Plum Island. He spent 5 years in Hyattsville, Maryland, in staff assignments in Emergency Programs, as well as in Animal Health Information. While in Hyattsville, Dr. King directed the development of the agency's National Animal Health Monitoring System. He left APHIS briefly to serve as the Director of the Governmental Relations Division of the American Veterinary Medical Association (AVMA) in Washington, DC, and served as the lobbyist for the AVMA on Capitol Hill.

Dr. King was in private veterinary practice for 7 years in Dayton, Ohio, and Atlanta, Georgia. As a native of Wooster, Ohio, Dr. King received his bachelor of science and doctor of veterinary medicine degrees from OSU in 1966 and 1970, respectively. He earned his master of science degree in epidemiology from the University of Minnesota and received his master's degree in public administration from American University in Washington, DC, in 1991. Dr. King is a board-certified member of the American College of Veterinary Preventive Medicine and has completed the Senior Executive Fellowship program at Harvard University. He served as president of the Association of American Veterinary Medical Colleges from 1999 to 2000 and was the vice-chair for the National Commission on Veterinary Economic Issues from 2000 to 2004. He has served on four NAS committees, including chairing the National Academies' Committee on Assessing the Nation's Framework for Addressing Animal Diseases. He is also Chair of the IOM Committee on Lyme Disease and Other Tick-Borne Diseases and for State of the Science, and he is also chairing the AVMA's Commission for AVMA Vision 2020. Dr. King is currently a member of the IOM Committee on Microbial Threats to Health, is a past member of FDA's Board of Scientific Advisors, and

is past president of the American Veterinary Epidemiology Society. He served as the chair for the national One Medicine Task Force for the AVMA, which helped start the country's One Health Initiative. Dr. King was elected as a member of the IOM of the National Academies in 2004.

Marion Koopmans, D.V.M., Ph.D., completed her training in veterinary medicine at the Utrecht University, Veterinary Faculty. She worked as associate professor at the same Faculty to become a specialist in large animal internal medicine and nutrition. In parallel, she completed her Ph.D. in veterinary sciences (virology, 1990), studying novel enteric viruses and their importance as pathogens for cattle. She continued to study enteric viruses during a fellowship and as visiting scientist at the CDC from 1991 until 1994, and returned to the Netherlands to become section chief of the enteric virus group at the National Institute of Public Health and the Environment (RIVM). She is coordinator of a European research and surveillance network on enteric viruses, and since 2000 holds the chair of the Virology Division of the Diagnostic Laboratory for Infectious Diseases at RIVM. Her responsibilities include reference diagnostics, syndromic surveillance, and emergency preparedness for viral diseases, including research aimed at improving the response capacity of a public health laboratory.

In 2006 she was appointed professor of public health virology at the Erasmus University in Rotterdam. Her research interests include enteric viruses, emerging disease preparedness, and infections at the human–animal interface, with a particular focus on unraveling mechanisms underlying possible emergence of new health threats and optimizing the early detection and response. She has authored more than 200 papers in peer-reviewed journals.

W. Ian Lipkin, M.D., is internationally recognized as an authority on the use of molecular methods for pathogen discovery. Dr. Lipkin has more than 30 years of experience in diagnostics, microbial discovery, and outbreak response, has mentored and trained more than 30 students and postdoctoral fellows, and leads a team of more than 65 investigators, postdoctoral fellows, and research and support staff at the Center for Infection and Immunity at Columbia University's Mailman School of Public Health. In the 1980s, Dr. Lipkin identified AIDS-associated immunological abnormalities and inflammatory neuropathy, which he showed could be treated with plasmapheresis, and demonstrated that early life exposure to viral infections affects neurotransmitter function. He was the first to use purely molecular methods to identify infectious agents, implicated West Nile virus as the cause of the encephalitis epidemic in New York in 1999, assisted the WHO and the Peoples Republic of China during the 2003 SARS outbreak, developed MassTag PCR and Greenchip technology, and pioneered the use of high-throughput sequencing in pathogen discovery. He and his team have discovered or characterized more than 400 infectious agents including Borna disease virus, West Nile virus, LuJo virus, human rhinovirus C, piscine reovirus, and canine hepacivirus.

Steve Luby, M.D., has overseen CDC activities in Bangladesh since 2004. He is seconded from CDC and posted into the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), where he heads the Centre for Communicable Diseases. Dr. Luby earned his medical degree from the University of Texas Southwestern Medical School at Dallas in 1986 and completed his internship and residency in internal medicine at the University of Rochester-Strong Memorial Hospital. He studied epidemiology and public health in the Epidemic Intelligence Service and the Preventive Medicine Residency of the CDC. From 1993 to 1998, Dr. Luby directed the Epidemiology Unit of the Community Health Sciences Department at the Aga Khan University in Karachi, Pakistan. Dr. Luby has authored more than 170 scientific articles, the majority concerning communicable disease epidemiology in low-income countries.

Mike Robach joined Cargill in January of 2004 to lead the company's global food safety and regulatory programs. In this role he leads Cargill's corporate efforts across food protection and security, quality assurance, animal health, and regulatory compliance. Mr. Robach started out his career with Monsanto Company, and prior to joining Cargill he headed up technical services for Wayne Farms LLC. Mr. Robach is a graduate of Michigan State University and Virginia Tech. He is a member of the Board of the Global Food Safety Initiative (GFSI), the American Meat Institute, the National Turkey Federation, GMA's Science Institute Executive Board, past Chairman of the U.S Poultry and Egg Association's Research Advisory Committee, a member of the International Association of Food Protection, the Institute of Food Technologists, and the American Society for Microbiology. Mr. Robach is the past President of Safe, Supply of Affordable Food Everywhere (SSAFE), a group of global food companies, non-governmental organizations, intergovernmental agencies, and universities working together to ensure the safety and security of the global food supply chain. He has worked closely with the OIE, FAO, USDA, FDA, and global governments regarding food safety policy, HACCP, and regulatory reform based on science. From 1995 through 2000, Mr. Robach was a member of the National Advisory Committee for Microbiological Criteria in Foods.

Robert Tauxe, M.D., M.P.H., is Deputy Director of the Division that is charged with prevention and control of food-borne, water-borne, and fungal infections at the CDC. The Division monitors the frequency of these infections in the United States, investigates outbreaks, and develops strategies to reduce the disease, disability, and deaths that they cause.

Dr. Tauxe graduated from Yale University, in New Haven, Connecticut, cum laude in 1975 and received his medical degree from Vanderbilt Medical School in Nashville, Tennessee. In addition, he holds a master's in public health degree from Yale University in New Haven, Connecticut. Dr. Tauxe completed an internal medicine residency at the University of Washington and is certified in internal

medicine. He then trained at the CDC in the Epidemic Intelligence Service for 2 years and joined the CDC staff in 1985.

His interests include bacterial enteric diseases, epidemiology and pathogenesis of infectious diseases, epidemiologic and clinical consequences of bacterial genetic exchange, antimicrobial use and resistance to antimicrobial agents, and teaching epidemiologic methods.

Dr. Tauxe's memberships include the American Epidemiology Society, the American College of Physicians, the American Society for Microbiology, and the American Academy of Microbiology; he is a Fellow of the Infectious Diseases Society of America and a member of the National Advisory Committee on Microbial Criteria for Foods. He has also served internationally in Belgium, Mali, Rwanda, Peru, and Guatemala and has supervised numerous domestic and overseas epidemiologic investigations.

His faculty appointments include the School of Public Health, Department of International Health, and the Department of Biology, both at Emory University, Atlanta. Dr. Tauxe has authored/co-authored 254 scientific journal articles, letters, and book chapters.

Michael R. Taylor, J.D., was named Deputy Commissioner for Foods at FDA on January 13, 2010. He is the first individual to hold the position, which was created along with a new Office of Foods in August 2009 to elevate the leadership and management of FDA's Foods Program. Mr. Taylor is a nationally recognized food safety expert, having served in high-level positions at FDA and the USDA, as a research professor in academia, and on several NAS expert committees.

As Deputy Commissioner for Foods, Mr. Taylor provides leadership and direction to all food programs in the Agency, including those managed by the Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM), and the foods-related programs of FDA's inspection and compliance arm, the Office of Regulatory Affairs (ORA).

Mr. Taylor returned to FDA in July 2009 as Senior Advisor to the Commissioner. Before that, he served as research professor, School of Public Health and Health Services, George Washington University. His research agenda focused on policy, resource, and institutional issues that affect the success of public health agencies in carrying out their prevention-related missions. Mr. Taylor received his law degree from the University of Virginia and his B.A. degree in political science from Davidson College.

Henrik C. Wegener, Ph.D., completed his master's in food science and a doctorate in microbiology from the University of Copenhagen, Denmark. He has been involved in food safety research since 1987. Initially he worked as a molecular microbiologist from 1987 to 1994, and then for 10 years he headed surveillance and epidemiological research at the Danish Zoonosis Centre. In 2004, he became head of the Department of Epidemiology and Risk Assessment, at the National

Food Institute in Denmark, and since 2006 he has been the Director of the Institute. Since November 2011 he has been the Chief Academic Officer of the Technical University of Denmark. He is adjunct professor of zoonoses epidemiology at the University of Copenhagen, and he has been Head of the WHO Collaborating Centre on Antimicrobial Resistance in Foodborne Pathogens and head of the European Food Safety Authorities Zoonosis Collaboration Centre. He was involved in the establishment of the WHO Global Foodborne Infections Network. Dr. Wegener has received the Alliance for the Prudent Use of Antibiotics—International Leadership Award to Preserve the Power of Antibiotics. He is an associate editor of the *Emerging Infectious Diseases Journal*, and he has published more than 100 scientific papers and book chapters.

Craig Wilson is Vice President, General Merchandising Manager of Quality Assurance and Food Safety for Costco Wholesale Corporation. Costco Wholesale Corporation operates membership warehouses that offer a selection of branded and private-label products in a range of merchandise categories.

Prior to joining Costco Wholesale, Mr. Wilson worked as a Special Projects Director for Frigoscandia Equipment Food Safety Systems for more than 20 years. During his time with Frigoscandia, he published numerous research papers in the areas of food safety and food processing and holds five patents, the most notable for steam pasteurization of food. He is the recipient of the Gia/Matek Excellence in Food Safety Award and serves on the Steering Committee for the National Food Safety Consortium as well as the Technical Committee of the GFSI.

Nathan Wolfe, D.Sc., is an epidemiologist who fights disease pandemics with an unprecedented early-warning system to forecast, pinpoint, and control new plagues worldwide before they kill millions. He is the Lorry I. Lokey Visiting Professor in Human Biology at Stanford University and the founder and CEO of Global Viral Forecasting (GVF), an independent research institute founded in 2008. His survey of diseases that have historically had the greatest impact on humanity revealed that most started with animals. Based on this, he created a global network of sites in viral hotspots where people are highly exposed to animals and are most at risk for early infection when viruses leap from animals to humans.

GVF, which coordinates more than 100 scientists and staff globally, spots viruses as soon as they surface by collecting and cataloguing blood samples, surveying wild animals, scanning urban blood banks, and documenting the transfer and distribution on disease. Data gleaned from a dozen field sites in Cameroon, China, Malaysia, and other countries have led to the discovery of a number of previously unknown infectious agents, notably simian foamy and T-lymphotropic viruses that emerged into humans from primate reservoirs.

Dr. Wolfe has received numerous awards including a Fulbright fellowship and an NIH Director's Pioneer Award and was chosen as a National Geographic

Emerging Explorer and World Economic Forum Young Global Leader. He was also named one of *Time Magazine's* 100 Most Influential People in the World for 2011. Dr. Wolfe has more than 80 scientific publications, and his work has been published in or covered by *Nature*, *Science*, *The New York Times*, *The Economist*, *NPR*, *The New Yorker*, and *Forbes*, among others. He has received support totaling more than \$30 million in grants and contracts from Google.org, NIH, NSF, the Bill & Melinda Gates Foundation, DOD, and others.

Cathie Woteki, Ph.D., is Under Secretary for the USDA's Research, Education, and Economics (REE) mission area, and the Department's Chief Scientist.

Before joining the USDA, Dr. Woteki served as Global Director of Scientific Affairs for Mars, Inc., where she managed the company's scientific policy and research on matters of health, nutrition, and food safety. From 2002 to 2005, she was dean of agriculture and professor of human nutrition at Iowa State University. Dr. Woteki served as the first Under Secretary for Food Safety at the USDA from 1997 to 2001, where she oversaw U.S. government food safety policy development and the USDA's continuity of operations planning. Dr. Woteki also served as the Deputy Under Secretary for REE at the USDA in 1996.

Dr. Woteki served in the White House Office of Science and Technology Policy as Deputy Associate Director for Science from 1994 to 1996. Dr. Woteki has also held positions in the National Center for Health Statistics of the U.S. Department of Health and Human Services (1983-1990), the Human Nutrition Information Service at the USDA (1981-1983), and as Director of the Food and Nutrition Board of the IOM (1990-1993). In 1999, Dr. Woteki was elected to the IOM, where she has chaired the Food and Nutrition Board (2003-2005). She received her M.S. and Ph.D. in human nutrition from Virginia Polytechnic Institute and State University (1974). Dr. Woteki received her B.S. in biology and chemistry from Mary Washington College (1969).

