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# Detecting and Mitigating the Environmental Impact of Fecal Pathogens Originating from Confined Animal Feeding Operations: Review

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# Detecting and Mitigating the Environmental Impact of Fecal Pathogens Originating from Confined Animal Feeding Operations: Review

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# Notice

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to the Agency's review and has been approved for publication as an EPA document.

# Foreword

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This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

**Sally Gutierrez, Director**
**National Risk Management Research Laboratory**

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# 1. Introduction and Overview

The trend in animal production has shown a dramatic shift in the last 50-60 years from small family farms and grazing operations towards large commercial confinement operations. Since 1982, animal production at these facilities has nearly doubled while at the same time they have become more spatially concentrated (U.S. Department of Agriculture, National Resources Conservation Services (USDA-NRCS), 2000). Recently, the U.S. Department of Agriculture (USDA) reported that more than 80% of all livestock revenues are generated in confinement facilities that account for a scant 18% of all livestock operations (USDA-NRCS, 2002). In fact, more than 43% of all beef cattle, dairy cattle, swine, and poultry are raised in the largest two percent of operations (Goellehon *et al.*, 2001). The concentration of animals into confinement facilities poses many environmental challenges, among which pathogenic microorganisms of fecal origin are of concern.

The U.S. Environmental Protection Agency (USEPA) defines a concentrated animal feeding operation (CAFO) as an animal feeding facility that houses more than 1,000 animal units (AU), has 300 to 1000 AU but meets certain conditions, or is designated a CAFO by the state (USEPA, 2001). The number of animal units are based on an equivalent number of beef cattle. Therefore, 1,000 AU equals 1,000 beef cattle, 700 mature dairy cattle, 2,500 swine, 5,000 ducks, 10,000 sheep, 55,000 turkeys, or between 30,000 and 100,000 laying hens or broilers depending on the animal waste management system employed. According to National Resources Conservation Service (NRCS) estimates, 11,398 CAFOs (>1000 AU) were in operation in the U.S. in 1997, and comprised five percent of all livestock facilities (USDA-NRCS, 2002). These CAFOs were largely commercial operations (94%) with large revenues. Total agricultural sales for 97.9% of CAFO owners exceeded $500,000 per year. In comparison, non-commercial livestock facilities (intermediate or rural-residence farms) earned 22.3% of all livestock revenue, generating on average $18,500 per farm. Figure 1 shows the distribution of confined poultry, swine, dairy cattle, and feed cattle operations in the U.S. in 1997.

Animal agriculture results in the production of copious amounts of manure, much of which is ultimately used as fertilizer for crops or spread onto land. On a per weight basis, livestock animals produce between 13 and 25 times more manure than humans. Comparing the most recent U.S. census data and USDA livestock reports, it can be estimated that animals produce somewhere between 3 and 20 times more manure than people in the U.S. each year, as much as 1.2 – 1.37 billion tons (wet weight) (American Society for Microbiology (ASM), 1998; USEPA, 2003; USEPA, 2004). This is enough to cover a land mass the size of Rhode Island with more than twelve inches of manure. Even moderate livestock operations can produce as much manure as a small sized city. For example, a 2,500-head dairy cattle operation can produce a waste load similar to a city of 61,000 people. Two important differences are that livestock CAFO animal wastes can be as much as 100 times more concentrated than human wastes, and the treatment of human wastes is required by law prior to discharge into the environment (USEPA, 2001).

Animal wastes contain zoonotic pathogens, which are viruses, bacteria, and parasites of animal origin that cause disease in humans. Diseases that can be caused by zoonotic pathogens include Salmonellosis, Tuberculosis, Leptospirosis, infantile diarrheal disease, Q-Fever, Trichinosis, Cryptosporidiosis, and Giardiasis to name a few. These diseases typically present as mild

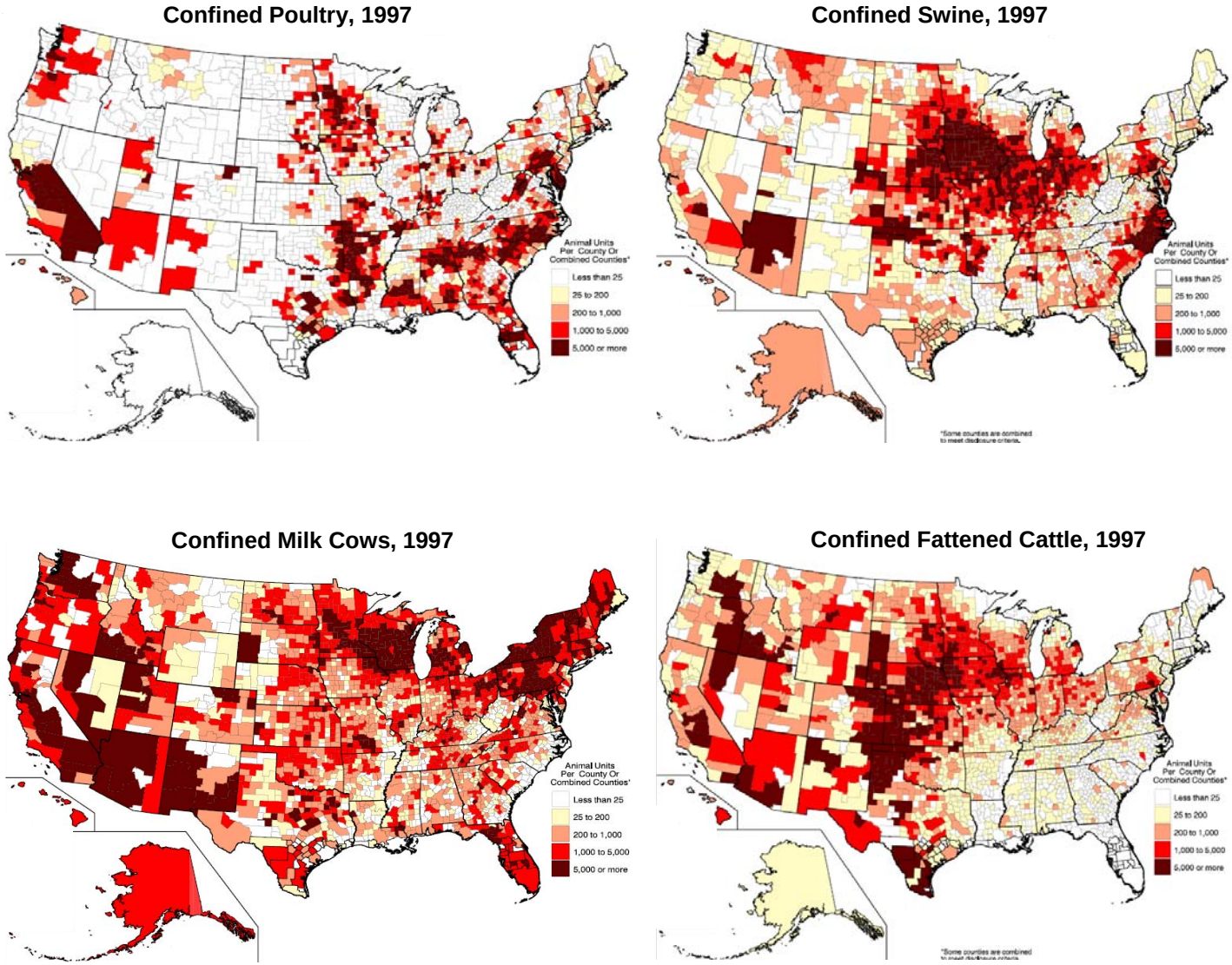


**Figure 1.** Confined swine, poultry, dairy cattle, and feed cattle per county in 1997 (adapted from USDA-NRCS, 2002).

diarrhea, fever, headaches, vomiting, and muscle cramps. In more severe cases, however, these diseases may cause meningitis, hepatitis, reactive arthritis, mental retardation, miscarriages, and even death, particularly in the immunocompromised. The dosing of livestock animals with copious amounts of antimicrobial agents for growth promotion and prophylaxis may promote antimicrobial resistance in pathogens, increasing the severity of disease and limiting treatment options for sickened individuals (Lee *et al.*, 1994; Marano *et al.*, 2000).

Zoonotic diseases from livestock animals, transmitted through air, water, and food, cause significant human suffering and economic losses in the U.S. every year (Schlech *et al.*, 1983; Besser *et al.*, 1993; MacKenzie *et al.*, 1994; Solo-Gabriele and Neumeister, 1996; Hoxie *et al.*, 1997; Mead *et al.*, 1999; Valcour *et al.*, 2002; Clark *et al.*, 2003). Increasing the concentration of animals in confinement facilities amplifies the potential for localized runoff and contamination, increasing the probability for accidental exposure of susceptible individuals. In fact, living near CAFO operations has been associated with significant deterioration in human health including increased gastrointestinal illness, headaches, sore throats, sinusitis, and childhood asthma (Wing and Wolf, 2000; Merchant *et al.*, 2005). There is increasing evidence that impoverished and nonwhite communities may be burdened with a disproportionate share of not only these negative health outcomes, but also pollution and offensive odors emanating from CAFO facilities (Wing *et al.*, 2000; Wilson *et al.*, 2002; Wing *et al.*, 2002). Based on studies in North Carolina, operations run by cooperate investors may be more likely to be concentrated in poor and nonwhite areas than operations run by independent growers (Wing *et al.*, 2000).

The USEPA recognizes the need to improve manure management practices at confined animal feeding operations (USEPA, 2003). Several other U.S. governmental entities, including the U.S. Department of Agriculture (USDA), U.S. Geological Survey (USGS), and Centers for Disease Control and Prevention (CDC), have also recognized the need for control of pathogens at CAFOs and have robust research and surveillance activities to improve the outcomes for public health and welfare in the U.S. Several recent advances in the fields of medicine, molecular microbiology, engineering, agronomy, and epidemiology are addressing issues pertinent to the control of pathogens from CAFOs at a rapid pace. However, reported literature and research activities can in some cases be divergent between some disciplines and repetitive between others. There is a lack of integration of both knowledge and skills necessary to drive the research in an appropriate direction. As stated by Landry and Wolfe (1999):

> *The range of disciplines conducting fecal bacteria research and the diverse nature of the literature are obstacles to application and synthesis of existing knowledge by animal waste managers and scientists.*

In this report, we synthesize the current state of knowledge regarding pathogen research as it relates to livestock CAFOs, including a summary of research ongoing at USDA and other federal agencies. Pathways for the release of zoonotic agents and antimicrobial-resistant bacteria endemic in animals raised in confinement and their potential to persist in different *milieus* are reviewed. We discuss the impact to the environment and public health and welfare posed by the release of these agents from CAFOs, as well as manure management practices that are employed to mitigate their release into the environment. The objectives of this review are to summarize pathogen issues with regard to livestock CAFOs and identify and discuss gaps in the research that need to be addressed to improve public health.

## 2. Pathogens

Livestock animals can harbor and shed viruses, bacteria, protozoan parasites, and helminthes that are pathogenic for humans, other domestic animals, and wildlife. Pathogens present in animal carcasses or shed in animal wastes may include rotaviruses, hepatitis E virus, *Salmonella* spp., *E. coli* O157:H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum*, and *Giardia lamblia* to name a few (Sobsey *et al.*, 2002). These zoonotic pathogens can exceed millions to billions per gram of feces, and may infect humans through various routes such as contaminated air, contact with livestock animals or their waste products, swimming in water impacted by animal feces, exposure to potential vectors (such as flies, mosquitoes, water fowl, and rodents), or consumption of food or water contaminated by animal wastes (Schlech *et al.*, 1983; Bezanson *et al.*, 1983; Hawker *et al.*, 1998; Valcour *et al.*, 2002; Armand-LeFevre *et al.*, 2005). The consequences of infection by pathogens originating from animal wastes can range from temporary morbidity to mortality, especially in high-risk individuals. Antimicrobial use in animal agriculture may exacerbate the problem by increasing the resistance of these pathogens to therapeutic drugs used to treat human disease.

It has been estimated that 61% of all human pathogens and 75% of emerging human pathogens are zoonotic (Mahy and Murphy, 1998; Murphy, 1998; Taylor *et al.*, 2001; Woolhouse *et al.*, 2002). The overwhelming majority of these pathogenic zoonoses that commonly infect humans are related to animal husbandry practices. Table 1 lists some of the zoonotic pathogens that may be of concern in animal agriculture. Many of these pathogens are endemic in livestock and difficult to eradicate from the animals or their production facilities (Sobsey *et al.*, 2002). For instance, a study of healthy swine on eight farms in Iowa and North Carolina revealed greater than 90% incidence of *Campylobacter coli* in all three growth stages (nursery, grower, and finisher) (Wesley *et al.*, 1998). Similarly, the prevalence of *Salmonella* spp. and *Campylobacter jejuni* have been reported to be as high as 100% in poultry operations, *Yersinia enterocolitica* as high as 18% in swine operations, and *Giardia lamblia* and *Cryptosporidium* spp. as high as 100% in cattle operations (Olson, 2003). The primary reservoir for *E. coli* O157:H7 was determined to be healthy cattle in one study in Canada, although this bacterium is also endemic to swine and sheep (Jackson *et al.*, 1998). In the U.S., *E. coli* O157:H7 infection was widely distributed across all 13 states at an average rate of 1.61% of all cattle when tested in 1994 (Dargatz, 1996). At slaughter, the prevalence of *E. coli* O157:H7 in Scottish cattle may be greater than 13% (Low *et al.*, 2005). Fratamico *et al.*, (2004) tested 687 swine fecal samples from swine operations in 13 of the top 17 swine-producing states and determined that 70% of the samples were positive for shiga-toxin (*stx 1* and *stx 2*) genes. Due to the endemic nature of zoonotic pathogens in livestock, there is a clear need for appropriate management practices at livestock facilities that are protective of human health and the environment and firmly grounded in risk analyses.

The risk of contracting disease following exposure to livestock wastes is dependent on the properties of the infectious agent, the exposed individual, the route of exposure, and the dose. There are a wide range of infective doses for different pathogens as shown (Table 1). For instance, severe gastrointestinal illness may require the ingestion of millions of *Yersinia enterocolitica* bacteria, or as little as 5 to 10 *E. coli* O157:H7 cells (PHAC, 2005). The infectious doses listed in Table 1 were established based on infectivity studies in healthy

individuals, and therefore, may not be particularly useful for establishing safe exposure limits for human health. Particularly susceptible individuals such as children, the elderly, or the immunocompromised, which represent nearly 25% of the U.S. population may succumb to infection at much lower doses than the general population (Naumova *et al.*, 2003). For instance, approximately 70% of the diarrhea-associated deaths in the U.S. each year occur among individuals 55 or older.

Regulatory limits on the concentrations of pathogens in the environment protective of human health have not been established. As such, pathogenic organisms are rarely monitored in waste streams from animal feeding operations. Difficulty in quantifying pathogens at relevant concentrations in environmental matrices, the large number of analytical tests that would be required to measure all of the zoonotic pathogens shed in livestock feces, and a lack of epidemiological data to establish appropriate and safe levels of pathogens in the environment have all led to this deficiency. Due to the difficulties in quantifying pathogens, indicators of fecal pollution, including coliform bacteria, fecal coliforms, *E. coli*, and/or Enterococci have been monitored in lieu of overt pathogens for more than 100 years (Smith, 1893; Allen *et al.*, 1952; Kirschner *et al.*, 2004; Byamukama *et al.*, 2005). Epidemiological evidence supports the relationship between the fecal indicator bacteria *E. coli* and enterococci and incidence of gastrointestinal illness following recreational water exposure, and provides the basis for local, state, and federal water quality regulations (USEPA, 1986). However, the works of several researchers has shown that these indicators are not reliable surrogates for many pathogens, including bacteria and most viruses and parasites (Seligmann and Reitler, 1965; Boring *et al.*, 1971; Wetzler *et al.*, 1979; Carter *et al.*, 1987; Geldreich, 1996; Ashbolt *et al.*, 2001; Grabow, 2001; Leclerc *et al.*, 2001; Tillett *et al.*, 2001; Hörman *et al.*, 2004; Harwood *et al.*, 2005). New approaches for detecting pathogens are needed to improve monitoring systems. There also remains a need for epidemiological data to enable the identification of appropriate and safe limits of pathogens in the air, drinking water, recreational water, and in food. Based on surveillance of water and foodborne outbreaks in the U.S., priority for standard methods and recreational and drinking water guidelines should be given to *Salmonella* spp., *Campylobacter jejuni*, *E. coli* O157:H7, *Cryptosporidium*, *Giardia*, and selected viral agents indicative of viral contamination. Priority should be established on the incidence of a particular illness due to a pathogen or the severity of the illness or possibly both.

**Table 1.** Selected zoonotic pathogens zoonoses that may be of concern for water quality near CAFOs †

| Infectious Agent | Infectious Dose | Incubation Period | Disease<br>*Symptoms* | Host Range | Reservoir |
|---|---|---|---|---|---|
| **Bacterial** | | | | | |
| ***Bacillus anthracis*** | 8000-50000<br>*(by inhalation)* | 2-5 days | **Anthrax, Wool sorter's disease**<br>*Cutaneous – skin lesions, death (5-20%)*<br>*Inhalation – respiratory distress, fever, shock, death*<br>*Intestinal – abdominal distress, fever, septicemia, death (rare)* | Humans, cattle, swine, goats, sheep, horses | Spores remain viable in soil contaminated by animal wastes for years |
| ***Brucella spp.*** | Unknown | Highly Variable 5-60 days | **Brucellosis, Undulant Fever, Bang's Disease, Malta Fever, Mediterranean Fever**<br>*Intermittent fever, headache, weakness, profuse sweating, chills, arthralgia* | Humans, cattle, swine, goats, sheep, deer, caribou, elk, dogs, coyotes | Cattle most common |
| ***Campylobacter jejuni*** | ≤500<br>*(by ingestion)* | 1-10 days | **Campylobacter enteritis, Vibrionic enteritis, Traveler's Diarrhea**<br>*Diarrhea, abdominal pain, malaise, fever, nausea, vomiting, septicemia, meningitis, Guillain-Barré syndrome, death (rare)* | Humans, cattle, swine, goats, sheep, poultry, rodents, birds, household pets, | Cattle, swine, sheep, poultry household pets, rodents, birds |
| ***Clostridium tetani*** | Toxin is extremely potent | 3-21 days | **Lockjaw, Tetanus**<br>*Painful muscular contractions, abdominal rigidity, spasm, death (30-90%)* | Humans, animals | Intestine of animals and humans, soil contaminated with animal feces |
| ***Coxiella burnetii*** | 10<br>*(by inhalation)* | 2-3 weeks | **Q fever, Query Fever, Rickettsia**<br>*Acute febrile disease – chills, headache, weakness, malaise, severe sweats, pneumonitis, pericarditis, hepatitis*<br>*generalized infections – endocarditis* | Humans, cattle, sheep, goats | Sheep, cattle, goats, especially at parturition |

† Hazen and Toranaos, 1990; WHO, 1993; DuPont *et al.*, 1995; Morris and Levin, 1995; Geldrich, 1996; ASM, 1998; Haines *et al.*, 2004; PHAC, 2005

**Table 1.** Selected pathogenic zoonoses that may be of concern for water quality near CAFOs (*Continued*)

| Infectious Agent | Infectious Dose | Incubation Period | Disease<br>*Symptoms* | Host Range | Reservoir |
|---|---|---|---|---|---|
| **Bacterial (*Cont.*)** | | | | | |
| **Enterohemorrhagic *Escherichia coli*** (*E. coli* O157:H7 and others) | 5-10 | 2-8 days | **EHEC, Verotoxin-produding *E. coli*, VTEC, Shiga toxin-producing *E. coli*, STEC**<br>*Hemorrhagic colitis, abdominal pain, bloody diarrhea, fever, hemolytic uremic syndrome, thrombocytopenic purpura, death (in children)* | Humans, cattle, swine, goats, sheep, poultry | Humans and livestock animals |
| **Enteropathogenic *Escherichia coli*** | $10^8$-$10^{10}$ in adults, Unknown in infants | 0.5-3 days | **Attaching and effacing *E. coli*, enteroadherant *E. coli*, infantile diarrheal disease**<br>*Watery diarrhea, fever, cramps, vomiting, bloody stool in some cases, serious disease in infants* | Humans (esp. infants), cattle, swine, goats, sheep, poultry | Humans and livestock animals |
| ***Leptospira* spp.** | Unknown, but may be as low as 3 | 4-19 days | **Leptospirosis, Weil's Disease, Canicola fever, Hemorrhagic jaundice, Mud fever, Swineherd's disease**<br>*Fever, headache, chills, muscle aches, vomiting, meningitis, rash, jaundice death (rare)* | Humans, cattle, swine, horses, dogs, rats, wild animals | Farm and pet animals, rats and rodents (urine and abortion products) |
| ***Listeria monocytogenes*** | Unknown, but likely less than $10^3$ | 3-70 days *(mean = 21)* | **Listeriosis, Listerella**<br>*Fever, muscle aches, nausea, diarrhea, headache, stiff neck, confusion, loss of balance, convulsions miscarriage or stillbirth, premature delivery, death in about 20% of all cases* | Mammals, birds, fish, crustaceans, and insects | Domestic and wild mammals, fowl, and humans (aborted fetuses of livestock animals) |
| ***Mycobacterium bovis* *M. tuberculosis*** | 10 *(by inhalation)* | 4-12 weeks | **Tuberculosis, TB**<br>*Fatigue, fever, cough, chest pain, hemoptysis fibrosis, irreversible damage to lungs* | Humans, cattle, swine, other animals | Humans, diseased cattle, swine, and other mammals |
| ***Salmonella* spp.** (non-*typhi* or *paratyphi*) | 100-1000 *(by ingestion)* | 0.25-3 days | **Salmonellosis, Acute Gatroenteritis**<br>*Abdominal pain, diarrhea, nausea, vomiting, dehydration, septicemia, reactive arthritis* | Humans, cattle, swine, poultry, horses, rodents, household pets | Humans, cattle, swine, poultry, horses, rodents, domestic pets |
| ***Yersinia enterocolitica*** | $10^6$ | 3-7 days | **Yersiniosis, enterocolitis, pseudotuberculosis**<br>*Diarrhea, acute mesenteric lymphadenitis mimicking appendicitis, fever, headache, anorexia, vomiting, pharyngitis, reactive arthritis* | Humans, swine, household pets | Primarily swine |

**Table 1.** Selected pathogenic zoonoses that may be of concern for water quality near CAFOs (*Continued*)

| Infectious Agent | Infectious dose | Incubation Period | Disease<br>*Symptoms* | Host Range | Reservoir |
|---|---|---|---|---|---|
| **Protozoans** | | | | | |
| ***Balantidium coli*** | Unknown, may be as low as 10-100 | 4-5 | **Balantidiasis, Balantidiosis, Balantidial dysentery**<br>*Diarrhea, dysentery, abdominal colic, tenesmus, nausea, vomiting, bloody and mucoid stools* | Humans, swine | Primarily swine, also rodents |
| ***Cryptosporidium parvum*** | 132 | 1-12 | **Cryptosporidiosis**<br>*Diarrhea, cramping, abdominal pain, weight loss, nausea, vomiting, fever*<br>*Prolonged symptoms and in some instances death in immunocompromised host* | Humans, small and large mammals, poultry, fish, reptiles | Humans, cattle, and other domestic animals |
| ***Giardia lamblia*** | 1-10<br>*(by ingestion)* | 3-25 | **Giardiasis, Lambliasis, "Beaver Fever"**<br>*Diarrhea, abdominal cramps, bloating, fatigue, weight loss, severe hypothyroidism, lactose intolerance, chronic joint pain* | Humans, wild and domestic animals, household pets | Humans, wild and domestic animals |
| ***Toxoplasma gondii*** | Unknown | 10-23<br>*(by ingestion)* | **Toxoplasmosis**<br>*Mild cases – diarrhea, localized lymphadenopathy, fever, sore throat, and rash*<br>*Severe cases – stillbirths, abortion, newborn syndrome, hearing and visual loss, mental retardation, dementia and/or seizures* | Humans, felines, most warm blooded animals and birds | Cats, cattle, swine, chicken, sheep, goats, rodents, and birds |

**Table 1.** Selected pathogenic zoonoses that may be of concern for water quality near CAFOs (*Continued*)

| Infectious Agent | Infectious dose | Incubation Period | Disease<br>*Symptoms* | Host Range | Reservoir |
|---|---|---|---|---|---|
| **Helminthes** | | | | | |
| ***Schistosoma* spp.** | Unknown | 14-42 | **Schistosomiasis, Bilharziasis, Snail Fever, Swimmer's Itch**<br>*S. mansoni and S. japonicum –diarrhea, abdominal pain, and hepatosplenomegaly*<br>*S. haematobium – urinary manifestation including dysuria and hematuria*<br>*Chronic infections may lead to liver fibrosis, portal hypertension, or colorectal malignancy* | Humans, cattle, swine, water buffalo, horses, rodents, and household pets | Humans, cattle, swine, water buffalo, horses, rodents, household pets |
| ***Trichinella spiralis*** | Unknown | 1-2 days for gastrointestinal symptoms<br>2-4 weeks for systemic symptoms | **Trichinelosis, Trichinosis, Trichiniasis**<br>*Malaise, nausea, diarrhea, abdominal cramping, muscular soreness, edema of upper eyelids, eosinophila, ocular pain, photophobi, pneumonitis, remittent fever, cardiac and neurologic complications or death* | Humans, swine, household pets, rodents, wild mammals, and marine mammals | Swine, household pets, rodents, wild animals |

**Table 1.** Selected pathogenic zoonoses that may be of concern for water quality near CAFOs (*Continued*)

| Infectious Agent | Infectious dose | Incubation Period *days* | Disease *Symptoms* | Host Range | Reservoir |
|---|---|---|---|---|---|
| **Viruses** | | | | | |
| **Hepatitis E Virus** | Unknown | 14-63 | **HEV** *Jaundice, anorexia, hepatomegaly, abdominal pain, nausea, vomiting, fever, Liver Failure; most severe hepatitis during pregnancy of all hepatitis viruses* | Humans, swine, rodents, chicken | Unknown – possibly in swine |
| **Influenza A virus** | 2-790 | 1-4 | **Flu** *Acute fever, chills, headache, myalgia, weakness, runny nose, sore throat, cough* | Humans, swine, horses, domestic and wild avian species | Humans, animal reservoirs (particularly swine) are suspected as sources of new human subtypes |
| **Lymphocytic choriomeningitis virus** | Unknown | 8-21 | **LCM, Lymphocytic meningitis** *Mild influenza-like illness or maningeal or meningoencephalomyelitic symptoms, Guillain-Barré type syndrome, orchitis or parotitis.* *In more severe cases, temporary or permanent neurological damage, abortion, congenital hydrocephalus, and mental retardation* | Humans, swine, household pets, rodents | Rodents, swine, household pets |
| **SARS Coronavirus** | Unknown | 6.4 (mean) | **SARS** *High fever, dry cough, dyspnoea, myalgia, diarrhea, vomiting, death (13.2% for infected individuals under 60, 43.3% for those over 60)* | Humans, swine chickens, ferrets, cats, macaques | Unknown – but animal reservoir is suspected |
| **West Nile Virus** | Unknown | 3-14 | **West Nile Encephalitis, Viral Encephalitis** *Sudden onset of flu-like illness, malaise, anorexia, nausea, vomiting, rash, and lymphadenopathy.* *More severe infections can result in aseptic meningitis or encephalitis, mental status changes, seizures, coma, severe neurologic disease, and death (4-11%)* | Mammal, reptilian, and avian hosts. Mammals generally considered dead-end hosts | Birds are the amplifying host |

# 3. Antimicrobial Resistance

Antimicrobial agents include all types of natural or synthetic substances capable of killing or inhibiting the growth of microorganisms. Antimicrobials include antibiotics, antivirals, antifungals, probiotics, disinfectants, sanitizers, food preservatives, antimicrobial pesticides/biocides, and wood preservatives among others (Health Canada, 2002). The proper use of antimicrobial agents is an integral component of good animal agriculture practices. However, their use may be exacerbated in large confinement facilities where animals are raised in close quarters and infection in one animal can rapidly spread through hundreds or even thousands of animals. Many times, infection of one animal leads to the treatment of many animals within the facility prophylactically (Shea, 2004). Additionally, antimicrobial agents have long been administered in sub-therapeutic (non-lethal) doses to livestock animals in their feed, with the ultimate goal of increased animal growth rates. Table 2 lists the antimicrobials used therapeutically and non-therapeutically (prophylaxis and growth promotion) in livestock animals in the United States.

The use of antimicrobial compounds in animal feed has increased more than 10-fold since the 1950s, as total U.S. production of antimicrobials increased from approximately 1 million pounds in 1950 to as much as 44 million pounds in 1986 (Levy, 1992; U.S. Congress, OTA, 1995; McEwen and Fedorka-Cray, 2002). The rise in agricultural use of antimicrobial agents is certainly related to changes in their production and availability, improvements in animal health practices, increasing need for therapeutic use as animals are confined into smaller and more densely packed housing units, a perceived need for prophylactic use due to close confinement and increased risk of the spread of disease, and realization of the financial benefits of shortening the time to reach market weight. According to Dewet *et al.*, (1997) farmers with large operations are much more likely than those with small farms to use antibiotics in feed supplements for growth promotion and prophylaxis. Of the large confinement operations, those working with veterinary consultants were twice as likely to use such feed additives. In a recent survey of antimicrobial treatment practices, approximately 83% of feedlots administered at least one antimicrobial to cattle in feed or water for prophylaxis or growth promotion (Animal and Plant Health Inspection Service, 1999). Precise figures on the use of antibiotics in animal agriculture are not available, but Table 3 shows some recent estimates by various sources. Although estimates shown in Table 3 vary, three facts remain: the use of antimicrobials in animal agriculture has increased substantially since the 1950s, copious amounts of antibiotics are used every year in livestock animals, and most of the antimicrobials used are for growth promotion and prophylaxis, not for the treatment of sickened animals.

## *3.1 Mechanisms of bacterial resistance*

Each class of antimicrobial compound operates at a specific site within the bacterial cell. Bacitracin, cephalosporins, penicillins, ionophores, and polymyxins attack cell walls and membranes. Aminoglycosides, chloramphenicols, and tetracyclines act on cellular components responsible for protein synthesis. Rifamycins, nalidixic acid, and quinolones act upon nucleic acids, and methotrezate and sulfonamides interrupt important biochemical pathways within the cell (Khachatourians, 1998). To combat the action of antimicrobial compounds, bacterial cells have adapted three primary mechanisms including reducing the accumulation of antimicrobial

**Table 2.** Selected Antimicrobial Agents Approved for Use in Animal Agriculture*

| Antimicrobial Class and Drug | Use in Animal Agriculture | | | Analogs Used for Human Therapy ‡ |
|---|---|---|---|---|
| | Animal Species | Therapeutic | Non-therapeutic† | |
| **Aminoglycosides** | | | | |
| Gentamicin | Cattle (Beef and Dairy), Horses, Swine, Poultry§ | | X | Amikacin, Gentamicin, |
| Neomycin | Cattle (Beef and Dairy), Sheep, Swine, Poultry | X | X | Neomycin, Streptomycin |
| Spectinomycin | Beef Cattle, Swine, Poultry | X | X | |
| Streptomycin | Cattle (Beef and Dairy), Swine, Poultry | X | X | |
| **Aminopenicillins** | | | | |
| Ampicillin | Cattle (Beef and Dairy), Horses, Swine | X | | Amoxicillin, Ampicillin, |
| Amoxicillin | Cattle (Beef and Dairy), Swine | X | | Amoxicillin-clavulanic acid, Pivampicillin |
| **Cephalosporins (*3rd generation*)** | | | | |
| Ceftiofur | Cattle (Beef and Dairy), Horses, Swine, Poultry, Sheep | X | X | Ceftriaxone, Cefixime, Cefotaxime, Ceftazidime, Ceftizoxime |
| **Fluoroquinilones** | | | | |
| Enrofloxacin | Beef Cattle, Poultry | X | | Ciprofloxacin, Difloxacin, Gatifloxacin, Levofloxacin, Moxifloxacin, Norfloxacin, Ofloxacin, Trovafloacin-Nalidixic acid |
| **Lincosamides** | | | | |
| Lincomycin hydrochloride | Swine, Poultry | X | X | Clindamycin, Lincomycin hydrochloride |
| **Macrolides** | | | | |
| Erythromycin | Cattle (Beef and Dairy), Swine, Poultry, Layers | X | X | Erythromycin, Azithromycin |
| Tylosin | Cattle (Beef and Dairy), Swine, Poultry | X | X | |
| Tilmicosin | Cattle (Beef and Dairy), Sheep, Swine | X | X | |

* US Congress OTA, 1995; Khachatourians, 1998; US GAO, 1999; NRC, 1999; Mellon *et al.*, 2001; Shea, 2003; Sayah *et al.*, 2005; USFDA, 2005

† Non-therapeutic uses include prophylaxis and/or growth promotion.

‡ Antimicrobials used in human medicine that are similar to or the same as antimicrobials used in animal agriculture.

§ Poultry = Broilers and/or turkeys; Fowl = Quail, pheasant, duck, and/or geese; Sheep = sheep and/or goats.

**Table 2 (cont.)** Selected Antimicrobial Agents Approved for Animal Agriculture*

| Antimicrobial Class and Drug | Use in Animal Agriculture | | | Used for Human Therapy ‡ |
|---|---|---|---|---|
| | Animal Species | Therapeutic | Non-therapeutic† | |
| **Penicillins** | | | | |
| Cloxacillin sodium | Dairy Cattle | X | | Ampicillin sublactam, Cloxacillin sodium, Penicillin G benzathine, Penicillin G potassium, Piperacillin Ticarcillin |
| Penicillin G procaine | Cattle (Beef and Dairy), Horses, Sheep, Swine, Poultry, Fowl | X | X | |
| Penicillin G benzathine | Beef Cattle, Horses | X | | |
| **Peptides** | | | | |
| Bacitracin | Cattle (Beef and Dairy), Sheep, Swine, Poultry, Layers | X | X | Bacitracin |
| **Sulfonamides** | | | | |
| Sulfadiazine | Horse | X | | sulfamethoxazole |
| Sulfadimethoxine | Cattle (Beef and Dairy), Horse, Poultry, Fowl, Fish | X | X | |
| Sulfamethazine | Cattle (Beef and Dairy), Swine, Poultry | X | X | |
| Sulfanitran | Poultry | | X | |
| Sulfaquinoxaline | Cattle (Beef and Dairy), Poultry | X | X | |
| Sulfathiozole | Swine | | X | |
| **Streptogramins** | | | | |
| Virginiamycin | Beef Cattle, Swine, Poultry | | X | Quinipristin, Dalfopristin |
| **Tetracyclines** | | | | |
| Chlortetracycline | Cattle (Beef and Dairy), Sheep, Swine, Poultry | X | X | Tetracycline hydrochloride, Doxycycline |
| Oxytetracycline | Cattle (Beef and Dairy), Sheep, Swine, Poultry, Fish, Honey bees | X | X | |
| Tetracycline hydrochloride | Cattle (Beef and Dairy), Horses, Sheep, Swine, Poultry | X | X | |

* US Congress OTA, 1995; Khachatourians, 1998; US GAO, 1999; NRC, 1999; Mellon *et al.*, 2001; Shea, 2003; Sayah *et al.*, 2005; USFDA, 2005

† Non-therapeutic uses include prophylaxis and/or growth promotion.

‡ Antimicrobials used in human medicine that are similar to or the same as antimicrobials used in animal agriculture.

§ Poultry = Broilers and/or turkeys; Fowl = Quail, pheasant, duck, and/or geese; Sheep = sheep and/or goats.

**Table 3.** Estimates of the use of antimicrobial agents in livestock animal production

| Total Mass Used | Specific Use | Source |
|---|---|---|
| 20 million pounds used annually | 20% for treating disease<br>80% for growth promotion and prophylaxis | Swartz, 1989 |
| 18 million pounds used in 1985 | 12.2% for treating disease<br>63.2% for prophylaxis<br>24.6% for growth promotion | U.S. Congress, OTA, 1995 |
| 17.8 million pounds used in 1998 | 83% for prophylaxis and treating disease<br>17% for growth promotion | Animal Health Institute, 2000 |
| 29.5 million pounds used annually | 7% for treating disease<br>93% for growth promotion and prophylaxis | Mellon *et al.*, 2001 |
| 14.4 million pounds used in 1997 | Not Reported | Silbergeld, 2004 |

agents within the cell, attacking and inactivating the antimicrobial compounds enzymatically, or altering, protecting, or replacing target cellular structures. Bacteria may gain these resistance mechanisms in three ways: (1) acquire resistance genes from the DNA of antibiotic producers and modify them such that they are optimized for resistance to the antimicrobial agent (2) mutate genes whose products play a role in physiological cell metabolism such that they attack or inactivate the antimicrobial agent, and/or (3) mutate genes whose products are the target structures of the antimicrobial compounds such that the target structures become resistant to the inhibitory effects of the respective antimicrobials (Schwartz and Chaslus-Dancla, 2001).

The initial development of antimicrobial resistance may be relatively slow as single point mutations that give rise to resistance genes are rare events ($10^{-9}$ to $10^{-8}$ per cell per generation) (Kelly et al, 1986; Freifelder, 1987; Smith et al, 1999). Once acquired, antimicrobial resistance traits can be rapidly transferred vertically through division of the host cell, and/or horizontally between different bacteria (both commensal and pathogenic) via transduction (a bacteriophage-mediated process), conjugation/mobilization (requiring contact between donor and recipient cell), or transformation (transfer of free DNA into competent recipient cells). In the mixed bacterial populations of animal and human skin and mucosa, conjugation and mobilization are considered to be of primary importance for the spread of resistance genes (Schwartz and Chaslus-Dancla, 2001) and may occur on the order of $10^{-5}$ to $10^{-4}$ per cell per generation (Summers, 2002). Transduction only occurs between bacteria of very similar species and genera as it is limited by host-specificity of bacteriophages, and therefore plays a lesser role in the spread of resistance traits in these milieus. Spread of resistance traits via transformation is considered to be very limited (Bennett, 1995).

The primary genetic elements involved in horizontal gene transfer include plasmids, transposons, and integrons/gene cassettes. Aside from the antimicrobial-resistance traits, plasmids and transposons may also carry genes (such as the *tra* gene complex) which allow them to move from one bacterial cell to another via conjugation or mobilization. Plasmids may serve as vectors for transposons and integrons/gene cassettes facilitating their horizontal transfer to competent cells. Transposons and integrons/gene cassettes can be transferred via transduction

when resistance genes are co-located with prophage genes that are not excised precisely from chromosomal DNA prior to packing into phage heads. Small plasmids may also be transferred via transduction if they are packed into bacteriophage heads instead of phage DNA during phage assembly (pseudophages), however this process is limited compared to conjugation and mobilization (Schwartz and Chaslus-Dancla, 2001). Once established, resistance genes may persist in commensal bacteria serving as a reservoir for rapid acquisition of antimicrobial resistance for any new pathogen that may inhabit the intestinal tract (Barza, 2002). Of particular interest are enterococci and *E. coli* that can play a major role in the transmission of mobile resistance genes (Salyers, 1995).

Antimicrobial-resistance in bacteria may be conferred by tandem arrays of genetically linked resistance genes borne by integrons or other transposons that can reside in the chromosome and on conjugative or mobilizable plasmids (O'Brien et al, 1985; Zhao *et al.*, 2001; Roe *et al.*, 2003). Adaptation of a bacterial cell to any given antimicrobial via gene transfer can thus result in selection for resistance to not only that specific agent, but also, by genetic linkage of resistance genes, to other antimicrobials (Summers, 2002). Antimicrobial resistance determinants are also often co-located with virulence determinants on mobile genetic elements. Treatment with antimicrobials for which resistance is conferred may result in the enrichment of more virulent bacterial strains in the selective environment. Epidemiological evidence from reported *Salmonella* and *Campylobacter* infections suggest that resistant strains are somewhat more virulent than susceptible strains, exhibiting prolonged or more severe illness (Travers and Barza, 2002). In a study of 67 individuals not treated with antimicrobials, diarrhea lasted longer when the isolates were ciprofloxacin-resistant (12 days) than when they were ciprofloxacin susceptible (6 days) (P=0.02) (Marano *et al.*, 2000). The likelihood of hospitalization and average length of hospital stay are significantly higher in those infected with antimicrobial-resistant organisms than those with susceptible stains (Lee *et al.*, 1994).

Resistance to one antimicrobial compound may also confer resistance to other antimicrobial compounds through similarity of the antimicrobial agents (Khachatourians, 1998). Cases of multi-drug resistance in bacterial zoonoses caused by structural similarity of human-use antimicrobials to those used in animal agriculture have been documented. Virginiamycin-resistant bacterial isolates from turkeys were found to be resistant to the structurally similar and clinically important human-use drugs quinipristin and dalfopristin (Feinman, 1998; Chadwick and Goode, 1997). Tylosin-resistant streptococci and staphylococci-resistant animal isolates were determined to be resistant to the structurally similar and clinically important human-use drug erythromycin, and were found not only in the livestock animals, but in their caretakers as well (Feinman, 1998; Chadwick and Goode, 1997). Virginiamycin and Tylosin are both used prophylactically and/or for growth promotion in beef and dairy cattle, swine, broilers, and turkeys. Table 2 lists human-use drugs that are structurally similar to several antimicrobial compounds used in animal agriculture.

## *3.2 Antimicrobial resistance in livestock animals*

The occurrence of antimicrobial-resistant bacteria tends to be rapid following introduction of antimicrobial agents into clinical or agricultural use. For instance, occurrence of tetracycline resistant bacteria was reported in 1956, four years following its introduction to clinical use and only eight years following its initial discovery. The time lag between introduction to clinical use and occurrence of antimicrobial resistant bacteria was 15 years for vancomycin, 4 years for nalidixic acid, 3 years for gentamicin, 3 years for fluoroquinolones, one year for erythromycin, and less than one year for streptomycin (Schwartz and Chaslus-Dancla, 2001). Although the latent period between the introduction of an antimicrobial and the emergence of resistance may vary, once the prevalence of resistance in a population reaches a certain level, reversal of the problem may be extremely difficult (Swartz, 2002). For example, fluoroquinolone-resistant Campylobacter were detected in 43-96% of market chickens from two producers more than one year after fluoroquinolones were no longer used in their poultry production (Price *et al.*, 2005).

Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging antimicrobial resistance (Schwartz and Chaslus-Dancla, 2001). Resistance of both commensal and pathogenic bacteria in livestock animals to antimicrobials of clinical importance is now commonplace and is related to their increased use for growth promotion and prophylaxis over the last 50 years (Shere et al, 1998; Maynard *et al.*, 2003). Hayes et al (2004) surveyed 541 *Enterococcus faecium* isolates from 82 farms within a poultry production region in the eastern United States. Sixty-three percent were resistant to quinipristin-dalfopristin and 52.7% were resistant to four or more antimicrobials. In a study of several swine farms in the United States, Jackson *et al.,* (2004) determined that Tylosin use for growth promotion resulted in erythromycin-resistance in 59% of enterococci isolates, compared to 28% at a farm where Tylosin was used for treatment of disease only, and 2% at a farm that did not use Tylosin. National surveillance of Salmonella in swine in the U.S. has revealed resistance to several important antimicrobials including tetracycline (50%), ampicillin (12%), sulfamethoxazole (23%), and streptomycin (23%) (NARMS, 1998). Hoyle *et al.*, (2004) studied ampicillin-resistant *E. coli* in calves in the United Kingdom and determined that ampicillin resistance peaked over 80% within 4 months, steadily declining to less than 10% as the calves aged to 8 months. Schroeder *et al.*, (2002) tested 752 *E. coli* isolates from humans and animals for resistance to several antimicrobials of clinical importance. Approximately half of the isolates displayed resistance to one or more antimicrobials including penicillins, sulfonamides, cephalosporins, tetracyclines, and Aminoglycosides, with the highest frequencies of antimicrobial resistance in humans and turkeys and the lowest in non-food animals. Sayah *et al.*, (2005) studied antimicrobial resistance patterns in livestock, companion animals, human septage, wildlife, farm environments (manure storage facilities, lagoons, and livestock holding areas) and surface water in the Red Cedar Watershed in Michigan. *E. coli* isolates from livestock showed resistance to the largest number of antimicrobials and multidrug resistance was most common in swine fecal samples. Resistance was demonstrated most frequently to tetracycline, cephalothin, sulfisoxazole, and streptomycin. Similarities in patterns of resistance in *E. coli* were observed in livestock animals and environmental samples taken from their respective farms. These authors suggest that farm environment samples may best describe potential contamination of nearby waters with antimicrobial-resistant bacteria.

## *3.3 Risk to public health*

Much concern surrounds the elevated use of antimicrobial agents in confinement facilities and, in particular, the use of antimicrobial agents at non-therapeutic doses in animal agriculture (American Academy of Microbiology, 1999, Mellon *et al.*, 2001). The use of antimicrobial agents inevitably selects for resistance of both commensal and pathogenic microorganisms exposed to the agents (Linton et al, 1975; Dawson *et al.*, 1984; Levy et al, 1976; Dunlop et al, 1998; Endtz *et al.*, 1991; Jacob-Rietsma *et al.*, 1994; Bager *et al.*, 1997; Low *et al.*, 1997; Tauxe, 1997; Gynn *et al.*, 1998; McEwen and Fadorka-Cray, 2002; Vasiľ *et al.*, 2002). The conditions of widespread, prolonged exposure to antimicrobial compounds at sublethal doses with little dose control in CAFOs may exacerbate their development. Once established, the movement of antimicrobial-resistant microorganisms from animal to animal or animal to animal care worker may be facilitated by the crowding of animals into confinements, often with suboptimal hygiene. The co-colonization of animal gastrointestinal tracts by antimicrobial-resistant commensal bacteria and bacterial pathogens may lead to further development of antimicrobial-resistant bacterial zoonoses (Kruse *et al.*, 1999). As much as 75-80% of an antibiotic may pass undigested through an animal, thus its waste may not only harbor high concentrations of antimicrobial-resistant bacteria, but also their resistance genes and raw (undigested) antimicrobial compounds (Campagnolo and Rubin, 1998). This waste is often stored in open air lagoons and/or spread on fields where these compounds, resistant organisms, and antimicrobial-resistance gene reservoirs may move into the environment via aerosolization, infiltration into the groundwater, or runoff into surface water resources.

Antimicrobial resistance in zoonotic pathogens is a serious threat to human health (Ghidán *et al.*, 2000; Cheng *et al.*, 2002; Travers and Barza, 2002). Many of the drugs are used in animal agriculture and human medicine are the same or very similar including, but not limited to, beta-lactams (penicillin, ampicillin, cloxacillin), tetracyclines, sulfonamides and potentiated sulfonamides, cephalosporins, and fluoroquinolones (McEwen and Fadorka-Cray, 2002). Exposure to zoonotic pathogens harboring resistance to antimicrobials of clinical importance may lead to diseases with few or no treatment options in humans. In cases where pathogens are resistant to administered antimicrobial compounds, vulnerability to infection can increase up to three-fold, primarily resulting from a transient decrease in an individual's resistance to colonization by the pathogen (Barza and Travers, 2002). Antimicrobial-resistant pathogens tend to be more virulent than their susceptible counterparts, causing more prolonged or severe illnesses (Marano *et al.*, 2000; Travers and Barza, 2002; Swartz *et al.*, 2002). There is circumstantial evidence that increased prevalence of antimicrobial resistance in human isolates may be linked to the use of antimicrobial agents in animal agriculture (Levy *et al.*, 1976; Jensen *et al.*, 1998; Swartz *et al.*, 2002; Silbergeld, 2004). Many cases of severe human disease caused by acquisition of antimicrobial-resistant zoonotic pathogens from animal agriculture have been documented (Levy *et al.*, 1978; Schlech *et al.*, 1983; Holmberg *et al.*, 1984; Morgan *et al.*, 1988; Besser *et al.*, 1993; Cieslak et al, 1993; Isaacson *et al.*, 1993; Lee *et al.*, 1994; MacKenzie *et al.*, 1994; Millard et al, 1994; Tschape *et al.*, 1995; Centers for Disease Control and Prevention, 1998; Jackson *et al.*, 1998; Crampin *et al.*, 1999; Huovinen, 1999; Wegner *et al.*, 1999; Franklin, 1999; Kruse, 1999; Health Canada, 2000; License *et al.*, 2001; Clark *et al.*, 2003).

# 4. Survival of Pathogens in the Environment

Pathogens at concentrated animal feeding operations may be present in animal wastes, water used for maintenance of livestock and animal housing units, soils where animal manures and wastewaters are spread, on crops grown in soils where manures were applied or where contaminated irrigation waters are used, and groundwater and surface waters contaminated by manure runoff. The survival of pathogenic organisms in the environment varies widely depending on the pathogen, environmental conditions, and the chemical, physical, and biological composition of milieu of interest. Enteric bacterial, viral, and protozoan pathogen inactivation in soil, water, crops, or manure may be affected by predation, competition, water stress/osmotic potential, temperature, UV radiation, pH, inorganic ammonia, and organic nutrients (Geldreich *et al.*, 1968; Davenport *et al.*, 1976; Crane and Moore,1986; Hurst *et al.*, 1989; Davies and Evison, 1991; Olson *et al.*, 1999; Sattar *et al.*, 1999; Burkhardt *et al.*, 2000; Davies-Colley *et al.*, 2000; Wait and Sobsey, 2001; Jamieson *et al.*, 2002; Ferguson *et al.*, 2003). The importance of each factor is strongly related to the milieu of interest. In general, the survival of pathogens is inversely related to predation, competition, temperature, UV radiation, water stress, and inorganic ammonia, except for *Cryptosporidium* oocysts and *Giardia* cysts, which have low survival at sub-zero (<-20ºC) temperatures (Van Donsel *et al.*, 1967; Zibilske and Weaver, 1978; Reddy *et al.*, 1981; Jamieson *et al.*, 2002; Ferguson *et al.*, 2003). The relationship of pathogen survival to pH and organic nutrients may be more complex. Under the right conditions, pathogens are capable of surviving in the environment for days to more than a year.

## *4.1 Manure and manure slurries*

Table 4 summarizes the survival of bacterial and parasitic pathogens noted in literature in manures and manure slurries. These nutrient rich environments may offer protection from environmental insults such as solar UV radiation, desiccation, and temperature fluctuations, promoting survival or even regrowth of pathogenic zoonoses. For instance, Muirhead *et al.*, (2005) determined that within cowpats, *E. coli* grew for 6 to 14 days instead of following a traditional logarithmic die off curve and Olson (2003) noted that the eggs of *Ascaris suum*, a common parasite in swine, are highly resistant to inactivation in feces, potentially remaining infectious for years. However, these environments may also me hostile, as they may harbor predators and competitors, or produce toxic components that may reduce pathogen viability. For instance, inorganic ammonia, naturally produced by hydrolysis of urea and in decomposing manure, can be biocidal at high concentrations, and has been exhibited to be directly proportional to *Cryptosporidium* oocyst inactivation (Jenkins *et al.*, 1998; Jenkins et al, 1999). As seen in Table 4, animal manures and manure slurries may remain significant reservoirs for environmental contamination by zoonotic pathogens for many months.

Bacterial pathogens may persist for long periods in animal manures under typical environmental conditions. This may be exacerbated when the temperatures are low, moisture remains optimal, and aeration is not used. For instance, *Salmonella* and *E. coli* O157:H7 have been noted to survive for 4-6 months in animal manures and manure slurries kept at 1-9ºC, up to 49 times longer than at 40-60ºC. Nicholson *et al.*, (2002) studied the survival of *E. coli* O157:H7,

**Table 4.** Survival of pathogenic zoonoses in livestock manures and manure slurries

| Environment | Temperature (°C) | Survival[†] (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Bacterial Pathogens[‡] | | | | | Parasites[§] | |
| | | *Salmonella* sp. | *Campylobacter* sp. | *Yersinia enterocolitica* | *E. coli* O157:H7 | *Listeria* sp. | *Giardia* | *Cryptosporidium* |
| ***Manure*** | | | | | | | | |
| Broiler Litter | 40-60 | 4 | 4 | | 4 | 8 | | |
| Cattle, beef or dairy | -20 to -4 | >180 | 56 | >365 | >100 | | <1 | >365 |
| | 1-9 | 196 | 21 | 100 | 130 * | | 7 | 56 |
| | 10-19 | | | | 45 | | | |
| | 20-29 | 65 * | 3 | | 90 | | 7 | 28 |
| | 30-39 | 48 | 7 | 30 | 49 | | 7 | 28 |
| | 40-60 | 4 | 4 | | 8 | 4 | | |
| | On farm (<23) | | | | 47 | | | |
| Swine | 40-60 | 16 | 2 | | 32 | 4 | | |
| Sheep | 1-10 | | | | >100 | | | |
| | 10-19 | | | | >100 | | | |
| | 20-29 | | | | 40 | | | |
| | On farm (<23) | | | | 630 | | | |
| ***Manure slurries*** | | | | | | | | |
| Cattle, beef or dairy | -20 to -4 | | | | 21 | | | |
| | 1-9 | 115 * | | | 150 * | | | |
| | 10-19 | | | | 40 | | | |
| | 20-29 | 89 * | 3 | | 103 * | | | |
| | 30-39 | 19 | | | 22 * | | | |
| | 40-60 | | | | <2 | | | |
| | On farm (5-20) | 93 | 32 | | 93 | 185 | | |
| Swine | 1-9 | 14 | | | | | | |
| | 20-29 | 8 | 2 | | | | | |
| | 30-39 | <8 | | | | | | |

† Longest survival time reported

‡ Bolton *et al.*, (1999); Kudva *et al.*, (1998); Wang *et al.*, (1996); Himathongkham *et al.*, (1999); Mitscherlich and Marth (1984); Guan and Holley (2003); Olson (2003); Tauxe (1997); Plym-Forshell (1993); Nicholson *et al.*, (2002)

§ Cole *et al.*, (1999); Robertson *et al.*, (1992); Fayer *et al.*, (1998); Olson (2003); Olson *et al.*, (1999)

* Calculated as 7 times the reported decimal reduction time (time required for 1-log reduction in pathogen concentration) assuming logarithmic die-off and based on a reported initial inocula of $10^6$-$10^8$ organisms per gram manure or milliliter slurry.

*Salmonella*, *Listeria*, and *Campylobacter* in dairy cattle, swine, and poultry manures stored at 40-60ºC and determined that aeration of the solid manures decreased survival times for *E. coli* O157:H7 and *Salmonella* by as much as 88%. These researchers noted a decrease in the survival of *E. coli* O157:H7 and *Salmonella* sp. when a higher dry matter content was maintained in the slurry. Kudva *et al.*, (1998) noted similar changes in *E. coli* O157:H7 in sheep manure, which survived for 630 days at temperatures below 23ºC when not aerated versus 120 days when aerated, the difference likely due to drying of the aerated manure.

Parasitic protozoan survival in animal manures may also be related to temperature, but the trends are not as strong as those reported for bacterial pathogens. This is likely due to their ability to form cysts and oocysts for protection from environmental pressures under the range of temperatures reported in Table 4. These parasites have been shown to be susceptible to temperature extremes, with reported survival of *Cryptosporidium* oocysts ranging from 1 hour at -70ºC, 1 day at -20ºC, one or more years at 4ºC, 3-4 months at 25ºC, 1-2 weeks at 35ºC, and just minutes at 64ºC (Fayer and Nerad, 1996; Finstein, 2004). *Cryptosporidium* oocysts in manures may also be susceptible to desiccation and bacterial degradation whereby warmer temperatures may accelerate the degradation process. A similar pattern exists for *Giardia* cysts, but they are inactivated more rapidly than *Cryptosporidium* oocysts and are less resistant to temperature extremes.

Information regarding the survival of zoonotic viruses in animal wastes is sparse. Although not shown in Table 4, zoonotic viruses in animal manures and manure slurries may exhibit long inactivation times that extend for weeks to months. Karetnyi *et al.*, (1999) determined that swine hepatitis E was detectable in positive stool samples for more than 2 weeks, regardless of whether the samples were maintained at -85ºC, 4ºC, or room temperature. Pesaro et al (1995) studied the survival of several viruses including picnoraviruses, rotaviruses, parvoviruses, adenoviruses, and herpes viruses as well as the coliphage f2 in nonaerated liquid and semisolid animal wastes. Ninety percent reduction in virus titer ranged from less than 1 week for herpes virus to more than 6 months for rotavirus, suggesting that a 4-$\log_{10}$ reduction in viruses may require storage for as much as two years for some pathogens. Although little information exists regarding the survival of viral pathogens in fecal environments, these studies show that under non-aerated conditions viruses may exhibit prolonged persistence in manure and manure slurries, suggesting a strong potential for viral pathogen contamination when manure is spread on land.

In general, pathogen survival in animal manures is dictated by the effects of aeration and temperature, whereby increased aeration and higher temperatures lead to more rapid die-off. Of the pathogens listed in Table 4, *E. coli* O157:H7, *Listeria* sp., and *Salmonella* sp. were the most persistent in manure and manure slurries regardless of the temperature. However, considering the work of Pesaro *et al.*, (1995), viral pathogens may persist much longer than the bacterial pathogens, and should be given more consideration in future studies.

Much of the work to date has concentrated on the survival of pathogens in cattle manures and manure slurries. However, based on the summary presented in Table 4, there seems to be dissimilarities in the survival of pathogens in different animal feces. This may be due to differences in the physical, chemical, or biological properties of the various animal manures, but could also be a result of the low numbers of studies on swine and poultry manures versus those

of cattle. The lack of studies on pathogen survival in swine and poultry manures impedes the development of safe management practices.

The survival of pathogens in animal manures and manure slurries is typically studied under controlled laboratory conditions. Kudva *et al.*, (1998) noted that survival of pathogens in laboratory studies were generally lower than those observed in field studies. For instance, these researchers determined that *E. coli* O157:H7 survived in sheep manure for 100d under a controlled (4-10ºC) laboratory setting versus 630 days when exposed to environmental (ambient) conditions (<23ºC). Based on their observations, laboratory experiments may not provide a reasonable estimate of pathogen survival in on-farm conditions. Future efforts should concentrate on measuring the survival of pathogens *in-situ*.

## *4.2 Natural Waters*

Manure runoff and wastewaters from concentrated animal feeding operations may contain pathogenic zoonoses and antimicrobial-resistant bacteria that can survive and proliferate in nearby natural waters. Runoff and wastewater discharges may also contribute both organic and inorganic nutrients that may encourage the growth and proliferation of indigenous or introduced pathogens (Grimes *et al.*, 1986). Table 5 summarizes the survival of bacterial and parasitic pathogens in dirty waters from livestock operations, natural waters, and drinking water as reported in literature. In these milieus, UV radiation, disinfectants, temperature, predators, and toxin producers generally challenge the survival of pathogenic zoonoses and antimicrobial-resistant bacteria (Chao *et al.*, 1988; Johnson *et al.*, 1997).

The survival of bacteria in natural waters may be longer than exhibited in manures or manure slurries. *Yersinia enterocolitica* exhibited the greatest survival among all bacterial pathogens considered in Table 5 whereas *Campylobacter* was the least. However, in a viable but not cultivable state, *Campylobacter* may survive for as much as 120 days at 4ºC. *E. coli* O157:H7 has also been noted to enter a viable but not cultivable state in water increasing the survival time over that reported in Table 5 (Wang and Doyle, 1998). Pathogens may also settle into streambed sediments, decreasing exposure to UV radiation and predators and increasing survival times over those reported in Table 5. For instance, Anderson *et al.*, (2005) determined that a 90% reduction in fecal coliforms in fresh waters required 4.2 days, whereas 50 days was required to achieve the same reduction in the underlying sediments. Although differences in the survival of *Enterococcus* spp. was observed, the trend was the same (1.4 days in water and 4.5 days in the underlying sediments), and held true for salt water environments.

*Cryptosporidium* oocysts may also be especially resistant in environmental waters, surviving for more than a year in optimal (low temperature) conditions. For instance, Robertson *et al.*, (1992) studied oocyst infectivity during incubation in cold river water and reported up to 66% viability at 33 days and 11% viability at 176 days. In another study, Medema *et al.*, (1997) determined that the time required for one-log reduction in *Cryptosporidium* oocyst infectivity in river water at 15ºC was 40-160d, whereas at 5ºC it was 100d. Even more extreme are viruses, which can persist for several years in the subsurface. Azadpour-Keeley *et al.*, (2003) reviewed the movement and longevity of viruses in the subsurface and suggested that soil environments may actually enhance viral survival. They report a wide variation in inactivation rates in different

**Table 5.** Survival of pathogenic zoonoses in soils, contaminated water-irrigated soils, and manure-amended soils

| Environment | Temperature (°C) | Survival[†] (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Bacterial Pathogens[‡] | | | | | Parasites[§] | |
| | | *Salmonella* sp. | *Campylobacter* sp. | *Yersinia enterocolitica* | *E. coli* O157:H7 | *Listeria* sp. | *Giardia* | *Cryptosporidium* |
| ***Soil*** | | | | | | | | |
| | -20 to -4 | >84 | 56 | >365 | >300 | | <7 | >365 |
| | 1-9 | 196 | 20 | >365 | 100 | | 49 | 56 |
| | 20-29 | >45 | 10 | 10 | >56 | | 14 | 28 |
| ***Dirty Water-Irrigated Soils*** [*] | | | | | | | | |
| | 0-22 | 120 | 120 | | 34 | 128 | | 30 |
| ***Farm-yard manure-amended soil*** | | | | | | | | |
| Cattle | | | | | | | | |
| Beef | 0-22 | 63 | 120 | | 64 | 120 | | 30 |
| Dairy | 0-22 | 120 | 64 | | 34 | 120 | | 30 |
| Poultry litter | | | | | | | | |
| Broilers | 0-22 | 32 | 16 | | 32 | >32 | | |
| Broilers & layers | 0-22 | 63 | 64 | | 32 | 56 | | 30 |
| Sheep | 0-22 | 120 | 34 | | 63 | 128 | | 30 |
| Swine | 0-22 | 120 | 34 | | 32 | 120 | | 30 |
| ***Manure slurry-amended soil*** | | | | | | | | |
| Cattle | | | | | | | | |
| Beef | 0-22 | 120 | 64 | | 32 | 120 | | 30 |
| Dairy | 0-22 | 120 | 63 | | 64 | 120 | | 30 |
| Swine | 0-22 | 299 | 36 | | 32 | 120 | | 63 |

[†] Longest survival time reported

[‡] Mubiru *et al.*, (2000); Mitscherlich and Marth (1984); Zibilske and Weaver (1978); Guo *et al.*, (2002); Chao *et al.*, (1988); Guan and Holley (2003); Olson (2003); Ciesak *et al.*, (1993); Nicholson *et al.*, (2002); Hutchinson *et al.*, (2004); Hutchinson *et al.*, (2005)

[§] Cole *et al.*, (1999); Robertson *et al.*, (1992); Fayer *et al.*, (1998); Olson (2003); Olson *et al.*, (1999)

[*] Dirty water from livestock operations.

soils at near-neutral pH suggesting it may take as little as 0.8 days to as many as 11 years to achieve 99.99% (4-$\log_{10}$) die off of some viruses in aquifers. Keswick *et al.*, (1982) report that survival of enteric bacteria and viruses were longer in groundwater than surface water, presumably due to lower temperatures and protection from sunlight and microbial antagonism.

Maintenance of antimicrobial-resistance in natural waters has not been studied extensively. In untreated seawater suspensions, Guardabassi and Dalsgaard (2002) noted that multiple antibiotic resistant *E. coli* and *Citrobacter freundii* survived and maintained their multiple resistance properties for more than 30 days, whereas a multi-antibiotic resistant *Acinetobacter johnsonii* survived and maintained its multiple resistance properties for 14 days. In untreated pond water suspensions, these authors noted survival times of 21 days (*E. coli* and *A. johnsonii*) and 28 days (*C. freundii*), while maintaining multiple resistance properties. This suggests that antimicrobial resistant microorganisms may survive for long periods upon discharge to aquatic environments and that stress and nutrient depletion may not affect the stability of their resistance phenotypes. The effect of low concentrations of antimicrobial compounds discharging to surface or ground waters via manure runoff, lagoon leakage, or wastewater discharge on the maintenance of antimicrobial-resistant phenotypes or genotypes has not been studied.

## *4.3 Manure-amended soil*

Table 6 summarizes the survival of bacterial and protozoan pathogens in soils. The survival times reported in Table 6 are more similar to those of manures and manure slurries and less than those exhibited in water. In general, it has been reported that survival of pathogens in soil increases when manures are incorporated into soils rather than unincorporated. For instance, Hutchison et al (2004) studied the die off of *Salmonella*, *Listeria*, *Campylobacter*, and *E. coli* O157 following application of manure to soil and incorporation of the manure upon application, one week following application, or no incorporation. The authors noted that die-off was similar in summer and winter months, but more rapid when the manure was not incorporated into the soil. The increased survival of pathogens incorporated into soils may be related to decreased exposure to UV radiation, temperature extremes, and desiccation and increased availability of nutrients. However, soils may harbor competitor organisms and predators that can reduce pathogen survival. Survival of pathogenic bacteria in soils may also be limited by low soil pH (Jamieson *et al.*, 2002) or freeze-thaw cycling. Jenkins *et al.*, (1999) determined that *Cryptosporidium* oocyst infectivity decreased from greater than 50% to less than 1% when exposed to freeze-thaw cycles in a soil environment. Walker et al (2001) noted that inactivation of *Cryptosporidium* oocysts during freeze-thaw cycling or heating was enhanced by increased osmotic stress (decreased water potential).

The most important factor affecting the survival of enteric pathogens in soils systems may be the moisture status, which is influenced not only by precipitation, but also by moisture retaining properties such as particle size distribution and organic matter content (Gerba *et al.*, 1975; Tate *et al.*, 1978; Kibbey *et al.*, 1978; Chandler and Craven, 1980; Crane *et al.*, 1981; Reddy *et al.*, 1981; Faust, 1982; Mubiru *et al.*, 2000, Entry *et al.*, 2000b; Jamieson *et al.*, 2002). For instance, Nicholson *et al.*, (2002) studied the survival of bacterial pathogens following land spreading and determined that there are some indications that pathogen survival is longer in clay loam grassland soil than in sandy arable soil. Burton et al (1983) determined that *Salmonella newport*

**Table 6.** Survival of pathogenic zoonoses in drinking water, livestock rinse waters, surface fresh waters, surface salt waters, surface water sediments, soils irrigated with livestock rinse waters, and ground waters

| Environment | Temperature (ºC) | Survival† (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Bacterial Pathogens‡ | | | | | Parasites§ | |
| | | *Salmonella* sp. | *Campylobacter* sp. | *Yersinia enterocolitica* | *E. coli* O157:H7 | *Listeria* sp. | *Giardia* | *Cryptosporidium* |
| ***Water*** | | | | | | | | |
| Drinking | 1-9 | 90 | 12 * | 90 | 90 | | 25 | |
| | 10-19 | | 12 | | | | | |
| | 20-29 | | 2 | | | | | |
| | 30-39 | | 1.5 | | | | | |
| Ground or Spring | -20 to -4 | | | | | | | |
| | 1-9 | | | 448 | | | | |
| | 10-19 | 152 | | | | | | |
| | 20-29 | | | | | | | |
| Surface | -20 to -4 | >180 | 56 | >365 | >300 | | <7 | >365 |
| | 1-9 | >180 | 12 ** | >365 | >300 | | 77 | >365 |
| | 10-19 | | | 14 | | | | |
| | 20-29 | >180 | 4 | | | | 14 | 70 |
| | 30-39 | | | 10 | 84 | | | |
| Dirty water †† | 5-20 | 32 | 16 | | 16 | 93 | | |

† Longest survival time reported

‡ Wang and Doyle (1998); Bolton *et al.*, (1999); Santo Domingo *et al.*, (2000); Mitscherlich and Marth (1984); Karapinar and Gonul (1991); Chao *et al.*, (1988); Buswell *et al.*, (1998); Rollins and Colwell (1986); Blaser *et al.*, (1980); Guan and Holley (2003); Olson (2003); Fayer *et al.*, (1998); Kenneth *et al.*, (1998); Ford, 1999; Nicholson *et al.*, (2002)

§ Cole *et al.*, (1999); Olson (2003); Robertson *et al.*, (1992); Fayer *et al.*, (1998); Olson *et al.*, (1999)

* In the presence of a biofilm, survival was as much as 29 days at 4ºC and 11 days at 30ºC

** Survival may be more than 120 days in a viable but not cultivable (VBNC) state

†† Dirty water from livestock operations

survived longer in soils with higher clay content, potentially owing to a higher concentration of organic matter and nutrients. Mubiro *et al.*, (2000) suggested that survival of *E. coli* O157:H7 may also be enhanced in soils of higher matric potential not only due to enhanced water holding capabilities, but also because these soils better retained nutrients. The addition of manure to the soils may enhance survival of pathogens such as *Campylobacter* spp. or *E. coli* O157:H7, possibly due to increased organic and inorganic nutrient availability (Gagliardi and Karns, 2000).

The effects of water/osmotic potential on microbial stress in soil environments may be exacerbated by specific properties of the pathogen of interest. Bacteria and viruses with a hydrophobic envelope tend to accumulate at the air water interface leading to increased inactivation (Johnson and Gregory, 1993; Thompson *et al.*, 1998; Thompson and Yates, 1999). The lack of a hydrophobic envelope may reduce attraction to the air-water interface, and thus may afford some protection from viral inactivation due to osmotic stress (Ferguson *et al.*, 2003). In soils, osmotic stress typically increases near the soil surface, and may lead to reduced pathogen survival (Gerba, 1999).

Even when not incorporated into soils, the survival of pathogens following application of manures to land may be lengthy. Hutchinson *et al.*, (2005) determined decimal reduction times (the time required for 1-$\log_{10}$ reduction) for *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *C. jejuni* of 1.31 – 3.20 days (mean) and *Cryptosporidium parvum* oocysts of 8-31 days following the application of livestock waste onto fescue plots (no incorporation). Most zoonotic agents declined below detectible levels by 64 days, except for *L. monocytogenes*, which persisted for up to 128 days in some plots. Potential mechanisms for pathogen reduction may have included, among others, desiccation, UV radiation, and runoff from the grasslands to nearby receiving waters.

Where food crops are grown in manured soils or using contaminated irrigation waters, pathogens can contaminate produce surfaces. The level and persistence of contamination may be related to the irrigation method (spray irrigation or surface irrigation) and time of contact of produce with contaminated soils. For instance, Ingham *et al.*, (2004) identified *E. coli* contamination on carrots, lettuce, and radishes up to 120 days following application of non-composted bovine manure as a fertilizer in fields in Wisconsin. Following growth in *E. coli* O157:H7 contaminated manure-fertilized soil; Johannessen *et al.*, (2005) detected *E. coli* O157:H7 on the stems, but not on the edible parts of lettuce. Solomon *et al.*, (2002) noted that spray irrigation following a single exposure to *E. coli* O157:H7 resulted in 90% of the lettuce being contaminated with *E. coli* O157:H7 and the contamination persisted for more than 20 days in 82% of the plants. Where surface irrigation was used under the same circumstances, only 19% of the lettuce was contaminated. Immersion of harvested lettuce heads in 200ppm chlorine solution for 1 minute did not eliminate all *E. coli* O157:H7 cells from infected lettuce, regardless of irrigation method. Guo *et al.*, (2002) investigated water and soil as reservoirs of *Salmonella* for contaminating mature green tomatoes, and determined that the population of *Salmonella* on tomatoes in contact with contaminated soil increased over 4 days by 2.5 $\log_{10}$ CFU per tomato during storage at 20ºC, and remained constant for an additional 10 days. In contrast, where tomatoes were not in contact with soil, but *Salmonella* were inoculated onto the fruit surface, the number of cells declined over 14 days by 4 $\log_{10}$ CFU per tomato when held at 20ºC. At day one, *Salmonella* was associated with the skin surface. As time of storage increased, more *Salmonella* cells were

associated with less accessible stem scar and subsurface areas of the tomatoes, which may render the fruits more resistant to disinfection with sanitizing agents.

## *4.4 Discussion*

Relatively few studies are available describing the survival of pathogenic zoonoses in environmental milieu, especially considering the broad range of properties of soils, manures, and waters that may potentially be contaminated. Much of the emphasis has been placed on cattle manures, manure-amended soils, and surface waters, with less emphasis on ground waters and manures from other livestock animals such as swine and poultry. In general, pathogenic zoonoses tend to survive longer cooler rather than warmer temperatures and in water rather than in manures or soils. This may be problematic as manures and soils are stationary whereas water is a significant transport medium for pathogens. Further, very few studies have been reported on the survival of viruses which is troubling because the relatively few studies that are available suggest that viruses are more persistent than bacteria and parasitic protozoa and can travel vast distances in both surface and ground waters. A significant limitation is the lack of information regarding the survival of antimicrobial-resistant bacteria in various milieus including the persistence of phenotypic and genotypic antimicrobial-resistance traits. Most studies reported in Tables 4-6 were carried out in the laboratory instead of *in-situ*, and only a few examined more than one environmental stressor simultaneously. The combined effects of multiple stressors in the natural environment or presence of additional growth and maintenance factors may limit or enhance pathogen survival in reference to lab-scale studies of single stressors (Crane and Moore, 1986; Robertson et al, 1992; Kudva *et al.*, 1998; Jenkins *et al.*, 1999; Friere-Santos *et al.*, 2000; Walker *et al.*, 2001).

Current methods for detecting pathogens in environmental systems may limit the ability to determine accurate survival times in difficult milieu. Specific soil or manure properties or survival strategies of the various pathogens may limit their detection with cultivation techniques. For instance, upon being stressed, bacteria may die or adapt using a number of mechanisms including formation of spores, formation of ultramicrobacteria, or entering viable but not cultivable (VBNC) states. Many of the bacterial pathogens can survive for much longer periods of time than indicated in Tables 4-6 in VBNC states (Wang and Doyle, 1998; Santo Domingo *et al.*, 2000; Rollins and Colwell, 1986). Better and more sensitive methods for pathogen detection in different media need to be developed to determine more accurately pathogen survival. Accurate information regarding the survival of pathogenic zoonoses and antimicrobial resistant bacteria is necessary for modeling their fate and transport from confined animal feeding operations. Based on available information, ensuring the safety of food crops and water resources may require management practices that eliminate pathogens in manures and other CAFO wastes prior to land application or discharge to natural waters.

# 5. Pathogen Movement – An Ecological Perspective

Figure 2 provides a partial picture of the potential routes of transmission of zoonotic pathogens from confined livestock animals to humans and the environment. The movement of pathogens onto, within, and off farms is a complex ecological issue owing to the continuous exchange of microbes between human and animal hosts and environmental reservoirs (Sobsey *et al.*, 2002; Summers, 2002). For instance, Herriott *et al.*, (1996) tested twelve herds and their feeds and water troughs as well as co-located (non-bovine) livestock, companion animals, wild birds, rodents and flies at dairies and feedlots in Idaho, Oregon, and Washington for the presence of *E. coli* O157:H7. *E. coli* O157:H7-positive cattle were identified in all 12 herds with a prevalence of 1.1-4.4% in dairies and 1.5-6.1% in feedlots. It was also detected in 1.3% of trough water samples, 2.0% of trough water biofilm samples, in a nearby horse, two dogs, pooled bird droppings, and composite fly samples. Considering antimicrobial resistance, the issue becomes more complicated as mobile genetic elements conferring resistance provide a distinct selective advantage in stressed environments such as the colonic tract of humans and animals being treated with antimicrobials. In these environments, proliferation of resistance traits among bacteria can be rapid and have lasting effects (O'Brien, 2002; Summers, 2002). Addressing the movement of pathogens between intensive livestock operations and the environment will require understanding of the ecological principle that everything is connected to everything else. The following is a discussion of some of the potential pathways for movement of zoonotic pathogens from livestock animals raised in confinement to humans and the environment.

## *5.1 CAFOs and Abattoirs*

The presence of zoonotic pathogens in CAFO environments may begin with the stocking of infected animals or with the use of selected feed products on the farm. Animal feeds and drinking water containing antimicrobial compounds may lead to the development and persistence of resistant bacterial zoonoses in livestock animals which may proliferate through the farm environment. Animal feeds can also be a direct source of zoonotic pathogens and antimicrobial-resistant bacteria for livestock animals (Curtain, 1984; Durand *et al.*, 1990; Izat and Waldroup, 1990; Gabis, 1991; Veldman *et al.*, 1995; Davies and Wray, 1997; Primm, 1998; Shirota *et al.*, 2001a,b). For instance, of ten feed ingredient piles at 12 commodity dairy feeding farms, Kidd *et al.*, (1999) identified two feeds contaminated with *Salmonella enteritidis*. Sixty two percent of Enterobacteriaceae isolates from the ten piles were ampicillin-resistant and 10% were tetracycline-resistant. Although feed can be contaminated on-farm, it may also arrive contaminated, as shown in a recent survey of 629 feed samples from 3 feed mills where 8.8% of feed mash samples and 4.2% of pelleted feed samples were positive for Salmonella (Jones and Richardson, 2004). Antimicrobial-resistant bacteria and other pathogens can also be present in trough waters (Marshall *et al.*, 1990; Herriott *et al.*, 2002; Kemp *et al.*, 2005) potentially resulting from either stocking troughs with contaminated water or through deposition of contaminated material into the water from an animal harboring the disease (via the saliva, mucosa, or feces). Antimicrobial compounds in the water and the presence of biofilms in which bacteria are in close contact may lead to proliferation of antimicrobial resistance within these microbial communities. The confinement of animals into dense units where trough waters are shared and where animals have increased contact with each other and their fecal matter may exacerbate the spread of pathogens from animal to animal.

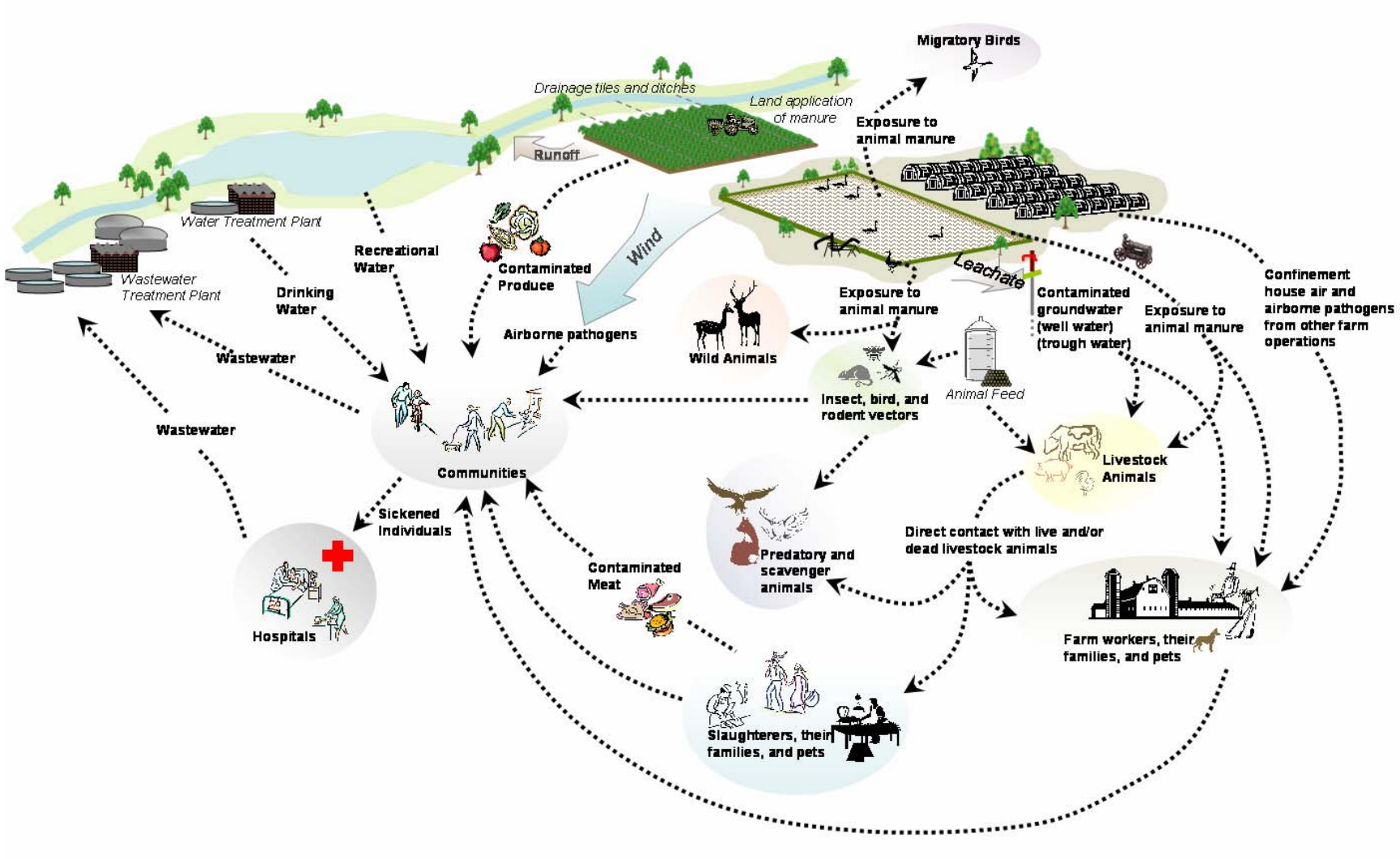


**Figure 2.** Movement of pathogens - an ecological perspective

Standing trough waters and animal feeds laden with antimicrobial compounds and pathogenic zoonoses as well as unsanitary conditions and poor manure management practices pose other problems for controlling the spread of disease. Animal and insect vectors may be attracted to feed piles, trough waters, fecal matter, manure treatment lagoons, treatment wetlands, or the dense animal populations present in CAFOs resulting in movement of pathogens on and between farms as well as off of farms and into human populations. Several studies have supported the movement of pathogens and antimicrobial resistant bacteria through animal vectoring. In a longitudinal study on Wisconsin farms, Shere *et al.*, (1998) reported that the use of antimicrobials subtherapeutically in animal feeds or trough waters and therapeutically for treatment of diarrhea correlated well to the emergence of antimicrobial-resistant *E. coli* O157:H7, which may have been transmitted through birds eating the animal feed and drinking contaminated trough waters. Nielsen et al (2004) screened 446 fecal samples at eight Danish cattle and swine farms and detected stx1 and stx2 genes in production animals, wild birds, and rodents suggesting transmission between the livestock animals and vectors. Halos *et al.*, (2004) detected the *Bartonella* citrate synthase gene in Hippoboscidae flies on wild roe deer, cattle, horses, and sheep in France suggesting that these flies may act as a vector for transmission of *Bartonella* between wild and domestic ruminants. Waldenström et al (2005) observed antimicrobial resistant *Campylobacter jejuni* in wild thrushes, shorebirds, and raptors in Sweden suggesting the spread of antimicrobial-resistant pathogens to wild birds. Raptors had the highest prevalence of antimicrobial-resistant strains, potentially from predation on infected animal vectors. On 12 diary and beef feedlots in Idaho, Oregon, and Washington, Herriott *et al.*, (2002) identified *E. coli* O157:H7 in composite fly samples and pooled bird droppings. Marshall *et al.*, (1990) inoculated pigs with an antimicrobial-resistant strain of *E. coli* and within a four month period was able to isolate the same strain from trough water, bedding materials, mice, flies, and a human caretaker.

Other studies may point to broader ecological implications of animal vectoring in the environment. Cole et al (2005) compared free-living Canadian geese in Craven county, Georgia that were using swine waste lagoons and surface waters adjacent to farm fields to Canadian geese in Griffin, Georgia, where there were crop fields, but no nearby animal production facilities. The proportion of *E. coli* isolates resistant to antimicrobial agents was significantly greater ($p=0.0004$) among Craven county geese (72%), where interaction with swine waste lagoons was observed, than in Griffin geese (19%). These researchers proposed that Canadian Geese may be acting as vectors for antimicrobial-resistance and resistance genes in agricultural animal-production environments. Their findings suggest the spread of pathogens and antimicrobial resistant bacteria from livestock operations may be vast considering potential migration of Canadian geese over hundreds of kilometers.

Other factors unique to concentrated animal feeding operations may encourage the spread of disease on farms. Several researchers have detected high levels of airborne bacteria ($2x10^3$ – $8x10^5$ CFU/$m^3$) in confinement house air including antimicrobial-resistant bacteria and other zoonotic pathogens such as *Enterococcus*, *Staphylococcus*, *Pseudomonas*, *Bacillus*, *Listeria*, *Salmonella*, *Campylobacter*, and *E. coli* (Cormier *et al.*, 1990; Cazwala *et al.*, 1990; Crook *et al.*, 1991; Heederick *et al.*, 1991; Predicala *et al.*, 2002). In houses of experimentally infected broiler chickens, Gast et al (2004) were able to detect *Salmonella* spp. in the air for four weeks post-infection, even when the litter was cleaned from the floors weekly. In a study of three

mechanically ventilated swine CAFOs, Zahn *et al.*, (2001) detected tylosin resistance in 80% of cultivable airborne bacteria. Chapin *et al.*, (2005) isolated 137 *Enterococcus* and staphylococci from the air within a concentrated swine feeding operation and screened them for resistance to erythromycin, clindamycin, virginiamycin, tetracycline, and vancomycin. 88% of the isolates expressed high-level resistance to at least two antibiotics and 84% to at least three antibiotics commonly used in swine production, but none were resistant to vancomycin, an antibiotic that has never been approved for use in livestock in the United States. Thirty seven percent of the isolates were resistant to virginiamycin, an analog to quinipristin-dalfopristin which is a drug of last resort for multidrug-resistant gram-positive infections characterized by glycopeptide-resistant *E. facium* and coagulase-negative staphylococci.

These findings have significant implications for the health of livestock animals, animal care workers, their families, and casual farm visitors, and to a lesser extent to nearby communities that may be susceptible to secondary infections via exposure to sickened animal care workers and their families. Chapin *et al.*, (2005) proposed a scenario by which airborne pathogenic zoonoses resistant to clinically important antimicrobials may spread from confined livestock animals to the public through exposure to sickened animal care workers and their families.

> *"Bacteria resistant to virginiamycin are often cross-resistant to quinipristin-dalfopristin, and a previous study has shown that transfer of streptogramin-resistant Enterococcus can occur between animals and humans in the livestock environment (Jensen et al., 1998)… Inhalation of air contaminated with multidrug resistant Enterococcus or streptococci could lead to colonization of both the nasal passages (Aubry-Damon, 2004) and the lungs of swine CAFO workers, potentially making the workers themselves reservoirs of antibiotic-resistant organisms. Co-exposures to other aerosols and gases in the swine environment such as organic dusts, molds, and ammonia have been shown to induce symptoms associated with chronic bronchitis, including a persistent cough characterized by expectoration (Mackiewicz, 1998). The presence of this type of cough can increase the potential for secondary spread of antibiotic-resistant organisms into the community, where additional individuals could serve as reservoirs of multidrug-resistant bacteria… Thus, the inhalation of virginiamycin-resistant gram-positive bacteria in the swine environment could contribute to the appearance of quinipristin-dalfopristin-resistant gram-positive infections in humans, leaving few or no treatment options for the affected individual(s)"* – Chapin *et al.*, (2005)

The more recent work of Armand-LeFevre *et al.*, (2005), who determined that a number of *Staphylococcus aureus* strains that caused infections in swine populations (including four methicillin-resistant strains) were also present in healthy swine farmer nasal cavities, but not in the nasal cavities of healthy non-farmer controls, further supports their hypothesis.

Many other pathways for infection of animal care workers with pathogenic zoonoses and antimicrobial-resistant bacteria exist including, but not limited to direct contact with infected animals, increased exposure to insect and wild animal vectors on the farm, exposure to animal excreta, handling animal carcasses, exposure to contaminated air from manure spreading, and drinking water from fecally-contaminated wells (Skilbeck and Miller, 1986; Everard *et al.*, 1989; Seuri and Granfors, 1992;Thomas *et al.*, 1994; Hogue *et al.*, 1997; Cole *et al.*, 2000; Barkocy-

Gallagher et al, 2001; Chomel, 2004). These exposures have manifested in increased illness in animal care workers, their families and pets, and casual farm visitors (Levy *et al.*, 1976; CDC, 2000), and have potentially spread into nearby communities based on empirical evidence reported in literature. For instance, McDonald *et al.*, (1997) genotyped vancomycin-resistant fecal bacterial isolates from swine, poultry, farm workers and their pets in Denmark and concluded that transmission had occurred between livestock animals, humans, and household pets. Hummel *et al.*, (1986) detected nourseothricin-resistance traits in 33% of fecal isolates of swine exhibiting diarrhea, 18% of fecal isolates from swine farmers and their families, and 16% fecal isolates from outpatients exhibiting diarrhea in communities adjacent to the swine farms. Nourseothricin was not used for treatment of human disease in the region, but was used for two years for promoting growth of swine on the farms.

The presence of antimicrobial-resistant bacteria may occur rapidly following the introduction of antimicrobials as growth promoters in feed animals. Levy et al (1976) determined that tetracycline-resistance in fecal isolates from chickens increased rapidly from 10% of animals excreting less than 0.1% of organisms resistant to tetracycline (baseline) to 90% of animals excreting 100% of organisms resistant to tetracycline within 2 weeks of introducing tetracycline to chicken feed, whereas no increase was observed in the control group. Further, multidrug resistance developed, even though only tetracycline was being supplemented in the feed. By 12 weeks, more than 60% of the animals from the experimental group excreted bacteria resistant to tetracycline plus one or more other antimicrobial compounds. More then 25% were resistant to 4 or more antimicrobials. After 4 months, antimicrobial resistance had spread from the experimental group to the control group, where a third of the chickens excreted bacteria of which more than 50% of isolates were tetracycline resistant. Within 6 months, antimicrobial resistance had also spread to the farm workers and their immediate families. More than 30% of fecal samples form farm workers and their families contained more than 80% tetracycline-resistant organisms, versus 6.8% from their neighbors. A 4-drug resistance pattern similar to that observed in the experimental chickens was observed in the farm workers and their families. Stopping the feed additives eventually reduced the incidence of tetracycline-resistant bacteria in the farm dwellers.

Livestock animals can also be a source of antimicrobial-resistant bacteria and pathogenic zoonoses such as *E. coli* O157:H7, *Salmonella* sp., and *Staphylococcus aureus* in abattoirs, which may slowly die off or in some instances regrow in the waste products (Hepburn *et al.*, 2002). When improperly handled, these wastes may potentially contaminate adjacent land and nearby watercourses or infect slaughters, and through secondary infections, their families and pets (Crawford *et al.*, 1969; Nesbakken, 1988; Molin *et al.*, 1989; Reboli and Farrar, 1989; Merilahti *et al.*, 1991; Seuri and Granfors, 1992; Huys *et al.*, 2005). In fact, as with animal care workers, epidemiological evidence supports transmission of these pathogens from livestock animals to humans in the abattoir environment, but suggests the infection rate is lower than that observed in farmers and their families. For instance, van den Bogaard *et al.*, (1997) phenotyped fecal *Enterococcus* spp. isolates from turkeys, turkey farmers, turkey slaughterers, and nearby residents of the turkey farms in Europe. Vancomycin-resistant *Enterococcus* (VRE) was detected in half of turkey samples, 39% of turkey farmers, 20% of turkey slaughterers, and 14%

In a recent study, van den Bogaard *et al.*, (2001) surveyed three poultry operations (broilers, turkeys, and laying hens) and five human populations (turkey farmers, broiler farmers, laying-hen farmers, broilers slaughterers, and turkey slaughterers) in the Netherlands for antimicrobial-resistant fecal *E. coli*. These researchers determined that 35% of isolates from laying hens were antimicrobial-resistant, as compared to 84% of the isolates from turkeys and 80% of the isolates from broilers. Similarly, 66% of *E. coli* isolates from turkey farmers, 60% from broiler farmers, 67% from turkey slaughterers, and 59% from poultry slaughterers were antimicrobial-resistant whereas only 45% of isolates from laying hen farmers were antimicrobial-resistant. Antimicrobial resistance patterns of the isolates were similar between turkeys, turkey farmers and turkey slaughterers, and in broilers, broiler farmers, and broiler slaughterers. Pulsed-field gel electrophoresis (PFGE) "fingerprinting" patterns of an *E. coli* isolate from a turkey was identical to one from a turkey farmer. Similarly, one isolate from a broiler chicken was identical to an isolate found in a broiler chicken farmer. Their results strongly indicate transmission of antimicrobial resistant bacteria between humans and poultry commonly occurs.

of area residents. VRE is one of the leading causes of nosocomial infections in the hospital environment. Nijsten *et al.*, (1994) determined that the resistance of fecal isolates to antimicrobial compounds was more prevalent in swine farmers than slaughterhouse workers and suburban residents in the same geographic region. In Japan, antimicrobial resistance of fecal microbes was also noted to be highest in swine farmers and elevated in slaughterhouse workers when compared to urban control cohorts (Saida *et al.*, 1981). Others have realized similar trends supporting the movement of antimicrobial-resistant bacteria from farm animal to farmer or slaughterer (Ozanne *et al.*, 1987; Levy, 1978; Marshall *et al.*, 1990).

## *5.2 Food*

It is well established that pathogenic zoonoses can cause human disease via consumption of contaminated meat products (Corpet, 1993; U.S. Congress, Office of Technology and Assessment, 1995; Milleman *et al.*, 2000). The amplified use of antimicrobial compounds in confinement animals for growth promotion and prophylaxis may exacerbate disease by reducing treatment options and potentially increasing the virulence of bacterial pathogens in meats. For instance, in 1995 fluoroquinolone antibiotics were approved for use in poultry for growth promotion and prophylaxis. In 1997, Smith *et al.*, (1999) screened chicken obtained from Minnesota shopping markets that originated from 15 abattoirs in nine states for *Campylobacter jejuni* and resistance to ciprofloxacin, an important human-use fluoroquinolone antibiotic of choice for presumptively treating severe bacterial food poisoning. Fourteen percent of the samples were contaminated with ciprofloxacin-resistant *C. jejuni*. During a similar period, statewide-surveillance indicated that fluoroquinolone-resistance increased from 1.3% of all human *C. jejuni* infections in 1992 to 10.2% in 1998 (Smith et al, 1999). In a more recent study, Wallinga *et al.*, (2002) surveyed 200 fresh whole market chickens and 200 packages of ground turkey from stores in Iowa and Minnesota and determined that 95% of whole chickens were contaminated with *Campylobacter* and 18% with *Salmonella*. Two percent of ground turkey samples and were contaminated with *Campylobacter* and 45% with *Salmonella*. Six percent of the *Salmonella* isolates were resistant to 4 or more antimicrobials, while 62% of the *Campylobacter* isolates were resistant to 1 or more antimicrobial compound including an 8%

prevalence of resistance to ciprofloxacin. Greater than ninety percent of enterococci isolated from the chicken or turkey were resistant to quinipristin-dalfopristin, an important antibiotic for the control of VRE infections in hospitals. In a similar study, Hayes *et al.*, (2003) screened 981 samples of raw retail meats including chicken, turkey, pork, and beef from 263 grocery stores in Iowa and found high levels of resistance to several antimicrobials in *Enterococcus* isolates. Their results indicate that antimicrobial-resistant *Enterococcus* spp. commonly contaminate retail meat products and that the antimicrobial resistance pattern of isolates from each meat product (poultry, pork, and beef) reflected well the use of approved agents in each food animal production class (broilers, swine, and beef cattle).

Dairy products may also be contaminated with pathogenic zoonoses and antimicrobial-resistant bacteria following direct contact of dairy cattle to contaminated sources in the farm environment and subsequent excretion from the udders of infected animals (Oliver *et al.*, 2005). For instance, Van Kessel *et al.*, (2004) surveyed 861 bulk tank milks on farms in 21 states and detected *Listeria monocytogenes* (6.5%) and several *Salmonella* serotypes (2.6%) including Montevideo, Newport, Muentster, Meleagris, Cerro, Dublin, and Anatum. Kim *et al.*, (2005) tested 316 bulk milk tank samples across the U.S. between January 2001 and December 2003 for *Coxiella burnetii*, the causative agent for Q-fever. These researchers detected *C. burnetii* in greater than 94% of bulk tank milk. Jayarao and Henning (2001) surveyed bulk tank milks from 131 dairy herds in South Dakota and Minnesota and detected *Campylobacter jejuni* (9.2%), shiga-toxin producing *Escherichia coli* (3.8%), *Listeria monocytogenes* (4.6%), *Salmonella* spp. (6.1%), and *Yersinia enterocolitica* (6.1%), with one or more species of pathogenic bacteria in 26.7% of the samples. Although pasteurization may reduce the incidence of disease in humans attributable to contaminated milk, Oliver *et al.*, (2005) argue that outbreaks of disease have been traced back to both unpasteurized and pasteurized milk, and that unpasteurized milk is often consumed directly by dairy producers, farm employees, and their families, as well as by their neighbors and raw milk advocates. In their bulk tank-milk study, Jayarao and Henning (2001) observed that 60% of the dairy producers drank unpasteurized milk, 27% of which contained one or more types of pathogenic bacteria. According to the model presented in Figure 1, disease contracted via this route may be spread to nearby communities via contact with infected individuals. It has also been noted that an even larger segment of the population may be directly exposed to contaminated dairy products via consumption of cheeses made from unpasteurized milk (Oliver *et al.*, 2005).

CAFOs produce massive quantities of manure, much of which is spread onto agricultural fields as fertilizer. Fecally-contaminted water, potentially resulting from runoff from manure-treated fields or discharge of wastes from agricultural operations, may be used to irrigate crops in arid regions of the United States. Direct contact with soils on which manure was applied and/or irrigation with fecally-contaminated water may result in contamination of produce such as lettuce, radishes, apples, and sprouts with pathogenic zoonoses including antimicrobial-resistant bacteria, especially where the edible parts are exposed to the soil or water (Besser *et al.*, 1993; Tschäpe *et al.*, 1995; Nelson, 1997; Taormina *et al.*, 1999). In a recent study, Ingham *et al.*, (2004) identified *E. coli* contamination on carrots, lettuce, and radishes up to 120 days following application of non-composted bovine manure as a fertilizer in fields in Wisconsin. In contrast, Johannessen *et al.*, (2005) did not detect *E. coli* O157:H7 on the edible parts of lettuce after growth in *E. coli* O157:H7-contaminated manure fertilized soil. Solomon *et al.*, (2002) noted

that spray irrigation following a single exposure to *E. coli* O157:H7 resulted in 90% of the lettuce being contaminated, persisting for more than 20 days in 82% of the plants. Where surface irrigation was used under the same circumstances, only 19% contamination was observed on the lettuce. Immersion of harvested lettuce heads in 200ppm chlorine solution for 1 minute did not eliminate all *E. coli* O157:H7 cells from infected lettuce, regardless of irrigation method. It has been suggested that some produce may absorb pathogens into their internal tissues through the root system, protecting them from cleaning procedures such as washing or irradiation. In a survey of fresh domestic produce conducted in the spring of 2000, the US Food and Drug Administration detected Salmonella on 2.6% of cantaloupe, 1.6% of cilantro, and 1.8% of lettuce originating from U.S. farms (US FDA, 2001).

## *5.3 Air*

Vast quantities of manure produced at CAFOs containing high levels of pathogenic microorganisms and antimicrobial-resistant bacteria are applied to agricultural lands each year. Viable bacteria and viruses become airborne from agricultural sprayers, pasturelands, and farm fields treated with manure, ultimately decreasing the quality of air near CAFOs. The upward flux of viable bacteria may be strongly related to plant cover and soil moisture condition. For instance, upward flux of viable bacteria from bare soil and various crops has been reported to increase an order of magnitude in dry soil over young corn in wet soil, another order of magnitude in a closed wheat canopy over dry soil, and four orders of magnitude between bare soil and an alfalfa field (Lindemann *et al.*, 1982). Although plants have been found to be a stronger source of bacteria than soil (Lindemann and Upper, 1985), specific agricultural practices that increase particle emissions may significantly impact bacterial loading to an airshed. Strong vertical temperature gradients, low relative humidity and low soil moisture may lead to increased emission of PM10 from agricultural fields during tilling (Holmen *et al.*, 2000; Clausnitzer and Singer, 2000). As dust may harbor viable bacteria, these factors may increase pathogen loading to an airshed. If pathogens survive in soils until harvest, it is possible that significant airborne spread may occur. It has been estimated that during harvest, up to 42% of bacterial loading in an airshed can be attributed to harvesting activities (Lighthart, 1984; Tong and Lighthart, 2000).

Upward flux of viable bacteria may also be related to temperature, exposure to solar radiation, protection from associated soil particles, and wind speed. Lindeman and Upper (1985) report that upward flux of bacteria over bean plants in Wisconsin occurred during the warmest parts of the day with a maximum around noon, especially on windy days, and was observed to cease when wind speeds were less than 1m/s. Tong and Lighthart (1999) suggest that peak concentrations of viable bacteria over agricultural lands in Oregon may occur in late afternoon, presumably due to less exposure to solar radiation or association with larger particles protective from the effects of the sun. Upward flux of viable bacteria over a high desert chaparral have been observed to peak late in the evening, with minimum viable bacterial concentrations at 13:20 hours and a maximum at 22:00 hours, presumably due to the strong effects of solar radiation (Lighthart and Schaffer, 1994). The effects of temperature on upward flux of bacteria may overcome the effects of solar radiation. Summer months have been associated with higher incidence of airborne viable bacteria, even though increased solar radiation may negatively influence bacterial viability due to UV damage or desiccation of bacterial cells (Tong and Lighthart, 1997; Tong and Lighthart, 2000).

Several studies have documented increased airborne pathogens directly attributable to the spread of human or animal manure on agricultural lands through spray irrigation with contaminated waters or deposition of animal placental and fecal wastes and subsequent distribution to downwind animal or human receptors (Boutin *et al.*, 1988; Hughes, 2003; Donnison *et al.*, 2005; Brooks *et al.*, 2005). Boutin *et al.*, (1988) identified bacterial counts as high as 2000 viable particles per cubic meter at the edge of applied areas following land spreading of cattle and pig slurry. Donnison *et al.*, (2005) studied the survival of *Bacillus subtilis* and *Serratia entomophila* in irrigation aerosols in spring and summer in New Zealand. Viable *B. subtilis* and *S. entomophila* corresponding to the respirable fraction of inhaled air were recovered at 100 m from a low pressure sprayer and 200 m from a high pressure sprayer. Brooks et al (2005) studied the aerosolization of *E. coli* and coliphage MS-2 from liquid biosolids applied from a spray tanker under hot (22-37.5C) and arid (5-15% relative humidity) conditions. At wind speeds between 0.7-6 m/s, these researchers could not detect aerosolized *E. coli* at distances as low as 2 m, but detected coliphage MS-2 at distances as far as 60 m. Paez-Rubio et al (2005) identified aerosolization as a potential mechanism for the dissemination of wastewater bacteria and other microorganisms at flood irrigation wastewater reuse sites. These researchers identified more than 1 billion enteric bacteria per cubic meter in downwind air samples. Airborne *Coxiella burnetii* associated with sheep operations and Picnoravirus from swine operations have been estimated to travel several kilometers in the air in concentrations sufficient to cause infectious disease in humans and animals (Henderson, 1969; Hugh-Jones and Wright, 1970; Smith *et al.*, 1993; Hawker *et al.*, 1998; Lyytikainen *et al.*, 1998).

## *5.4 Recreational and Drinking Water*

The USEPA's 1998 National Water Quality Inventory indicates that agricultural operations, including animal feeding operations, are the most common polluters of rivers and streams, contributing to the impairment of 59% of those surveyed. Agricultural operations also have significant impacts on lakes, ponds, reservoirs, and estuaries, contributing to the impairment of more than 3,590,000 acres of these valuable water resources (USEPA-NACAC, 2005). Figure 3 illustrates the relationship between confined livestock animals in the U.S. in 1997 and impairment of surface waters indicated in the 1998 National Water Quality Inventory. In 1998, pathogens (microbial indicators, not overt pathogens) were the most common water pollutant contributing to 7,742 impairments (14.24% of surveyed waters). Sources of these microorganisms may have included wastewater and storm water outflows, the spreading of biosolids and animal manures on agricultural lands, and wild animals. However, the sheer quantities of animal wastes generated and spread onto land compared to those of other sources suggests animal agriculture may be the dominant contributor. Pathogenic microorganisms continue to pose a major challenge to the quality of U.S. waters, contributing to a total of 7,894 impairments (13.16% of surveyed waters) on the USEPA's 2002 National Water Quality Inventory.

A survey of literature regarding overt pathogens in agricultural waters and drinking water sources suggest that the National Section 303(d) listings may underscore the actual extent of microbial contamination in agricultural watersheds resulting from livestock activities. For instance, two surveys of source waters for surface water treatment plants in 29 states resulted in detection of *Cryptosporidium* oocysts in 55% and 87% of the waters tested. Similarly, *Giardia*

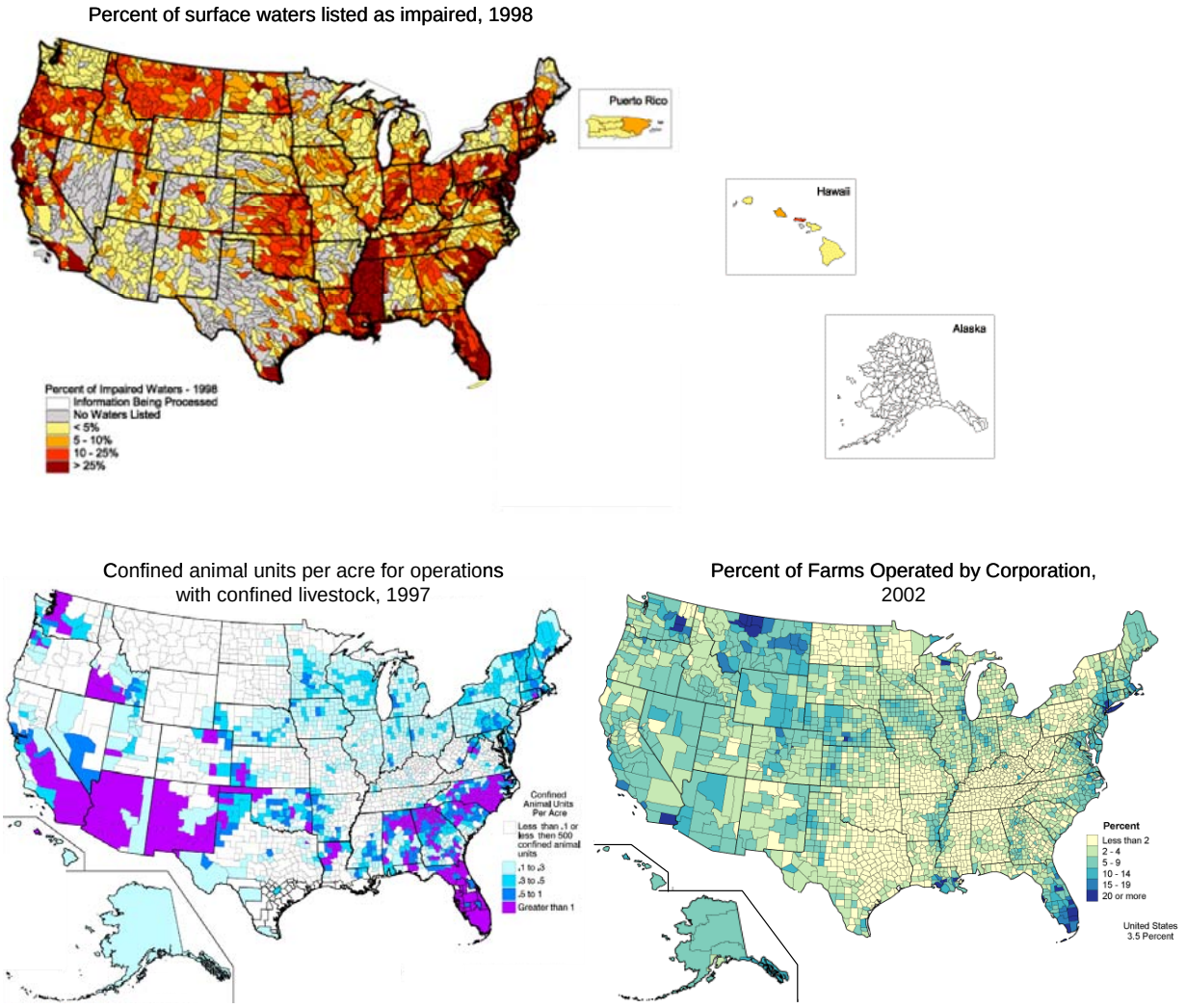


**Figure 3.** The impact of confined animal feeding operations on agricultural watersheds (adapted from USDA-NRCS, 2002; USEPA, 1998)

cysts were detected in 16% and 81% of the waters tested (LeChevallier *et al.*, 1991; Rose *et al.*,1991). *Mycobacterium avium*, potentially from cattle, swine, and broiler operations, have been detected in several marine waters, rivers, lakes, streams, ponds, and spring waters (AWWARF, 1997; Ichiyama *et al.*, 1988; Falkinham *et al.*, 2001; LeChevallier, 1999). A high prevalence of *Campylobacter* spp. in environmental water samples in a dairy farming area in the United Kingdom including 56.7 % of running waters (streams and ditches) and 45.9% of standing waters (ponds) was recently noted (Kemp *et al.*, 2005). Swine farming activities have been implicated in the contamination of at least one major Canadian river by enteroviruses (Payment, 1989), and have also been correlated to the presence of *Cryptosporidium parvum* oocysts, *Yersinia enterocolitica*, and *Salmonella* spp. in nearby drainage canals, groundwater wells, and surface waters where lagoon and spray systems are used (CDC, 1998). Groundwater surveys in Ontario Canada indicated that wells located near manure application areas were at higher risk for fecal bacterial contamination, and the level of contamination was inversely correlated to the distance the wells were from animal feedlots or exercise yards (Conboy and Goss, 2002).

The delivery of zoonotic pathogens to environmental waters following manure application is dependent on several factors including, but not limited to, the initial and persisting pathogen load, properties of the pathogen of interest, the soil and vegetation type, travel distance/time to the receiving water, pathogen inactivation by various environmental stressors, and potential engineered or natural barriers to pathogen transport. When manure is spread onto land, overland flow of pathogens to water bodies may occur via attachment to applied waste products, attachment to soil particles, or movement in the free form (Tyrrel and Quinton, 2003, Muirhead et al, 2005). Leachate from manure-amended fields and poorly designed manure holding lagoons may inundate natural soil barriers resulting in contamination of underlying groundwater (Jones, 1980; Kowel, 1982; Natsch *et al.*, 1996; Jogbloed and Lenis, 1998). Within the soil profile, movement of pathogenic zoonoses may be limited by soil moisture and solid-phase interactions (adsorption-desorption) or facilitated by the presence of macropores from burrowing animals, fractured media, or plant roots (Ferguson *et al.*, 2003). Contaminated groundwater may be captured by drainage tiles that discharge to surface waters, bypassing overland treatment in natural or engineered vegetative or riparian buffers. Alternatively, pathogens may enter groundwater where they may potentially migrate towards wells or natural springs that may be used for drinking water. The delivery of an infective dose to a susceptible individual will depend not only on the transport properties of pathogens, but also on the time required for pathogen inactivation due to environmental stressors or predation in surface and groundwater resources.

The concentration of pathogens reaching subsurface tile drains that discharge to nearby streams often exceeds drinking water supply and recreational use standards (Warnemuende and Kanwar 2000). Tile drainage has been noted to be a significant pathway for pathogens to enter surface waters from manure-treated fields, especially during periods of wet weather (Dean and Foran, 1992; Joy *et al.*, 1998; Geohring *et al.*, 1999; Hunter *et al.*, 2000; Monaghan and Smith, 2005). Evans and Owens (1972) noted that approximately 0.05% if *E. coli* applied with swine manure applications to a sandy clay loam pasture could be recovered in the tile drainage water. In a study of swine operations in Iowa and Missouri, Karetnyi *et al.*, (1999) identified swine hepatitis E in a tile outlet draining a field to which manure had been applied. Evans and Owens (1972) determined that fecal bacteria present in swine waste slurries could be detected in the tiles draining the pasture to which the waste was applied within a few hours of application.

Where pathogens bypass tile drainage systems or tile drainage is non-existent, significant contamination of groundwater resources may occur. The transport of pathogens that have infiltrated the soil profile depends strongly on adsorption-desorption interactions. Key characteristics of pathogenic zoonoses related to adsorption-desorption phenomena include size, surface electrostatic properties, cell wall hydrophobicity, and the presence of flagella (Gerba, 1984; Dowd *et al.*, 1998; Heise and Gust, 1999). For viruses, attachment to soil particles is rapid, and may be increased by low pH or high ionic strength groundwater or by high soil organic carbon content (Gerba, 1981; Gerba *et al.*, 1978; Goyal and Gerba, 1979; Taylor *et al.*, 1980; Moore *et al.*, 1981; Taylor et al, 1981; Moore, 1982; Singh *et al.*, 1986; Bales *et al.*, 1991; Bales *et al.*, 1993; Sakoda *et al.*, 1997). At the neutral pH of most groundwater, organic carbon content of the soil may dominate retardation of viral particles. Retardation of bacterial particles in saturated porous media may also be dominated by organic-carbon partitioning, but can also be a product of straining or simple filtration (Heise and Gust, 1999). Straining and filtration may be

even more significant for the larger protozoan parasites such as Cryptosporidium oocysts and Giardia cysts. Once contaminated, restoration of water quality in contaminated aquifers is very slow (Olson, 2003).

In packed sand columns, it has been demonstrated that Cryptosporidium oocysts, although initially filtered, may exhibit time-dependent detachment leading to a constant low-level elution from porous media (Harter *et al.*, 2000). Free oocysts have been observed to move in pore water without retardation suggesting potential for considerable transport in aquifers considering their long survival times (Brush *et al.*, 1999; Harter *et al.*, 2000). Similarly, viruses become attached to sediments near the source of contamination and leach slowly into the groundwater. Therefore, even single contamination events may provide a lingering source of viral contamination to groundwater (de Borde *et al.*, 1998b). Viruses have been shown to be able to travel considerable distances through the subsurface depending on their size, adsorption characteristics, and degree of inactivation (Keswick and Gerba, 1980; Dowd *et al.*, 1998). For instance, enteric viruses, some of which may remain infective for more than 9 months, have been observed to move up to 1000-1600 m per year in channelized limestones and several hundred meters per year in glacial silt-sand aquifers with travel times similar to bromide tracers (Skilton and Wheeler, 1988; Bales et al, 1995; Bosch, 1998; de Borde et al; 1998a; de Borde et al; 1999). Bacterial pathogens may similarly move considerable distances as indicated in a study by Withers *et al.*, (1997), who identified groundwater contamination by *E. coli* from an unlined cattle waste lagoon 76 m below ground surface and 80 m downstream the lagoon in the United Kingdom.

In a collaborative study performed at 9 Swine CAFOs in Iowa employing lagoon and spray systems, the CDC tested the swine waste lagoons and several selected points near the agricultural facilities for pathogenic zoonoses. They identified elevated concentrations of *E. coli* (≤380,000 per 100mL), *Enterococcus* sp. (≤1,900,000 per 100 mL), *Salmonella* sp. (≤9,300 per 100 mL), and *Cryptosporidium parvum* oocysts (≤2250 per liter) in the swine waste lagoons. *C. parvum* oocysts were detected in monitoring wells nears the swine waste lagoons of three CAFOs (9-15 oocysts per L) and in the river adjacent one CAFO (6 oocysts per L). A single *Yersinia* sp. was detected in an agricultural drainage ditch draining the spray field at one facility. Elevated *E. coli* were detected in the agricultural drainage wells (300-740/100mL), drainage ditches (520-3,700/100mL), monitoring wells (10-390/100mL), and drainage tile inlet/outlets (10-2,900/100mL). Similarly, *Enterococcus* sp. were detected in the agricultural drainage wells (4,500/100mL), drainage ditches (610-13,000/100mL), monitoring wells (80-910/100mL), and drainage tile inlet/outlets (30-2,400/100mL). *Campylobacter* sp. were not detected at any of the sampling points. Of the 18 *E. coli*, 3 *Salmonella* sp., and 20 *Enterococcus* sp. isolates tested for antimicrobial resistance, 16 *E. coli*, and all 3 *Salmonella* and 20 *Enterococcus* sp. were resistant to one or more antimicrobials commonly used in swine management practice as feed supplements and therapeutics (16 total including fluorfenicol, tetracycline, sulfamethoxazole, ampicillin, streptomycin, apramycin, bacitracin, lincomycin, penicillin, synercid, kanamycin, cephalothin, amoxicillin-clavulanic acid, ceftiofur, chloramphenicol, and gentamicin). Eight *E. coli* and all 3 *Salmonella* sp. and 20 *Enterococcus* sp. were multi-drug resistant (2-11 antimicrobials) (CDC, 1998).

A significant limitation of the National 303(d) listings is the lack of monitoring for antimicrobial-resistant bacteria. Antimicrobial-resistant bacteria are generally shed in animal feces, but may also be present in the mucosa of livestock animals. The massive use of antibiotics in animal agriculture pose a great risk as antimicrobial-resistant bacteria shed in animal wastes and stored in lagoons or spread onto land may eventually find their way to the aquatic environment (CDC, 1998; Levy, 1998; Chee-sanford *et al.*, 2001). For instance, Chee-Sanford *et al.*, (2001) detected all eight classes of tetracycline-resistance genes in two swine waste lagoons and the underlying groundwater up to 250 meters down-gradient the lagoons. Tetracycline resistant bacterial isolates from groundwater harbored a *tet*(M) gene identical to that detected in the swine waste lagoons. Resistance genes from antimicrobial-resistant bacteria in contaminated discharge waters can be transferred to otherwise susceptible bacteria living in unpolluted aquatic habitats, encouraging the spread of antimicrobial resistance in environmental waters (Guardabassi and Dalsgaard, 2002; Gurdin *et al.*, 2002). The extent of proliferation may be limited by the distance from the discharge point.

Antimicrobials in livestock animals are primarily removed in the urine and bile, either unchanged or in metabolite form, and therefore can directly contaminate environmental waters. Once in the environment, antimicrobial compounds and their metabolites may degrade rapidly (tetracyclines, penicillins, and fluoroquinolones) or persist (macrolides and sulfonamides), resulting in long-term contamination near animal confinement operations. For instance, Campagnolo *et al.*, (2002) detected several antimicrobials used in animal agriculture in animal waste lagoons (2.5-1000 µg/L) and in monitoring wells, field drainage tiles, springs, streams, and rivers (0.06-7.6 µg/L) proximal to confined animal feeding operations in Iowa and Ohio. The use of antimicrobials in animal agriculture most certainly contributed to the frequent detection of antimicrobials in a recent U.S.G.S. survey of rivers and streams of the United States (Kolpin *et al.*, 2002). Although the presence of a pharmaceutical residues and their metabolites in potable water sources present their own ecological challenges (Goni-Urriza *et al.*, 2000; Zuccato *et al.*, 2000; Hirsch *et al.*, 1999; Halling-Sorensen *et al.*, 1998; Daughton *et al.*, 1999), their typical concentrations in environmental waters are usually far below (approximately 1000-fold) those that would selectively enrich for resistant bacteria. Resistant bacteria found in surface waters are likely to have originated from wastewater or manure runoff from antimicrobial-rich settings such as animal feeding operations or wastewater treatment plants or subsequently contaminated animal vectors (Levetin, 1997; Stetzenbach, 1997; Summers, 2002).

Although low environmental concentrations of antimicrobials may not be adequate to enrich for resistant strains of bacteria, their role in the proliferation and maintenance of antimicrobial-resistance genes in these complex milieus is uncertain. For example, Gurdin et al (2002) screened isolates of *E. coli* and enterococci from swine farm wastes, and environmental isolates of *E. coli*, enterococci, *Kleibsiella*, and *Aeromonas* in surface waters upstream and downstream of study farms for antimicrobial resistance. These researchers observed that the diverse resistance patterns exhibited by rural background surface water isolates likely reflected human and animal impacts. In contrast, bacteria isolated downstream from swine farms exhibited increased antimicrobial-resistance that reflected the swine waste isolates. Sixty seven percent of Aeromonas and 12% of enterococci isolates upstream the study farms were resistant to erythromycin, whereas 91% of Aeromonas and 30% of enterococci isolates down-stream the study farms were resistant. Antimicrobial residues were also more likely to be detected

downstream rather than upstream swine farms. However, antimicrobial resistance did not always correlate to detection of residues. Swine farms were shown to be capable of contributing resistant enteric bacteria that act as reservoirs for the spread of resistance traits to susceptible bacteria, and antimicrobial residues which may encourage the maintenance and spread of the resistance traits. More work is needed to clearly identify threshold concentrations of antimicrobial residues in environmental waters that encourage the spread of antimicrobial resistance.

## *5.5 Hydrologic events*

Once in natural water bodies, viral particles, bacteria, and protozoan cysts and oocysts may attach to larger particles such as organic matter or soils and settle into the sediments of streams or reservoirs. Due to their size, settling of free particles may be limited. Their association with sediments may offer some protection from environmental stressors such as solar and UV radiation, pH extremes, desiccation, antibiotics, and predators leading to increased survival (Gerba and McLeod, 1976; Smith *et al.*, 1978; Roper and Marshall, 1979; Bitton and Marshall, 1980; LaBelle and Gerba, 1980; Schaiberger *et al.*, 1982; Metcalf *et al.*, 1984; Rao *et al.*, 1984; Long and Davies, 1993). .As such, the sediments of natural water bodies may act as reservoirs for pathogenic zoonoses and antimicrobial-resistant bacteria discharged from CAFOs (Hendricks, 1971; Grimes, 1975; Gerba *et al.*, 1977; Davies *et al.*, 1995). For instance, in estuary waters, Metcalf *et al.*, (1984) detected enteroviruses and rotaviruses in 14 and 50% of two water samples but 72 and 78% of their respective sediments contained these viruses. In 20-70% of surface waters, it has been observed that viruses occur as solid-associated particles, and may be present in high concentrations in bed sediments when compared to overlying water even at vast distances from the original source of contamination (Ferguson *et al.*, 2003).

The movement of pathogens from CAFO operations can be exacerbated by rainfall, which may stimulate the release of pathogens from otherwise stable manure-treated fields or fecal pats leading to increased overland transport, discharge to surface waters by drainage tiles, or infiltration into groundwater resources (Kress and Gifford, 1984; Mawdsley *et al.*, 1996a,b; Hunter *et al.*, 2000; Ogden *et al.*, 2001; Davies *et al.*, 2004; Monaghan and Smith, 2005). Often, stream flow increases significantly during hydrologic events, stirring up bedded sediments and further increasing pathogen concentrations, especially in shallow surface waters (Ferguson *et al.*, 2003). For example, Ferguson (1994) determined that an increase of 1-cm in rainfall increased *Cryptosporidium* oocysts in the Georges River by 24%. Atherholt *et al.*, (1998) demonstrated a positive correlation between parasitic protozoan concentrations in the Delaware River Watershed and precipitation events. Kistemann *et al.*, (2002) measured *E. coli*, fecal streptococci, *Clostridium perfringens*, *Cryptosporidium*, and *Giardia* in the tributaries of 3 drinking water reservoirs during normal and wet weather events and noted a 1-2 $\log_{10}$ increase in bacterial and parasitic microbial concentrations during runoff compared to normal conditions. Crowther *et al.*, (2002) observed highly significant positive correlations between concentrations of coliforms, *E. coli*, and enterococci in two watersheds in the United Kingdom during hydrologic (high flow) events and land use/management variables associated with intensive livestock farming. High flow conditions were associated with a greater than 10-fold increase in geometric mean fecal indicator concentrations (coliforms, *E. coli*, and enterococci) potentially due to storage and resuspension of viable organisms in channel bed sediments. Kunkle (1972) noted a marked

dependence of bacterial concentrations on stream flow in the Sleepers River Basin near St. Johnsbury, Vermont, and emphasized the importance of stream surveillance that accounts for the hydrology involved. Joy *et al.*, (1998) reported bacterial contamination of surface water due to the application of liquid manure by accepted practices over a two year period. Drainage tiles were determined to deliver significant amounts of bacteria to surface waters, which was exacerbated by rainfall shortly following manure application.

Extreme precipitation may pose more significant problems for CAFO operators as lagoons and other engineered manure management systems such as vegetative buffers, infiltration basins, and constructed wetlands may be challenged by the level of flooding associated with these events. Passive manure management systems are typically designed for 20-50 year flood events, and may be overtopped during more rigorous flooding. Flood waters may engulf vegetative buffers allowing direct contact with animal wastes applied to fields. Flooding may also engulf animal confinement houses drowning animals and transporting raw fecal material and animal carcasses downstream. Waste management systems that do not fail will experience elevated discharge, reducing their efficacy as a barrier to pathogens. Because of the potential liability associated with overtopping or failing waste lagoons during flooding, many CAFO operators opt to spray down their lagoons during heavy rainfall in lieu of violating freeboard limits (Wing *et al.*, 2000). Significant environmental damage associated with intentional and accidental release of manures and other potentially infectious materials from CAFO operations during flooding events has been documented and the danger still persists (Taylor, 1999; Mallin, 2000; Scmidt, 2000; Wing *et al.*, 2002). In 1999, Hurricane Floyd flooded several CAFOs and caused extensive environmental damage to river and coastal waters in North Carolina. During this event, it was estimated that dozens of animal waste lagoons were breached and more than 100,000 hogs, 2.4 million chickens, and 500,000 turkeys drown in the flood waters. Wing *et al.*, (2002) estimate that greater than 240 CAFOs still operate within the region flooded by this category 3 hurricane.

# 6. Public Health Outcomes

Pathogens may enter and proliferate in a farm environment through the stocking of new animals, exposure to airborne pathogens from an upwind source, contaminated trough water or feed, insect or rodent vectors, human-to-animal and animal-to-animal transmission, to name a few. Concentrating animals in confinement with suboptimal hygiene may encourage the spread of disease within farms. As discussed eariler (Section 4: Survival of Pathogens in the Environment), the survival of zoonotic pathogens in animal manures and the environment can range from days to years depending on the pathogen, the medium, and environmental conditions. Where animal wastes are improperly managed, there exists potential for the movement of pathogens off farms and into nearby water, land, and air. Uncontrolled releases of pathogens may occur via runoff, aerosolization, or infiltration into soils and groundwater, especially when manure is spread onto land. Stored animal feeds and manure can attract animal vectors that can spread disease within a farm, to nearby farms or communities, or, in the case of migratory birds, over large distances spanning hundreds of kilometers. Animal care workers are exposed to elevated levels of pathogens in confinement house air and through direct contact with livestock and animal manures, leading to an increased incidence of illness and spread of disease to their families and communities. A similar trend is seen in abattoir workers and their families due to the proliferation of pathogens within slaughterhouse environments. Contamination of produce or meat products with zoonotic pathogens may further spread disease within human populations. Even where extensive management practices are in place, exposures can and do occur. The outcomes of these exposures are animal and human disease, sometimes with serious consequences.

## *6.1 Waterborne and Foodborne Outbreaks*

The impacts animal feeding operations may have on public health are evident in surveillance of waterborne and foodborne outbreaks in the U.S. reported by the Centers for Disease Control and Prevention (CDC). Table 7 summarizes the CDC outbreak data between 1991 and 1997. During this period, there were more than 3,900 reported outbreaks infecting more than 500,000 individuals. Based on reported data, foodborne outbreaks were 8.3 times more likely to be reported than waterborne outbreaks. However, waterborne outbreaks tend to affect larger numbers of individuals per incident, most likely because communities share drinking water resources and recreational waters. Between 1991 and 1997, the number of infected individuals per waterborne outbreak was 35 times larger than for foodborne outbreaks (2-3 times larger discounting the *Cryptosporidium* outbreak in Milwaukee in 1993 that infected more than 400,000 individuals). Of the outbreaks of known etiology reported from 1991-1997, slightly less than half (48%) of the recreational water outbreaks and nearly two thirds (66%) of the outbreaks associated with untreated drinking water were caused by zoonotic pathogens. During the same period, 82% of the foodborne outbreaks of known etiology were caused by zoonotic pathogens. The pathogens most often

**Of the outbreaks of known etiology reported from 1991-1997, slightly less than half (48%) of the recreational water outbreaks and nearly two thirds (66%) of the outbreaks associated with untreated drinking water were caused by zoonotic pathogens. During the same period, 82% of the foodborne outbreaks of known etiology were caused by zoonotic pathogens.**

associated with outbreaks include *Giardia*, *Cryptosporidium*, *Campylobacter*, *Salmonella*, and toxigenic *E. coli* (including *E. coli* O157:H7, *E. coli* O126:NM, and *E. coli* O121:H19). As noted above, all of these microbial agents are endemic in cattle, swine, and poultry flocks, and all are characterized by a low infectious dose.

Although the number of outbreaks and cases of illness reported to the CDC due to recreational and drinking water exposure, as well as foodborne sources, are massive, they greatly underscore the true incidence of disease caused by these sources. A complex chain of events must occur in order for a foodborne or waterborne disease outbreak to be reported to the CDC's foodborne and waterborne outbreak surveillance systems. A break at any point in the chain results in an unreported incident. Significant limitations to the reporting system begin at infection, as there is a continuum of disease from asymptomatic infection and mild illness to death. Illness can be sporadic in the population following exposure, and most sickened individuals seek medical attention only in severe cases. Outbreaks that are most likely to be brought to the attention of public health authorities include those that are large, such as interstate or restaurant-associated outbreaks, or those that can cause serious illness, hospitalization, or death. The identification of the source of infection in many cases is difficult and may be compounded by the long incubation periods of some agents, as noted in Table 1 (Section 2: Pathogens). For instance, the illness following exposure to *Brucella* spp. may manifest in as little as five days or as much as 60 days, a time in which the number of potential vehicles of transmission may be massive. Even where cases may be simple, reporting may be limited. Reporting of outbreak data is at the discretion of the states, many of which do not have adequate monitoring and reporting systems in place, primarily due to lack of financial resources to implement such systems. Outbreaks reported in the foodborne and waterborne outbreak surveillance summaries are a small and variable fraction of all outbreaks and cases that occur in the U.S. every year. They do not include those caused by secondary infections, animal contact infections, airborne infections, or many of the other pathways discussed above. As a result, the true incidence of illness that may be caused by zoonotic pathogens remains largely unknown. Table 8 shows the estimated total yearly incidence of disease caused by selected pathogens in the U.S. (Mead *et al.*, 1999). Based on these estimates, zoonotic pathogens may be responsible for as much as 90% of bacterial and parasitic infections of known etiology.

The actual incidence of waterborne and foodborne disease is certainly much higher than that reported in annual surveillance activities. For instance, Mead *et al.*, (1999) estimated that foodborne disease causes 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the U.S. each year. The American Society for Microbiology (1998) reported that 900,000 illnesses and 900 deaths each year may be caused by waterborne microbial infections following recreational water contact. Morris and Levin (1996) estimated that disease-causing microbes in drinking water alone may cause 7.66 million illnesses and 1,200 deaths each year. Based on these estimates, acquiring infection by a foodborne organism may be 10-84 times more likely than for waterborne infections (either through recreation or drinking contaminated water). However, actual studies suggest that the risks associated with drinking water that meets federal standards are understated. The reasons for this are unclear, but may be related to the perception that water is "clean". There may be a tendency of individuals and medical practitioners to identify food as a source of contamination when the vehicle of transmission is unclear, especially when the etiological agent is not identified. In any case, evidence from studies of several water

**Table 7.** Water and foodborne outbreaks in the U.S. reported by the CDC (1991-1997).

| Etiologic Agent | **Waterborne** Outbreaks (*Cases*) | | | | | | **Foodborne** Outbreaks (*Cases*) | **Total** Outbreaks (*Cases*) |
|---|---|---|---|---|---|---|---|---|
| | Drinking Water | | | Recreational Water | | Total Waterborne | | |
| | Untreated | Treated | Unknown | Natural | Man-Made | | | |
| **Bacteria** | | | | | | | | |
| *Bacillus* spp. | | | | | 1 (*20*) | 1 (*20*) | 22 (*969*) | 23 (*989*) |
| *Brucella* spp. | | | | | | | 1 (*19*) | 1 (19) |
| *Campylobacter* spp. | 5 (*253*) | 1 (*32*) | 2 (*274*) | | 1 (*6*) | 9 (*565*) | 38 (*773*) | 47 (1338) |
| *Clostridium* spp. | | | | | | | 107 (*4991*) | 107 (4991) |
| *E. coli* (toxigenic) † | 4 (*747*) | 5 (*90*) | 2 (*9*) | 32 (*476*) | | 43 (*1322*) | 90 (*3312*) | 133 (4634) |
| *Legionella spp.* | | 6 (*80*) | | | 1 (*149*) | 7 (*229*) | | 7 (229) |
| *Leptospira spp.* | | | | 3 (*402*) | | 3 (*402*) | | 3 (402) |
| *Listeria monocytogenes* | | | | | | | 3 (*100*) | 3 (100) |
| *Mycobacteria spp.* | | | | | 1 (*5*) | 1 (*5*) | | 1 (5) |
| *Plesiomonas shigelloides* | | 1 (*60*) | | | | 1 (*60*) | | 1 (60) |
| *Pseudomonas spp.* | | | | 1 (*50*) | 63 (*1090*) | 64 (*1140*) | | 64 (1140) |
| *Salmonella* spp. | | 2 (*749*) | 1 (*84*) | | 1 (*3*) | 4 (*836*) | 560 (*35861*) | 564 (36697) |
| *Shigella* spp. | 4 (*496*) | 4 (*109*) | | 13 (*1256*) | 5 (*120*) | 26 (*1981*) | 48 (*1671*) | 74 (3652) |
| *Staphylococcus spp.* | | | | | 1 (*3*) | 1 (*3*) | 57 (*1950*) | 58 (1953) |
| *Streptococcus* spp. | | | | | | | 3 (*228*) | 3 (228) |
| *Vibrio* spp. | | | 2 (*114*) | | | 2 (*114*) | 9 (*50*) | 11 (164) |
| *Yersinia enterocolitica* | 1 (*2*) | | | | | 1 (*2*) | 2 (*27*) | 3 (29) |
| Other bacterial | | | | | | | 6 (*609*) | 6 (609) |
| **Protozoa** | | | | | | | | |
| *Cryptosporidia spp.* | 2 (*141*) | 8 (*407701*) | 3 (*162*) | 6 (*654*) | 45 (*12494*) | 64 (*421152*) | | 64 (421152) |
| *Giardia spp.* | 8 (*61*) | 16 (*2218*) | 1 (*4*) | 6 (*85*) | 5 (*187*) | 36 (*2555*) | 7 (*79*) | 43 (2634) |
| *Niagleria fowleri* | 1 (*2*) | | | 29 (*29*) | | 30 (*31*) | | 30 (31) |
| **Helminthes** | | | | | | | | |
| *Schistosoma spp.* | | | | 11 (*234*) | | 11 (*234*) | | 11 (234) |
| *Trichinella spiralis* | | | | | | | 3 (*60*) | 3 (60) |
| **Virus** | | | | | | | | |
| Adenovirus 3 | | | | 1 (*595*) | | 1 (*595*) | | 1 (595) |
| Hepatitis A | 2 (*56*) | | | | | 2 (*56*) | 38 (*1262*) | 40 (1318) |
| Norovirus | 6 (*882*) | 4 (*1804*) | 2 (*665*) | 8 (*391*) | 3 (*60*) | 23 (*3802*) | 10 (*1483*) | 33 (5285) |
| Uncharacterized | | 1 (*70*) | | | | 1 (*70*) | 24 (*2104*) | 25 (2174) |
| **AGI and Other Unknown‡** | 21 (*2731*) | 34 (*11997*) | 6 (*634*) | 16 (*1176*) | 10 (*268*) | 87 (*16806*) | 2461 (*51731*) | 2548 (68537) |

† Includes *E. coli* O157:H7, *E. coli* O121:H19, and *E. coli* O26:NM

‡ AGI= Acute gastrointestinal illness of unknown etiology; also includes other illnesses of unknown etiology

**Table 8.** Estimated number of total cases, hospitalizations, and fatalities that may occur annually in the U.S. by selected etiological agent as reported by Mead *et al.*, (1999).

| Etiologic Agent | Total Cases | Hospitalizations | Fatalities |
|---|---|---|---|
| **Bacteria** | | | |
| *Brucella* spp. | 1,554 | 122 | 11 |
| *Campylobacter* spp. | 2,453,926 | 13,174 | 124 |
| *Escherichia coli* O157:H7 | 73,480 | 2,168 | 61 |
| Enterohemorrhagic *Escherichia coli* (non-O157:H7 STEC) | 36,740 | 1,084 | 30 |
| *Listeria monocytogenes* | 2,518 | 2,322 | 504 |
| *Salmonella* spp. | 1,412,498 | 16,430 | 582 |
| *Yersinia enterocolitica* | 96,368 | 1,228 | 3 |
| **Protozoans and Helminthes** | | | |
| *Cryptosporidium parvum* | 300,000 | 1,989 | 66 |
| *Giardia lamblia* | 2,000,000 | 5,000 | 10 |
| *Toxoplasma gondii* | 225,000 | 5,000 | 750 |
| *Trichinella spiralis* | 52 | 4 | 0 |

systems meeting federal drinking water standards suggests that as much as 6-40% of gastrointestinal illness in the U.S. may be drinking water related (Payment *et al.*, 1991; Golstein *et al.*, 1996; Cottle *et al.*, 1999; Morris *et al.*, 1996; Schwartz *et al.*, 1997; Schwartz *et al.*, 2000; Levin *et al.*, 2002). For instance, in a study conducted in Contra Costa County, California, reverse osmosis drinking water treatment systems (half sham and half real) were installed on the taps of more than 400 participants (50% sham and 50% real). Participants with true systems had 20.4% less gastrointestinal illness episodes than those who used tap water meeting all federal and state drinking water treatment standards (Colford *et al.*, 2002). These results are similar to those of earlier Canadian studies (Payment *et al.*, 1991a,b; Payment, 1994; Payment *et al.*, 1994; Payment *et al.*, 1997), and indicate that infections acquired through contaminated drinking water may approach those acquired through consumption of tainted food.

## *6.2 Specific Cases*

Although the scale of infections caused by zoonotic pathogens remains unclear, the transmission of pathogenic zoonoses from livestock animals to humans and other negative public health outcomes resulting from living in proximity to confinement animals has been clearly documented in reported literature. Both epidemiological studies (See sidelights.) and specific incidences reported in the U.S. and other high income countries, such as the United Kingdom (UK), Canada, The Netherlands, and Japan, have implicated livestock animals and their wastes as the source of illness and other health outcomes. Animal manures in particular have been implicated as the source of pathogens in several waterborne outbreaks (Jackson *et al.*, 1998; Crampin *et al.*, 1999; License *et al.*, 2001; Health Canada; 2001). When manure has been implicated as the source of outbreak, the consequences have been severe. For instance, manure runoff contaminating groundwater near a municipal well in Walkerton, Ontario, Canada resulted in an outbreak of *E. coli* O157:H7 and *Campylobacter* spp. in May, 2000 that caused 2,300 illnesses and 6 deaths (Valcour *et al.*, 2002; Clark *et al.*, 2003; Federal-Provincial-Territorial

Committee on Drinking Water, 2005). Solo-Gabriele and Neumeister (1996) describe a *Cryptosporidium* outbreak in Corrollton, GA in 1989 in which manure runoff was suspected to have been the cause of over 13,000 illnesses. Richardson *et al.*, (1991) and Atherton *et al.*, (1995) describe *Cryptosporidium* outbreaks in Swindon, Oxfordshire, and Bradford UK in 1989 and 1994, respectively, in which storm runoff from farm fields was suspected to have been the cause of 641 illnesses. MacKenzie *et al.*, (1994) describe a *Cryptosporidium* outbreak in Milwaukee, Wisconsin in 1994 in which 87 deaths and over 400,000 illnesses were attributed to animal manure and/or human excrement contaminating the water supply.

Merchant et al. (2005) studied the association between farm living and the prevalence of asthma outcomes. Children living on swine farms were more likely to have asthma outcomes, and the prevalence was more dramatic where antibiotics were added to feed. Nearly 43% of children on farms with less than 500 pigs had asthma or asthma indicators. This number climbed to 46% on farms with more than 500 pigs. However, 55.8% of children living on hog farms where antibiotics were added to feed experienced asthma or asthma indicators . This compared to 26.2% prevalence in children on farms that did not raise hogs. The study indicated that 33.6% of children not living on a farm and not around swine had at least one indicator of asthma. Although farms that use antibiotics tended to be larger, the research team concluded that antibiotic exposure may also have played a role in the development of childhood asthma.

Animal manure has also been implicated as the source of many foodborne outbreaks, mostly resulting from contaminated produce (Schlech *et al.*, 1983; Morgan *et al.*, 1988; Besser *et al.*, 1993; Cieslak et al, 1993; Millard et al, 1994; Tschape *et al.*, 1995). Manure-contaminated produce (fruit and vegetables, including juices and salads) tends to result in more illnesses per outbreak than those associated with contaminated meat. This is because fertilizing fields with manure or irrigating with fecally-contaminated water results in larger numbers of potentially infectious products that are eaten raw in most cases. For instance, Fukushima *et al.*, (1995) describe an outbreak of *E. coli* O157:H7 in Sakai City, Japan in which animal manure-contaminated alfalfa sprouts were suspected of causing 12,680 illnesses, 425 hospitalizations, and 3 deaths. Outbreaks of *E. coli* O157:H7 have also been associated with the consumption of manure-contaminated apple cider (Bresser *et al.*, 1993) and potatoes (Levy *et al.*, 1978). In contrast, contaminated meats, which may result from infected animals or contamination at the abattoir, are generally cooked, destroying pathogens and leading to more sporadic incidence of illness per outbreak. However, the number of outbreaks and total number of cases

Wing and Wolf (2000) surveyed residents of three rural communities, one in the vicinity of a 6000-head hog operation, one in the vicinity of two intensive cattle operations, and a third without livestock operations using liquid waste management systems. Residents in the vicinity of the hog operation were 7.6 times more likely to report occurrences of headaches, 5.2 times more likely to experience runny noses, 3.6 times more likely to have sore throats, 4.7 times more likely to excessively cough, 3.0 times more likely to have bouts of diarrhea, and 5.6 times more likely to have burning eyes than residents of the community without intensive livestock operations. All results were adjusted for sex, age, smoking, and work outside the home.

associated with contaminated meat are higher than produce. According to surveillance of outbreaks in the U.S. between 1990 and 1998, contaminated produce accounted for about 24% of the outbreaks and 41% of the cases (Griffiths, 2000).

Although proper cooking can eliminate most pathogens from meat products, contaminated meat remains a significant link between humans and pathogenic zoonoses. Outbreaks of enterohemmhorhagic *E. coli* in the U.S. between 1982 and 2002 can be attributed primarily to contaminated meat (41%), followed by produce (21%), person to person contact during illness (14%), contaminated drinking or recreational water (9%), and directly contacting infected animals or their wastes (3%) (Rangel *et al.*, 2005). The emergence of many antimicrobial-resistant zoonotic pathogens in human populations has been linked to the consumption of food animals and dairy products. For instance, Holmberg *et al.*, (1984) attributed a 6-state outbreak of multi-drug resistant *Salmonella newport* to consumption of beef from a feedlot that was using subtherapeutic doses of chlorotetracycline as a growth promoter. The emergence of multidrug-resistant *Salmonella typhimurium* DT 104 in 1988 in cattle in the UK was rapidly followed by its detection in meat (Threlfall *et al.*, 1997) and later in humans, presumably via the consumption of contaminated beef, pork sausages, and chickens. Between 1990 and 1995, human illnesses in UK attributed to *S. typhimurium* DT 104 increased from 259 to 3837 (Lee *et al.*, 1994). The emergence of fluoroquinolone-resistant pathogens in the Netherlands rapidly followed its introduction as veterinary drug in chickens and humans. Enrofloxacin-resistant *Campylobacter* in poultry increased from 0-14% between 1982 and 1989, while resistant *Campylobacter* causing human infections rose from 0-11% (Endtz, 1991). As poultry are a primary reservoir for *Campylobacter* spp., the use of fluoroquinolones in the poultry industry was implicated as the vehicle for human-acquired enrofloxacin-resistant *Campylobacter*.

Airborne zoonotic pathogens from animal feeding operations may also infect humans and other livestock animals. As noted above, pathogens and antimicrobial-resistant bacteria have been detected at elevated concentrations in confinement house air (Cormier *et al.*, 1990; Cazwala *et al.*, 1990; Crook *et al.*, 1991; Heederick *et al.*, 1991; Zahn *et al.*, 2001; Predicala *et al.*, 2002; Gast et al 2004; Chapin *et al.*, 2005). Farm workers exposed to confinement house air are much more likely than the general population to acquire infections of the lungs and sinuses (Mackiewicz, 1998; Aubry-Damon, 2004; Armand-LeFevre *et al.*, 2005), and the potential for secondary infection of nearby populations is high. Airborne zoonotic pathogens may also travel over vast distances downwind of an infected livestock source. Henderson (1969) and

Smith et al. (1993) and Hawker et al. (1998) describe an outbreak in the West Midlands, UK in which airborne transmission of *Coxiella burnetii* was identified as the causative agent of 147 illnesses. Outdoor lambing and calving was performed on farms south of the urban area. Strong gales blew towards the urban area on a single day approximately three weeks prior to the onset of illness. *Coxiella burnetii* is known to multiply to very high concentrations in the placenta of sheep which, following deposition on the ground during outdoor birthing, can dry out allowing bacterial release with airborne particulates (Welsh et al., 1958; Jones and Harrison, 2004). The mean incubation period for *Coxiella burnetii* in humans has been shown to be 20 days, consistent with the period of time between the day of strong gales and the peak onset of symptoms in the outbreak (Aitken et al., 1987).

Hughes and Wright (1970) describe a series of airborne picnoravirus outbreaks (foot and mouth disease) in pigs, cattle, and sheep in Worcester, UK in 1967, in which infected animals at three pig farms were suspected as the cause. Casal *et al.*, (1997) estimated that the airborne dispersion could have transported an infectious dose of this virus from the three source swine farms to cattle as far as 7 km away. However, secondary infection from cattle or sheep was unlikely to affect cattle or sheep more than 200 m away (Donaldson *et al.*, 2002). It has been estimated that picnoravirus can be transported in the air over distances as great as 60 km overland and 300 km over seas (Gloster *et al.*, 1982; OIE, 2005). Lyytikainen *et al.*, (1998) describe an outbreak of Q-fever in a small rural community in Germany in which airborne transmission of *Coxiella burnetii* from a nearby infected flock of 1,000-2,000 sheep may have caused 45 illnesses over a four month period. Outdoor calving was performed on the farm, and the wind blew from the farm towards the town 57% of the time during the course of the outbreak. Both picnoraviruses and *Coxiella burnetii* may be shed in animal feces suggesting that these organisms, among others, could be dispersed over vast distances following spray irrigation of animal manures onto croplands.

## *6.3 Antimicrobial Resistance*

Antimicrobial-resistant bacteria and other zoonotic pathogens from CAFOs often infect humans, many times with serious consequences. Evidence in the reported literature overwhelmingly supports this conclusion and includes direct epidemiological studies, temporal evidence of the emergence of resistance in livestock animal populations prior to the emergence in human populations, trends in resistance among human isolates that mimic the use of antimicrobials in livestock animals, and studies that show farmers, slaughterers, and their family members are much more likely than the general population to acquire antimicrobial zoonoses. Antimicrobial resistance can limit treatment options in sickened individuals and increase the number, severity, and duration of infections (FAAIR Scientific Advisory Council, 2002). Varma *et al.*, (2005) evaluated *Salmonella* outbreaks in the U.S., and determined that among 32 reported outbreaks between 1984 and 2002, 22% of 13,286 people in ten *Salmonella*-resistant outbreaks were hospitalized compared with 8% of 2,194 people in 22 outbreaks caused by pansusceptible strains. These differences are not only the consequence of limited options for antimicrobials, but are also related to the increased virulence often associated with antimicrobial-resistant organisms. For instance, Lee *et al.*, (1994) determined that individuals infected with resistant organisms were ill 25% longer and were significantly more likely to be hospitalized than those infected with pansusceptible strains. Those

Bezanson et al (1983) describe the infection of a newborn child with a multidrug-resistant strain of *Salmonella* ser. typhimurium resulting in septicemia and meningitis. The source of infection was the child's asymptomatic mother, who acquired the bacterium through ingestion of unpasteurized milk and passed it to her child during delivery in the hospital. Illness in the newborn child manifested within 24 hours, and within 72-96 hours had spread to several other infants in the hospital nursery. In another case described by Lyon et al. (1980), a multidrug-resistant strain of *Salmonella heidelberg* was spread from an asymptomatic mother to newborn child during delivery via cesarean section. The mother was a farmer who shortly before delivery had been working with calves from an infected herd. Three infants in the hospital nursery were infected with the organism and developed bloody diarrhea.

infected with resistant strains were hospitalized on average ten days versus eight days for those infected with susceptible strains, even though most subjects in both cases were treated with an antimicrobial to which the infectious agent was susceptible. The difference in hospitalization rates likely reflects the higher virulence of the resistant infectious organism, and, to a much lesser extent, an inappropriate first choice of antimicrobial for treatment. Resistance to antimicrobial agents, resulting from their extensive use in animal agriculture, may result in tens of thousands of additional infections by zoonotic pathogens compared to what would be experienced with pansusceptible strains. This may result in more than ten thousand additional days of hospitalization, and hundreds of thousands of excess days of diarrhea in the U.S. each year (Barza and Travers, 2002; Travers and Barza, 2002)

## *6.4 Hydrologic Events*

Hydrologic events ranging from mild rainfall to flooding can increase the movement of pathogens from CAFOs or manure-amended fields to waters that are likely to come into contact with people. Serious public health consequences of the increased pathogen load, especially during flooding events, are common (Isaacson *et al.*, 1993; MacKenzie *et al.*, 1994; Health Canada, 2000; CDC, 1998). Several studies in low income countries have reported increases in morbidity and/or mortality following flood events due to cholera, cryptosporidiosis, nonspecific diarrhea, poliomyelitis, rotavirus, and typhoid and paratyphoid (Fun *et al.*, 1991; van Middelkoop *et al.*, 1992; Katsumata *et al.*, 1998; Biswas *et al.*, 1999; Sur *et al.*, 2000; Mondal *et al.*, 2001; Kunji *et al.*, 2002; Kondo *et al.*, 2002; Heller *et al.*, 2003; Vollard *et al.*, 2004). The increased relative risk (RR) or odds ratio (OR) of contracting disease during flooding in these cases ranged from 1.39 to 4.52. Significant increases in vector- and rodent-borne diseases were also observed (Trevejo *et al.*, 1998; Han *et al.*, 1999; Sanders *et al.*, 1999; Sarkar *et al.*, 2002; Leal-Castellanos *et al.*, 2003).

The increased risk of contracting disease post-flood in high income countries such as the U.S., UK, and Australia exist, but are less pronounced (Ahern *et al.*, 2005). Bennet *et al.*, (1976) observed hospital visits by the flooded to more than double in the year following an event in Bristol, UK, in 1968. These researchers also observed a 50% increase in mortality among the flooded, mostly in the elderly. Reacher *et al.*, (2004) interviewed 467 households following a flood in Lewes, UK, and observed a slight increase in gastrointestinal illness in those whose homes were flooded. In Brisbane, Australia (1974), a flood led to increased morbidity, but not mortality, in the flooded group (Price, 1978; Abrahams *et al.*, 1976). However, Handmer and Smith (1983) noticed no flood-related increase in hospital admissions during flooding in Lismore, Australia the same year. In a cohort study of 1,110 people in a U.S. Midwestern community, Wade *et al.*, (2004) reported an increase in the incidence of gastrointestinal illness during a flood event in April and May of 2001. The increase in gastrointestinal illness was pronounced in persons with potential sensitivity to infectious gastrointestinal agents and those who came into contact with the flood water, especially children. Heather *et al.*, (2004) noted the significance of heavy rainfall in the Walkerton, Canada outbreak of *E. coli* O157:H7 and *Campylobacter*. The rainfall was equivalent to a 60-year event, and it was suggested that this extreme precipitation may have mobilized animal wastes and led to the outbreak. Curriero *et al.*, (2001) studied the link between reported waterborne disease outbreaks in the U.S. between 1948 and 1994 and extreme precipitation events. These authors found a strong correlation between

rainfall and disease. Disease due to surface water contamination primarily occurred during the month of the precipitation event, whereas disease associated with groundwater contamination occurred two months following extreme precipitation events.

Serious health outcomes from flooding events can and do occur in the U.S. and may be unfairly weighted against the underprivileged. The recent flooding of New Orleans, Louisiana following hurricane Katrina, a category 4 event, resulted in the exposure of tens of thousands of people to floodwaters laden not only with chemical wastes, but also decomposing bodies, animal carcasses, sewage, and animal wastes. *E. coli* concentrations in these waters reached as high as 42,000 per 100 mL, hundreds of times higher than levels associated with gastrointestinal illnesses that result from "recreational contact". Those unable to escape the city prior to the hurricane were primarily the underprivileged, and illness was exacerbated by the lack of availability of medical provisions and personnel. The eye of the hurricane traveled through Mississippi, the fourth largest poultry-producing state in the U.S., with the highest rainfall amounts (ranging between 12.5-22.5 centimeters, falling at a rate of 1-2 cm per hour) tracking over the central and northeastern portions of the state. As can be seen in Figure 4, these regions are associated with the bulk of large concentrated swine feeding operations in the state of Mississippi. The pollution from these operations has been previously reported to disproportionately affect impoverished and African-American peoples (Wilson *et al.*, 2002). The full breadth of public health outcomes from this hurricane, as well as potential environmental injustice resulting from the flooding, have yet to be fully understood. These events, however, signify the need for water quality officials to seriously consider precipitation events during planning.

## *6.5 Economic considerations*

Infection by zoonotic pathogens results not only in extensive human suffering, but also significant economic loss. For instance, the Milwaukee outbreak of Cryptosporidiosis in 1993 cost the community as much as 96.2 million; 31.7 million in medical costs and 64.6 million in lost productivity (ASM, 1998; Corso *et al.*, 2003; Water Health Connection, 2005). The Walkerton, Ontario outbreak of *E. coli* O157:H7 and Campylobacteriosis in 2002, with 2300 cases and 6 deaths, cost the community an estimated 40 million in lost productivity, medicine, and hospitalization costs. The American Society for Microbiology estimates that even a mild case of diarrhea may cost $330 in lost work productivity and over-the-counter medicines (adjusted to 2005 dollars). More severe cases were estimated to cost up to $9,500 per person for medical diagnosis and treatment. Considering the number of illnesses that may be experienced in the U.S. each year, foodborne illnesses may amount to three billion dollars per year due to hospitalization and more than 20 billion in lost work productivity and over-the-counter medicines, a significant portion of which may be due to transmission of disease from livestock animals. Similarly, waterborne illnesses may result in a total of two to twenty billion dollars in costs annually (Garthright *et al.*, 1988; Hardy *et al.*, 1994; Gerba, 1996; Liddle *et al.*, 1997; Fleisher *et al.*, 1998; Scott *et al.*, 2000; Dwight *et al.*, 2001; Fruhwirth *et al.*, 2001; Corso *et al.*, 2003). Considering the annual healthcare costs of managing antimicrobial resistance, which may be in the range of 4-30 billion dollars (Khachatourians, 1998; American Academy of Microbiology (AAM), 1999; Montague, 2000), the annual costs associated with illness caused by

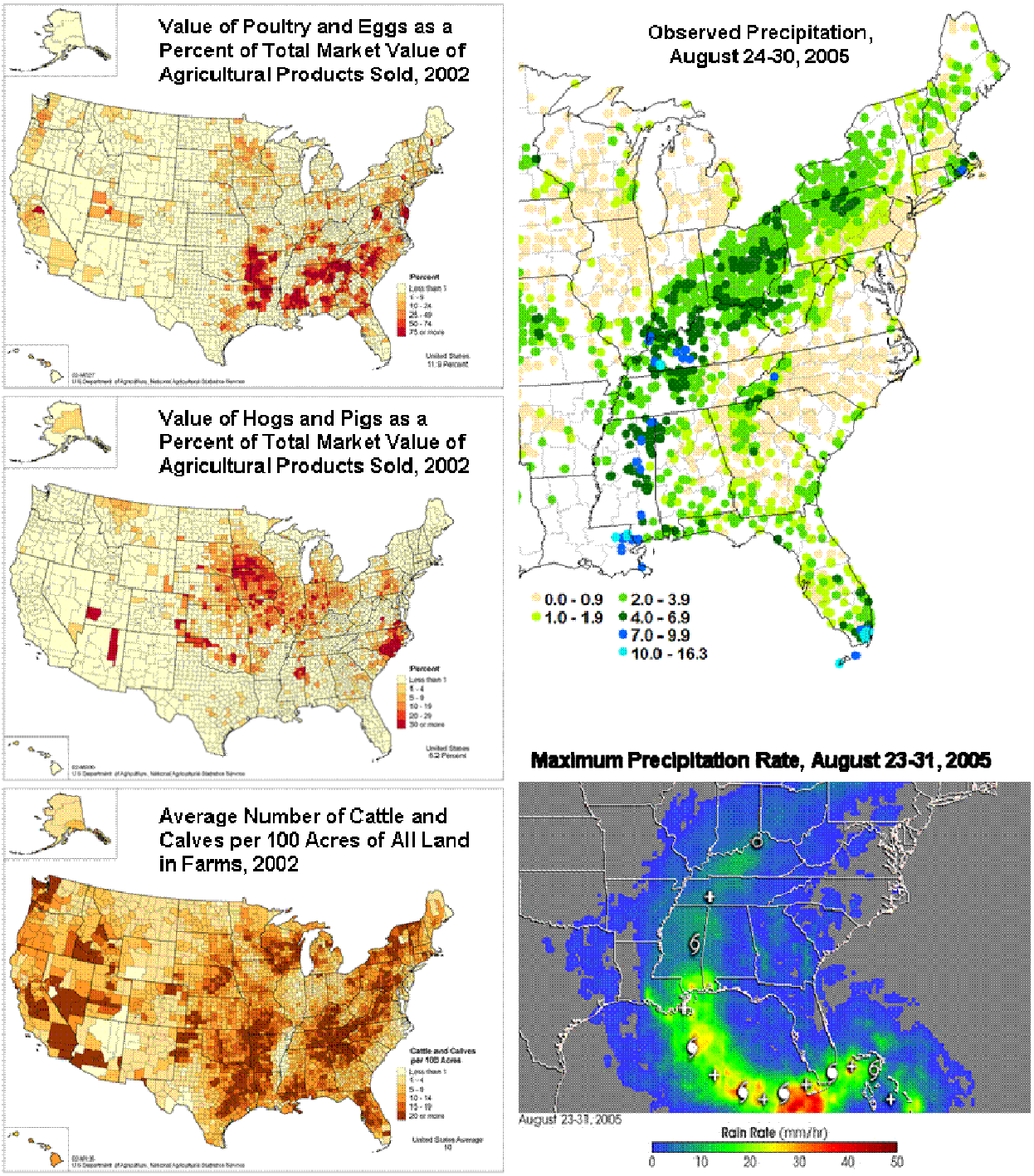


**Figure 4.** Distribution of livestock animals in regions impacted by Hurricane Katrina, August, 2005 (adapted from USDA, 2002; National Oceanic and Atmospheric Administration, 2005; National Aeronautics and Space Administration, 2005).

zoonotic pathogens and antimicrobial resistant bacteria from livestock operations may be staggering.

Significant economic losses may also be incurred by the closing of beaches when waters cannot meet USEPA recreational water guidelines. Of the thousands of beach closings every year, more than 80 percent are due to excessive levels of bacteria (ASM, 1998). A beach closing due to bacteria indicates that levels were excessive the day prior to the closing, during which time thousands of individuals may have been exposed to contaminated water. Dwight *et al.*, (2001) estimated the economic burden from illness associated with recreational coastal water pollution at Newport and Huntington Beaches, Orange County, California alone to be 3.3 million per year. Considering the thousands of beaches closed every year, economic losses may be in the billions of dollars.

## *6.6 Discussion*

Both waterborne outbreaks and those associated with fresh produce have been on the rise in recent decades and will likely continue to increase as surveillance is improved. Although the source of contamination in many of these outbreaks remain unreported, poor manure management in livestock operations most assuredly plays a significant role as alternative sources of contamination are limited in scale compared to manure applications and typically much less infectious in character. The annual costs of infectious zoonotic diseases in the U.S. may reach into the tens of billions of dollars considering both food and waterborne illnesses. These estimates exclude such costs as death, pain and suffering, lost leisure time, financial losses to food establishments, legal expenses, and long-term health outcomes due to infections that may result in degenerative diseases or cancer. The economic burden of pathogenic zoonoses has been shifted from corporate farms who fail to use appropriate manure management at the source of disease to sickened individuals and businesses that experience decreased revenues due to beach closures and lost productivity. Economic burdens of CAFO pollution may be especially shouldered unfairly by minority groups and the poor, as evidenced by recent works describing environmental injustice surrounding the swine industries of North Carolina and Mississippi (Wing *et al.*, 2000; Wilson *et al.*, 2002). It is at present unclear how new molecular microbiological technologies such as microbial source tracking will affect litigation and potential liability of CAFO operators in future disease outbreaks.

# 7. Emerging Technologies: Monitoring Pathogens in the Environment

Concentrated animal feeding operations may release pathogens into the environment through a variety of mechanisms that may result in extensive human suffering and economic loss. Current surveillance activities may be inadequate to identify fully the scope of problems surrounding the release of overt pathogens from CAFOs to the environment. For instance, surface water quality surveillance in the U.S. relies on the quantitative detection of bacterial indicators of fecal pollution including *E. coli* and enterococci rather than direct identification of selected etiologic agents associated with disease in humans. Although related to gastrointestinal illness following recreational water contact, these indicators may not be reliable surrogates for all bacterial pathogens and most parasites and viruses. Human illness can occur even when the concentrations of *E. coli* and enterococci indicate that bathing waters are safe. The use of microbial indicators as surrogates for pathogens continues because infectious concentrations of pathogens in waters may be low and difficult to detect, and standard methods for analysis do not exist for many pathogens.

The February 28, 2005 ruling by the 2nd U.S. Circuit Court of Appeals required the USEPA to identify and characterize the performance of animal waste management practices and barrier technologies that specifically address contamination of the nation's waters by pathogens emanating from CAFOs. Transport properties and the virulence of various pathogens vary to a wide degree, and may be poorly represented by the bacterial indicators *E. coli* and enterococci (Ferguson *et al.*, 2003). Thus, this requirement signals the need for new and improved pathogen detection technologies. New approaches to water quality monitoring and emerging technologies enabling the identification of overtly pathogenic agents in natural waters and their source will greatly improve human health and welfare and increase the biosecurity of our natural resources.

Considering the many potential exposure routes following release of pathogenic zoonoses from CAFO facilities, identification of the risks associated with pathogens emanating from concentrated animal feeding operations may require technologies that enable the measurement of overt pathogens in air, drinking and recreational water, meat and produce, soil and sediments, and feces of various animals among others. For foods, standard methods for analysis for a small number of zoonotic pathogens already exist (FDA, 2005). For other matrices, such as soil and sediments, drinking water, and natural waters, methods are lacking or have not been standardized. Methods reported in literature include classical cultivation approaches, and more recently, identification and quantification of agents via the detection of surface antigens or nucleic acids. In either case, the detection of zoonotic pathogens with infectious doses as low as a few ingested or respired particles presents specific challenges including concentration of large environmental samples, removal of inhibitory compounds from sample concentrates, detection of viability, detection of multiple agents in a limited sample, and long analysis periods, especially for cultivation-based approaches. Considerable advances in emerging nucleic acids and sensor technologies are reducing analysis times from weeks to hours in some instances, but present trade-offs in terms of the costs and technical expertise required to apply the technologies. The intent of this review is not to provide a complete description of all of the emerging detection technologies and report their application, but rather to identify their strengths and limitations and discuss the challenges posed by their use.

## *7.1 Sample Processing*

Appropriate sample processing is critical to detecting pathogenic agents at concentrations relevant to their infectious dose in environmental matrices. For some media that may contain high numbers of pathogens but are extremely heterogeneous such as animal manure, obtaining an appropriate sample may hinge on careful compositing procedures. For instance, Pearce *et al.*, (2004) examined the distribution of *E. coli* O157 in bovine fecal pats and determined that the density of O157 in the pats was highly variable, differing by as much as 76,800 CFU/g between samples of the same fecal pat. These researchers determined that most positive samples bordered the detection limit, and that testing of only 1 g per pat (as is commonly performed) may result in as much as 20-50% false-negatives. For other media such as air, sample processing may need to be particularly careful regarding stressing organisms in the sampling device.

The low infectious dose associated with many etiological agents leads to the need to concentrate copious quantities of air, food, or water into smaller volumes amenable to detection with classical cultivation or the newer molecular microbial methods. Several mechanisms have been used to concentrate these agents to detectable numbers including filtration, immunocapture, and enrichment. However, some of these methods may concentrate inhibitory and/or interfering compounds with the agent of interest, while others require additional analysis time or alter the sample from its initial state. Thus, the use of any of these mechanisms may present tradeoffs in downstream analysis, potentially affecting analytical detection limits, analysis times, or the number of agents that can be detected from a single sample. At present, standard methods for the concentration of viruses from water samples rely on electrostatic capture from 100 or more liters of water onto positively-charged filters followed by elution, precipitation, and resuspension in a small volume of sodium phosphate buffer (USEPA, 1993). Concentration of the protozoan parasites *Giardia* and *Cryptosporidium* require filtration of ten or more liters of water through a depth filter followed by elution, centrifugation, and immunomagnetic separation (USEPA, 2001). Concentration of bacterial pathogens is usually performed by membrane filtration, although turbidity of water can severely inhibit the volume of water that can be passed through the filter.

None of the accepted concentration techniques listed above is applicable to all of the various groups of etiologic agents (viruses, bacteria, protozoans). The detection of several agents may require the collection of multiple large volume samples from a single location and concentration by a number of techniques. To overcome these limitations, newer methods for sample concentration applicable to all classes of etiologic agents have been proposed. Most notably, hollow-fiber ultrafiltration has been used to simultaneously concentrate viruses, bacteria, and protozoan parasites from water samples as large as 100 L to volumes as low as 250 mL with recovery efficiencies on the order of 20-92% (Juliano and Sobsey, 1997; Kuhn and Oshima, 2001; Olsezewski *et al.*, 2001; Evans-Strickfaden *et al.*, 1996; Simmons *et al.*, 2001; Morales-Morales *et al.*, 2003; Ferguson *et al.*, 2004). Subsequent analyses with small portions of a single eluent can lead to detection of several pathogens at environmentally-relevant concentrations (Olsezewski *et al.*, 2001; Morales-Morales *et al.*, 2003). Hollow-fiber ultrafiltration may also have the added benefit of allowing small or water soluble inhibitors of nucleic acids techniques to pass into the permeate, rather than co-concentrate with the pathogens in the retentate, prior to sample analysis (Wilson, 1997).

## 7.2 Conventional Cultivation and Nucleic Acids Approaches

Conventional cultivation methods for the detection of bacterial pathogens usually require several steps including (1) sampling and release of bacteria from the environmental matrix, (2) pre-enrichment in non-selective broth to allow small numbers of stressed bacterial pathogens to recover and grow prior to applying further environmental stress in selective broths, (3) transfer to selective broth to enrich low numbers of pathogens and reduce competitor bacteria, (4) inoculation of a selective solid medium to identify presumptive positive colonies, and (5) biochemical and/or serological confirmation of presumptive-positive colonies. Most probable number (MPN) techniques can be used to arrive at a quantitative result (USEPA, 2005). Depending on the number of steps required; confirmation of the presence of specific pathogens by conventional methods may take as few as two days to two weeks or more.

Nucleic acid technologies follow the same framework for detection as cultivation methods, but detection or quantitation can occur prior to or following pre-enrichment in nonselective media or further enrichment in selective broths. Nucleic acid technologies are also commonly used in lieu of biochemical and/or serological confirmation for presumptive colonies, or to acquire more detailed genomic information on bacterial isolates, such as possession of antimicrobial-resistance or virulence traits. Enrichment broths (both selective and nonselective) for nucleic acids techniques are used not only to increase the numbers of pathogens, thereby improving detection, but also to dilute potential inhibitors of the polymerase chain reaction (PCR). Immunomagnetic separations have also been used to separate etiological agents from large volumes and/or samples with inhibitory agents prior to or following enrichment steps.

The detection limit of nucleic acid assays is usually dependent on the amount of time available for analysis. In general, if the etiological agent of interest is in high concentration or the medium is relatively clean (such as drinking water), short analysis times of less than 6-8 hours can be realized, as inhibitors may be in low concentration relative to pathogens in the original sample retentate. In more turbid samples with low numbers of infectious agents, such as stream waters, analysis times can extend from a day to as much as four days. Extensive sample processing and selective enrichments may be required to achieve detection limits relevant to the infectious dose. Tables 9 and 10 list several studies that have used nucleic acids techniques for the detection of etiological agents.

As can be seen in Table 9, nucleic acids techniques may provide very sensitive detection of selected etiological agents in clean samples, such as air or drinking water, even without enrichment. For turbid environmental samples, such as surface water, feces, or soils, nucleic acid technologies may have detection limits several orders of magnitude higher than cultivation techniques (See Table 9). This is because PCR inhibitors, such as humic substances associated with many environmental samples, are co-extracted with the etiological agent prior to detection. Even with inhibitors present, nucleic acid technologies may occasionally yield more sensitive results than cultivation-based techniques. For instance, Inglis and Kalischuk (2004) used nested real-time SYBR Green-PCR to quantify *Campylobacter lanienae* in cattle feces without enrichment. These researchers were able to detect *C. lanienae* at concentrations as low as 250 CFU/g feces in less than 4 hours, a level more sensitive than could be achieved with cultivation-based methods that required 2 days for presumptive results. However, in most instances,

**Table 9.** Sample times and detection limits of several nucleic acids-based techniques for detecting pathogens in different matrices without enrichment.

| Etiologic Agent | Matrix | Time *hours* | Detection Limit | Reference |
|---|---|---|---|---|
| **No Enrichment** | | | | |
| Anthrax Spores | Air | 1-2 | 1 spore/100 L | Makino and Chuen (2003) |
| *E. coli* | Drinking Water | 5 | 1 CFU/L | Abd El-Haleem *et al.*, (2003) |
| *Salmonella* | Drinking Water | 5 | 1 CFU/L | Abd El-Haleem *et al.*, (2003) |
| *E. coli* O157:H7 | Ground Water | 3 | 20,000 CFU/100 mL | Vaughn *et al.*, (2003) |
| Hepatitis A | Sewage effluent | <24 | 1,000,000 PFU/mL | Jean *et al.*, (2001) |
| Rotavirus | Sewage effluent | <24 | 3,000 PFU/mL | Jean *et al.*, (2002) |
| Hepatitis A | Produce | <24 | 1,000,000 PFU/surface | Jean *et al.*, (2001) |
| *Campylobacter* spp. | Meat † | <24 | <100,000 CFU/10 g | Uyttendale *et al.*, (1995-1997) |
| *Listeria monocytogenes* | Meat | 4 | 100 CFU/g | Rodríguez-Lázaro *et al.*, (2004) |
| *E. coli* O157:H7 | Cattle Feces | 4 | 26,000 CFU/g | Ibekwe and Grieve (2003) |
| *Campylobacter jejuni* | Cattle Feces | 4 | 3,000 CFU/g | Inglis and Kalischuk (2004) |
| *Campylobacter lanienae* | Cattle Feces | 4 | 250 CFU/g | Inglis and Kalischuk (2004) |
| *Clostridium difficile* | Human feces | 1 | 50,000 CFU/g | Bélanger *et al.*, (2003) |
| *Salmonella* spp. | Biosolids | 24 | $10^6$ CFU/g | Burtscher and Wuertz (2003) |
| *Staphylococcus aureus* | Biosolids | 28 | $10^6$ CFU/g | Burtscher and Wuertz (2003) |
| *E. coli* O157:H7 | Soil | 4 | 26,000 CFU/g | Ibekwe and Grieve (2003) |
| *E. coli* O157:H7 | Soil | 4 | 35,000 CFU/g | Ibekwe *et al.*, (2002) |
| **No Enrichment, Immunomagnetic Separation** | | | | |
| *Cryptosporidium parvum* | Clean Water | <24 | 50 Oocysts/100 L | Baeumner *et al.*, (2001) |
| *Cryptosporidium parvum* | Turbid Water | <24 | 50 Oocysts/100 L | Baeumner *et al.*, (2001) |
| Hepatitis A | Ground Water | 6-12 | 20 PFU/20 mL | Abd El Galil *et al.*, (2004) |
| Enterohemorrhagic *E. coli* | Chicken Rinsate | <24 | 55 CFU/mL | Call *et al.*, (2001b) |
| *Campylobacter jejuni* | Chicken Feces | 6 | 230 CFU/g | Rudi *et al.*, (2004) |
| *Campylobacter jejuni* | Chicken Ceces | 6 | 2,000 CFU/g | Rudi *et al.*, (2004) |

† Meat = beef, poultry, or pork

improved sensitivity over cultivation-based techniques will not be realized without further sample processing.

Immunomagnetic separation methods (IMS) have been used successfully in several studies to concentrate etiological agents prior to or following sample concentration. These methods use paramagnetic particles coated with antibodies specific to the pathogen of interest to bind the agent and remove it from the sample matrix in a concentrated form via magnetic attraction of the pathogen-paramagnetic particle complex (Campbell and Smith, 1997; Bukhari *et al.*, 1998; Rochelle *et al.*, 1999). The separation of the agent of interest from the environmental sample reduces the presence of inhibitory substances improving detection by PCR. For instance, Abd El Galil *et al.*, (2004) developed a protocol for using combined Immunomagnetic Separation-Molecular Beacon-Reverse Transcription-PCR to detect Hepatitis A virus in groundwater samples. These authors concentrated 100 liters of groundwater using an electropositive

microporous (1-MDS) filter followed by elution in beef extract, centrifugation, and resuspension of the pellet in 20 mL sodium phosphate buffer (final pH=7.4). Immunomagnetic separation with two-hour incubation was used to recover the virus followed by extraction of viral RNA, reverse transcription, and real-time PCR with a molecular beacon probe. As few as 20 plaque-forming units (PFU) per 20 mL groundwater concentrate were recovered by these methods. Immunomagnetic separation methods may be attractive where selective enrichment cannot be used effectively.

Selected amplification facilitators or specific DNA treatments may also be used to reduce (but not eliminate) the effects of inhibitory compounds on PCR (Satoh *et al.*, 1998; Abu Al-Soud and Rådström, 2000; Böddinghaus *et al.*, 2001). Common facilitators may include bovine serum albumin (BSA), the single-stranded DNA-binding T4 gene 32 protein (gp32), betaine, and several proteinase inhibitors, all of which work to a varying degree depending on sample type (Abu Al-Soud and Rådström, 2000). Of the facilitors used, BSA is the most common, and tends to work with samples from a wide variety of origins, including blood, human and animal feces, surface and ground waters, soils and sediments, and meat. For instance, Rudi *et al.*, (2004) applied integrated cell concentration and DNA purification using immunomagnetic beads with real-time (TaqMan) PCR to detect and quantify *Campylobacter jejuni* in chicken fecal samples. These researchers could detect as few as 1,000-10,000 CFU/g feces in untreated samples, but with 0.4% BSA in the reaction mix, could reduce PCR inhibition caused by the fecal extract, so that they could reduce their detection limit to as low as 230-2,300 CFU/g feces. Since the mode of action of many facilitators is similar (removal of inhibitors), their benefits are not additive (Abu Al-Soud and Rådström, 2000).

To improve detection of bacterial pathogens in difficult matrices, especially for particularly infectious agents that may be of interest at very low concentrations, enrichments can be used to revive stressed bacteria and increase their numbers prior to detection. Enrichments have the added benefit of diluting PCR inhibitors prior to detection by nucleic acid techniques, resulting in lower detection limits than can be realized by direct sampling (See Table 10). The enrichment of bacterial pathogens presents a trade-off: selective media may enrich one pathogen at the expense of other agents of concern. Several enrichment broths may be required to detect several agents. However, in some cases, nonselective broths may improve the detection of many bacterial pathogens following a single enrichment. For instance, Nam *et al.*, (2004) evaluated the use of universal pre-enrichment broth (UPB) versus selective enrichment broths [lactose broth, modified trypticase soy broth (plus novobiocin), and *Listeria* enrichment broth] for detection of *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes* from dairy fecal slurry, lagoon water, drinking water, silage/feed, trapped rats, bird droppings, calf fecal swabs, milking parlor floor swabs, bulk tank milk, and in-line milk filters. These researchers observed no differences between growth in UPB and selective media using pure cultures of the three pathogens, either individually or mixed. However, slightly better recovery of pathogens from environmental samples was observed when UPB was used for the initial enrichment step, and transfers were made from the single pre-enriched sample to selective media. UPB supported the growth of all three pathogens to levels detectable by culture techniques within 24 hours from the different environmental matrices.

**Table 10.** Sample times and detection limits of several nucleic acids-based techniques for detecting pathogens in different matrices following enrichment.

| Etiologic Agent | Matrix | Time *hours* | Detection Limit | Reference |
|---|---|---|---|---|
| **Nonselective Enrichment** | | | | |
| *E. coli* O157:H7 | Drinking Water | <24 | 100 CFU/100 mL | Campbell *et al.*, (2001) |
| *E. coli* O157:H7 | Surface water | <24 | 600 CFU/100 mL | Campbell *et al.*, (2001) |
| *E. coli* O157:H7 | Apple Juice | 15 | 100 CFU/100 mL | Fortin *et al.*, (2001) |
| *Salmonella* spp. | Produce | 20 | 4 CFU/25 g | Liming and Bhagwat (2004) |
| *E. coli* O157:H7 | Milk | 10 | 100 CFU/100 mL | Fortin *et al.*, (2001) |
| *Salmonella* spp. | Milk | 24 | Equal to cultivation [§] | Malorny *et al.*, (2004) |
| *Salmonella enteritidis* | Egg | <48 | <10 CFU/25 g | Cook *et al.*, (2002) |
| *Salmonella typhimurium* | Oysters | <24 | 100 CFU/g | Lee *et al.*, (2003) |
| *Vibrio* spp. | Oysters | <24 | 100 CFU/g | Lee *et al.*, (2003) |
| *Salmonella* spp. | Chicken Rinsate | 24 | Equal to cultivation | Malorny *et al.*, (2004) |
| *E. coli* O157:H7 | Meat [†] | 8-10 | 580 CFU/g | Sharma *et al.*, (1999) |
| *Salmonella* spp. | Meat | <24 | 1500 CFU/25 g | Cheung *et al.*, (2004) |
| *Salmonella* spp. | Meat | 24 | Equal to cultivation | Malorny *et al.*, (2004) |
| *E. coli* O157:H7 | Cattle Feces | 8-10 | 1200 CFU/g | Sharma *et al.*, (1999) |
| *Salmonella* spp. | Biosolids | 24 | 10 CFU/g | Burtscher and Wuertz (2003) |
| *Staphylococcus aureus* | Biosolids | 28 | 10 CFU/g | Burtscher and Wuertz (2003) |
| *E. coli* O157:H7 | Soil | 10 | 10000 CFU/g | Campbell *et al.*, (2001) |
| *E. coli* O157:H7 | Soil | 14 | 6 CFU/g | Campbell *et al.*, (2001) |
| *E. coli* O157:H7 | Soil | 24 | 2 CFU/g | Campbell *et al.*, (2001) |
| *E. coli* O157:H7 | Soil | <24 | <10 CFU/g | Ibekwe and Grieve (2003) |
| *E. coli* O157:H7 | Soil | <24 | <10 CFU/g | Ibekwe *et al.*, (2002) |
| **Selective Enrichment** | | | | |
| *Campylobacter jejuni* | Surface Water | 72 | Equal to cultivation | Sails *et al.*, (2002) |
| *Campylobacter coli* | Surface Water | 72 | Equal to cultivation | Sails *et al.*, (2002) |
| *E. coli* O157:H7 | Surface Water | <48 | 120 CFU/100 mL | Müller *et al.*, (2003) |
| *Listeria monocytogenes* | Produce | <48 | <10 CFU/10 g | Uyttendale *et al.*, (1995-1997) |
| *Listeria monocytogenes* | Dairy Products | <48 | <10 CFU/10 g | Uyttendale *et al.*, (1995-1997) |
| *Listeria monocytogenes* | Dairy Products | <72 | <10 CFU/60 g | Blais *et al.*, (2001) |
| *Salmonella* spp. | Milk | 24 | Better than cultivation [§] | Kessel *et al.*, (2003) |
| *Listeria monocytogenes* | Eggs | <72 | <10 CFU/60 g | Blais *et al.*, (2001) |
| *Vibrio parahaemolyticus* | Oyster | 24 | Better than cultivation | Blackstone *et al.*, (2003) |
| *Campylobacter* spp. | Meat | <48 | 10 CFU/10 g | Uyttendale *et al.*, (1995-1997) |
| *Listeria monocytogenes* | Meat | <48 | <10 CFU/10 g | Uyttendale *et al.*, (1995-1997) |
| *E. coli* O157:H7 | Sewage Sludge | <48 | 120 CFU/100 mL | Müller *et al.*, (2003) |
| *Listeria monocytogenes* | Biosolids | 28 | 10 CFU/g | Burtscher and Wuertz (2003) |
| *Listeria monocytogenes* | Biosolids | 28 | 10 CFU/g | Burtscher and Wuertz (2003) |
| *Yersinia enterocolitica* | Biosolids | 28 | 10 CFU/g | Burtscher and Wuertz (2003) |
| *Salmonella* spp. | Biosolids | 24 | 10 CFU/g | Burtscher and Wuertz (2003) |

[†] Meat = beef, poultry, or pork

[§] Nucleic acid techniques provided a result equivalent to or better than cultivation methods based on trials in actual samples

Several studies have documented improvements in detection of bacterial pathogens using nucleic acids techniques that can be realized using enrichments in both non-selective and selective broths. Ibekwe and Grieve (2003) used real-time TaqMan PCR to with a detection limit of 26,000 CFU *E. coli* O157:H7 per gram of soil without enrichment. Using a 16 hour pre-enrichment in modified Luria-Bertani broth (containing vancomycin, cefoxime, and cefsulodin); these researchers could reduce their detection limits to less than 10 CFU per gram soil. Burtscher and Wuertz (2003) evaluated the use of PCR for the detection of *Salmonella* spp. *Listeria monocytogenes, Yersinia enterocolitica,* and *Staphylococcus aureus* in biosolids from anaerobic digesters and aerobic composters following one- and two- step enrichment (24-48 hours) in selective broths. These researchers were able to detect less than 10 CFU per gram of waste for each organism following enrichment, versus $10^6$-$10^7$ CFU per gram of waste without enrichment.

Nucleic acids techniques may also be useful as a surrogate for biochemical confirmation or for genotyping environmental isolates following detection with cultivation techniques, potentially saving days in analysis time. Müller *et al.*, (2003) tested sewage sludges and river waters for *E. coli* O157:H7 using combined cultivation and nucleic acids techniques. These researchers filtered 100 mL river water samples through 0.45 µm nitrocellulose membranes then enriched the retentate in peptone-saline water (PSW) supplemented with vancomycin-cefixime-cefsulodin solution for six hours. Similarly, 100 µL sewage sludge was enriched for six hours directly in the antibiotic-PSW solution. Immunomagnetic separation was used to isolate *E. coli* O157:H7 from a small portion of the enrichment media, and the paramagnetic bead-bacteria complexes were further enriched on selective media for 24 hours. Suspect colonies were investigated with PCR targeting genes associated with Shiga-like toxins 1 and 2, attachment and effacement, and enterohaemolysin. With these methods, the researchers could detect as few as 120 CFU/100 mL in sewage sludge and river samples in less than 48 hours.

Several researchers have also noted that PCR detection of antibiotic-resistance traits is more rapid and sensitive, and potentially more cost-effective, than culture or selective media. This is attractive for clinical diagnosis and surveillance (Lévesque *et al.*, 1995; Briggs *et al.*, 1999; Paule *et al.*, 2001; White *et al.*, 2001; Paule *et al.*, 2003; Blickwede and Schwartz, 2004; Sundsfjord *et al.*, 2004; Shamputa *et al.*, 2004; Jalava and Marttila, 2004). However, in some cases, resistance to antimicrobials can be phenotypically observed with the lack of detection of antimicrobial resistance determinants (Patel *et al.*, 1997). This may occur, as antimicrobial resistance may be conferred by several different genes, not all of which have been characterized. Because of the potential risks associated with misdiagnosis of disease, resistance screening by molecular methods in a clinical setting should be used as a compliment to classical phenotypic approaches.

Other problems may arise when using nucleic acids techniques if researchers are not careful with their methods. Of particular concern is establishing detection limits for nucleic acid techniques for pathogens in food, clinical diagnostic samples, and environmental matrices. Detection limits should be established with samples that closely mimic the matrix of interestenvironmental conditions. If detection limits are reported that over-estimate the efficacy of the method, their use for surveillance or diagnosis may put the public at risk. For instance, Lyon (2001) developed a real-time (TaqMan) PCR method to detect *Vibrio cholerae* O1 and O139 in raw oysters without enrichment. This researcher spiked 25 g oyster homogenates with a single inocula

(approximately 6.2 x $10^6$ *V. cholerae* O1 and 6.7 x $10^6$ *V. cholerae* O139), then serially diluted with alkaline-peptone water to 6 $\log_{10}$ the original concentration. Because both organisms could be detected in the most dilute samples, a detection limit of 6-8 CFU/g oyster was reported. However, by diluting their samples up to six-fold in alkaline-peptone water instead of unspiked raw oyster homogenate, they may have diluted out a significant amount of PCR inhibitors for their most dilute samples. The true detection limits of this assay are unclear.

## *7.3 Pathogen Viability*

For pathogenic zoonoses in different environmental matrices to pose a threat to human health, they must be in a viable state. The detection of viability by either cultivation-based techniques or nucleic acids approaches, however, may not be straight-forward. Cultivation techniques require the viability of microorganisms to yield a result. However, viable-but-nonculturable (VBNC) cells can remain undetected and may complicate interpretation of results. Aside from a clear definition of what constitutes a VBNC state (Barer and Harwood, 1999; Kell *et al.*, 1998; Keer and Birch, 2003; Besnard *et al.*, 2000; del Mar Lleó *et al.*, 2000; Grey and Steck, 2001b; Nilsson *et al.*, 1991; Turner *et al.*, 2000: Bogosian *et al.*, 2000), it still remains unclear as to whether cells in a VBNC state are pathogenic (Barer *et al.*, 2000; Grimes *et al.*, 1986; Steinert *et al.*, 1997; Grey and Steck, 2001a; Cappelier *et al.*, 1999). What is known, however, is that degradation of nucleic acids in VBNC cells may proceed at much slower rates than in killed cells. In fact, some studies have indicated that the pool of messenger RNA (mRNA) may stabilize within VBNC cells rather than continually degrade (Thorne Williams, 1997; Smuelders *et al.*, 1999). Indeed, VBNC bacterial pathogens may harbor genes encoding antimicrobial resistance and other virulence mechanisms for long periods of time after entering a VBNC state (Chaiyanan *et al.*, 2001), serving as a potential reservoir for virulence determinants in the environment. It is clear that molecular methods cannot differentiate between viable and VBNC pathogens when nucleic acids persist in the cells (Thorne and Williams, 1997; Smeulders *et al.*, 1999; Lázaro *et al.*, 1999; del Mar Lleó *et al.*, 2000; Weichart *et al.*, 1997).

Nucleic acid techniques may yield more rapid and sensitive results than cultivation-based techniques for detecting pathogens in different matrices, but there still remains a question as to what a positive PCR result means. Presence of DNA is not a reliable indicator of bacterial viability (McCarty and Atlas, 1993; Masters *et al.*, 1994; Deere *et al.*, 1996; Hellyer *et al.*, 1999). Ribosomal RNA has been shown to be a better indicator of bacterial viability due to a more rapid degradation than DNA upon cell death, but may not be reliable in all cases (McKillip *et al.*, 1999; Villarino *et al.*, 2000l McKillip *et al.*, 1998; Meijer *et al.*, 2000; Tolker-Nielsen *et al.*, 1997). Because of its extremely short half-life following cell death (seconds), mRNA may be the most reliable nucleic acid for indicating cell viability (Keer and Birch, 2003). However, it has been shown that mRNA may still persist. Therefore, care must be taken to design probes and primers that target regions of mRNA more susceptible to degradation (Cook, 2003).

Detecting mRNA requires a higher level of technical expertise than standard DNA-based methods, and does not lend to direct quantitation of pathogens in a sample as multiple and variable quantities of mRNA may be present in a single cell. Quantitative results may, however, be achieved using MPN techniques. Two methods commonly used for detecting ribonucleic acids are reverse-transcriptase PCR and nucleic acid sequence-based amplification (NASBA).

Enrichment of environmental or food samples prior to detection may enhance method sensitivity and aid in the detection of viable versus non-viable cells. Considering the potential of VBNC pathogens to act as environmental reservoirs for virulence determinants, as well as the ability of nucleic acids techniques to detect these cells, molecular methods may offer a distinct advantage over more classic cultivation-based assays for the protection of human health and the environment.

## *7.4 Emerging Surveillance Technologies*

Considerable advances in nucleic acids and sensor technologies continue at a rapid pace (Walker, 2002; Dunbar *et al.*, 2003; Petrenko and Vodyanoy, 2003; Turnbough, 2003; Olsen *et al.*, 2003; Greene and Voordouw, 2003; Grow *et al.*, 2003; Unnevehr *et al.*, 2004; Panicker *et al.*, 2004; Raymond *et al.*, 2005). Of the emerging nucleic acids technologies, perhaps the most promising for surveillance and biosecurity are microarrays. Call *et al.*, (2003) and Ye *et al.*, (2001) describe the emerging use of microarrays and their potential for pathogen detection and genotyping. Microarrays are essentially a large set of very small southern blots, an array of many nucleic acid probes complimentary to discrete gene sequences, bound to a solid or semi-solid matrix, usually a modified glass surface. Because of the miniscule size of the blots (100-200 µm diameter "spots" separated from neighbors by typically 200-500 µm), thousands of sequences can be screened on a single array of less than four square centimeters. Target nucleic acids, which are typically, but not necessarily, PCR products, are challenged by the microarray under stringent hybridization conditions. Targets are usually prepared prior to hybridization with fluorescent labels or incorporating specific chemistries such as biotin-streptovidin that permit detection with a secondary fluorescent marker. Once post-hybridization steps are complete, arrays are catalogued using high resolution laser- or filter-based scanners and charged-coupled device (CCD) imaging. Based on hybridization patterns between the spotted arrays and the nucleic acids targets, the genotype of the original pathogen or the presence of specific pathogens in complex samples can be identified.

Microarrays are presently limited to endpoint detection, rather than quantification, of specific microbial targets. Although in some instances they can be used to detect nucleic acids isolated directly from complex matrices, the sensitivity of microarrays severely impedes their use for pathogen detection at the very low concentrations of interest in environmental samples without a specific nucleic acid amplification strategy (Call *et al.*, 2003). When coupled with nucleic acid amplification techniques, microarrays have been used successfully to detect enterohemorrhagic *E. coli* in chicken rinsate at concentrations as low as 55 CFU/mL. Perhaps more promising, microarrays can be used to rapidly genotype specific pathogens with greater sensitivity (Chizhikov *et al.*, 2001; Call *et al.*, 2001b; Johnson and Stell, 2000; Bekal *et al.*, 2003). Whole and partial genome microarrays for many pathogens are also commercially available, allowing for "fingerprinting" of microbial pathogens by establishing patterns unique to particular species that may further enable genotyping studies. By challenging these arrays with nucleic acids of a wide variety of sources, very small sets of unique markers for specific pathogens may be identifiable, better enabling environmental pathogen detection.

## 7.5 Discussion

Pathogen detection is performed routinely for meat, produce, seafood, milk, occasionally for biosolids from human municipal treatment facilities, but rarely for environmental matrices such as animal manure and their treated residuals, environmental waters, air, soils, and sediments. The recovery and detection of pathogens in environmental matrices are imperative to identify the extent to which these agents are removed, inactivated, or persist in livestock animal waste treatment processes and management systems at CAFOs. Conventional cultivation-based and newer nucleic acids-based approaches to detect or enumerate etiological agents in some environmental matrices are available. However, these methods are not amenable across different groups of pathogens or matrices and can complicate environmental sampling. Very limited standardization of pathogen detection methods exists, except for in the case of foods (Association of Analytical Communities (AOAC), 2005; FDA, 2005; USDA, 2005). The applicability of the standard methods for detecting pathogens in food to other matrices such as feces, water, and soil has not been established. Standard methods with the required sensitivity for detecting pathogens at relevant concentrations in environmental milieus are sorely lacking, especially for hyper endemic or emerging pathogens such as *E. coli* O157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Campylobacter jejuni*, swine hepatitis E virus and the protozoan parasites *Giardia lamblia* and *Cryptosporidium parvum* (Sobsey *et al.*, 2002). The efficacy of animal waste management systems for removing zoonotic pathogens and antimicrobial-resistant bacteria from waste streams at CAFOs remains uncertain.

Aside from assessing the efficacy of livestock animal waste management systems, the recovery and detection of pathogens in water is imperative to protecting human health and the environment. The wise old adage of indicator organisms is becoming outmoded, as their reliability to predict all waterborne outbreaks is uncertain, their results come “a day late and a dollar short”, and newer technologies are becoming available that negate the need to rely solely on bacterial indicators of pathogenicity. Improving surveillance activities in recreational and drinking waters will require these new methods for detecting pathogens to be available in near real time. However, this may be hindered by specific physical or chemical properties of environmental waters that, combined with low concentrations of pathogens, may increase sample processing times. In general, the cleaner the sample, the more methods will be effective for rapid pathogen detection. For clean matrices such as drinking water, sample concentration methods alone may be sufficient to yield results directly usable by both cultivation and direct molecular detection. For more challenging matrices, such as ground and surface waters, significant sample clean-up may be required to remove inhibitors prior to processing with molecular methods.

As discussed above, the detection of etiologic agents in environmental waters is problematic, not only due to very low (but potentially significant) concentrations, but also due to a lack of methods with the required sensitivity and competing methods that are not amenable across different groups of pathogens. As such, there is a need to develop a unified and automated system for the detection of all waterborne pathogens (Straub and Chandler, 2003). It has been suggested that such technologies rely on nucleic acids analyses because they are amenable to automation and are at present the most promising for rapid and specific quantitation of viable microbial pathogens (Jothikumar *et al.*, 1998; Levin *et al.*, 2002; Straub and Chandler, 2003). Hollow-fiber ultrafiltration systems can concentrate all classes of pathogens in a single step and

can be reused (Kuhn and Oshima, 2001; Olszewski *et al.*, 2001). Therefore, these filters may serve well as a basis for sample concentration in such a system. More recently, renewable surface technologies for automated sample processing coupled with microarray technologies have also shown promise as a basis of such a system (Chandler *et al.*, 2000a,b). Nucleic acids technologies, however, are still primarily in the hands of researchers and beyond the scope of all but the most highly trained staff and most affluent utility laboratories (Levin *et al.*, 2002). Significant investments need to be made in the development of simple and reliable technologies that are less technically-demanding.

Several pertinent issues need to be addressed before nucleic acids technologies are exclusively used as standard methods for surveillance activities, such as monitoring recreational and drinking water quality. First and foremost, there remains a need for regulatory establishment of acceptable concentrations in environmental matrices to determine the relevancy of detection limits established in these studies. Method development and standardization cannot proceed until target detection limits that reflect true risks of illness are established. These limits need to identify target concentrations based on epidemiological studies of health risk rather than indicate that pathogens should not be detected in a given sample volume. Regulatory guidelines should also clearly indicate standards regarding acceptable recoveries from environmental samples during concentration, analysis sensitivities, and standard errors. This is because sample concentration methods may present a wide variation in recovery of etiological agents from environmental waters, and regardless of the sample concentration methods used, PCR-based detection systems must confront a number of front-end challenges inherent to complex environmental waters that may reduce the sensitivity of the assay (Chandler, 1998; Loge *et al.*, 2002; Call *et al.*, 2003). As noted by others, a positive detection is relatively simple to interpret. However, knowledge of assay sensitivity, which varies from sample to sample, is critical to interpreting negative results (Loge *et al.*, 2002; Call *et al.*, 2003). It is possible that specific sample properties, such as the presence of PCR inhibitors, may affect the sensitivity of an assay thereby resulting in a false-negative result. The probability of false negative results will increase with lower numbers of pathogens per sample. Regulatory guidelines should consider these limitations in order to reduce the public health impacts of false-negative results. Good sampling designs need to consider how much sample is processed, the efficiency of pathogen isolation, the efficiency of nucleic acid extraction, and the effect of co-precipitating factors that inhibit PCR (Loge *et al.*, 2002; Call *et al.*, 2003).

Considering that acceptable regulatory concentrations of specific pathogens in environmental matrices will likely be very low, it is unlikely that current technology would be able to detect pathogens in real-time. Measurement of pathogens to satisfy what would be regulatory levels may still take 24-48 hours as they will require enrichment to increase pathogen numbers or reduce co-precipitating factors. This may also pose challenges as several different enrichment media may be necessary to detect several different pathogens. The only true method to alleviate the need for enrichment to detect low numbers of pathogens in environmental samples would be intensive sample concentration. Unless significant advances are made in sample concentration, nucleic acid extraction methods, and nucleic acid clean-up to remove inhibitory compounds, real-time pathogen detection will remain unrealistic. This will remain a critical issue for biodefense applications where near real-time identification of etiological agents may be imperative to protecting human health.

# 8. Microbial Source Tracking

Microbial source tracking (MST) is a set of methodologies by which the animal or human source of fecal pollution in a contaminated water body may be identified. MST technologies rely on phenotypic and genotypic differences in fecal microorganisms shed in the wastes of animals and humans that make them unique to a particular animal host (host-specific). These differences may arise due to variations in growth environments and the selective pressures of various animal guts such as differences in diet, antimicrobial treatments, temperatures, pH, and more. Upon release into the environment, it is assumed that these organisms remain unchanged and migrate with fecal pollution. Their detection in concert with indicators of fecal pollution or overt pathogens is thus assumed to be indicative of the animal source.

Knowledge of the source(s) of microbial contamination in water bodies and their relative load contribution helps to focus remedial efforts and resources in the right direction at an earlier time. Source identification may also enable investigation of best management practice (BMP) effectiveness leading to improvements in total maximum daily load (TMDL) development and implementation. There are many potential sources of bacteria extant in watersheds, and it is important to be able to sort out the source of observed contamination so that an evaluation of the effectiveness of control strategies can be made. MST may also improve enforcement activities when discharges exceed permitted levels.

In MST, the selection of the right indicator is important, since it is the single element which provides the measurable parameters to determine the origin of the pollution. Both phenotypic and genotypic technologies have been developed primarily using fecal coliforms, *E. coli,* fecal enterococci, fecal streptococci and viruses. Some of these technologies are library-dependent; they rely on comparing fingerprint databases, either phenotypic or genotypic, of microorganisms from known sources to the fingerprints of unknown samples. These may include antibiotic resistance analysis (ARA), carbon utilization profiles (CUP), repetitive extragenic palindromic PCR (rep-PCR) DNA fingerprinting, randomly amplified polymorphic DNA (RAPD) analysis, amplified fragment length polymorphism (AFLP) analysis, pulse field gel electrophoresis (PFGE), and ribotyping (Harwood, 2000; McClellan *et al.*, 2001; Hagedorn *et al.*, 2003; Carson *et al.*, 2003; Ting *et al.*, 2003; Leung *et al.*, 2004; Scott *et al.*, 2004; Webster *et al.*, 2004). Other MST technologies are library-independent; they rely on the conservation of unique genetic identifiers inherent to a specific fecal microorganism endemic to the members of a single animal species (the in-group) that are different from the genetic identifiers of the same or different fecal microorganisms in other animals or humans (the out-group). Examples of library-independent MST technologies include gene-specific PCR, 16S rRNA gene clone libraries, and target-specific PCR-based methods (Bernhard and Field, 2000; Khatib *et al.*, 2002; Khatib *et al.*, 2003; Field *et al.*, 2003; Simpson, *et al.*, 2003; Bonjoch *et al.*, 2004; Scott *et al.*, 2004; Simpson *et al.*, 2004; Suerinck *et al.*, 2005).

At present, MST studies have primarily employed library-dependent methods including ARA, AFLP, CUP, and ribotyping (EPA, 2005). Library-independent methods, especially gene-specific and target-specific PCR, have been the focus of recent literature as extensive libraries are not needed for their application, and they can be easily and rapidly applied to source identification studies. Potentially, phenotypic and genotypic methods could complement each other according to training, equipment, and funding available. This section highlights the more

common methods of analysis that provide information about the source of microbial contamination and the methods used to analyze the data obtained by these methods. A full review of all available MST technologies is beyond the scope of this review. An excellent resource for further information regarding MST, its application, promises, and limitations is available in the USEPA Microbial Source Tracking Guide Document (2005).

## *8.1 Antibiotic Resistance Analysis (ARA)*

The antibiotic-resistance analysis (ARA) method has gained popularity over the last decade because it is readily applicable and simple to use. However, the classification accuracy is usually lower than that of the molecular methods at the level of individual species. When animal species are grouped into larger animal categories like human, livestock, and wildlife, the accuracy improves notably to values of 95% or more. This method has been reported to provide sensible classifications of known and unknown fecal isolates and to resolve MST queries to satisfaction in various case studies (Parveen *et al.*, 1997; Hagedorn, 1999; Harwood *et al.*, 2000).

ARA is based on the following two premises (1) the use of antibiotics in humans and animals can result in antibiotic-resistant bacteria, and (2) differences in the selective pressures resulting from dosing with different types and concentrations of antimicrobials, as well as different growth environments in various animal intestines, result in unique patterns of antibiotic resistance specific to different animal types. The development and study of such specific patterns are the basis of this methodology, which uses fecal coliforms, *E. coli*, enterococci, or fecal streptococci as indicators. The laboratory procedure requires only conventional microbiology training and techniques. Therefore, it is cost-efficient and can be rapid when performed by an experienced research team. It begins with the recovery of the fecal bacteria from samples, mostly by membrane filtration and incubation in or on selective media. It continues with the inoculation of the isolated fecal bacteria onto agar or into broth medium containing a number of antibiotics at increasing concentrations. Lastly, the results are evaluated by comparing the antibiotic resistance profiles of the polluted water to the reference source library profiles.

The key to success of this method is having a representative source library with an acceptable *average of correct classification* (ARCC). Most important is to perform a cross-validation test before using the library to classify unknown isolates. The cross-validation test can be done with hold-out isolates or with new known isolates that are submitted as unknowns to the statistical software (Harwood *et al.*, 2000). The rate of correct classification from this test should not be significantly different from the original rates obtained when the library was initially classified. Similarly, it is important to use the antibiotics and the concentrations that provide the more accurate classification of the library isolates. For instance (as noted above), several antimicrobials used in animal agriculture have human-use analogs. Resistance that develops in livestock animals may therefore confer resistance to human antimicrobials and vice-versa. Therefore, preliminary tests are recommended prior to the analysis of the water isolates (Hagedorn *et al.*, 1999). While this method does not classify individual animal species very accurately, it has been used with satisfaction for human, poultry, livestock and wildlife categories (See examples below). In most cases, this is sufficient and supports the development of a restoration strategy.

Several examples of the use of ARA for MST exist. Parveen, *et al.*, (1997) used ARA methodology to differentiate point-source (PS) from nonpoint-source (NPS) *E. coli* from the Apalachicola Bay, Florida. They used 765 isolates and obtained average MAR indexes of 0.25 for PS and 0.13 for NPS. PS isolates showed higher resistance to single antibiotics and to combinations of antibiotics than NPS isolates. Sixty-five resistance patterns were observed for PS isolates, compared to only 32 patterns for NPS isolates, when cluster analysis was used. Wiggins (1996) developed a protocol to generate more extensive AR profiles by using various concentrations for each antibiotic tested. He also introduced the use of a reference source library and the multivariate analysis of variance discriminant function analysis to the studies of MST by ARA. He studied 193 water fecal streptococci, with a source library of 1,435 fecal streptococci isolates against a battery of five antibiotics at four concentrations each. An ARCC of 72% was obtained when the source categories were analyzed at the species level and 82% when some species were pooled into "poultry" and "beef" categories. In general, increasing the number of antibiotics used in an analysis increased the ARCC that could be achieved.

Others have further validated the ARA method by using additional statistical analysis. Hagedorn (1999) created separate source databases with 7,058 and 892 isolates with ARCCs of 87% and 88%, respectively, which increased to 97% and 95% after pooling species into poultry and beef. They used discriminant analysis to classify 4,615 water isolates from Page Brook River and obtained 82% beef, 7.3% deer, 5.6% waterfowl, and 0% human, and 5.3% unknown. Cluster analysis was also used, which generated very good separation between sources with high antibiotic resistance including chicken, dairy cattle and human clusters. Among beef cattle and deer, which had low levels of resistance, there was separation, but the deer tended to sub-cluster within the larger beef cow isolate cluster. Most of the unknown source isolates were grouped in beef cow, deer, and waterfowl clusters, whereas none grouped in the human cluster. Based on the results of this work, cattle access to the stream was reduced by fencing.

Harwood *et al.*, (2000) obtained ARCCs of 64% and 62 % with a source database of 6,144 fecal coliform and 4,619 fecal streptococci isolates. Similar to Wiggins (1996) and Hagedorn (1999), the ARCCs improved to 75% and 72%, respectively, after pooling the species into human and animal groups. Fecal coliforms from cattle were classified correctly at a higher rate than those of fecal streptococci. Conversely, fecal streptococci from humans were correctly classified at a higher rate than those from fecal coliform isolates. Overall, the fecal coliform database had a significantly greater ARCC than the fecal streptococci database. Spearman's ranked correlation using the percentage of correctly classified isolates versus the corresponding number of sampling events resulted in a significant negative-correlation between sampling events and the percentage of correctly classified isolates for the fecal coliform database, but not for the fecal streptococci database. They analyzed 91 fecal coliform isolates from surface water receiving effluent from faulty septic systems, 81 of which were classified into the human category. Similarly, 38 of 51 fecal streptococci from the same samples were categorized as human. After the septic systems were repaired, only 7.8% of fecal coliforms and 1.2% of fecal streptococci were classified as human.

Graves, *et al.*, (2002) constructed a library of 1,174 enterococci isolates, and with two categories (human and nonhuman) achieved an ARCC of 96%. By splitting nonhuman sources into livestock and wildlife, they were able to achieve an ARCC of 92%. They analyzed 2,012

enterococci isolates from a stream that drains a watershed with large populations of livestock and wildlife and that passes through a community of 82 homes served by individual septic systems. The yearly average classification was 10% human, 40% wildlife, and 50% livestock. Burnes (2003) analyzed 800 fecal coliform isolates from Big Creek, a mixed-use watershed, against a source library of 1,125 fecal coliform isolates (human and non-human categories, ARCC=94%). He found that human sources contributed greater than 50% of the base flow fecal coliforms in urbanized areas. Chicken and livestock appeared to be responsible for the base flow fecal coliforms found in rural reaches of the stream. Hydrologic events changed the contribution of each source to the stream such that fecal coliform pollution was 16% attributable to domestic sources, 21% attributable to wildlife, and up to 60% attributable to chickens and other livestock sources.

## *8.2 Ribotyping*

Ribotyping is a DNA fingerprinting method that exploits small differences in 16S and 23S rRNA-coding regions of bacterial DNA to identify genetic relationships between unknown bacteria and a set of known index organisms (Grimont and Grimont, 1986; Stull *et al.*, 1988; Graves *et al.*, 1999). This method works on the premises that (1) multiple copies of the genes encoding 16S and 23S rRNA may appear within the bacterial genome with different flanking restriction site locations, (2) there is variability amongst 16S and 23S rRNA genes, and (3) there is variability in the intergenic spacer region between 16S and 23S rRNA genes. Ribotyping involves the culturing of a bacterium followed by DNA extraction and purification. Subsequently, the DNA is digested with one or more enzymes and the digestion products are separated by gel electrophoresis. DNA bands are typically transferred onto a nylon membrane and challenged by hybridization analysis with a chemically-labeled nucleic acid probe. The probes may be generated from an index bacterium, such as a particular strain of *E. coli*, by reverse transcribing the 16S and 23S rRNA and labeling the cDNA with a chemical labeling scheme. Because of small differences in the restriction sites of different bacteria, the resulting band patterns from the hybridization analysis will be distinct. This pattern is called a ribotype. By comparing ribotypes of unknown samples to a library of known samples challenged by probes from the same index organism, genetic and evolutionary relationships can be discerned. Ribotypes may be translated to a binary code facilitating a discriminant statistical analysis to aid in interpretation of results (Grimont and Grimont, 1986; Parveen, 1999; Carson *et al.*, 2001).

In early years, ribotyping was used in epidemiological studies to characterize bacteria such as *E. coli, Salmonella enterica* and *Vibrio cholerae* (Stull *et al.*, 1988; Olsen *et al.*, 1992; Popovic *et al.*, 1993). Gradually, ribotyping found application in multidisciplinary areas such as plant pathology, animal science, food technology, and MST (Nassar *et al.*, 1994; Nagai *et al.*, 1995; Kilic *et al.*, 2002; Scott *et al.*, 2004). By comparing libraries of ribotypes grouped by animal source, some researchers have noted that distinct bands unique to specific sources may emerge from non-distinct bands, thus facilitating the identification of the source of pollution in unknown samples. For instance, Parveen (1999) tested the applicability of this methodology to predict the source of *E. coli* pollution in the Apalachicola Bay, Florida. They analyzed a library of 238 *E. coli* isolates and found that discriminant analysis of the ribotype profiles showed an ARCC of 82%. A total of 97% of nonhuman and 67% of the human isolates were correctly classified. Carson *et al.*, (2001) extended the application of ribotyping to distinguish *E. coli* from humans

and seven nonhuman hosts. When ribotypes from a library of 287 *E. coli* isolates obtained from humans, cattle, pigs, horses, chickens, turkeys, migratory geese, and dogs were used, the ARCC was 73%. By reducing the discrimination to human and nonhuman sources, these authors were able to achieve an ARCC of 97%. Using a library of 160 *E. coli* isolates, Scott *et al.*, (2004) was able to employ ribotyping to identify animals as the primary source of pollution in a water way near Charleston in South Carolina. Prior to this investigation, a significant human input was suspected. Kuntz *et al.*, (2003) successfully combined targeted sampling protocols with ribotyping to identify the source of fecal contamination of Sapelo River in Georgia. *E. faecalis* DNA fingerprints in the river were a 43% match to those in a nearby wastewater lagoon, suggesting that fecal contamination of the river originated from the wastewater treatment facility.

Other studies have used ribotyping successfully but have noted caveats in its application. For instance, Hartel (2002) studied 568 *E. coli* isolates from different locations in Georgia and Idaho to determine the geographic variability of *E. coli* from different animal species. They found that the percentage of ribotype sharing within an animal species increased with decreased distance between geographic locations for cattle and horses, but not for swine and chicken. The data suggested that the ability of libraries to classify unknown isolates is good provided both library and unknown isolates belong to the same geographic area. Wheeler *et al.*, (2002) explored the potential of *E. faecalis* as a human fecal indicator for MST using ribotyping. He analyzed fecal samples from humans and a variety of livestock, domestic, and wildlife and found that the host range of *E. faecalis* was limited to dogs, humans, and chickens and the ribotypes clearly distinguished between human and chicken hosts. The dog isolates were apparently eliminated when a protocol to quickly isolate *E. faecalis* was used. Hartel *et al.*, (2003) noted that ribotypes of *E. coli* isolates from wild deer was significantly affected by their diets. Wild deer exhibited 35 *E. coli* ribotypes, whereas penned deer generated only 11. Although issues of geographic stability, host range of target bacteria, and host stability of ribotypes may be of concern in some instances, ribotyping remains a preferred and well-accepted method for MST. From the results above, it appears to be a reliable tool to discriminate between pollution sources and provide valuable information for water management purposes.

## *8.3 Amplified Fragment Length Polymorphisms (AFLP)*

AFLP is a DNA-fingerprinting method based on the detection of characteristic differences in the fingerprints of two genomes resulting from polymorphisms, insertions, and deletions that occur within or immediately adjacent to restriction sites. In this method, genomic DNA is extracted from the target cells and digested with a pair of restriction enzymes. Linkers specific to the restriction enzyme sites chosen are ligated to the DNA fragments providing the sequences for hybridization of PCR primers in the amplification steps. As large numbers of different fragments can be generated during digestion (more than $10^6$ fragments per digestion of a genome of $10^9$ base pairs (bp)), selective DNA amplification is used to limit the number of amplification products. Selective amplification can be achieved through a variety of mechanisms including 3'-extensions to one or both linkers, "pre-amplification" with only one primer complementary to one of the restriction enzyme sites, inclusion of 3'-extensions on the "pre-amplification" primer or one or both AFLP-PCR primers, and labeling only one of the AFLP-PCR primers. Amplified products are separated on a denaturing polyacrylamide gel in an automated DNA sequencer, and "fingerprints" are captured by specialized software which can scan the fingerprints for

discriminatory bands. Evaluation of the results by various statistical analyses can be performed provided the DNA banding patterns are converted to binary form.

AFLP has proved to be highly discriminatory and reproducible when compared to other molecular typing techniques. During the last decade, it has become a reliable tool to classify bacteria to the strain level, as well as to perform genetic mapping of higher organisms (Janssen *et al.*, 1996; Desai *et al.*, 1998; Savelkoul *et al.*, 1999; Iyoda *et al.*, 1999; and Zhao *et al.*, 2000). Two reported evaluations of AFLP as a tool for MST stand out in literature. Both investigations compared AFLP to other fingerprinting techniques and utilized *E. coli* as the indicator organism. In the first of these studies, Guan *et al.*, (2002) studied a collection of 105 *E. coli* isolates from the feces of cattle, poultry, swine, deer, goose, moose, and human samples and compared AFLP to the ARA and the 16S rDNA methods. The results indicated that AFLP was significantly more effective than the other two methods. Ninety-four percent of the livestock isolates, 97% of the wildlife isolates, and 97% of the human isolates were correctly classified by AFLP. In comparison, 46% of livestock isolates, 95% of wildlife isolates, and 55% of human isolates were correctly classified by ARA, while 16S rDNA-based techniques resulted in 78%, 74%, and 80% correct classification, respectively. Although additional isolates in the source library may improve the ARCCs of the ARA and 16S rDNA methods, the resolution achieved by AFLP with a small library was impressive in this instance.

In another study, Leung *et al.*, (2004) used Shiga-toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and non-pathogenic *E. coli* from cattle, swine, and human sources from very diverse geographic areas, including the U.S., Canada, Europe, and Australia, to construct source libraries. A multiple-response permutation procedure analysis of the data obtained with AFLP indicated that the seven groups defined by host-pathogenicity combinations (bovine STEC, bovine ETEC, bovine non-pathogenic *E. coli*, human STEC, human ETEC, human non-pathogenic *E. coli* and swine non-pathogenic *E. coli*) were significantly different. Subsequently, stepwise discriminant function analysis was used to select 39 discriminant DNA bands distinguishing the host specificity of the *E. coli* strains for the analysis. The overall cross-validation classification efficiency was 93.6% with 91.4% of human, 90.6% of bovine, and 97.7% of swine isolates being classified into their correct host types.

AFLP also distinguished the non-pathogenic *E. coli* from STEC and ETEC, and was able to classify the strains based on both host specificity and virulence (Leung *et al.*, 2004). Stepwise discriminant function analysis selected 41 DNA bands to classify the isolates based on pathogenicity with an overall cross-validation classification efficiency of 99.1% (100% of non-pathogenic *E. coli*, 100% of STEC, and 90.9% of ETEC correctly classified). Fifty DNA bands were selected by that same means to differentiate the seven host-pathogenicity combinations (bovine VTEC, bovine ETEC, bovine non-pathogenic *E. coli*, human VTEC, human ETEC, human non-pathogenic *E. coli* and swine non-pathogenic *E. coli*) with an average cross-validation classification efficiency of 86.4% (individual group classification efficiency ranging from 50 to 100%). Despite the wide geographic origin of the *E. coli* strains in this study, AFLP was capable of differentiating the *E. coli* strains with a high rate of correct classification from the various hosts.

Like most methods used in MST, AFLP needs the development of a reference source library of the indicator organism. Based on the reports above and the fact that AFLP screens the entire genome, this tool has great potential for MST. However, reports of case studies with successful application of AFLP are necessary to further MST using AFLP.

## *8.4 Host-specific molecular biomarkers*

Host-specific molecular biomarkers for MST studies, target-specific PCR-based methods, are attractive because they offer rapid analysis (no source library or cultivation are needed) and greatly reduced cost. They are technically less demanding than most alternative MST techniques. Several bacterial targets have been proposed in the literature for MST applications including *Bacteroides*, *Bifidobacterium*, *Streptococcus* Lancefield Group D, and *Rhodococcus coprophilus* (Whitehead and Cotta, 2000; Vancanney *et al.*, 2002; Bernhard *et al.*, 2003; Bonjoch *et al.*, 2004; USEPA, 2005). However, since each marker is specific to a single animal host, a combination of markers may be required to fully identify potential sources of contamination. Because no cultivation is required, these methods are applicable to detection of several biomarkers in a single sample, which may strengthen the argument for source identification (USEPA, 2005). For instance, samples with positive results for several human-specific biomarkers, such as human-specific *Bacteroides* spp. (Bernhard *et al.*, 2003), both the human-specific primer pairs for *Bifidobacterium adolescentis* and *Bifidobacterium dentium* (Bonjoch *et al.*, 2004), and the human-specific *Enterococcus* spp. *esp* markers (Scott *et al.*, 2005), but negative for the cattle-specific *Bacteroides* (Bernhard *et al.*, 2003) or *E. coli* LTIIa toxin (Khatib *et al.*, 2002), would strongly implicate a human rather than cattle source. At present, *Bacteroides* markers are the most commonly used host-specific molecular biomarkers for MST (Bernhard *et al.*, 2003; Seurinck *et al.*, 2005; USEPA, 2005). However, the use of these markers for resolving watershed-scale microbial pollution is unknown.

The development of the human- and ruminant-specific *Bacteroides* biomarkers is described by Bernard and Field (2000a,b). These researchers created 16S rDNA clone libraries of members of the *Bacteroides-Prevotella* group from human and cow fecal samples. Individual and pooled clones were examined by length heterogeneity (LH)-PCR and terminal-restriction-fragment–length-polymorphism (T-RFLP) techniques. Sequencing and phylogenetic analysis demonstrated that the human-specific sequences clustered together and were closely related, but not identical, to sequences of *Bacteroides vulgatus*, which is commonly found in human feces. The cattle-specific sequences formed the new gene clusters CF123 and CF151. All human- and cattle-specific genetic markers were found in DNA extracted from river and estuary water contaminated with fecal pollution.

Primer sets were developed to amplify specific sequences within the *Bacteroides-Prevotella* host-specific gene clusters (Bernhard and Field, 2000a,b): one forward primer specific for the human-specific gene cluster HF8, two forward primers for the cattle-specific gene clusters CF123 and CF151, and a general *Bacteroides-Prevotella* reverse primer for use with all three forward primers. These primers were successfully used to amplify 16 human and 19 cow fecal 16S rDNAs (Bernard and Field, 2000b). In subsequent work, Bernhard *et al.*, (2003) used these primers to test 22 water samples from Tillamook Bay and the results were congruent with land use. For example, the human specific primer pair, HF183F/Bac708R, amplified only DNA from

waters around urban areas and sewage treatment plants, demonstrating specificity for human *Bacteroides* DNA. The cattle specific primers, CF128F/Bac708R and CF193F/Bac708R, amplified DNA from waters primarily near rural areas. However, these primers amplified other ruminant DNA as well. Therefore, positive results with these primers should be scrutinized against land use and ruminant wild-life populations to prevent misinterpretation of results. Dick *et al.*, (2005) have also recently published host-specific primers for swine and horse.

Relatively few other host-specific molecular biomarkers have been reported in literature (Khatib *et al.*, 2002a,b; Nebra *et al.*, 2003; Bonjoch *et al.*, 2004; Scott *et al.*, 2005). Most of these markers target 16S rDNA, but some markers are emerging that detect alternative regions of the bacterial genome, such as virulence genes (Khatib *et al.*, 2002a,b; Scott *et al.*, 2005). The efficacy of these and other biomarkers have not been well established. Therefore, interpretation of the presence of these molecular markers in different *milieus* may be complicated. Because of the small number of environmental samples studied so far, host-specific molecular biomarker technology needs further exploration in case studies to show field-applicability.

## *8.5 Discussion*

Several methodologies are available for MST studies. As shown by the publication evidence, ARA represents a good tool for MST validated by various applications in real-life case studies. However, specific considerations may limit its usefulness including human-livestock analogs, and transfer of resistance traits in different *milieus*. Ribotyping is probably the most field-tested method among the molecular methodologies used for MST. From the results above, it appears to be a reliable tool to discriminate between pollution sources and provides valuable information for water management purposes. Although ribotyping may generate high ARCCs, it has also been associated with a high cost and may be labor intensive, requiring long studies to achieve results (Hartel *et al.*, 2003). AFLP technologies are emerging as a promising tool for MST. These methods offer a high degree of specificity, as they can screen the entire genome instead of selected regions such as the 16S and 23S rDNA screened by ribotyping. However, considerable technical expertise and expense may be needed to fully utilize the technique. Other molecular methods, although sophisticated and capable of measuring parameters with high resolution, are in various stages of development. More research is needed for these methods to become accessible for a broader population of users. As of today, these techniques have mostly been used in feasibility studies with a small amount of isolates. Full-scale watershed studies are needed to assess the potential of these technologies for future use.

Several factors may complicate the use of MST technologies in contaminated watersheds. Poor survival of reference organisms in the environment may result in little or no detection, limiting the ability of the various methods to identify the source of fecal contamination (Simpson *et al.*, 2003; U.S.EPA, 2005). Even those reference organisms that are reasonably hearty may exhibit variable survival times for different phenotypes or genotypes dependent on the environmental milieu. This may lead to changes in genotypic and/or phenotypic signatures of the overall populations and divergence from fingerprints in source libraries established with the raw fecal material. For instance, Anderson *et al.*, (2005) studied decay rates for fecal bacterial indicator organisms (fecal coliforms and *Enterococcus* spp.) originating from dog feces, wastewaters, and soil in freshwater and sediment and saltwater and sediment. These researchers observed variable

decay rates based on fecal bacterial source, environment, and even ribotype. These changes may complicate interpretation of environmental fingerprints and undermine source tracking efforts, potentially resulting in misinterpretation of environmental data.

Another serious complicating factor is that the transport properties of different bacteria may vary several-fold depending on the specific microbial agent and the *milieu* (Ferguson *et al.*, 2003). If transport and survival of the index organism(s) used to identify fecal pollution source(s) do not match that of pathogens emanating from potential sources, MST may yield questionable information. For instance, Simpson *et al.*, (2003) could not establish a relationship between the molecular fingerprints of 16S rDNA fecal *Bacteroides* clones in a large horse manure pile immediately adjacent to a receiving stream and downstream water as close as 5 m from the manure pile. In contrast, other researchers have noted significant overland and downstream transport of antibiotic-resistant bacteria and several bacterial, viral, and parasitic pathogens, such as *Cryptosporidium parvum, Salmonella* spp., swine hepatitis E, and *Yersinia enterocolitica*, for several hundred meters from concentrated animal feeding operations (CDC, 1998; Karetnyi *et al.*, 1999; Gurdin *et al.*, 2002). Further, if several different bacterial index organisms are used for source identification, as suggested by the USEPA (2005), the transport properties of the individual agents need to be clearly defined to interpret what differences in the level of detection for different animal sources mean. MST studies need to identify the distance at which selected biomarkers may be detectable from their pollution source(s) and whether or not this may be indicative of the fecal pathogens emanating from the same source.

# 9. Treatment Technologies and Management Practices

As animal agriculture has evolved to larger facilities with large numbers of animals in limited spaces, the problems associated with manure handling have grown. A single swine, beef, dairy, or poultry facility can produce waste equivalent to a small city. The waste is, for the most part, untreated and spread into the environment with little control on dissemination of microorganisms in the waste. All animal manures contain microorganisms, some of which are pathogenic to humans and other animals. Zhao *et al.*, 1995 surveyed dairy herds in 14 states to determine the prevalence of *E. coli* O157:H7. Their results indicated that *E. coli* O157:H7 was present in about 5% of each herd.

Prior sections of this report have enumerated the pathogenic organisms in manure and detailed the illnesses associated with them. Environmental problems originate when the manure containing pathogens is distributed into the open environment with no effort made to reduce the content of pathogens or limit their movement in the environment. Wind, surface flow, and subsurface flow can all carry enough pathogens to receiving waters to exceed water quality standards. In many cases, streams and lakes are used for recreation, and the people using them can be exposed to infection without knowing that they have been exposed. Knowledge of the survival and transport of potential pathogens in the environment is critical for implementing corrective actions on the landscape to limit people's exposure to pathogens.

Microorganisms can move in the environment in several ways. Organisms can move with any dust produced in animal housing, feedlots, or manure spreading. Other airborne transport can happen as liquid waste is spread by spraying as an irrigation process, spraying from an application vehicle, or agitation of lagoons prior to spraying. After manure has been applied to a field surface, microbes can move with water when rainfall exceeds the infiltration rate, thereby creating runoff. Rainfall impact dislodges the organisms from soil or manure particles, and flowing water transports them to receiving waters. Another path for movement of organisms is through subsurface drainage. Microorganisms can enter worm burrows or root channels and move downward in the soil profile as the water flows to groundwater or to drainage tile. If groundwater is shallow, it is possible for serious contamination to occur in wells situated too close to application sites or CAFO installations.

Tile drains can short circuit groundwater recharge by intercepting water and diverting it to streams before it can percolate through the soil. Water that infiltrates the soil down to tile depth is usually below the majority of the root zone and thus not available for plant uptake. Tile drains can accelerate the movement of nutrients and bacteria into receiving waters (Joy *et al.*, 1998, McLellan *et al.*, 1993). Hunter *et al.*, 2000 found that sheep grazing in pastures in England could adversely affect water quality even though the animal population was quite low, one animal per square kilometer basis. Tile drains and open ditches were conduits for microorganisms from pastures to streams. Janzen *et al.*, 1973 found that water quality in streams near dairy farms frequently exceeded coliform limits due to bacterial contamination. About 42 % of the farms were responsible for exceeding standards. Some research has shown that waste applied to the surface of a tiled field can enter the tile quickly after a rainfall. The width of the zone affected by tile at the soil surface is on the order of a meter. In fields that have tile drains, the spacing of tile lines is on the order of 25 or more yards depending on the soil type. Coarser grained soils will allow wider spacing of tile lines than fine grained soils. Each tile line will drain infiltrating water down to its level after a rain event. A portion of the infiltrating water will drain below

the tile level and enter groundwater as recharge. There is a possibility that groundwater can be contaminated by manure spread on the surface.

As shown earlier in this report, beef, dairy, poultry and swine operations have become fewer in number and larger in animal populations. The result is to produce more manure in limited areas with little opportunity apply it to land at low enough levels to reduce pathogenic organisms to background levels. Microorganisms in manure produced in large CAFOs pose a serious risk to water quality for recreation, human health, and possibly to nearby farms by spreading disease. One of the most feared occurrences in the agricultural community is an outbreak of disease among farm animals. Recent outbreaks of hoof and mouth disease in the United Kingdom led to multiple billion dollar losses (Ferguson *et al.*, 2001). Reducing the presence of potential pathogens in wastes applied to land will go a long way to improving the safety of farms from disease. Reduction of the bacterial population in water can also improve downstream biosecurity of adjacent farm operations. Manure management practices and potential treatment technologies can be applied to reduce the number of microorganisms distributed into the open environment. There is a great need to implement microorganism reduction techniques to animal waste to prevent detrimental environmental effects.

## *9.1 Manure management: active and passive systems*

Common practices used for managing manures in the U.S. include passive and active approaches. The passive systems include lagoons, storage prior to disposal, vegetated buffer strips, constructed wetlands, separation of different ages of animals, and land application. The passive systems do involve manipulation of manure to move it and eventually land apply the materials, but do not require more than minimal operator input. Active systems include composting, anaerobic digesters, aerobic digesters, and actively operated lagoons. Active systems require more operator attention, such as turning compost windrows, monitoring digesters, and mixing lagoons. In both cases, the key factor is operation with minimum input of labor and capital.

Lagoons are large excavations that may or may not be lined with plastic or clay that receive liquid wastes from animal confinement buildings. Lagoons can be single or multiple cell designs. A passive lagoon is effectively anaerobic due to the large load of organic matter flowing into the lagoon and limited aeration from diffusion and wind. Multiple cell designs can have anaerobic cells, followed by increasingly aerobic cells as the organic content of the waste stream decreases by settling and degradation by microorganisms. Lagoons can also be modified by adding covers to collect methane, or as a permeable cover to oxidize ammonia to nitrate that can be reduced to nitrogen gas in the microenvironment of the cover.

In some cases, usually dairy, beef, and poultry operations, manure is simply scraped into piles and held until a convenient time for disposal by land application. Separation of animals into age groups has also been shown to be effective in reducing specific pathogens, especially *C. parvum* (Atwill *et al.*, 1999). Young animals frequently shed large numbers of oocysts, and older animals do not. Manure from calves can be collected and treated separately from the larger quantities of manure from older animals (Hutchinson *et al.*, 2005). The costs of treatment are lower due to the much smaller mass to treat. In some cases, manure is periodically removed from the animal confinement buildings and directly land applied with no treatment. Simple holding of waste for greater than 90 days will achieve bacterial reductions of >90% (Thayer *et al.*, 1974).

Vegetated buffer strips (VBS) are placed along the downslope sides of fields where wastes are applied to intercept runoff water. As the water flows across the buffer strips contaminants are retained in the vegetated area. The vegetation slows water velocity, allowing settling of particles and infiltration of water into the soil. A well-designed VBS will reduce the quantity of microorganisms leaving a field during runoff events. Many studies have shown greater than 50% reduction of bacterial populations between water entering a VBS and water leaving a VBS. While this reduction may not fully achieve primary contact standards, it is a step in the right direction. Key factors in VBS success are the width of the VBS, slope of the soil, type of soil, and degree of vegetative cover. Good buffers are usually about ten meters wide, with slopes less than 8%, and have about 90% coverage with vegetation. Other management options can help lower the numbers of organisms being applied to the fields. Microorganisms can be retained in strips and wetlands to a significant degree; however, reduction of loads to meet water standards has proven elusive. Many VBS studies show reductions of organisms reaching streams by as much as 90+% (Coyne *et al.*, 1998). The difficulty arises in that reducing populations 90% (e.g., $1x10^6$ to $1x10^5$ per 100 mL) can still leave more organisms in the water than standards allow (Coyne *et al.*, 1995). Table 11 summarizes research done on the trapping of microorganisms in VBS systems and wetlands which primarily treat surface water flow and some shallow ground water.

Active manure management systems involve more operator participation to maintain functionality. Composting requires attention to carbon to nitrogen ratio, moisture, and periodic aeration. In composting, the degradable organic matter is consumed by microorganisms reducing the mass of material. During the process, the temperature of the compost pile will rise to over $50^o$ C. Under these conditions, pathogenic organisms cannot survive. Composting is very effective in reducing pathogenic organism content of wastes (Olson, 2003). Adequate disinfection requires that conditions of specified time at specified temperatures be met.

Anaerobic digesters can be either plug-flow or mixed reactors. In both cases, the easily degraded organic matter in the waste stream is consumed, reducing the oxygen content of the reactor to methanogenic conditions. Generation of methane can partially offset the costs of reactors and maintenance by providing electricity and / or hot water for the farm. Anaerobic digesters can be operated at ambient (20-30$^o$C), mesophilic (30-37$^o$C), or thermophilic (45-55$^o$C) temperatures. The efficiency of the reaction and reduction of pathogens is different under the different temperature conditions. The thermophilic reactors are more efficient in production of methane and in destruction of pathogenic organisms (Sobsey *et al.*, 2002). However, they are more susceptible to upsets. Aerobic reactors actively incorporate oxygen into the reactor fluid with the goal of maintaining aerobic metabolism by the microorganisms. Aerobic reactors can also operate at ambient, mesophilic, and thermophilic temperatures. The benefit of an aerobic reactor lies in odor reduction and greater carbon mass reduction. Pathogenic organisms are also reduced in aerobic reactors, with greater reductions occurring at higher temperatures (Hill, 2003). In an earlier study, Munch *et al.*, 1987 determined the decimation times for several pathogens in cattle and swine manure slurries from five herds in the temperature ranges 18-20$^o$C and 6-9$^o$C. The results are shown in Table 12. As can be seen in these results some organisms are relatively poor in survival. In general, colder environments favor survival, and aeration favors the reduction of microorganisms. Other organisms, especially fecal streptococci and *E. coli*, can have very long decimation times at cool temperatures. Management practices that address the resistant organisms should at the same time reduce the less-resistant organisms.

**Table 11.** Summary of microorganism retention in vegetated buffer strips and wetlands.

| Type | Width | Slope | *Protozoan Parasites* (*Cryptosporidium or Giarda*) | Viruses | Fecal Indicator Bacteria | | Reference |
|---|---|---|---|---|---|---|---|
| | *(meters)* | *(%)* | | | Coliforms | Streptococci | |
| ***Vegetative Buffer Strips*** | | | | | | | |
| Grass | 1 | 5-20 | 35->99% | | | | Atwill *et al.*,2002 |
| Grass | 1.1 | 5-20 | 90-99% | | | | Tate *et al.*,2004 |
| Grass | 1.5 | 10, 20 | >99% | | | | Davies *et al.*, 2004 |
| Grass | 2 | 8-10 | | | 370-600 † | | Pote *et al.*, 2003 |
| Grass | 3 | 3.3 | | | 90% | >95% | McCaskey *et al.*, 1971 |
| Grass | 4.5 | | | | 75% | 68% | Coyne *et al.*, 1998 |
| Grass | 5 | 8 | | | >95% ‡ | | Collins *et al.*, 2005 |
| Grass | 6.1 | 3 | | | 6,000 † | | Busheé *et al.*, 1998 |
| Grass | 9 | 9 | | | 43-74% | | Coyne *et al.*, 1995 |
| Grass | 9 | | | | 91% | 74% | Coyne *et al.*, 1998 |
| Grass | 22 | 10-30 | >99% | | | | Tate *et al.*, 2000 |
| Grass | 70 | | | | 2,900-10,000 † | 4,800-17,000 † | Heinonen-Tanski *et al.*, 2001 |
| Grass | NR § | 1.5-4.5 | 99.4- 98.3% | | | | Trask *et al.*, 2004 |
| Grass plus forest | 30 | | | | >90% | | Entry *et al.*, 2000 |
| Corn and grass | 41 | | | | 69% | 70% | Young *et al.*, 1980 |
| Grass and barley stubble | 600 $m^2$ | | | | >93% | | Fenlon *et al.*, 2000 |
| ***Wetlands*** | | | | | | | |
| Wetland | 100 | | | 85% | | | Chendorian *et al.*, 1998 |
| Wetland | NR | | 87% and 64% §§ | | 99% | | Ferguson *et al.*, 2003 |
| Wetland | 2 cell | | | | 96-97% * | | Sobsey and Hill, 2002 |
| Wetland | 3 cell | | | 85% | | | Chendorian *et al.*, 1998 |
| Wetland | 4 cell | | | | 99-99.9% | | Behrends *et al.*, 1999 |

† CFU per 100 mL in runoff
‡ under low flow conditions
§ NR = not reported
* in each cell
§§ For 87% for *Cryptosporidium*, 64% for *Giardia*

**Table 12.** Bacterial decimation times in aerated and non-aerated manure slurries in weeks.

| Organism | Aerated | | Non-aerated | |
|---|---|---|---|---|
| | 7 ºC | 20 ºC | 7 ºC | 20 ºC |
| **Fecal streptococci** | | | | |
| Cattle | 6.3-18.5 | 2.5-3.9 | 12.1 | 4.1-6.9 |
| Pig | 19.2 | 5.1-6.7 | 21.9 | 5.5-7.0 |
| **Overall** | **12.0** | **5.4** | **21.4** | **5.7** |
| ***Escherichia coli*** | | | | |
| Cattle | 1.4-1.8 | 0.7-2.2 | 3.4-6.9 | 1.6-4.5 |
| Pig | 1.7-2.7 | 0.7-1.7 | 3.4-17.2 | 1.3-1.9 |
| **Overall** | **2.1** | **1.5** | **8.8** | **2.0** |
| ***Salmonella typhimurium*** | | | | |
| Cattle | 1.3 | 0.5 | 4.7 | 1.9 |
| Pig | 1.6 | 0.7 | 5.8 | 1.8 |
| **Overall** | **1.6** | **0.6** | **5.9** | **2.0** |
| ***Staphylococcus aureus*** | | | | |
| Cattle | NR † | NR | NR | NR |
| Pig | 1.8-2.4 | 0.5-1.1 | 2.3-7.5 | 0.8-1.2 |
| **Overall** | **2.6** | **0.7** | **7.1** | **0.9** |
| ***Yersinia enterocolitica*** | | | | |
| Cattle | NR | NR | 0.9 | NR |
| Pig | 0.6-0.7 | 0.3 | 1.0-1.5 | 0.5 |
| **Overall** | **0.7** | **0.3** | **1.6** | **0.6** |

† NR = Not Reported

The pathogen reduction effectiveness of different manure management practices is shown in Table 13. In most cases, potential pathogens are reduced in common practices by 2 $\log_{10}$ orders or 99%. While this is important, it is not enough to achieve acceptable water quality standards in receiving waters. Lagoon effluent, surface runoff water, tile drain water, or digester effluent may need to have 4 to 6 $\log_{10}$ orders of organism reduction to meet water quality standards.

Olsen and Larsen, 1987, identified bacterial decimation times of several pathogenic organisms in meso and thermophilic anaerobic digesters. Their results are shown in Table 14. The important factors were the species of bacteria and temperature, but not the source of manure, reactor process (batch or continuous), gas produced, ammonia content, or pH.

Combining management practices has been shown to accomplish greater reductions of pathogenic organisms than single practices. Among the practices tested are multicell lagoons with constructed wetlands (Ibekwe *et al.*, 2002), multicell lagoons followed by constructed wetlands (Sobsey and Hill, 2002), solid separation prior to wetlands (Hill *et al.*,1999), lagoons followed by constructed wetlands followed by infiltration basins (Lorimor *et al.*, 2003), solids separation followed by composting of solids and treatment of the water, digesters followed by constructed wetlands (Bicudo and Goyal, 2003), animal diet manipulation to reduce pathogen

**Table 13.** Microorganism inactivation by different management techniques.

| Etiologic Agent | Composting | Anaerobic | Aerobic | Solar | Diet Separation | Reference |
|---|---|---|---|---|---|---|
| **Bacteria** | | | | | | |
| *Campylobacter* spp. | T [†] | | T | | X [§] | Olson, 2003; Hutchinson *et al*., 2005 |
| *E. coli* | T | T, M * | T | X | X | Olson, 2003; Davies-Colley *et al.*, 1999; Collins *et al.*, 2005; McCaskey *et al.*, 1998; Martin *et al.*, 2003; Shaw *et al.*, 2004; Hutchinson *et al.*, 2005; Schamberger *et al.*, 2004; Wright *et al.*, 2003 |
| | T | | | | | |
| *Listeria* spp. | T | T | T | | X | |
| *Salmonella* spp. | T | T | T | | X | Losinger, 1995 |
| *Yersinia enterocolitica* | T | T | T | | | |
| **Protozoan Parasites** | | | | | | |
| *Cryptosporidium* | T | T | T | X | X | Olson, 2003; Mendez-Hermida, 2005; Whitman *et al.*, 2004 |
| *Giardia* | T | T | T | | | Olson, 2003 |
| **Viruses** | T | T, X | | | | Monteith *et al.*, 1986 |

[†] T = Thermophilic process, yields virtually complete reduction of pathogens

[§] X = Approximately 90% reduction

* M= Mesophilic process, yielded approximately 99.9% reduction

**Table 14.** Bacterial decimation times in anaerobic digesters. [†]

| Etiologic Agent | Decimation Time | |
|---|---|---|
| | Days at 35 ºC | Hours at 53 ºC |
| *Erysipelothrix rhusiopathiae* | 1.8 | 1.2 |
| *Escherichia coli* | 1.8 | 0.4 |
| *Salmonella dublin* | 2.0 | 0.6 |
| *Salmonella typhimurium* | 2.4 | 0.7 |
| *Staphylococcus aureus* | 0.9 | 0.5 |
| *Streptococcus faecalis* | 2.0 | 1.0 |
| Group D streptococci | 7.1 | |
| Fecal coliforms | 3.2 | |
| Total coliforms | 3.1 | |
| *Clostridium perfringens* (spores) [§] | Not inactivated | Not inactivated |
| *Bacillus cereus* (spores) | Not inactivated | Not Inactivated |

[†] Adapted from Olsen and Larsen, 1987

[§] C. perfringens was still present after 300 days at 35ºC and 180 days at 53ºC

loads, and separation of animals into different buildings at susceptible life stages (Shaw *et al.*, 2004). North Carolina State University has compared conventional lagoons followed by sprayfields with solids separation followed by a constructed wetland. The second treatment system reduced coliforms and *E. coli* by 3 to 4 $\log_{10}$, while the first reduced coliforms and *E. coli* by 1 to 2 $\log_{10}$. Multicell lagoons have been shown to reduce potential pathogens in wastes by about 99 % in each cell. With two or three cells in series, microbial populations can be reduced to acceptable water quality levels. New York City has published a multiple barrier approach to protecting source water watersheds (New York City and the Watershed Agricultural Council, 1996). The approach starts with good animal husbandry, including herd health, separation of age groups, sanitation improvements, and crop system changes. Szostakowska *et al.*, 2004 reported on the presence of *C. parvum* and *G. lamblia* in cattle barn flies and landfill flies. The flies from the barn had a greater load of infective cysts than the flies from the landfill. These studies show the importance of controlling the spread of pathogenic organisms in the environment by nonagricultural vectors. The second stage is improving barnyards, manure handling, application timing, soil management, and composting. The third stage improves stream corridors, adds vegetated buffer strips, adds stream crossings for pastured animals, fences animals away from streams, and adds watering stations remote from streams. Milne (1976) showed that livestock in proximity to a stream increased the nutrient and organism load in the stream. Fecal coliforms and fecal streptococci were found in the stream above water quality standards. Jellison *et al.*, 2002 found major sources of *Cryptosporidium* spp. in a watershed to be from wildlife and cattle. *C. parvum* was found in cattle and deer. One example of a method of runoff control is simply fencing livestock away from streams (Line *et al.*, 2000; Owens *et al.*, 1996). In both cases, fencing cattle away from streams led to significant reductions of nutrients and sediments entering the streams. Owens *et al.*, 1996 also showed that 1% of storm flow accounted for 27% of the sediment losses. Peak losses were in May and June.

## *9.2 Disussion*

Conventional manure management techniques do reduce the populations of pathogenic microorganisms. The extent of the reduction for most techniques is on the order of 90-99%. The pathogenic organisms originate in the digestive tracts of warm blooded animals, so it is not surprising that conditions in the open environment are inimical to their survival. Important factors in organism survival are nutrient content of the waste, organic content of the waste, temperature, and the species of microorganism. Organic acids, ammonia, and pH changes can also act to reduce the survival of microbial populations. Competition and predation can also affect the population of pathogens in waste materials. Once the manures are spread on fields, microbes can move with air, surface and subsurface flow. VBSs will retain large fractions of microbial populations, but not enough to allow discharge into receiving streams. Solar radiation and timing of manure application with regard to rainfall have a significant effect on microorganism survival. Soil management techniques are also important in planning for reduction of pathogen reduction. Surface application of manure will take advantage of solar radiation as a disinfection technique, but ammonia losses to the atmosphere may be increased. Injection of liquid waste will retain ammonia as a fertilizer, but pathogen survival is enhanced when organisms are protected from drying and the effects of sunlight. The most effective methods for manure management that also control pathogenic organisms are composting and thermophilic digestion. In both cases, the temperature of the process is adequate to destroy many pathogenic organisms. Composting is probably the least costly process to use. It does require attention to solids content, moisture content, and C: N ratios. Properly done, composting can yield a value-added product that can be marketed to the public. This does require development of a marketing plan. High temperature digestion will also destroy pathogens. Under anaerobic conditions, methane can be recovered and used to offset the cost of building and running the digester. High temperature aerobic digesters destroy pathogens and reduce the carbon mass that must be handled, but operational costs are likely to be high and are hard to justify.

The best methods for reducing pathogen loads in manures will combine more than one management tool to achieve reduction of microbial populations to levels that will meet water quality standards. One example of a combined management process is separation of solids from the waste stream, composting of the solids, and digestion of the liquid portion followed by a constructed wetland. This process would reduce pathogen loads in the waste so that land application of either the solid or liquid phase would pose little or no risk to receiving waters. Another example of a combined treatment system is an anaerobic digester, followed by solids separation and constructed wetlands for treatment of the liquid phase of the waste. Multicell lagoons, followed by constructed wetlands, are another form of combined systems. Wastes should not be applied to land unless two or three management/treatment steps are used before land application. VBS should also be present at the edges of the fields used for application of the wastes. Waste management will include management of the animals in terms of diet and housing sanitation. Animal producers will need to work with agricultural researchers and planners to reduce the dissemination of pathogenic microorganisms from their facilities. Achieving these reductions need not require high cost technologies. In some cases, the reductions can be achieved by modifying existing facilities and perhaps adding additional processes, such as wetlands. Adding baffles to lagoons to increase the length of the flow path is one such modest cost option.

Changing the manure management process at CAFOs will require that each facility examine its manure handling process and look for ways to incorporate more steps that reduce pathogen loads. The USDA Natural Resources Conservation Service publishes conservation standard practices that can be applied to manure management problems (http://www.nrcs.usda.gov/technical/Standards/). Collaboration between producers, conservation officers, and environmental advisors can lead to great improvements in the handling of animal manures in the U.S.

# 10. Ongoing research at the EPA and Other Federal Agencies

The presence of CAFOs and the associated wastes are a topic of interest for several agencies of the U.S. government. Natural resources in the U.S. that are potentially affected by the presence of CAFOs and the attendant manure include, but are not limited to the air, water, and soils. In particular, the United States Department of Agriculture (USDA) has a significant interest and commitment to animal issues, both from a production and an environmental perspective. Within the USDA, two organizations have a dominant interest in manure-related research including the Agricultural Research Service (ARS) and the Cooperative State Research, Education, and Extension Service (CSREES). ARS has a formal research area known as National Program 206 (NP206) that is tasked with manure related research with a wide array of topics of interest to the EPA. CSREES sponsors research by funding of universities and other organizations. Research goals encompass atmospheric emissions, nutrient management, pathogens, and pharmaceutically active chemicals, and byproducts. All phases of animal production will enter into aspects of manure management from feed formulas to field application of manure. In addition, the Natural Resources Conservation Service (NRCS) funds implementation of conservation practices through a variety of programs. NRCS does not conduct research directly. In the conservation activities of NRCS, there are several different programs established to help livestock producers improve the environmental performance of their operations. These programs are either cost-sharing or outright grants to help producers mitigate environmental impacts of livestock operations. One of the larger programs is the Environmental Quality Incentive Program (EQIP) that provides low interest loans and cost sharing to producers that install conservation practices on their property. The EQIP funding level was about $1.5 billion in Fiscal Year 2005 (http://www.nrcs.usda.gov/programs/2005_allocations/2005_allocations.html).

Another agency that conducts research into microbial problems related to CAFOs is the United States Geological Survey (USGS). The USGS conducts water quality research across the U.S. and complements USDA research in several ways. USGS emphasizes assessment of water bodies and the impacts of animal waste in karst terrain and ground and surface waters. USGS tends to place its research in a watershed context while USDA tends to place its research into a location specific context. Both approaches are needed to fully address the impact of animal waste on water resources in the U.S. See Table 15 for a listing of some current microbiological research carried out by USGS.

The Centers for Disease Control (CDC) has conducted research on the public health effects of animal waste in the environment. The areas of interest to CDC include antibiotic resistance, bacterial populations, and nitrates in water. The research conducted by CDC has been severely limited due to budget cutbacks. There is an important need for epidemiological analysis of microorganisms originating from animal waste to determine if human health is at significant risk. Waterborne disease is believed to be greatly under-reported (Morris and Levin, 1996; American Society for Microbiology, 1998). Many cases of gastroenteric illness that could be attributed to contaminated water are likely to go unreported by people because they simply do not associate swimming in streams, lakes, and ponds with the onset of symptoms. It is more likely for people to assume that a gastroenteric illness was associated with foods consumed during recreational activities.

**Table 15.** Studies carried out or in progress in the United States Geological Survey

| Location | Media † | Analytes | Waste Type | Observations |
|---|---|---|---|---|
| Missouri | GW | Nutrients, Fecal Bacteria | Poultry | Wells showed contamination with no history of manure in the area |
| Delaware | SW | | Poultry | |
| Arkansas, California, Missouri, Colorado, Virginia | SW | Bacteria, Viruses, Protozoa, Nutrients | Various | Presence in streams, fate and transport, methods, isotopes |
| Arkansas | GW | Nitrate, Bacteria | | Effects in karst terrain, spring resurgences, nitrate from septic systems, highest bacteria in initial flow |
| Not identified | Feed | *E. coli*, *Salmonella* | Cattle | Resistance development, cost analysis |
| Not identified | GW, SW | Viruses | Swine | Survey of waste from hog to stream, survival |
| Iowa | GW, SW | Nutrients, Bacteria | Swine | Effect of CAFO on GW,SW |
| Missouri | SW | Bacteria | Poultry | Coliforms in Shoal creek watershed |
| Not identified | GW, SW | Antibiotics | | Methods, presence of antibiotics in water |
| Iowa | SW | Antibiotics | | Survey of streams for antibiotics |
| Florida | GW | Nutrients, Bacteria | Dairy | Survey of wells for bacteria and nitrate, downstream had elevated levels of both. |
| Central Appalachia | GW | Bacteria | Dairy | Land use effects, seasonal variation, soil-water effects, BMP effects |
| New Mexico | GW | Nitrate | Dairy | 94 dairies surveyed, high nitrate found at many |
| Missouri | GW | Viruses, Bacteria | Various | Survey of wells, few had contaminants, positives in areas of high agriculture |
| Michigan | GW, SW | Bacteria | Various | Models currently inadequate to describe transport, no well contamination |
| Five States, eastern US | GW | Nitrate, Phosphorus | | Permeability versus runoff |
| Not identified | GW, SW | Nutrients, Bacteria | Various | |
| Michigan | SW | Bacteria | Wild Birds | Birds were dominant sources, antibiotic resistance patterns, rainfall increased counts in 48-72 hours depending on wind, collection time affected counts. |
| Nebraska | SW, wetlands | Bacteria, Nutrients | Swine | Decline of contaminants through wetlands before a wildlife refuge. |

† GW = Groundwater; SW = Surface water

The various agencies with interests in microorganisms associated with animal waste have a broad range of topics that they are pursuing. A key topic for all agencies is development and validation of methods for the identification and enumeration of potential pathogens in the different media associated with manure in the environment. The methods for total and fecal coliforms and enterococci are mature, but largely limited to water. There are few methods suitable for a broad range of media such as soil, sediment, lagoons, manure, and water. New methods need to be developed especially to identify overt pathogens in different media. Assuming that a given method is credible, the survival and transport of microorganisms in the environment becomes the next major topic of research. Currently, there is limited research on the movement of microorganisms in the environment. Do they move with soil particulates? What affects movement of organisms in the environment? Are they independent of soil? How long do different organisms survive in the environment? What are safe limits of the different organisms in recreational waters? Beyond the questions raised here are larger questions of how to control the content of microorganisms in animal waste. What effect do animal diets have on microbial populations in the manure? What effect does manure storage have on pathogen populations? Is pathogen regrowth a significant problem?

In addition to development of methods for enumerating pathogenic organisms in environmental samples, there are several other common topics of interest to USDA, USGS, CDC, and EPA. These topics include survival and transport of organisms in the environment, source identification and tracking, and antibiotic resistance characteristics of the microorganisms. Do tile drain lines enable transport of bacteria into receiving waters? What effects do different soil types have on microorganism survival? What effect does timing of manure application to soil have on microbial populations? What is the effect of solar radiation on bacterial survival? What effect does rainfall have on transport of microorganisms from application sites to nearby streams? What effects do different Best Management Practices (BMPs) have on the movement of microorganisms in the environment? BMPs commonly include vegetated buffer strips, constructed wetlands, runoff retention basins, infiltration basins, terracing, injection of waste into the soil rather than broadcast application, and more. The development of models of microbial behavior in the environment is a topic of interest to the different Agencies because good models can help to conserve resources and assist in planning for Total Daily Maximum Load (TMDL) implementation and assessing plans for placement of new animal operations on the landscape.

Research carried out by the different agencies is performed on many different scales. Laboratory scale studies are performed to develop new detection and enumeration methods, measure movement of microorganisms through small soil columns under controlled conditions, develop source-tracking techniques, and evaluate small model digester performance. The work done to develop new methods for detecting and estimating microbial populations uses several approaches: 1) Culture-based methods are being refined to be more selective and to reduce the number of steps or time required to provide data for analysis; 2) antimicrobial compound resistance patterns are used as one approach to identify organisms originating either from humans or animals; 3) genetic analysis techniques are also being developed to discriminate human from nonhuman isolates to help identify sources of organisms in water. Another goal is to develop robust methods that can detect and estimate the population of specific organisms in the environment and track them to their source. The benefit of source identification is that corrective actions can be focused more effectively on specific problems rather than being applied

to a broad area. Laboratory-scale work is also done to conduct analyses of samples from different environments using legally standard methods. Beyond the laboratory, field experiments are conducted at plot-scale, field-scale and watershed-scale. The plot and field-scale experiments usually evaluate the effects of application rate, application timing, rainfall effects on transport, and survival of microorganisms in the field. Also included at this scale are effects of vegetated buffers, wetlands, and other field management practices that can impact the transport and fate of microorganisms. Watershed-scale examination of effects of CAFOs on microbial populations in waters is perhaps the most difficult to carry out. Examination of waters for populations of total or fecal coliforms only reveals if the waters meet standards or not. The current methods simply do not allow for estimation of the contribution of human versus other animal inputs. Similarly, the inputs of wildlife cannot be separated from domestic animals or humans using total or fecal coliform methods. Consequently, installation of management practices at one CAFO may reduce that facility's input to the stream, but have little effect on the total load of microorganisms. Much work needs to be done to adequately model microorganism behavior in the environment and to identify critical control points. A means for separating the total microbial load into its important components needs to be developed and validated to enable estimation of the maximum load for individual water bodies. Associated with this work is a need to estimate or identify the health risk of fecal organisms in water.

The majority of the research conducted by USDA has dealt with the control and retention of plant nutrients in manures. The nitrogen and phosphorus content of manure represents a valuable resource for fertilization of agricultural land. Similarly, the organic matter content of manure is a valuable soil conditioner. In recent years, the ARS has added a significant amount of research on the microbial content of manures with regard to the presence of pathogenic microorganisms, the transport of organisms in the environment, and the survival of organisms in the environment (Table 16). When farms were smaller and had small numbers of livestock present, the manure produced was largely used as a soil amendment and fertilizer. In most cases, the small farm manure load would have been indistinguishable from the background of wildlife sources. The advent of large animal production units has altered the quantity of manure generated in small land areas. It is common now to have poultry houses with over 100,000 birds and swine houses with more than 1,000 animals in a building. Beef feedlots and dairy facilities can also have very large numbers of animals present. The amount of land available within economical transport distances for application of manure is also limited. The result is that too much manure is applied to too little land, leading to the possibility for serious runoff losses of nutrients and potential pathogens.

The importance of understanding microbial behavior in the environment cannot be overestimated. The health of humans and animals can be seriously affected by microorganisms that are commonly present in manures. If one farm has animals that are shedding pathogens in their manure, that manure can be a source of infection for other farms, recreational water users, and possibly municipal water supplies downstream of the farm. The true risks remain largely unknown because there is little information on the presence and survival of pathogens in animal waste after it enters the environment. USDA research on the microorganisms in manure is addressing this concern in studies from laboratory to entire watershed studies. The indicator organisms (coliforms and Enterococci) are useful for screening of waters for the presence of fecal contamination, but are limited in revealing the presence of pathogens.

**Table 16.** Studies carried out or in progress by the United States Department of Agriculture, National Program 206 [†]

| Location | Media [§] | Analytes | Waste Type | Observations |
|---|---|---|---|---|
| Georgia | SW, Soil | Bacteria | Poultry | Survival and transport of pathogens |
| North Carolina | SW | Nutrients, Bacteria | Swine | Advanced waste treatment system evaluation |
| | SW | Bacteria | Cattle | Runoff content of bacteria, vegetative treatment system |
| Virginia | SW | Viruses | Dairy | |
| Maryland | SW | *Cryptosporidium parvum* | | Modeling, runoff, transport |
| Chesapeake | SW | Bacteria, Protozoa | | |
| Illinois | SW | Nutrients, Bacteria | | Integrated waste systems |
| Wisconsin | SW | Bacteria | Dairy | |
| California | GW | Nitrate, Bacteria | Dairy | Dairy lagoon water site |
| Idaho | SW | Nutrients, Bacteria | Swine, Poultry, Fish | Gases, PM2.5, management effects, percolation |
| Texas | SW | Bacteria, Protozoa | Cattle | Best Management Practice effects, method recovery efficiency |
| Texas | SW | Nutrients, Bacteria | Cattle | Commercial additive effects |
| Texas | SW | Nutrients, Bacteria | Poultry, Swine | Transport in soil columns |
| Kentucky | SW | Bacteria | Swine | Survival of pathogens |
| Kentucky | GW, SW | Bacteria | Swine | Runoff, antibiotics, treatment methods |
| Idaho | SW | Bacteria | | Transport, riparian buffer effects, at different scales |
| Maryland | SW | Bacteria | | BMP effectiveness |
| Pennsylvania | SW | Nutrients, Bacteria | Various | BMP placement, stream processes |
| Iowa | SW | BMP Effectiveness | Various | BMP effectiveness, crop effects |
| Iowa | SW | Iowa | | Tile water, soil effects, survival |
| Colorado | GW | Nutrients, Bacteria | Human | |
| | SW | Nutrients, Bacteria | Dairy | Pond effects on removal |
| Mississippi | SW, Wetlands | Nutrients, Bacteria | | Pollutant removal at edge of field |
| Virginia | SW | Nutrients, Bacteria | | Methods, source ID, *E. coli* O157 prevalence |

[†] There are also several projects not associated with a specific state that are examining fate and transport of microorganisms in the environment. These projects also examine the factors affecting microorganisms and their movement. Pathogen identification, antibiotic resistance, modeling, composting, wetlands, management practices, and animal diet effects are among the research topics.

[§] GW = Groundwater; SW = Surface water

**Table 17.** Studies carried out or in progress by USDA or cooperating Universities listed in the CRIS database [†]

| Location | Media [§] | Analytes | Waste Type | Observations |
|---|---|---|---|---|
| Texas | SW, sediments | *E. coli*, *Salmonella* | Cattle | 5 to 7 types of *E. coli* dominate each group. |
| North Carolina | SW, constructed wetlands | Nitrification | Swine | |
| Georgia | SW, riparian buffers | Bacteria | Swine, Poultry | Buffers can be effective in removing bacteria |
| Louisiana | SW | Bacteria | Dairy | *E. coli declines with time after application.* |
| California | Multiple | Bacteria | Dairy | *E. coli* can survive 45 days after application |
| California | SW | *E. coli* O157:H7 | | Pathogen transport |
| California | Air, SW | Protozoa, Bacteria | Dairy | Protozoans increased, bacteria decreased after application |
| California | Food surfaces | Bacteria | | Method development to measure populations |
| Colorado | Manure piles, compost | Bacteria, Antibiotic resistance | Horse, cattle, poultry | |
| Georgia | SW, riparian buffers | Bacteria | Dairy, swine, alligator, poultry | Buffers are effective with swine waste, upland cropping was effective with poultry and dairy waste |
| Georgia | SW | Bacteria, nutrients | Dairy, Swine, Poultry | Buffers alone are not adequate, multiple cropping and forest help limit loads. |
| Georgia | SW | Bacteria | Poultry | Composting, UV, Chemical treatment effects on survival |
| Georgia | SW, GW, soil | Bacteria, Hormones, Protozoa | Poultry | E. coli not best source tracking organism, protozoa can penetrate soil to depth, small ponds can reduce organism load, tillage, temperature, texture were important |
| Georgia | SW, soil | Bacteria, Hormones | Poultry | Watershed, landscape scale, methods, filtering by plants |
| Georgia | Compost | Bacteria | Various | Compost has to be well managed to reduce pathogen levels |
| Hawaii | SW | Bacteria | Various | Multiple scales, bacterial reduction |
| Idaho | SW | Bacteria | Various | Use of flocculants as a treatment |
| Idaho | SW | Bacteria | Various | Diet modification effects |
| Idaho | SW | Bacteria | Various | Landuse and coliform levels |
| Illinois | Various | Bacteria | Swine | Feed and odor, antibiotic resistance |

[†] Some of the entries may duplicate entries in Table 2.

[§] GW = Groundwater; SW = Surface water

**Table 17.** Studies carried out or in progress by USDA or cooperating Universities listed in the CRIS database (continued) †

| Location | Media § | Analytes | Waste Type | Observations |
|---|---|---|---|---|
| Indiana | Tile drain | Bacteria | Various | DOC and pathogen transport, Effect of manure on bacterial survival |
| Iowa | SW | Bacteria | Swine | Bacteria at different places in waste streams, diet effects |
| Iowa | Soil, manure | Bacteria | Swine | Control strategies, native community effects on manure bacteria |
| Kentucky | Multiple | | Various | Waste management in karst areas |
| Louisiana | SW | Bacteria | Dairy | Differentiation of sources |
| Maryland | SW | Bacteria | Dairy, swine | Multiple research areas to reduce bacteria and recover value from manure |
| Maryland | Milk | Bacteria, Viruses | Dairy | 3 to 8 % of milk tanks had contamination |
| Maryland | Water, air, manure | Bacteria | Dairy, beef | Land use and buffers affect organisms, methods, source ID |
| Maryland | SW | *E. coli* O157, *C. parvum* | Dairy, beef | O157 is more diverse than previously known, DOC enables percolation of pathogens, urban water has greater E. coli, oysters can be 90% contaminated |
| Maryland | SW | Bacteria, Nutrients | Dairy | Algal treatment of dairy waste retained nutrients |
| Maryland | Soils | Bacteria | | Manure particles reduce attachment and enable percolation of bacteria |
| Minnesota, Wisconsin | Soils | Bacteria, Antibiotics | Beef, swine, turkey | Tillage, soil type had large effects on resistance, transport |
| Mississippi | Soil | Bacteria | Poultry, swine | Feeding study, methods, survival, phage control |
| Nebraska | SW | Bacteria | Beef | Runoff control, compost, vegetative treatment area |
| Nebraska | SW, sediment | Bacteria, protozoa, Phage | Beef | Survival, wetland, runoff, methods |
| New York | SW | *C. parvum* | Various | Transport models, vegetation, soil type, slope, management practices |
| North Carolina | SW | Bacteria | Swine, poultry | Diet, new waste systems, survival after treatment and application |
| North Carolina | Wetlands | Nutrients, Metals, Bacteria | Swine | Continuous marsh reduced nutrients better than other patterns, water depth was important, solid liquid separation |

† Some of the entries may duplicate entries in Table 2.

§ GW = Groundwater; SW = Surface water

**Table 17.** Studies carried out or in progress by USDA or cooperating Universities listed in the CRIS database (continued) †

| Location | Media § | Analytes | Waste Type | Observations |
|---|---|---|---|---|
| North Carolina | Various | Nutrients, Bacteria | Swine | Nitrification, denitrification, phosphorus recovery |
| Oklahoma | Soil | Bacteria, Metals | | |
| Pennsylvania | SW, soil | Bacteria | | Management practice effects, wetlands, hydrogen production |
| South Carolina | Wastewater | Nutrients | | Nitrification denitrification |
| South Carolina | Wastewater, wetlands, | Nutrients, Bacteria | Swine | Waste treated with different materials and practices for the recovery of nutrients and reduction of pathogens. |
| Texas | | Bacteria | | Develop phage as a bacterial control technology for waste |
| Texas | Soil. Irrigation water | Bacteria, Protozoa, | | Multiple aspect study examining many aspects of animal waste in the environment. |

† Some of the entries may duplicate entries in Table 2.
§ GW = Groundwater; SW = Surface water

Considering that microorganisms originating in animal waste represent a significant risk to people and animals, methods to reduce the microbial load of waste are important. There is a great need to develop manure management procedures that will reduce the load of microorganisms before waste is allowed to enter the open environment. Anaerobic digestion is one technique with promise to be cost neutral or beneficial due to the use of generated methane as a fuel source. Aerobic digestion is a net cost, but reduces odors and microorganisms. Composting reduces odors and microorganisms and produces a potentially salable product. Composting may be practical if markets can be developed. There are other approaches that generate activated carbon, pelletized fertilizers and other products. Combinations of waste management methods may also be used to reduce microorganism loads before waste disposal. Storage of wastes for six months has shown reduction of bacterial populations. Storage in concert with another management practice may be able to reduce loads of organisms to the point where application to land would pose little fecal load runoff potential. The important factor is that any treatment approach has to be economically feasible in comparison to existing manure management practices.

A key task to be completed is integration of the various government agency research activities. The benefit of integration will be to maximize efficiency of planned research by expanding the scope of work, avoiding duplication of effort, and sharing of information across interest groups. EPA is establishing a scientist to scientist level series of workgroups with the goal of integrating work across agencies. Other goals include enabling scientists to participate in larger projects than any individual could manage alone and prepare documents that are useful to producers at the farm level for implementation of environmentally sound practices. Collaboration with the USDA and Extension services will facilitate these goals.

# 11. Summary and Outstanding Issues

Bacteria, viruses, and parasites that can cause disease in humans are endemic in livestock animals. The confinement of animals into densely-populated feeding operations exacerbates the spread of disease and encourages the use of antimicrobial agents for both prophylaxis and to increase animal growth rates, resulting in the emergence of antimicrobial-resistant bacteria. These zoonotic pathogens may proliferate in confinement houses and are shed in animal wastes that, in most cases, are stored and eventually spread onto land. Exposure to antimicrobial-resistant bacteria and other zoonotic pathogens may occur through direct contact with livestock animals, breathing confinement house air, contact with insect and animal vectors, recreational or drinking waters contaminated with manure runoff or leaking manure storage pits, eating produce from manure fertilized fields, and secondary infection from exposed individuals. Several mechanisms are in place to prevent the spread of disease from livestock animals to humans and may include animal stocking techniques, animal waste treatment practices to destroy pathogens (such as composting and thermophilic anaerobic digestion), storage of animal manure to reduce pathogen concentrations prior to spreading, barriers (such as wetlands and buffer strips) to control runoff from manured fields, and surveillance of our nation's food and waters for pathogenic organisms. However, from reported literature, it is clear that exposure to zoonotic pathogens cause significant human suffering and economic losses in the billions of dollars annually due to lost productivity, treatment of disease, and beach closures. Because of the continuing human disease caused by zoonoses contaminating food and water resources in the U.S., we believe that the current environmental regulations and conventional animal manure management practices are inadequate for protection of human health and the environment.

The USEPA and other governmental entities including the USDA, USGS, and the CDC are actively working towards resolving the threat to human health and welfare posed by antimicrobial-resistant bacteria and other zoonotic pathogens that may be released into the environment from CAFOs. As can be seen in this review, the outstanding issues regarding the fate and transport of zoonotic pathogens are vast; addressing these issues will require the expertise of all of these agencies and the many disciplines they represent. Of particular concern is the synthesis of information generated in these studies into a comprehensive and usable package, so that resources can be pooled to arrive at a more complete and usable plan.

Much work is still needed to fully address issues surrounding the contamination of our environment and with antimicrobial-resistant bacteria and zoonotic pathogens originating from livestock animals. Based on our review, we recommend that the pathway forward involve not only value-added research, but also policy changes that are consistent with current limitations on the use of human waste biosolids as fertilizers.

## *11.1 General recommendations*

Animal agriculture produces copious amounts of manure, most of which is stored untreated and spread onto land. Based on available manure management technologies, ensuring the safety of food crops and water resources will require active treatment practices that greatly reduce or eliminate pathogens in manures and other CAFO wastes prior to land application or discharge to natural waters. At present, animal manures applied to land as a fertilizer are not regulated in terms of pathogen reduction. This lack of regulation is at odds with requirements for the application of biosolids originating from human septage (USEPA, 2003). Consider:

- Even moderately-sized concentrated animal feed operations, such as a 2,500 dairy cattle operation, may produce as much manure as a city of 61,000 people. Serious fines for environmental pollution and lawsuits would result if a city of that size spread all of its sewage onto land without treatment.
- Animal manures and other animal wastes may contain high concentrations of pathogens, hormones, antimicrobials and other pharmaceutically active compounds, metals, nutrients, and other chemicals, similar to human sewage.
- Animal manures can be as much as 100 times more concentrated than human sewage, as human wastes are diluted with other domestic wastewaters prior to treatment.
- Because of their concentrated form, animal manures have a higher demand for oxygen, higher nutrient content, and higher concentration of pathogens than human septage on a per weight basis.
- Every year animals raised in CAFOs produce three times as much manure as humans in the U.S.

Regulatory bodies should carefully weigh the full costs associated with zoonotic disease, which are estimated to reach into the billions annually, when considering difficult decisions regarding the regulation of livestock animal wastes. Several cost effective options for animal waste treatment can be implemented at CAFO facilities that would reduce pathogens to safe levels prior to application as a fertilizer. The most effective and cost-efficient methods for achieving these ends may be composting or thermophilic anaerobic digestion with recovery of methane that can be used as a fuel. However, circumstances specific to each animal confinement facility would need to be considered when choosing appropriate manure treatment systems. These active treatment systems should be used in concert with management practices to reduce pollution of water bodies by treated manure fertilizers, such as vegetative filter strips, terraced landscapes, and constructed wetlands.

Of great concern is the continued use of antimicrobials in animal agriculture for growth promotion and prophylaxis. Many of the drugs used to promote growth in animal agriculture are the same as or very similar to human medicines, and result in the shedding of high concentrations of antimicrobial resistant bacteria that may infect humans and other animals. Antimicrobial resistant zoonotic pathogens are a serious threat to human health (Ghidán *et al.*, 2000; Cheng *et al.*, 2002; Travers and Barza, 2002), and billions of dollars are spent in the U.S. every year treating diseases resistant to antimicrobials and managing the spread of resistance in hospital environments. The benefits of growth promotion in livestock animals are certain, and at

present, difficult to offset completely with market alternatives (Harper, 2004; Gill, 2005). However, a combination of education of owner/operators, alternative feed additives, and improved and more sanitary animal husbandry practices are promising for achieving this end (Gill, 2005). Regulatory agencies should fully weigh the costs and benefits of continued use of antimicrobial compounds in animal agriculture for growth promotion and consider the phased removal of these feed additives from the market in favor of alternative technologies. Tighter regulation of the use of antimicrobial compounds for prophylaxis should also be considered.

## *11.2 Recommendations for Future Research*

Significant progress has been made to date to address the release and movement of microorganisms from CAFOs and fields fertilized with their manure byproducts. Research has ranged from bench studies on pathogen survival to investigations of specific management practices for impeding the movement of fecal indicator bacteria to receiving waters and specific surveys of pathogens and antimicrobial-resistant bacteria near CAFO facilities. Current research is exploring new and innovative ways to detect and quantify pathogens in soils, manures, and natural waters that are enabling more specific characterization of animal waste management practices and technologies performance. These techniques have also opened the door for development of improved monitoring and surveillance systems that may revolutionize the way we look at water quality. Some of these new technologies are progressing rapidly towards the end of being able to identify with great accuracy the source of pathogenic agents in recreational and drinking water resources that may cause disease. Other advances are being made in the development of cost efficient and reliable livestock animal waste treatment technologies that may ultimately reduce the burden of zoonotic disease in the U.S.

Although advances are being made, significant amounts of work are still required to fully address the issues surrounding antimicrobial resistant bacteria and other zoonotic pathogens from CAFO facilities. There is a need for fundamental information on specific etiological agents pertinent to their movement and inactivation in manures, soils and sediments, and natural waters. There remain questions as to what levels of these agents are acceptable in natural systems such that the risk of contracting disease upon accidental exposure is low. New models that can predict with accuracy the fate and transport of pathogens in the environment following the application of manure fertilizers to land are needed to identify potential control points to locate new operations safely and in a sustainable manner. In addition, integrated systems that can monitor our nation's water resources in real-time for threats that may be posed by zoonoses and other biological agents are needed to improve biosecurity. All of these research needs are integral to improving human health and welfare in the U.S., especially in areas of intensive livestock farming. The following is a top ten list of research needs to address the pathogen issue and reach this goal.

1. *There is a need for standardized methods of analysis for zoonotic pathogens in animal manure, soil and sediments, wastewater, recreational water, and drinking water.*

   Standard methods with the required sensitivity for recovering and enumerating pathogens at environmentally relevant concentrations in animal manures, soils, wastewaters, recreational water, and drinking water are sorely lacking, especially for hyper-endemic or emerging pathogens. These methods are needed to (a) identify the extent to which these agents are removed, inactivated, or persist in animal waste treatment processes and

management systems at livestock operations, (b) determine the survival of these agents in manures, soils, sediments, and natural waters to improve our ability to predict their fate and transport in the environment, and (c) improve surveillance and biosecurity of our nation's recreational and drinking water resources.

2. *There is a need for rapid methods of analysis for pathogens in recreational and drinking water to improve surveillance and biosecurity of our nation's water resources.*

   Microbiological water quality surveillance in the U.S. relies on the detection of bacterial indicators of fecal pollution. Although epidemiologically related to gastrointestinal illness, these indicators do not fully describe the risks associated with recreational or drinking waters contaminated with some bacteria and most viruses and parasites. Furthermore, since conventional cultivation methods take 18-24 hours to yield a presumptive-positive result, a positive result today means that everyone drinking the water or swimming in it yesterday was exposed to unacceptable levels of fecal pollution.

   As such, there is a need to develop rapid and reliable methods for the detection of fecal bacterial indicators and overt pathogens in recreational and drinking waters. A tiered approach to rapid monitoring methods may be the most reasonable, starting with indicators and then adding pathogens as methods become available. It has been suggested that such technologies rely on nucleic acids analysis because the tests lend themselves to automation and are at present the most promising for rapid and specific quantitation of both fecal indicator organisms and viable microbial pathogens (Jothikumar *et al.*, 1998; Levin *et al.*, 2002; Straub and Chandler, 2003).

   Current technologies for rapid detection of the fecal bacterial indicators are being field tested against proven cultivation methods to develop guidelines for improving recreational water quality monitoring by the USEPA and CDC (USEPA, 2005). However, the near real-time detection of overt pathogens with very low infective doses, such as *E. coli* O157:H7, *Campylobacter jejuni*, and *Cryptosporidium* will require significant advances in technologies to concentrate these agents from large volumes of potentially dirty water. In particular, there is a need for effective and reliable sample concentration technologies capable of co-concentrating viruses, bacteria, and parasites into clean samples amenable to detection with nucleic acids technologies. At present, hollow fiber ultrafiltration systems may be the most promising to this end. However, the retention of a variety of pathogens from different waters by these filters needs testing and validation.

   Ultimately, the development of a unified and automated system for the detection of all waterborne pathogens is needed (Straub and Chandler, 2003). Hollow fiber ultrafiltration devices, renewable surface technologies for automated sample processing, and microarray technologies have shown promise as a basis of such a system (Chandler *et al.*, 2000a,b). However, such a system should be constructed in a way that it is simple, reliable, and technically less demanding than current nucleic acids technologies. The need for rapid pathogen detection technologies will remain a critical issue for biodefense where real-time identification of etiological agents may be imperative to protecting human health.

3. *There is a need for epidemiological data to establish regulatory guidelines for pathogens in manure, wastewater, recreational water, and drinking water.*

   Regulatory guidelines on the concentrations of pathogens in the manure, wastewater, recreational water, and drinking water protective of human health do not exist because (a) there is a lack epidemiological data to ascertain the risks of illness associated with exposure, and (b) there remain questions as to what level of risk is acceptable. There remains a need for epidemiological data to enable the identification of appropriate and safe limits of pathogens in manure, drinking water, recreational water, and in food. Based on surveillance of water and foodborne outbreaks in the U.S., priority should be given to *Salmonella* spp., *Campylobacter jejuni*, *E. coli* O157:H7, *Cryptosporidium*, *Giardia*, and viral agents such as swine hepatitis E virus.

4. *There is a need to identify inactivation kinetics of zoonotic pathogens in manures, soils, and environmental waters.*

   Relatively few studies are available describing the survival of zoonotic pathogens in environmental matrices, especially considering the broad range of properties of soils, manures, and waters that may potentially be contaminated. A significant limitation is the lack of information regarding the survival of antimicrobial-resistant bacteria in various *milieus*, including the persistence of phenotypic and genotypic antimicrobial-resistance traits. Most studies on the survival of pathogens have been carried out in the laboratory instead of *in-situ*, and only a few examined more than one environmental stressor simultaneously. Accurate information regarding the survival of pathogenic zoonoses and antimicrobial resistant bacteria is necessary for modeling their fate and transport from CAFOs.

   Based on these limitations, the following needs have been identified:

   - Comprehensive studies that examine the combined effect of several stressors simultaneously on the survival of zoonotic pathogens and antimicrobial-resistant bacteria in manures, soils, and surface water sediments, and natural waters are needed. Stressors that should be considered include:
     - Biological factors, such as antagonism, competition, and predation;
     - Physical factors, such as temperature, soils and sediment properties, and solar radiation;
     - Growth factors, such as pH and availability of nutrients.
   - There is a need to identify the effect of the retention of some pathogens on soils and sediments on survival in various matrices.
   - There is a need for small-scale studies to determine the concentration of antimicrobial compounds needed for an organism to maintain antibiotic resistance and the number of growth cycles that lead to the loss of the resistance trait.

5. *There is a need for fundamental research to characterize the transport of zoonotic pathogens over land, through soils and ground water, and in surface water bodies.*

   The movement of antimicrobial-resistant bacteria and other zoonotic pathogens from animal wastes through the environment is a complex issue. Research is needed to address significant data gaps regarding the properties of etiological agents that may affect their retention or mobilization in soils and stream bed sediments. In order to better address the transport of pathogens in the environment, several needs must be met, including:

   - Characterization of the properties of zoonotic pathogens that may affect their fate and transport in the environment, which, if understood, would allow them to be incorporated into existing hydrologic and geogaphical information systems (GIS)-based transport models.
   - Identification of the particle sizes with which zoonotic pathogens may be transported in the environment.
   - Identification of the potential effects of soil and sediment retention of some pathogens on overland transport and resuspension in stream bed sediments.
   - Verification that batch and column studies performed in the laboratory to determine pathogen fate and transport properties accurately describe field observations.

6. *There is a need for research to characterize the movement of antimicrobial-resistant bacteria and other pathogenic zoonoses into the environment following land application of animal manures with particular attention paid to the effects of hydrologic (rainfall) events.*

   Information is lacking regarding the concentrations of antimicrobial-resistant bacteria and zoonotic pathogens in the environments proximal to CAFOs and fields where their manures are applied. However, on a larger scale, significant microbial contamination in agricultural watersheds has been observed by the USEPA. Rainfall has been noted to increase concentrations of fecal indicator bacteria in agricultural watersheds, and much of the outbreaks of waterborne disease in the U.S. and Canada have been linked to heavy rainfall events.

   Surveillance of pathogens and antimicrobial resistant bacteria near several CAFOs with different confinement animals and manure management practices is needed to ascertain the potential pathways for pathogen transport from manured fields. Monitoring plans should also consider sampling at the sub-watershed and watershed scales. Field studies are needed to identify the role of drainage tiles and overland transport of pathogens to receiving waters during rainfall events. Continuous or event-triggered sampling devices should be used so that events are not missed. Samples should be taken following manure application and 24 to 48 hours after a substantial rainfall. Future studies should also consider management records on the use of antimicrobials on each specific farm that may be helpful to correlate farm practices with findings obtained through the studies.

7. *There is a need for continued research into methods for tracking fecal pollution in natural waters to its source.*

   Much of our nation's water resources are impaired due to high concentrations of fecal microorganisms. In many instances, the source(s) of fecal contamination are unclear. MST is an emerging technology that identifies the animal origin of fecal bacterial pollution. However, many caveats to the use of MST still exist, and much work is needed to improve these technologies for more widespread application. Some of the data gaps for the various methods include:

   - Poor survival of MST reference organisms in the environment may result in little or no detection, limiting the ability to identify the source of fecal contamination. Reference organisms need to be chosen so that they are useful at a distance from the potential source.
   - Variability in the survival of different phenotypes or genotypes of MST reference organisms in environmental matrices that may lead to divergence from host-specific fingerprints in source libraries need to be clearly defined. Limitations to interpretation of MST results dependent on these findings need to be documented.
   - Variability in transport and survival of the index organism(s) used to identify fecal pollution source(s) and pathogens of the same source that may lead to misidentification of the source of disease. Studies are needed to ascertain whether or not reference organisms are reliable indicators of pathogen transport. It may be that several reference organisms are needed to describe the full suite of pathogens that may contaminate water bodies.
   - Variability in the transport properties of different index organisms needs to be clearly defined to enable interpretation of MST results.
   - Advances in MST technology need to be made to reduce the time of analysis, the level of expertise required, and the cost. Host-specific molecular biomarkers offer the most promise for achieving this end, but significant advances in their development must be achieved before they are off-the shelf ready.
   - MST techniques need more field-scale testing to prove their utility in varied circumstances. Full-scale watershed type studies are needed to assess the potential of these technologies for future use.
   - There is a need for different levels of analytical methods to address microorganism tracking from simple indicators to methods for exact pathogen and source identification.
   - Additional research is needed in the area of spatial and temporal variability for library-independent MST methods.

8. *Fundamental studies on the efficacy of various manure management practices including uncertainty in their performance are needed.*

   Many management practices have been proven effective for reducing the discharge of stressors such as nutrients and sediment runoff to surface waters. However, the efficacy of different management practices for impeding the movement of zoonotic pathogens and antimicrobial-resistant bacteria to receiving waters following land application of animal manures remains uncertain. Based on studies using fecal indicator organisms, these practices may reduce the discharge of pathogenic microorganisms. However, the reductions associated with most practices are only on the order of 90-99%, a scant number considering that animal manures may contain billions to trillions of bacteria, viruses, and parasites per gram. Therefore, although specific management practices such as vegetative buffer strips will retain large fractions of microbial populations, they will not retain them well enough to protect receiving streams from contamination.

   There is a need to identify the performance of common barrier technologies such as infiltration basins, wetlands, and buffer strips for the retention and inactivation of pathogenic organisms. Studies should address retention in the context of factors related to the design of the systems such as size, slope, solids or hydraulic residence time, vegetation, undercutting by tile drainage, etc. Studies are also needed to address the impacts of rainfall on management practice performance. Of particular interest is exploration of a multibarrier approach versus single barrier BMPs.

   Aside from barrier technologies, there is a need to verify and field test manure treatment technologies like anaerobic digestion, not only for pathogen reduction, but also to identify the potential for fuel recovery. These technologies should be compared and contrasted to conventional manure storage technologies in terms of stressor reduction and cost. Vector attraction reduction and pathogen regrowth in treated materials should also be explored.

   Many of these questions are being addressed in the areas of public wastewater treatment and biosolids from public treatment works. Analogies for manure treatment and runoff barrier technologies for pathogens, as well as vector attraction reduction, may be drawn from the extensive pool of research available within the biosolids community. However, livestock animal wastes tend to be more concentrated than human sewage; thus, treatment solutions for human wastes need to be feild-tested for application at CAFOs. The most readily applicable technologies may be those for pathogen reduction in biosolids, but liquid separation and treatment may need to be performed prior to application of solids treatment technologies.

9. *Models are needed to better predict site-specific optimal manure treatment technologies and runoff management practices for pathogen and other stressor reductions*

   Better models of microbial behavior in the environment are needed to assist in planning for TMDL implementations and assessing plans for placement of new animal operations on the landscape. Of particular interest would be lifecycle assessment models capable of analyzing the effects of different treatment technologies and management practices. Models should be capable of predicting potential outcomes regarding not only pathogens but other stressors such as nutrients and pharmaceutically active compounds. Best possible treatment and management practice combinations, as well as sustainable livestock populations based on environmental and human health outcomes, should be predicted considering uncertainty in the performance of the various treatment technologies and management practices. Models that integrate the fate and transport of antimicrobial-resistant bacteria and zoonotic pathogens may be different from present models in many ways. The issues of multi-drug resistance, microbial reservoirs, horizontal gene transfer of resistance determinants, and the ranges of infectious doses resulting from various host characteristics are not part of current models for chemical risk assessment. These factors need to be integrated into CAFO models. Further, particular attention should be given to the relationship between pathogens and organic matter, sediments, and nutrients, particularly in terms of survival and facilitated transport during hydrologic (rainfall) events. These models will ultimately need to be proven at the sub-watershed and watershed scales.

10. *There is a need to improve the coordination of research activities and dissemination of technical information, methodologies, and new technologies between research scientists of the various agencies and to a vast array of end users such as educators, regulators, and CAFO owners and operators.*

    There is a confounding level of technical literature relevant to pathogens and livestock animals dating back more than 100 years, and literature propagates at an astounding rate. Researchers in the fields of engineering, microbiology, agronomy, epidemiology and infectious diseases, as well as the geological sciences and others, are conducting a wide variety of studies on pathogens and/or fecal bacteria relevant to CAFO issues. Interpreting the literature is difficult not only due to the massive amounts of technical information available, but also due to the diverse nature of these disciplines. As such, there remains a need for better integration of the various government research activities. The benefit of integration would include (a) pooling of resources, (b) broadening of technical expertise, (c) maximizing efficiency, (d) expanding the scope of work that can be performed, (e) avoiding duplication of effort, and (f) sharing information across interest groups. Without significant interdisciplinary integration and cooperation, the assimilation of available information into a comprehensive and meaningful form for the waste managers, educators, and regulators is unlikely.

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