Fate and Transport of Zoonotic Bacterial, Viral, and Parasitic Pathogens During Swine Manure Treatment, Storage, and Land Application

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Foreword

The Board of Directors of the Council for Agricultural Science and Technology (CAST) approved entering into a collaborative agreement with the National Pork Board to conduct a critical literature review on the topic "Fate and Transport of Zoonotic Bacterial, Viral, and Protozoan Pathogens during Swine Manure Storage, Treatment, and Land Application." An eminent group of 10 experts was chosen as the writing task force, led by Dr. Dana Cole, Department of Environmental Health Sciences, College of Public Health, University of Georgia, Athens, and cochaired by Dr. Jan Vinjé, Respiratory and Enteric Viruses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia.

The task force prepared an initial draft document and revised subsequent drafts, under the leadership of Dr. Cole. Six highly qualified scientists were invited to review and comment on the document. The authors, as well as the CAST Editorial and Publications Committee and the CAST Executive Committee, reviewed the final draft. The CAST staff provided editorial and structural suggestions and prepared the final document for transmittal to the National Pork Board. The task force authors are responsible for the document's scientific content.

On behalf of CAST, we thank the cochairs, authors, and invited reviewers who gave of their time and expertise to prepare this report as a communication of scientific information to the agricultural community and to the public. We also thank the employers of the scientists, who made the time of these individuals available at no cost to CAST.

CAST appreciates the opportunity to work collaboratively with the National Pork Board on this important publication.

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Concerns have been voiced about the potential for pork production facilities to disseminate pathogenic microorganisms into the surrounding environment. The Pork Checkoff's environment and pork safety committees direct research programs that address issues concerning the potential implications of pathogens related to pork production. These committees felt that a comprehensive literature review to assess the data and determine researchable knowledge gaps would be valuable. The review would be used as input into developing research priorities, to assess potential risks of pork production and to identify potential interventions as appropriate.

The Pork Checkoff approached CAST to conduct such a literature review. The scope of the review was defined as the fate and transport of zoonotic pathogens following manure storage and land application. This covered zoonotic bacteria, viruses and parasites.

The Pork Checkoff recognizes the effort of the authors to develop a comprehensive, objective review of published literature that is applicable to U.S. pork production. The authors selected the literature cited and the data presented. The conclusions drawn are those of the authors.

The Pork Checkoff staff appreciates the opportunity to work with CAST on this project.

Paul Sundberg DVM

Paul Sundberg, DVM, PhD, DACVPM
Vice President, Science and Technology

1.
INTRODUCTION: OVERVIEW OF SWINE MANURE MANAGEMENT SYSTEMS AND FACTORS AFFECTING THE SURVIVAL AND ENVIRONMENTAL TRANSPORT OF MICROBES

Animal manure management systems in the United States are designed to store, treat, and land apply solid, semisolid, slurry, or liquid manure (urine and fecal material) on agricultural fields following removal from the animal environment. Manure processed in swine management systems is usually in liquid (1–4% solids), slurry (4–15% solids), and semisolid forms, and land application most often involves spreading on fields as fertilizer (Copeland and Zin 1998; Dickey, Brum, and Shelton 1981; Hill 2003). The majority of these management systems are designed to reduce the concentrations of microbes that may be found in swine manure by 90–99% or more (Sobsey et al. 2005), and prevent off-farm transport of manure materials (i.e., nondischarge systems). The majority of microbes contained in swine manure are not pathogenic to humans (i.e., zoonotic). Nonetheless, the effectiveness of swine manure management systems to prevent environmental contamination with human pathogens is a concern, because there are several putative

Table 1.1 Waste management technologies used in swine production systems

System	Functional classification	Estimated percentage of U.S. swine facilities using system (USDA 2002b)[a]
Confinement building under slat scrape, gravity drainage or flush system	Removal of semisolid, liquid, or slurried wastes from animal environment	Not reported
Below ground (deep pit) slurry storage	Storage of wastes	57.2%
Solids separator	Physical treatment of wastes by removing solid fraction of slurried or semisolid wastes from liquid fraction	14.6%
Single nonaerated lagoon	Storage and biological treatment of slurried or liquid wastes	22.8%
Multistage nonaerated lagoon system	Storage and serial biological treatment of slurried or liquid wastes	38.6%
Aerated lagoon	Storage and biological treatment of slurried or liquid wastes	0.4%
Anaerobic digestor	Biological treatment of liquid and slurried wastes; methane production for energy recovery	Not reported
Composting (including vermiculture)	Biological treatment of solid or semisolid fraction of wastes	6.7%
Surface or subsurface flow constructed wetlands	Biological treatment of liquid wastes	Not reported
Surface spreading or sprayfield irrigation	Disposal of treated solid, semisolid (surface spreading), or liquid wastes (sprayfield irrigation)	61% Broadcast/solid spreader 49.1% Slurry spreading 11.2% Irrigation
Subsurface soil injection	Disposal of treated slurried wastes	34.3%

[a]Some facilities may use more than one management technology, allowing for total percentage to exceed 100%.

environmental transmission pathways by which these zoonotic pathogens may be transported to water resources. Consequently, several studies have addressed the level of environmental protection afforded by swine manure management technologies and the potential for environmental transport of zoonotic pathogens.

Manure treatment systems may include multiple mechanisms of physical, biological, or chemical treatment of manure. Most treatment technologies used in swine production, however, rely on physical and biological treatment of manure to decrease nutrient and microbial concentrations before removal from the system. Table 1.1 provides some of the more commonly used waste management systems in swine production systems.

MICROBIAL SURVIVAL IN SWINE MANURE MANAGEMENT SYSTEMS

The inactivation rate of viruses in nonaerated swine manures was evaluated by Pesaro, Sorg, and Metzler (1995). Inactivation of virus in swine manure (mixed feces and urine) followed a first-order kinetic process and ranged from 8.5 d for 90% inactivation (D_{90}) of virus to 71.2 d, depending on whether viruses were exposed to manure constituents that flowed through 15 nanometer (nm) membrane pores or not. Virus inactivation, however, did not seem to be influenced by pH or average temperature directly. Instead, virus inactivation seemed to be more dependent on uncharacterized virucidal factors that were allowed to pass through the pores. These factors seemed to be temperature-dependent. Significant differences were observed among different viruses in the overall inactivation rates and in the role of manure-associated virucidal factors on viral inactivation. Herpes virus exhibited the most rapid inactivation with a D_{90} of less than 2.5 d. In contrast, adenovirus was most resistant to inactivation when not exposed to the virucidal factors in the manure ($D_{90}=111$ d). Adenoviruses were most susceptible to the unknown virucidal factors compared with all other viruses, exhibiting a D_{90} of 37.3 d when exposed to the pored membrane. Parvovirus exhibited the most consistent D_{90} (26–29 d) under all observed conditions. This study was well designed and executed

and demonstrated concepts of viral survival in swine manures that also may apply to other types of microbial organisms: (1) temperature effects on virus inactivation may not be direct but reflect temperature-dependent processes in swine manure that affect viral survival and (2) the survival properties of different viruses are very variable, preventing extrapolation of findings from one virus strain to another.

Sobsey and colleagues (2005) evaluated microbial reductions achieved by several swine manure management technologies. In this study, single nonaerated lagoon systems were considered "conventional" treatment systems, and reductions achieved by these systems were compared with several "investigational" treatment systems. It was observed that the initial concentrations of recovered microbes in treatment influent varied significantly among farms as a result of differences in farm size and production type. Consequently, all comparisons of microbial survival in manure treatment systems were based on log_{10} reductions in recovered microbes. The two nonaerated lagoon systems differed in the log_{10} microbial reductions achieved by half a log_{10} or more for many of the evaluated microbes. In addition, the relative reductions in microbes did not follow the same trends in the two systems. For example, in lagoon #1 *Salmonella* die off (nearly 2 log_{10}) was slightly greater than *Escherichia coli* (about 1.8 log_{10}), whereas in lagoon #2 *Salmonella* die off (less than 0.5 log_{10}) was substantially lower than *E. coli* (over 1 log_{10}). In lagoon #2, *Salmonella* survival was the greatest for all microbes recovered. In fact, on some sample dates, treated manures from lagoon #2 exhibited higher concentrations of *Salmonella* than the manure influent. Survival of *Clostridium perfringens* (a fecal indicator organism used to model parasite survival) was high, exhibiting an average reduction of less than 1 log_{10} compared with influent concentrations in both lagoons. Viral die off (1.1–1.5 log_{10}) was higher than *C. perfringens,* but slightly lower than bacterial die off. Composting solid swine manure for 30 d resulted in reduced survival of *C. perfringens* and virus (over 2 log_{10} reductions) compared with nonaerated lagoons, but bacterial survival was better in this system. Composting solid swine manure for 30 d followed by storage of the composted manure for another 30 d, however,

significantly reduced the survival of all microbes (*E. coli* and *C. perfringens* were lowered nearly 4 $\log_{10}$, virus was lowered over 2.5 $\log_{10}$, and *Salmonella* was lowered slightly over 1 $\log_{10}$).

Removal of the solid fraction of swine barn flush liquids reduces the concentrations of microbes. Vanotti and colleagues (2005a, b) observed 0.5 $\log_{10}$ (*Salmonella)* to 1 $\log_{10}$ (enterococci) reductions in bacteria as a result of solids separation in two different manure management systems. It is unknown from these reports, however, how much of this reduction represents physical removal of organisms versus reduced survival as a result of changes in chemical oxygen demand, temperature, nitrogen, or pH. Following solids removal, the liquid portion of swine manure can be further treated using constructed wetlands. In field studies, Sobsey and colleagues (2005) found that constructed wetland treatment of swine manure liquids resulted in *E. coli* reductions of over 4 $\log_{10}$, *C. perfringens* reductions of 4 $\log_{10}$, and virus and *Salmonella* reductions of nearly 3 $\log_{10}$. These were significantly higher microbial reductions than those achieved by the nonaerated lagoon systems. Hill and Sobsey (2003) reported the results of a study of a field-scale, two-cell surface flow constructed wetland system and laboratory-scale surface flow and subsurface flow constructed wetland systems. In the first cell of field treatment, *E. coli* concentrations were reduced 1.1 $\log_{10}$, but enterococci only were reduced 0.7 $\log_{10}$. Observed *Salmonella* reductions were only 0.4 $\log_{10}$ in the first cell, but treatment in the second cell reduced *Salmonella* concentrations 1.5 $\log_{10}$. *C. perfringens* and virus (coliphages) were reduced in the first cell 1.2 $\log_{10}$ and 1.0 $\log_{10}$, respectively. Similar reductions were observed in the laboratory-scale surface flow constructed wetland where greater reductions in bacteria were observed (2.9 $\log_{10}$ for *Salmonella* and 3.9 $\log_{10}$ for *E. coli*), but reductions were significantly related to the loading rate of the system (inverse correlation between microbial reductions and loading rate). In addition, the hydraulic residence time of the manure liquid was more than twice as long in the subsurface flow wetland reactor. The reduction of microbes varied substantially between the field-scale constructed wetland system described by Sobsey and colleagues and the one described by

Hill and Sobsey. The first (Sobsey et al. 2005) achieved over 3 log10 reductions for all studied microbes after treatment in a two-cell system, whereas the other system (Hill and Sobsey 2003) achieved less than 2 $\log_{10}$ reductions after treatment in two cells. Descriptive data on the two systems were not comparable to elucidate reasons for the observed treatment differences in the two systems. The first system was much larger than the second, however, measuring 8 acres and having an estimated daily flow of 8,000 gallons/d compared with 121m^2 for the second system. In addition, Hill noted that *Salmonella* concentrations varied substantially between sampling dates in the second system, and enterococci concentrations were sometimes higher after treatment than in influent in both systems (Hill and Sobsey 2003; Sobsey et al. 2005). These observations underscore the fact that variability within and between manure management systems prevent comparisons across studies.

In general, the more treatment technologies used by a manure management system, the lower the microbial survival. As the comparisons of the microbial survival in the two nonaerated lagoon systems (Sobsey et al. 2005) and the two constructed wetland systems (Hill and Sobsey 2003; Sobsey et al. 2005) illustrate, however, the capacities of manure management systems and the reductions in microbial concentrations achieved by manure treatment can vary widely. Because each report of manure management system effectiveness relies on only one representative system at a time, it is impossible to quantitatively interpret the observations across systems or to compare studies.

MICROBIAL SURVIVAL AND TRANSPORT IN THE ENVIRONMENT

Swine manure storage and treatment facilities are largely subsurface. To protect the surrounding soil environment from seepage, an organic (compacted soil and/or bentonite) or inorganic (synthetic or concrete) liner is placed at the base of the storage/ treatment structure. Similarly, subsurface injection of liquid manure into the soils of application fields sometimes is performed to maximize soil retention

and protect against surface runoff of land-applied manure. Studies of soil cores beneath organic-lined or unlined (built before 1990) lagoons have found evidence that swine lagoon liquor can leak from lagoons under some conditions (Ham and DeSutter 2000). Nonetheless, passage through soil is a very effective barrier to the environmental survival and transport of microorganisms under most conditions (Ferguson et al. 2003; Hijnen et al. 2005). Several factors that impact pathogen survival in the soil matrix have been described: retention (sorption/desorption) by soil particles, inactivation, and predation by environmental microbes (Ferguson et al. 2003). Recently, an additional influence on pathogen survival has been identified. The survival of the intracellular pathogens *Salmonella* and *Campylobacter spp.* within environmental protozoa such as *Tetrahymena* and *Acanthomoeba* may increase environmental persistence by protecting the organisms from predation (Brandl et al. 2004; Snelling et al. 2005). In addition, although survival characteristics alone may determine the risk of environmental transport among viral and protozoal pathogens, the regrowth of bacterial pathogens in the environment may increase the risk of transport following seepage from swine manure management systems. Gagliardi and Karns (2000) introduced *E. coli* with a specific plasmid to three

types of loamy soils (silt, clay, and sand) and found that cell numbers increased in the soil leachate following simulated rainfall. Increases in bacterial concentrations ranged from 1.3 to 30 times the initial inoculum size in intact and disturbed soils. Because the study organism was distinct from potential environmental sources, it was shown that *E. coli* may not only survive in the soil matrix, but may expand in number.

Drivers of microbial transport within subsurface soil systems include microbial cell length, soil pore size, organic content, charge characteristics, and hydraulic conductivity (Gannon, Manilal, and Alexander 1991; Lance, Gerba, and Melnick 1976; Santamaría and Toranzos 2003). In general, clay soils increase microbial survival by protecting cells from predation but delay transport because of increased retention (Santamaría and Toranzos 2003). Sandy soils decrease survival due to low organic content, but increase the rate of transport because of higher hydraulic conductivity properties (Hijnen et al. 2005; Santamaría and Toranzos 2003). Even if conditions favor pathogen survival and transport to groundwater, however, it has been estimated that a travel time of 50 d or more will inactivate most enteric pathogens other than spore-forming bacteria or protozoa (Hijnen et al. 2005).

Krapac and colleagues (2002) monitored groundwater around two deep concrete-walled swine manure storage pits in Illinois to evaluate the potential survival and movement of fecal bacteria from the storage facilities into groundwater. Shallow wells (6–9 m) within 15 m of the deep manure storage pits yielded culturable fecally-associated bacteria, but deep wells 33–82 m did not have similar contamination. Background wells located 50–100 m away from the storage pits, however, also contained fecally associated bacteria. There was no indication that differences in the frequency of detection between monitoring wells and background wells were statistically significant. Consequently, it is unknown whether the fecal bacteria found in shallow wells near the pits were actually from the swine manure pits. In addition, no climate or soil saturation data were provided to evaluate the conditions of microbial survival and transport, nor was there any indication of the groundwater flow rate.

A similar study of lagoon seepage was done by Chee-Sanford and colleagues (2001). The first farm was a 5-year-old 4,000 pig finishing operation that consisted of a two-stage waste-handling system: a concrete settling basin collected solids and the remaining liquid was passed into an unlined lagoon. The authors noted that no effort was made to compress the soil during lagoon construction. The second farm was a 7-year-old 1,200 sow farrowing and nursery operation. The facility used a single-stage, unlined lagoon and used lagoon liquid to flush manure pits below the confinement buildings. This study isolated tetracycline-resistant bacteria from the lagoons of both swine facilities and from the groundwater wells on the farm properties. According to the report, tetracycline-resistant bacteria were found on the first farm only somewhat consistently in a well that was 5.2m in depth within 80m southwest of the lagoon. Evaluated isolates from this well contained tetracycline resistance genes (tet(M)) that also were found in bacteria in the lagoons. Because groundwater flow on this farm was reportedly in a northwesterly direction, it is not clear whether the bacteria isolated from this well were transported from the lagoon. Sporadic isolations of tetracycline-resistant bacteria were found on this same farm in the groundwater of three additional wells, but in only one

of these wells was an isolate found with a tetracycline resistance gene matching those found in the lagoon. This second well was 8.1m in depth and was over 80m southwest of the lagoon. Again, because it was not apparent that the groundwater sampled from this well was downgradient of the lagoon, it is not clear whether the isolate came from the lagoon. At the second farm, sporadic isolations of tetracycline-resistant bacteria were found in two wells that were both downgradient from the farm lagoon. Only one of these isolates contained the tetracycline-resistance gene found in the lagoon (the well was within 50m of the lagoon and 4.1m in depth). All of the tetracycline-resistant bacteria found in the groundwater were not identified. Of the two bacteria containing tetracycline-resistance genes found in the lagoon that were identified, neither were species commonly associated with swine manure or identified in the farm's lagoon. Although it could not be determined whether the resistant bacteria found in the groundwater were transported from the farm lagoons, Chee-Sanford and colleagues (2001) suggested that either genetic elements were independently moved from the lagoons into the soil matrix and groundwater, or interactions between environmental bacteria and lagoon bacteria resulted in movement of resistance genes among bacteria in the environment.

When liquid or solid manures are surface-applied to crops or fields as fertilizer, a number of factors increase the potential for survival and movement of swine manure-associated zoonotic pathogens in the environment. The survival of land-applied microbes is influenced by the inherent environmental survival characteristics of the microbe, the amount of ultraviolet (UV) radiation, the humidity or moisture of the environment, and the environmental temperature. Factors that influence the transport of surface-applied microbes in the environment include the residence time of land-applied manures before a rainfall sufficient to cause runoff, the application rates of swine manures to fields, and the physical characteristics (i.e., solid or liquid) of the applied manures (Crane et al. 1983).

Anderson and Sobsey (2006) compared *E. coli* recoveries and antimicrobial resistance patterns in the groundwaters of two farms housing swine and two farms raising crops. Monitoring wells on the swine

farms were under fields where sprayfield application of swine lagoon liquid was done, and up- and downgradient from the swine lagoons (only one swine farm had a monitoring well upgradient from the swine lagoon). Although a significantly higher number of *E. coli* were recovered from the swine farm groundwaters compared with the crop farms, the majority of isolates recovered from swine farm groundwaters (79%) were from one farm that had evidence of aquifer contamination resulting from a piezometer. When the results from this farm were not included in the analysis, there was no statistically significant difference in the *E. coli* recoveries from the groundwater of the other swine farm and the reference farms.

A controlled field experiment by Muirhead, Collins, and Bremer (2006) evaluated the effect of flow rate and ground tillage on the overland flow of *E. coli*. In this experiment, runoff was simulated on a 5m down slope (slope angle was not specified) on plots with intact, grassed soil and plots turned with a spade and then cultivated with a rotary hoe. Tap water was used initially to model runoff flow rates of 2, 6, and 20L/min., and *E. coli* levels were observed to increase in the captured runoff with increased flow distance. This suggested that background *E. coli* may contribute several logs of bacteria/100mL runoff, especially at low flow rates where more water infiltrates the soil matrix. Dairy cattle manure was diluted with water to make a liquid with an average *E. coli* concentration of 3.9 x 105 most probable number (MPN)/100mL (% solids of final effluent not provided) and was applied to the plots at the top of the slopes at the desired rate until captured runoff represented a saturation-excess state. As expected, low flow conditions (2L/min) and cultivated plots were most effective at retaining *E. coli* in the soil matrix, exhibiting a logarithmic trend of microbial reduction with distance. At the low flow rate of 2L/min, a 40% reduction in the bacterial concentrations was observed on cultivated plots 5m from the application point. In contrast, flow rates of 6L/min or more on intact, grassed soil plots did not exhibit logarithmic removal of *E. coli* and less than 10% of *E. coli* was removed at 5m. In addition, 80% of *E. coli* recovered in the runoff was transported on particles less than 20mm in diameter rather than larger, dense particles. The generalizability

of these observations to natural conditions is dubious, as this study represents extreme runoff conditions because manure effluent was used to simulate overland flow—thus eliminating the effects of microbial dilution, extended contact time of microbes with soil, UV radiation, desiccation, and temperature on the microbial concentration of runoff.

This chapter has provided a brief overview of the determinants of microbial survival and transport in swine production facilities. The following chapters will review the body of research investigating the fate and transport of enteric microbes and known pathogens in more detail and will provide the basis for the discussion of the research gaps and future studies required to systematically assess the risk of environmental transport of zoonotic pathogens from the swine production environment.

2.
BACTERIAL HAZARDS ASSOCIATED WITH SWINE MANURE

In 1999, the U.S. General Accounting Office (GAO) reported on waste management practices used in animal agriculture (GAO 1999), and Humenik and colleagues (2004) published a summary of environmentally superior technologies in swine production facilities. Whereas many of these practices emphasized limiting nutrient loading, runoff, and other ecologically sound practices, none specifically addressed the control of zoonotic pathogens, even though the U.S. Environmental Protection Agency (EPA) cited bacteria as one of the top three sources of impairment in rivers and estuaries (USEPA 1998).

Zoonotic bacterial pathogens that have been associated with swine manure include *Salmonella* spp., *Mycobacterium* spp., *Escherichia coli, Brucella* spp., *Bacillus anthracis, Leptospira* spp., *Chlamydia* spp., *Listeria monocytogenes, Campylobacter* spp., *and Yersinia* spp. These pathogens may be transmitted either through direct contact with the manure or indirectly through the environment (Pell 1997; Strauch and Ballarini 1994) (Table 2.1). But the most frequently studied enteric pathogens occurring in swine manure are *Salmonella, E. coli, Campylobacter, Listeria,* and *Enterococcus.* Lack of data on other bacterial pathogens in swine manure is due to the difficulty in culturing and identifying them; for example, *Mycobacterium* spp. grow extremely slowly and require specialized media and procedures, and *Chlamydia* spp. are obligate

intracellular pathogens that require cell culture to propagate. Understanding the implications of the persistence of swine-associated zoonotic pathogens during storage, treatment, and land application is important for assessing and controlling their presence in the environment.

Because the epidemiology (i.e., occurrence) of the most common zoonotic bacterial enteric pathogens are well described elsewhere, this review will focus on the persistence of the best characterized bacterial pathogens (*Salmonella, E. coli, Campylobacter, Listeria,* and *Enterococcus)* contained in stored swine manure, the effects of land application, their survival in soil, the effects of runoff events, and their presence in water. Table 2.1 presents a summary of data on zoonotic pathogen prevalence and survival. As this table demonstrates, studies vary widely in reported

presence and survival of zoonotic pathogens, depending on studied growth conditions, sensitivity of culture media, and swine production system. Data supporting the prevalence in swine manure is the most abundant and comes from survey types of studies. There is little information on the survival of these pathogens in swine manure representing on-farm conditions where urine and feces are being added on a continuous basis, as most studies take samples away from the storage unit and hold them in laboratory conditions.

Unfortunately, survival studies of zoonotic pathogens from swine manure on plants, in the soil, and in water are limited. Effects of soil type, pH, and moisture content on swine manure pathogens have not been studied systematically. There is a need for good hypothesis-driven research on swine manure pathogens' prevalence and survival beyond the typical survey work

Table 2.1 Reported bacterial zoonotic pathogens found in swine wastes

Bacterial Pathogen	Prevalence in Swine Wastes (samples)	Prevalence in Stored Wastes	Survival on Plants	Survival on Soil	Survival in Water	References
Salmonella	7.9–100%	5.2–22%	16–63 d	16–120 d	5-21 wk	Côté and Quessy 2005; Guan and Holley 2003; Hutchison et al. 2005a, b; Jones et al. 1976; Nicholson, Groves, and Chambers 2005; Rostagno et al. 2005; USDA 2005
Enteropathogenic E. coli (EPEC)	0–22%	15.5–24%	16–63 d	16–99 d	3 mo	Hutchison et al. 2005a, b; Jones et al. 1976; Nicholson, Groves, and Chambers 2005
Campylobacter	13.5–73.9%	10.3% (one study)	16–63 d	8 to >32 d	2 d to > 60 d	Brandl et al. 2004; Guan and Holley 2003; Hutchison et al. 2005a, b; Lund 1996; Nicholson, Groves, and Chambers 2005; USDA 2005
Yersinia enterocolitica	0–65.4%	0% (one study)	Unknown	10 d	6–448 d	Bhaduri, Wesley, and Bush 2005; Bhaduri and Wesley 2006; Guan and Holley 2003; Gütler et al. 2005; Lund 1996
Listeria	16–19.8%	0–19%	42–128 d	Up to 120 d	7–56 d	Hutchison et al. 2005a, b; Nicholson, Groves, and Chambers 2005; Van Renterghem et al. 1991

currently in the literature. Further research is needed for land application of manure with regard to pathogens in bioaerosols, transport into/through soil, and the potential to enter water via infiltration or run off. Although the enteric pathogens have been most studied to date, more research is needed on levels of other zoonotic pathogens in swine manure as well as their survival and dissemination in soil and water. More information is needed on how different climate and soil factors affect the ability of these bacteria to persist and transport through soil and water.

STORED SWINE MANURE

Fresh swine manure typically contains more than 10^{10} bacterial cells/gram (g), but the pathogenic concentrations typically are much lower, often less than 10^4 bacterial cells/g (Sobsey et al. 2005). The low concentrations—combined with the difficulty in growing some species under laboratory conditions, the presence of competing nonpathogenic species, and the labor intensity of culture methods—make enumeration and differentiation of many of these pathogens challenging. There still is need for data on the levels and persistence of zoonotic bacterial pathogens in stored animal manures, manure slurries, and wastewaters to assess the ecological impact of swine manure application, to identify effective manure treatments or management practices for pathogen reduction, and to decrease potential human and animal exposures.

The majority of the research on pathogens in swine feces and manure has focused on *Salmonella* and *E. coli*. During the U.S. Department of Agriculture's (USDA) National Animal Health Monitoring System (NAHMS) study of swine farms, data were collected on the prevalence of *Salmonella* and pathogenic *E. coli* (USDA 2002a). In 2000, 2.3% of breeding females, 6.6% of weaned, and 8.4% of grow/finish pigs were positive for *Salmonella*. Weaned pigs also were susceptible to *E. coli* diarrhea (24.0%), whereas 45.2% of suckling pigs had *E. coli* (colibacillosis) (USDA 2002a). The zoonotic nature of these infectious *E. coli* organisms was not verified. More recently, the Animal and Plant Health Inspection Service (APHIS) monitored 60 swine farms in five states (Iowa, Minnesota, North Carolina, Texas,

and Missouri) for an expanded number of enteric bacteria in feces (APHIS 2005). After four quarters of monitoring (July 2003 through June 2004), the average percentages of positive fecal samples were as follows: *Salmonella,* 9.9%; *Campylobacter,* 73.9%; generic *E. coli,* 90.8%; and *Enterococcus,* 71.8% (numbers for *E. coli* and *Enterococcus* include nonpathogenic strains). The predominant serovars of *Salmonella enterica* isolated were Derby, Typhimurium (Copenhagen), Heidelberg, Mbandaka, Give, Typhimurium, and Worthington (APHIS 2005).

The percentage of *Salmonella*-positive farms varies among research reports, with some lower and others higher than those reported for NAHMS farms. Letellier and colleagues (1999) surveyed swine herds in Québec, and of the five finishing units surveyed, all had *Salmonella* varying from 21.1 to 100% of samples positive in each unit. When an additional 41 farms were sampled, 70.7% had at least one sample positive for *Salmonella*. In studies of North Carolina swine herds (Davies et al. 1997a, 1997b, 1998), 84–100% of swine herds may have *Salmonella* isolated, and the prevalence of shedding by individuals can be as high as 84% in some management systems. These data were obtained for single-time-point samples. Ghosh (1972) followed *Salmonella* shedding in feces at one farm over 2 years (yr) and reported that 53% of fecal samples were positive.

Many different *Salmonella* serovars have been isolated from swine herds (Davies et al. 1998; Funk et al. 2001; Ghosh 1972; USDA 1997). Although at one time it was thought that a single serovar was associated with one herd, results of serotyping isolates have demonstrated that one herd may carry many serovars. Ghosh (1972) found five different serovars on one farm. Recently, Rostagno and colleagues (2005) reported isolation of multiple serovars from individual swine fecal samples: 37% of samples contained two serovars, 25% had three, 5% had four, and from one sample, five serovars were isolated. Whereas some serovars tend to be more prevalent, there is a great deal of variation among regions, farm-to-farm, and animal-to-animal. Although the full implications for observed serovar variation within and between farms are not well understood, the observed differences in the rates of human illness associated with different serovars, combined with differences in

phenotypic and genotypic antimicrobial resistance characteristics among serovars isolated from the same swine manure sources, suggest that some serovars associated with swine manure potentially pose a greater public health risk than others (Cole 2002; Gebreyes et al. 2004; Gebreyes and Thakur 2005).

Although enterohemmorhagic *E. coli* (EHEC) has received most of the attention lately, other pathogenic *E. coli* also may be present in swine feces or wastes including enterotoxigenic *E. coli,* enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli.* The pathogenicity of most *E. coli* strains, however, seems to be extremely host specific (Nataro and Kaper 1998). Whereas EHEC have been isolated from pigs, most were not in serogroups known to cause disease in humans (Beutin et al. 1993, 1995; DesRosiers et al. 2001; Nagy, Wilson, and Whittam 1999). Nagy, Wilson, and Whittam (1999) found four EHEC serogroups O157 in 43 *E. coli* isolates from pigs. In comparing 1,352 human fecal isolates with 620 porcine fecal isolates, only one serotype of O8:H19 was found in both human and porcine isolates, but they had different pathotypes (DesRosiers et al. 2001). No porcine isolates were identified from the O157 serogroup, and one was identified as an O91 that has been associated with

hemorrhagic colitis in humans.

Most pig *E. coli* serotyped as EHEC isolates did not produce verotoxin or hemolysin (Beutin et al. 1993), and those pig isolates with EHEC O157:H7-associated plasmid were negative for hemolysin (Beutin et al. 1995). In other countries, non-O157:H7 hemorrhagic E. coli have been associated with pigs, and these may be more important zoonotic agents in pigs (Guan and Holley 2003). Because of the host specificity exhibited by most pathogenic *E. coli* found in swine manure, it seems that their presence in feces or wastes may not pose a significant human health risk. Lu and colleagues (2002) evaluated the diversity of *E. coli* at a farrow-to-finish swine operation found in fresh feces and aerated stored manure (2 samples taken 3 months [mo] apart). The fresh feces had 19 unique *E. coli* genotypes, whereas the first and second manure samplings had 39 and 22 unique genotypes. The dominant fecal genotypes were found in both manure samples, but each sample had many unique genotypes. Furthermore, within the manure storage tank there was special variation in the *E. coli* genotypes found (Lu et al. 2002). Unfortunately the authors did not determine whether any of these *E. coli* genotypes represented zoonotic pathogens.

Little research has been published on the load of potentially pathogenic *E. coli* in swine feces and stored manure. When 33 samples were taken from swine manure handling systems on farms across 7 states, 100% of samples were positive for STII toxin gene from enterotoxigenic *Escherichia coli* when at least 35 isolates were screened (Khatib, Tsai, and Olson 2003). Samples from 5 pig manure lagoons were evaluated for the frequency of enterotoxigenic and enterohemorrhagic toxin-associated genotypes of *E. coli* (Chern, Tsai, and Olson 2004). All pig manure samples were positive for STII toxin gene (associated with enterotoxigenic *E. coli)*, with a frequency range of 1 gene per *E. coli* to 1 gene per 33 *E. coli* (Chern, Tsai, and Olson 2004). Additional assessment of the presence of genes associated with pathogenic *E. coli* in feces and wastes would be beneficial, inasmuch as *E. coli* remains one of the predominant indicator organisms isolated from swine manure, and information regarding the proportion of isolates that may represent human pathogenesis is relevant to determining potential human risks.

Few studies have assessed the presence of zoonotic pathogens in stored swine manure. When pig manure slurries from 54 farms were surveyed, 22% were positive for *Salmonella* and 24% were positive for EPEC (Jones et al. 1976). At least 10 *Salmonella* serovars were isolated, and pathogen concentrations were approximately 10^3 bacteria/milliliter (ml). Hill and Sobsey (2003) consistently detected *Salmonella* at four of four swine farms investigated in North Carolina, at concentrations of 2.2 to 2.4 $\log_{10}$ MPN/100 ml in flushed swine lagoon liquid. Reductions of 1 to 2 $\log_{10}$ (90 to 99%) in *Salmonella* concentrations were measured for each swine lagoon used to treat the flushed swine manure. Anugwa and colleagues (1989) recovered *Campylobacter* at an average of 2×10^6 cells/g feces, dry weight, and Enterobacteriaceae at 5 x 106 cells/g feces, dry weight. Hutchison and colleagues (2005a) investigated the prevalence of zoonotic pathogens in farm wastes in Britain. One hundred eighty-four pig manure samples (126 fresh and 58 stored) were tested between 2000 and 2003 and evaluated for *E. coli* O157, *Salmonella, Listeria,* and *Campylobacter.* Pathogens were detected in pig manure samples as follows: 11.9 and 15.5% were positive for *E. coli* O157; 7.9 and 5.2% for *Salmonella;*

19.8 and 19.0% for pathogenic *Listeria;* and 13.5 and 10.3% for *Campylobacter,* in fresh and stored manure, respectively (Hutchison et al. 2005a). Manure samples from 13 piggeries in Queensland, Australia, were sampled and examined for thermotolerant coliforms: *E. coli, Camplylobacter jejuni, C. coli,* and *Salmonella* (Chinivasagama et al. 2004).

In general, the closer to the animals the waste effluent was, the higher the level of pathogens. *Salmonella* detection was relatively low; only three farms had counts higher than 11 MPN/100 ml. All farms were positive for *Campylobacter,* with counts ranging from less than 30 to 1.1×10^4 MPN/100 ml in anaerobic lagoons following screen removal of manure solids. Counts of thermotolerant coliforms and *E. coli* in lagoons were higher and averaged between 10^5 to 10^6 colony-forming units (CFU)/100 ml (Chinivasagama et al. 2004). Van Renterghem and colleagues (1991) reported 16% of fresh pig feces were positive for *Listeria monocytogenes,* but it was not detected in stored manure slurries. In a survey of enterococci presence in four European countries (Sweden, Denmark, Spain, and England) 97% of pig manure samples were found positive for enterococci (both pathogenic and nonpathogenic species), and colony counts were between 10^4 and 10^5 CFU/ml (Kühn et al. 2003).

Much of the data on the persistence of pathogens in swine manure are limited by modeling pathogen survival in a static system. Most research on persistence of pathogens in manure and wastewaters removes the manure from on-farm storage, adds a specific number of pathogenic bacterial cells, and then monitors the concentration of pathogenic bacteria in the manure over time. There is evidence that pathogens do not exhibit the same survival properties under controlled laboratory conditions; in fact, survival times in the laboratory may be decreased compared with field conditions (Rogers and Haines 2005). Furthermore, manure storage and treatment on-farm is a dynamic system with continual inoculation of fresh feces and urine; therefore, more research is needed to study pathogen survival in continuous, dynamic manure treatment processes. Chern, Tsai, and Olson (2004) speculated that the difference in occurrence of enterotoxigenic genes in swine manure between

sampling times at least 3 mo apart was likely due to differences in animal shedding. Nicholson, Groves, and Chambers (2005) monitored solid pig manure inoculated with *E. coli* O157, *Salmonella, Listeria,* and *Campylobacter* in 10-m³ piles either composted or not composted. Maximal pathogen survival was 32 and 4 d for *E. coli* O157, 16 and 4 d for *Salmonella,* 4 and 4 d for *Listeria,* and 2 and 2 d for *Campylobacter,* in composted and noncomposted farmyard manures, respectively. Interestingly, the concentrations of pathogens reported by Hutchison and colleagues (2005a) did not differ significantly between the fresh and stored manures, even though fresh manure was added to 81% of stored pig manures continually.

LAND APPLICATION

The potential for pathogen transmission associated with land application of swine manure comes from the formation of bioaerosols, transfer to crop plants, and runoff events. Formation of bioaerosols during manure application to the soil is dependent on the method of application used, air temperature, humidity, and wind speed. Although there is concern about pathogen transmission during land application, the limited data suggest that the risks are low for the general public (Gerba and Smith 2005; Pillai et al. 1996). Pillai and colleagues (1996) investigated airborne bacteria during land application of human dewatered sewage sludge and found that even though indicator and pathogenic bacteria were detected at relatively high levels in sludge piles (1.1×10^8 MPN of fecal coliforms/g wet sludge; 3.5×10^6 MPN of fecal streptococci/g wet sludge; and 2.9×10^4 MPN of *Salmonella*/g wet sludge), none of these could be detected in the air downwind from either the sludge loading site (where sludge was loaded into spreading hoppers using a front-end loading) or the sludge application site. Only *Clostridium* was detected at approximately 5×10^2 CFU/m3 on two of four sampling times in the air downwind of the sludge loading site. Lewis and Gattie (2002) expressed concern about aerosolized pathogens because of the aggregation of bacteria with organic matter making isolation difficult. This aggregation also extends the time enteric organisms can survive in the environment.

The risk of pathogen transmission from swine manure is much greater through survival on plants and from runoff events than from bioaerosols. The Centers for Disease Control and Prevention (CDC) estimates that 12% of foodborne outbreaks result from consumption of contaminated plant material that may or may not have been contaminated by manure (Herikstad et al. 2002). Both *E. coli* and *Salmonella* outbreaks have been linked to plant contact with animal manure. Unfortunately, data on the survival of bacterial pathogens transmitted from manure to plants are limited. The EPA (1992) gives survival times of pathogens on plant surfaces as 6 mo for absolute maximum and 1 mo for common maximum survival. Hutchison and colleagues (2005b) reported the maximum survival times for pathogens contained in livestock manure after spreading on fescue plots. Manure samples (5 cattle, 2 pig, 1 poultry, 1 sheep) were inoculated with *Salmonella enterica* serovar *Typhimurium, Campylobacter jejuni, Listeria monocytogenes,* and *E. coli* O157 at approximately 10^6 CFU/g of manure. The length of time for a 1-log decrease (10^1) ranged from 1.3 to 1.9 d for *S. enterica,* 1.7 to 3.1 d for *C. jejuni,* 1.7 to 3.2 d for *L. monocytogenes,* and 1.3 to 1.7 d for *E. coli* O157. The longest survival times ranged from 16 to 63 d for *S. enterica, E. coli* O157, and *C. jejuni;* and 42 to 128 d for *L. monocytogenes* (Hutchison et al. 2005b).

Although the levels of pathogens rapidly declined on these pastures, there still was a risk for reinfection of animals on these plots. Dong and colleagues (2003) demonstrated that *Salmonella* strains and *Klebsiella pneumoniae* could colonize the interior of alfalfa roots and hypocotyls and that these bacteria and *E. coli* O157 could colonize the rhizosphere of the alfalfa when measured 5 d postinoculation. Colonization levels depended on the inoculation dose, but were sufficient to cause human disease for the salmonellae. Whereas *Campylobacter jejuni* could not grow on lettuce or spinach leaves, at 10°C it was cultured from radish roots and the spinach rhizosphere for 23 and 28 d, respectively (Brandl et al. 2004). Thirty percent of European farmland and crops that did not have manure applied were positive for enterococci; when manure was applied, however, enterococci were detected on 44% of farmland

and 48% of crops (Kühn et al. 2003). Furthermore, the concentrations of enterococci were much higher when manure was applied (from approximately 10 CFU/g for unfertilized land and crops to 100 CFU/g).

Runoff events may occur when rainfall closely follows land application of manure. Surface water may be contaminated by rainfall-induced runoff after manure application (Tyrrel and Quinton 2003). Increasing the time between application and rainfall decreases the risk of pathogen transport via runoff. In their review, Tyrrel and Quinton (2003) theorized that pathogens from manure spread onto soil or crop surfaces would be more likely transported with water than pathogens from manure incorporated into the soil, but they indicated a lack of evidence as to whether soil incorporation or injection of manure decreased pathogen runoff.

Using a swine manure *E. coli* isolate as an indicator organism, Saini and colleagues (2003) found the length of time between swine manure application (top applied in no-till, tilled and incorporated into soil) and rainfall events affected the leaching of the *E. coli* with 10 to 316-fold reduction in CFU/ml leachate when the first rainfall was 8 d postapplication rather than 4 d and a further 0 to 50-fold reduction with the increase to 16 d postapplication rainfall over the 8 d. Concentrations of the *E. coli* strain in the leachate after first rainfall event at 4 d were similar for all manure application types. The concentration of the bacterium in the leachate of successive rainfall events at days 8 and 16 was lower than the concentration in the 4-d leachate. But there was not an increase in the cumulative amount of the *E. coli* collected with variations in multiple rainfall events on the different applications (Saini, Halverson, and Lorimor 2003), indicating that the majority of the bacteria was retained in the soil. The total amount of the *E. coli* strain recovered in the tilled and no-till treatments was 1 to 3% of the inoculum and 10 to 13% in the incorporated manure application. Using fecal coliforms and fecal streptococcus as indicator organisms in a multiyear study to investigate bacterial survival in shaded exposed outdoor soil plots, Van Donsel, Geldreich, and Clarke (1967) found that the soil survival times varied due to season of the year. Both the fecal coliforms and streptococci had a 90% loss of

cell counts of less than 4 d in the summer, which was the only season where the coliforms had longer survival times than the streptococci. During the spring, fecal coliforms lost 90% of cell counts in less than 10 d but fecal streptococci required about 15 d. In the autumn and winter seasons, fecal coliforms required between 10 and 15 d to reach the 90% decrease in cell counts but for the fecal streptococci the times ranged from 15 to 20 d (Van Donsel, Geldreich, and Clarke 1967). Both fecal coliforms and streptococci were isolated from storm-water runoff when soil counts were at least 10^4 CFU/g soil. The authors noted that soil moisture, soil pH, and temperature affected the ability of both indicator organisms to survive. Contrary to these results, Malik, Randall, and Goyal (2004) did not detect *Salmonella entirica* serotype Anatum in the spring leachate samples after winter application (pre-freeze up) of swine manure containing the *Salmonella* strain was applied to tile-drained clay loam soil plots by injection application. Indicator organisms including fecal coliforms had similar concentration in the leachate from both control and manure-applied plots; the source of these bacteria on the control plots was suspected to be rodents, birds, and other animals (Malik, Randall, and Goyal 2004).

Shehane and colleagues (2005) discussed linkages between rainfall and runoff to waterborne disease outbreaks; more than 50% of waterborne disease outbreaks were preceded by precipitation events. The authors determined the levels of fecal coliforms, enterococci, and *Clostridium perfringens* in the St. Johns River in Florida over a 24-mo period. Increases in these indicator organisms correlated with rainfall: increased rainfall resulted in increased concentrations of indicator organisms. Source tracking indicated that <21% of fecal coliforms was from domestic animals (chicken, cows, and dogs) and ≥85% was due to wild animals (Shehane et al. 2005). Evans and Owens (1972) demonstrated increased concentrations of coliforms and enterococci in a subsurface drain during high rates of drain discharge for an experimental field with swine manure application. Kühn and colleagues (2003) found that 100% of farm runoff water from fields that had swine manure applied between 1 to 60 d previously was positive for enterococci.

Research investigating the survival of zoonotic

pathogens on plant surfaces is limited; recent outbreaks of enteric disease related to consumption of produce, however, have increased research on this topic. The survival of swine manure zoonotic pathogens on plant surfaces is of interest. Hutchison and colleagues (2005b) found that when experimentally inoculated pig manure slurry was applied to fescue grass pasture, *Lysteria monocytogenes* was detected for 63 d, *E. coli* O157 for 32 d, and *Salmonella* strains and *Campylobacter jejuni* were detected for 16 d. When farmyard manure from pigs was applied to the same grass pasture, *L. monocytogenes* and *E. coli* O157 survivals were the same, but the *Salmonella* strains and *C. jejuni* survival increased to 32 d (Hutchison et al. 2005b). Using *Listeria innocua* and *Clostridium sporogens* as surrogates of pathogenic bacteria, Girardin and colleagues (2005) found that *C. sporogens* spores survived very well in the soil with under a 10 CFU/g of soil decrease after 100 d and were detected on parsley grown in that soil at between 100 and 1000 CFU/g throughout cultivation. On the other hand, *L. innocua* decreased from 4.0×10^5 to <10 CFU/g soil during the first 30 d and was only detected on the parsley during that time (Girardin et al. 2005). Interestingly, the *Listeria* survived better when introduced to the field in winter compared with spring. *Campylobacter jejuni* was detected on spinach and lettuce leaves for 9 d postinoculation and survival was higher on wounded spinach leaves (Brandl et al. 2004). On radish root, C. jejuni was able to survive at least 23 d and could survive over 28 d on the roots of spinach plants. These results led the authors to postulate that most *C. jejuni* strains may not survive well on plant surfaces but would survive better in plant rhizospheres due to the nature of those environments (Brandl et al. 2004). *E. coli* and *Salmonella* were able to survive in sandy loam and loamy sand soils when applied in a contaminated swine manure, *E. coli* for over 2 mo in both soils, and *Salmonella* for 54 d in loamy sand and 27 d in sandy loam (Côté and Quessy 2005). Even with this long survival in the soil, neither pathogen was detected on pickling cucumbers grown in those soils.

Survival in Soil

Survival of pathogens from swine manure in soil is complex, affected by pathogen survival characteristics,

soil type, soil moisture, soil temperature, soil organic matter content, rainfall, humidity, air temperature, sunlight exposure, salt concentrations, whether manure is incorporated into the soil and how it is incorporated, initial concentration of pathogenic organisms, and the native soil microbial community (Jones 1986; Santamaria and Toranzos 2003). With nearly an infinite number of combinations of these factors, prediction of pathogen survival on a given field is nearly impossible. General survival times of pathogens in soil are estimated to be 1 yr as an absolute maximum with 2 mo as a common maximum survival time (USEPA 1992). Nicholson, Groves, and Chambers (2005) found that maximum pathogen survival in soils after land application of pig manure was 16 and 32 d for *E. coli* O157 and *Salmonella,* 8 and >32 d for *Listeria,* and 4 and 16 d for *Campylobacter,* in sandy arable soil and clay loam grassland soil, respectively. These pathogens tended to survive longer in the clay loam soil than in the sandy arable soil. Monitoring *Enterobacteriaceae* in clay loam soil with swine manure applied over a 3-yr period, de Freitas and colleagues (2003) found that *Enterobacteriaceae* concentrations tended to increase with increasing rates of manure application. Average *Enterobacteriaceae* concentrations were different across the 3 yr (0.4×10^3 CFU/g soil in Year 1, 5.4×10^3 CFU/g soil in Year 2, and 0.7×10^3 CFU/g soil in Year 3, averaging counts from 150 and 300 kg nitrogen/hectare application rate plots).

Incorporation and timing of incorporation of swine manure into arable sandy loam soil in spring or winter altered the survival rate of enteric bacterial pathogens (Hutchison et al. 2004). Immediate incorporation with spring application of liquid swine manure increased the time for a log reduction of *Salmonella sp.* (2.44 versus 1.89 and 0.79 d), *E. coli* O157 (4.89 versus 0.97 and 1.76 d), *Listeria* sp. (1.13 versus 0.66 and 0.84 d), and *Campylobacter* sp.(1.03 versus 0.63 and 2.26 d for immediate versus delayed and unincorporated manure). The reduction times were slightly different with winter application: *Salmonella* sp. (5.20 versus 2.00 d), *E. coli* O157 (1.86 versus 1.54 d), *Listeria* sp. (1.71 versus 1.21 d), and *Campylobacter* sp. (1.14 versus 0.92 d for immediate versus unincorporated manure). Results were similar for farmyard manure (feces and

bedding) (Hutchison et al. 2004). The seasonal effects were different for the various pathogens; survival of *Salmonella* in the soil was enhanced with winter application of the liquid swine manure. The maximum isolation time for these pathogens was 120 and 56 d for *Salmonella* sp., 16 and 16 d for *E. coli* O157, 120 and 120 d for *Listeria* sp., and 34 and 36 d for *Campylobacter* sp. in sandy loam soil after application of swine farmyard manure and liquid manure, respectively.

Recent research links survival of pathogens in the soil with exposure of fruits and vegetables. Research of environmental mechanisms for manure-derived bacterial contamination of plants demonstrated that bacterial-feeding nematodes can uptake pathogenic bacteria and then deposit them on plants. Under laboratory conditions, *Caenorhabditis elegans* was
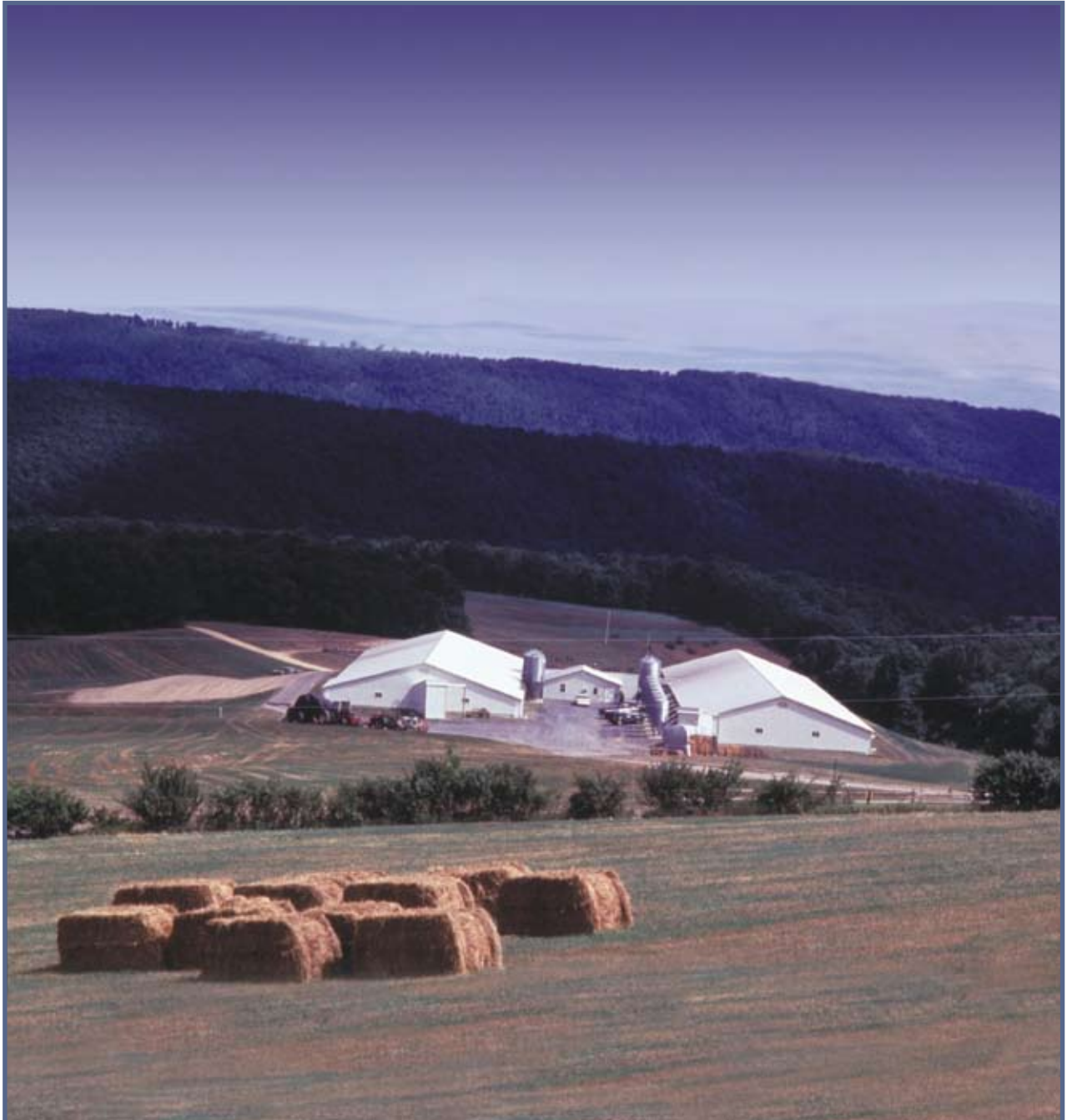
strongly attracted to *E. coli* OP50, *Salmonella enterica* Typhimurium, *Listeria welshimeri,* and *Bacillus cereus;* more than 90% of worms were located in the bacterial colonies after 6 min (Anderson et al. 2003). The nematodes then were able to disperse the bacteria onto uninoculated agar plates at the approximate rates of 60 colonies/worm with *E. coli* OP50; 70 colonies/ worm with *S. enteric* Typhimurium; 950 colonies/ worm with *L. welshimeri;* and 40 colonies/worm with *B. cereus* after 24 hr. When this same nematode's attraction was determined for seven strains of *E. coli* O157, eight serotypes of *Salmonella,* and six strains of *L. monocytogenes,* Caldwell and colleagues (2003) found that for almost all strains examined, most worms had migrated to the bacterial colonies within 20 min. These authors also found that *S. enterica* serovar Poona was present more rapidly on cantaloupe rinds on soil inoculated with both *C. elegans* and *S. enterica* Poona than when the soil only had been inoculated with *S. enterica* Poona. Other work determined that a different nematode (*Diploscapter* sp. strain LKC25) was attracted to, ingested, and dispersed *S. enterica* serovar Poona, *E. coli* O157:H7, and *L. monocytogenes.* Attraction of *Diploscapter* to *L. monocytogenes* lagged behind its attraction to *Salmonella* and *E. coli;* 85% of worms were associated with the *Salmonella* and *E. coli* after 60 minutes (min), at which time only about 65% of worms were associated with the *Listeria* (Gibbs et al. 2005). Worms shed approximately 10^4 cells/worm of *Salmonella* and *E. coli* and 10^2 cells/worm of *Listeria* during 24 hr.

WATER QUALITY EFFECTS

Pathogenic bacteria can survive in water for various lengths of time. Coliform bacteria and enterococci have been detected in groundwater at 10^5 to 10^6 CFU/ml and reported to survive for 400 d when the temperature is 4–8°C (Nevecherya et al. 2005). Bacteria may be filtered out or absorbed by some soils under some conditions, but it is unclear whether this filtration can be predicted or if it is sufficient to protect groundwater from contamination (Macler and Merkle 2000). Nevecherya and colleagues (2005, note original publication is in German) cited work that determined water containing 10^4 *E. coli*/ml moved

through fine sand at a rate of 3.5 centimeters (cm) /d. Furthermore, viable bacteria were detected in the water after passing through the sand for 3 mo. Inactivation rate factors (% decrease in bacterial counts per day) for some bacteria in groundwater at 10°C were 5.2 and 6.9%/d for *E. coli,* 5.6 and 3.4%/d for *Salmonella enterica* Typhimurium, 1.7%/d for *Yersinia enterocolitica,* 2.9%/d for *Streptococcus faecalis,* and 0.5%/d for *Clostridium perfingens* (summary of German data in Nevecherya et al. 2005). Whereas inactivation rate factors commonly are believed to be constant, experimental data demonstrate that these inactivation rates can vary with time; initial values for inactivation rate factors are much larger than those at later times (Nevecherya et al. 2005).

Jones (2001) reviewed campylobacters in water, sewage, and the environment. In fresh water streams and rivers, the major sources of *Campylobacter* contamination are agricultural slurries, wastes, and municipal sludge following land application or leakage from these sources. Contaminated groundwater could create a vicious cycle for animal production, with animal manure as the source of groundwater contamination and the contaminated groundwater introducing pathogens into animals (Jones 2001). Birds and sewage effluent probably are the source of most enteric pathogens in coastal waters; in estuaries, however, it is likely that agricultural animal sources make significant pathogen contributions. Blaser and colleagues (1980) evaluated the survival of different *Campylobacter* fetus strains in stream water at two temperatures. When the stream water was kept at 25°C the organisms died within 4 d, but when the water temperature was 4°C the *Campylobacter* survived for between 1 and >4 weeks (wk) (Blaser et al. 1980).

To explore the relationship between *Salmonella* and *E. coli* O157:H7 prevalence in surface waters and animal agriculture, Johnson and colleagues (2003) did multiple samplings at ten different sites within the Oldman River Basin (Alberta, Canada) during 1999 and 2000. Livestock facilities in the watershed included beef and dairy farms; farrow-finish, weaning, and feeder hog farms; and broiler, layer, and turkey farms. Sampling sites included three with no animal manure units (AMU), one with low density AMU, two with medium- density AMU, one with high-density AMU, two

storm drains, and one in the City of Lethbridge. The percentage of samples positive for *Salmonella* averaged 16.7% with no AMU, 20.0% with low AMU, 11.4% with medium AMU, 33.0% with high AMU, 52.5% for the storm drains, and 4.6% in Lethbridge. *E. coli* O157:H7-positive samples averaged 10.1% with no AMU, 0% with low AMU, 3.8% with medium AMU, 0% with high AMU, 0% for the storm drains, and 9.1% in Lethbridge. No direct correlation between bacterial prevalence and manure production was identified, and the authors postulated that factors influencing bacterial runoff as well as time, amount, and frequency of manure applications may have influenced the surface water contamination for these pathogens (Johnson et al. 2003). Pathogenic bacteria may have survival mechanisms in water similar to the nematodes in soil. Signoretto and associates (2004) found, during an 18-mo survey of lake and seawater, that *Enterococcus faecalis* was either attached to plankton or free in water, but not both in lake water; and in seawater, most cells were attached to plankton rather than in the water. In a follow-up study, attachment of *Enterococcus faecalis* to plankton induced the viable but nonculturable state, indicative of an undetected bacterial reservoir (Signoretto et al. 2005).

In river water inoculated with different *Salmonella* strains and held at room temperature, direct counts of the bacteria did not change over a 45 day period, even though plate counts (on Trypticase Soy Agar plates) of the bacteria decreased of from 10^8 to 10^3 CFU/ml untreated river water, although 1 strain decreased to about 10 CFU/ml (Santo Domingo, Harmon, and Bennett 2000). This finding illustrates a caveat: evaluations of bacteria using culture methods potentially can underestimate the load of pathogens in a given sample, because the bacteria can enter a state in which they are not cultured by the standard methods. Rollins and Colwell (1986) reported a similar condition in *Camplylobacter jejuni* where the bacterium, which had been inoculated into stream water and held at 4°C, was not detected using plate counts after 10 d but viable campylobacters were sustained for over 4 mo at concentrations similar to the original inoculum. A number of factors seem to be involved in the survival of *Campylobacter* species in water including temperatures below 16°C and the degree of oxygenation (Buswell

et al. 1998). These authors also demonstrated that incorporation into biofilms doubled *Campylobacter* survival times.

Summary

Determining the environmental fate of bacterial pathogens from swine manure is extremely difficult. Biological variables include pathogen shedding by individual pigs; microbial interactions within stored manure; inoculation of stored manure each time a pig sheds pathogens; interactions with plants, nematodes, organic matter, and soil microorganisms after land application; and water organic matter, aquatic plants, and plankton. Physical variables include type of manure storage, temperature and humidity during storage, soil type, temperature, moisture, water pH, salinity, and rainfall events (Table 2.2). Fecal shedding of pathogens has been the aspect of this topic that has been most studied, yet understanding still is limited. Although some research indicates that pathogens in swine manure do not survive long once they are applied to the soil, other data contradicts this with relatively long survival times in soil and water. There is a great need for good hypothesis-driven research to determine the factors that affect the environmental survival and persistence of zoonotic pathogens contained in swine manures.

Table 2.2 Examples of the effects of biological and physical variables on bacterial pathogens in wastes

	Variable	Significant Conditions	Effect on Pathogens	References
Physical	Temperature	1–9°C vs. 40–60°C	Increased survival of *Salmonella* and *E. coli* O157:H7 at lower temperatures	Rogers and Haines 2005
		8°C vs. 20°C vs. 37°C	Increasing survival of *Salmonella* as temperatures were lowered	Guan and Holley 2003
	Moisture	2% DM vs. 7% DM	Increased survival of *E. coli* O157 and *Salmonella* at higher moisture content. No effect on *Listeria* and *Campylobacter* survival	Nicholson, Groves, and Chambers 2005
	Aeration	Conditions not specified	Decreased survival of *Salmonella* and *E. coli* O157:H7 in solid manures when manures were aerated	Rogers and Haines 2005
	Turning solid manure heap	Time interval not provided	Turning swine manure heap increased survival of *E. coli* O157 and *Salmonella,* but had no effect on *Listeria* and *Campylobacter* survival	Nicholson, Groves, and Chambers, 2005
	Removal of solids from liquid fraction	Removal of 98% of total suspended solids from swine wastewater	Reduced *Salmonella* 0.5 to 1 $\log_{10}$	Vanotti et al. 2005a
	Sunlight	Ultraviolet-B λ=285-315	Inactivates *E. coli* (indicator bacteria, pathogenic *E. coli* not measured)	Hill 2003
Biological	Anaerobic mesophilic lagoon treatment	Not applicable	Reduces *Salmonella* 0.8-1.8 $\log_{10}$ and enterococci 1.8 $\log_{10}$	Hill and Sobsey 2003; Vanotti et al. 2005a, c
	Actinomyces bacteria	Antibiotic production	Antagonistic to *Salmonella* spp.	Hill 2003
Chemical	Removal of biological nitrogen	Denitrification resulting in removal of 95% of TKN	Reduction of *Salmonella* by 2.4 $\log_{10}$ and enterocci by 4.1 $\log_{10}$	Vanotti et al. 2005a, c
	High pH	Average pH value over 10	Reduction of *Salmonella* and enterococci by over 1.5 $\log_{10}$	Vanotti et al. 2005a, c

3.
COMMON VIRUSES OF SWINE

INFLUENZA

Influenza viruses are enveloped, segmented, single-stranded (ss), negative-sense ribonucleic acid (RNA) viruses belonging to the family *Orthomyxoviridae* (Lamb and Krug 2001). The *Orthomyxoviridae* family consists of four genera: influenza A virus, influenza B virus, influenza C virus, and thogotovirus (Lamb and Krug 2001). Swine influenza virus belongs to the influenza A virus genus. Type A influenza viruses cause disease in lower mammals, birds, and humans (Wright and Webster 2001). Although type B influenza viruses primarily have been associated with disease in humans, there is evidence for rare type B influenza infections outside humans reported in the United Kingdom (U.K.) and China in pigs that were shown to possess antibodies against influenza B virus (Brown, Harris, and Alexander 1995; Mu et al. 1988), and a single report of an infection in a marine mammal (Wright and Webster 2001). Type C influenza viruses are isolated only occasionally, and although they can infect humans, dogs, and swine, there is little evidence that they circulate widely in swine (Brown, Harris, and Alexander 1995; CDC 2005; Matsuzaki et al. 2002; Youzbashi et al. 1996). Because swine influenza is caused primarily by influenza A viruses, this genus will be discussed in detail in this chapter.

The influenza A virus genome consists of eight RNA genes (Lamb and Krug 2001) that encode for 11 different viral proteins (Chen et al. 2001; Gibbs et al. 2003). Two of the genes—hemagglutinin (HA) and neuraminidase (NA)—encode for surface glycoproteins that project from the viral envelope, and because they possess distinct antigenic properties, they are used to subtype influenza viruses into 16 HA types (1–16) (Fouchier et al. 2005) and 9 NA types (1–9) (Lamb and Krug 2001). Two internal genes—nucleoprotein and matrix—are conserved highly within the three types (A, B, and C) of influenza viruses and often are targeted in detection assays.

Influenza A viruses are named by their HA and NA type, (e.g., H1N1), and often are given "strain" names that include their genus or type, host species if other than human, location of isolation, arbitrary laboratory number, and year of isolation (e.g., A/Swine/Iowa/15/1930).

Epidemiology

Swine influenza virus (SIV) has evolved from a seasonal disease caused by a stable genotype to a year-round endemic respiratory disease caused by multiple genotypes undergoing continual change (Erickson and Gramer 2003). These changes are caused by two mechanisms: antigenic shift (or reassortment) and antigenic drift (Lamb and Krug 2001). Antigenic shift is a dramatic change that occurs when virus gene segments are exchanged between two viruses infecting the same host cell (Hilleman 2002). Antigenic drift is a more subtle change that occurs through accumulations of point mutations made during replication of the virus genome (Schweiger, Zadow, and Heckler 2002). For 80 yr, pigs in North America had only one endemic strain of SIV, classical H1N1, until 1998 when a human reassortant H3N2 SIV was detected in U.S. swine (Brown 2000). In 1998, reassortant H3N2 SIVs emerged in the swine population that either were double reassortant with human and avian strains of influenza (A/Sw/NC/98) or triple reassortment with human, avian, and swine influenza strains (A/Sw/TX/98). In early 1999, the HA of the preexisting classical H1N1 SIV reassorted with H3N2 SIV virus to create a second reassortant virus, H1N2 (Karasin, Olsen, and Anderson 2000). Control then was complicated further as evidenced by multiple outbreaks of swine influenza resulting from H1N2 infection despite possible preexisting vaccinal immunity against classical H1N1 SIV (Erickson and Gramer 2003; Karasin, Olsen, and Anderson 2000). Further reassortment occurred in late 2002 when both the HA and NA of H3N2 SIV were replaced by the classical H1 and N1 genes, thereby creating a reassortant novel H1N1 SIV with avian internal (PA and PB2) genes (Webby et al. 2004).

Pigs possess respiratory epithelial cell receptors that are permissive to infection by both avian and mammalian influenza viruses (Ito et al. 1998). These cellular receptors are carbohydrate and not protein based. This is significant because the use of a carbohydrate receptor by a virus usually results in a broad host range (Young 2001), with influenza viruses spreading from their aquatic bird reservoir to pigs, horses, sea mammals, and humans as a key example (Webby and Webster 2001). Once the virus binds to the receptor and enters the cell, virus replication occurs in the pigs' respiratory tract and new virus particles are produced after a short incubation period of 12 to 24 hours (hr) (Urman, Underdahl, and Young 1958). As a result, respiratory epithelial cells become necrotic and slough into the alveolar, bronchial, or tracheal lumens (Urman, Underdahl, and Young 1958). Clinical signs of pneumonia—including dyspnea, tachypnea, coughing, nasal discharge, lethargy, and fever—occur shortly after infection. Virus is excreted in nasal secretions and aerosolized because of coughing. Individual pigs will shed virus for approximately 5 to 7 d (d) postinfection (pi). Immunity develops, which seems to be incomplete but effective at decreasing viral levels to undetectable rates or levels insufficient at causing overt respiratory disease. Once immunity develops, lung lesions and clinical signs eventually resolve (Urman, Underdahl, and Young 1958).

Zoonotic Potential

Influenza virus is a zoonotic agent that can be transmitted easily between animals and humans (Castrucci et al. 1993; Webby and Webster 2001). The reservoir for influenza viruses is aquatic birds (Webster et al. 1992), and the broad host range of influenza viruses includes humans, pigs, birds, marine mammals, horses, mink (Webster 1997), cats (Thanawongnuwech et al. 2005), and dogs (Crawford et al. 2005). Experimentally, rabbits, ferrets, and mice are infected readily and used as laboratory models but are not likely to be natural hosts (Lamb and Krug 2001).

The influenza pandemic of 1918 was caused by an H1N1 virus that spread from an avian species to humans and pigs (Tumpey et al. 2005). Reassortant human and avian influenza viruses resulted in human pandemics including the 1957 H2N2 Asian and the 1968 H3N2 Hong Kong flu pandemics (Webby and Webster 2001). More recently, highly pathogenic H5N1 avian influenza viruses have caused human fatalities and are the focus of much global attention (Ferguson et al. 2005; Perdue and Swayne 2005; Tam 2002).

Infection of humans with SIV has occurred sporadically (Alexander and Brown 2000; Dacso et al. 1984; Top and Russell 1977; Wells et al. 1991), causing clinical disease of varying severity and variable transmissibility, but occasionally, some occurrences have been fatal. There is strong evidence that swine veterinarians, swine farmers, and meat-processing workers are at increased risk of SIV infection compared with people who have no exposure to swine (Myers et al. 2006; Olsen et al. 2002). Prevention of infection is advisable, and public health guidelines were published in 2004 by the National Pork Board (Olsen 2004).

Inactivation

As enveloped viruses, influenza viruses are sensitive to heat, lipid solvents, detergents, irradiation, and oxidizing agents. The influenza viruses are considered environmentally labile outside the host (Quinn et al. 2002). The CDC recommends sterilization of human H2N2 influenza virus-infected materials using gravity displacement autoclaves set at 121°C and 15 to 19 pounds of pressure for 20 min or in dry-heat ovens set at 170°C for 1 hr, 160°C for 2 hr, or 121°C for at least 16 hr (CDC 2005). Alternatively, the CDC recommends chemical disinfection with a 1:10 dilution of household bleach or with any of several of theEPA's List H Registered Antimicrobial Products for Medical Waste Treatment (USEPA 2005). The list contains several products including those with calcium oxide, sodium chloride, sodium dichloro-s-triazinetrione (e.g., swimming pool chlorine), glutaraldehyde, and quaternary ammonium compounds as active ingredients. For disinfection of avian influenza viruses (AIV), studies have shown that phenolic disinfectants (Tek-Trol and One-Stroke Environ), quaternary ammonia compounds (Lysol No-Rinse sanitizer), peroxygen compounds (Virkon-S), and sodium hypochlorite (household bleach) are effective at inactivating AIV at recommended concentrations (Suarez et al. 2003).

Effectiveness of any disinfectant can be reduced in the presence of organic matter that alters the pH and/or the temperature. For example, chlorine inactivation of human picornaviruses (poliovirus) in urban wastewater effluent was shown to be relatively unaffected by fluctuations in temperature, but sensitivity to chlorine was altered at different pH levels (Hajenian and Butler 1980). Inactivation of influenza virus in the presence of organic matter is discussed in the section "Environmental Survival."

Presence and Fate

Because influenza virus infections in pigs primarily are respiratory infections, and few reports exist supporting theories of influenza viremia or systemic infection in pigs (Wallace and Elm 1979), much of the focus regarding transmission and spread should be on respiratory tract secretions. When aerosolized in small particle size, influenza viruses of swine origin decayed to undetectable levels by virus isolation (VI) at 15 hr postdelivery into a laboratory chamber containing air at 15% humidity and held at 21°C (Mitchell, Guerin, and Robillard 1968). When the influenza virus-infected respiratory secretions settle they can collect on the equipment, the floors, and in the manure or slurry. Researchers in Denmark studying SIV survival in slurry detected virus for as long as 9 wk at cold temperatures (5°C), decreasing to 2 wk at 20°C, approximately 24 hr at

35 to 40°C, approximately 150 min at 50 °C, and 60 min at high temperatures (55°C) (Haas et al. 1995). Studies on AIV survival in chicken farm environments also have been done. Researchers found that H7N2 AIV was inactivated in chicken manure in less than 7 d at ambient temperatures of 15 to 20°C (Lu et al. 2003). Direct correlations between AIV survival and SIV survival should be made with caution, because the virus is shed in both the feces and respiratory secretions of birds (Perdue and Swayne 2005) and only in respiratory secretions in swine. Influenza virus will survive for short periods on equipment and can be removed by any of the several methods discussed in the previous section.

HEPATITIS E VIRUS

Swine hepatitis E virus (HEV), a novel virus closely related genetically and antigenically to human HEV, was discovered serendipitously and characterized by Meng and colleagues (1997) from the acute phase sera of naturally infected pigs in the United States. Swine HEV infection was reproduced experimentally in specific-pathogen-free (SPF) pigs (Halbur et al. 2001; Kasorndorkbua et al. 2002; Meng et al. 1998b; Williams et al. 2001). Pigs experimentally and naturally infected by swine HEV remain clinically normal but develop microscopic lesions of hepatitis in liver biopsies (Halbur et al. 2001; Meng et al. 1997). The biological characteristics of swine HEV largely are unknown

because of the lack of a cell culture system to propagate the virus. The well-described human HEV is a small, nonenveloped, single-stranded, positive-sense RNA virus. The viral genome contains three partially overlapping open reading frames (ORFs 1, 2, and 3), and short 5' and 3' noncoding regions (NCR) (Huang et al. 1992). The HEV recently was classified in the family Hepeviridae, genus Hepevirus (Emerson et al. 2004). The complete genome of swine HEV is approximately 7.2 kb in length (Meng et al. 1998a) with, as in human HEV, three ORFs, a 5' NCR and a 3' NCR. The ORF1 is predicted to encode the nonstructural proteins, whereas ORF2 encodes the immunogenic capsid protein. The ORF3, a small ORF of less than 400 nucleotides in length, overlaps ORF2, and its function is unknown.

Swine HEV infection in pigs is ubiquitous worldwide; swine HEV now has been identified from pigs in more than a dozen countries (Arankalle et al. 2003; Cooper et al. 2005; Garkavenko et al. 2001; Huang et al. 2002; Meng 2000, 2005a, b; van der Poel et al. 2001; Wang et al. 2002; Yazaki et al. 2003). In some herds in the United States, approximately 60–100% of pigs are infected (Meng et al. 1997). Most pigs older than 3 mo of age have antibodies to HEV, whereas pigs younger than 2 mo of age generally are seronegative. Swine HEV RNA generally is detectable in sera and feces from pigs of 2 to 4 mo of age, but rarely from adult pigs, indicating that active swine HEV infection occurs between 2 to 4 mo of age (Cooper et al. 2005; Huang et al. 2002; Yazaki et al. 2003). Genetic analyses showed that swine HEV isolates

Textbox 3.1 There exist at least four major genotypes of human HEV worldwide: genotype 1 (epidemic strains from Asia), genotype 2 (a single epidemic strain from Mexico), genotype 3 (strains from sporadic cases worldwide), and genotype 4 (strains from sporadic cases in China, Japan, and Taiwan) (Emerson and Purcell 2003; Huang et al. 2002; Meng 2003, 2005a); however, all known HEV genotypes seem to belong to a single serotype. All swine HEV isolates identified worldwide thus far belong to either genotype 3 or 4 (Cooper et al. 2005; Meng 2003, 2005a, b).

identified from pigs worldwide belong to either genotype 3 or 4 (Cooper et al. 2005; Huang et al. 2002; Meng 2005a, b). Genotypes 3 and 4 HEV strains primarily are responsible for sporadic cases of hepatitis E in humans, whereas genotypes 1 and 2 strains mainly are responsible for hepatitis E epidemics (Textbox 3.1; Emerson and Purcell 2003; Meng 2003, 2005a, b).

Zoonotic Potential

Hepatitis E, an enterically transmitted form of hepatitis, is an important public health disease in many developing countries of Asia and Africa (Emerson and Purcell 2003; Meng 2003; Purcell and Emerson 2001). Cases of acute hepatitis E also have occurred, sporadically, in patients from industrialized countries including the United States (Clemente-Casares et al. 2003; Erker et al. 1999; Hsieh et al. 1999; McCrudden et al. 2000; Mizuo et al. 2002; Schlauder et al. 1998; Takahashi et al. 2003; van der Poel et al. 2001; Yazaki et al. 2003). The mortality rate in infected patients is generally low (<1%), but it could be as high as 25–30% in infected pregnant women (Khuroo and Kamili 2003; Kumar et al. 2004; Purcell and Emerson 2001). The main route of transmission for HEV is believed to be fecal–oral. The disease is generally endemic, and the rare epidemics usually are associated with feces-contaminated water in countries with poor sanitation conditions.

Although only sporadic cases of acute HEV have been reported in humans from the United States and other industrialized countries, HEV antibodies are prevalent in a significant proportion of healthy people in these countries (Clemente-Casares et al. 2003; Mast et al. 1997; Meng 2000; Meng et al. 1999, 2002; Thomas et al. 1997). Presence of antibodies, however, demonstrates exposure, not necessarily infection or disease. The existence of a population of anti-HEV positive individuals in industrialized countries could be explained by subclinical infection of humans with swine HEV. However, city dwellers in the United States and many other industrialized countries, who have no significant exposure to pigs except possibly as food, are also found positive for anti-HEV. Therefore, other animal species may also serve as reservoirs for HEV. The recent discoveries of animal strains of HEV—swine HEV from pigs (Meng et al. 1997) and avian HEV from chickens (Haqshenas et al. 2001; Huang et al.

2004)—and their demonstrated ability to infect across species (Meng et al. 1998a; Sun et al. 2004) has led to the hypothesis that hepatitis E is a zoonotic disease.

Cross-species infections of human and swine HEVs have been documented. It has been demonstrated that a genotype 3 human HEV (strain US-2), but not genotype 1 or 2, is transmissible readily to SPF pigs (Halbur et al. 2001; Meng et al. 1998a, b); the inoculated pigs rapidly became viremic, seroconverted, and spread the virus to naïve pigs through direct contact. Conversely, the genotype 3 swine HEV has been shown to infect both rhesus monkeys and a chimpanzee, the surrogate of man (Meng et al. 1998a). Although both rhesus monkeys and the chimpanzee infected with swine HEV did not develop clinical signs of hepatitis, microscopic lesions of hepatitis were evident in liver biopsies of infected monkeys. In addition, the infected rhesus monkeys had elevation of serum levels of liver enzymes near the time of seroconversion. A genotype 4 strain of swine HEV also has been shown to infect nonhuman primates (Arankalle 2005). It seems that the genotypes 1 and 2 epidemic strains of HEV may have a more limited host range than do genotypes 3 and 4 strains, inasmuch as all swine HEV isolates identified worldwide thus far belong to either genotype 3 or 4 (Cooper et al. 2005; Meng 2003, 2005a, b).

Increasing evidence indicates that hepatitis E is a zoonotic disease and that pigs (and maybe other animal species) are reservoirs for HEV (Meng 2005a). In the United States, the viruses recovered from two hepatitis E patients—one in Minnesota (US-1) and one in Tennessee (US-2)—are most closely related to the genotype 3 strain of swine HEV recovered from a pig in Illinois (>97% sequence identity in ORFs 1 and 2) (Erker et al. 1999; Meng et al. 1998a; Schlauder et al. 1998). In Taiwan and China, genotype 4 strains of human HEV identified from hepatitis E patients are related very closely to swine HEV isolates identified from pigs in the same regions (Hsieh et al. 1999; Wang et al. 2002; Wu et al. 2002). Similarly, in Japan, genotypes 3 and 4 swine HEV isolates recovered from Japanese pigs were closely related to the sequences of genotypes 3 and 4 strains of human HEV from Japanese hepatitis E patients (Nishizawa et al. 2003; Takahashi et al. 2003). Sporadic cases of acute hepatitis E in Japan were linked

to the consumption of undercooked or uncooked pig or wild boar livers (Matsuda et al. 2003; Mizuo et al. 2005; Tamada et al. 2004; Yazaki et al. 2003). Swine HEV RNA was detected in approximately 2% of the packaged raw pig livers sold in local grocery stores in Japan. Most importantly, the sequences of seven swine HEV isolates recovered from packaged pig livers from local grocery stores were related very closely, or were identical in a few instances, to the viruses recovered from human hepatitis E patients. In the United States, genotype 3 swine HEV RNA was detected in approximately 11% of pig livers sold in local grocery stores and the contaminating virus in the pig livers remained infectious (Feagins et al. 2007). However, the contaminating HEV in commercial pig livers can be easily inactivated by traditional cooking methods such as frying or boiling (Feagins et al. 2008). These data provided convincing evidence that swine are reservoirs for HEV, at least for genotypes 3 and 4 strains.

Pig handlers have been shown to be at increased risk of zoonotic HEV infection. In a large well-controlled seroepidemiological study involving 465 swine veterinarians, Meng and colleagues (2002) found that swine veterinarians in the United States were 1.51 times (using genotype 3 swine HEV antigen, p = 0.03) or 1.46 times (using genotype 1 human HEV antigen, p = 0.06) more likely to be positive for HEV antibodies than age- and geography-matched normal U.S. blood donors. Swine veterinarians who reported having needle sticks while working with pigs were approximately 1.9 times more likely to be seropositive than those who did not. Also, individuals from major swine states seem more likely to be seropositive than those from traditionally nonswine states. Similarly, Drobeniuc and colleagues (2001) reported that approximately 51% of swine farmers from Moldova were positive for HEV antibodies, whereas only 25% of control subjects were seropositive. In North Carolina, swine workers were shown to have a 4.5-fold higher HEV antibody prevalence rate than control subjects (Withers et al. 2002). In Taiwan, approximately 27% of pig handlers were positive for HEV antibodies compared with only 8% of control subjects (Hsieh et al. 1999). Taken together, these data provide compelling evidence that (1) hepatitis E is a zoonotic disease, and (2) pigs are a reservoir of genotype

3 and genotype 4 strains of HEV, although not the sole reservoir of all HEV.

Inactivation

Only limited information is available regarding HEV resistance to inactivation by physical, chemical, and environmental forces. Under laboratory conditions, HEV is sensitive to CsCl gradient centrifugation and low-temperature storage (Bradley et al. 1987; Purcell and Emerson 2001). Iodinated disinfectants and autoclaving normally will destroy the virus (Balayan 1997; Schlauder and Dawson 2003). HEV virions are reportedly stable when exposed to trifluorotrichloroethane (Ticehurst 1991). Like other nonenveloped small RNA viruses, however, HEV can survive harsh environments. The fecal–oral route of transmission indicates that HEV is resistant to inactivation by acidic and mild alkaline conditions in the intestinal tract. It recently has been shown that HEV is more heat labile than the hepatitis A virus (HAV), another enterically transmitted hepatitis virus (Emerson, Arankalle, and Purcell 2005). When fecal suspensions of wild-type HM175 strain of HAV or Sar-55 strain of HEV were diluted in a phosphate-buffered saline (PBS) buffer and compared in the same test by heating for 1 hr at 45, 50, 56, 60, 66, or 70°C, HAV was inactivated only 50% at 60°C but was almost totally inactivated at 66°C. In contrast, HEV was approximately 50% inactivated at 56°C and almost totally inactivated (96%) at 60°C. The in vitro results were recently confirmed by an in vivo pig study demonstrating that incubation of infectious HEV at 56°C for 1 hr did not abolish the infectivity of the virus (Feagins et al. 2008).

Presence and Fate

As fecal–oral transmitted diseases, waterborne epidemics are the characteristic of hepatitis E outbreaks in humans (Purcell and Emerson 2001). Like human HEV, swine HEV is thought to be transmitted via the fecal–oral route under natural conditions. It has been demonstrated that infected pigs shed large amounts of viruses in feces (Halbur et al. 2001; Meng et al. 1998b; Williams et al. 2001), which likely is the main source for virus spread within a herd. Fecal shedding of HEV in pigs, ranging from 3 to 8 wk, lasts much longer than viremia, and the infectious virus titer in feces also is higher than that in blood (Halbur et al. 2001; Kasorndorkbua et al. 2002, 2004; Meng et al. 1998b). It generally is believed that pigs become infected through ingestion of feces-contaminated water or feed or through direct contact with infected pigs. Current swine production procedures often flush water through confinement housing either in open gutters or under slatted floors, providing the opportunity for either direct exposure of pigs to feces (open gutter system) or indirect exposures via droplet aerosols (slatted floor system) (Dickey, Brumm, and Shelton 1981). But under experimental conditions, infection of pigs via the oral route of inoculation with an infectious stock of swine HEV proved to be more difficult than via the intravenous route (Kasorndorkbua et al. 2004).

Because of the ubiquitous nature of swine HEV in pigs and the large amount of viruses excreted in feces, swine manure in swine production areas could contaminate irrigation and drinking water in nearby wells, rivers, ponds, or coastal water with concomitant contamination of produce or shellfish (Smith et al. 2001). Consumption of contaminated shellfish has been implicated in sporadic cases of acute hepatitis E (Cacopardo et al. 1997; Koizumi et al. 2004). It also has been shown that, in HEV endemic regions, the use of river water for bathing, waste disposal, and drinking purposes is a significant risk factor (Sedyaningsih-Mamahit et al. 2002). In Southeast Asia, a unique riverine ecology of HEV transmission has been reported. Epidemic foci centered in riverine environments (Corwin et al. 1999), the epidemic peaked with heavy rains and flooding, and the attack rate was significantly higher in villages supplied with river water than in those relying on wells or ponds for water supply (Bile et al. 1994). In these particular studies, the precise origin of the HEV was undetermined.

Hepatitis E virus strains of both human and swine origin have been detected in raw sewage water (Clemente-Casares et al. 2003; Jothikumar et al. 1993; Pina et al. 1998, 2000), and the recovered virus from sewage water still is infectious in nonhuman primates. Pina and colleagues (2000) found that the E11 strain of HEV—likely of swine origin, recovered from sewage water of pig slaughterhouses in Spain—has a close

genetic relation to two Spanish strains of human HEV. The HEV strains recovered from raw sewage water in Washington D.C. are more closely related genetically to the prototype U.S. swine HEV than to the US-1 and US-2 strains of human HEV (Clemente-Casares et al. 2003). In India, it has been shown that the HEV antibody positivity was significantly higher (P < 0.01) in sewage workers (57%) than in controls (19%). A significant rise in HEV antibody positivity (P < 0.05) was found in sewage workers working for more than 5 yr (Vaidya et al. 2003). These data suggest that raw sewage water contaminated with swine HEV could be a source for human infection.

Swine HEV has been detected in swine manure and wastewater associated with hog operations (Karetnyi et al. 1999) and in concrete pits and earthen lagoons of swine manure storage facilities (Kasorndorkbua et al. 2005). Fresh feces, manure slurry (from earthen lagoons and/or concrete pits), and drinking and surface water samples were collected from 28 pig farms in the Midwestern United States. All samples were tested for HEV RNA by reverse transcription-PCR (Kasorndorkbua et al. 2005). Of the 22 farms where pit samples were accessible, 15 contained HEV, and of 8 farms that had lagoons, 3 contained HEV. However, none of the water samples collected on or near the pig farms tested HEV positive. Subsequent pig inoculation experiment revealed that the HEV in pig manure slurry was infectious when inoculated intravenously into pigs (Kasorndorkbua et al. 2005). Therefore, swine manure land application and runoff could be the source for water contamination and subsequent contamination of produce or shellfish (Smith et al. 2001), thus leading to potential transmission of HEV to humans. It is not known, however, how long the virus can survive in the swine manure and remain infectious or what effect the manure storage and treatment will have on the infectivity of HEV. There also is a potential risk of transmitting swine HEV either through drinking feces-contaminated water or consuming contaminated food. In Turkey, individuals using untreated wastewater for irrigation were found to have a significantly higher HEV antibody prevalence rate (34.8%) than the controls with the same socioeconomic status and age (Ceylan et al.

2003). In Japan, Yazaki and colleagues (2003) reported that sporadic cases of acute human hepatitis E were associated with ingestion of contaminated pork livers and intestines.

Although infection does not always equal illness, the demonstrated ability of cross-species infection by swine HEV does raise a public health concern, especially for the high-risk group (swine veterinarians and other pig handlers, pregnant women, and pig xenograft recipients). A vaccine against HEV is not yet available. The experimental recombinant HEV vaccines seem to be effective; their efficacy, however, must be evaluated thoroughly for protection against the emerging strains of HEV, including genotypes 3 and 4 swine HEV (Purcell et al. 2003). A simple preventive measure for pig handlers is to wash hands thoroughly after handling pigs and to avoid drinking water of unknown purity. Although swine HEV seems to be nonpathogenic in pigs, it is not known if concurrent infections of swine HEV with other swine pathogens could have any synergistic effects. Currently, there is a lack of information on the fate of HEV in swine manure. Future work is warranted to assess the survivability of HEV in swine manure and in different environmental regimens.

ENTERIC CALICIVIRUSES (NOROVIRUSES AND SAPOVIRUSES)

Caliciviruses are small, nonenveloped, ss, positive-sense RNA viruses. The icosahedral viral capsid (27–40 nm in diameter) is composed of a single major (VP1) capsid protein, and it contains genomic RNA of 7–8 kb in length, excluding the poly A tract (Green, Chanock, and Kapikian 2001; Mayo 2002). The family *Caliciviridae* now is divided into four genera based on sequence identities and genome organization: *Norovirus* (NoV), *Sapovirus* (SaV), *Vesivirus,* and *Lagovirus.* Viruses in the *Vesivirus* (feline calicivirus and vesicular exanthema of swine virus) and *Lagovirus* genera (rabbit hemorrhagic disease virus and European brown hare syndrome virus) do not infect or cause diarrhea in humans, whereas viruses in the *Norovirus*

and *Sapovirus* genera cause diarrhea in humans and animals and are referred to as human or animal enteric caliciviruses (Green, Chanock, and Kapikian 2001).

The NoV genus is divided into five genogroups (GI, GII, GIII, GIV, and GV), and each genogroup is subdivided further into a number of genotypes (8, 17, 2, 1, and 1, respectively) based on phylogenetic tree topology and distance analysis of the capsid protein (Zheng et al. 2006). Human noroviruses (HuNoVs) can be grouped to GI, GII, and GIV, whereas GIII includes bovine noroviruses (BoNoV) (Dastjerdi, Snodgrass, and Bridger 2000; Dastjerdi et al. 1999; Liu et al. 1999; Smiley et al. 2003). Three separate genotypes of porcine noroviruses (PoNoVs) have been found to be closely related to GII HuNoVs (Wang et al. 2004) whereas the recently discovered murine norovirus can be grouped in GV (Karst et al. 2003). Using the same criteria and methodology, viruses in the Sapovirus genus can be divided into five genogroups (GI–GV) with each genogroup subdivided in 3, 3, 1, 1, and 1 genotypes, respectively, based on distance and phylogenetic analysis of the capsid sequence (Farkas et al. 2004; Schuffenecker et al. 2001). Human sapoviruses (HuSaVs) belong to GI, GII, GIV, and GV, whereas to date porcine sapoviruses (PoSaVs) have been found to belong uniquely to GIII.

Zoonotic Potential

Low infectious doses (Green, Chanock, and Kapikian 2001), prolonged asymptomatic shedding (Graham et al. 1994; Patterson, Hutchings, and Palmer 1993; Rockx et al. 2002), environmental stability (Barker, Vipond, and Bloomfield 2004; Berg et al. 2000; Duizer et al. 2004; McDonnell et al. 1997; Rzezutka and Cook 2004), and great strain diversity (Ando, Noel, and Fankhauser 2000; Farkas et al. 2004) increase the risk of infections by this virus family. Moreover, the identification of closely related animal enteric caliciviruses in pigs and the existence of recombinants within PoNoV, HuNoV, and also HuSaV (Jiang et al. 1999; Katayama et al. 2002; Katayama et al. 2004) raise concerns for possible human infections or coinfection of animals or humans with human and animal enteric caliciviruses.

Norovirus

Animal enteric caliciviruses cause gastroenteritis in calves and pigs and also have been isolated from healthy pigs. Porcine noroviruses were detected in Japan and Europe and recently in the United States (Wang et al. 2004). In Japan, PoNoV was detected in fecal contents of 4 of 1,017 normal slaughtered pigs, whereas a study performed in the Netherlands showed that 2% of 100 pooled samples from 3- to 9-mo-old pigs were positive for PoNoVs (Sugieda et al. 1998; van der Poel et al. 2000). In the United States, PoNoV was reported recently (Wang et al. 2004) in 6 of 275 fecal samples collected from three farms and one slaughterhouse in Ohio, two farms in North Carolina, and one farm in Michigan between December 2002 and June 2003. The newly identified PoNoVs belong to three different genotypes within GII, the most widely detected NoV genogroup in humans.

As part of this study, the authors also reported that PoNoVs were detected only in fecal samples collected from the finisher pigs, but not in nursing, postweaning pigs or sows. Most positive samples in this study were from healthy animals, suggesting that, as previously observed for HuNoV infections (Rockx et al. 2002), asymptomatic shedding of PoNoV occurs in adults, contributing to virus persistence in the field. Moreover, identification of genetically closely related caliciviruses in humans and animals and studies of the seroprevalence and antigenic cross-reactivity between them suggests possible zoonotic transmission. In a survey of antibodies against SW918 (a PoNoV GII strain detected in Japan), the authors reported that 83% of sera from domestic pigs in the United States showed positive reactions, and 52% reacted with HuNoV GII. Surprisingly, 63% of sera also reacted with Norwalk virus (NV), a HuNoV GI (Farkas et al. 2005). Viruses in these two genogroups do not cross-react, and no PoNoV GI has been detected in swine. Recently, it was observed that convalescent serum from gnotobiotic (Gn) pigs inoculated with PoNoV GII cross-reacts with virus-like particles (VLPs) of several HuNoV GII strains, confirming antigenically similar NoVs in pigs and humans and suggesting their possible interspecies transmission (Wang et al. 2004).

Sapovirus

Sapoviruses in humans primarily have been associated with acute gastroenteritis in young children (Chiba et al. 2000). The PoSaV/Cowden strain, first reported by Saif and colleagues (1980), was based on its calicivirus morphology by electron microscopy (EM) of diarrheic stools of pigs in the United States. In a small-scale survey of SaVs in three Ohio swine farms (Guo and Saif, Personal communication), PoSaV was detected in 51% of 156 fecal samples, mainly from nursing pigs (48%) and postweaning pigs (75%), but not from adult pigs in one farm. The PoSaV was detected in 100% of diarrheic nursing pigs and 50% of normal nursing pigs, and in 89% of diarrheic postweaning pigs and 69% of normal postweaning pigs in the same farm. In this farm, 80–100% of pigs in all age groups were seropositive for PoSaV antibodies, indicative of high exposure rates. These limited findings suggest that PoSaV may be a major cause of postweaning and nursing pig diarrhea, but subclinical PoSaV infections also occur. The PoSaV has emerged as an important pathogen associated with diarrhea and subclinical infections among pigs of all ages. (Barry, Alfieri, and Alfieri 2008; Jeong et al. 2007; Martella et al. 2008; Wang et al. 2006)

Inactivation

Enteric viruses are acid stable and can survive in the gastrointestinal tract. Most viruses remain infectious after refrigeration and freezing and also retain their infectivity after heating to 60°C for 30 min. Because enteric caliciviruses (NoVs and SaVs), with the exception of PoSaV/Cowden strain (Guo et al. 2001), cannot be propagated in cell culture, most attempts to determine inactivation profiles of caliciviruses have been conducted with cultivable caliciviruses such as feline enteric coronavirus (FeCV) and canine calicivirus (CaCV).

Chlorine

Chlorine-based disinfectants are considered the most effective against enteric viruses. But several studies using FeCV show that caliciviruses are relatively resistant to chlorine (Doultree et al. 1999; Gulati et al. 2001). Complete inactivation of FeCV and CaCV

was reported only at chlorine levels of 3,000 parts per million (ppm) (or higher) for 10 or 30 min at room temperature (Duizer et al. 2004).

Temperature

The NV virus remains infectious for volunteers after heating at 60°C for 30 min (Green, Chanock, and Kapikian 2001). Allwood and colleagues (2003) compared the survival rates of FeCV at 4, 25, and 37°C for up to 28 d in dechlorinated water. Their results showed that a 90% decrease in infectious titer was achieved at 3 d at 37°C, but the value increased to 5.2 d at 25°C (room temperature) and 7.3 d at 4°C. Similar results were obtained by Kadoi and Kadoi (2001) when survival of different FeCV strains was assayed in marine water at 4, 10, and 20°C. After application of contaminated manure to land, the potential for environmental contamination may exist, including possible spread to other areas resulting from increased rainfall, overflow, or aerosol. (Tyrrel and Quinton 2003) Although the virus concentration will be lower in water, the low infectious dose of HuNoV (as low as 10–100 particles) (Moe et al. 1999) and its ability to survive increase the risk of outbreak when contaminated water sources are used in food processing or as public water supplies (Hoebe et al. 2004; Ueki et al. 2004).

Ultraviolet Irradiation

Most UV inactivation studies have not been conducted using monodispersed viruses and therefore may give a biased idea of the true inactivation kinetics. One study using FeCV as an indicator organism showed an infectivity reduction of 90% with a UV dose of 480 joule (J)/m^2, four times more than the dose required for hepatitis A and double the amount required for 90% inactivation of poliovirus (Nuanualsuwan et al. 2002).

pH

In human volunteer studies, NV (the prototype HuNoV) was shown to retain its infectivity after exposure to pH < 3 for 3 hr at room temperature (Dolin et al. 1972), and an outbreak strain was protected after exposure to high and low pHs (pH 2, 3, 10, and 12) for 30 min at 37°C. Recent research demonstrates that the newly discovered cultivable murine norovirus shows

similar resistance to extreme pHs and may be a better surrogate for the HuNoVs than the frequently used FeCV (Cannon et al. 2006). Organic acids are unlikely to have any effect on the viability of these viruses during short contact times (Seymour and Appleton 2001).

Presence and Fate

Limited data are available on the behavior of viruses or their possible surrogates in manure and soil. In the particular case of animal enteric caliciviruses (NoVs and SaVs), at least two factors should be highlighted. First, these viruses and their presence and prevalence only recently have been reported; and second, and perhaps more importantly, there is a lack of cell culture for assessment of virus infectivity for human NoVs and SaVs. The latter deficiency necessitates assessment of the environmental survival of these viruses through a surrogate virus such as FeCV using lab-scale assays. There are two difficulties with this approach: the first is to understand whether a nonenteric virus—such as FeCV—adequately reflects the stability of the surrogate enteric virus. A good correlation was observed when FeCV and HuNoVs were inactivated by heat, UV, or free chlorine, but only HuNoVs remained infectious when the pH was lower than 3 (Dolin et al. 1972; Duizer et al. 2004). The second difficulty results from the fact that the true environmental scenario cannot be replicated fully in a laboratory, because multiple and simultaneous factors affect virus survival in the field.

Land application of agricultural manure occurs worldwide, and pathogens present in manure can affect soil and water integrity. Different environmental factors affect the fate and transport of pathogens from manure into soil and water. In lab-scale experiments, microbial concentrations initially decline with time when added to a solution, but thereafter remain basically constant as indicated by studies of Slomka and Appleton (1998) using seawater. This study showed that a 20-fold decrease in FeCV infectivity occurs on addition to seawater, but no significant decrease occurs in the next 24 hr. But in the field this also depends on the equilibrium of the microorganism between water and soil, and furthermore on the soil composition. This involves the presence of salts, organic matter, and pH. Microorganisms exist in a state of zero charge when the pH reaches a characteristic value called the Isoelectric point (pI), and this value varies for each microorganism. Microorganisms with high pI tend to absorb to surfaces to a higher extent than those with low pI. The pI of phage MS2 (pI 3.9) is similar to the pI of hepatitis A (pI 2.8), and lower than the value for poliovirus-1 (pI 7.2). Studies performed in 1995 (Sobsey, Hall, and Hazard 1995) demonstrated that absorption of poliovirus-1 to soil columns was higher than MS2 and hepatitis A. The pI of NV (HuNoV GI) determined from VLPs produced in the laboratory was estimated to be 4.9 (Redman et al. 1997). Based on this value, NV is expected to be more absorptive than MS2, but less so than poliovirus-1. The study performed by Meschke and Sobsey (1998) on the absorption of NV, poliovirus-1, and phage MS2 in six different soils confirmed that NV is less absorptive than poliovirus-1, suggesting that it will be easier to remove NV than poliovirus-1 from sediments. Prediction of absorptive properties based on pI values refers to overall virus charge under a given pH, but not to local areas of charge of the virion. Therefore, as demonstrated by Redman and colleagues (1997) for MS2 and NV-VLPs, depending on the pH of the environment, viruses with higher pI may display less absorption than viruses with lower pI.

It is generally accepted that very low or very high pH may decrease pathogen viability. In the case of NoVs, however, it has been demonstrated that pH lower than 3 or higher than 10 will not affect virus stability (Duizer et al. 2004). Moreover, evidence suggests that adsorption of viruses to particulate matter and sediments confers protection against the inactivating influences of pH.

Solar radiation promotes inactivation of viruses through visible and shortwave UV components. Again, lab-scale experiments showed that although differences exist between UV inactivation of surrogates of NoVs (FeCV and CaCV) in suspensions or on a dried surface, inactivation also is achieved in the presence of high organic material because RNA is the target. If UV is compared with ionizing radiation such as gamma rays, the former is more effective in the presence of solutes that can react with free OH radicals (De Roda Husman et al. 2004). But in contrast to studies of water sanitation, the effects of these radiations have not been studied extensively for animal manure.

Viruses may be released from the host in an aggregated state. In general, it is assumed that aggregated viruses are more resistant to inactivation than single virus particles. In the case of NoVs, this has been demonstrated for chlorine inactivation (Thurston-Enriquez et al. 2003), because each virus particle within the aggregates must be inactivated before the whole aggregate is considered inactivated. Adsorption to sediments may decrease because of the aggregated state, which could be beneficial in terms of limiting local soil contamination, but these particles can be transported easily by the air or rainfall to other points (Tyrrel and Quinton 2003; Hutchison, Avery, and Monaghan 2008).

Virus-like particles as laboratory surrogates for enteric caliciviruses have been used to assay reduction of NoVs in soil columns under different conditions that resemble those in the field (different soil composition, groundwater, and rainwater) (Meschke and Sobsey 1998). The NV VLPs have been used successfully to study accumulation of NV in digestive tissues of shellfish (Loisy et al. 2004). The results were not only useful to improve shellfish safety, but also indicated that VLPs behave similarly to native virus and could be used as surrogates, at least in lab-scale experiments. Although these experiments and results can be extended to estimate and understand what hypothetically would happen in the field, the true scenario where all the previously mentioned factors interact needs to be evaluated.

At least 18 environmentally superior technologies recently have been developed for the treatment of animal manure to decrease its impact on the environment and public health (Humenik et al. 2004). For animal enteric caliciviruses, the first study to investigate the effect of environmental technologies on the fate of these pathogens in animal manure under field conditions was performed recently (Costantini et al. 2007). In this study, the occurrence of PoSaV and PoNoV first was assessed in fresh feces of swine; then the effects of different animal manure management and treatment technologies on their survival was evaluated by RT-PCR or enzyme-linked immunosorbent assay (ELISA) (Guo et al. 2001; Wang, personal communication). Because enteric caliciviruses are not cell-culture-adapted, ELISA and RT-PCR results were

Table 3.1. Detection of animal enteric viruses in pre- and post-treatment (Costantini et al. 2007)

Management system and treatment technology	Enteric viruses							
	PoNoVs[a]		PoSaV[b]		RV-A[b]		RV-C[b]	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Conventional swine operation	-	-	+	+	+	+	-	-
Aerobic up flow biofiltration system	-	-	+	-	+	+	+	+
Constructed-wetland system	+	-	+	-	+	+	-	-
Super soil system	+	-	+	-	+	+	+	+
High rise hog building	+	-	+	-	+	+	+	+
Ambient temperature anaerobic digester	-	-	+	-	+	-	+	-

a Determined by RT-PCR with specific primers
b Determined by RT-PCR with specific primers and ELISA

combined to provide estimate prevalence and treatment effects. The presence of infectious particles could be overestimated if ELISA and RT-PCR were positive, because inactivated particles still could be detected by both techniques. ELISA-negative and RT-PCR-positive or ELISA- and RT-PCR negatives also could result in underestimation of the presence of infectious particles, because the numbers of infectious particles may be lower than the detection limit of one or both techniques, respectively. Only in case of ELISA-positive and RT-PCR-negative might it be considered that infectious particles may not be present and that the positive result on ELISA may result from detection of soluble proteins. A final definitive answer cannot be provided without an infectivity assay; therefore, for highly critical samples, it is possible to assess infectivity in vivo using calicivirus-seronegative-susceptible conventional pigs or Gn pigs. In the previously cited study (Costantini

et al. 2007), the authors evaluated five different environmental technologies and a conventional swine operation where storage and treatment of manure was in wastewater lagoons (Table 3.1). PoSaVs were detected in pretreatment fresh feces from each system consistent with the high prevalence of PoSaVs among all ages of swine, and its RNA was detected posttreatment only in lagoons in the conventional swine operation, but it was not detected after the other five treatments, suggesting that all the applied technologies will decrease the virus detection rates to undetectable levels by the detection techniques applied. PoNoVs were detected initially from fresh feces in three systems, but they were not detected after treatment in any system (Table 3.1). These results provide new and promising data about the impact of each animal manure management system on the persistence of animal enteric caliciviruses in the treated manure. It cannot be definitively stated that a risk of transmission does not exist, however, because at least for HuNoVs, the dose required for infectivity is very low (in the range of 10 to 100 particles) (Moe et al. 1999). The presence or absence of infectious virus and the risk of transmission will be determined more accurately by in vitro and in vivo infectivity assays, respectively. But because of the lack of a cell culture that can assess in vitro infectivity of most enteric caliciviruses, answers to this question remain uncertain.

ROTAVIRUS

General Virology

Rotaviruses (RV) belong to family Reoviridae and possess a segmented double-stranded (ds) RNA genome consisting of 11 segments. RV particles consist of a triple-shelled capsid with two viral proteins (VP): VP4 and VP7 comprising the outer capsid, and one inner capsid (VP6) surrounding the core (VP2) layer. The 11 ds RNA segments encode six structural and six nonstructural proteins (Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994). Segment six encodes VP6, which is the basis to classify RVs into seven serogroups (A–G) (Saif and Jiang 1990; Saif, Rosen, and Parwani 1994). Segments seven, eight, or nine encode VP7, and segment four encodes VP4, both of which independently induce neutralizing antibodies (Kapikian, Hoshino, and Chanock 2001;

Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006). Rotaviruses are the leading cause of acute viral gastroenteritis in the young of both avian and mammalian species, including pigs and humans (Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006). Groups A, B, and C (RV-A, RV-B, and RV-C, respectively) infect humans and animals, whereas groups D, E, F, and G (RV-D, RV-E, RV-F, and RV-G, respectively) have been found only in animals (Saif and Jiang 1990; Yuan, Stevenson, and Saif 2006). Group A is responsible for 12 to 71% of diarrhea episodes in developed and developing countries, respectively (Kapikian 1996). The RV-A, RV-B, and RV-C infect pigs (Geyer et al. 1996; Kim et al. 1999; Saif and Jiang 1990; Saif et al. 1980; Yuan, Stevenson, and Saif 2006), and both RV-A and nongroup A can be detected in the same herd (Geyer et al. 1995; Janke et al. 1990; Kim et al. 1999). The RV-A are the main agents of viral diarrhea in piglets, accounting for 53% of preweaning and 44% of postweaning diarrhea in swine (Atii, Ojeh, and Durojaiye 1990; Fitzgerald et al. 1988; Gatti et al. 1993; Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006).

Classification and cross-protection between strains of RV are based on the outer capsid proteins, VP4 and VP7. The VP7 (major outer surface component) is a glycoprotein, whereas VP4, or RV surface spike, is a protease-sensitive protein. The glycoprotein (G) type is defined by VP7, whereas the protease (P)-sensitive type refers to the VP4 protein (Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994). For RV-A, 15 G serotypes/genotypes (G1–G14) have been identified. The G-types 1–6, 8–10, and 12 were isolated from human infections, whereas the main G-types in pigs are 3, 4, 5, and 11 (Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994; van der Heide et al. 2005; Winiarczyk et al. 2002; Yuan, Stevenson, and Saif 2006). To date, at least 11 P serotypes (determined by neutralization assay with polyclonal or monoclonal antibodies) and 22 different P genotypes (determined by hybridization and sequence analysis) have been described for RV-A (Hoshino and Kapikian 1996; Hoshino, Jones, and Kapikian 2002; Liprandi et al. 2003; Martella et al. 2001; Okada et al. 2000). Because a complete correlation between P serotypes and P

genotypes does not exist, a different designation has been adopted with open numbers for P serotypes, and numbers between brackets for P genotypes. Among human rotavirus (HRV) strains, P1, 2, 3, 4, 5, and 11 were detected, whereas P2 and 9 were detected in pigs (Estes and Cohen 1989; Hoshino et al. 1984; Martella et al. 2001). Among the 22 P genotypes identified, P[4], P[6], P[8], P[9], P[10], and P[12] are associated with HRV, and P[6], P[7], P[14], and P[19] with pigs (Burke, McCrae, and Desselberger 1994; Gouvea, Santos, and Timenetsky 1994a; Huang, Nagesha, and Holmes 1993; Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006; Zaberezhny, Lyoo, and Paul 1994). For HRV, P[4], P[6], P[8], and P[9] correspond to serotypes P1B, P2A, P1A, and P3, respectively, whereas in pigs, P[6] and P[7] belong to P2B and P9, respectively (Saif, Rosen, and Parwani 1994; Yuan, Stevenson and Saif 2006). Among the most common human strains are P1A[8]G1, P1B[4] G2, P1A[8]G3, and P1A[8]G4, whereas among porcine strains, P2B[6]G4 and P9[7]G5 are the most prevalent (Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006). But typical human and bovine G- and P genotypes have been described in pigs that suggest the possibility of transmission of RVs between species (Martella et al. 2001; Saif, Rosen, and Parwani 1994; Teodoroff et al. 2005; Winiarczyk et al. 2002; Yuan, Stevenson, and Saif 2006; Zaberezhny, Lyoo, and Paul 1994).

Detection

Rotaviruses can be detected in feces of infected people or pigs by several techniques such as RT-PCR, nested or seminested PCR, polyacrylamide gel electrophoresis (PAGE), EM, immune electron microscopy (IEM), immunofluorescence (IF), VI, latex agglutination (LA), and ELISA (Iturriza-Gomara, Green, and Gray 2000; Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006). During an acute RV infection, approximately 10^8 to 10^{12} viral particles/ml are excreted. In these circumstances, diagnosis by ELISA (sensitivity of 10^5 to 10^6 viral particles/ml) (Gilchrist et al. 1987; Rubenstein and Miller 1982) and rapid tests such as LA with a sensitivity of 4×10^6 up to 2×10^7 infectious particles/ml fecal suspension are useful.

Although all these tests have shown high specificity and sensitivity for the detection of RV in clinical samples, in delayed sampling or environmental samples where viruses are not replicating, the amount of virus usually is under the detection level for these techniques. In this scenario, molecular techniques or techniques that combine molecular with virus replication approaches are needed. The RT-PCR, nested PCR, and seminested-PCR techniques have been developed to detect RV-A, RV-B, and RV-C and also to differentiate RV-A G- and P-types (Gouvea et al. 1991; Gouvea, Santos, and Timenetsky 1994a, b; Racz et al. 2000; Villena et al. 2003; Winiarczyk et al. 2002). To compare cell culture and nested-PCR, the presence of RV in cell culture and fecal samples was assayed. The detection limit for virus in cell culture supernatants was 3×10^{-2} tissue cultures infections dose (TCID)$_{50}$ by RT-PCR and 3×10^{-3} TCID$_{50}$ by nested-PCR (Elschner et al. 2002). An immunochromatographic test for the detection of RV-A also showed high sensitivity (89%) and specificity (99%) compared with ELISA for feces of different species (de Verdier Klingenberg and Esfandiari 1996).

For environmental samples, most studies have focused on detection of RV in water as a source of infection. The critical point in this type of sample is to concentrate the virus, and several methods have been assessed (Abbaszadegan, Stewart, and LeChevallier 1999; Brassard et al. 2005; Caballero et al. 2004; Gratacap-Cavallier et al. 2000; Hot et al. 2003; Kittigul et al. 2000; van Zyl et al. 2004). After concentration is achieved, detection of RV has been performed by RT-PCR, seminested PCR, and flow cytometry (FC) (Abad, Pinto, and Bosch 1998; Bosch et al. 2004; Fout et al. 2003; van Zyl et al. 2004). All techniques mentioned earlier do not differentiate between infectious or noninfectious particles; however, this is very important to assess the risk of disease transmission from environmental samples. In general, the detection limit for ELISA is 10^5 particles/ml, and RT-PCR assays detect RNA from 10^{1-3} particles/ml. Cell culture propagation has been shown to detect 10^{0-1} RV infectious particles/ml, which does not differ from RT-PCR and could be used as a substitute technique but is more time consuming and requires maintenance of cell lines. Detection of RNA in a sample, however, does not

always indicate presence of infectious virus. Although it is considered that RNA will degrade in the absence of the protective core protein, no information is available about the time required for this in various types of samples.

Epidemiology

Groups A, B, and C RV infect pigs and humans (Geyer et al. 1995; Kapikian, Hoshino, and Chanock 2001; Kim et al. 1999; Martella et al. 2001; Saif and Jiang 1990; Teodoroff et al. 2005; Winiarczyk et al. 2002; Yuan, Stevenson, and Saif 2006). Both RV-A and nongroup A have been detected in the same herd (Atii, Ojeh, and Durojaiye 1990; Fitzgerald et al. 1988; Gatti et al. 1993).

Group A

Group A RV is the main agent of viral diarrhea in piglets, accounting for 53% of preweaning and 44% of postweaning diarrhea in swine (Will et al. 1994). One report attributes 89% of all RV diarrhea in commercial pig operations to RV-A (Gouvea et al. 1991). Diarrheic animals shed virus in high titer in feces (10^7–10^8 infectious doses/g feces). In pigs, the infection is an age- and husbandry-associated disease. Thus RV has been associated with acute diarrhea in pigs weaned at 2–8 wk of age and during different stages of the suckling period, but not usually during the first 7–10 d of life (Bohl et al. 1978; Nagy et al. 1996; Wieler et al. 2001; Woode 1982). This difference has been attributed to the level of passively transferred antibodies that remains high during the first week of life, and to passive antibodies in colostrum and milk maintained in the gut. The occurrence of RV was significantly higher in 22- to 28-d-old pigs than in younger pigs during diarrhea outbreaks in 24 farms in Germany (Wieler et al. 2001). Nagy and colleagues in Hungary (1996) reported an 18.6% prevalence of RV-A in postweaning pigs with diarrhea. In studies by Janke and colleagues (1990), RV-A was detected in 76.4% of nursing pigs and 40.9% of weaned pigs during a diarrhea outbreak in a conventional swine operation. Increased numbers of outbreaks or outbreaks in pigs less than 1 wk old can occur, if one or more risk factors such as the introduction of a new RV strain, primiparous sows with qualitatively and quantitatively poorer colostrum, or poor farm management practices are present on

the farm. Farm expansion, early weaning, and all-in/all-out production were associated with increased numbers of outbreaks in Ontario between 1994 and 1998 (Dewey et al. 2003). A nonpreviously circulating RV strain, primiparous sows, and high population density were considered as the three major risk factors that contributed to an outbreak of diarrhea by RV-A affecting pigs from birth up to 28 d of age (53% of up to 1-wk-old pigs, 60% of 8- to 21-d-old pigs, and 52% of more than 21-d-old pigs) in Brazil (Barreiros et al. 2003).

Group B and C RVs

The RV-B and RV-C have been identified in humans and pigs. Geyer and colleagues (1996) reported RV-B in 4.6% and RV-C in 10.8% by PAGE of samples collected from 1- to 43-d-old pigs with diarrhea. In the study by Janke and colleagues (1990), RV-B and RV-C were detected in 7.4% and 7.5% of nursing pigs and in 18.2% and 22.7% of weaned pigs, respectively, by PAGE. In the United States, RV-C was identified in fecal samples collected from finishing pigs with diarrhea by IEM, cell-culture IF, and RT-PCR (Kim et al. 1999).

Group C RV was the cause of enzootic neonatal diarrhea in a swine herd in Quebec reported in 1990. During the outbreaks of diarrhea, the morbidity rate was 100% and case fatality rates were 5 to 10% among 1- to 2-day-old piglets (Morin, Magar, and Robinson 1990). Group RV-B and RV-C were reported as a cause of 12% of 120 outbreaks that occurred in Quebec during 1 yr in 2-d- to 5-wk-old pigs (Magar, Robinson, and Morin 1991).

Most RV-B or RV-C in humans have been isolated cases, but outbreaks have been reported worldwide (Bridger, Pedley, and McCrae 1986; Castello et al. 2000; Gabbay et al. 1999; Jiang et al. 1995; Maunula, Svensson, and von Bonsdorff 1992; Penaranda et al. 1989; Rasool et al. 1994; Tsunemitsu, Jiang, and Saif 1992). But the finding of a low prevalence of antibody to RV-C in humans (Nilsson, Sigstam, and Svensson 2000; Saif and Jiang 1990; Tsunemitsu, Jiang, and Saif 1992) and a higher prevalence in pigs (Bridger and Brown 1985; Saif and Jiang 1990; Tsunemitsu, Jiang, and Saif 1992) has led to the suggestion that RV-C could be a zoonotic infection of humans (Will et al. 1994).

Coinfections

A number of authors have reported coinfections of pigs and humans with different RV groups or different G- and P-genotypes within RV-A. Janke and colleagues (1990) reported coinfection in 8.8% of samples from nursing pigs and in 18.2% of samples from weaned pigs. Coinfection of RV-A and RV-C and RV-C and HuNoV GII was detected in children younger than 3 yr of age (Phan et al. 2004). Mixed infections among G1, G2, G3, G4, and G9 have been described in humans in India and in Europe (Fischer et al. 2005; Jain et al. 2001).

Zoonosis

Zoonoses are diseases that under natural conditions are transmitted from animals to humans. The close contact between workers and animals on the farm and the fact that viruses can survive in the environment and be transmitted to humans—directly by air, water, and soil, or indirectly by food—raises concerns for possible interspecies transmission. The presence of RV in livestock is a potential public health problem whose significance is increased by the detection in humans of serotypes and genotypes of animal strains and vice versa. The G3, G4, G5, and G11 types and P2B[6], P9[7], P[14], and P[19] types are the most common in pigs (Burke, McCrae, and Desselberger 1994; Desselberger, Iturriza-Gomara, and Gray 2001; Gouvea, Santos, and Timenetsky 1994b; Huang, Nagesha, and Holmes 1993; Kapikian, Hoshino, and Chanock 2001; Saif, Rosen, and Parwani 1994; Yuan, Stevenson, and Saif 2006; Zaberezhny, Lyoo, and Paul 1994). But human G- and P-types such as G1, G2, G9 (Bellinzoni et al. 1990; Ciarlet and Liprandi 1994; Racz et al. 2000; Santos et al. 1999), P[8], and P[6] (Racz et al. 2000; Santos et al. 1999) and bovine G- and P-types such as G6, G8, G10 (Gouvea, Santos, and Timenetsky 1994b; Palombo 2002; Pongsuwanna et al. 1996; Racz et al. 2000), and P[1], P[5], and P[11] also have been detected in pigs (Gouvea, Santos, and Timenetsky 1994a; Martella et al. 2001; Pongsuwanna et al. 1996). Human RV strains commonly found in animals have been isolated

from children in developed and developing countries. Desselberger, Iturriza-Gomara, and Gray (2001) and Palombo (2002) reviewed these findings indicating that G3 (usually found in cats, dogs, pigs, mice, rabbits, and horses), G5 (pigs and horses), G6, G8, and G10 (cattle), and G9 (pigs and lambs) have been isolated from humans worldwide.

Moreover, in the last 3 yr, evidence for the presence of three different porcine strains circulating in humans has been reported. A P[8]G5 strain was reported in children in Cameroon (Esona et al. 2004), whereas the presence of a G4 strain similar to porcine strains was detected in children in Mexico (Laird et al. 2003). In 2004, a human strain with all segments (except for VP7) more similar to a porcine strain than to human strains was reported (Varghese et al. 2004), whereas Teodoroff and colleagues (2005) indicated that the most common G9 strain circulating in pigs in Japan was related closely to the G9 strain circulating in humans. The emerging G9 strain may have been transmitted to humans from animals, because it has been found in lambs and pigs (Fitzgerald et al. 1995; Santos et al. 1999). These uncommon HRVs may have developed as a whole virus or as a reassortant between human and animal strains during coinfection of a single cell in animals or humans. Similar to the observations for influenza virus, different strains can simultaneously infect one species, rearrange their genomes by exchanging genome segments, and emerge as a new strain pathogenic for the original host or for other species.

Controlled experiments have demonstrated that RV circulating in one species can, under the right conditions, emerge as pathogenic reassortants for other species. As an example, RV PP-1 strain (P[7] G3) emerged after passage of a bovine fecal sample in Gn pigs. The PP-1 strain was pathogenic for pigs but not for calves (El-Attar et al. 2001). After the original bovine fecal sample was serially passaged in Gn calves, however, the emergent strain CP-1 (P[5]G3) was pathogenic for calves but not for pigs (Bridger and Pocock 1986). Serial passage in cell culture yielded the reference P[5]G6 bovine strain. These results support the idea that RV exists as a population of reassortants from which a new strain can emerge under appropriate conditions (Gouvea and Brantly 1995). El-Attar and

colleagues (2001) proposed that the original bovine fecal sample contained at least P[5] and P[7] and at least G6 and G3, and that the three different strains emerged under different passage/host conditions.

Environmental Survival

Studies of environmental survival of pathogens under field conditions can be performed only if contamination levels are naturally high, or if the pathogen is introduced into the environment. Pesaro, Sorg, and Metzler (1995) worked with different nonaerated liquid and semiliquid animal manure to study in situ inactivation of RV which required almost 4 mo, with a decay rate of less than 1 $\log_{10}$ in 6 wk. In an attempt to estimate the impact of RV contamination on land via cattle slurries, it was estimated that 1 m^2 of land could be contaminated with 2.5 x 10^4 infectious particles, after land application of slurry previously stored for 3 mo on an average farm (according to U.K. regulations). The authors also estimated that in a nonfavorable scenario for virus spread, where animal RV will have limited specificity for humans and humans would be partially protected from previous virus exposure, 5,000 human infections per yr still may develop from contact with calves on dairy farms in the U.K. (Cook et al. 2004). Similar estimations can be made for swine herds, considering that animals are handled by workers during the nursing and weaning time, manure is stored in anaerobic lagoons, and high population density favors disease spread (Gray et al. 2007).

Only in exceptional situations will permission be given to do field studies that require seeding the environment with a pathogen. Therefore, a surrogate organism or recombinant particle that models the fate and transport of the pathogenic organism without increasing the risk of disease for the population is desirable. Caballero-Hernández and colleagues (2004) evaluated RV VP2-VP6 recombinant 2/6 VLP as a surrogate for HRV in different environmental scenarios. Although results did not always have 100% correlation between 2/6 VLP and infectious virus, surrogate particles always were equal or more resistant to different treatments. After 1 mo in seawater at 20ºC, both had the same decay rates; in the presence of 0.2 milligrams

(mg) of free chlorine/l, no differences were observed, but the surrogate 2/6 VLPs remained detectable longer than infectious virus when the free chlorine concentration was 1mg/l. The authors also investigated UV inactivation in fresh water and seawater, observing that, again, recombinant 2/6 VLP surrogates were less susceptible to UV inactivation than infectious viruses.

Cook et al. (1990) suggested that RV may be airborne. Aerosolized virus can be produced during manure storage, in pig units with forced air ventilation, or after pressure cleaning of pens or floors with regular or recycled water (Pillai 2007). Several authors studied the survival of RV in the air, and although differences in the results were reported, the general conclusion was that RV can survive in the air long enough to increase the risk of human infection (Ansari, Springthorpe, and Sattar 1991). When in the air, RV could be disseminated in the farm and into the nearby population directly or indirectly by air or water (Brooks et al. 2005). Low relative humidity and rainfall were associated with an increased number of outbreaks among pigs in Venezuela (Utrera et al. 1984). Gratacap-Cavallier and colleagues (2000) observed that RV isolated from drinking water in houses of children with recurrent diarrhea resulting from HRV were of human and animal origin. Although the RV strains isolated from children and from drinking water differed, the authors suggested that consumption of contaminated water could increase the risk of infection or coinfections. Nevertheless, RV detected in drinking water could originate from animal farms (Ferguson et al. 2003). It also is possible that, as proposed by Gouvea and Brantly (1995) and El-Attar and colleagues (2001), different G-types may coexist in the water, and a new human reassortant strain may emerge after coinfection of humans with multiple RV strains of animal or human origin.

Most studies have focused on the detection of RV in fresh fecal samples in swine barns. In attempts to evaluate other potential vehicles of transmission, samples of dust, dry feces, and effluent were collected from a pig farm and examined for RV-A by ELISA, EM, and infectivity of MA-104 cells. The authors found that samples from farrowing and weaning houses were positive by ELISA before and after cell culture, indicating that not only were viral particles present, but

they also were infectious. Moreover, this study showed that infectious virus also was also present in sewage from the farrowing house and in samples collected from a weaning house not used for 3 mo (Fu, Hampson, and Blackmore 1989). Their study showed that RV can survive for 4 mo with a decline rate of 0.5 $\log_{10}$ TCID$_{50}$ each month, similar to the data presented by Pesaro, Sorg, and Metzler (1995).

Overall, there are at least four major conclusions to emphasize from these studies. First, animal RVs present in the farm are shed in high concentration; second, they have high environmental stability in manure, air, soil, and water; third, they can be disseminated directly or indirectly to other geographical points inside or outside the farm; and, finally, evidence is accumulating in recent years using newer molecular diagnostic techniques to support the potential zoonotic transmission of RVs. Recently, as is the case for other enteric viruses, the effect of environmental technologies on the fate of these pathogens in animal manure under field conditions is under scrutiny. Previous studies focused on anaerobic inactivation of animal viruses in pits, one of the most commonly used systems at that time (Pesaro, Sorg, and Metzler 1995). As indicated by the authors, at least 4 mo were required to inactivate RV, with a potential risk of environmental contamination as a consequence of pit breaks, infiltration into soil, or dissemination from the surface.

Recently, superior environmental technologies were developed to decrease the impact of environmental contamination by different treatments, including high temperature anaerobic digester, biofiltration, solid separation, etc. (Humenik et al. 2004). In an attempt to study RV survival after application of these technologies, the presence of RV in fresh feces of swine and their survival after treatment was assessed by ELISA and RT-PCR (Costantini et al. 2007). The authors evaluated five different environmental technologies, including a conventional swine operation with an anaerobic lagoon system (Table 3.1). The RV-A were detected in pretreatment fresh feces from each farm, whereas RV-C were detected in pretreatment feces from four of five farms using this technologies. After treatment, only RV-A and RV-C RNAs were detected in four of five and three of four technologies, respectively. Differences between the detection level by

ELISA (10^5 particles/g) and RT-PCR (10^{1-3} particles/g) could result in positive samples in those systems that tested negative by ELISA. However, neither infectious particles were detected by CCIF, nor were clinical signs or seroconversion detected in inoculated Gn pigs. These results indicate that only RV-A/C RNA, but no viral infectivity, was detected after treatment, suggesting that all techonologies were effective reducing virus infectivity when evaluated by CCIF and Gn pig inoculation.

Inactivation

Rotaviruses are nonenveloped viruses, resistant to inactivation by ether, chloroform, detergents, many chemical disinfectants, and antiseptics (Abad, Pinto, and Bosch 1998). Phenols, formalin, chlorine, and ethanol (95%), however, have been shown to be effective (Sattar et al. 1994; Yuan, Stevenson, and Saif 2006).

Chlorine

Chlorine is considered among the most effective disinfectants against RV in drinking water and wastewater. Several studies have demonstrated that chlorine inactivation is dose-, pH-, exposure time-, and virus type-dependent (Abad et al. 1994; Ojeh, Cusack and Yolken 1995; Vaughn, Chen, and Thomas 1986). The inactivation of simian RV SA-11 and HRV Wa by chlorine was compared at 4ºC at different pHs and doses by Vaughn, Chen, and Thomas (1986). Viruses did not show significant differences in behavior. Both viruses usually were more readily inactivated at pH 6.0 than at pH 8.0 when low chlorine concentrations (0.05 to 0.2 mg/l) were used. A complete ($5 \log_{10}$) reduction of both was obtained within 20 seconds (sec) at all pH levels when chlorine concentrations were increased to 0.3 mg/l. Little inactivation was observed when copper and silver ions, in combination with low levels of free chlorine, were assayed in water (Abad et al. 1994). In their studies of RV inactivation, Ojeh, Cusack, and Yolken (1995) evaluated the efficiency of chlorine on infectivity and its correlation with the presence of viral RNA. The authors observed that 2,500 ppm chlorine completely destroyed the infectivity of RV as well as viral RNA amplifiable by PCR.

Ultraviolet and Gamma Radiation

Ultraviolet inactivation has been shown to be effective for inactivation of RV (Battigelli, Sobsey, and Lobe 1993; Ojeh, Cusack, and Yolken 1995; Smirnov et al. 1991). The irradiation completely destroyed the infectivity of RV as well as viral RNA amplifiable by PCR, as indicated by Ojeh, Cusack, and Yolken (1995). The effects of UV irradiation also were evaluated by Battigelli, Sobsey, and Lobe (1993) with RV in phosphate-buffered water. The 99.9% inactivation dose for SA-11 was 42 milliwatt (mW) sec/cm², almost three times higher than the dose required to achieve 99.9% inactivation for hepatitis A. But all these studies were done using virus in buffered solutions, and the influence that the presence of solid organic material may have had, such as in fecal suspensions or food, has not been evaluated.

Temperature

Survival and inactivation of RV have been studied at different temperatures and under different substrate compositions (buffered solutions, food, and feces) (Benkaddour et al. 1993; Ramos et al. 2000). SA-11 RV infectivity remained after incubation for 1 hr at 37°C, 24–8 hr at 25°C, or 5 min at 50°C. In addition, it also was stable after milk pasteurization (15 sec at 80°C) (Benkaddour et al. 1993; Kapikian, Hoshino, and Chanock 2001).

Rotavirus also maintained its infectivity for 7–9 mo at room temperature (18–20°C). Ramos and colleagues (2000) analyzed the stability of porcine rotavirus (PRV) in feces. Fecal samples were kept at 10°C without any preservative for 32 mo. After that, the integrity of viral RNA was demonstrated by PAGE and RT-PCR, and correlated with virus infectivity by inoculation of MA-104 cells. Of these samples, 31% still were positive by PAGE after 32 mo; 50% of these PAGE-positive samples retained infectivity in cell culture and 60% were positive by RT-PCR.

pH

Rotaviruses are stable at a pH range from 3 to 9, and infectivity is relatively stable within this pH range (Kapikian, Hoshino, and Chanock 2001; Yuan, Stevenson, and Saif 2006). In vitro, initial binding is sodium dependent, pH insensitive between 5.5 and 8.0, and dependent on salic acid residues on the membrane (Kapikian, Hoshino, and Chanock 2001); however, pH indirectly affects inactivation by other methods.

Other Methods

Several other methods have been tested for inactivation of RV (Chen and Vaughn 1990; Kawana et al. 1997; Pontes et al. 2001; Walker and Toth 2000). The nonionic disinfectants, sodium hypochlorite and formaldehyde, did not inactivate RV in feces, whereas 95% ethanol was effective. Infectivity of SA-11 RV for MA-104 cells was maintained after treatment of RV with organic solvents, repeated freezing and thawing cycles, and sonication (Kapikian, Hoshino, and Chanock 2001).

An interesting approach for inactivation was proposed by Pontes and colleagues (2001). The authors showed that inactivation of RV by high pressure was effective without loss of immunogenicity based on neutralization titer in plaque-reduction assays, antigen titer in ELISA, and direct interaction with the particle, as measured by gel-filtration chromatography. After pressure treatment, the particles were recovered with slight structural changes in VP4 compared with urea denaturation and controls.

Another alternative development was a pilot study conducted to determine if bacterial proteases could inactivate RV. Alcalase was the most effective among several proteases tested, but the results showed that this protease was able to inactivate RV to a certain degree depending on pH, temperature, and protease concentration. At pH 6.0 and 25°C (similar to field conditions), 1% alcalase reduced the SA-11 RV titer by 2.75 $\log_{10}$ in 24 hr, and by 3.25 $\log_{10}$ in 120 hr. But it is necessary to investigate inactivation under field conditions in which several substrates can compete for the same enzyme (Walker and Toth 2000).

The inactivation of HRV Wa and simian RV SA-11 by chlorine dioxide was investigated at 4°C in phosphate-carbonate buffer at pH 6.0 to 8.0. Both viruses were rapidly inactivated, within 20 sec under alkaline conditions (pH 8.0), with chlorine dioxide concentrations ranging from 0.05 to 0.2 mg/l. Similar reductions of infectivity required additional exposure time of 120 sec at pH 6.0, and inactivation was moderate at neutral pH (Chen and Vaughn 1990).

Kawana and colleagues (1997) studied the inactivation of a range of enveloped and nonenveloped viruses by povidone-iodine (PVP-I) and other commercially available antiseptics in Japan. Rotavirus was inactivated by PVP-I drug products benzalkonium chloride and benzethonium chloride within a short period of time, but it was not inactivated by chlorhexidine gluconate or alkyldiaminoethyl-glycine hydrochloride.

Inactivation of RV has been achieved by different methods with different efficiency. Although some of these methods such as chlorine, UV, and high pressure have been shown to be effective under controlled conditions, experiments are needed to assess their effectiveness under field conditions.

SWINE VESICULAR DISEASE VIRUS

General Virology

Swine vesicular disease virus (SVDV) is an enterovirus in the family Picornaviridae, antigenically related to Coxsackie virus type B5. The virus is nonenveloped, 20–30 nm in size, and contains ss RNA.

Epidemiology and Disease

Swine vesicular disease is a contagious disease of swine. Transmission is by direct and indirect contacts and by feeding uncooked garbage to pigs. The virus may also enter the host via minor abrasions on the feet. Although SVDV is an enterovirus, fecal transmission is uncommon. Clinical signs consist of lameness, fever, and vesicular lesions on snout, coronary bands, and interdigital space. Vesicles may also be found on teats and buccal cavity. These vesicular lesions are indistinguishable from those produced by foot and mouth disease virus, vesiculat stomatitis virus, and vesicular exanthema virus.

Detection

Differential diagnosis with other vesicular diseases is important and can be accomplished only in the laboratory. Virus isolation, antigen-capture ELISA, and EM have been used. Indirect evidence of virus infection can be obtained by detection of antibodies in serum using a virus-neutralization test.

Zoonotic Potential

Human Coxsackie virus type B5 is antigenically related to SVDV. A large outbreak of gastroenteritis in a Swiss village in 1998 was attributed to contamination of drinking water with noroviruses and enteroviruses. RT-PCR products obtained by the use of enterovirus primers revealed high-sequence similarity with SVDV (Hafliger, Hubner, and Luthy 2000). Persons working with infected pigs can harbor the virus in their nasal passages and human infection has been observed (House and House 1999). Clinical signs in humans are similar to those produced by coxsackie viruses minus the vesicular lesions. In fact, SVDV is very closely related to coxsackie

virus B5. In an outbreak of waterborne disease due to contaminated sewage in Switezerland, Norovirus and enteroviruses were detected by reverse transcription-polymerase chain reaction (RT-PCR) in one of two drinking water samples. The enteroviral amplicon showed high sequence similarity with SVDV, indicating the potential of SVDV to cause gastroenteritis (Hafliger, Hubner, and Luthy 2000).

Environmental Survival

Contact with contaminated environment has been shown to cause rapid spread of disease among a susceptible population of pigs (Dekker et al. 1995). Drying the virus at high relative humidity kills it faster than drying at low relative humidity (Batty et al. 1979). The presence of salts and organic material also may influence virus survival. For example, drying of virus suspended in distilled water resulted in a rapid kill as compared with virus suspended in tissue culture fluid. Because of the cytotoxic nature of slurry, it may not be possible to detect the presence of SVDV in pig slurry unless cytotoxic substances are removed by treatment with chemicals such as Freon (Turner, Williams, and Wilkinson 1999). Turner and colleagues (1999) studied the inactivation of SVDV in a pilot-scale treatment plant that treated pig slurry continually at a rate of up to 100 l/hr. The plant functioned by heating the slurry, maintaining at least 99.99% of the slurry at the required temperature for at least 5 min, and then recovering the heat to raise the temperature of the incoming slurry. The SVDV was inactivated in pig slurry (pH 7.5–8.0) to below detectable levels at 50–55°C. In acidified slurry (pH 6.4), on the other hand, heating to 55–60°C was needed for inactivation.

In another study, Turner and Williams (1999) compared chemical treatment and heat treatment for the inactivation of SVDV in pig slurry. The addition of NaOH or $Ca(OH)_2$ at different concentration/time combinations at 4 and 22°C was examined, as was virus stability at different temperature/time combinations. In slurry, SVDV inactivation required at least 2 min at 65°C. The addition of 1.0% (w/v) NaOH or $Ca(OH)_2$ was not effective against SVDV at 22°C after 30 min, but the addition of 1.5% (w/v) NaOH or $Ca(OH)_2$ caused inactivation of SVDV at both 4 and 22°C (Turner and Williams 1999).

Inactivation

The SVDV is resistant to physical and chemical agents and may survive for up to six months in the barn environment. It can survive at 3.9-9.1 pH for 38 days under refrigeration conditions. The virus can also survive processing of pork products.

4.
FATE AND TRANSPORT OF ZOONOTIC PARASITIC PATHOGENS

There are several helminth worm and protozoal parasites of swine that are infectious to humans, but human infection usually results from ingestion of raw or undercooked meat rather than from exposure to infected feces. Among the protozoal and helminth parasites known to infect both swine and humans, there is uncertainty regarding host specificity and parasite prevalence that complicates understanding of the human health risk associated with zoonotic parasites of swine in the United States. For example, molecular analysis of *Giardia* sp. suggests that this protozoal parasite may have a moderate level of host specificity (Caccio et al. 2005; Thompson 2004). Of the seven *Giardia* molecular assemblages, only two—A and B—are associated with human infection. Although *Cryptosporidium parvum* has been isolated from 155 host species and was once thought to have little host specificity, recent molecular analysis of isolates has revealed significant differences and the identification of new host-adapted species (Fayer 2004). Even host-adapted strains, however, exhibit significant zoonotic transmission in most instances.

A study of *Ascaris* infections in endemic areas found that zoonotic infection of humans from swine reservoirs comprised an insignificant proportion of human infections (Anderson and Jaenike 1997). But *Ascaris* cross-infection between humans and pigs is considered a major source of human infection in nonendemic areas such as North America (Anderson 1995; Anderson and Jaenike 1997). Differences in pathogen host specificity, host susceptibility, and endemic pathogen prevalence among study sites limit the ability to quantify the human health risk associated with parasites potentially found in swine manure in the United States (see Textbox 4.1). Because animals are housed mostly on concrete instead of soil, helminth parasites are considered well controlled. Consequently, only the nematode Ascaris and relevant protozoal parasites will be discussed here.

Textbox 4.1 The ability of nematode and protozoal parasites to form thick-walled eggs, cysts, or oocysts enhances their survival during storage, treatment, and disposal of animal wastes. Compared with bacteria and viruses, whose environmental survival is measured in days and weeks, parasites may survive for many months (Fayer 2004; Rogers and Haines 2005).

ASCARIS SUUM

Ascaris is one of the most common worms infecting humans worldwide. Approximately 25% of the global population is infected with this parasite (O'Lorcain and Holland 2000). Human *Ascaris* infection is associated with *A. lumbricoides,* and pig infection with *A. suum.* These two worms have identical life cycles and generally are very similar, so there is some controversy over whether they truly represent different species or are host-adapted subpopulations (Nejsum et al. 2005a; O'Lorcain and Holland 2000). Nonetheless, host specificity has been demonstrated, in that *A. suum* parasites will not reach maturity in the human intestine, and molecular studies have demonstrated that human infection with *Ascaris* worms molecularly identical to pig worms seems to represent a cross-infection (Anderson and Jaenike 1997). *Ascaris suum* remains one of the most common helminth parasites of pigs. Although intensive management and anthelmintic therapy have decreased the incidence of *A. suum* in swine significantly (Roepstorff 1997), its fecundity and environmental persistence prevent its complete eradication from modern swine herds.

There are few published studies of *A. suum* prevalence in North America. A 5-yr study (1977–1981) of a single total confinement herd in Georgia found that *Ascaris* prevalence was highest in gilts, ranging from 33.3 to 86.2% in gestation-age gilts compared with 6.4 to 29.2% in sows (Marti and Hale 1986). In 1988, a U.S. survey found that 70% of farms had evidence of

this parasite (Kennedy, Marchiondo, and Williams 1988). Significant progress in animal management and husbandry has been made since the time of these studies, so the current prevalence is likely much decreased. More recently, an abattoir study in Canada found evidence of current or past infection with *Ascaris* worms in 44 to 57% of carcasses examined (Wagner and Polley 1997). Although the prevalence of past or present infection seemed largely unchanged in 1997 from a previous study in 1980, the presence and intensity of infection with adult worms was significantly lower and likely reflected interrupted life cycles as a result of intensive management. No management system data was collected in the Canadian study, however. Consequently, it is difficult to estimate the potential impact this parasite may have in U.S. swine herds with current data. But European studies (Joachim et al. 2001; Roepstorff 1997; Roepstorff et al. 1999) describe intensive housing systems similar to those found in the United States, suggesting that this parasite is very likely to still be significant in U.S. swine herds.

Epidemiology

Because of the high global human worm burden resulting from *A. lumbricoides,* studies of the transmission of *A. suum* from pigs to humans are conflicting, depending on the country of origin of human samples. For example, a study in Denmark examined the burden of human ascariasis attributed to infection with *A. suum* from several countries, including Denmark, Bangladesh, and Guatemala, and found in the epidemiological analysis that Danish patients with ascariasis were nine times more likely to live in rural areas than in urban (Nejsum et al. 2005b). In addition, more than 80% of patients had contact with pigs or pig manure. Genetic analysis of the *A. suum* isolates from humans and pigs in Denmark revealed 97% homology between the worm populations, and both of these populations were distinct from worms isolated from patients in other countries. A U.S. report also found that human infection with ascarids molecularly related to those isolated from pig populations was related to contact with pigs—five of eight infected people in this study kept pigs (Anderson 1995). In contrast to the Denmark and U.S. findings, a study that

examined worms isolated from Guatemalan patients found that the majority of human *Ascaris* isolates were not molecularly similar to pig isolates (Anderson and Jaenike 1997). The conflicting results of these studies suggest that in areas of the world where the human worm burden attributable to *A. lumbricoides* is high, zoonotic transmission of *A. suum* does not represent a significant proportion of human infection with ascarid worms. In European and North American countries, however, *A. lumbricoides* prevalence is very low, and pig-to-human transmission of *A. suum* may play a more significant role in human ascariasis (Anderson 1995; Anderson and Jaenike 1997; Murrell et al. 1997).

Fate and Transport

The risk of human exposure to *A. suum* eggs depends on the parasite burden in the herd and the persistence of infective eggs during manure storage, treatment, and disposal. European studies indicate that parasite prevalence between swine herds is highly variable (De Bie 2003), and most positive farms do not have significant worm burdens in their herds (Roepstorff et al. 1999). Because comparable studies have not been done in the United States, it is unknown if this data reflects U.S. swine herds. It is likely, however, that there remain U.S. swine herds with parasite burdens and among these the environmental persistence of *A. suum* eggs may pose a hazard, both because female worms may shed up to 2 million eggs per day (Roepstorff 2003) and because eggs are very hearty in the environment.

According to the 2000 USDA NAHMS study of swine facilities, approximately 51% of operations store swine manure in underground pits (USDA 2002b). After 4 wk of storage in untreated slurry, 80% of *A. suum* eggs were still able to develop; egg viability decreased under these conditions to 40% at 8 wk and 0% at 16 wk (Gaasenbeek and Borgsteede 1998). Under laboratory conditions, eggs survived over 85 d in manure slurries at 8°C and over 65 d at 18°C (Bürger 1982). Storage of liquid manures in tanks in Germany found viable eggs after 365 d, however. Egg viability under anaerobic lagoon storage conditions (used by nearly 23% of U.S. swine operations) seems to be more favorable to egg survival (USDA 2002b). Gaasenbeek

and Borgsteede observed enhanced survival of *A. suum* eggs under anaerobic conditions compared with untreated slurry—reporting 80% viability after 12 wk (Gaasenbeek and Borgsteede 1998). Juris and colleagues had similar results, reporting more than 80% viability of eggs after 20 d of anaerobic stabilization (Juris et al. 1996) in tanks designed to simulate anaerobic lagoon stabilization. The effectiveness of ensiling the solid fraction of separated swine manure was examined by Caballero-Hernandez and colleagues as an alternative technology that might decrease the infectivity of *A. suum* eggs (Caballero-Hernández et al. 2004). Ensiling of manure had no effect on the observed egg count, and nearly 70% of recovered eggs remained viable after 56 d of treatment.

To examine the possibility of environmental transport after land application of swine manure, egg survival in swine slurries land-applied on outdoor plots under varying conditions of sun and simulated rainfall was assessed (Gaasenbeek and Borgsteede 1998). Eggs were collected from naturally infected pigs at a slaughterhouse in the Netherlands and inoculated into tubes containing pig slurry. Tubes were placed on 1m2 plots and treated to artificial rainfall that reflected the long-range average for the country. Survival of parasite eggs was highest on wet, shaded plots, with at least 90% egg viability at 8 wk. On sunny (temperature did not exceed 25°C), dry plots, egg survival was lowest, and a 90% loss of viability was observed between 2 and 8 wk. This study also found that increased relative humidity (77.5% and 100%) during the experiment favored egg survival. The study did not provide specific data on rainfall parameters or specific conditions of shade or sun, so it is unknown which part of the United States is most represented by the experimental conditions of the study. A study in Norway found that viable eggs could be recovered from soils amended with sewage sludge for more than 810 d (Bürger 1982). Application of untreated swine slurries to soil revealed that *A. suum* eggs remained in the most superficial layers of the soil column and were vulnerable to runoff (Papajova et al. 2002).

A. suum egg survival characteristics in untreated slurries and in anaerobic lagoons suggest that a significant proportion of excreted viable eggs may be land-applied on farms using this management practice to dispose of manure and utilize manure nutrients. Once in the field, eggs may remain on the superficial layers and on vegetation for several weeks. Furthermore, during periods of rainfall, and the greatest potential for runoff, egg survival is greatest. Most research on this subject has been done in Europe, however, so it is unknown how this compares to conditions in the United States.

CRYPTOSPORIDIUM

Cryptosporidium describes a genus of protozoan parasites that infect a wide range of vertebrates. There are several *Cryptosporidium* species, most of which are host-adapted, but there are zoonotic strains of *C. parvum* that are associated with outbreaks in several mammalian hosts. *Cryptosporidium* is an intracellular parasite that typically infects epithelial cells of the small intestine. But infection sites outside the intestinal tract can occur. The life cycle is direct, meaning a period of development outside the host is not required and oocysts are infective immediately when passed from infected hosts. Autoinfections also can occur (Fayer 1997). Fewer than ten oocysts may be sufficient to initiate infection in susceptible hosts (Caccio et al. 2005; Okhuysen et al. 2002).

Cryptosporidium sp. are transmitted via contaminated feed and water. Opportunities for human infection exist during exposure to infected livestock, their manure, or contaminated water. Oocysts are environmentally stable; consequently, fecal contamination of the environment can result in waterborne dissemination of oocysts and in human outbreaks associated with drinking and recreational waters. Cryptosporidiosis is a common cause of protozoal diarrhea in humans worldwide. In 1993, *Cryptosporidium* was responsible for the largest waterborne disease outbreak in the United States since monitoring began. Although a livestock source was suspected initially, molecular analysis of isolates revealed homology with a human strain (Caccio et al. 2005; Zhou et al. 2003).

Cryptosporidiosis is reported in swine. Although diarrhea has been the primary clinical sign, many infected pigs have concurrent infections with other

enteric pathogens; some pigs apparently do not seem to show any signs of infection. Typically, cryptosporidiosis is an infection of young animals. Published reports document that *Cryptosporidium* sp. commonly infect nursing and weaned pigs more than sows (Xiao, Herd, and Bowman 1994). In Ontario, Canada, 5.3 % of 3,491 pigs primarily 1–12 wk of age were infected (Sanford 1987). Based on these data it is possible that farrowing houses are more likely to be found as sources of *Cryptosporidia* oocysts compared with sites housing feeder pigs or mature breeding stock.

Epidemiology

The current state of knowledge of *Cryptosporidium* is incomplete. This is because of the large number of mammalian species that can become infected with *Cryptosporidium* sp. and the evolving understanding of host specificity. New molecular methods are elucidating relationships between animal hosts and species of *Cryptosporidium* (Caccio et al. 2005), but there is no question that some species of *Cryptosporidium* in domesticated animals are associated with human infection. For example, young dairy calves are well documented as a source of *C. parvum,* which can cause human sickness. The incidence of infection is high in young dairy calves, and the potential for human exposure is significant among dairy calf handlers and veterinary students working with dairy calves. But the zoonotic potential with most other species of *Cryptosporidium,* including those of swine origin, is much less clear.

Two species of *Cryptosporidium* are reported to occur in humans, *C. hominis* and *C. parvum*. Humans are the major reservoir for *C. hominis,* and this species tends to account for most human outbreaks worldwide. *C. parvum* is clearly a zoonotic species and usually is associated with cattle. Increasing reports of *C. parvum* in certain regions of the world may result, in part, from the intensive husbandry practiced for ruminants and the associated high concentrations of young animals at these feeding operations (Xiao et al. 2004).

In a molecular comparison of *Cryptosporidium* isolates from a variety of hosts, there was evidence of a geographically widespread but swine-specific strain

of *C. parvum* (Morgan et al. 1998). Nonetheless, two genotypes (Type 1 and 2) of *Cryptosporidium* have been reported in swine and also have been found in humans. But pig genotype 1 has a very low prevalence in humans and is unlikely to emerge as a major human pathogen (Xiao et al. 2004). More recently, Xiao and colleagues (2006) studied *Cryptosporidium* genotypes obtained from unseparated pig slurry in storage tanks on pig farms in Northern Ireland. Polymerase chain reaction (PCR) and sequencing of PCR products revealed 3 genotypes of *Cryptosporidium* in the slurries: *C. muris,* pig genotype II, and *C. suis.* Only *C. muris* and *C. suis* have been isolated from human cases. It is noteworthy that piglets have been infected with *C. hominis,* but caution should be used in interpreting the significance of finding parasites traditionally associated with animals in humans and vice versa. Although an animal source of *C. parvum* often is suspected to be the source of *C. parvum* or other zoonotic *Cryptosporidium* spp. found in humans or the environment, many human infections are traced back to human sources (Xiao et al. 2004).

Fate and Transport

In a Canadian study, *Cryptosporidium* was found on four of six swine farms, with an overall prevalence of 11% among the evaluated animals (Olson et al. 1997). No information about the management systems used by the studied farms was provided. Although no published studies have examined the concentration of *Cryptosporidum* oocysts in swine manure, Thurston-Enriquez, Gilley, and Eghball (2005) referenced unpublished data reporting 20 to 90 oocysts found per gram of swine lagoon wastewater. The *Cryptosporidium* oocyst is a resistant parasitic stage and can retain its viability in typical environmental conditions. Compared with *Giardia, Cryptosporidium* is much more resistant to decay over a wide range of temperatures. *Cryptosporidium* can remain viable for more than 6 mo at 20°C (Fayer 2000; Gajadhar and Allen 2004), and at 25–30°C, infectivity can be retained for up to 3 mo (Fayer 2000). Even at -20°C, *Cryptosporidium* oocysts can remain viable for up to 8 hr, and oocysts held at -5°C were infectious for up to 2 mo (Fayer 2000). When *Cryptosporidium* oocysts were inoculated into pig slurries and persistence measured in unstirred tanks,

the time required for a 1-log reduction in oocysts ranged from 133 to 345 d in summer and 217 to 270 d in winter (Hutchison 2005c). This is considerably longer than the previous persistence estimates of 28 d in manure at 20–37°C and more than 12 wk in frozen manure (Olson et al. 1999). *Cryptosporidium* oocysts have been shown to attach readily to solid particles, with 30% attaching instantaneously to particles in secondary human sewage effluent and 75% attaching after 24 hr (Ferguson et al. 2003). How this compares with swine lagoon effluent is unknown, however, because treatment processes of human sewage are different from swine manure management systems.

Rainfall has been shown to be a significant factor in the release and environmental transport of waste-associated oocysts. With the application of *Cryptosporidium* oocysts to soil blocks followed by intermittent irrigation under laboratory conditions, researchers have found that oocysts can move through some soils for more than 70 d, and most oocysts remained in the upper 2 cm of the soil block (Fayer 2000). In a field trial, land application of lagoon wastewaters to 1.5-m² plots followed by simulated rainfall and collection of runoff waters resulted in the recovery of up to 2.2 x 10^6 oocysts (Thurston-Enriquez, Gilley, and Eghball 2005). Subsurface flow wetland treatment of manure reportedly reduces *C. parvum* oocysts 1.0–3.1 $\log_{10}$ per meter (m) of vegetated buffer (Ferguson et al. 2003). If runoff of oocysts from land application fields results in water contamination, oocysts survive in water up to 10 wk at 20–30°C and more than 12 wk in frozen water at 4°C.

GIARDIA INTESTINALIS

Giardia describes a genus of flagellate protozoan parasites of the small intestine that infects a wide range of vertebrates. There are three main species described: *Giardia angilis, Giardia muris,* and *Giardia intestinalis* (Eligio-García and Cortes-Campos 2005). *Giardia intestinalis* also is known as *Giardia duodenalis* and *Giardia lamblia* and is the species known to infect humans (Ali and Hill 2003; Eligio-García and Cortes-Campos 2005). The life cycle of *Giardia* is direct, meaning cysts of *Giardia* are infective immediately when excreted by infected hosts. The life cycle is short; cysts

appear in the feces within a week or two after infection in dogs, where the life cycle is better understood than in swine (Bowman, Lynn, and Georgi 1999).

Giardia sp. are known to infect both young and adult swine. A Canadian survey reported that four of six swine confinement farms were positive for *Giardia,* with an overall prevalence rate of 3% in young animals and 18% in adult swine (Olson et al. 1997). This study used only one sample point; thus the prevalence reported likely is underestimated because excretion of parasite stages from infected hosts can be intermittent. In Ohio, *Giardia* infections were detected in both weaned and nursing pigs (Xiao, Herd, and Bowman 1994). *Giardia* prevalence on farms varies depending on the stage of production. For example, a Canadian study detected *Giardia* in 3.8 and 9.8% of piglets and weaners, respectively, in 10.8% of growers but 15% of finishers, and 5.7% and 4.1% of boars and sows, respectively (Olsen et al. 2000). Control and prevention of both *Giardia* and *Cryptosporidium* infections in pigs are complicated by the short life cycle, long survival time of the infective stage in the environment, and the potential for rapid reinfection in contaminated housing of confined livestock.

Transmission between animal and human hosts typically occurs through ingestion of fecally contaminated water, which can come from a variety of mammalian fecal sources. *Giardiais* is a common cause of protozoal diarrhea in humans worldwide, infecting approximately 2.8 million people annually (Ali and Hill 2003).

Epidemiology

Recent progress in the molecular biology of *Giardia intestinalis* has shed considerable light on the epidemiology of this protozoal pathogen. Although several classification schemes have been described, the most commonly used molecular classification of *G. intestinalis* is the designation of seven assemblages, A–G, that can be distinguished by the ribosomal ribonucleic acid and elongation factor 1 alpha genes (Ali and Hill 2003; Eligio-García and Cortes-Campos 2005). Only *G. intestinalis* assemblages A and B have been associated with human infections and are considered zoonotic (Ali and Hill 2003), but two distinct clusters have been characterized in assemblage A—A-I and

A-II—and A-II has been isolated only from humans, suggesting a human reservoir for this group (Eligio-García and Cortes-Campos 2005).

Because molecular characterization of *G. intestinalis* strains has identified some host specificity among genetically similar groups, the role of animals (including swine) in transmitting *G. intestinalis* to humans remains unclear. The infective dose is small, perhaps as few as 1 in 10 cysts (Caccio et al. 2005). No reports have confirmed transmission to humans from swine or their manure, but *G. intestinalis* assemblage A has been isolated from pigs (Ey et al. 1997; van Keulen et al. 2002), including at least one isolate that was molecularly indistinguishable from human isolates (Ey et al. 1997), suggesting a potential for human illness after exposure to swine manure or waste-contaminated media.

Fate and Transport

The concentration of *Giardia* cysts in swine lagoon wastewaters can be as high as 1,075 cysts/g (Thurston-Enriquez, Gilley, and Eghball 2005), but survival of *Giardia* cysts seems to be highly temperature dependent (Olson et al. 1999). For 90% degradation of cysts inoculated into mixed human and swine manure at 5°C, 129 d were required, but only 4 d were required at 25°C (Deng and Cliver 1992). The researchers also noted that swine manure seemed much more toxic to cysts than human sewage effluent—cysts exhibited 90% die-off in 8.5 d in swine manure compared with 28.6 d in human effluent—and speculated that this difference might be attributable to increased bacterial degradation in swine manure relative to human effluent. Research in cattle manure has shown *Giardia* cysts to be noninfective within 1 wk after freezing at -4°C and infective for only 1 wk at 4° and 25°C. *Giardia* cysts are sensitive to freezing of soil, becoming noninfective after only 7 d at -4°C, but *Giardia* cysts were recoverable from soils maintained at 4°C for up to 8 wk. Soils maintained at 25°C inactivated *Giardia* cysts within 1 wk, but *Giardia* seems to be effectively retained in soil columns: sandy soils reduced cysts over 7 logs, and gravel soil rarely resulted in breakthrough recovery of cysts (Hijnen et al. 2005).

In a study of transport of *Giardia* cysts during runoff events (Thurston-Enriquez, Gilley, and Eghball 2005), up to 3.58×10^6 cysts could be recovered from 0.75-m x 2-m field plots after simulated rainfall. In water, cysts survive less than 14 d at 25°C but up to 77 d at 4–8°C (Olson et al. 1999). Research suggests, however, that human sewage effluents may represent the most significant source of infectious *Giardia* cysts in water (Thompson 2004).

5.
CONCLUSIONS AND RECOMMENDATIONS

Several biological and physical variables drive the still poorly described ecology of microbes in swine management systems and the environment. An evidence-based, systematic evaluation of studies characterizing the presence and abundance of zoonotic pathogens in swine manure systems and their relative contributions to the environment must be qualitative rather than quantitative in nature. Against the backdrop of a rapidly evolving and diverse industry and the continuous development of new scientific methodologies is a complex ecology that cannot be adequately characterized under controlled, bench top conditions or studied adequately under field conditions. The wide variety of microbes, the animal and manure management practices, and the environmental factors that influence the presence, persistence, survival, and transport of pathogens result in an inestimable number of combinations of potential pathogen fates in the environment.

Field studies that have attempted to identify transport of microbes from swine manure management systems through the environment have largely failed to confirm that the source of microbes was indeed the swine manure. In addition, dilution of pathogens in environmental media under natural conditions results in concentrations that are likely to be too low to recover except by molecular methods, and these cannot distinguish between viable and nonviable organisms. Although high concentrations of pathogens may be added to environmental media in controlled field studies to study these under more natural conditions, the risks associated with intentional introduction of pathogens to the environment are prohibitive to the conduct of research. Consequently, bench top studies using artificial environments are designed to study the specific environmental effects on pathogens under very controlled conditions, but these fail to adequately capture the myriad processes that influence microbial fate and transport in the natural world. Nonetheless, research has characterized many of the drivers of microbial survival and transport and has provided much useful information on the ecology of microbial environments in swine manure treatment systems and the environment. In light of the scientific gaps in the estimation of the human health risks associated with swine-related zoonotic pathogens, the following recommendations for future research directions are offered.

1. Develop sensitive and quantitative methods of microbial recovery from manure management systems—with an emphasis on methods that recover multiple classes of pathogens at the same time.

The sensitivity of microbial recovery methods is highly variable among studies. For example, in the study of *Giardia* cyst degradation in mixed swine wastes by Deng and Cliver (1992), the limit of detection was 10^3 cysts/ml. In contrast, the limit of detection of rotavirus in water by reverse transcriptase-polymerase chain reaction has been shown to be as high as 10^{1-3} ribonucleic acid particles/ml or as low as 10^{0-1} infectious particles/ml by cell culture (Fout et al. 2003; van Zyl et al. 2004). In the case of *Giardia*, the limit of detection was well above a biologically significant level, but the rotavirus limit of detection by cell culture was possibly at or below an amount with biological significance. In addition, swine manure contains numerous zoonotic pathogens, and recovery methods that could simultaneously isolate multiple classes of pathogens would lead to a more complete understanding of both the microbial ecology and attendant risks of exposure to swine manure. Hollow fiber ultrafiltration has shown good recovery efficiencies for multiple classes of microbes from water (Hill et al. 2005), and microarray technologies have proved useful in the simultaneous identification of molecular fragments from a variety of microbes in a water sample (Maynard et al. 2005). Significant work needs to be done, however, to validate these methods on highly contaminated samples such as swine manure or contaminated water.

2. Continue molecular characterization of pathogens from both animal and human sources to identify important zooonotic pathogens in swine manure and in the environment.

The extensive body of literature cited in this report illustrates the dramatic progress in the identification and quantification of zoonotic pathogens in swine manure management systems achieved through the proliferation of new molecular methods of microbial characterization. Notably, studies of swine HEV (Clemente-Casares et al. 2003) and *Cryptosporidium* (Hunter et al. 2007) have shed considerable light on the significance of agents previously thought to be species specific that may be in swine manure management systems and transported in the environment. Likewise, molecular methods of microbial characterization contribute to the ability of field studies to better distinguish swine manure sources of environmental zoonotic pathogens from human sources. As molecular methods continue to be used to characterize specific pathogen strains, the significance of specific viruses, bacteria, and parasites in swine manure management systems and the environment may be elucidated.

3. Develop methods to source-track microbes in environmental soils, water, and irrigated produce.

Many studies rely on the recovery of microbial indicators of fecal pollution to characterize sources of environmental pollution. These indicators are used because isolation of the various individual pathogens, in the absence of source data, is not feasible. Authors of several papers, however, have demonstrated significant differences in the presence, transport characteristics, and environmental survival between fecal indicators and pathogens, and also between closely related bacteria (Guan and Holley 2003; Rogers and Haines 2005; Sobsey et al. 2005). These observed differences limit the ability of indicator microbes, and other closely related bacteria, to estimate the rate of off-farm transport of pathogenic microbes. Furthermore, without methods to accurately source-track pathogenic microbes in the environment, studies cannot

determine which of several potential rural sources (e.g., human sewage, livestock farms, or wildlife) is contributing to observed contamination.

4. Design studies examining the environmental and ecological conditions that contribute to off-farm transport of zoonotic pathogens.

Numerous factors influencing pathogen survival and transport have been identified. The differences in results between laboratory studies and those done under natural environmental conditions, however, underscore the relative inability to characterize the complex ecological interactions present in natural systems. Future studies might use methods of characterizing microbial community structures under varying biological, physical, and chemical conditions (Rogers and Haines 2005) to better characterize the conditions that lead to enhanced survival of pathogens in the environment. Likewise, the role of other environmental drivers could be characterized better by examining the relative roles of soil types, manure application intensity, and rainfall under varying intensities and soil saturation conditions.

5. Design and conduct quantitative risk assessments for common zoonotic pathogens found in swine manure.

As stated earlier, designing comprehensive scientific studies to address the complexities of pathogen survival and transport in environmental systems often is prohibitive. Consequently, methods of stochastic risk modeling are gaining wide acceptance in environmental risk analyses. The microbial quantitative risk assessment (QRA) framework provides a mechanism whereby the body of scientific data can be incorporated into a science-based, theoretical model to provide a quantitative estimate of microbial loads in vulnerable environmental systems. Once a zoonotic pathogen has been identified for study, an exposure assessment is done by building a compartmental model of the fate and transport of the microbe from the reservoir to the environmental media under study (e.g., surface or groundwater). Published

studies and other sources of scientific data are used to estimate microbial distributions in each compartment and to describe the mathematical relationships that characterize the fate of microbes between compartments. Table 5.1 outlines a risk assessment framework for estimating the environmental burden of zoonotic pathogens from swine facilities.

An advantage of stochastic modeling is the ability to quantitatively assess and analyze the roles of both data uncertainty and variability on risk estimates. During the hazard identification and exposure assessments, gaps in the scientific database can be identified, and the impact of these gaps on the estimate of the burden of zoonotic pathogens in the environment can be evaluated. The results of a QRA can be used to identify research needs that will contribute most effectively to understanding the environmental contamination risks associated with swine production facilities. As additional scientific data become available, they can be incorporated into the model easily.

A disadvantage of the QRA framework is that it reduces complex ecological systems to a relatively simple model of microbial fate and the resultant human exposure. Consequently, risk estimates may not prove to be robust as the understanding of microbial ecology and environmental systems increases. Nonetheless, the QRA can be used to estimate the environmental burden of zoonotic pathogens and the attendant risks that are too low to be measured by field studies and can help to identify specific research needs.

In spite of a technologically advanced industry that places emphasis on animal health and management, zoonotic pathogens are not likely to disappear from swine manure management systems. Although bench top and environmental field data suggest that there may be a biologically significant level of viable pathogens in the environment that may be associated with modern swine production, these levels often are too low to quantify easily. In this setting, quantitative risk assessment may serve to bridge the gap between bench top studies and environmental science to provide an estimate of the risk that is difficult to assess using traditional field science methods.

Table 5.1 Components of a Quantitative Risk Assessment Framework to estimate the human health risks associated with zoonotic pathogens in the swine production environment

Steps in QRA	Conceptual model	Variables
Hazard identification	Characterization of zoonotic pathogens in swine manure	• Stage of production • Disease status of animals
Exposure assessment	Swine waste as a source of zoonotic pathogens	• Microbe characteristics • Stage of production • Prevalence and density of pathogen in untreated wastes • Pathogen reductions achieved by waste storage and treatment technology
	Movement from waste-holding facilities to environmental media	• Storage and treatment methods • Ecologically-significant processes among environmental microbes and pathogens • Pathogen characteristics • Soil characteristics • Environmental conditions
Dose-response assessment	Biologically-relevant level of pathogen in environment	• Pathogen characteristics • Proximity of vulnerable resources
Risk characterization	Environmental risk resulting from swine-manure-associated contamination	• Frequency and duration of environmental contamination • Pathogen characteristics

APPENDIX A: ABBREVIATIONS AND ACRONYMS

AIV	avian influenza virus
AMU	animal manure units
APHIS	Animal and Plant Health Inspection Service
BoNoV	bovine norovirus
CaCV	canine calicivirus
CDC	Centers for Disease Control and Prevention
CFU	colony-forming units
cm	centimeter
d	day
ds	double-stranded
EHEC	enterohemmorhagic *E. coli*
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EPA	Environmental Protection Agency (see also USEPA)
EPEC	enteropathogenic *E. coli*
FC	flow cytometry
FeCV	feline enteric coronavirus
FMDV	foot and mouth disease virus
G	glycoprotein
g	gram
GAO	General Accounting Office
Gn	gnotobiotic
HA	hemagglutinin
HAV	hepatitis A virus
HEV	hepatitis E virus
hr	hour
HRV	human rotavirus
HuNoV	human norovirus
HuSaV	human sapovirus
IEM	immune electron microscopy
IF	immunofluorescence
J	joule
LA	latex agglutination
m	meter
mo	month
mg	milligram
min	minute
ml	milliliter
MPN	most probable number

mW sec/cm²	milliwatt per second
NA	neuraminidase
NAHMS	National Animal Health Monitoring System
NCR	noncoding regions
nm	nanometer
NoV	norovirus
NV	Norwalk virus
ORF	open reading frames
P	protease
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pI	isoelectric point
pi	postinfection
PoNoV	porcine norovirus
PoSaV	porcine sapovirus
ppm	parts per million
PRV	porcine rotavirus
PRV	pseudorabies virus
PVP-I	povidone-iodine
QRA	quantitative risk assessment
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RV	rotavirus
SaV	sapovirus
sec	second
SIV	swine influenza virus
SPF	specific-pathogen-free
ss	single-stranded
SVDV	swine vesicular disease virus
TCID	tissue cultures infections dose
UK	United Kingdom
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
UV	ultraviolet
VI	virus isolation
VLP	virus-like particle
VP	viral protein
wk	week
yr	year

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