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PATHOGENS IN ANIMAL WASTES AND THE IMPACTS OF WASTE MANAGEMENT PRACTICES ON THEIR SURVIVAL, TRANSPORT AND FATE

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INTRODUCTION AND BACKGROUND

Concerns about potential animal waste pollution of the environment have focused mainly on water, and the potential impacts of nitrogen, phosphorous, and turbidity (suspended solids). However, contemporary issues associated with potential pollution impacts of livestock operations now include microbial pathogens, gaseous emissions (such as ammonia), and odors (odorants). Increased awareness of zoonoses (pathogenic microbes of animal origin) in animal wastes is now recognized as a public health concern, especially because of the occurrence of waterborne disease outbreaks apparently caused by fecal contamination of manure origin (for example, in Walkerton, Ontario, in 2000). Identification and characterization of zoonotic animal pathogens is one of the key steps in reducing potential human exposures via water and other routes (foods, air and soil). Various bacteria, viruses, and protozoa exist in apparently healthy animals, but upon transmission to humans these pathogens can cause illness and even death. Exposure of humans to these disease-causing pathogens of animal origin can occur via occupational exposure, water, food, air or soil. Some of the important pathways for pathogen transmission to humans are shown in Figure 1.

The fecal wastes and other wastes (such as respiratory secretions, urine, and sloughed feathers, fur or skin) of various agricultural (livestock) and feral animals often contain high concentrations of human and animal pathogens (disease-causing microorganisms) (Strauch and Ballarini, 1994). Concentrations of some pathogens occur at levels of millions to billions per gram of wet weight feces or millions per ml of urine. Per capita fecal production by agricultural animals such as cattle and swine exceeds that of humans. Furthermore, the trend for production facilities to harbor thousands to tens of thousands of animals in relatively small spaces results in the generation of very large quantities of concentrated fecal and other wastes that must be effectively managed to minimize environmental and public health risks.

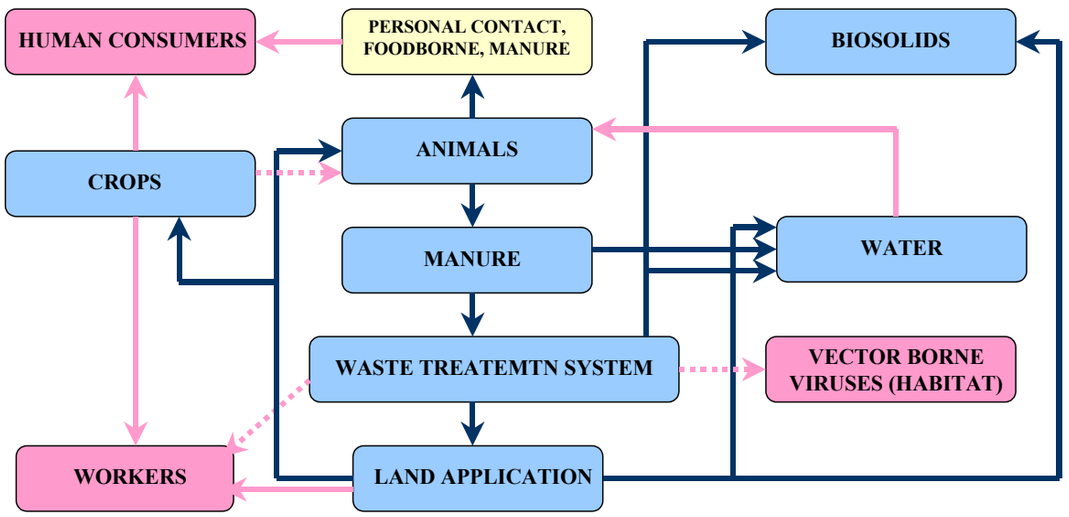

Figure 1. Sources and transmission pathways of pathogens to humans from animal agriculture.

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Until recently, little effort has been made to characterize the reductions and fate of actual pathogens in animal waste treatment processes and manure management systems. Most studies have relied on measuring the reductions of common fecal indicator bacteria, such as fecal coliforms, that are commonly present at high concentrations in the intestinal tracts and feces of animals, including humans. However, it is not clear that the reduction and fate of such fecal indicator bacteria adequately predict the responses of all types of pathogens, including viruses and parasites. Furthermore, not all fecal coliform bacteria arise from feces, and they can have non-fecal environmental sources. Such non-fecal sources of these indicator bacteria can make it difficult to follow the fate of pathogens in animal waste management systems both on and off of farms.

Relatively little has been done to determine the extent to which true human and animal pathogens, including viruses and parasites, persist in animal waste management systems and enter the non-farm environment to contaminate water, land or air. While some animal waste treatment processes and management systems have been designed to operate at conditions capable of inactivating or removing pathogens (e.g., composting and other thermophilic biological processes), the extent to which they actually reduce these pathogens has not been adequately studied and characterized. For other animal waste management systems based on treatment processes not specifically designed for extensive pathogen removal or inactivation, such as various mesophilic biological processes, the extent to which they remove or inactivate pathogens either has not been studied or has been studied only to a limited extent.

Many true pathogens of agricultural animals are difficult to detect and quantify, especially for their infectivity. Detecting pathogens by their infectivity or cultivability is important for decision-making about pathogen risks to human and animal health because only live or infectious pathogens pose health risks. Dead or inactivated pathogens can still be present in animal waste and their treated solid or liquid residuals, and they may be detectable by some analytical methods (e.g., microscopy, nucleic acid amplification by PCR, immunoassays, etc.). However, detection of these dead or inactivated pathogens is a "false-positive" result because they no longer pose human or animal health risks. Detection of infectious pathogens requires the use of recovery and isolation methods employing multiple steps of cultivation for bacteria, cell cultures or experimental animals for cultivating viruses, and microscopic methods or cell cultures coupled with microscopic or nucleic acid methods to detect and quantify parasites. These methods are technically demanding, relatively sophisticated, slow to produce results and costly. Furthermore, they are not practical for routine monitoring and surveillance. For this reason, there is an interest in knowing if certain indicator microorganisms in animal wastes are capable of providing a more practical, convenient, rapid and less expensive approach to monitor the efficacy of treatment and management systems for their ability to reduce and contain pathogens.

Pathogens in Animal Wastes

Animal pathogens posing potential risks to animal and possibly human health include a variety of viruses (Table 1), such as swine hepatitis E virus (Halber et al., 2001; Meng et al., 1997), bacteria (Table 2), *Salmonella* species (Davies et al., 1997), and parasites (Table 3) such as *Cryptosporidium parvum* (Pell, 1997; Sischo et al., 2000; Slifko et al., 2000). Some of these pathogens, such as the ones just mentioned, are endemic in commercial livestock and are difficult to eradicate from both the animals and their production facilities. Because these pathogens are so widely prevalent in animals, they are often present in fresh animal manure and other animal wastes. Therefore, the pathogens in animal manure and other wastes pose potential risks to human and animal health both on and off animal agriculture production facilities if the wastes are not adequately treated and contained (Crane et al., 1983; Graczyk et al., 2000). Manure and other animal waste management technologies must be capable of reducing and containing these pathogens in order to prevent or minimize human and animal exposures to them that would pose health risks (Cole et al., 1999, 2000; Darwin and Yukifumi, 1998). The purpose of this report is to review: (1) the types of pathogens potentially present in the manure of agricultural animals, (2) the levels of some important microbial pathogens and indicators for them that have been detected in animal wastes, (3) the potential for off-farm release or movement of pathogens present in manure and other wastes under current or proposed management practices, and (4) the extent to which these pathogens are reduced by currently used and candidate manure treatment and management technologies.

In addition to the ability of many animal pathogens to pose health risks to exposed humans and animals, there are also growing concerns about the presence of high concentrations of antibiotics and antibiotic-resistant bacteria in agricultural animal manures (Witte, 1998; Meyer et al., 2000). Antimicrobials are widely used therapeutically and subtherapeutically in animal production for disease prevention and growth promotion, respectively. Subtherapeutic antimicrobial use is associated with increased antibiotic resistance (AR) and multiple AR in enteric bacteria in swine and other animals (Langlois et al., 1978; Levy, 1978; Gellin et al., 1989; Mathew et al., 1998, 1999; Moore et al., 1996) and other livestock (Khachatourians 1998; Davies et al., 1999). Furthermore, *E. coli* has been implicated in AR gene transfer to other enteric bacteria (Abdul and Venable, 1986). Enteric bacteria with AR genes can spread from farm animals to other animals and to farm workers (Marshall et al., 1990; Nijsten et al., 1994; 1996; Ozanne et al., 1987; Saida et al., 1981). Research and outbreak data have shown that AR *Salmonella* have lower infective doses and cause increased incidence of human salmonellosis (Cohen and Tauxe, 1986). Therefore, the presence of antibiotic resistant bacteria in animal manures is another potential health risk of concern from both on-farm exposure and off-farm contamination.

Of the many potentially antimicrobially resistant bacteria, *Salmonella* spp. are a particular concern. This is because they are important human pathogens, they are widespread in agricultural animals and they have developed a wide range of antimicrobial resistance of considerable public health concern. The prevalence of resistance varies among the different *Salmonella* serotypes (MacDonald, 1987; Cohen and Tauxe, 1986; NARMS, 2000). For example, the 2000 report of the National Antimicrobial Resistance Monitoring System (NARMS) found that 96% of human Braenderup isolates were pansusceptible to tested antimicrobials but only 51% of Typhimurium isolates were pansusceptible (NARMS, 2000). In addition, host factors play a significant role in the probability of infection with a resistant strain. For example, prior or concurrent antimicrobial usage is one of the most consistent risk factors for human infection with resistant *Salmonella* (Cohen, 1986; Riley, 1984; Holmberg, 1984; MacDonald, 1986). The use of antimicrobials seems to convert asymptomatic colonization to symptomatic infection and lowers the infectious dose required for resistant *Salmonella* illness. Consequently, the etiologic fraction of resistant infections resulting from antimicrobial usage is estimated to range from 16 to 64%, depending upon the *Salmonella* serotype (Cohen, 1986). Other risk factors associated with human infection by a resistant strain include age of 60 years or older, regular antacid usage, and Hispanic origin (Riley, 1984). A recent Danish study also found that infection with MAR Typhimurium was associated with at least a fivefold increase in mortality compared to matched population-based controls (Helms, 2002). Although associated with increased virulence, resistant *Salmonella* appear to be less infectious than susceptible organisms and may require a selective advantage to cause disease (Riley, 1984).

Because non-Typhi salmonellosis is considered a zoonotic disease, livestock are believed to be the main reservoir of resistant organisms. Food-borne outbreaks of resistant *Salmonella* organisms have been documented (MacDonald, 1987; Cohen, 1986; Molback, 1999; Maguire, 1993), and two studies of retail meats found contamination with resistant *Salmonella* (White, 2001; Zhao, 2002). Consequently, the association between resistant isolates found in food animals and humans has been the focus of numerous studies. Food animal *Salmonella* isolates exhibit a high prevalence and similar spectrum of resistance as human clinical isolates (Cruchaga, 2001; Cohen, 1986; Helmuth, 2000; Angulo, 2000). For example, results from the 2000 NARMS demonstrate that the predominant resistance patterns found among both human and livestock *Salmonella* isolates include tetracycline, sulfamethoxazole, streptomycin, and ampicillin (NARMS 2000; USDA 2000). The prevalence of resistance to tetracycline among animal Typhimurium isolates is reportedly as high as 86%, and to sulfamethoxazole 88% (USDA 2000).

Perhaps the best example of a resistant *Salmonella* pathogen whose zoonotic transmission has been well-described is MAR phage type DT104. This organism is a pathogen of both animals and humans and is characterized by a pentaresistance pattern to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Other resistance patterns have also been found in association with DT104 (Glynn, 1998; Davis, 1999; Ribot, 2002; Ridley, 1998). DT104 emerged worldwide in the 1980s but did not become a significant cause of human disease until the 1990s. The increased incidence in human DT104 infection corresponded with an increased incidence in cattle in the U.S. and multiple livestock species in Great Britain (Davis, 1999; Glynn, 1998; Hogue, 1998).

Studies in Great Britain and the U.S. have found that contact with sick cattle or meat and dairy products are the main risk factors for human disease (Glynn, 1998; Hogue, 1997). A study of 46 outbreaks of DT104 in Great Britain found that 78% of the human outbreaks resulted from food-borne exposures and 15% resulted from direct contact with farm animals. Similarly, studies in the U.S. indicate 10% of human cases may result from direct contact with animals (Hogue, 1997).

Resistance genes coding for specific resistance patterns are molecularly indistinguishable between human and animal isolates, indicating circulation of resistance genes between animal and human populations (Davis, 1999; Angulo, 2000; Piddock, 2002). A study of MAR *Salmonella enterica* Typhimurium isolates from humans and a variety of farm animals found several phage types associated with similar MAR patterns, and the same gene cassettes accounted for the observed resistance patterns (Daly, 2000). Similarly, another study found that epidemiologically unrelated resistant *Salmonella* isolates from humans and animals all shared an identical plasmid-mediated CMY-2 gene (Winokur, 2000).

HUMAN PATHOGENS PRESENT IN ANIMAL AGRICULTURE PRODUCTION SYSTEMS

Viruses

A variety of different viruses can be present in animal fecal wastes and manures (Table 1). Especially important are a variety of enteric and respiratory viruses, including animal enteroviruses, rotaviruses, adenoviruses, hepatitis E viruses, caliciviruses, reoviruses, parvoviruses and other non-enveloped viruses. The animal viruses in Table 1 are primarily of concern to agricultural animal health and productivity because the animal diseases they caused are responsible for high morbidity and mortality and reduced food animal production. However, the impacts of some of these viruses, such a swine HEV and caliciviruses, on animal health and productivity are uncertain and perhaps have minor impacts. Because many of these viruses are non-enveloped, they are relatively persistent in the environment and resistant to treatment processes. However, some enveloped viruses also can be present in animal manures at high concentrations, and they may persist for considerable periods of time in the manure and in treatment and storage processes. Many of these enveloped viruses of concern are not endemic in the U.S. Vesicular Stomatitis Virus is an enveloped virus endemic in the U.S., but it is not present at high concentrations in manure. Instead, it is transmitted primarily by direct contact with infected animals, by aerosols and perhaps by the bites of flies.

Some of the viruses in Table 1, notably the caliciviruses, rotaviruses, myxoviruses and hepatitis E viruses, are or may be capable of infecting humans. On rare occasions some viruses, notably myxoviruses such as swine influenza virus, have caused human illness. The extent to which animal caliciviruses, rotaviruses and animal HEV strains pose risks to human health remains uncertain but appears to be low in terms of documented risks of severe illness. However, epidemiological investigations of the human health risks of these viruses are limited. Therefore, the extent of such risks remains uncertain and needs further study.

Infections and diseases caused by human enteric and respiratory viruses that are transmitted through fecally contaminated food and water have been well documented. However, the transmission of fecally associated viruses of animal origin to a human host is not common. This is may be the result of the relative specificity of the viruses to their host. Three virus groups associated with fecal-oral transmission, astroviruses, reoviruses and rotaviruses, infect a wide range of hosts. However, rotaviruses and astroviruses are thought to be relatively host specific (Matsui, 1996). Some reoviruses, notably type 3, have a wide host range including humans. Recently, hepatitis E viruses of swine and possibly other animal species have become of increasing concern with respect to human health because these viruses are prevalent in swine and present in swine wastes. Swine HEV has been shown to be experimentally transmissible to primates and human HEV is infectious for swine. Furthermore, swine and human HEV strains are genetically very similar on a country or regional basis. For example, in the U.S., human and swine HEV are very similar genetically and the same is true for the human and swine HEVs in Taiwan (Erker et al., 1999; Hsieh et al., 1999; Shlauder, 1998; Wu, 2000). To date, swine, sheep, and rats have been found to be carriers

Table 1. Some important animal viruses potentially present in animal manure.

Virus or Virus Group	Taxonomic Group	Animal Hosts	Disease in Animals	Human Infection/ Disease	Trans-mission Routes	Presence in USA	Presence in Manure
Enteroviruses	Picornaviridae	Bovine, porcine, avian	Yes in some	No, but needs study	Fecal-oral and respiratory	Yes for some, no for others (FMDV)	Yes
Caliciviruses	Caliciviridae	Bovine, porcine, avian	Yes in some	No, but needs study	Fecal oral and respiratory	Yes, some	Yes
Reoviruses	Reoviridae	Wide host range for some	Yes in some	Yes/No	Fecal-oral; respiratory?	Yes, some	Yes
Rotaviruses	Reoviridae	Found in many animals	Yes in some	No, but needs study	Fecal-oral; respiratory?	Yes, some	Yes
Adenoviruses	Adenoviridae	In many animals	Yes in some	No, but needs study	Fecal-oral and respiratory	Yes, some	Yes
Herpes viruses	Herpesviridae	In many animals	Yes, some	No, but needs study	Respiratory	Yes, some	Yes
Myxoviruses	Myxoviridae	In many animals	Yes in some	Yes, some; no, others	Respiratory	Yes, some	Yes
Pestiviruses	Pestiviridae	In many animals	Yes in some	No	Fecal-oral and respiratory	Yes, some	Yes, some
Corona-viruses	Coronaviridae	In many animals	Yes in some	No	Respiratory	Yes	Yes
Hepatitis E virus	Uncertain	Swine, rat, chicken, maybe others	Yes, but mild effects	Maybe	Respiratory and enteric?	Yes	Yes
Vesicular stomatitis virus	Rhabdovirus	Cattle, horses, swine; others	Yes	Yes, occu-pationally	Contact with infected animals	Yes	Maybe

of hepatitis E but only swine have been implicated in possible zoonoses (Harrison, 1999; Kabrane-Lazizi, 1999; Meng, 1998). In general, it is believed that animal-to-human transmission via animal wastes is not as high a risk as bacterial transmission. However, animal-to-animal transmission or herd-to-herd transmission of viruses is a concern.

Rodent urine and feces contain viruses that may be transmitted to humans via aerosols such as arenavirus (located in West Africa) and hantavirus. Aphthovirus (foot and mouth disease) and swine vesicular disease virus (SVDV), members of the *Picornaviridae* family, are transmitted to humans through aerosols containing the virus or ingestion of contaminated food products from infected cattle and pigs (Morgan-Capner, 1998). However, the disease incidence is low and these viruses are not considered important risks due to eradication and importation restriction measures. Vaccinations for aphthovirus and SVDV are an effective means of control for these diseases within the animal population. However, some of these viruses are of concern as bioterror agents, and greater vigilance is now being exercised to prevent their introduction and spread in the U.S.

Zoonotic transmission of animal viruses to humans via the fecal-oral route is believed to be limited in the U.S. However, some enteric viruses, such as swine HEV, are of increased concern in this respect because of serological evidence for higher levels of infection in swine workers than in the general population or in similar, but non-animal, agriculture occupations. Virus transmission between animals and humans also may occur through direct contact with the infected animal or by

Table 2. Factors influencing survival of viruses and other pathogens in animal wastes and other media.

Factor	Physical Effects
Heat or thermal effects	Increasing inactivation at higher temperature; pasteurize
Desiccation or drying	Increased inactivation at lower moisture content or relative humidity
Aggregation	Clumping protects viruses from inactivating agents
Encapsulation or embedding	Viruses within membranes or larger particles are protected from inactivation
	Chemical Effects
Hydrogen ions; pH	Viruses survive best near neutral pH and worst at pH extremes
Ammonia	NH_3 has virucidal activity; manifest at higher pH (>pH 8)
Enzymes	Proteases and nucleases contribute to virus inactivation
	Biological Effects
Microbial activity	Biological treatment and microbial activity/metabolism in soils, sediments, water; several contributing mechanisms
Proteolytic activity	Proteolytic enzymes inactivate/denature virion proteins
Microbial predation	Engulfment, ingestion, etc. by protozoa, helminths, etc.

a vector such as ticks or mosquitoes. Therefore, viral zoonoses also are a concern for occupational workers who come into direct contact with animals and their waste. For example, poxviruses associated with sheep are commonly transmitted to humans working with domestic sheep (Robinson, 1983). Prevention of zoonotic viruses via direct contact is accomplished by good hygienic and sanitation practices.

Persistence and Survival of Viruses and Other Pathogens

A variety of physical, chemical and biological factors can influence the persistence and stability of viruses in animal waste treatment and management systems. Some of the key factors are listed in Table 2. Many if not all of these same factors also influence the survival of bacteria and parasites in animal wastes and environmental media. Virus survival in animal manures is probably most directly influenced by temperature, pH (either very high or very low levels), microbial activity, ammonia and indirectly by solids-association and other physical conditions of viruses (aggregation, encapsulation or embedding, etc.). Differences in the values of or conditions for these variables have been shown to dramatically influence virus survival in manures, biosolids and other matrices. However, it is not possible to rank these factors for their effects on virus survival, because the nature and magnitude of their effect depends on the actual level or state of the factor and the levels or states of the other factors.

Overall, viruses survive longer than bacteria in the environment. For example, enteric viruses have been observed to survive for greater than 6 months in semiliquid cattle manure (Pesaro et al., 1995). Further details of virus persistence in manure and animal waste treatment systems are provided in later sections of this report.

Bacteria

Many of the important bacteria of animals that can potentially pose risks to human health are shown in Table 3. Many of these bacteria are known human pathogens. However, some are of low risk to human human health and others are not commonly found in agriculturally important animals. Details of some of the most impotant bacteria, their sources, human health risks and effects, their occurrence and persistence in animal wastes and the methods for preventing and controlling human exposure are briefly described below.

Aeromonas hydrophila

Aeromonas species are Gram-negative, facultatively anaerobic bacteria widely distributed in soil and fresh and brackish water worldwide. *Aeromonas hydrophila*, the most prevalent cause of human infection in the U.S., is primarily considered a free-living organism in fresh water. Reported human and animal infections occur, especially in immunocompromised and other more susceptible hosts. Gastrointestinal, skin and wound infections in humans have resulted from exposure to the organism in fresh and brackish water environments by ingestion, bathing and other contact with

Table 3. Some important bacteria potentially present in animals and their wastes.

Genus and Species	Animal Hosts	Disease in Animal Hosts	Human Infection and Disease	Transmission Routes	Presence in USA?	Presence in Manure?	Non-Fecal Sources?
Aeromonas hydrophila	Many	Usually no	Yes, but only virulent strains	Water, wounds, food	Yes	Yes	Yes
Arcobacter butzleria	Many	Yes, often	Yes	Direct contact, maybe food and water	Yes	Yes	No
Bacillus anthracis	Goats; others	Yes	Yes	Aerosols, skin (wounds), ingestion	Yes	Yes	Yes
Brucella abortus	Cattle	Yes	Yes	Direct contact, food, air, water	Yes, rare	Yes, rare	No
Campylobacter jejuni	Poultry, other fowl	No	Yes	Food and water	Yes	Yes	Maybe
Chlamydia psittaci	Parrots; other fowl		Yes	Direct contact; airborne	Yes	Unlikely	No
Clostridium perfringens	Many	Sometimes	Yes	Food, wounds	Yes	Yes	Yes, soil and sediments
Clostridium botulinum	Many	Sometimes	Yes	Food	Yes	Maybe	Yes, soil and sediments
Coxiella burneti							
Escherichia coli	All mammals	No	Yes, pathogenic strains	Food and water	Yes	Yes	No, but natural occurrence in tropics
Erysipelothrix rhusiopathiae	Swine, other animals, fish and shellfish	Yes, sometimes	Yes, rare	Direct contact, skin abrasions	Yes	Yes	Yes, infected animals
Francisella tularensis	Ovines, other animals, ticks, deerflies	No	Yes	Direct contact, fomites	Yes	Yes	Animal tissue
Leptospira interrogans and other species	Many animals	No	Yes	Direct contact	Yes	Yes	Urine
Listeria monocytogenes	Many animals	No	Yes	Food, water, fomites			Soil, vegetation
Mycobacterium tuberculosis	Rare; some animals	?	Yes	Respiratory exposure	Yes	Yes	No
Mycobacterium paratuberculosis	Some animals	?	Yes	Respiratory	Yes	Yes	No
Salmonella species	Many animals	No	Yes	Food, water, fomites	Yes	Yes	No
Yersinia pestis	Rats, squirrels, other animals	No	Yes	Flea bite, direct contact	Yes	Yes	Animal tissue
Yersinia enterocolitica	Swine, other animals	No	Yes	Direct contact, food, water	Yes	Yes	Possibly environmental sources
Yersinia pseudotuberculosis	Swine, rodents, birds						

contaminated waters. *Aeromonas* spp. also have been isolated from red meat, poultry, fish, vegetables, and unpasteurized milk. Therefore, the potential for outbreaks due to contamination of food also is a risk. *A. hydrophila* has been isolated from the feces of several agricultural animals and is assumed to be present in the manures and other fecal wastes of these animals. *Aeromonas* is also commonly present in natural aquatic habitats and aquaculture systems for finfish and shellfish. This potentially constitutes other possible routes of transmission.

Aeromonas hydrophila occurs as a minor part of the total bacterial flora in the intestine in cows and pigs. However, of the different Aeromonads isolated in cattle feces, *A. hydrophila* is the major species. *A. hydrophila* was reported to be the second most common Aeromonad in pig fecal samples (Gray, 1989), and it also has been isolated from sheep, poultry products, hens, turkeys, and ducks (Cenci, 1991; Cwikova, 1993; Khurana, 1997). Domestic dogs and cats were recently shown to be carriers of *A. hydrophila* (Ghenhesh, 1999). It is currently unknown how these animals acquired *A. hydrophila;* however, drinking water sources for the animals have been implicated. Even though a variety of animals carry this organism, their occurrence in feces, manures and other animal wastes in uncertain, but may be very low. The risks to human and animal health from various *Aeromonas hydrophila* strains may differ depending on their carriage of virulence factors, including exotoxins (haemolysins, cytotoxins, enterotoxins), haemagglutinins, and invasions. Many environmental strains may lack these virulence factors and therefore be less likely to cause human infection and illness. The extent to which virulent *A. hydrophila* are associated with the wastes of agricultural animals is poorly understood and requires further study.

Arcobacter

Arcobacter species previously known as *Campylobacter cryaerophila* are now classified as a distinct genus due to their ability to tolerate aerobic conditions and grow at 15°C, which is lower than the growth conditions of *Campylobacter* species. In humans, *A. butzleria* causes enteritis and at times may cause the infected host to become septic. *A. butzleria* has been isolated from healthy and symptomatic cattle, pigs, poultry, and sheep. *Arcobacter* infection impacts farms by causing abortions and enteritis in the animals. One study showed 43% of aborted pigs contained *Arcobacter* (Wesley, 1993). The primary carrier of *Arcobacter* is poultry, and in one study *A. butzleria* isolated from 97% of the carcasses (Wesley, 1997). In healthy swine, 40% (n = 1102) of fecal samples taken contained *Arcobacter* (Wesley, 1997); however, only 5% of the carcasses at four different pork-processing plants contained *A. butzleria* (Collins, 1996), thus suggesting consumption of undercooked poultry poses the greatest risk.

The possible transmission routes are through food and water; however, transmission has yet to be completely elucidated. *A. butzleria* has been isolated from well water in Idaho indicating a route of exposure to the human population (Rice, 1999). However proper chlorination of drinking water kills this pathogen. In the same study by Rice, 0.45 mg/L free chlorine was added to well water and an approximately 5-log reduction was obtained after 60 seconds (Rice, 1999). Proper treatment of water sources is necessary since a survival time of 16 days in groundwater was observed (Rice, 1999). Irradiation was shown to effectively reduce *Arcobacter* levels, however, higher doses are required as compared to *Campylobacter* (Wesley, 1997). These studies indicate that food processing plants and unchlorinated waters appear to be significant routes of exposure for *Arcobacter* to humans. Although studies have yet to be conducted, abattoir workers may be at risk. Thus properly cooked foods, good hygiene, and treated water supplies may serve as effective controls for this pathogen.

Bacillus anthracis (Anthrax)

Bacillus anthracis is a Gram-positive, spore-forming, rod-shaped bacterium that tends to grow in long chains. The bacterium commonly occurs in wild, domestic and agricultural animals, including cattle, sheep, goats, buffaloes, pigs, antelopes, and other herbivores. It is the cause of anthrax, a disease that can take several different forms, depending on the route of exposure leading to infection. Pulmonary anthrax, also known as wool sorter's disease, is a respiratory infection caused by inhaling anthrax spores. Historically, exposure has come from handing contaminated animals hides, hair, wool and other contaminated animal materials. This disease starts as a mild respiratory illness and can progress rapidly (within a few days) to severe respiratory disease with edema of the chest and neck and ultimately to death. Cutaneous anthrax occurs when the spores enter a break in

the skin and produce a local lesion that eventually forms a black scab in a week to 10 days. In a small proportion of cases the disease progresses to a systemic illness than can result in death if untreated. A third type of anthrax, the gastrointestional form, can occur from the ingestion of the spores, usually in raw or undercooked meat. *Bacillus anthracis* is also of concern as a weapon of bioterrorism, as the spores can be cultured, dried and dispersed by the air to cause human exposures resulting in disease and death.

Spores of *Bacillus anthracis* are very hardy, relatively resistant to physical, chemical and biological treatments and capable of persisting for long periods of time (decades) in the environment. *Bacillus anthracis* spores typically are not present in the intestinal tracts of human and animals or fecally shed in large quantities unless the animals have a developed a systemic infection. Animal infection usually results from grazing on contaminated land or less commonly by inhalation or through wounds. Uninfected animals do not harbor anthrax and therefore do not shed the bacteria in their wastes or manures. However, infected and diseased bacteria can have relatively high levels of anthrax in their blood (1 billion cells per ml), which can result in high concentrations of vegetative cells and spores on fur and skin, in secretions and in carcasses. Therefore, infected carcasses must be disposed of properly to prevent further environmental contamination and spread to other animals or humans. Anthrax spores can be spread by flooding pastures with contaminated water or disposing of infected carcasses in streams or ponds. Marshes and wetlands and flooded areas can become contaminated with anthrax form infected animals and can become sources of exposure leadng to infection. Contaminated hay has been responsible for outbreaks of anthrax in animals. To prevent the spread of anthrax it is recommended that infected carcasses be cremated (burned to ashes) or buried several feet deep and covered with lime or quicklime before covering with soil.

Brucella (Brucellosis)

Brucella species are found worldwide and are a particular concern to agriculture due to the negative economic impacts in the industry from loss of milk and meat as a result of abortions (Roux, 1979). Brucellosis is no longer very common in the U.S., with about 100 to 200 cases annually. Not all *Brucella* species cause infection in humans. However, *B. melitensis*, *B. abortus*, and *B. suis* infections in humans can cause severe flu-like symptoms that can last for months or years. Symptoms include headache, fever, profuse sweating, constipation, cough, asthenia, and arthromyalgia. Without treatment, the infection progresses and becomes localized to one area such as osteo-articular, neurological, pulmonary, ocular, or digestive regions of the body.

In cattle, *Brucella* concentrates in the placenta causing in abortions and in some cases sterility. Pigs, dogs, goats, poultry, and sheep also can serve as symptomatic or asymptomatic reservoirs (Roux, 1989; Forbes, 1990; Plommet, 1998). Up to 10^{12} organisms/g have been associated with placenta and fluids (Alexander, 1981). *Brucella* is excreted through the urine, milk, and products used for conception such as semen. Therefore, *Brucella* can be present in the manure of infected animals. However, *Brucella* transmission to humans from infected animals occurs through contaminated milk, direct contact, aerosols, food, or ingestion of contaminated drinking water. (Cole et al., 1999; Chomel, 1994). *Brucella* growth is affected primarily by low temperatures and acidic conditions. The organism does survive at high temperatures in various media including feces and soil. Survival in cow feces at room temperature was documented for 122 days but in environmental waters survival did not exceed 10 days at 25°C (Mitscherlich, 1984).

Control essentially lies in eliminating animal reservoirs, vaccination, and pasteurization of milk because *Brucella* survives in milk, urine, feces, water and soil for months (Roux, 1989). Hygiene and sanitation practices such as washing hands will limit infections via occupational exposure. Vaccination of herds has been shown to be a significant control measure when accompanied by elimination of infected animals in the herd (Carpenter, 1987; Roux, 1979). Veterinary medical intervention has essentially eliminated *Brucella* in agricultural animals in the U.S. Eradication of the disease in the U.S. is being undertaken through the Brucellosis Eradication Program. From 1956 to 1998, the number of known brucellosis-affected herds decreased from 124,000 to 15. However, bison and elk in the northern Rocky Mountain states are reservoirs for the diseases and pose a threat for reintroduction of brucellosis into domestic livestock. Efforts are under way to develop *Brucella* vaccines for bison and elk.

Campylobacter

Of the different species of *Campylobacter*, *C. jejuni* and *C. coli* are considered of major public health importance. *C. jejuni* is among the most common causes of diarrheal disease in the U.S., and this high risk has been attributed to the relatively low human infectious dose of this organism. It is estimated that as few as 100 to 500 organisms have a high probability of infecting a susceptible human host (Robinson, 1981). Symptoms of *Campylobacter* intestinal illness or "campylobacteriosis" in humans include abdominal pain, diarrhea, flu-like symptoms, headache, and fever. The disease is self-limiting in immunocompetent individuals. Treatment of *Campylobacter* infections usually consists of rehydration therapy. However, Guillain-Barre Syndrome (GBS) and Miller Fisher syndrome (MFS) are neurological complications that may on rare occasions occur in some individuals (Mishu, 1993; Nachamkin, 1998). When nucleic acid "fingerprinting" methods have been used to analyze *Campylobacter jejuni* isolates from chickens and humans, similar banding patterns have been found. This indicates that *Campylobacter* can cause zoonotic infections. Additionally, *Campylobacter* strains associated with GBS and MFS in humans have also been found associated with chickens (Duim, 2000). *Campylobacter* inhabits the intestinal tracts of cattle, chickens, sheep, cats, dogs, and pigs, but does not usually cause disease in these animals. High concentrations of *Campylobacter* are present in the feces and manures of infected animals. Poultry have the highest carriage rates while carriage in horses is almost non-existent and in dogs and cats it is low. The major route of transmission is through under-cooked meat that has become fecally contaminated during slaughter and processing. Fewer outbreaks occur from red meat than from poultry, which has been attributed to lower percentages of red meats being contaminated compared to poultry. A study found 3.5% of beef samples tested positive for *Campylobacter* while 59% of the poultry samples were positive (Skirro, 1998). Also, 80% of poultry purchased by consumers was found to be contaminated with *Campylobacter*. Even though only low percentages of beef samples have been reported to be contaminated with *Campylobacter*, a research study surveying 31 farms found 80.6% of the farms were positive for *Campylobacter jejuni*. Therefore, dairy cattle farms have been considered a potential source for waterborne transmission (Wesley, 2000). Indeed, dairy cattle manure has been implicated in groundwater contamination leading to waterborne outbreaks (CDC, 1999; Stanley, 1998). Direct transmission of C*ampylobacter* has been reported between humans and pets, in occupational situations such as poultry handling, and between family members, but is not the predominant mode of transmission (Saeed, 1993). Abattoir workers who shed *Campylobacter* in their feces usually will not pass the disease onto their family (Skirro, 1998). Humans are not the typical host for these bacteria but they may be asymptomatic carriers and may excrete these bacteria for up to 69 days (Kapperud, 1992). Reducing colonization of *Campylobacter* within agricultural animals focuses on reducing exposure of the animals to *Campylobacter* from infected or contaminated drinking water sources. Reducing human exposure has focused on improved food hygiene and sanitation, including terminal irradiation during processing and public education regarding the proper cooking of foods.

Because antibiotics are used therapeutically and subtherapeutically in animals, such use has led to resistance in *Campylobacter*. In 1999, antibiotic resistance to fluoroquinolone resulted in 18% of human *Campylobacter* infections (http://www.cdc.gov.ncidod/dbmd/diseaseinfo/campylobacter_t .htm). In Spain and the Netherlands, antibiotic resistance rates of 50% have been found (Skirrow, 1998).

Recent research suggests that *Campylobacter jejuni* enters a viable but non-culturable state. One study showed that culturability was lost at approximately 120 days in PBS incubated at 4°C and between 6 and 8 days at 20°C. However, when respiration and cell integrity were used as measures of viability, survival continued for an additional 7 months at 4°C but only 15 days at 20°C (Lazaro, 1999). Generally, survival is greatest at lower temperatures, and therefore the greatest risk of infection may be from refrigerated meats and poultry. At 4°C, *Campylobacter* could be cultured from feces for 3 weeks, in water for 4 weeks and in urine for 5 weeks (Blaser, 1980). *Campylobacter* does not survive within the pH range of 1-4 or at temperatures greater than 47°C (Ebigwei, 1993).

In the feces and liquid manure of broiler chickens, cattle, and swine, *Campylobacter* survival in moist conditions was approximately 3-4 days. Under dry conditions, survival ranged from a few minutes to a few hours (Mitscherlich, 1984). In moist heat at 55°C, *Campylobacter* survives for

less than 1 minute. Meat contaminated with *Campylobacter* saw a 7-log reduction post irradiation, thus showing irradiation to be an effective means to control *Campylobacter* (Collins, 1996).

Chlamydia

Of the three species of *Chlamydia*, only *Chlamydia psittaci* causes zoonotic infections in humans. Birds and humans display clinical symptoms. Infection occurs through the inhalation of the bacteria from infected birds or their droppings. Asymptomatic infection in humans occurs but human-to-human transmission is not common. Symptoms in humans include flu-like symptoms or pneumonia. Turkeys, chickens, pigeons, and wild birds are the primary reservoirs, but cats carry two strains: *Chlamydia felis* and *Chlamydia psittaci*. Both may pose a threat to human health due to their endemicity within the cat population throughout the world (Everett, 2000; Yan, 2000). Other animals affected by exposure to *Chlamydia psittaci* are cattle, sheep, and pigs, resulting in late-term abortions (Potter, 1979). Occupational exposure poses the greatest risk in the human population. Control is through reducing exposure to birds and their droppings or reducing *Chlamydia* in the host by antibiotics (Schachter, 1989).

In urine and feces, *Chlamydia psittaci* only survives a day or two up to 11 days, respectively. However, survival is longer in media of non-fecal origin. In bird feed, a survival time at room temperature of up to 2 months was reported and in frozen meat up to 50 days. The limiting factor for growth of this organism is pH where growth occurs between 6.5 and 7.5 (Mitscherlich, 1984).

Clostridium perfringens

Like other species of Clostridia, such as *C. botulinum* (a cause of food poisoning) and *C. tetani* (the cause of tetanus), *C. perfringens* is a Gram-positive, spore-forming, anaerobic bacterium. *C. botulinum* is an important cause of foodborne disease from improper preparation, handling and storage of foods, and *C. tetani* is the cause of deep wound infections resulting in the systemic disease tetanus. The spores of Clostridia are extremely hardy, resistant to various physical, chemical and biological treatment processes and can persist in various environments and media for long periods of time, as long as decades. *Clostridium perfringens* is present in the human and animal enteric tract, is fecally excreted by humans and a wide range of animals, and can be found in soil, animal manure, and sewage, and fecally contaminated raw meat and poultry. Concentrations of *C. perfringens* in animal feces and manures are relatively high (more than 10,000 organisms per gram) and more persistent than other enteric bacteria. *C. perfringens* can cause enteric illness in agricultural animals, including overeating disease in goats and calfhood scours.

C. perfringens are genetically diverse. They can produce a number of different toxins that occur with different and characteristic frequencies in various host animals and are responsible for the disease symptoms occurring in human and animal hosts. *C. perfringens* endotoxin type A is the most important human pathogenic strain. Illnesses caused by *Clostridium perfringens* also depend on the type of exposure. Foodborne *C. perfringens* food poisoning is caused by ingestion of endotoxin-producing vegetative cells in improperly stored foods such as stews, soups ands gravies. Symptoms are mainly abdominal pain, diarrhea and sometimes nausea starting 8-24 hours after eating the food. *C. perfringens* also causes a deep wound infection known as gas gangrene. The bacteria grow rapidly and produce toxins, primarily in muscle tissues, which can lead to bacteremia, shock and death, if untreated.

Because *C. perfringens* spores are very hardy and resistant to treatment, they are reduced less than other enteric bacteria in animal waste treatment processes and manure management systems. If the spores are land-applied, they can persist in soils and on vegetation for long periods of time. However, appreciable (>99.9%) reductions of *C. perfringens* can be achieved in various animal waste treatment processes and systems, especially if the systems employ multiple steps of biological treatment, high pH (lime or alkaline stabilization) or high temperatures (composting or thermophilic digestion).

Coxiella burnetii

Coxiella burnetii is the bacterium (actually a rickettsial agent) responsible for Q fever. Q fever typically is a zoonotic disease transmitted primarily by inhalation exposure. Abattoir workers, veterinarians, farm workers and others who come in contact with infected animals, primarily cattle, sheep and goats, are at the highest risk of exposure and infection. Outbreaks in the U.S. and elsewhere occur in populations associated with livestock. Infections may cause influenza-like symp-

toms, pneumonia, fever, or may progress to endocarditis or hepatitis. In addition to cattle, sheep and goats, other reservoirs for *Coxiella* include pigs, horses, dogs, cats, buffaloes, pigeons, geese, and wild birds (Marrie, 1998). Animal carriers are generally asymptomatic. *Coxiella* is transmitted to humans via aerosols or ingestion when products or other vehicles (air, fomites, foods) are contaminated with placental or birth fluids, colostrums, or milk (Norlander, 2000). The organism is excreted into the milk, urine and feces of infected animals. Therefore, the fecal-oral route may also be implicated in infection because fecal samples from sheep have been positive for *Coxiella* by PCR (Berri, 2000). *Coxiella* survives on surfaces at 15-20°C for 7-10 months, fresh meat for 1 month and 40 months in skimmed milk at room temperature (Christie, 1974). Long survival times are attributed the bacteria's ability to form spores and persist in a dry state. In one study, *Coxiella* was shown to survive at room temperature in dried feces for 586 days; however, shorter survival times at higher temperatures have also been reported (Mitscherlich, 1991).

Organisms are excreted in milk, urine, and feces of infected animals and a number of prevention and control measures are recommended for *Coxiella burnetii*. These include: public (and especially worker) education on sources of infection, proper handling and disposal of placenta, birth products, fetal membranes, and aborted fetuses at facilities housing sheep and goats, restricting access to barns and laboratories used in housing potentially infected animals, use of only pasteurized milk and milk products, use of appropriate procedures for bagging, autoclaving, and washing of laboratory clothing, locating facilities housing high risk animals away from populated areas, routinely testing animals for antibodies against the agent, and quarantining imported animals. A vaccine is available but is not used in the U.S.

Escherichia coli

Escherichia coli (*E. coli*) are native inhabitants of the gastrointestinal tract of all warm-blooded mammals. A subset of this group, diarrhetic *E. coli,* consists of Enteropathogenic (EPEC), Verotoxigenic (VTEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), and Enteroaggregative (EaggEC) *E. coli*. Only ETEC and VTEC are associated with animals and humans. ETEC are known to be species-specific because specific receptors in the host organism are required for bacterial attachment (Nagy and Fekete, 1999). However, specific serotypes of VTEC organisms are associated with zoonoses in humans. Verotoxigenic *E. coli* (VTEC) organisms represent over 100 serotypes, which serotype O157:H7 is the most recognized. This organism has received the most attention due to the severity and frequency of the documented outbreaks caused by cattle fecal contamination in water and food products (CDC, 1999). However, other *E. coli* serotypes such as O26 and O111 also cause disease in humans (Jackson, 1998; Blanco et al., 1995). Cattle serve as the asymptomatic carrier of the O157:H7 serotype. Other animals including pigs, dogs, cats, rabbits, goats, and sheep also carry various VTEC serotypes (Beutin, 1993; Gannon, 1988; Hammermueller, 1995; Kudva, 1997). Disease manifestations in humans include bloody diarrhea, severe abdominal cramps, hemolytic uremic syndrome (HUS), or death. Control measures as with all bacteria of fecal origin include good hygiene and adequate water and food quality to prevent the transmission of the disease.

Extensive work regarding the survival and quantities of *E. coli* in the environment has been performed because these bacteria are the current preferred indicator of fecal pollution in drinking water and fresh recreational bathing water. *E. coli* are present in human and animal feces at concentration of about 1 billion organisms per gram. They are also present at high concentrations (up to 1 billion per 100 ml) in manure and other animal fecal wastes. The majority of *E. coli* strains in the feces and fecal wastes of humans and animals are non-pathogenic inhabitants of the human and animal intestinal tract. However, pathogenic strains of *E. coli* can infect the intestinal tracts of humans and animals and be present in high concentration in animal feces. In particular, some pathogenic strains of *E. coli*, such as O157:H7, have become common and predominant residents of the intestinal tracts of cattle, are fecally shed at high concentrations and therefore abundant in the manure from these animals. This has become a cause of public health concern because of the potential for human infection and illness from fecally contaminated food and water and the documented water- and food-borne outbreaks that have occurred from animal fecal sources of contamination.

The survival of *E. coli* in feces, wastes, water and food has been extensively studied. Concern over verotoxigenic *E. coli* lead researchers to study its survival in bovine feces. *E. coli* seeded into feces at 10^5 *E. coli*/g survived up to 12 weeks at 25°C, 18 weeks at 15°C and 14 weeks at 4°C.

Similarly *E. coli* seeded at 10^1 *E. coli*/g survived up to 4 weeks at 5°C and 25°C and up to 5 weeks at 15°C (Fukushima, 1999). In seawater, a study using *E. coli* K12 showed that *E. coli* decreased to non-detectable levels within 6 days at 15 and 20°C but at lower temperatures survived longer (Sorensen, 1991).

E. coli survives in feces at room temperature for 42-84 days but at lower temperature can survive up to 160 days. However. in manure liquid *E. coli* has been reported to survive as little as only 2 days at 20°C (Mitscherlich, 1984). *E. coli* growth does not occur at pH levels less than 3.6 or under high saline conditions. Factors that significantly limit growth of *E. coli* are water activity, temperature and acidity. However, acid tolerant strains of O157:H7 have been identified that survive at pH 2.5 for 5 hours (Benjamin, 1995). These findings have lead to the reevaluation of treating meat with lactic acid for destruction of this organism.

Erysipelothrix rhusiopathiae (Erysipeloid)

Erysipelothrix rhusiopathiae is a facultatively anaerobic, nonmotile, non-spore-forming, Gram-positive, rod-shaped bacterium that is spread through exposure to contaminated animals and their discharges, including feces, containing the organism. The organism does not normally inhabit the intestinal tract of humans but is often associated with disease in pigs and poultry. In swine, erysipelas occurs in three forms: acute septicemia in suckling pigs with sudden death; diamond skin disease with various-sized rash lesions on the abdomen, ears and snout, or endocarditis or polyarthritis. Infected pigs often have symptoms that include high fevers, painful movement due to infected joints and a skin rash of raised, pink to purple, rhomboid lesions ranging in size from <1 mm to about 10 cm in width. Similar lesions can occur in infected humans, and the bacteria can also spread to the bloodstream causing damage to the heart valves, or sudden death. Arthritic changes can occur from joint infection in humans. In sheep and calves the illnesses include post-dipping laminitis, polyarthritis caused by the organism entering through wounds or nonhealed umbilicus. In fowl, *Erysipelothrix* causes septicemia, and in cattle the diseases are polyserositis, arthritis and septicemia.

Erysipelothrix rhusiopathiae survival in manure has been documented for 37 days at 35-37°C and up to 180 days at 2-5°C. However, in sewage survival time is much shorter but still 14-21 days at 18-20°C (Mitscherlich, 1984). Generally, most survival times for this organism are dependent on temperature. The higher the temperature the shorter the survival in the environment. The people at greatest risk are abattoir workers and others associated in agricultural or meat handling occupations. It is rare in the U.S. with one case per year (Smith, 1998). Reducing occupational exposure to diseased animals through good hygiene or covering exposed skin are the most effective controls. As for other pathogens, workers should wear protective clothing (gloves) when handling animals, their wastes or body tissues. After animal contact, preventive hygiene and sanitation should include hand washing with soap and water and proper disposal or cleaning and disinfection of clothing and protective wear.

Francisella tularensis

Francisella tularensis is a small, facultative, Gram-negative rod-shaped bacterium that causes a plague-like disease known as tularemia. The bacterium is hardy and capable of surviving for weeks at low temperatures in water, moist soil, hay, straw or decaying animal carcasses. *F. tularensis* is carried by many species of wild rodents, rabbits, beavers, and muskrats in North America. The highly virulent strain occurring in ticks and rabbits is found only in North America. The route of infection determines the type of disease. Inhalation results in pulmonary symptoms while ingestion causes ulceroglandular symptoms. The overall mortality rate for the strain has been 5-15%, but in pulmonic or septicemic cases of tularemia without antibiotics treatment the mortality rate has been as high as 30-60%. With antibiotic treatment, mortality rates in the U.S. have been 2%. The infectious dose has been documented as low as 10 organisms (Tarnvik, 1989).

Rabbits, rats, lemmings, squirrels, dogs and muskrats carry *Francisella*. This pathogen is transmitted to humans primarily by ticks or other vectors. However, occupational contact with carcasses infected with *Francisella*, airborne exposure due to dust contaminated with excrement of infected rodents, waterborne outbreaks, and foodborne outbreaks have been documented as other routes of exposure (Pearson, 1998). Water contaminated with carcasses of infected lemmings was positive for *Francisella*, which resulted in human infections (Berdal, 2000). However, these routes of expo-

sure are secondary to vector-borne exposure. Francisella has been shown to survive at 4°C for 5 months in water. In contaminated water samples held at 7°C, *Francisella* survived between 23-35 days. In well water seeded with *Francisella* and held at 21-24°C survival was 12 days. Control of *Francisella* is achieved by protecting food and water from exposure to rodents and chlorination of water. Tularemia is primarily a disease of hunters, because they have the most contact with wild animals, including the dressing of rabbits and other game, and they are most likely to be exposed to the insect vectors. In high risk populations, such as microbiology laboratory workers, vaccination is effective for protection against the disease (Tiggert, 1962), and a live, attenuated vaccine is available for laboratory personnel. However, a vaccine it is not yet available to the general public in the U.S.

Leptospira species

Leptospires are long, thin motile spirochetes (spiral-shaped) bacteria that can cause the disease leptospirosis. They may be free-living or associated with animal hosts, and they survive well in fresh water, soil, and mud in tropical areas. Leptospirosis is a disease that heavily impacts the cattle and swine industries but can also be carried by sheep, dogs, and horses (Ciceroni, 2000; Steger-Lieb, 1999). Rats serve as an important reservoir on farms and in urban areas (Dalu, 1997; Vinetz, 1996). Primarily young animals and pregnant or lactating animals show clinical signs of infection. Two manifestations that impact the cattle industry are stillbirths or abortions and "milk drop syndrome." Milk drop syndrome is distinguished by a loss or drop in milk production along with the utter appearing as if milking already occurred (Ellis, 1998). Late-term abortions occur in pigs and sheep.

In humans, leptospirosis is marked by acute onset of headache, muscle pain, fever, rash, and photophobia. Depending on the strain causing the infection, symptoms may also include renal failure, liver failure, or death if untreated. The majority of infections occur in developing countries, but 100-200 cases are reported annually in the U.S., primarily in Hawaii. High risk groups for exposure are workers in rice fields, sugar cane plantations, mines, sewer systems, and slaughterhouses; animal caretakers and veterinarians; and travelers to tropical parts of the world involved in recreational activities in fresh water (e.g., rafting, kayaking, and swimming).

Transmission to humans occurs primarily through contact with animal urine or afterbirth. Two main risk factors for workers in an occupational situation were smoking and drinking while working with infected animals and their wastes, indicating the importance of hygienic practices in prevention (Campagnolo, 2000). Sewer workers also showed high seroprevalence levels from contact with contaminated sewage, thus indicating other routes of transmission (De Serres, 1995). Waterborne transmission was documented in Illinois from swimming in a pond contaminated with leptospirosis. The apparent sources were nearby areas containing cattle and wildlife (Jackson, 1993). Environmental conditions determine whether leptospires survives in water.

Leptospires will not survive desiccation, low temperatures, exposure to heavy metals except iron, or a pH of lower than 6.8 (Mitscherlich, 1984). The best protection against this organism is to avoid contact with infected animals or fluids that may carry the bacteria. Streptomycin has proven to be an effective control measure for infection. Hand washing and other sanitation and hygiene measures also serve as an effective control for infection from this organism.

Listeria monocytogenes

Both agricultural animals and humans are susceptible to *Listeria monocytogenes*. a Gram-positive, rod-shaped bacterium that is widely present in the environment. Infection can result in disease that may cause abortions of fetuses, encephalitis, and septicemia. Listeriosis in humans affects mainly the immunocompromised, elderly, or pregnant women, with fatality rates of in 20-40% (McLauchlin, 1998). In the U.S., an estimated 2,500 persons become ill with listeriosis each year, of which about 500 die.

Animals can carry *L. monocytogenes* without appearing ill The main route of transmission to humans is foodborne, with meat, cheese and other dairy products of highest risk. Vegetables can become contaminated from the soil or from manure used as fertilizer. The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing, such as soft cheeses and cold cuts. Unpasteurized (raw) milk or cheeses and other foods made from unpasteurized milk may contain the bacterium.

Direct transmission from animals to veterinarians and poultry workers has been documented while direct human-to-human transmission is rare to non-existent. Listeria is shed in the feces of symptomatic and asymptomatic animals but has also been isolated from healthy humans and animals and various food and environmental samples. Sheep, cows, goats, pigs, chickens, turkeys, pheasants, seagulls, sparrows, rats, rabbits, cats, dogs, ducks, pigeons and other wildlife are potential carriers of *Listeria* (Ryser, 1991). Occupational workers are thought to become infected from animals via direct contact. Slaughterhouses, food, or water sources have been linked to *Listeria* contamination (Autio et al., 2000; El-Shenawy, 1998; Vallabhbhai, 1999). Data suggests that main sources of contamination for *Listeria* are the animals themselves and their food products (such as raw milk) or human origin from food handlers (Autio et al., 2000; El-Shenawy, 1998). Therefore, efforts must be made to determine if the animal and its products or the food handler transferred *Listeria* to a food or water vehicle. *Listeria* is sensitive to the antibiotics ampicillin, penicillin and erythromycin. Treatment with these antibiotics is successful but *Listeria* has some resistance to tetracycline and is resistant to cephalosporins and fluoroquinolones.

Listeria is commonly present in soil and survives in topsoil for 12 days with sun exposure and 182 days if there is no sun exposure. It is also found in high concentrations in manure and sewage sludge. In cattle feces at 5°C, *Listeria* was found to survive 182-2190 days and in liquid manure 36 days in the summer and 106 days in the winter (Ryser, 1991). *Listeria monocytogenes* was able to grow for a period of 2 days in fresh chicken manure at 20°C with a resulting 1-2 log unit increase in CFU (Himathongkham and Riemann, 1999). After 6 days of storage, the number of *L. monocytogenes* did not decrease below initial levels. However, drying the manure to a moisture content of 10% followed by exposure to ammonia gas in an amount of 1% of the manure wet weight decreased *L. monocytogenes* by 4 $\log_{10}$. Like most enteric bacteria, control is through proper hygiene, sanitation, including cooking of meats, and pasteurization of dairy products. Lactic acid prevents growth of *Listeria.*

Salmonella

Salmonella bacteria can be considered as belonging to main groups with respect to human health risks: typhoidal *Salmonella* (*S. typhi* and *paratyphi*) that case enteric fevers ("typhoid fever") and non-typhoidal *Salmonella*, which cause primarily gastrointestinal illnesses. *S. typhi* and *paratyphi* are strictly human pathogens while all of the other *Salmonella* infect a variety of animals as well as humans. The main carriers of zoonotic infection are poultry, cattle, sheep, and pigs. Clinical signs of disease are not usually present in poultry but the other animal reservoirs may or may not suffer from diarrhea or fever. The main symptoms resulting from salmonellosis infection in humans are diarrhea, abdominal pain, and fever. Nausea, muscle pain and vomiting may also accompany the infection. Because non-typhoid *Salmonella* infect the intestinal tracts of animals, they are shed at high concentrations in the feces of infected animals and often are present at high concentrations in animal manures and other animal fecal wastes.

The human infectious doses of *Salmonella* can be either high ($>10^5$ organisms for a high probability of infection) or low (10-100 cells for a high probability of infection), depending on *Salmonella* species, serotype and strain. One study found a human infectious dose of less than 10 cells (D'Aoust, 1985). Studies also have determined that as the dose increases, so does the severity of the illness (Glynn and Bradley, 1992 and Mintz 1994).

Transmission of *Salmonella* to humans primarily occurs via undercooked or raw meats and poultry. Animal carcasses, raw or undercooked eggs, milk and other dairy products can all serve as potential vehicles for transmission. Another source of human salmonellosis is fecally contaminated drinking water. Although food handlers are implicated in outbreaks of salmonellosis, they are usually not the cause when strict hygienic practices are followed (Cruickshank, 1987). Outbreaks from food preparation are usually associated with poor hygiene and sanitation practices or cross-contamination of food from another *Salmonella* source. Unlike other bacterial infections, *Salmonella* transmission may also occur between people either directly by the fecal-oral route or indirectly via inanimate but shared objects. Therefore, secondary spread of *Salmonella* is another source of human infection.

Salmonella outbreaks continue to occur in the U.S. and antibiotic resistant strains of *Salmonella* are contributing to a reemergence of salmonellosis. A emerging *Salmonella* strain of concern is

multiple-antibiotic-resistant *Salmonella enterica* Typhimurium phage type DT104. This organism is a pathogen of both animals and humans and is characterized by a pentaresistance pattern to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Other resistance patterns have also been found in association with DT104 (Glynn, 1998; Davis, 1999; Ribot, 2002; Ridley, 1998). DT104 emerged worldwide in the 1980s but did not become a significant cause of human disease until the 1990s. The increased incidence in human DT104 infection corresponded with an increased incidence in cattle in the U.S. and multiple livestock species in Great Britain (Davis, 1999; Glynn, 1998; Hogue, 1998). Studies in Great Britain and the U.S. have found that contact with sick cattle or meat and dairy products are the main risk factors for human disease (Glynn, 1998; Hogue, 1997). A study of 46 outbreaks of DT104 in Great Britain found that 78% of the human outbreaks resulted from foodborne exposures and 15% resulted from direct contact with farm animals. Similarly, studies in the U.S. indicate 10% of human cases may result from direct contact with animals (Hogue, 1997). In 1993, 8.3% of the patients with clinical symptoms of *Salmonella* DT104 died (Wall, 1994) and this pathogen has been responsible for at least 5 outbreaks in the U.S. (Meng, 1999). In a Missouri community, a waterborne *Salmonella typhimurium* outbreak resulted in the hospitalization of over 2% of the 625 people affected, and nearly half of those hospitalized died (Kramer, 1996). In 1990, only 7% of *Salmonella typhimurium* were DT104, whereas in 1996 28% of the *Salmonella* isolates were identified as this strain (Meng, 1999). *Salmonella* DT104 has been found in cattle, swine, sheep, chickens, turkeys, ducks, horses, goats, cats, dogs, elk, coyotes, ground squirrels, chipmunks, coyotes, mice, geese and game birds (Besser, 1997). Mortality rates of 50-60% have been reported for animals with clinical symptoms (Hogue, 1997).

In feces *Salmonella* survived for 96-190 days at room temperature. In manure, at 10°C and 45°C *Salmonella* survived for 14 and 35 days, respectively while in liquid manure at 10°C survival was 140 days (Mitscherlich, 1991). *Salmonella* inoculated into cattle slurry were observed to survive for 2-4 months when storage temperatures were 20ºC or less (Jones, 1976). Based on the medium, survival of *Salmonella* varies widely but the predominant limiting factors for growth are temperature, acidity, and moisture.

Many control approaches for *Salmonella* in humans and animals have been utilized. Antibiotic treatments eliminate the clinical symptoms but do not always eliminate the carrier state of the bacteria in the host. Vaccinations are partially effective at protecting poultry and cattle from disease, but they do not eliminate infections or the carrier state in animals (Barrow, 1990). Gamma radiation of meat has shown to be effective at destroying *Salmonella* (Dempster, 1985). Good hygiene and sanitation practices appear to be effective at reducing *Salmonella* exposure due to food handling in the kitchen and they prevent or reduce cross contamination. Also, cooking meats destroys the pathogen and storing of meats below 4°C prevents *Salmonella* growth. However, *Salmonella* continue to be widely present in animal agriculture production facilities and are readily detected in manures and other animal fecal wastes.

Yersinia

In terms of human health risks, *Yersinia* bacteria can be divided into two main groups: *Yersinia* that cause plague (*Yersinia pestis*) and non-plague *Yersinia* species. Non-plague *Yersinia* consist of two main species: *Y. enterocolitica*, and *Y. pseudotuberculosis*. *Yersinia* species are found worldwide. *Yersinia enterocolitica* affects both sexes and all ages, whereas *Y. pseudotuberculosis* tends to affect young males at a greater rate (Butler, 1998). Yersinias are not normal inhabitants of the human intestinal tract. *Y. enterocolitica* illness in humans (or yersiniosis) typically manifests as abdominal pain, fever, and diarrhea. However, the illness leads to sepsis when the host is immunocompromised or iron levels in the body are overloaded (Bottone, 1997). An infectious dose of 10^9 organisms was assessed in an adult human volunteer studies, indicating the need for relatively high doses of the bacteria to pose an appreciable health risk (Morris, 1976). The course of *Yersinia* infection and symptoms lasts 1-3 weeks, with continued shedding of the bacteria several weeks after the symptoms have ceased (Marks, 1980). *Yersinia enterocolitica* has been detected in swine and other animal feces and, therefore, can be present in the wastes of swine and perhaps other livestock animals. *Y. pseudotuberculosis* causes an appendicitis-like syndrome and fever but is self-limiting in humans.

The major reservoirs for *Y. enterocolitica* are pigs, rodents, rabbits, chickens, waterfowl, sheep, goats, cattle, horses, dogs, and cats (Bottone, 1999). *Y. pseudotuberculosis* has the same reservoirs as *Y. enterocolitica* but also is associated with turkeys, ducks, geese, pigeons, pheasants, and canaries (Butler, 1998). Animals serve as asymptomatic carriers, although disease has been documented in dogs and sheep, and mastitis in cattle. Human-to-human transmission of yersiniosis by secondary spread is not significant, even though the bacteria are shed for several weeks. However, blood-borne transmission of the bacterium is a concern. Transfusion of blood tainted with *Yersinia* has caused shock and death in patients (Morbidity Mortality Reports, 1991).

There are both pathogenic and non-pathogenic strains of *Y. enterocolitica*, and there are also several serotypes that differ in prevalence and human health significance in the U.S. and other parts of the world. Pathogenic strains of *Yersinia enterocolitica* possess both plasmid and chromosomal virulence factors that are responsible for the ability of these bacteria to cause human disease. Both pathogenic and non-pathogenic *Yersinia enterocolitica* are widely distributed in the environment. Therefore, it is important to distinguish the pathogenic from the non-pathogenic strains, because only the pathogenic strains pose human health risks.

Yersinia enterocolitica is primarily transmitted via food-borne exposure, although waterborne outbreaks have also been reported (Schiemann, 1990). *Y. enterocolitica* detected in stream water have been linked to swine farms. Isolates from the stream water had the same DNA fingerprint (banding pattern by pulse-field gel electrophoresis) as isolates from swine feces from a local farm (Pilon, 2000).

Y. enterocolitica is not adversely affected by antibiotics but antibiotic therapy does reduce complications of illness in older children. Under the age of six, antibiotics are not effective (Hoogkamp-Korstanje, 1993; Pham, 1991). Additionally, antibiotic susceptibility and resistance appears to be serotype dependent. However, all biotypes from one study were susceptible to choramphenicol, ciprofloxacin, gentamicin, tetracycline, and trimethoprim (Pham, 1991). Vaccines have not been developed for this pathogen and control has primarily focused on hygiene and sanitation measures. Avoiding contact with the intestinal contents and the oral cavity of slaughter animals by abattoir workers and complete cooking of meat in the home are recommended prevention measures. Yersinia isolation and growth from clinical, food and environmental samples is affected by temperatures greater than 44°C and acidity with a pH lower than 4 (Mitschlerich, 1984). *Yersinia* are isolated most efficiently by cold enrichment in either non-selective or selective broth media, followed by plating and colony isolation on *Yersinia*-selective agar media, followed by biochemical, serological and virulence factor characterization.

Yersinia pestis is the cause of plague, a disease that has two main forms: bubonic and pneumonic. Bubonic plague is transmitted primarily by the bite of a rat flea and results in an illness characterized by fever, swollen lymph nodes, and a painful swelling (called a bubo), usually in the groin. In a large proportion of untreated cases, bacteria spread to the bloodstream (septicemic plague) causing septic shock and death. A small percentage of plague cases are manifest as pneumonia (respiratory illness) or pneumonic plague, in which symptoms are fever, chest tightness, cough with sputum, then shock and death. Both forms of the disease are often fatal, unless treated with antibiotics.

Rats and rat lice are major carriers of *Yersinia pestis*, but rabbits, squirrels, prairie dogs, mice, coyotes and wolves also can be infected. Agricultural animals are not carriers and they are rarely infected. In the U.S. about a dozen human cases of plague are reported annually, and these are usually the result of contact with rats, other carrier animals or their infected fleas. Respiratory or inhalation exposure also can occur from droplets of infected animals experiencing respiratory symptoms, especially cats. Prevention and control of the plague is by rat and other vector control and related public health measures, including flea control of domestic pets and avoiding contact with dead carrier animals, such a rodents, squirrels and prairie dogs. Occupational exposures of animal workers and non-occupational exposures of humans coming in contact with animal reservoirs or their fleas are high risk groups in the U.S. Overall, the risks of plague in animal agriculture are very low. However, *Y. pestis* also is a bioterrorism agent that can be spread by aerosols of the bacterium grown in culture or by introduction into agricultural animals.

Mycotic Agents and Mycoses

Fungal infections or mycoses from exposure to animal waste are not usually a major public health concern. This is because most fungal infections are a result of exposure to one's own fungal microflora on or in the body, or to soil or other environmental samples laden with high concentration of mycotic agents. However, some animals can carry disease-causing mycotic agents in the feces thus facilitating transmission to humans. Most people do not show clinical symptoms from mycotic infections but immunocompromised populations are affected and should be of concern for exposure. Mycoses can result from exposures to soils contaminated with feces of animals, primarily birds.

Histoplasmosis capsulatum tends to be associated with soils contaminated with bird or bat feces even though birds do not carry this fungus. Chicken houses or areas where birds roost are places where the risk of exposure may be greatest. Clinical symptoms consist of lesions on the lungs. *Histoplasmosis* primarily occurs in Canada and the central and eastern U.S. In endemic areas, asymptomatic infections can occur in up to 90% of the population but 50% prevalence is more typical (Clinkenbeard, 1989). Control consists of spraying contaminated soils with formaldehyde and avoiding inhalation of dust contaminated with bird feces (Halde, 1989; Lloyd, 1998).

Pneumocystis carinii has a wide host range and recent molecular analysis revealed host specificity (Dei-Cas, 1998; Mazars, 1998). Thus, contrary to previous associations with zoonotic transmission, animals do not serve as a reservoir for human infection. Transmission of *Pneumocystis* within the human population is unknown and this disease is of greatest treat to the immunocompromised population.

Cryptococcus neoformans is a yeast that causes pneumonia primarily within the immunocompromised population. *C. neoformans* is associated with bird droppings, primarily pigeon droppings, in soils, where concentrations can exceed 1 million per gram. Transmission between bird feces and humans has been documented (Nosanchuk, 2000; Clauson, 1996). Also, this organism has been linked to pneumonia in cattle, buffalo, sheep and goats, documenting its importance to the health of farm animals (Pal, 1989).

Overall, mycotic agents do not appear to pose particular risks associated with animal waste management treatment processes and systems. However, the ubiquitous presence of mycotic agents, some of which are pathogens for immunocompromised persons, requires special measures to prevent such highly susceptible populations from being exposed to them in both rural and urban settings.

Parasites (Protozoans and Helminths)

Many of the important parasites of animals that can potentially pose risks to human health are shown in Table 4. The two broad taxonomic groups of parasites are the protozoans, which are eukaryotic, single-celled microbes, and the worms, which are actually multicellular animals. The protozoans are divided into several taxonomic groups: ciliates, amoeba, flagellates, coccidians (which have both sexual and asexual stages in their life cycle) and the microsporidia. The worms are also divided into several taxonomic groups, but the only ones considered here are the nematodes or roundworms. Many of the parasites shown in Table 4 are known human pathogens. However, some are of low risk to human health and others are not commonly found in agriculturally important animals. Detals of some of the more important parasites, their sources, human health risks and effects, their occurrence and persistence in animal wastes and the methods for preventing and controlling human exposure are provided below.

Ascaris and Ascariasis

Ascaris is not as common within the U.S. or Western Europe as it is in developing countries, where it remains a major cause number of infections and infestations worldwide (an estimated 25% of the world's population is infected). *Ascaris* infections are accompanied by abdominal pain, nausea and vomiting. Two categories of *Ascaris* can infect humans: *A. lumbricoides* (human ascarid) and *A. suum* (swine ascarid). These are among the largest human parasites. The adult females can measure up to 18 inches long; males are generally shorter. The adult worms live in the small intestine and eggs are passed in the feces. A single female can produce up to 200,000 eggs per day. About two weeks after passage in the feces the eggs contain an infective larval or juvenile stage,

Table 4. Some important parasites potentially present in animals and their wastes.

Parasite	Taxonomic Group	Animal Hosts	Disease in Animals?	Human Infection/ Disease	Transmission Routes	In USA?	In Manure ?
Ascaris suum	Helminth, nematode	Swine	Yes	Yes	Ingestion of water, food, soil	Yes	Yes
Balantidium coli	Protozoan, ciliate	Swine, other animals	No	Yes	Contact, ingestion of water and soil	Yes	Yes
Cryptosporidium parvum	Protozoan, coccidian	Many animals	Yes	Yes	Ingestion of water	Yes	Yes
Giardia lamblia	Protozoan, flagellate	Many animals	Yes	Yes	Ingestion of water	Yes	Yes
Microsporidia	Protozoans, microsporidia	Many animals	No	Yes, immunocompromized	Ingestion, possibly water	Yes	Yes
*Pneumocystis carinii**	Fungus; similar to protozoans	Environment and many animals	Yes	Yes, immunocompromized	Inhalation	Yes	Yes
Toxoplasma gondii	Protozoan, coccidian	Felines	Yes	Yes	Ingestion of feces, food, water	Yes	Yes, if an infected host

*Previously considered a protozoan parasite, but now known to be a mycotic agent.

and humans are infected when they ingest such infective eggs. The eggs hatch in the small intestine, the juvenile penetrates the small intestine and enters the circulatory system, and eventually the juvenile worm enters the lungs. In the lungs the juvenile worm leaves the circulatory system and enters the air passages of the lungs. The juvenile worm then migrates up the air passages into the pharynx where it is swallowed, and once in the small intestine the juvenile grows into an adult worm. Migration of the worm through the body may cause intestinal obstruction, jaundice or pancreatic. The origins or sources of *Ascaris* infections in the U.S. are not clear. Some research suggests that infections in the U.S. are of swine origin. Genetic analysis showed two distinct worm populations; one associated with humans and the other associated with swine (Anderson, 1995). However, another study also proposed that human infections were of pig origin (Maruyama, 1996). Thus *Ascaris* may be a trans-species infection and should be analyzed to determine which type of *Ascaris* caused the infection.

Ascaris was once common in U.S. swine herds with up to 60% prevalence (Kennedy, 1988). Female *Ascaris* can produce 200,000 eggs/day thus can significantly impact environmental quality (Murrell, 1998). In a study by Reimers, a sewage treatment system with abattoir waste input contained 81,800 eggs/kg while sewage treatment systems receiving little to no abattoir waste contained 7,900 eggs/kg (Reimers, 1981). This indicates abattoir waste contains a significant number of eggs serving as a potential source for human exposure. Because of the persistence of the eggs, *Ascaris* is a difficult parasite to control in swine. However, the availability of effective drugs and the trend towards raising swine indoors under more hygienic conditions in the U.S. have led to significant reductions in the prevalence of this parasite. Currently available and FDA-approved drugs in the U.S. are effective in treating and preventing infections when used strategically. Information about the extent of Ascaris presence in animal wastes is limited. However, *Ascaris* eggs were identified in only 1% of samples from manure storage lagoons in Canada, and the investigators concluded that there is a minimal risk of human infection from environmental contamination of water or soil by hog manure (Guselle and Olson, 1999).

Ascaris eggs are resistant to disinfection, survive sewage treatment facilities, and can survive in soil for years (Weatherly, 1992). Due to the high prevalence, resistance to environmental stress, and production of eggs, *Ascaris* should be considered a potential threat to populations who directly contact swine manure and when untreated manure is applied to crops. Special attention to *Ascaris* con-

trol in human and swine waste treatment processes and management systems is recommended as this is perhaps the only effect means for control. More research is needed to determine the distribution of *Ascaris* infection in humans and swine the U.S. and the extent to which *Ascaris* eggs are removed and destroyed by waste treatment processes and systems.

Balantidium coli

Balantidium coli is a ciliated protozoan found in the intestines of humans and commonly in pigs. This protozoan appears to be a rarely reported human disease in North America, and its potential to be transmitted from pigs to humans remains controversial (Acha and Szyfres, 1987). *Balantidium coli* has occasionally caused death in humans if untreated. It can be treated with tetracycline. Approximately 1 in 5 humans who become infected develop symptoms and all ages are susceptible to infection. Although pigs are considered the primary carriers, rats and primates may also serve as carriers. Close contact with pigs is required for transmission and infection. The prevalence of *Balantidium coli* amongst pigs has been reported historically at 50-100% (Knight, 1978). Transmission of the organism can occur via fecally contaminated food or water or through direct contact and ingestion of feces with the organism. Good hygienic practices are effective for the control of *Balantidium coli*. This organism is not considered a significant health threat in the U.S. but it could be a concern for those working directly or are in close contact with pigs or their feces.

Cryptosporidium parvum

Cryptosporidium parvum is one of the best-known parasitic protozoa due to a large (estimated 400,000 cases) outbreak from contaminated drinking water in Milwaukee in 1993. *C. parvum* is considered to be a significant public health concern for humans. It is a coccidian protozoan with both sexual and a sexual stages in its life cycle. The parasite is fecally excreted as an oocyst, about 3-8 micrometers in diameter, that is fully developed (sporulated) and infectious. The oocyst is very hardy and stable and can persist in the environment for months. A wide range of animals serve as hosts including cattle, sheep, goats, horses, pigs, cats, dogs, deer, rodents, and rabbits. Cattle are considered the primary source for zoonotic transmission. In animals and humans, asymptomatic infection is common; however, disease may occur in both healthy or immunocompromised people and animals. Symptoms in humans are characterized by watery diarrhea, vomiting and abdominal cramps, but infection is self-limiting in healthy people, with a duration of illness lasting days to a few weeks. In immunocompromised hosts the disease can persist and eventually result in death. *Cryptosporidium parvum* consists of at least two major strains: one primarily associated with animals ("cattle" strain) and the other associated with humans ("human" strain). The taxonomy of *C. parvum* is still being determined, but it appears that this species of *Cryptosporidium* is actually a complex or super-group of closely related, but genetically distinguishable, strains or biotypes. The prevalence of zoonotic infections from the animal strains may be lower (10-54%) than infections from the human strain (Caccio, 2000). However, outbreaks may be of human or animal origin. *Cryptosporidium parvum* is infectious for humans at low doses. One strain has a 50% human infectious dose estimated to be 9 oocysts. Fecal contamination of food or water has received the most attention as sources of exposure, but direct person-to-person and animal-to-person transmission has been reported. The most effective controls for *Cryptosporidium* are to prevent the oocysts from entering water systems and to avoid ingestion of fecally contaminated products.

Infected animals such as newborn calves can excrete large numbers of *Cryptosporidium* in their feces, up to 10^{10} oocysts per day, for periods of 3-12 days (Anderson, 1981). Fecal shedding by humans is similar to that by animals, with shedding of up to 10^5-10^7 oocysts per gram of feces (Goodgame et al., 1993) for periods of days to weeks (more than 50 days) (Jokipii and Jokopii, 1986). *Cryptosporidium* has been detected in human sewage influent in the U.S. and Europe in concentrations ranging from 30-76,000 per liter (Robertson, 1995; Rose, 1986). In slaughterhouse effluent, animal housing areas, and poultry slaughterhouse effluent, 93, 10^5, and 293 oocysts/L were detected, respectively (Robertson, 1999).

Cryptosporidium is very stable in environmental waters and highly resistant to various types of waste treatment processes. The primary factor which influences survival is temperature. Survival is longer at lower temperatures and oocysts are killed rapidly a higher temperatures. At temperatures of 55°C or higher, *C. parvum* oocysts are rapidly inactivated (within minutes) in water and wastes (Barbee, 1997). At -4°C, *Cryptosporidium* survives for greater than 12 weeks in water and feces

while in soil it survived for 10 weeks. However, when the temperature was 25°C survival times decreased to 4 weeks in soil and feces but remained for 10 weeks in water (Olson, 1999). *Cryptosporidium* survives longer in fresh water than in seawater (Simmons 2001). Upon desiccation, *C. parvum* oocysts survive only 2-4 hours (Casteel, 1998; Robertson, 1992). In marine waters, sunlight impacts the number of oocysts that survive, with the time for 90% reduction in seawater exposed to sunlight to be 48 hours (Johnson, 1997). Free ammonia, which is present in wastes and other organic samples at higher pH levels (pH >8.5), contributes to *Cryptosporidium* inactivation. *Cryptosporidium* is highly resistant to chlorine disinfection and the most effective control for this organism by sewage or water treatment is coagulation, lime treatment (softening or alkaline stabilization) and filtration. However, ozone and UV disinfection are effective for control of *Cryptosporidium* in drinking water and wastewater (Belosevic et al., 1997; Shin et al., 2001).

Cyclospora, Isospora, and Eimeria

Cyclospora, *Isospora*, and *Eimeria* have all been implicated in outbreaks related to food contaminated with feces. *Cyclospora*, *Isospora,* and *Eimeria* are coccidian protozoan parasites associated with humans or animals; however, the various strains appear to be species-specific. *C. cayetanensis,* which causes a diarrheal disease in humans, was not associated with domesticated pigs, cattle, horses, goats, dogs, cats, chickens, ducks, turkeys, pigeons, or guinea pigs in Haiti though humans were infected (Eberhard, 1999). This suggests that humans are the sole source of *Cyclospora* and colonization or carriage does not occur in animals. Various species of *Eimeria* are important pathogens of agricultural animals that result is mortalities and reduced production. However, these animal *Eimeria* do not infect humans and therefore appear to pose no risks to humans from animal waste management systems. *Isospora belli* is a human-specific pathogen that is a health risk to immunocompromized persons. Other *Isospora* species of animals apparently do not infects humans, and therefore animal fecal wastes do not pose human health risks from these agents. Overall, it appears that these coccidian parasites have sufficient host range specificity that the animal coccidians (*Eimeria* species and *Isospora* species) do not infect humans and the human coccidians (*Cyclospora cayetanensis* and *Isospora belli*) do not infect animals. Therefore, based on current information, these coccidian parasites are not zoonotic risks to human health from agricultural animal wastes and animal waste management systems.

Giardia

Giardiasis considered one of the most prevalent parasitic infections in the world. The disease is more common in developing nations where a lack of sanitary conditions exist. The route of exposure is fecal-oral and infections occur in people of all ages. In humans, asymptomatic infections are common especially in children. Symptomatic infections manifest as watery diarrhea, cramps, bloating, and flatulence. Animals can be either asymptomatic carriers or have symptomatic infections. The human pathogenic *Giardia* species is *Giardia lamblia*, which has a very wide host range in feral, domestic and agricultural animals. Other *Giardia* species, such as the rodent species *Giardia muris*, are not human pathogens. Waterborne and foodborne outbreaks of giardiasis have resulted from animal fecal contamination, as documented by the identification of the fecal waste source and its presence in fecally contaminated water or food incriminated in the outbreaks.

Recent molecular methods are now employed to definitively link *Giardia lamblia* sources with outbreaks. *Giardia* from beavers was implicated in an outbreak in British Columbia where contaminated water served as the route for human infection. Genetic analysis of the cysts showed that those infecting humans and beavers were the same (Slifko, 2000). The importance of identifying *Giardia* genotypes in animals also is important in *Giardia* infections of calves and dogs. Both carry two genotypes: one that can be transmitted to humans and the other solely associated with infection of the specific animal host (Thompson, 2000). Thus, the presence of *Giardia* in an animal does not conclusively establish it as the source of human infection.

Giardia are divided into species-specific strains which remain stable across geographic regions, and consist of two main groups: assemblage A and assemblage B (Thompson, 2000). Assemblage A and B of *Giardia* are the species which pose a threat for zoonoses. Potential reservoirs for infection are cats, dogs, birds, cattle, horse, sheep, pigs, beavers, and seals (Olson, 1997; Thompson, 1998; Deng, 2000). Besides animals serving as reservoirs for certain species of *Giardia*, person-to-person transmission is also of great significance. The risk of transmission from dogs and cats is considered

low compared to human sources of exposure. The highest prevalence of *Giardia* in cattle occurs in young animals, with 14-100% of cattle less than 6 months of age being infected. Along with age, maternity management and season also influence infection in cattle, with decreased risk of infection when calves are feed fresh colostrums and during the winter months (Wade, 2000).

Giardia lamblia can be shed at high concentrations for days in the feces of infected animals, such as calves. Therefore, *Giardia* cysts can be present at potentially high concentrations in animal manures and other animal fecal wastes. Concentrations of *Giardia* were assessed in slaughterhouse effluent, housing areas, and poultry slaughterhouse effluent. In slaughterhouse effluent and animal housing areas *Giardia* was found at 42 cysts/L and 10 cysts/L, respectively, but were not detected in poultry slaughterhouse effluent in the study (Robertson, 1999). Reported *Giardia* levels have ranged from 10,000 to 100,000 cysts/L in untreated sewage, 10 to 100 cysts/L in treated sewage, and 10 or few cysts/L in surface water sources and tap water. In human sewage, *Giardia* is commonly detected ranging from 4-40,000 per liter of influent (Robertson, 1995). Cysts have also been detected in cisterns and in wells contaminated by surface water or sewage. Levels are generally higher in water sources influenced by agriculture (e.g., cattle or dairy farming) or municipal and residential wastewater discharges

The survival of *Giardia* cysts in the environment is significantly affected by temperature, with greater survival at lower temperatures. Cyst viability is also appreciably reduced by freezing and thawing, but a small fraction of cysts can withstand a single freeze-thaw cycle. Cysts can survive for 2 to 3 months in water temperatures of less than 10°C, and at 21°C, cysts have remained viable for about one month. *Giardia* cysts are rapidly killed at higher temperatures, with complete inactivation in 10 minutes at a water temperature of 54°C. Cysts are killed almost immediately by boiling.

Chemical prophylaxis is effective at controlling *Giardia* infections in humans. Recently vaccines have been developed for animals which reduce the number of cysts shed and the duration of the shedding (Olson, 2000). Control measures primarily are proactive and directed at hygiene and sanitation practices. Prevention of food and water sources from human and animal feces containing cysts is an effective control measure. Isolation of infected animals, such as calves, and containment of their feces is also an effective measure to reduce environmental contamination. Better sanitary conditions and reduced crowding also reduces person-to-person transmission. Further details of the methods to control *Giardia* cysts in animal wastes by treatment and management systems is provided in later sections of this report.

Microsporidia

The microsporidia include over 143 genera more than 1,200 species as obligate intracellular parasites that infect a wide range of vertebrate and invertebrate hosts. Microsporidia are characterized by the production of environmentally resistant spores that vary in size, depending on the species. Microsporidia cause disease in AIDS patients, but usually are asymptomatic in immunocompetent populations. *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi* and several other microsporidia are detected frequently in AIDS patients (Mota, 2000). Some domestic and wild animals may be naturally infected with microsporidian species infecting humans, specifically *Encephalitozoon cuniculi*, *E. intestinalis*, and *E. bieneusi*. Transmission of microsporidia occurs by the fecal-oral route; waterborne transmission has been proposed but not yet definitively proven as a route of transmission for human infection (Dowd, 1998). Microsporidia have a broad host range including humans, animals, insects, and birds. Direct zoonotic transmission to humans has not been documented. However, recent genetic analysis showed that *Enterocytozoon bieneusi* is highly prevalent amongst swine. Genotypes of swine and cat isolates were related to the genotypes of human isolates whereas dog isolates were not genetically similar (Mathis, 1999). Currently zoonotic transmission and waterborne routes of exposure are only speculative and more research is needed on the sources of human exposure to the pathogens. Microsporidia can survive for years in cold temperatures (4°C) but *E. cuniculi* are susceptible to inactivation in water at >60°C for 5-10 minutes and to oxidant disinfection (Koudela, 1999; Labeau, 1998; Waller, 1979). These results suggest that these organisms can be adequately controlled by appropriate water and waste treatment processes. However, further research is needed to determine if agricultural animals are sources of human exposure

to human pathogenic microsporidia and to document the response of these agents to various animal waste treatment processes and management systems.

Toxoplasmosis

Toxoplasmosis gondii is a coccidian protozoan primarily carried by cats and other felines. *T. gondii* is associated with a wide range of intermediate hosts including goats, chickens, pigs, sheep, dogs, and cattle. However, the definitive host is cats because this is the host in which the organism completes its life cycle. In healthy, immunocompetent humans, *T. gondii* infection is generally asymptomatic. However, in pregnant women without prior exposure to toxoplasmosis infection there is a risk of illness not only in the woman but also in the developing fetus, in which infection causes a variety of birth defects, with ocular diseases being the most common. In immunocompromised people, this organism causes illness and an appreciable risk of death. In animals, *T. gondii* impacts the agricultural industry by causing death of the adult, death of the neonatal animal, or abortions. Only cattle and horses do not show clinical signs of infection but can carry the organism.

Transmission of *T. gondii* to humans can occur when intermediate hosts become infected and humans eat undercooked meat infected with the parasite or when there is exposure to and ingestion of sporulated oocysts in cat feces, either from direct contact or from fecally contaminated food or water. Of all the possible foodborne sources, pork is the primary vehicle of transmission to humans, due to the greater amounts of consumption as compared with sheep, goat, or horse. In other countries consumption of other types of meat causes these food sources to be of greater risk (Dubey, 1994).

Occupational workers are also at risk of acquiring *Toxoplasma* infection. On an Illinois swine farm the seroprevalence of *T. gondii* was 31% with the greatest risk factors for transmission being lack of hygienic practices (Weigel, 1999). Prevalence of *T. gondii* on swine farms in New England ranged from 4-100% of the herd (Gamble 1999). Immunization of cats is considered the best approach for reducing the prevalence of the oocysts since toxoplasmosis completes its life cycle only in cats. Good hygiene practices and fully cooking meats are also important for reducing human infection (Dubey, 1998). The extent of human exposure to *T. gondii* from animal wastes is not known, but is expected to be low for most agricultural animals because they are not the definitive host excreting cysts. However, the potential presence of *T. gondii* in the tissues of agricultural animals means that animal mortalities must be properly disposed of to reduce human contact and environmental contamination with the oocysts.

T. gondii oocysts are shed at high concentrations by infected cats for periods of days to weeks, with daily shedding of more than 10 million oocysts per day. The shed oocysts are not immediately infectious and must mature by sporulating (a process that takes several days to weeks) to become the infectious form. *T. gondii* are very environmentally resistant and can survive for months to years under a variety of environmental conditions, including freezing, drying and moderate temperatures. The cysts are rapidly inactivated (within minutes) at temperatures of 66°C or higher. Tissue cysts of *T. gondii* are not rapidly and extensively inactivated by freezing to modest temperatures (above -10°C), but they are rapidly inactivated by freezing to low temperatures (below -10°C). Oocysts are resistant to most healthcare disinfectants, and their response to water and wastewater disinfection processes has not been determined. Information on the survival and persistence of *T. gondii* oocysts in animal waste treatment processes and management systems is lacking and requires further study.

FECAL INDICATOR ORGANISMS

Detection of every potential pathogen in animal wastes and environmental samples is unfeasible and impractical, due to the many different kinds of pathogens, the lack of reliable and sensitive methods for their detection, the inability of some methods to detect infectious microbes, and the lengthy time and high costs for such analyses. Therefore, indicator organisms have been adopted as models for pathogenic microorganisms. Indicator organisms warn of the potential presence of pathogenic microorganisms and are measured to determine both the levels of fecal contamination and the efficacy of treatment processes to reduce microbes. Indicator organisms share characteristics with some pathogenic microbes, but those in current use resemble only the pathogenic enteric

bacteria, such as *Salmonella*. Current indicators are not widely available for enteric viruses or parasites, but some indicator organisms for the classes of pathogens have been proposed and are becoming more widely used, at least for research. Specific criteria have been established for indicator organisms and are as follows: (1) present whenever pathogens/pollution are present and absent when pathogens/pollution are absent, (2) have similar survival time as the pathogen, (3) occur in greater number than pathogens, (4) must be similarly resistant to disinfectants and to stressed environmental conditions, (5) enumeration and detection is performed quickly and easily, (6) have characteristics which allow for specific identification, (7) distribution is random in a sample, and (8) are indicative of the relative risks to human health caused by pathogens (Berg, 1978; Bitton, 1983).

Traditional indicators of fecal pollution in water are total coliform and fecal coliform bacteria. Total coliforms consist of the following groups of bacteria: *Escherichia, Klebsiella, Citrobacter,* and *Enterobacter*. *Escherichia* and some thermotolerant *Klebsiella* represent fecal coliforms and are associated with the intestinal tracts of humans, warm-blooded mammals, and birds. Fecal coliforms are characterized by their ability to ferment lactose at 44.5°C. *Klebsiella*, *Citrobacter,* and *Enterobacter* are not only associated with fecal material but found to occur in the environment, therefore, they are not specific indicators of fecal pollution (Feachem et al., 1983). *Escherichia coli* is specifically associated with fecal material and is present when fecal material is present and absent when fecal material is absent. Therefore, *E. coli* serves as a specific indicator of fecal pollution and the possible presence of pathogenic microorganisms. However, *E. coli* is less persistent than some of the pathogenic enteric bacteria, viruses and protozoans. Therefore, the absence of *E. coli* in wastes, water and other samples is not definitive evidence of the absence of and low health risks from human enteric pathogens.

With increased knowledge of other groups of enteric pathogens like protozoa and viruses, it is now apparent that *E. coli* and other coliform bacteria do not always serve as the best indicator. Therefore, coliphages, viruses of *E. coli* bacteria, and *C. perfringes* spores (hardy organisms capable of persisting in a variety of environmental conditions and treatment processes as do parasites) have been utilized as indicators of viruses and protozoa, respectively. Most contemporary research regarding the efficiency of treatment processes and the persistence, transport and fate of pathogens through the environment has focused on these organisms, in addition to fecal coliforms and *E. coli*.

Culturing of indicator organisms alone cannot identify the sources of fecal pollution. However, the development of source identification methods are based on the utilization of indicator organisms to identify the sources of fecal pollution (Harar, 2002a,b; http://soils1.cses.vt.edu/ch/biol_4684/bst/BST.html). Although much research remains to be performed and one method does not answer all questions, interested parties can select a microbial source tracking method that best addresses their questions of concern. The different tests are based on either culturing species-specific microorganisms, antibiotic resistance patterns for bacteria, molecular fingerprinting or genotyping of bacteria or coliphage, and chemical indicators. Toxin gene biomarkers utilize a species-specific DNA sequence from *E. coli* toxin genes. This method predicts the presence/absence of a source and have been developed for human, cattle, and swine fecal pollution at this time (Khatib and Olson, 1999; Khatib, 2000; Oshiro and Olson, 1997). Chemical indicators such as fecal sterols are highly specific but can be stable for years in the environment, thus not differentiating between recent and historical events of pollution (Nichols et al., 1993, 1996). Species-specific microbial methods are less labor-intensive but may not always be culturable. Microbial methods are also not always geographically representative (Evison, 1975; Sinton et al., 1998). Antibiotic resistance patterns are effective at differentiating animal vs. non-animal sources (Kruperman et al., 1983; Parveen et al., 1997). Molecular methods utilize different target organisms to produce a database of banding patterns from known sources. Banding patterns of environmental isolates are then compared to the database to determine the source (Bahirathan et al., 1998; Simmons, 1998; Parveen, 1999). The main issue antibiotic resistance and fingerprinting methods face is geographic representativeness and representation within the sample, because only a small portion of the colonies are screened. Thus, a large number of colonies needed to be screened per sample. The advantage of these methods is that a broad range of species can be captured. Genotyping of male-specific coliphage effectively differentiates sewage versus non-sewage sources but further refinement for species identification is currently under investigation (Hsu et al., 1996).

PATHOGEN REDUCTIONS DURING WASTE TREATMENT

Commercially raised farm animals can be infected with numerous pathogens that are also pathogenic to humans (Cole et al., 1999). Thus, farm animals can shed human pathogens which can subsequently be found in animal waste that is further managed on the farm. Waste management techniques that may be effective for reducing pathogen concentrations in farm animal waste are discussed in this paper. Improved animal management and housing techniques can also be effective in reducing pathogen levels in animal waste. These techniques are not addressed in detail in this paper, but generally include vaccination, prophylactic antibiotic therapy, animal diet modifications (Kudva et al., 1997; Cray et al., 1998), on-farm hygienic and sanitation measures (van de Giessen et al., 1998), herd management (Davies et al., 1997a, 1999, 2000) and housing designs (Davies et al., 1997b).

The persistence and fate of pathogens in animal waste treatment processes and management systems have not been adequately characterized and quantified. Only limited studies have been reported and most have been laboratory studies. Most studies have attempted to quantify reductions of microbial infectivity (inactivation) in animal manure slurries or mixtures of these with other constituents under controlled temperature conditions and maintenance of either aerobic or anaerobic conditions. Studies on the fate of pathogens after land application of animal manures, liquids or solids have not been reported (or at least could not be readily found in the peer-reviewed literature). Some of the main factors influencing virus reductions in animal manure treatment processes and the estimated virus reductions by these processes are summarized in Table 5. The term "reduction" includes pathogen inactivation (loss of infectivity) and well as physical removal of the microbe. Some processes cause primarily pathogen inactivation, such as thermophilic processes. Others cause both inactivation and physical removal, such as many of the mesophilic biological processes (e.g., lagoons and constructed wetlands). Estimates of pathogen reductions are uncertain and based on limited lab studies or pilot field studies with few pathogens, including indicator microbes (primarily fecal coliform bacteria), being investigated. More specific information from individual studies reported in the literature is presented in the text following the table.

Table 5. Summary of animal waste treatment processes and pathogen reductions.

Treatment Process	**Estimated Virus Reduction (log_{10})**
Physical	
Heat/Thermal Processes	
Mesophilic	Typically, 1-2
Thermophilic	Typically, >4
Freezing	Variable; depends on temperature, type of waste and pathogen
Drying or desiccation	Typically >4 at <1% moisture; Typically <1 at >5% moisture
Gamma Irradiation	Typically >3
Chemical	
High pH (>11)	Inactivation at high pH, e.g., alkaline/lime stabilization; typically, >3
Low pH (<2 to <5)	Inactivation at low pH; acidification: Typically, <2
Ammonia	Inactivation at higher pH (>8.5) where NH_3 predominates
Biological Processes	
Aerobic, mesophilic	Typically 1-2
Aerobic, thermophilic (composting)	Typically >4
Anaerobic, mesophilic	Typically 1-2
Anaerobic, thermophilic	Typically >4
Silage treatment, mesophilic	Variable
Land application	Highly variable and largely unknown; potentially high

MANURE SOLIDS WASTE

Manure can contain high concentrations of pathogens (Overcash et al., 1983; Kudva et al., 1998; Zhao et al., 1995). Over time, a decrease in pathogens in untreated manure will occur without intervention but the die-off of microbes may not be extensive and depends on factors such as microbe type, manure physico-chemical characteristics and environmental conditions. *E. coli* O157:H7 has been found to survive for over a year in non-aerated sheep manure (Kudva et al., 1998). *Salmonella* inoculated into cattle slurry were observed to survive for 2-4 months when storage temperatures were 20ºC or less (Jones, 1976). Enteric viruses have been observed to survive for greater than 6 months in semiliquid cattle manure, with lower virus survival times observed for samples having lower fractions of manure solids (Pesaro et al., 1995). *Ascaris* eggs can survive for greater than two years in biosolids held at 4°C (O'Donnell, 1984). Increased pathogen inactivation rates in manure can be achieved using manure treatment techniques, especially those that involve aeration or elevated temperatures. When air drying alone is used as a method for sludge treatment, the sludge must rest on sand beds for a minimum of 3 months with 2 months at temperatures above 0°C (Haug, 1992). This process only inactivates bacteria while viruses and helminths remaining detectable. The time required in various processes and their effectiveness for reductions of pathogens influence the development of methods to achieve pathogen reductions in manure or animal waste treatment systems. Treatment methods selected are based on the potential for pathogen exposure to humans, management of byproducts, the types of waste treated, cost, ease of application, and area required for the process.

Dry Techniques: Composting

Composting depends primarily on indigenous microorganisms to degrade manure waste materials under aerobic and warm conditions. Yet factors such as the heterogeneity of the material, the moisture content, and temperature stability and uniformity throughout the process determine the success for reducing pathogens. Additionally, aeration, presence of bulk organic material (e.g., wood chips, bark mulch, etc.), carbon/nitrogen content, and pH contribute to the efficiency of indigenous microbes in breaking down the waste materials.

Three main types of composting are utilized: pile, windrow, and in-vessel. The pile process consists of mixing the waste with a bulking agent that encourages aeration. Air is blown into the mixture for approximately 21 days followed by a 30-day curing period (Bitton, 1999). During the windrow process, the sludge is also mixed with a bulking agent and then piled into rows 1-2 m high. The rows are turned every few days for 30-60 days. In-vessel composting consists of mixing sludge in a composting vessel while air is forced through the waste materials.

Some agricultural practices utilize composted materials to amend soils with nutrients or conditioners, therefore much of the research on composting is related to the nutrient and soil conditioning properties of the biosolids. Concern about the possible survival of pathogens in composted materials has led to research on the survival of pathogens in these materials, transport of pathogens through manure-amended soils, and the possibility of runoff from land application fields. Pathogens originally present in the feed stock materials may remain in the finished compost if the composting process is not adequately controlled and monitored. Additionally, certain bacterial pathogens present in the original biosolid materials, such as *Salmonella*, may undergo proliferation during or at the end of the composting process, if the reactor or storage conditions are not adequately controlled. The superficial layers of a compost pile will not reach elevated temperatures unless the material is constantly or periodically turned. Under such circumstances, improper temperature regimes within the compost pile can lead to pathogen regrowth due to the sudden increase in available carbon and nitrogen nutrients. Optimization of composting conditions is necessary in an effective method for the reduction of bacteria, viruses, protozoan cysts, and helminth eggs. In a static pile process, a temperature of 55°C or higher must be maintained for 3 days or longer to achieve effective pathogen inactivation (Bitton, 1999). Frequent turning or mixing, on a daily to weekly basis, is important for providing oxygen to the aerobic compost microorganisms, keeping temperatures elevated, and removing excess moisture. However, composting conditions that are effective for inactivating some pathogens (e.g., pathogenic bacteria such as *Salmonella*) may not be effective for inactivating more persistent pathogens (e.g., helminths and viruses).

Viruses, helminth eggs and protozoan parasite cysts and oocysts are generally thought to be the most resistant pathogens to typical composting conditions, but can be completely inactivated when composted at >60°C for 1 hour (Feachem, 1983). In one study, fecal coliforms, fecal streptococci and *Salmonella* were reduced by 5 $\log_{10}$, 4 $\log_{10}$, and 3 $\log_{10}$, respectively, after 14 days of treatment in a combined windrow-static pile composting system (Ponugoti, 1997). An aerobic static pile compost system treating swine manure was reported to reduce enterococci concentrations by 4 $\log_{10}$ after 15 days of treatment (Bhamidimarri and Pandey, 1996).

Salmonella is the focus of many composting studies due to its ability to survive and regrow in composted materials (Sidhu, 1999). The windrow process effectively eliminates *Salmonella* when temperatures of 64-67°C were maintained for 21 days (Tiquia et al., 1998). However, *Salmonella* regrowth can occur if there is incomplete disinfection of the biosolids during the composting process. It is thought that this regrowth is facilitated by the inactivation of competitive, non-pathogenic native microflora in the biosolids (Sidhu et al., 2001). Additionally, storage alone or after composting does not ensure elimination of *Salmonella* in the compost. Composting of sludge for two weeks has been reported to be seven times more effective for inactivating *Salmonella* than sludge storage for 117 weeks (Sidhu et al., 2001). Although windrow composting can be effective for reducing *Salmonella* concentrations, research has shown that more persistent pathogens, such as *Giardia* cysts can remain at relatively high concentrations (200-600 cysts/g dry weight) in windrow-composted material (Gibbs et al., 1998). Gibbs et al. (1998) found that storage of the windrow compost for an additional 30 weeks was needed to reduce *Giardia* cyst concentrations to below their detection limit.

Although mechanical agitation of biosolids during the various stages of composting is crucial to maintaining effective composting conditions, the mixing process may aerosolize pathogens. Milner et al. (1980) reported that mechanical agitation of compost material was a major source of airborne emissions. Neef et al. (1999) monitored airborne microorganisms for 12 months around two different composting facilities (open and closed) in Germany and found that the emission levels of aerosolized molds were higher around the closed composting facility as compared to the open facility. These researchers suggested that additional fungal microbes such as *Saccharopolyspora* spp. and *Thermoactinomyces* spp. should be included in air monitoring programs of composting facilities. Although there are studies documenting the presence of *Aspergillus fumigatus* spores around composting facilities, few studies have been published regarding the presence of specific pathogens such as *Salmonella* spp. Millner et al. (1997) have reported finding airborne *A. fumigatus* spores in close proximity to a composting facility. Nersting et al. (1991) reported that total numbers of microorganisms in composting plants can range between a 500 CFU/m^3 and 105 CFU/m^3 while Gram-negative bacteria can range between 200 CFU/m^3 and 50000 CFU/m^3. The increased occupational risk to compost workers in such settings have been reported (Bunger et al., 2000; Douwes et al., 2000). Douwes et al. (2000) recently reported on the upper airway based occupational exposure of compost workers to microbial agents (endotoxin and beta 1,3 glucan). Bunger et al. (2000) have also reported that high exposure to bioaerosols in compost workers is significantly associated with higher frequency of health complaints and diseases as well as higher concentrations of specific antibodies against molds and actinomycetes. Thus, while composting can be an effective method for reducing pathogen concentrations in animal manure, appropriate process controls and safety measures should be maintained to protect worker health and minimize environmental contamination in the vicinity of composting facilities.

Manure Slurry Treatment Techniques

Manure and other animal wastes are often removed from production areas using water. The resultant manure slurry can then be stored prior to land application, or further treated. Anaerobic lagoons are a popular alternative for liquid manure storage and biological treatment. Due to concerns regarding the extent of waste treatment in anaerobic lagoons, gas emissions from lagoons, and the potential for waste releases during lagoon failures, substantial effort is being focused on developing and evaluating alternative treatment systems for animal waste. Available information is often insufficient to fully evaluate the efficacy of many of these treatment techniques for reducing pathogen concentrations in manure slurry. However, comparatively more relevant data may be available from research on these techniques for treating municipal wastewater.

Physical Treatment Techniques

Physical treatment techniques for flushed manure include sedimentation, screening and filtration to separate solid particles from bulk liquid. Filtration is not a common physical separation technique for animal waste management systems, but techniques such as sand filtration and drying beds may be effective for separating solids in flushed animal waste. However, labor requirements to maintain and operate such systems may be a limiting factor. The primary purpose of sedimentation and screening in animal waste management systems is to reduce the organic and solids loadings to subsequent treatment systems, thereby improving their potential performance and minimizing operation and maintenance problems. Increasingly, solids separation is being investigated for recovery of biosolids as commercial fertilizer or soil amendment. Little data is available to estimate potential pathogen reductions in solids separation units for animal waste. In municipal waste systems, many studies have investigated pathogen removals in primary sedimentation tanks. In general, removals of viruses, bacteria, and protozoa can all be expected to be less than 90% (1 $\log_{10}$) (Feachem et al., 1983; Gerba, 1981; Wheater et al., 1980). For the most part, the solids separation process does not destroy pathogens but only partitions them into the two resulting waste streams, solids and liquids, with about equal numbers in both of them.

Biological Treatment Techniques

Lagoons traditionally are built for wastewater to flow through at specified retention times, resulting in the decrease of biological oxygen demand (BOD), chemical oxygen demand (COD), various nutrients such as phosphorous and nitrogen, and pathogens. Lagoons, also referred to as stabilization ponds in municipal treatment systems, vary based on the type of oxygen environment maintained throughout the reactor water column. Three primary classifications exist for lagoons: aerobic lagoons, anaerobic lagoons, and facultative lagoons. Aerobic lagoons are those in which measurable levels of dissolved oxygen are present throughout the lagoon depth. In multi-cell municipal waste lagoon systems, the final lagoon is often naturally aerobic. In lagoon systems such as those typically found in concentrated animal feeding operations (CAFOs), organic loadings to the lagoons are sufficiently high such that aerobic conditions in the lagoons can only be achieved by forced introduction of air into the lagoons (e.g., by mixing, air blowers, bubble aerators). Facultative lagoons maintain aerobic and anaerobic zones simultaneously. The anaerobic zone occurs deeper in the lagoon while the aerobic zone is closer to the surface. A zone that oscillates between anaerobic and aerobic classifies this type of lagoon as facultative. Anaerobic lagoons are those in which anoxic conditions are maintained throughout the lagoon depth, with the exception of the small surface layer in contact with ambient air. In general, greater pathogen reductions are expected in aerated or facultative lagoons, due to conditions related to the higher metabolic activity present in aerobic biological treatment systems and the toxic effects of oxygenic compounds (Klock, 1971; Marais, 1974; Curtis et al., 1992).

The treatment purpose for a lagoon varies from treating sludge and wastewater to serving as storage or overflow area for other treatment systems. Several considerations must be given prior to utilizing lagoons as a method for waste treatment. Land area, climate, loading rates and subsurface soil conditions are major considerations for lagoon design (Haug et al., 1992).

Anaerobic Lagoon Treatment

Lagoon storage is a common management technique for flushed animal waste because of its cost effectiveness. Lagoons receiving untreated flushed animal excreta (manure and urine) are typically anaerobic throughout their reactor water volume due to the high organic loading that they receive. Under normal conditions, non-aerated animal waste lagoons built and operated according to Natural Resource Conservation Service (NRCS) Code 359 are anaerobic and have hydraulic residence times (HRTs) of over 3 months. Anaerobic swine waste lagoons have been shown to reduce enteric bacterial and viral indicator microbes by 1-2 $\log_{10}$, but concentrations of fecal coliform concentrations on the order of 100,000 cfu/100 mL remain in swine lagoon liquid (Hill and Sobsey, 1998). *Salmonella* and fecal coliforms have been found at geometric mean concentrations of 140-260 MPN/100 mL and 320,000-820,000 CFU/100 mL, respectively, in anaerobic swine waste lagoons (Hill, 2001). *C. parvum* oocysts have been found at concentrations of 1200-2200 oocysts/L in commercial swine waste lagoons in Iowa (CDC, 1998). Overall, reductions of enteric bacteria and viruses in anaerobic animal waste lagoons can be substantial (up to 3 $\log_{10}$), but the effectiveness of

these systems is not consistent (e.g., reductions below 1 $\log_{10}$) and can be affected by seasonal changes in climatic conditions like ambient temperature (Hill, 2001).

Multiple Lagoon Systems

It is well established for municipal waste stabilization pond systems that the use of multiple ponds in series is more effective for reducing nutrient and enteric microbe concentrations than the use of a single pond with the same overall reactor volume (Mara, 1976). Recently, researchers have linked the concept of multiple lagoon systems to recommendations they made for revisions to the World Health Organization's microbiological quality guidelines for the use of treated wastewater in agriculture (Blumenthal et al., 2001). Fecal coliform reductions as high as 4-6 $\log_{10}$ have been achieved in municipal stabilization pond systems having 4-5 ponds in series and overall HRTs of approximately 20-30 days, a range of HRTs that is substantially lower than is typical in CAFO lagoons (Pearson et al., 1996; Dixo et al., 1995; Mara and Silva, 1979). *Salmonella* were found to be reduced by 2-4 $\log_{10}$ in a series of 3 municipal waste lagoons, compared to reductions of 1-2 $\log_{10}$ in single-stage ponds operated at the same HRT as the in-series system (Sandhya and Parhad, 1988). Swine waste lagoon systems having two lagoons in series have been found to achieve substantially higher reductions of *Salmonella* and enteric microbial indicators than single-lagoon systems. Additional reductions of up to 3 $\log_{10}$ can be achieved in secondary lagoons, depending on enteric microbe type and ambient conditions (Hill, 2001).

Aerated Lagoons and Oxidation Ponds

Aeration of manure slurry, whether in traditional lagoons or alternative designs such as oxidation ponds, can substantially increase metabolic activity beyond the extent achievable in anaerobic systems. In addition to increased nutrient removal, it is likely that aeration of flushed waste would increase pathogen reductions. Enteric viruses, *Salmonella*, *Yersinia* and *E. coli* have been shown to be more effectively reduced in aerated pig or cattle slurry than in non-aerated slurry (Derbyshire and Brown, 1979; Lund and Nissen, 1983; Munch et al., 1987). Fecal coliform and fecal streptococci reduction rates have been observed to be higher in municipal oxidation ponds than in non-aerated stabilization ponds (Bahlaoui et al., 1997). Viruses can be reduced by 2-$\log_{10}$ in aerated ponds, but treatment efficiencies are temperature dependant. In cool weather, the time to achieve a 2-log reduction of viruses was found to be five times as long as in warm weather (Bitton, 1999). Bacteria can be reduced by 1-2 $\log_{10}$ in oxidation ponds, with the primary factors influencing inactivation being hydraulic residence time, reactor pH, temperature, predation, and sunlight (Bitton, 1999). Most of the data available regarding pathogen reductions in aerated lagoons and oxidation ponds is from studies of municipal waste treatment systems. Insufficient data is currently available to fully evaluate the effectiveness of these aeration technologies for achieving pathogen reductions in CAFO wastes.

Anaerobic Digestion

Anaerobic digestion is a well-established treatment method for animal wastes and domestic wastewater sludge, and is often used when a design goal is the production of methane gas for energy recovery. Anaerobic digesters can be operated at controlled mesophilic (~ 35ºC) or thermophilic (>50ºC) temperatures, or operated under uncontrolled ambient temperature conditions (e.g., in-ground anaerobic digestion). Under highly controlled conditions of pH, retention times, and anoxia, digestion of organic materials results in the production of methane and carbon dioxide, the dewatering of solids, and the breakdown of large particulate matter. Anaerobic digestion produces nutrient-rich products for land applications, but pathogen reductions are also achieved by this treatment technique.

Mesophilic Anaerobic Digestion

Mesophilic temperatures are generally within the tolerance ranges of most enteric microbes and therefore are not the direct cause of pathogen inactivation during mesophilic anaerobic digestion. There are numerous biologically mediated factors that contribute to pathogen reductions during mesophilic anaerobic digestion. In general terms, the effectiveness of mesophilic digestion for reducing pathogens is greater when temperatures are higher and HRTs are longer. In an anaerobic digester fed cattle slurry at an operating temperature of 28ºC and an HRT of 24 days, *S. typhimurium*, *Y. enterocolitica*, *L. monocytogenes*, and *C. jejuni* reductions were modest: 0.7, 1.4, 0.9 and 0.1 $\log_{10}$ (Kearney et al., 1993). The same researchers found that 90% (1.0 $\log_{10}$) of the *Cryptosporid-*

ium oocysts were inactivated when the digester temperature was 35°C (Kearney et al., 1993). A greater reduction of *C. parvum* oocysts (3 $\log_{10}$ total inactivation) was reported by Stadterman et al. (1995) for an anaerobic digester operated at 37°C and an HRT of 24 hours. Removal of *Ascaris* ova has been demonstrated for mesophilic anaerobic digestion at 25°C, but the HRT of the treatment process was very long (16 months) in order to achieve effective removal (Reimers, 1981).

Other research indicates that mesophilic anaerobic digestion can be somewhat effective for reducing pathogen concentrations in animal waste, especially when digestion is coupled with sedimentation. *E. coli*, *S. typhimurium*, and *S. faecalis* have been reported to be reduced by 0.1-0.8 $\log_{10}$ during anaerobic digestion without sedimentation. The number of organisms removed increased to 1.5 $\log_{10}$ when sedimentation was added prior to anaerobic digestion (Olsen, 1988). Although enteric bacteria are likely to be the microbes reduced to the greatest extent during mesophilic anaerobic digestion, research indicates that antibiotic-resistant enteric bacteria survive longer under these conditions than non-antibiotic-resistant strains (Abdul and Lloyd, 1985). It is likely that enteric bacteria in CAFO animal waste will have heightened antibiotic resistance if subtherapeutic antibiotics are used for animal production on the farm. Ambient temperature, in-ground anaerobic digesters (sometimes referred to as "covered lagoons") are being investigated for treatment of animal waste and energy recovery. As part of their research on an in-ground anaerobic digester receiving untreated swine waste, Cheng et al. (1999) reported a 2.5-log reduction for *E. coli* in the reactor.

Thermophilic Anaerobic Digestion

Pathogen reductions are generally greater at the higher temperatures at which thermophilic anaerobic digesters are operated than at lower, mesophilic temperatures. In similarly loaded municipal anaerobic digesters operated in parallel at an HRT of 20 days, enteroviruses were reduced by 3.3 $\log_{10}$ under thermophilic conditions (at 49ºC), but only by 1.1 $\log_{10}$ under mesophilic conditions (at 35ºC) (Berg and Berman, 1980). Anaerobic digestion at 55°C is effective at inactivating viable *Listeria monocytogenes* and *Salmonella typhimurium* after 2 hours of incubation (Burtscher et al., 1998). Various conditions were assessed in this study, which concluded that the temperature effect was the primary mechanism for inactivation of the microbes. In the same study, viable pathogens were detected under similar conditions at 37°C. Other studies support the effect of temperature on microbial inactivation. Reductions of 4 $\log_{10}$ for enterococci, porcine parvovirus and bovine enterovirus were achieved in 1.1, 11-54, and <0.5 hours, respectively, when manure was anaerobically digested at 55ºC (Lund et al., 1996).

Aerobic Digestion

Aerobic digestion of flushed manure is often used for odor control, nitrogen management and biodegradation of organics, but pathogen survival is an important component to consider. Aerobic digestion breaks down sludge in a reactor supplied with oxygen. This process is usually conducted for 60 days at 20°C or for 15 days at 35°C. The effectiveness of aerobic digestion for reducing pathogens is largely temperature- and time-dependent. Martin et al. (1990) developed a general model for the relationship between time and temperature for aerobic digesters. Their model indicates that digestion should occur for 60 days at 15°C, 40 days at 20°C, 29 days at 30°C or 25 days at 40°C. This data, as well as other studies on anaerobic digestion, show that temperature is the single most important factor for pathogen inactivation in aerobic digesters.

Mesophilic Aerobic Digestion

Mesophilic aerobic digesters operate at temperatures below 45°C, and thus rely more on time-sensitive biological and chemical processes to achieve pathogen reductions than do thermophilic aerobic digesters, for which temperature is the primary mechanism for pathogen inactivation. Mesophilic aerobic digestion in one study of 12 different municipal wastewater treatment plants was reported to reduce fecal coliform, fecal streptococci and *Salmonella* concentrations by 0.68 $\log_{10}$, 0.84 $\log_{10}$ and 0.68 $\log_{10}$, respectively. Further reductions of these microbes of 1-2 $\log_{10}$ was achieved when the digested solids were treated in a lagoon (Ponugoti, 1997). In general, mesophilic aerobic digestion can be expected to reduce enteric bacteria and viruses by 1-2, 1-2 and <1 $\log_{10}$, respectively, at digester HRTs of 10-60 days (Farrah and Bitton, 1984; Martin et al., 1990; Ponugoti et al., 1997). However, mesophilic aerobic digestion is not likely to be effective for inactivating helminth ova (e.g., *Ascaris*) unless long HRTs are employed (Marti et al., 1980; Reimers et al., 1986).

Thermophilic Aerobic Digestion

In a study by Kabrick and Jewel (1982), enteric bacteria were reduced by 2-5 $\log_{10}$ more during thermophilic aerobic digestion than during mesophilic anaerobic digestion. Thermophilic aerobic digestion was also found to reduce viruses to non-detectable levels in digested sludge. At temperatures ≥60°C, thermophilic aerobic digestion can achieve complete inactivation of *Ascaris* eggs as long as the residence time is >1 hour (Kabrick, 1982). In general, thermophilic aerobic digestion can reduce enteric bacteria, viruses and parasites by 3-8 $\log_{10}$, 4-8 $\log_{10}$ and 3-6 $\log_{10}$, respectively, in swine waste slurry and municipal sludge (Ugwuanyi et al., 1999; Kabrick and Jewell, 1982; Boehm, 1984; Burden and Ginnivan, 1978). Although high pathogen reductions can be achieved using thermophilic aerobic digestion, the energy requirements to maintain the target temperature and aerobic environment can make this approach costly. Research on combined thermophilic aerobic digestion and mesophilic anaerobic digestion indicates that effective pathogen reduction can be achieved with this approach, while incurring lower energy costs than would be required if only thermophilic aerobic digestion were used (Pagilla, 2000). A 5-log reduction of fecal coliforms in swine waste was obtained by using this combined system (Pagilla, 2000).

Activated Sludge

Traditional activated sludge systems consist of an aeration tank, where influent is mixed with an aerated suspension of microorganisms, followed by a sedimentation tank, where the waste is clarified and a portion of the settled solids returned to the aeration tank. The activated sludge process can also be performed in a single tank, known as a "sequencing batch reactor" (SBR). A typical activated sludge system can be expected to reduce bacteria, viruses, protozoan parasites and helminth ova in wastewater by approximately 80-99+, 90-99, 80-99 and 0-90%, respectively (Feachem et al., 1983; Bitton, 1999; Schwartzbrod et al., 1989). *C. parvum* oocysts have been shown to be removed by 80-92% in activated sludge systems (Villacorta-Martinez de Maturana, 1992; Stadterman, 1995). In an intermittent-aeration conventional activated sludge system treating swine waste, fecal coliforms and fecal streptococci were reduced by 2.8 and 3.0 $\log_{10}$, respectively, at a system HRT of 17.5 days (Bicudo and Svoboda, 1995). An SBR treating swine waste was shown to be capable of reducing fecal coliforms by 2-4 $\log_{10}$ (APWMC, 1998). Activated sludge systems have been observed to remove >90% of rotaviruses and coliphages (Bitton, 1999). Thus, activated sludge systems are not likely to completely remove or inactivate pathogens in wastewater, but can be expected to reduce their concentrations substantially.

Biofiltration

Biofiltration is a general term for wastewater treatment processes that use high reactor specific-surface areas (e.g., on plastic media) to establish biofilms which metabolize organic compounds and nutrients, and contribute to pathogen reductions. Biofiltration can be performed aerobically by introducing air into the reactors, or anaerobically. Anaerobic biofilters may also be referred to as modified types of anaerobic digesters (e.g., fixed-media, fixed-film, fixed-bed). An aerobic biofilter system treating flushed swine waste was shown to be capable of reducing *Salmonella*, fecal coliforms and coliphages by 1-2 $\log_{10}$ at an HRT of 1 day, although the effectiveness of the system was significantly affected by seasonal fluctuations in reactor temperature (Hill et al., 2002). Little data is available regarding pathogen reductions in anaerobic biofilters, but it appears that enteric microbe reductions in these systems may be less than in aerobic biofilters, unless longer HRTs are provided (Harrison et al.,1999; Cullimore and Viraraghavan, 1994).

Constructed Wetlands

Constructed wetlands are engineered wetland systems for the improvement of water or wastewater quality. Biological treatment occurs in constructed wetlands due to the biological activity of microorganisms and vegetation. Physical and chemical processes also affect nutrient and pathogen removal in these treatment systems. Fecal coliforms have been shown to be removed by 90-99.9% in surface flow and subsurface flow constructed wetlands treating municipal wastewater (Ottová et al., 1997), dairy wastewater (Tanner et al., 1998; Newman et al., 2000) and swine wastewater (Hill and Sobsey, 1998; Hill et al., 2001). Coliphage and protozoan parasite (*C. parvum* and *G. lamblia*) concentrations can be reduced by 95-99% and 58-73%, respectively, during wastewater treatment in constructed wetlands (Gerba et al., 1999; Gersberg et al., 1987). Helminth ova have been reported to be removed by approximately 90% in subsurface flow wetlands at short retention times

(1-4 hours) (Mandi et al., 1996). Important factors for wastewater treatment in constructed wetlands include temperature (Reddy et al., 2000; Hill, 2001) and retention time (Tanner et al., 1995; Hill, 2001). In addition, it appears that pathogen reductions in subsurface flow wetlands are greater than in similarly loaded surface flow wetlands (Hill, 2001), but long-term clogging of subsurface flow wetlands is a potential limiting factor for their use for CAFO waste treatment.

Overland Flow

The overland flow technique is typically performed by applying wastewater to the upper portion of a sloping, grass-covered field of low permeability soil and allowing the wastewater to flow as a sheet through the grass to runoff collection ditches at the bottom of the slope. Treatment occurs in the surface soil and water film flowing over the overland flow area. This wastewater treatment approach is simple, but relatively land-intensive, and may only be capable of achieving moderate reductions in nutrients and pathogens (Hawkins et al., 1995; Hunt et al., 1979). In a study of an overland flow system treating swine lagoon liquid, average reductions of fecal coliforms and other bacterial and viral indicator microbes were reported to be between 0.1 and 0.6 $\log_{10}$ when the system was operated at a hydraulic loading rate of 3 cm/d (Hill and Sobsey, 1998).

Disinfection and Chemical Treatments

There are a variety of chemical and physical approaches that can effectively disinfect CAFO wastewater and manure slurry. Many of these approaches have been evaluated primarily for municipal waste treatment systems, but may find utility as part of CAFO waste management systems. Factors affecting the efficacy of disinfection approaches include suspended solids, dissolved organics, inorganic materials, pH, and temperature. Chlorine and chlorine dioxide efficacy are affected by the pH, while techniques such as ozone and UV are affected by the presence of suspended solids, dissolved organic matter and other reduced compounds (Safe Drinking Water Committee, 1980).

Chlorine

Chlorination is the standard method for disinfection of water. The main disadvantage of chlorination is the production of eco-toxic and carcinogenic by-products when chlorine reacts with organic matter in the water. Chlorination is very effective against bacteria but less effective against viruses and protozoan parasites. Up to a 5 $\log_{10}$ reduction of *E. coli* can be achieved when these microbes are exposed to free chlorine at a concentration of 0.2 mg/L for at least 15 minutes (Sobsey, 1989). A study performed on *Cryptosporidium* exposed to free chlorine showed no to little reductions of oocysts (Venczel et al., 1997). For Giardia, 90-99% reductions in cysts have been observed for water treated with free chlorine (Jarroll et al., 1981, Rice et al., 1982, Leahy et al., 1987, Hibler et al., 1987). Free chlorine (0.5 mg/L) greatly reduces viruses by 99.9 to 99.99%. The study included male-specific phage virus MS2 and human viruses, rotavirus and hepatitis A (Sobsey et al., 1988; Grabow et al., 1983). As for any chemical disinfectant, the efficacy of chlorination can be substantially impacted by the presence of dissolved compounds and suspended organic and inorganic matter, which may exert a chlorine demand and therefore reduce the effective chlorine concentration to which microbes are exposed. Without extensive pre-treatment, it is likely that manure-derived wastewaters will have substantial interfering constituents that will make chlorination ineffective or impractical.

Ozone

Ozone (O_3) is produced when electricity is passed through oxygen (O_2). The ozone gas is bubbled through the water resulting in the oxidation of living organisms. This method is highly destructive to living tissues and can be toxic to humans. Ozone was at first used for taste and odor control in drinking water but was found to effectively reduce pathogens when low turbidity and dissolved organic matter is present in the water. Ozone can reduce pathogen concentrations when other methods of disinfection are not effective. However, organic materials interfere with ozonation and thus pretreatment is needed prior to an effective ozonation process. Ozone applied to create a residual concentration of 0.4 mg/L was found to reduce *E. coli* by 99.99% in secondary effluent (Lazarova et al., 1997). In other studies with *E. coli* and *Salmonella* seeded into activated sludge, ozone effectively reduced these organisms by 3.5 and 4 logs, respectively (Farooq et al., 1983). Farooq et al. (1983) also reported a 2.7 $\log_{10}$ of poliovirus. *Giardia* cysts have been reported to be removed by 99.9% with a residual of 0.3 mg/L (Lazarova, 1999). Some research has been con-

ducted on ozonation of animal waste slurry. Watkins et al. (1997) found that an ozone concentration of 2.0 g/L reduced *E. coli* concentrations by 3 $\log_{10}$ in swine waste slurry. Wu et al. (1998) measured average *E. coli* and coliphage reductions of approximately 1.4 and 0.6 $\log_{10}$, respectively, in swine waste slurry treated at an ozone concentration of 1.0 g/L. In general, ozonation can be effective for disinfecting wastewater, but infrastructure, energy requirements, maintenance demands and costs are factors that will limit the use of this technology on farms.

Chlorine Dioxide

Chlorine dioxide is the product of a chlorate or chlorite salt and an acid, such as hydrochloric acid. The advantage over chlorination is that the typical carcinogenic compounds formed from reactions of free chlorine with natural organic matter in water and wastewater are not formed. However, unlike chlorine, chlorine dioxide dissipates relatively quickly, and therefore residuals are not present at high levels after treatment. The inactivation of pathogens by chlorine dioxide depends on the pH. Higher pH levels result in greater inactivation of pathogens (Safe Drinking Water Committee, 1980). Chlorine dioxide inactivates a wide range of protozoan cysts and oocysts as well as viruses (Lazarova, 1999). Up to 4-5 $\log_{10}$ reductions of fecal bacteria have been achieved with 2-4 mg/L of chlorine dioxide at contact times of 5-15 minutes (Dernat et al., 1997). Human enteric viruses are generally reduced by 99-99.9 % when exposed to chlorine dioxide (Sobsey, 1989). Cysts of the protozoan parasite, *Giardia lamblia*, and oocysts of *C. parvum* have been reported to be reduced by 99% in several studies of chlorine dioxide disinfection (Sobsey, 1989; Belosevic et al., 1997).

Ultraviolet Light (UV) Irradiation

UV disinfection inactivates microorganisms by causing damage to the nucleic acid (DNA or RNA) of the microbes.. UV disinfection only decreases pathogens at the point of exposure to UV, and no disinfection residual is present. If continued disinfection after treatment is required, additional chemical disinfectants like chlorine will need to be added. Of the types of pathogens, viruses are the most resistant to UV disinfection, whereas enteric bacteria and protozoa are susceptible (Turner, 1997; Sobsey, 1989). Reductions of 2-5 $\log_{10}$ have been reported for with the coliphage, MS2, at UV doses of 100-200 mJ/cm^2 (Braunstein et al., 1996, Lazarova et al., 1999, Nieuwstad et al., 1991). However, a 2 $\log_{10}$ reduction of enteroviruses was documented even when low UV doses (32 mJ/cm^2) were used (Lazarova et al., 1999). In secondary and tertiary treated wastewater, a 3-5 $\log_{10}$ reduction in fecal coliforms has been shown at doses of 30-45 mJ/cm^2 (Lazarova, 1999). Although typically thought of as being environmentally-persistent pathogens, protozoan parasites have been shown to be highly susceptible to inactivation by UV irradiation (Campbell et al., 1995; Craik et al., 2001; Shin et al., 2001; Linden et al., 2002). UV irradiation has not been extensively researched for disinfection of animal waste slurries, but research by Hill et al. (2002) indicates that enteric bacteria and viruses can be reduced by 2 $\log_{10}$ in treated and untreated flushed swine waste subjected to low-pressure UV radiation at an incident dose of 60 mJ/cm^2 (Hill et al., 2002). UV disinfection may, therefore, be an effective treatment technique for reducing pathogen concentrations in CAFO wastewater, while retaining the nutrient content of the wastewater for crop production.

Lime Stabilization

Lime [$(Ca(OH)_2$] can be used to "stabilize" and disinfect wastewater, manure slurry, sludge or manure by increasing the pH of the waste to beyond the tolerance ranges of most enteric microbes (e.g., pH 11-12). Lime stabilization is the addition of lime to manure, solids or sludge to produce a pH of 12 during 2 hours of exposure. Lime is less effective against *Ascaris* eggs than it is against other pathogens (Haug et al., 1992). However, research suggests that complete inactivation of *Ascaris* eggs can be attained after storage for 3 months in 10% quick lime with a pH >12 (Eriksen, 1995). Lime mixed with pulverized fly ash was found to be effective at disinfecting sludge produced by municipal waste treatment plants. *Clostridium perfringens*, *E. coli*, *Aeromonas hydrophila, Yersinia enterocolitica*, *Salmonella typhimurium*, *Salmonella typhi*, and *Campylobacter jejuni* were inactivated when stored for 7 days with a pH of at least 11 (Boost, 1998).

Lime stabilization appears to completely inactivate viruses after 12 hours of contact (Sattar, 1976). Poliovirus 1 in municipal sludge was reduced by 99.99% at 43 °C with a contact time of 100 minutes (Ward, 1977). Another study investigating lime treatment of raw municipal wastewater found that at a pH of 11.5 and contact time of 8.5 hours, a lime treatment system removed 91% of

total solids and 99.99, 99.8, ~100, and 97.9% of fecal coliforms, *Salmonella* spp., helminth eggs and protozoan cysts, respectively (Mara and Pearson, 1992). A study performed on pig slurry seeded with bovine and porcine enteroviruses found that the viruses were not detected after at least 24 hours of exposure to lime (Turner, 1997). Research on lime disinfection of pseudorabies virus in pig slurry demonstrated that lime significantly increased the inactivation rate for this virus (e.g., 4.5 $\log_{10}$ in 3 hours and pH 11-12 vs. 2.5 $\log_{10}$ in 14 days without lime addition) (Koch and Euler, 1984). Similar pH inactivation responses are likely for other important viral pathogens such as Foot-and-Mouth Disease Virus (Parker, 1971) and Swine Vesicular Disease Virus (Herniman et al., 1973). Lime treatment can be expected to be an effective disinfection technique for CAFO manures, solids and wastewater, although there is insufficient data for some pathogens (e.g., protozoan parasites and helminths) to evaluate its potential effectiveness for all the pathogen types that may be present in CAFO wastes.

Pasteurization

Pasteurization is a process where sludge is maintained at a temperature of 70°C for 30 minutes. This process effectively reduces pathogens, although maintaining high temperatures increases energy costs for farms. This method is effective at reducing all pathogens but much of the research has focused on thermal treatments in the food industry. Further research would be needed to determine whether this is an economically viable alternative for CAFO waste management systems.

ANIMAL WASTE DISPOSAL OR RECYCLING OPTIONS

Land Application

Land application of animal manure, sludges or flushed waste makes use of the inorganic and organic nutrients in animal waste to condition farm land soil, improve its nutrient content and grow crops. Most research has focused on the application of treated human sewage to land. However, fecal wastes, whether human or animal, may contain pathogens which pose risks to the general population, agricultural workers, or near-by farms if they become exposed to due to run-off, food contamination, or direct contact. For municipal waste, the rate of land application is regulated by state and federal standards for sludge quality, and loading rates are based on metals, organic content and pathogens (40 CFR Part 503). Microbial quality standards have been established in the federal regulations for unrestricted use of municipal sludge [Class A standards (per gram dry weight): < 1,000 fecal coliforms/g, < 3 salmonellae/4g, < 1 enteric virus/4g, and < 1 helminth ova/4g] and restricted use of sludge (Class B standards: < 2,000,000 fecal coliforms/g dry weight). Operational criteria have also been established for treatment technologies meeting the Class A and Class B standards. Other guidelines for the microbiological quality of wastewater used for agriculture have recently been proposed which recommend that wastewater applied to agricultural land contain no more than 1,000 to 100,000 fecal coliforms/100 mL or 0.1 to 1 intestinal nematodes (e.g., *Ascaris*) (Blumenthal et al., 2000). These wastewater concentrations are generally lower than those found in traditional lagoon treatment systems (Hill and Sobsey, 1998; Hill, 2001). Therefore, animal agricultural systems for land application currently allow pathogen levels in excess of the levels recommended for agricultural use of domestic or municipal wastes. This matter needs immediate attention because there are potentially big differences in the allowable or recommended pathogen loadings to land between human and animal wastes, despite the high concentrations of human pathogens that can be present in land applied animal wastes.

For land application of animal waste, recommended waste loading rates have been established by the NRCS as Conservation Practice Standard Code 633 [Waste Utilization (Acre)] based on agronomic (i.e. nutrient) loading rates. Pathogen concentrations or loading rates are not criteria in NRCS Code 633. However, studies of pathogen concentrations in animal waste have shown that high concentrations of pathogens can be present in manure from CAFOs (Duim, 2000, Hill, 2001; Kudva et al., 1998). Waste loading rates and seasonal climate conditions are important considerations for minimizing the transport of pathogens from land application fields to ground water and surface water resources.

Information on the persistence of pathogens in land applied wastes and the factors influencing their persistence and transport are limited and incomplete. Of the factors influencing pathogen survival, UV and drying or desiccation have been specifically studied. Bacteria are highly susceptible

to UV light and desiccation in the environment. Bicknell (1972) showed that cattle grazed on a pasture 2-3 weeks after anaerobically digested human sludge was applied did not become ill. However, if no time passed prior to grazing on a pasture with human sludge, 30/90 cattle became ill with *Salmonella*. Bacterial pathogens tend to degrade in soils and their transport through soil is more limited than for viruses. Most fecal coliform bacteria are retained within the first 60 cm of soil however, but can be transported vertically as deep as 91 meters (Burge, 1978). It has been thought that high doses of bacteria are necessary to cause infection, therefore only gross pollution in water or food poses human health risks from most bacterial strains. This perception is now known to be incorrect for some fecal bacterial pathogens present in animal manure. For example, *E. coli* O157:H7, has an infectious dose of approximately 10-100 organisms, and it has been responsible for waterborne outbreaks attributed to cattle manure (http://www.attorneygeneral.jus.gov.on.ca/html /cad/walkertonrpt1.htm.

Depending on the extent of pathogen inactivation in manure or slurry treatment systems, pathogens may be present in land applied waste at concentrations of concern. When waste is applied to land, these microbes may be transported to ground water along with rainwater infiltration, or be transported to surface water along with runoff from the application fields. Viruses and protozoans have greater potential for off-farm transport and human health impacts than bacterial pathogens. Viruses, due to their small size and relatively greater persistence than bacteria in the environment, can readily pass through subsurface soils to contaminate surficial ground water aquifers (Keswick, 1982). Virus transport varies depending on soil type and adsorption to soil may range from 45-99.9% (Meschke, 1998). Protozoan cysts, though larger than viruses and bacteria, may be able to contaminate on- or off-farm water resources through ground water infiltration or surface runoff because they are highly stable and resistant to environmental degradation. Although helminth ova are also highly stable under environmental conditions, their relatively larger size (larger than protozoan cysts and oocysts) reduces the likelihood that these microbes may contaminate ground water, though runoff from land application fields is still a concern.

During land application, pathogens may become airborne and transported long distances as "bioaerosols." Research suggests that bioaerosols from municipal waste land application sites can increase the risk of infection to on-site workers (Dowd et al., 2000). Enteric viruses and bacteria have been isolated in municipal waste aerosols at distances of 60 m from spray irrigation sites (Brenner et al., 1988; Camann et al., 1988). Enteric microbes have also been isolated from aerosols during spray irrigation of animal waste at cattle and swine farms, at distances of up to 130 m (Boutin et al., 1988). These studies thus suggest that there are possible health risks from bioaerosols generated during land application of animal wastes. However, it is important that epidemiological studies be conducted alongside microbial monitoring studies so that the reliability of risk estimates can be verified using epidemiological data. Bioaerosol sampling used in conjunction with aerosol transport models can be used to estimate exposure during inhalation. This in turn could be used in dose-response models (for a given microorganism) to determine the risks of infection (Haas et al., 1999). Based on actual field sampling data, Dowd et al. (2000) have developed projections of concentrations of organisms per cubic meter (m^3) as predicted by point source and area source models for distances ranging from 100 to 10,000 meters.

Alternative strategies in biosolids and compost management to limit the spread of pathogens via aerosols may include: reducing mechanical agitation of open feed stocks and composted materials, improved facility design and siting to control off-site emissions, bioaerosol dispersion control, and maximizing buffer distances between compost facilities or land application of manure and population centers. Implementation of such control measure should reduce human and animal exposure to pathogen-laden bioaerosols.

Sprayfields

Spray from sprinklers may aerosolize pathogens and non-infectious microorganism thereby resulting in human exposure. In one study, *Salmonella* was detected in 4 of the 15 air samples while *C. perfringes* was detected in 11/15 samples (Dowd, 1997). Survival of the pathogen determines how far from the spray source the pathogen is detected. Factors that affect the dispersion and survival of aerosolized microorganisms include solar radiation, humidity, and atmospheric stability. Temperature and wind velocity did appear to be significant factors affecting the fate of the microorganisms (Teltsch et al., 1978). Low humidity and high solar radiation inactivated bacteria where

as high humidity and darkness enhanced the survival of the microbes. These data suggest that climate and time of day should be considered when utilizing wastewater for spray irrigation. Some research indicates that enteric viruses may travel further from the spray source than bacteria (e.g., *Salmonella* or total coliforms) due to their generally lower inactivation rates in aerosols (Teltsch et al., 1980). The highest concentrations of pathogens may occur up to 50 m from spray source (Sorber et al., 1976; Sorber et al., 1984; Teltsch and Katzenelson, 1978). Coliphage and coliform-like bacteria have been isolated up to 563 m and 198 m from the spray source (Bausum et al., 1982; Sorber, 1976). In one study on spray irrigation of cattle and swine slurry, spray irrigation was shown to disseminate fecal coliforms and fecal streptococci in aerosols up to 145 m downwind of the spraying gun. Bacterial loadings in aerosols generated by a "flail spreading device" and slurry spreading tanker were lower than for high-pressure reel guns at distances greater than 50 m (Boutin et al., 1988).

Although enteric microbe transport in spray irrigation aerosols has been documented, the risk of human infection due to exposure to aerosolized animal wastewater may not be significant. A study of municipal wastewater spray irrigation in Israel reported increased risks of infection for residents of communal settlements practicing wastewater irrigation (Katzenelson et al., 1976). However, subsequent epidemiological studies suggest that municipal wastewater spray irrigation poses no increased risk of infection for exposed populations. In one study, infection from enteric viruses was not significantly higher in populations living near spray irrigation fields than control populations (Ward et al., 1989). In another study, there was no acute infection in occupational workers, but higher antibody levels for enterovirus and coxsackievirus were detected (Linneman et al., 1984).

Spray irrigation is not a method of treatment for wastewater but an approach for water reuse and recycling nutrients. Insufficient research has been conducted specific to wastewater quality and spray irrigation at CAFOs to determine the extent of pathogen transport through spray field aerosolization or the risks posed by this waste management process for nearby residents.

AEROSOLIZATION OF PATHOGENS

With the use of spray irrigation and waste treatment systems to recycle waste, aerosolization of pathogens has become a concern. This section covers the concepts and state of the knowledge regarding pathogen aerosolization as it relates to waste management. Bioaerosols are defined as a collection of aerosolized biological particles. The composition, size and concentration of the microbial populations comprising the bioaerosol vary with the source, dispersal mechanisms in the air, and more importantly, the environmental conditions prevailing at the particular site. Bioaerosols generated from water sources (such as during splashing and wave action) are different from that generated from soil or non-aqueous surfaces in that they are usually formed with a thin layer of moisture surrounding the microorganisms. They often consist of aggregates of several microorganisms (Wickman, 1994). Bioaerosols released into the air from soil surfaces such as those surrounding biosolid and composting facilities are often single units or associated with particles. In many instances the presence of these particulate matter serve as "rafts" for microorganisms (Lighthart and Stetzenbach, 1994). The extent of dispersal (transport) and the settling of a bioaerosol are affected by its physical properties and the environmental parameters that it encounters while airborne. The size, density, and shape of the droplets/particles comprise the most important physical characteristics, while the magnitude of air currents, relative humidity and temperature are the significant environmental parameters. (Lighthart and Mohr, 1987; Pedgley, 1991). It must be emphasized that bioaerosols vary greatly in size ranging from 0.02 to 100 μm in diameter.

Aerosols can be launched from either "point" sources, "linear" or "area" sources. A biosolid pile is an example of a point source while an agricultural field that has been spread with biosolids is an example of an area source (Dowd et al., 2000). The transport of bioaerosols can be defined in terms of distance and time. Submicroscale transport involves very short periods of time under 10 minutes, as well as relatively short distances under 100 m. This type of transport is common within indoor environments. Microscale transport ranges from 10 minutes to 1 hour and from 100 m to 1 km and is the most common and significant type of bioaerosol transport from a human health standpoint. Mesoscale and macroscale transport refers to longer duration bioaerosol transport patterns (Hugh-Jones and Wright, 1970). The diffusion of bioaerosols during their transport is one of the primary

means by which their concentration decreases. Atmospheric turbulence significantly influences the diffusion of bioaerosols. Thus, with biosolid-based composting, the storage conditions of the feedstock, the length and size of the open windrows, the atmospheric conditions during storage and windrow "turning," and the distance to the closest population center have to be taken into careful consideration when evaluating whether composting associated bioaerosols can have public health implications.

Inhalation, ingestion and dermal contact are routes of human exposure for aerosolized microorganisms. Large aerosolized particles are lodged in the upper respiratory tract (nose and nasopharynx). Particles < 6μm in diameter are transported to the lung with the greatest retention of 1-2 μm-sized particles in the alveoli (Randall et al., 1966; Sattar and Ijaz, 1987; Salem and Gardner, 1994). Human microbial pathogens such as *Legionella pneumophila*, *Mycobacterium tuberculosis* and Hantavirus infections are known to be aerosol transmitted and are capable of causing severe infections. Asthma, hypersensitivity pneumonitis and other respiratory illnesses are also associated with exposure to bioaerosols containing respiratory pathogens. The typical route of exposure for organisms that are primarily associated with intestinal infections such as *Salmonella* spp., *Campylobacter* spp, and enteric viruses is based upon the inhalation of bioaerosols containing these pathogens, which are then deposited in the throat and upper airway and swallowed (Wathes et al., 1988). Additionally, the inhaled enteric pathogens may establish throat and respiratory infections that can in turn, increase the risk of swallowing an infectious dose (Clemmer et al., 1960). This could possibly explain why the infectious dose of enteric organisms is lower when these organisms are inhaled as opposed to ingestion (Darlow et al., 1961).

A number of publications over the last few years have documented that aerosolization of microbial pathogens is strongly linked to waste application practices, biosolids handling, wind patterns and micrometeorological fluctuations (Brenner et al., 1988; Lighthart and Schaffer, 1995; Pillai et al., 1996; Dowd et al., 1997). The very process of "turning over" or mechanical agitation of biosolids material at the initial stages of the composting process or during the process itself can generate large amounts of microbial pathogens. Studies conducted around biosolids land application processes have shown that when the biosolids material is physically agitated *Salmonella* and fecal indicator viruses can be released into the surroundings (Dowd et al., 1997). At an arid location in the U.S., Dowd and coworkers detected bioaerosols averaging 300 most probable number (MPN) of *Salmonella* cells /m^3 or air at biosolids loading and application sites. The levels of fecal indicator viruses averaged around 1000 virus particles (PFU)/m^3. On occasions, *Salmonella* at levels up to 3000 MPN/m^3 were detected four miles down-wind. The detection of microbial pathogens at distances away from the point source is indicative how wind gusts and wind patterns can transport bioaerosols over distances. The amount of pathogens bioaerosolized and transported are dictated by the source material, wind patterns and mechanical agitation of the biosolids material. In addition to bacteria, poultry litter contains bacteriophages (Pillai et al., 2000), and fungi. Both bacteria and fungi produce endotoxins that have been identified to be key respiratory irritants. Thus, any off-site migration of bioaerosols and dust will potentially lead to the dissemination of either specific microbial pathogens or endotoxins. Lutgring et al. (1997) have shown that airborne microbial counts in poultry processing plants were highest around the shackling areas and decreased towards the packaging areas.

Not only do outdoor aerosols carry pathogens but indoor particulate matter carries human health concerns. Seedorf et al. (1998) have reported on the concentration and emission of endotoxins in different types of animal (cattle, pig and poultry) facilities in N. Europe. Cattle had the lowest concentration, while the highest concentration was detected within poultry houses. Endotoxin concentration was generally higher in the day than in the night. The high daytime concentration implied that it could be a significant occupational health issue. The mean emission rates from poultry houses were higher than that found in cattle barns. The indoor concentration of heterotrophic bacteria were however higher in cattle barns as compared to poultry houses. Airflow within poultry houses have been shown to influence the transmission of *Salmonella enteriditis* between birds (Nakamura et al., 1997). The contamination of feed and water never preceded the appearance of positive fecal droppings suggesting that bird inhalation was the primary route.

Koerkamp et al. (2000) recently reported that environmental and nuisance problems occur in Europe from poultry operations. They listed odor concentration, ammonia concentration, noise,

dust, pathogen concentration in dust, and sulfur gas as the key air quality parameters. Ellen et al. (2000) have reported that dust concentrations in poultry houses varied from 0.02 to 81.3 mg/m^3 for inhalable dust and from 0.01 to 6.5 mg/m^3 for respirable dust. Houses with caged laying hens showed the lowest dust concentration (< 2 mg/m^3) while dust concentrations in other housing systems were often four or five times higher. The most important source of dust was found to be feathers and fecal material. Thus dissemination and depositing of pathogens by aerosols are other areas of research where the magnitude of impact needs to be discovered.

MICROBIAL DETECTION ANALYSIS TECHNIQUES

The detection and characterization of microbial pathogens in animals and wastes depends significantly on the "age" of the waste material in question. Previous studies have shown that the concentration of the pathogen can decrease depending on the environmental conditions that the waste material is exposed to. Thus, it is theoretically easier to detect specific pathogens from fresh wastes because the numbers of organisms in fresh wastes are generally higher than stored wastes. However, for an "aged" waste, the assay has to be not only sensitive to detect the low numbers of target organisms but has to be able to detect organisms that may have undergone stresses such that they are no longer culturable or detectable if conventional methods are employed. Thus, in such situations it is quite impossible to know what the actual detection sensitivity of an assay (molecular or conventional) is or whether the sample does indeed contain any pathogen. Selection of a measurement technique requires identification of the questions to be answered and timeliness of the results. One of the major constraints in microbial monitoring is the speed of detection. Conventional plating method for the detection of microbial organisms is time consuming and may take up to 2 weeks or even more for confirmation. Delays in confirmation of pathogens with adverse effects on humans sometimes cannot be tolerated, such as during outbreak investigations.

Methods employed for microbial detection represent a myriad of culture, molecular, and chemical techniques. Mass spectrometry, high performance liquid chromatography, and radioimmunoassay, although rapid, require extensive sample preparation, specialized skills and instrumentation, are limited to detection of single pathogenic species and do provide information on viability or infectivity. Molecular methods targeting nucleic acids, such as DNA/RNA fingerprinting, hybridization, or PCR, are still more expensive than traditional culture techniques, may be labor intensive and time consuming and also do not provide definitive information on viability and infectivity. Although molecular methods such as PCR are theoretically very sensitive, may not always be applicable to the samples of interest. Inhibitory components in the sample may prevent the enzymatic process needed to amplify the target nucleic of the microbe, thus leading to false negative results. Additionally, molecular techniques do not indicate the infectious state of the organism if culturing is not performed prior to analysis. To address these problems, hybrid protocols that involve a combination of culture-based enrichment and molecular assays have been developed and are now commercially available (BAX assays, Qualicon, DE). These assays have now been approved for use in food samples. Although not yet commercially available, researchers have also applied the same culture enrichment and molecular assay technique to water and wastes. However, commercially available assays for on-farm use have not become a reality yet. To develop assays that are applicable to animal waste management, concerted efforts have to be undertaken to ensure that the protocols are tested (to the extent possible) on naturally contaminated samples, on samples that have varying numbers of target organisms, on samples that show marked differences in physical and chemical composition, and wastes from different animal species. Sampling regimes and protocols have to consider the heterogeneity of the sample and the potentially "patchy" distribution of the pathogens (Ricke et al., 1998). There is a need for the development of rapid and effective sample processing protocols to detect specific pathogens that may be in low numbers and tightly bound to particulates in waste (Pillai and Ricke, 1995).

Three main steps are necessary for detection of pathogens in waste and environmental samples: capturing, concentrating or culturing of pathogen in a representative, detecting, identify or quantifying the pathogen, and finally characterizing or confirming the identity and properties of the pathogen. Culture techniques typically used for bacterial detection are less expensive but have a limited range for the types of organisms that can be cultured at this time. The issue of viable but

non-culturable microorganisms is still a major limitation of culture-based analytical methodologies. Microbial cells during transport, deposition and sampling are exposed to a variety of inactivating/desiccating which could injure the bacterial cells (Terzieva et al., 1996; Lange et al., 1997). These "injured" cells maybe incapable of being cultured on routine microbiological media. Thus, assays relying on culture-based enumerations can be underestimating the actual number of viable cells within waste and environmental samples. Molecular methods are an alternative to culturing since many bacteria cannot be cultured and molecular methods increase the specificity of detection. Capturing of bacteria for molecular methods can be by filtration or centrifugation of a water sample or extracting from solid samples, and then either direct nucleic acid extraction or isolation on media prior to nucleic acid extraction.

Though molecular biology-based assays such as gene probe hybridization and gene amplifications have the promise to detect and characterize specific microbial groups within waste and environmental samples, the methods still suffer from some technical shortcomings such as inhibitory sample effects, sample processing deficiencies and laborious protocols and possible laboratory-based contamination (Alvarez et al., 1995; Pena et al., 1999). Droffner and Brinton (1995) have reported on the detection of *Salmonella* –specific nucleic acids within thermophilic compost piles suggesting that microbial nucleic acids can be resistant to degradation even at the elevated temperatures found within compost piles. The detection of stable nucleic acid sequences does not imply viable or infectious organisms and so, caution is required when interpreting the public health significance and health risks of molecular analyses, such as gene probe hybridizations and gene amplifications.

Because viruses and parasites in many samples occur in low numbers, concentration of the sample is required prior to molecular or culture analysis. The method of concentration may consist of either capturing microbes on a filter, concentrating by size exclusion filtration, antibody capture, flocculation or centrifugation (Graczyk, 1997a, Lechavallier, 1995, Nieminski, 1995, Schaeffer, 1996). Size exclusion filtration selects for larger particles to remain in the sample while allowing smaller molecules such as water pass through the filter. Antibody capture uses an antibody specific for a microbe to retain the specific microbe on a solid phase, such as a column. The microbe is subsequently released from the column by elution or extraction.

The concentrated sample or captured pathogens are then subjected to a method for detection. The detection methods consist of either an antigen/protein based detection system (i.e. ELISA), molecular detection system (i.e. PCR or hybridization) or culturing method (e.g., infection of cell monolayers). Some viral (hepatitis E virus) and protozoan (*Giardia lamblia*) pathogens at this time cannot be cultured. Therefore, microscopic, immunological or molecular methods are the only ones available of detection.

Detection by of viruses and parasites by most available methods identify only at the major group or genus level. Therefore, additional tests are needed to determine the species. Integrated cell culture PCR (ICC-PCR) is a promising application for the detection and identification of viable oocysts and infectious viruses in environmental samples. The major disadvantage of these methods is that they are not adapted for on-site detection at this time and require days for analysis. An increasing demand for high-throughput screening in the clinical and pharmaceutical industries has produced several technological developments in methods for detecting and analyzing biomolecules, many of which could be applied to the detection of pathogens in the agriculture environment. These emerging technologies include real-time polymerase chain reaction (PCR) and hybridization, flow cytometry, molecular cantilevers, matrix-assisted laser desorption/ionization, immunomagnetics, artificial membranes, ELISA (enzyme-linked immunosorbent assay), gas-phase detection (electronic nose), spectroscopy, and evanescent wave technologies (Food Manufacturing Coalition, 1997). For farm and environmental applications, three technologies have potential applications: electronic nose, spectroscopy, and evanescent wave technology, particularly the surface plasmon resonance (SPR) technique.

On-Farm Verification of Microbial Reduction by Corrective Measures

Electronic nose instrumentation has advanced rapidly during the past 10 years, with the majority of the applications in the foods and drink industry (Kress-Rogers, 1997) and for environmental monitoring (Keller et al., 1994). Electronic noses have also been reported to identify microorganisms. Gardner et al. (1998) reported their electronic nose to be able to identify and differentiate in

vitro bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* with nearly 100% accuracy. Alocilja et al. (1999) have shown the effectiveness of electronic nose in recognizing early infection of potato tubers from the bacterium *Erwinia carotovora.* Younts et al. (1999) have shown that an electronic nose can differentiate between *E. coli* O157:H7 from non-O157:H7 strains.

An electronic nose is a device usually consisting of metal oxide gas sensors coupled with an artificial neural network (ANN). The gas sensors detect the gases and generate a gas signature or pattern; the ANN interprets the pattern. An artificial neural network (ANN) is an information processing paradigm that is inspired by the way biological nervous systems, such as the brain, process information. It is composed of a large number of highly interconnected processing elements (neurons) working in unison to solve specific problems. ANNs, like people, learn by example. An ANN is configured for a specific application, such as pattern recognition, data classification, and forecasting, through a learning process. The most important advantage of ANNs is in solving problems that are too complex for conventional technologies. ANNs have already been successfully applied to various areas of food safety and medical diagnosis, such as diagnostic aides, biochemical analysis, image analysis, and drug development. The ANN approach to food and water safety information processing has several benefits: (1) It is trained by example instead of rules; (2) It is automated; (3) It eliminates issues associated with human fatigue; (4) It enables rapid identification; (5) It enables analysis of conditions and diagnosis in real time; (6) It is reagentless; and (7) It is inexpensive.

The electronic nose technology can be used to develop a "microbial sniffer" to provide an economically viable, reagentless, easy-to-use tool for identifying possible sources of contamination in the farm before infection spreads to unmanageable proportions or enters the food or water supply. Recently, a technique has been developed to detect the gastric ulcer-causing bacteria *Helicobacter pylori* in humans and rhesus monkeys through breath samples (Logan, 1993; Stadtlander and Stutzenberger, 1995). Diagnosis through this simple, noninvasive, inexpensive technique has been 85-100% accurate.

Real-Time Measurement Techniques To Support Microbial Survival, Transport, and Fate Analysis

Evanescent wave technology is a state-of-the-art system that couples antigen-antibody interactions with signal generation. The system used is based on the principle of surface plasmon resonance (SPR). SPR is an optical surface sensing technique that can be used to probe refractive index changes that occur within the immediate vicinity of a sensor surface. A surface plasmon is a charged density wave that oscillates along the surface of a metal. A metal film is layered over a high refractive index medium such as glass or epoxy. Using the Kretschmann geometry, light can be coupled into the surface plasmon mode and monitored as the analytical signal. In this geometry, light passes through a prism and is incident (at a certain angle of incidence) onto a thin metal film. An evanescent wave propagates through the metal and excites surface plasmons on the other side of the film, which is immersed in the liquid sample. SPR can be achieved by varying the frequency of the light, or by varying the angle of incidence. Either way, at some point resonance occurs: the reflected intensity of the light drops dramatically. The position of the SPR is extremely sensitive to the refractive index of the sample, which is unique to different types of bacteria (Spreeta Technology, 1998). Many medical applications, such as the human enzyme creatine kinase (CK), the anticonvulsant drug phenytoin, human chorionic gonadotrophin (hCG), and various other biological contaminants in matrices such as blood, serum, plasma, saliva and urine have been measured with success using SPR (Wortberg et al., 1997). This technology is currently being used in bringing medical diagnostics out of the laboratory to the point-of-care (Spreeta Technology, 1998). Detection can be performed in a matter of minutes (Meeusen, 2000; Meeusen et al., 2000). Recent developments in SPR technology have produced inexpensive (less than $3,000) miniaturized units that can be held in the palm of a person's hand, allowing for easy and convenient use in the field and point-of-diagnosis. The potential for using the technology for on-farm and onsite analysis is tremendous.

Chemiluminescence is one optical technique that can be used to detect the presence of pathogens in the food matrix. Chemiluminescence is the production of light by a chemical reaction in which a substrate reacts with an activating enzyme producing an instantaneous release of light energy. It uses quantitative measurements of the optical emission from excited chemical species to determine

analyte concentration. Chemiluminescence is usually emission from energized molecules instead of simply excited atoms. The bands of light determined by this technique emanate from molecular emissions and are therefore broader and more complex than bands originating from atomic spectra. Furthermore, chemiluminescence can take place in either the solution or gas phase. Chemiluminescence takes its place among other spectroscopic techniques because of its inherent sensitivity and selectivity. It requires no excitation source (as does fluorescence and phosphorescence), only a single light detector, no monochromator, and often not even a light filter. Its strength lies in the detection of electromagnetic radiation produced in a system with very low background. Because the energy necessary to excite the analytes to higher electronic, vibrational, and rotational states does not come from an external light source like a laser or lamp, the problem of excitation source scattering is completely avoided.

Most chemiluminescence techniques need only a few chemical components to actually generate light. Luminol chemiluminescence (Nieman, 1989), which has been extensively investigated, and peroxyoxalate chemiluminescence (Givens and Schowen, 1989; Orosz et al., 1996) are both used in bioanalytical methods. In each system, a "fuel" is chemically oxidized to produce an excited state product. In many luminol methods it is this excited product that emits the light for the signal. In peroxyoxalate chemiluminescence, the initial excited state product does not emit light at all but reacts with another compound; it is this fluorophore which becomes excited and emits light. The oxalate reactions require a mixed solvent system (buffer/organic solvent) to assure solubility of the reagents, optimized pH, and allow compatibility with the analytes.

In luminol chemiluminescence system, the chemiluminescent emitter is a "direct descendant" of the oxidation of luminol (or an isomer like isoluminol) by an oxidant in basic aqueous solution. Hydrogen peroxide is probably the most useful oxidant in luminol chemiluminescence; however, other oxidants have also been used, such as hypochlorite (Cunningham et al., 1998) and iodine (Seitz, 1981). The presence of a catalyst is paramount to this chemiluminescent technique as an analytical tool. Many metal cations catalyze the reaction of luminol, H_2O_2, and OH^- in aqueous solution to increase light emission or at least to increase the speed of the oxidation to produce the emitter and therefore the onset and intensity of light production.

The availability of highly specific and sensitive detection tools may not be the final answer. The assays should be cost-effective and simple so that they can be employed at the production farm level. The profit margin at the production level is so narrow that any assay unless it is cheaper than the conventional methods will not be employed. Alternatively, these assays could be used by the animal or bird breeder. An issue that is a major impediment to the adoption of molecular pathogen detection techniques is the lack of vertical integration within the industries. Except for the poultry industry, which is highly integrated, the rest of the animal industry is not vertically integrated and so the adoption of these methods by small-scale growers and producers is highly uncertain. It is, however, obvious that DNA or RNA based assays will not totally replace culture-based methods. Nevertheless, molecular assays will significantly improve the questions and answers that one can obtain from such pathogen detection assays. More research is needed in this area to integrate detection technologies that can be utilized at the farm level.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

A variety of different kinds of pathogens are likely to be present in animal manures and other wastes. Some of the important pathogens potentially present in animal manures are not present in the U.S., but there are growing concerns that non-endemic pathogens may be introduced either accidentally or deliberately into U.S. animal populations. New pathogens continue to be discovered in agricultural animal populations and the host ranges of these pathogens are uncertain. There are concerns that some of these recently discovered animal pathogens may be able to infect humans or that they have the potential to recombine with human pathogens and produce new strains capable of infecting humans. Particular pathogens of concern in this regard are hepatitis E virus and orthomyxoviruses (influenza viruses).

The reductions of pathogens by animal waste treatment processes and animal waste management systems have not been extensively studied. Therefore, there are considerable uncertainties about the extent to which pathogens survive waste treatment processes, are released into the environment and

are available to be transported off of farms. Off farm contamination can potentially occur inadvertently, such as in unplanned and uncontrolled releases by runoff, aerosolization or infiltration into soils and ground water, or it can occur purposefully when biosolids and other manure residuals are transported off of farms to be land applied, marketed or for other beneficial uses. The extent to which pathogens are removed and inactivated in various waste treatment processes and management systems is uncertain and needs further investigation.

The reductions of some pathogens by some animal waste treatment processes have been determined in laboratory and pilot scale field studies. In general, thermophilic processes, such as pasteurization, thermophilic digestion and composting are capable of producing extensive (>4 $\log_{10}$) pathogen inactivation, and therefore, resulting treated residuals are likely to contain only low pathogen concentrations. Further studies are recommended to better characterize pathogen inactivation in thermophilic processes for manure treatment and to define the optimum conditions to achieve extensive pathogen reductions.

Drying of some animal manures is a widely practiced management approach in some places. However, little is known about the extent to which pathogens are inactivated in manure drying processes or during dry storage because there have been few if any studies to document their effectiveness. Desiccation or drying to very low moisture levels (<1%) has been shown to result in extensive (>4 $\log_{10}$) inactivation of pathogens in municipal biosolids and in soils. Therefore, studies are recommended to determine the rate and extent of pathogen inactivation in drying and desiccation processes for animal manures.

Most mesophilic biological treatment processes for animal manures are not likely to reduce pathogen levels by more than 1-2 $\log_{10}$ or 90-99%, unless several treatment reactors or processes are used in series. Therefore, treated manures, effluents or biosolids from such processes may still contain high concentrations of pathogens. The fate of these pathogens in subsequent management operations, such as land application or prolonged storage, is uncertain and has not been adequately determined. Therefore, further studies on effectiveness of mesophilic treatment processes in reducing pathogens and on the fate of pathogens in these post-treatment management processes are recommended.

Chemical treatments of animal manures are typically by lime or other alkaline treatment. Such treatment is widely practiced for municipal biosolids but less so for animal wastes. Alkaline stabilization for pathogen inactivation has been highly effective in municipal biosolids and promising results have been obtained when it has been applied to animal biosolids. Therefore, further studies are recommended to better characterize pathogen inactivation by alkaline treatments of animal biosolids with respect to solids composition, pH and storage and handling conditions.

The ultimate fate of pathogens in animal manure management systems remains uncertain, especially for large scale, multi-stage systems involving treatment or storage followed by land application at production facilities with large numbers of animals and minimum acreage (confined animal feeding operations). Because of the magnitude of the quantities of animal wastes generated by these facilities and the potentially high pathogen loadings that can result if the treated manure residuals still contain high pathogen concentrations, further investigation of the fate of pathogens in these systems and their surrounding environments is recommended.

Definitive or reference methods to recover and detect many of the pathogens in animal manures and their treated residual solids and liquids have not been reported, especially for emerging pathogens, such as hepatitis E virus, bacteria such as *E. coli* O157:H7 and *Yersinia enterocolitica*, and parasites such as *Giardia lamblia* and *Cryptosporidium parvum*. Therefore, the extent to which these pathogens are removed or inactivated in animal waste treatment processes or management systems remains uncertain due to limitations associated with the pathogen recovery and detection methods. The development, evaluation and application of reliable, sensitive and affordable methods to recover and detect pathogens in animal manures and their treated residual solids and liquids is recommended.

In principle, methods are available to recover and detect some indicator microbes in animal manures and their treated residual solids and liquids. However, the methods for some indicators, such as bacterial viruses (coliphages) and spores of *Clostridium perfringens*, have not been adequately verified and collaboratively tested in these types of samples. Such verification and performance characterization studies are recommended. Also recommended are comparative studies on the re-

moval, inactivation and fate of indicator microbes and animal pathogens in manure treatment processes and management systems. If such studies show that the indicator microbes reliably reflect or predict the responses of the animal pathogens in manure treatment processes and management systems, it then becomes possible to use them in practical, rapid and affordable monitoring and surveillance activities to assess treatment process and system performance and the pathogen quality of the treated residuals.

RESEARCH NEEDS

1. Land application of manure and pathogen transport and survival (run-off and infiltration)—Holistic approach including relationship with nutrient loading.
2. Analyze optimal conditions for land application of manure (loading rates, seasonality, climatic effects etc.).
3. Survival and fate of all categories of organisms in treatment technologies and assess ease of use of technologies on farms.
4. Integrated technology approach to provide cost effective reduction of indicator organisms and pathogens.
5. Identify microbial assays for use to determine real-time performance of treatment technologies and streamline technologies for pathogen specific detection.
6. Clear identification and delineation of occupational health risks as compared to public health risks and effects on management practices.
7. The influence of regional climatic and soil factors that influence the dissemination of microbial pathogens, endotoxins in air, soil and water needs to be carefully identified for the poultry, swine and beef cattle industries.
8. Integration of microbiological monitoring data with GIS data sets so that predictive or forecasting capabilities become available.
9. Develop streamlined samplers and sampling designs so that microbiological information can be obtained from the air, soil, water and foods.
10. Develop better and more importantly, user-friendly mathematical models and tools to identify the pathogen exposure routes and quantitate the possible health risks.

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