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Zoonotic Pathogens in Domestic Livestock Manure

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Science to Social Issues

Contents

Introduction

Fecal waste from domestic livestock such as cattle, swine and poultry raised in contained facilities is excreted onto the floors of the animal stalls or pens. Accumulation of this fecal material acts as a collection basin for pathogens which may be spread between animals (Taylor et al., 2001). Disposal of domestic livestock fecal material may comprise of storage in lagoons or waste piles with the ultimate application to the soil surface and incorporation into the soil. These fecal wastes may also enter water systems by the direct contamination of the water or through the seepage or surface runoff (Jones, 1999; Graczyk et al., 2000).

In the rearing of domestic livestock on range or pasture, manure may not be concentrated in one area as with confined livestock. These animals can also contaminate water by defecation in unprotected surface water, through surface runoff and as a result of seepage of water through soil that contains an excessive amount of animal feces (Larsen et al., 1994; Graczyk et al., 2000; Donham, 2000). The potential for this environmental pollution is present and growing because of the concentration of production into fewer large-scale units, not the increase in total numbers of animals (Donham, 2000). Large-scale production facilities have the potential to cause serious environmental contamination as a result of the amount of manure produced at one site.

Currently, there are 1415 infectious organisms (bacterial, viral, parasitic and fungal) known to be pathogenic (an organism that causes clinical disease) to humans and 616 such pathogens that cause disease in livestock. Of the livestock pathogens, 243 (39.4%) are known zoonotic agents. Zoonotic pathogens are those that can be transmitted between animals and humans, potentially causing clinical disease in humans (Cleaveland et al., 2001). These zoonotic pathogens are more likely to be transmitted by indirect contact via food or an environmental reservoir, providing many opportunities for the pathogen to infect other species, while direct contact via wounds, sexual contact, vertical transmission or inhalation provides limited opportunity to infect other species (Taylor et al., 2001). On the whole, zoonotic pathogens are twice as likely associated with emerging diseases than non-zoonotic pathogens (Taylor et al., 2001). Presently, 29 (4.7%) of 196 livestock pathogens have been deemed as being emerging zoonoses with viruses and protozoa more likely to be emerging than other infectious microorganisms (Taylor et al., 2001).

Given that there are a limited number of domestic livestock pathogens in manure, water and soil that have the potential to infect humans and other domestic animals, the focus of this review will entail bacterial and parasitic pathogens. These pathogens are of the greatest concern to the public, who is exposed to these zoonotic pathogens through consumption of fecal contaminated food or water.

1.1 Bacterial Pathogens

Some bacteria harbored by domestic livestock and wildlife, such as *Salmonella* spp. have been known for many years to cause serious infection in humans. More recently, other pathogenic bacteria like *Escherichia coli* O157:H7, *Campylobacter jejuni, Listeria monocytogenes*, *Yersinia enterocolitica* and *Mycobacterium* spp. have emerged as important sources of infection for humans.

1.1.1 *Salmonella* spp.

Salmonella is a bacterium that is widespread in the intestines of most wild and domestic birds, reptiles and mammals, including humans and domestic livestock. Salmonellosis is caused by many species of *Salmonella*; however the pattern of distribution might differ for individual *Salmonella* serotypes. For example, *S. typhimurium* infects all animal species and has a worldwide distribution. On the other hand, the patterns of host adapted serotypes like *S. choleraeusuis* (porcine), *S. dublin* (bovine) and *S. enteritidis* (poultry) are patchy and generally match the patterns of distribution of the hosts to which they are adapted (Ekperigin & Nagaraja, 1998). Despite, the vast array of *Salmonella* species, salmonellosis is predominately characterized by three main clinical symptoms: septicemia, acute enteritis and chronic enteritis (Ekperigin & Nagaraja, 1998). *Salmonella* gains entry into an animal host through direct contact with feces and indirectly from contaminated food or inanimate objects. The most common entrance way into the host is through the host's oral cavity; however, minute pores in the shells of freshly laid eggs provide an important portal of entry into poultry (Tauxe, 1997; Ahl & Buntain, 1997; Ekperigin & Nagaraja, 1998). After entry, the invading *Salmonella* is either overtaken by the host's defenses and

destroyed or expelled or succeeds in overcoming the host's defenses and establishes itself within the host. If infection does not progress into salmonellosis, the *Salmonella* organism may remain in the gastrointestinal tract as part of the host's commensal flora and may be shed in the feces, contaminating the environment and providing a source of infection for other animals (Ekperigin & Nagaraja, 1998).

Salmonellosis in domestic livestock has been implicated with a recent increase of human salmonellosis (Tauxe, 1997). Feces of infected animals have been known to contaminate animal feed, water, milk, fresh and processed meats, and plant and animal products (Ekperigin & Nagaraja, 1998; Ahl & Buntain, 1997). In Alberta, the prevalence of *Salmonella* in yearling beef cattle within a feedlot has been reported at 1.1% and 0% in cows (Sorensen et al., 2002). The prevalence of *Salmonella* in yearling and cow samples at slaughter was found to be 0.2% and 0%, respectively (Van Donkersgoed et al., 1999). The prevalence of *Salmonella* in slaughter weight beef cattle in Prince Edward Island was found to be 4.6% with a significantly higher rate of infection in fasted (7.5%) than non-fasted (1%) cattle (Abouzeed et al., 2000). In Quebec, the prevalence of *Salmonella* isolated from swine feces ranges from 8-25% (Letellier et al., 1999b; Letellier et al., 1999a). The prevalence rate of *Salmonella* infection in chicken cecal contents has been found to be as high as 33% (Abouzeed et al., 2000).

1.1.2 *Escherichia coli* O157:H7

Enteropathogenic *Escherichia coli* are present in the feces of humans, wildlife and domestic livestock. Although, *Escherichia coli* may be found in feces, water and soil, only a small proportion (< 1%) of the bacteria are harmful strains. Most strains of *Escherichia coli* inhabiting the intestines of healthy humans, domestic livestock and wildlife are harmless, and in fact are a beneficial component of the natural intestinal flora (Riemann & Cliver, 1998).

There are several pathogenic strains of *Escherichia coli*; one of the best known and of zoonotic concern is *Escherichia coli* O157:H7. *Escherichia coli* O157:H7 and some other pathogenic strains produce toxins that can cause serious human illness. All *Escherichia coli* are classified on the basis of the presence or absence of surface antigens (O, H, K) and a numerical system that distinguish between harmless and harmful bacteria (Riemann & Cliver, 1998). Pathogenic *Escherichia coli* like *Escherichia coli* O157:H7 are also classified according to their ability to produce toxins, attach and invade host intestinal epithelial cells (Riemann & Cliver, 1998).

Escherichia coli O157:H7 has been isolated from the feces of healthy cattle (Hancock et al., 1997; Zhao et al., 1995; Laegreid et al., 1999). The shedding of *Escherichia coli* O157:H7 in cattle has been associated with season, age of animal, diet, geographical location, population density and management conditions (Van Donkersgoed et al., 2001; Van Donkersgoed et al., 1999; Schurman et al., 2000; Wilson et al., 1992). In Canada, the prevalence of verotoxin producing *Escherichia coli* O157:H7 isolated from cattle feces has been reported from 0.4% in Prince Edward Island, 10 - 25% in Ontario, and 0.8 - 7.5% in Alberta (Van Donkersgoed et al., 2001; Van Donkersgoed et al., 1999; Schurman et al., 2000; Wilson et al., 1992). The prevalence of *E. coli* O157:H7 in healthy pigs has been reported between 0.4% and 7.5% and up to 3.8 % of pork meat samples (Read et al., 1990; DesRosiers et al., 2001). Pigs rarely excrete verotoxin producing *Escherichia coli* O157:H7 that are harmful to humans (DesRosiers et al., 2001). Verotoxin producing *Escherichia coli* O157:H7 has yet to be isolated from chickens. *Escherichia coli* O157:H7 has been reported in wild animals such as deer (MMWR, 1996).

1.1.3 *Campylobacter* spp.

Campylobacteriosis is caused by a spiral-shaped bacterium, *Campylobacter* that is common in humans and domestic livestock. *Campylobacter jejuni* is the predominant species associated with cattle and poultry, whereas *Campylobacter coli* is primarily found in swine (Altekruse et al., 1998; Rosef et al., 1983; Altekruse et al., 1994). These species of *Campylobacter* colonize the intestines of poultry, cattle and pigs and are generally not pathogenic to livestock and are considered commensal bacteria (Altekruse et al., 1998). *Campylobacter jejuni* infection rates of domestic livestock such as cattle and chickens can be as high as 47% and 36%, respectively, with *C. coli* reported to range from 46 – 92% of pigs (Nielsen et al., 1997; Harvey et al., 1999; Wesley et al., 2000). In humans, campylobacteriosis is one of the most common bacterial causes of diarrheal illness in North America, infecting over 4 million people per year (Altekruse et al., 1998).

Most human outbreaks of campylobacteriosis are associated with the consumption of fecal contaminated unpasteurized milk or surface water (Altekruse et al., 1998). The sporadic cases of campylobacteriosis are often associated with the mishandling and consumption of fecal contaminated undercooked poultry and/or poultry products and/or the cross-contamination of foods by raw poultry (Tauxe, 1997; Finch & Blake, 1985; Altekruse et al., 1998; Altekruse et al., 1994). Beef and pork products other than milk are less frequently involved in foodborne infections (Lammerding et al., 1988). These foodborne infections are associated with fecal contamination of meat and the failure to adequately cook the meat product.

1.1.4 *Yersinia enterocolitica*

Yersinia enterocolitica is a bacterium that has been demonstrated in the feces of pigs and cattle (Cole et al., 1999; Gourdon et al., 1999). *Yersinia enterocolitica* O:3 is the most common strain that causes diarrheal illness in humans and has been isolated from the tonsils, oral cavity, intestines and feces of up to 83% of healthy pigs (Cole et al., 1999; Pilon et al., 2000). In Quebec, the prevalence of *Yersinia enterocolitica* isolated from pig feces ranges from 13 – 21% (Pilon et al., 2000; Letellier et al., 1999b). Although, there are no documented reports of direct transmission of *Y. enterocolitica*, pigs have been implied in human infections (Anderson et al., 1991). Farmers and slaughter house workers are considered at risk of developing of developing yersiniosis (Cole et al., 1999). *Yersinia enterocolitica* has been isolated from bulk milk tanks and is frequently associated with undercooked pork (Tauxe, 1997; Jayarao & Henning, 2001).

1.1.5 *Listeria monocytogenes*

Listeria monocytogenes is part of the normal intestinal flora of the distal portion of the intestinal tract of numerous animal species including domestic livestock such as cattle, pigs and chickens and also exists as a plant saprophyte (Weber et al., 1995; Cooper & Walker, 1998). The incidence of *L. monocytogenes* in cattle feces ranges from 6 to 51% whereas, the incidence of *L. monocytogenes* in pigs and chickens is lower, 2-6% and 0-33%, respectively (Unnerstad et al., 2000; Skovgaard & Norrung, 1989; Weber et al., 1995; Skovgaard & Morgen, 1988). Listeriosis usually occurs in sporadic cases; however outbreaks in domestic livestock have been associated with silage (Cooper & Walker, 1998; Fenlon et al., 1996). In humans, outbreaks of listeriosis have been foodborne, linked to contaminated cheeses and processed meats (Cooper & Walker, 1998; Donnelly, 2001; Farber et al., 1996). Clinical symptoms of listeriosis in humans are usually flu-like symptoms; however, encephalitis, abortion and stillbirth can be associated with infection (Cooper & Walker, 1998).

1.1.6 *Mycobacterium* spp.

Mycobacterium spp. is a group of bacteria that is associated with tuberculosis. Tuberculosis is for the most part a respiratory disease and transmission of infection within and between animal species is mostly through the airborne route; however, spread may occur indirectly from contaminated pastures, water and fomites (Wedlock et al., 2002; O'Reilly & Daborn, 1995). Bacteria can also be shed in the milk and feces of animals (O'Reilly & Daborn, 1995).

Mycobacterium bovis has a wide host range which includes cattle, humans, pigs and cervids, whereas *Mycobacterium paratuberculosis* can infect humans, cattle, sheep and goats (Bakker et al., 2000).

Mycobacterium bovis can cause tuberculosis in both cattle and in humans. In humans, it causes a disease which is indistinguishable with respect to pathogenesis, lesions and clinical findings to that caused by *Mycobacterium tuberculosis* (Wedlock et al., 2002). *Mycobacterium bovis* shows a high degree of virulence for both humans and cattle, in contrast to *M. tuberculosis* which is infectious for humans but not for cattle (Moda et al., 1996). In cattle, the disease is chronic and progressive over a number of years leading to the development of lesions in the lungs and thoracic lymph nodes and impaired lung function (Wedlock et al., 2002). Human infections of *M. bovis* usually occur by inhalation of aerosols or through the consumption of *M. bovis* contaminated milk, resulting in respiratory and non-respiratory tuberculosis, respectively (Wedlock et al., 2002). Non-respiratory tuberculosis typically involves the lymph nodes, intestinal tract, the meninges or gives rise to chronic skin tuberculosis, whereas respiratory or pulmonary tuberculosis involves the lung. Cases of human disease caused by *M. bovis* show regional variability depending on the presence or absence of the disease in the cattle population, the social and economic situation, the standard of food hygiene and effective preventative measures (O'Reilly & Daborn,

1995; Moda et al., 1996). In Canadian cattle, the incidence rate of *M. bovis* appears to increase with age; however the number of infected herds has decreased in recent years (Essey & Koller, 1994; Munroe et al., 2000). The incidence rate of *M. bovis* in pigs usually reflects the local levels of *M. bovis* tuberculosis in cattle (O'Reilly & Daborn, 1995). Livestock in Canada are considered tuberculosis free and the occasional case is associated with contact between domestic animals and isolated herds of wildlife (Bison) where the disease is considered endemic. Indeed, in Canada, tuberculosis in wildlife is also contained to a few isolated areas and the spread is continually monitored.

Mycobacterium paratuberculosis infection in cattle causes paratuberculosis or Johne's disease. The disease is chronic, progressive and incurable with symptoms of diarrhea, wasting and weight loss (Bakker et al., 2000). As the infection progresses, *M. paratuberculosis* is excreted in the milk and feces, and the bacteria spread through the blood to the internal organs (Collins, 1997). As a result, raw products from infected cattle like milk, meat and feces may contain *M. paratuberculosis* (Collins, 1997). It is transmitted mainly in the feces to young animals by infected adults that may or may not have clinical signs (Whittington & Sergeant, 2001). In humans, *M. paratuberculosis* has been linked to Crohn's disease which causes chronic inflammation and thickening of the intestine (Bakker et al., 2000; Herman-Taylor et al., 2000; Selby, 2000). The prevalence of *Mycobacterium paratuberculosis* antibodies in beef cow-calf operations is less than 1% whereas, the seroprevalence in Canadian dairy cattle ranges from 2 to 6% (Dargatz et al., 2001; VanLeeuwen et al., 2001; McNab et al., 1991).

1.2 Parasitic Pathogens

There are a large number of animal parasites that can cause infections in humans; however most of these are present in developing countries and in tropical and subtropical areas. The parasites of domestic livestock manure that need to be considered are *Giardia duodenalis, Cryptosporidium parvum* and *Ascaris suum.*

1.2.1 *Giardia duodenalis*

Giardiasis is caused by a microscopic flagellated protozoan parasite called *Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*). The life cycle of *Giardia* consists of two forms, motile trophozoites and highly resistant cysts (Wolfe, 1992; Juckett, 1996; Marshall et al., 1997). Trophozoites are the forms that divide within the lumen of the small intestine (Wolfe, 1992). Major hosts for this parasite are domestic livestock (cattle, sheep, pigs, horses), though humans, dogs, cats and some species of wildlife can also harbor the parasite (Olson et al., 1997b; Xiao, 1994). *Giardia* colonizes the small intestine of humans and livestock and can lead to moderate-to-severe diarrhea. Giardiasis is frequently subclinical in humans and usually subclinical in livestock. It occurs in all host age groups, but is most often present in the young due to poor sanitation and/or lack of innate immunity (Xiao, 1994). *Giardia* is predominantly transmitted through fecal-oral routes; however, waterborne and foodborne transmission have been reported (Slifko et al., 2000). Water contamination with *Giardia* has been associated with agricultural runoff (Ong et al., 1996).

Giardia duodenalis is common in cattle and pigs worldwide (Xiao, 1994). In Canada, the prevalence of *Giardia* in cattle is age dependent, ranging from adult cattle (10%) to calves (100%) (Buret et al., 1990; O'Handley et al., 1999; Olson et al., 1997a; Olson et al., 1997b). In a large Alberta study involving 2669 fecal samples and 90 hog farms, *Giardia* was documented in 80% of the farms and 11% of the fecal samples collected (Guselle & Olson, 2001). *Giardia* cysts were identified in 14.0% weaners, 20% growers and 16% finishers. In all other age groups, the occurrence of *Giardia* was 2.5% or less (Guselle & Olson, 2001). *Giardia* cysts have been shown to degrade in hog liquid holding tanks and therefore, it is unlikely the distribution of liquid manure poses a serious threat for contamination of surface water (Guselle & Olson, 2001).

1.2.2 *Cryptosporidium parvum*

Cryptosporidium parvum is a small coccidial protozoan parasite that colonizes the small intestine of vertebrate hosts throughout the world (O'Donoghue, 1995). This parasite has intestinal forms that multiply within the intestinal epithelium and environmentally resistant oocysts, which are shed in the feces. The pathogenic significance of this *C. parvum* was underestimated until the 1970's when infections were implicated as causative agent of diarrhea (O'Donoghue, 1995; Mosier & Oberst, 2000). Over the last 30 years, this parasite has been identified in severe intestinal disease

in both humans and animals (O'Donoghue, 1995; de Graaf et al., 1999). *Cryptosporidium parvum* infects humans and a wide range of domestic animals (cats, dogs, cattle, pigs, sheep) and wild animals(Holland, 1990; de Graaf et al., 1999; Fayer et al., 2000). Infections in animals and humans can be asymptomatic; however, in the young, elderly and immunocompromised, severe watery diarrhea may develop. Clinical signs usually coincide with oocyst excretion and typically persist for 7-14 days. Infected hosts can excrete between 109 and 1010 oocysts (Smith & Rose, 1998).

As with *Giardia*, this parasite can be transmitted by the fecal-oral, waterborne and foodborne routes; however, *Cryptosporidium* appears to be more likely associated with waterborne outbreaks (Rose & Slifko, 1999; Slifko et al., 2000; Smith & Rose, 1998; Fayer et al., 2000; Graczyk et al., 1997). Fecal contamination of water by human sewage and domestic livestock manure has lead to waterborne outbreaks of cryptosporidiosis (Graczyk et al., 1997; Smith & Rose, 1998). The source of the outbreaks is frequently undetermined but molecular epidemiology has enabled the tracing of some outbreaks. Most waterborne outbreaks have been traced to human sewage contamination of drinking water such as the events that occurred in Milwaukee, Wisconsin and North Battleford, Saskatchewan. However, livestock manure has been associated with waterborne cryptosporidiosis in Canada and throughout the world. Foodborne outbreaks have been associated with direct fecal contamination of food and the use of contaminated water in food processing (Slifko et al., 2000; Millard "et al., 1994; Laberge et al., 1996; Orlandi et al., 2002; Swartz, 2002).

Cattle infections with *C. parvum* are similar to *Giardia* infections in that cryptosporidiosis is age dependant (Anderson, 1998; Atwill et al., 1999; Xiao & Herd, 1994). In Alberta, the prevalence of *C. parvum* in cattle can be as high as 100% in calves less than a month of age and decreases as the animals mature (O'Handley et al., 1999; Olson et al., 1997b). Although there are limited studies, *Cryptosporidium* has been demonstrated in pigs throughout the world (Xiao et al., 1994; Olson et al., 1997b; Wieler et al., 2001; Quilez et al., 1996; Izumiyama et al., 2001). In a Canadian study, *Cryptosporidium parvum* was identified in 3 of 4 sampling sites with an overall prevalence of 11% (Olson et al., 1997b). In a larger Alberta study involving 90 farm sites, the greatest overall fecal prevalence of *Cryptosporidium* in pigs was 3.2%. In this same study, *C. parvum* was more prevalent in the younger market age animals (weaners, growers and finishers) rather than the mature breeding stock (Guselle & Olson, 2001).

Cryptosporidium andersoni was reported in poorly doing Idaho feedlot cattle (Anderson, 1987; Anderson, 1998). It has now been identified in most continents (Anderson 1991; Bukhari et al., 1996; Olson et al., 1997; Fayer et al., 2000). *C. andersoni* has recently been recognized as a separate species different from *C. muris* based upon the molecuar sequence of variable regions within its genome and by its inability to infect several rodent species (Lindsey et al., 2000). *C. andersoni* usually infects calves greater than 3 months of age and this infection can persist for years if not life long (Ralston et al., 2001). Abomasal *Cryptosporidium* invades the peptic and pyloric glands causing dilation of the glands, hypertrophy of the gastric mucosa and thinning of the epithelial lining as demonstrated. Functionally this leads to impairment of protein digestion by increasing gastric pH and inhibition of proteolytic function of pepsin (Anderson 1987; Anderson 1998; Anderson, 1990). One would expect animals on high protein rations, such as finishing feedlot calves and lactating dairy cows, to be most affected by *C. andersoni* infection. We have identified *C. andersoni* in up to 83% of cattle at the time they enter the feedlot but only approximately 5% become chronically infected (Ralston et al., 2001). *Cryptosporidium andersoni* infections can cause moderate to severe impairment of weight gain and decreased feed efficiency in feedlot cattle (Anderson, 1987; Anderson, 1990; Esteban and Anderson, 1995; Ralston et al., 2001). There can be a reduction in performance of up to 50% in feedlot cattle and dairy cattle infected with *C. andersoni* produce significantly less milk (approximately 3.2 kg/day). The effects of *C. andersoni* on range cattle are unknown but the known nutritional and milk production effects of this abomasal parasite would be expected to affect growth in nursing range calves. *C. andersoni* does not appear to cause infections in humans but it may be confused with *C. parvum* and lead to cattle being implicated in human infections.

Two other species of *Cryptosporidia, Cryptosporidium meleagridis* and *Cryptosporidium baileyi*, may infect the intestinal tract, bursa of Fabricius and cloaca of chickens (de Graaf et al., 1999). The fecal prevalence of *C. meleagridis* and *C. baileyi* in chickens has been reported as high as 27% (Ley et al., 1988; Rhee et al., 1991).

1.2.3 *Ascaris suum*

Ascaris suum is the large intestinal roundworm of pigs, whereas *Ascaris lumbricoides* is the large intestinal roundworm of humans that is especially common in the tropics and subtropics. It is debatable if they are the same or separate species for there are major difficulties distinguishing them (Crompton, 2001). Pigs and humans can become infected by ingestion of eggs that have developed to an infective stage in the environment. In pigs, *Ascaris* infections can cause liver lesions or "milk spots", leading the condemnation of livers at slaughter. Transient pneumonia may be caused by the migration of the larvae into the lungs. More importantly, large worm loads in the small intestine may lead to intestinal disturbances resulting in poor feed conversion and slower weight gains (Roepstroff & Nansen, 1998). In humans the accidental ingestion of infective eggs can lead to larval migrans which is the migration of the larvae into the tissues (Peng et al., 1996; Maruyama et al., 1996). Such infections in humans are not commonly reported, as they are in most cases asymptomatic and when they occur are very mild and non-specific. There have been no reports of human larval migrans of *Ascaris suum* in Alberta. It appears the risks of this infection in humans are extremely low.

In Saskatchewan, examination of up to 50% abattoir pig livers demonstrated scarring associated with ascarid larvae (Wagner & Polley, 1997). Environmental resistant *Ascaris suum* eggs can survive for two-to-four weeks in dry conditions, while under a moist, cool environment they can survive for over eight weeks (Gaasenbeek & Borgsteede, 1998). The routine use of anthelminths and indoor containment, which breaks the parasite life cycle, has dramatically reduced the prevalence of *Ascaris* infections in pigs. *Ascaris* was identified at 56% of 90 Alberta farms but in only 9.9% of collected fecal samples. It was present in 21% and 11% of the collected finisher and grower fecal samples, respectively and was recovered in less than 10% in all other age group fecal samples. *Ascaris* tends to be identified more frequently on farms that kept dry sows outdoors on soil (Guselle and Olson, 2001).

2 Transmission from Agricultural Lands

For the most part the transmission of manure pathogens from agricultural land occurs with the application of manure to soil and storage of manure on soil with the eventual entry of manure into a surface or groundwater source. The transmission by this route requires several steps to be successfully accomplished: 1) the pathogen is able to survive in the feces and primary processing (composting, lagoon/holding tank fermentation), 2) a viable pathogen in manure to be applied to the land is in sufficient amounts to cause a human infection if it is ingested, 3) the pathogen is able to survive the degradation/killing process of soil microorganisms, temperature, desiccation and light, 4) the pathogen is able to leave the soil through surface water runoff or permeate through the soil, 5) the organism is able to survive in the water and water treatment procedures (filtration, chlorination), and 6) there is a sufficient number of viable organisms in the water to cause an infection in humans.

Although the transmission process is complex and the risk is low, there is a definite potential for microbial contamination of ground and surface waters from livestock operations (Donham, 2000). Typically, in cattle operations which use range and pasture, collection and treatment of fecal waste typically is not performed (Cole et al., 1999). The accumulation of fecal waste can be avoided by not overstocking pasture land, keeping grazing evenly distributed, rotating any feeding, grazing and shading equipment and not restricting grazing on areas which could contaminate surface water that would be consumed by humans. Management of fecal waste is crucial when storm water runoff from areas that do not maintain vegetative cover can reach receiving water (Cole et al., 1999). Confinement and semi-confinement livestock operations (hog and cattle feedlot) generate liquid and solid wastes that are disposed by distribution and incorporation on land. These are potential sources of contamination of surface waters and ground waters (Cole et al., 1999; Jones, 1980).

Whether a pathogen reaches the surface waters or groundwater and is transported to drinking water wells depends on a wide range of factors. These factors include the concentration of the pathogens, the survival of the pathogens, the number of pathogens leached from the manure-soil interface, the degree of removal

through the soil zones and the hydraulic gradient (Straub et al., 1993). Other important considerations are the weather and the time of the year. The extent to which the aforesaid factors affect the probability of the pathogens entering and contaminating water has yet to be determined.

One main concern with land-applying animal manure is that the fecal pathogens will reach groundwater. Pathogens that reach ground or surface water on a farm potentially may be recycled by crop irrigation and may infect animals or humans through the ingestion of viable organisms on the a crop. Rainfall may result in pathogen spread into soil or surface water by runoff from stored or unincorporated manure or by leaching through soil profiles (Gagliardi & Karns, 2000).

Due to their size and electrical properties, bacteria such as *E. coli* are able to migrate through soil much more rapidly than parasites like *Cryptosporidium* and *Giardia* (Robertson & Edberg, 1997). Whether *Escherichia coli* O157:H7 reaches soil by either application of manure or via runoff from a point source, the organism can travel below the top layers of the soil for more than 2 months after initial contact (Gagliardi & Karns, 2000; Stoddard et al., 1998).

In the case of slurry storage structures, the majority of these structures only pose a risk of water pollution because of structural or operator failure (Gooddy et al., 2001). One United Kingdom study has found that the risk of ground water contamination of *Escherichia coli* O157 and *Cryptosporidium* from earth-based stores for livestock manure is minimal (Gooddy et al., 2001). On the other hand, contamination of groundwater supplies with domestic livestock feces has been implicated in several cases of *E. coli* O157:H7 as well as *Campylobacter* infections (Ackman et al., 1997; Jackson et al., 1998; Center for Disease Control and Prevention, 1999; Duke et al., 1996).

Runoff and infiltration studies of fecal bacteria have indicated that livestock feces landing near a stream have a much smaller potential for water quality impact than does manure landing directly in the stream (Larsen et al., 1994). This was indicated by the dramatic effect that a narrow, 0.61 meter buffer strip has on reducing both fecal bacteria runoff and infiltration (Larsen et al., 1994). It is also suggested that the use of off-stream watering devices, water gaps or fencing that prevents animals from depositing manure into streams can dramatically reduce the presence of manure borne pathogens in the stream (Larsen et al., 1994).

The movement of larger pathogens like *Giardia* and *Cryptosporidium* through soils varies. Following irrigation over 21 days in soil with zero gradient, over 70% of *Cryptosporidium* oocysts can be recovered from the top 2 cm of the soil and oocyst recovery from the leachate is minimal (Mawdsley et al., 1996a). At a gradient of 7.5% however, the movement of *Cryptosporidium* in runoff was demonstrated for at least 21 days and in one case an excess of 70 days from the time of inoculation (Mawdsley et al., 1996b). Although, *Giardia* and *Cryptosporidium* both have been recovered from ground waters, the sources of the pathogens remain unknown (Hancock et al., 1998). It is believed that the primary modes by which parasites like *Giardia* and *Cryptosporidium* are transported to surface water are via the drainage from manure storage areas, direct contact by cows with water, runoff of fields on which manure has been spread and wash from manure-laden soil (Graczyk et al., 2000; Jellison et al., 2002; Sischo et al., 2000; Ong et al., 1996; Duke et al., 1996; Kistemann et al., 2002).

Contaminated irrigation water and manure-amended soils are also sources of transmission of pathogens from agricultural lands. Irrigation waters are likely to become contaminated either by introduction of manure or by surface runoff (Thurston-Enriquez et al., 2002; Francis et al., 1999; Beauchat & Ryu, 1997). Parasites like *Giardia* and *Cryptosporidium* have been isolated from irrigation waters in the United States and Norway and have been subsequently isolated from fruits and vegetables (Thurston-Enriquez et al., 2002; Robertson & Gjerde, 2001). *Listeria* and other pathogenic bacteria have been reported from irrigation waters (Beauchat & Ryu, 1997). In some instances, *Salmonella* and *Ascaris* eggs have been recovered from over half of the manure contaminated irrigated water samples. Only 1 of 97 vegetables irrigated with the water yielded *Salmonella*, but *Ascaris* eggs were isolated from 2 out of 34 vegetable samples (Beauchat & Ryu, 1997).

Application of manure to soil is another way in which bacteria and parasites can contaminate fruits and vegetables. Both *Escherichia coli* O157:H7 and *Cryptosporidium* have been involved in outbreaks linked to unpasteurized apple juice (Cody et al., 1999; Millard et al., 1994; MMWR, 1996). In these situations, the source of contamination for the apples was animal manure (wild deer in orchards). In some instances, the contaminated soil can adhere to produce. *Salmonella* is able to infiltrate tissues of tomatoes during contact with contaminated soil (Guo et al., 2002). Both *Salmonella* and *E. coli* can contaminate

root and leaf vegetables grown in soils amended with bovine manure (Natvig et al., 2002). It has been suggested that non-composted bovine manure applied to soil in the spring, allowing more than 120 days between manure application and vegetable harvest, will ensure that root and leaf vegetables are free from these pathogenic bacteria (Natvig et al., 2002).

3 Survival of the Pathogens in the Environment

To assess the threat posed by different microorganisms in manure, pathogen survival in manure as it is usually handled on farms must be evaluated. Survival of parasitic and bacterial pathogens is dependent on the manure source, temperature, pH, dry matter content, age and chemical composition of the manure as well as the microbial characteristics (Pell, 1997). It is clear that the holding of manure as slurry, as a solid or as compost before it is distributed, results in a significant reduction in pathogen concentration. Most pathogens are also able to survive freezing or low temperatures for extended periods of time. The ability of the aforementioned bacterial and parasitic pathogens to survive in various agricultural environments under diverse conditions will be discussed.

3.1 Bacterial Pathogens

3.1.1 *Salmonella* spp.

Most *Salmonella* needs a temperature of 35°C to 37°C and a neutral pH for optimal growth. Some types of Salmonella need lower or higher temperatures (5°C or 47°C) and lower or higher pH (4.5 or 9.0) for survival. It has been shown to survive freezing and long-term frozen storage as well as drying (Ekperigin & Nagaraja, 1998). In water with a high degree of organic pollution, *Salmonella* survival is restricted to less than 24 hours whereas, in drinking water with a low amount of organic pollution, survival could reach up to 30 days (Pokorny, 1988; Santo Domingo et al., 2000). A standard chlorination as provided in conventional water treatment will eliminate *Salmonella* from water (Sobsey, 1989). *Salmonella* can survive in cow manure slurry for up to 5 weeks and in solid cow manure for up to 3 weeks (Himathongkham et al., 1999a). In poultry manure slurry and poultry manure *Salmonella* can survive up to 2 weeks and 22 weeks, respectively (Himathongkham et al., 2000). Eight-day storage of *Salmonella* in chicken manure with a water activity of 0.89 results in a million-fold reduction of the bacterium (Himathongkham et al., 1999b). Destruction of *Salmonella* in chicken manure is also greatly enhanced by drying manure to 10% moisture followed by the application of 1% ammonia gas (Himathongkham & Riemann, 1999). *Salmonella* can survive for 26 days and 85 days in the solid fraction of pig slurry under summer and winter conditions, respectively (Placha et al., 2001). The longest period of survival of *Salmonella* in liquid pig slurry is 56 days at 4°C (Ajariyakhajorn et al., 1997). *Salmonella* can be detected in the soil for up to 14 days after the application of *Salmonella*-contaminated swine slurry to agricultural soil (Baloda et al., 2001). In other agricultural situations, *Salmonella* can survive for at least 45 days in moist soil (Guo et al., 2002). In composted pig and cattle manure, *Salmonella* survives for a maximum of 48 hours at 45°C (Lung et al., 2001; Forshell & Ekesbo, 1993) .

3.1.2 *Escherichia coli* O157:H7

Although the major cause of *Escherichia coli* O157:H7 infection is fecal contaminated meat, manure may contaminate soil and water leading to waterborne outbreaks of *Escherichia coli* O157:H7 infections (Tauxe, 1997; Slutsker et al., 1998; Tuttle et al., 1999; Chalmers et al., 2000). *Escherichia coli* O157:H7 is fairly environmentally resistant and has extended survival time in water, feces and soil. In water, *E. coli* O157:H7 survival is greatest at 8°C for 91 days and least at 25°C for 49 days (Wang & Doyle, 1998). Similarly, in dairy cattle drinking water, *E. coli* O157:H7 was found to survive better and longer at a colder temperature (5°C) than at a warmer temperature (15°C) (Rice & Johnson, 2000). Chlorine levels typically maintained in water systems will inactivate *E. coli* O157:H7 from drinking water (Rice et al., 1999). *Escherichia coli* O157:H7 survives best in manure incubated without aeration at temperatures below 23°C and survives for at least 100 days in manure frozen at -20°C or in manure incubated at 4 or 10°C for 100 days (Kudva et al., 1998). *E. coli* O157:H7 can survive in a manure pile for 21 months and can persist in a cattle manure slurry for over 3 months (Kudva et al., 1998; McGhee et al., 2001; Himathongkham et al., 1999a; Himathongkham et al., 2000). Destruction of *E. coli* O157:H7 is enhanced by drying the manure to a 10% moisture content followed by exposure to 1% ammonia gas (Himathongkham & Riemann, 1999). In the composting process of cow manure, *Escherichia coli* O157:H7 survives for less

than 72 hours at 45°C (Lung et al., 2001). This bacterium can survive in the soil surface of grazing land for up to 99 days and can persist for more than 200 days in manure-amended soil at 21°C (Bolton et al., 1999; Jiang et al., 2002). *Escherichia coli* O157:H7 is also able to leach through the top layers of soil for more than 2 months after the initial application of manure and/or slurry (Gagliardi & Karns, 2000).

3.1.3 *Campylobacter* spp.

Campylobacter jejuni and *Campylobacter coli* are heat tolerant bacteria that grow best at 42°C, a temperature that is close to that in the intestine of warm-blooded animals. The bacteria are sensitive to stresses including freezing, temperatures below 30°C, drying, acidic conditions (pH ≤ 5.0) and salinity (Altekruse et al., 1998). Campylobacters are frequently identified in raw surface waters and the survival of these bacteria in stream water is extended in cold (6°C) water (Terzieva & McFeters, 1991; Duke et al., 1996; Chynoweth et al., 1998). Formation of bacterial aggregates with other *Campylobacter* species or normal water bacteria also extends the survival time in water (Chynoweth et al., 1998; Thomas et al., 1999). Campylobacters have been associated with several outbreaks from drinking water (Jones & Roworth, 1996; Pebody et al., 1997; Sobsey, 1989). *Campylobacter* spp. are sensitive to chlorine; a standard chlorination procedure will eliminate the organisms (Sobsey, 1989). In 4°C cattle feces, *Campylobacter jejuni* is able to survive for a maximum of 20 days (Valdes-Dapena Vivanco & Adam, 1983). It has a much longer endurance in mesophilic anaerobic digestion for it takes 438 days to reduce the population by 90% (Kearney et al., 1993a).

3.1.4 *Listeria monocytogenes*

Some pathogenic bacteria like *Listeria monocytogenes* are able to grow under a wide range of conditions; thus, when dispersed in the environment their control is difficult (Pell, 1997). In addition to having a wide host range, *Listeria monocytogenes* can live naturally in plant and soil environments and in poorly fermented silage (Driehuis & Oude Elferink, 2000). The ability of this bacterium to grow at a wide range of temperatures (3 to 42°C) and pH (≤ 5.5 to 9.0) and in high (up to 12%) salt concentrations makes control difficult (Cooper & Walker, 1998). *Listeria monocytogenes* is also resistant to environmental influences like freezing, thawing, desiccation and high temperatures including short-time pasteurization of milk at 71.7°C for 15 seconds or 62.8°C for 30 minutes (Cooper & Walker, 1998).

Listeria monocytogenes has been detected in manure three weeks after storage; however, two months after inoculation of stored liquid pig manure, stored liquid cattle manure and soil with *L. monocytogenes*, the bacterium could not be traced in any of the environments (Van Renterghem et al., 1991). One similar study found that *L. monocytogenes* survival is much like other bacteria, declining more rapidly in 17°C beef cattle slurry than at 4°C beef cattle slurry (Kearney et al., 1993b). On the other hand, time to reduce *Listeria monocytogenes* by 90% was 37.5 days in anaerobic semi-continuous digestion and 12.3 days in anaerobic batch digestion which is significantly higher than the 90% reduction time of *Escherichia coli, Salmonella typhimurium*, and *Yersinia enterocolitica* (Kearney et al., 1993b). In chicken manure, *Listeria monocytogenes* is able to grow for 2 days at 20°C (Himathongkham & Riemann, 1999). Destruction of *L. monocytogenes* is greatly enhanced by the drying of the manure to 10% moisture content and exposure to ammonia gas (Himathongkham & Riemann, 1999). Exposure of *L. monocytogenes* to chlorinated 4°C water is detrimental to the survival of the bacteria; however, exposure to 47°C water favors the growth when subsequently stored at 10°C (Delaquis et al., 2002).

3.1.5 *Yersinia enterocolitica*

Yersinia enterocolitica is a cold-loving bacterium that can grow at temperatures from 0°C to 45°C with optimal growth at 20-30°. It is able to multiply at low temperatures (2-4°C) that are usually used to store perishable food items (Ganeshkumar & Singh, 1994). Duration of *Yersinia enterocolitica* survival in beef cattle slurry is temperature dependent with the bacterium surviving for 12.8 days at 17°C and 20.8 days at 4°C. Under conditions of anaerobic digestion the time to reduce *Yersinia enterocolitica* by 90%, ranged from 0.7 days during batch digestion to 2.5 days during semi-continuous digestion (Kearney et al., 1993b). In agricultural surface water *Yersinia enterocolitica* is able survive for more than 4 days at both 6 and 16°C water (Terzieva & McFeters, 1991). *Yersinia enterocolitica* O:3 disappears rapidly in natural soil at 4°C and 20°C and from river water at 20°C (Tashiro et al., 1991).

3.1.6 *Mycobacterium* spp.

Mycobacterium paratuberculosis like *Listeria monocytogenes* is able to grow under a wide variety of conditions and is ubiquitous the environment (Pell, 1997). In cattle and swine slurry and a mixture of equal parts of both stored under anaerobic conditions at 5°C and 15°C, *Mycobacterium paratuberculosis* can survive at 5°C for 252 days in all 3 types of slurry (Jorgensen, 1977). At 15°C, *Mycobacterium paratuberculosis* can survive for 98 days in cattle slurry, 182 days in pig slurry and 168 days in mixed slurry (Jorgensen, 1977). The survival of this bacteria in soil appears to be related to soil type and the prevalence of *M. paratuberculosis* in cattle. This association has been positively correlated with acidic soil and increased soil iron content (Johnson-Ifearulundu & Kaneene, 1997; Johnson-Ifearulundu & Kaneene, 1999). The stamina of *M. paratuberculosis* in surface water has been tested under standard water treatment processes with various chlorine concentrations (0.5, 1.0, 2.0 microg/ml) and two contact times (15 and 30 minutes). *Mycobacterium paratuberculosis* cannot be killed at the applied chlorine concentrations and contact times (Whan et al., 2001).

Mycobacterium bovis that is shed in the feces of infected cattle may be capable of surviving for 176 days at 5°C in stored cattle slurry (Scanlon & Quinn, 2000). However, it has been indicated that *M. bovis* can survive for 4 weeks in non-sterile dry and moist soils held under 80% shade, in the darkness and in the laboratory (Duffield & Young, 1985). In this same study, *M. bovis* was not recovered after 4 weeks in dry or moist soils exposed to sunlight or from cattle feces held under any conditions. *Mycobacterium bovis* was not isolated from any substrate at 8 weeks or up to 32 weeks after inoculation (Duffield & Young, 1985).

3.2 Parasitic Pathogens

3.2.1 *Giardia lamblia*

The environmentally resistant form of *Giardia lamblia* is the cyst. *Giardia* cysts are non-infective in water, cattle feces, and soil following 1 week of freezing at -4°C and within one week at 25°C. At 4°C *Giardia* cysts are infective for 11 weeks in water, 7 weeks in soil and 1 week in cattle feces (Olson et al., 1999). *Giardia* cysts have been shown to be viable for up to 84 days in cold river and lake water (deReginer et al., 1989). *Giardia* cyst concentrations can be lower in chlorinated than raw water (Isaac-Renton et al., 1996). Anaerobic and primary wastewater treatment can be used to reduce *Giardia* cyst counts by as much as 76%; however anaerobic sludge digestion is ineffective at reducing *Giardia* cyst numbers (Chauret et al., 1999; Hu et al., 1996; Payment et al., 2000). Soil amendment is necessary to reduce *Giardia* levels in anaerobically digested sludge (Hu et al., 1996). In mixed human and swine manure slurry, the survival of *Giardia* cysts is temperature dependent; 5°C cysts survive for more than 156 days while cysts at 25°C survive for less than week (Deng & Cliver, 1992).

3.2.2 *Cryptosporidium parvum*

Cryptosporidium parvum oocysts are more environmentally resistant than *Giardia* cysts. At -4°C and 4°C the oocysts survive in soil, water and feces for more than 12 weeks with degradation of oocysts accelerating in these environments at 25°C (Olson et al., 1999). In cattle manure piles, that reach and maintain temperatures between 35 and 50°C, oocyst infectivity declines significantly within 70 days (Jenkins et al., 1999). Thermophilic aerobic digestion and sludge pasteurization at 55°C are effective treatments to inactivate *Cryptosporidium* oocysts (Whitmore & Robertson, 1995). *Cryptosporidium* oocysts can be inactivated by freezing at -70°C for 1 hour and -20°C for 24 hours but remain viable for up to 8 weeks when stored at -5°C (Fayer et al., 1996; Fayer et al., 1998). On the other end of the spectra, *Cryptosporidium* loses its infectivity by heating at 55°C for 30 seconds, 60°C for 15 seconds and 70°C for 5 seconds (Fujino et al., 2002; Fayer, 1994). *Cryptosporidium* oocysts are strongly resistant to most of the commonly used disinfectants and chlorination of drinking water is not sufficient to prevent an infection (Fayer, 1997). *Cryptosporidium* oocysts are also able to endure the silage fermentation process (Merry et al., 1997).

3.2.3 *Ascaris suum*

Due to its thick and resistant shell, which protects against adverse environmental conditions such as desiccation and chemicals, *Ascaris* may survive in the environment for up to 7 years (Roepstroff & Nansen, 1998; Ghiglietti et al., 1995). Under optimal environmental conditions of temperature and humidity, the eggs can become infective within 1-3 months (Roepstroff & Nansen, 1998). *Ascaris suum* egg degradation is faster in the summer than in the winter and when placed on short grass than when buried in soil (Larsen & Roepstroff, 1999). *Ascaris suum* can survive for 2 to 4 weeks in dry conditions while under a moist cool environment they can survive for over eight weeks (Gaasenbeek & Borgsteede, 1998). In stored pig slurry, *Ascaris* eggs are no longer viable beyond 16 weeks; however, 20 days of anaerobic mesophilic digestion of *Ascaris* contaminated pig slurry has little effect on viability (Gaasenbeek & Borgsteede, 1998; Juris et al., 1996; Johnson et al., 1998). After 33 months of storage in 4°C sludge, *Ascaris* eggs remain viable and infective, whereas most *Ascaris* eggs stored at 25°C are rendered nonviable at 12 months (O'Donnell et al., 1984). In compost, *Ascaris* eggs can survive for 3 weeks at 30°C but are destroyed at 37°C for 31 days (Tharaldsen & Helle, 1989).

4 Human Health Effects and Risk Factors

Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from the exposure to a hazard, in this case a bacterial or parasitic pathogen. Some of the important information to be including in risk assessments are: the pathogenicity of the bacteria or parasite including disease incidence and severity in humans, the route of infection, the infectious dose, the prevalence and/or incidence of the pathogen in food and/or water, predisposing health risks and preventative measures (FAO/WHO, 1995). Sufficient information on risk factors is required to provide a reliable risk assessment. This is not possible with the current state of knowledge of manure pathogens but the risk factors of each of the zoonotic pathogens will be discussed below.

4.1 Bacterial Pathogens

4.1.1 *Salmonella* spp.

Most *Salmonella* infections do not occur in recognized outbreaks, rather as sporadic infections (Olsen et al., 2000). *Salmonella* can be found in or on a wide variety of foods from animal origin as well as fruits and vegetables and water (Olsen et al., 2001).

Salmonellosis causes an acute gastroenteritis, acute infectious disease with the sudden onset of abdominal pain, diarrhea, nausea and vomiting. Dehydration may be severe in infants and the elderly. Salmonellosis may progress to more serious septicemia, including focal infections, abscesses, endocarditis, pneumonia; it may also cause typhoid like enteric fever; some cases develop reactive arthritis which may become chronic. *Salmonella* has an incidence rate of 18 cases per 100,000 population annually in the United States (Swartz, 2002). Deaths associated with salmonellosis are approximately 600 per year (Mead et al., 1999b; Olsen et al., 2001). Risk factors include age such as the very young or very old or debilitation and immunosuppression (Mead et al., 1999b).

The minimum infectious dose for many pathogens including *Salmonella* depends on factors like the virulence of the bacterium, the type and amount of food/water consumed, the levels of the bacterium in the food/water and the health status of the host (Farber et al., 1996). The infective dose of *Salmonella* for humans ranges from 10 to 1000 organisms (Blaser & Newman, 1982).

Occurrence of *Salmonella* in food varies and depends on the type of food. The actual occurrence of *Salmonella* in eggs is unknown, but the incidence of salmonellosis from ingesting raw or undercooked eggs is 2.2 cases per 100,000 (MMWR, 2000). The prevalence of *Salmonella* on fresh retail meats can range from a low of 2% on beef to a high of 4% on chicken (Zhao et al., 2001; Zhao et al., 2002). In Alberta, the prevalence of *Salmonella* spp. in ground beef is 1.3% (Sorensen et al., 2002). *Salmonella* has also been found on a variety of different produce (Ooi et al., 1997). The bacterium has been a source of several outbreaks involving fresh sprouts (Mohle-Boetani et al., 2001; Mahon et al., 1997; van Duynhoven et al., 2002; Taormina et al., 1999). Fresh tomatoes have also been a source of several outbreaks salmonellosis (Hedberg et al., 1999). Both watermelons and cantaloupes have also been sources of *Salmonella* outbreaks (Mohle-Boetani et al., 1999; Guo et al.,

2002). Waterborne outbreaks of *Salmonella* can also be associated with fecal contamination of the water supply (Kramer et al., 1996; Angulo et al., 1997). In 2000 and 2001, *Salmonella* was isolated from 6.7% (54/802) and 12.7% (104/822) raw river and irrigation water collected from the Oldman River in Southern Alberta, respectively (Gannon, 2002). The most common serotype isolated, *S. rublislaw,* is rarely reported in animals and humans in Alberta. The source of the *Salmonella* has yet to be determined.

The presence of *Salmonella* on a wide variety of food items suggests that the individuals at greatest risks are the young, elder and the otherwise immunocompromised. Preventative measures can be to properly wash all produce that may have been in contact with manure or manure-amended soil, to avoid eating raw or undercooked meat or eggs, and to prevent cross-contamination of foods (Zhao et al., 2001; MMWR, 2000; Beauchat & Ryu, 1997).

4.1.2 *Escherichia coli* O157:H7

Outbreaks of *Escherichia coli* O157:H7 can be both foodborne and waterborne and are a result of fecal contamination. Foodborne outbreaks have been associated between *E. coli* O157:H7 and meat; this was first illustrated with the consumption of undercooked ground beef and has subsequently been linked to other food products and untreated water (Riley et al., 1983; Bell et al., 1994; Rice, 1992; Olsen et al., 2002; Tauxe, 1997).

Symptoms of *E. coli* O157:H7 infection range from mild to severe bloody diarrhea (Sussman, 1997). Infections can cause serious disease and death in both children and the elderly. In severe cases usually involving children, hemolytic uremic syndrome and kidney failure can result. Occasionally, in adults the infection can progress to thrombotic thrombocytopenic purpura, a condition like hemolytic uremic syndrome but with neurological complications (Sussman, 1997). In the United States, the prevalence of *E. coli* O157:H7 in humans is around 0.4% (Slutsker et al., 1997).

The infective dose for *E. coli* O157:H7 is low and ranges from 4 to 24 organisms, which allows water to act as an efficient vector (Strachan et al., 2001; Olsen et al., 2002). One third of the reported *E. coli* O157:H7 waterborne outbreaks reported in the United States are caused by contaminated drinking water (Olsen et al., 2002). Many of these outbreaks are associated with small drinking water systems that may be less likely to adequately chlorinate and to do routine monitoring for contamination (Olsen et al., 2002). In 2000 and 2001, *E. coli* O157:H7 was isolated from 1.3% (11/802) and 1.9% (16/822) raw river and irrigation water samples collected from the Oldman River in Southern Alberta (Gannon, 2002). The source of the *E. coli* O157:H7 was believed to be cattle but it was not established.

Traditionally foodborne outbreaks have been associated with contaminated ground beef; more recently, some foodborne outbreaks been associated with manure contamination of produce (Mohle-Boetani et al., 2001; Hilborn et al., 1999; Ackers et al., 1998; Breuer et al., 2001; Riley et al., 1983; Bell et al., 1994). The prevalence of pathogenic *E. coli* O157:H7 from fresh retail chicken, turkey, beef, and pork ranges from 12 to 39% (Zhao et al., 2001). It is suggested that raw retail meats and produce may be vehicles for transmitting foodborne diseases like *E. coli* O157:H7. The consumption of unwashed produce and undercooked meat products and cross-contamination during food handling must be avoided (Zhao et al., 2001; Thunberg et al., 2002).

4.1.3 *Campylobacter* spp.

Campylobacter has been associated with outbreaks of both foodborne and waterborne campylobacteriosis (Nachamkin & Blaser, 2000). Most outbreaks are associated with raw milk or surface water, whereas sporadic illnesses are often associated with mishandling and consumption of undercooked poultry or cross-contamination of foods by raw poultry (Nachamkin & Blaser, 2000; Altekruse et al., 1998; Tauxe, 1997).

Campylobacter enteritis is a self-limited diarrheal disease with abdominal pain, fever and general malaise (Nachamkin & Blaser, 2000). Reactive arthritis is a benign sequela of campylobacteriosis that occurs in about 1% of the population. Pain and incapacitation may last for months (Altekruse et al., 1998). Guilain-Barre Syndrome is a more severe post-infection sequela. It is a neurological syndrome that occurs once in every 1000 cases of campylobacteriosis (Altekruse et al., 1998; Nachamkin & Blaser, 2000). Early symptoms are burning sensations and numbness that can progress to flaccid paralysis.

Campylobacteriosis has a high incidence rate of 22 cases per 100,000 adult population, however incidence rate is even greater in children under 1 year at 53 per 100,000 population (Nachamkin & Blaser, 2000). Mortality rates in developed countries are 2-3%,

but can be as high as 15% in developing nations (Nachamkin & Blaser, 2000; Altekruse et al., 1998). Fatal outcomes are rare and usually confined to elderly patients or those already suffering from another serious disease. Major risk factors for campylobacteriosis are age and immunosuppression (Nachamkin & Blaser, 2000).

The infectious dose for *Campylobacter jejuni* in humans is low ranging from 500 to 800 organisms (Black et al., 1988; Robinson, 1981). Of course this is dependant on factors like the virulence of the bacterium, the type of food/water consumed, the levels of *Campylobacter* in the food/water and the health of the host (Farber et al., 1996).

Since *Campylobacter* is susceptible to chlorine, waterborne outbreaks have occurred in municipal water systems as a result of a break in chlorination or when a non-chlorinated groundwater supply becomes fecal contaminated (Nachamkin & Blaser, 2000; Center for Disease Control and Prevention, 1999). Among foodborne outbreaks raw unpasteurized milk is the most common source of foodborne outbreaks. The milk is usually contaminated with cattle feces (Nachamkin & Blaser, 2000; Center for Disease Control and Prevention, 2002). A broad variety of other foods have been implicated in outbreaks and related to cross-contamination events in the kitchen, raw meats, particularly poultry, to a variety of other foods (Nachamkin & Blaser, 2000; Zhao et al., 2001). The prevalence of *Campylobacter* spp. on raw retail meats can range from < 1% on beef samples to as high as 71% of chicken samples (Zhao et al., 2001).

4.1.4 *Listeria monocytogenes*

In humans, outbreaks of listeriosis have been foodborne, linked to contaminated cheeses and processed meats (Cooper & Walker, 1998; Donnelly, 2001; Farber et al., 1996). *Listeria monocytogenes* can be found in or on a wide variety of foods including fresh produce (Farber & Peterkin, 1991; Norrung et al., 1999; Thunberg et al., 2002).

Clinical symptoms of listeriosis in humans are usually flu-like symptoms including diarrhea, however, encephalitis, abortion and stillbirth can be associated with infection (Cooper & Walker, 1998). Listeriosis occurs infrequently, between 2 to 7 cases per million population, and between 20 to 40% of the cases are fatal (Farber et al., 1996). Major risk factors include immunosuppression, pregnancy, and age (Farber et al., 1996). With the broad-based prevalence in the food system, together with the high mortality rate of listeriosis, implies that *L. monocytogenes* represents an important, emerging hazard to human health.

The potential effects of exposure to *L. monocytogenes* are wide ranging in severity; listeriosis is the common precursor. Efforts at hazard characterization have concentrated on establishing a dose response model, however there is no experimental dose response data on humans available for *Listeria*. Therefore the minimum infectious dose (MID) for *L. monocytogenes* for humans is unknown (Farber et al., 1996). The minimum infectious dose for many pathogens including *L. monocytogenes* depends on factors like the virulence of the strain, the type and amount of food/water consumed, the levels of the organism in the food or water and the state of the host health (Farber et al., 1996). For normal individuals an estimated infectious dose of *L. monocytogenes* is 107 to 109 and in high-risk people 105 to 107 (Farber et al., 1996; Farber & Peterkin, 1991).

The average incidence data for *L. monocytogenes* is 4.4 and 1.2 % for meats and dairy products, respectively. The levels of *L. monocytogenes* present in pate and soft cheese can be as high as 106 to 107 organisms per gram (Farber et al., 1996; Farber & Peterkin, 1991). *Listeria* has been found on a varietyof different produce from both supermarkets and farmers markets. *Listeria monocytogenes* was found on 18% of the field cress samples and 50% of the potatoes sampled (Thunberg et al., 2002). It is not unusual to find *Listeria* on fresh produce, for it is widespread in nature and has been isolated from manure-fertilized soil and vegetation. Since *Listeria* have been shown to survive and propagate on fresh vegetables their presence may pose a public health risk (Farber et al., 1989; Carlin et al., 1995).

4.1.5 *Yersinia enterocolitica*

Outbreaks of yersiniosis have been linked to both foodborne and waterborne. The risk of yersiniosis is increased by contact with untreated water and consumption of contaminated pork (Satterthwaite et al., 1999). Yersiniosis has also been linked to the consumption to raw unpasteurized milk (Ganeshkumar & Singh, 1994; Ackers et al., 2000).

Infection with *Y. enterocolitica* can cause a variety of symptoms depending on the age of the person infected. Infection with *Y. enterocolitica* occurs most

often in young children (Ganeshkumar & Singh, 1994; Bottone, 1997). Common symptoms in children are fever, abdominal pain, and diarrhea, which is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms, and may be confused with appendicitis (Ganeshkumar & Singh, 1994; Bottone, 1997; Bottone, 1999). In a small proportion of cases between the ages of 20-60, complications such as skin rash, joint pains, or spread of bacteria to the bloodstream can occur (Swaminathan et al., 1982). In the United States, approximately 1 per 100,000 persons confirmed *Y. enterocolitica* infection occurs each year. Mortality related to *Yersinia enterocolitica* infection is <1% of all cases (Mead et al., 1999a; Swartz, 2002). Children are infected more often than adults, and immunosuppression is a risk factor (Bottone, 1997; Bottone, 1999).

The minimal infective dose of *Yersinia enterocolitica* is around 106 organisms (Bottone, 1997). The incidence of *Yersinia enterocolitica* in pork can range from 25% to 92%, depending on the type of pork product sampled (Fredriksson-Ahomaa et al., 1999; Nielsen & Wegener, 1997). The prevalence of *Yersinia enterocolitica* in raw surface waters and on produce has not been established. The prevalence of *Yersinia enterocolitica* in bulk tank milk is approximately 6% (Jayarao & Henning, 2001). Due to the high incidence of *Y. enterocolitica* on pork and the persistence of the organism in raw milk and surface waters, precautions should be taken to avoid eating raw or undercooked pork and to consume only pasteurized milk or milk products and chlorinated water. Cross-contamination of foods can be prevented by using separate cutting boards and thorough cleaning of implements used with meat (Bottone, 1997).

4.1.6 *Mycobacterium* spp.

There have been no reported foodborne or waterborne outbreaks of *Mycobacterium bovis* or *Mycobacterium paratuberculosis* in humans. As stated previously, *Mycobacterium bovis* is a reportable disease in Canada and this country is considered free of this pathogen in domestic livestock.

The main source of *Mycobacterium bovis* infection for humans is through the consumption of unpasteurized milk and milk products or inhalation of aerosols (Ashford et al., 2001; Wedlock et al., 2002). However, *M. bovis* can survive in the soil, on fomites, and in feces for days to months and may be considered an source of infection for humans (Ashford et al., 2001). The infectious dose for *M. bovis* is only 10 organisms. Tuberculosis caused by *M. bovis* is indistinguishable, clinically, radiologically and pathologically from disease caused by *M. tuberculosis*, a human pathogen that can cause tuberculosis. The true incidence of and prevalence of human disease caused by *M. bovis* are unknown. However, it is estimated that in Europe and North America that 0.5 to 1.0 % of human tuberculosis is a result of *M. bovis* (Ashford et al., 2001). The lack of information on the prevalence of *M. bovis* infection in humans makes the gauging the weight of the zoonotic potential of bovine tuberculosis difficult. However, bovine tuberculosis should be considered potential threat to human health in endemic areas of the world, especially to those who work in close contact with cattle and to immunosuppressed individuals (Wedlock et al., 2002; Ashford et al., 2001).

Paratuberculosis or Johne's disease in cattle results from an infection with *Mycobacterium paratuberculosis*. The main route of transmission of the bacterium is through the ingestion of contaminated milk or milk products and through fecal contamination of food and/ or water (Collins, 1997; Whittington & Sergeant, 2001). Paratuberculosis is present worldwide; however, its actual prevalence in humans is unknown (Bakker et al., 2000). *Mycobacteria*, in particular, *M. paratuberculosis* is one of several microbial agents implicated in Crohn's disease. Crohn's disease is a chronic inflammatory disease of the small intestine affecting people during their teens and twenties. Patients usually suffer from diarrhea, weight loss, and abdominal pain (Chiodini & Rossiter, 1996; Selby, 2000). The prevalence of Crohn's disease is 10-50 per 100,000 people with 1-5 new case per 100,000 population per year (Selby, 2000). Like *M. bovis* the lack of information regarding the prevalence *M. paratuberculosis* and the true etiologic agent involved in Crohn's disease makes it difficult to determine the health risks involved with the organism. Possible health risks are to those in close contact with cattle feces and to immunosuppressed individuals but this is yet to be established.

4.2 Parasitic Pathogens

4.2.1 *Cryptosporidium parvum*

Outbreaks of *Cryptosporidium* can be both waterborne and foodborne; however, waterborne transmission of this parasite is more common (Slifko et al., 2000; Rose & Slifko, 1999). The foodborne infections are associated with the use of contaminated water in food processing. The disease is called cryptosporidiosis with symptoms of 7 to 14 days of watery diarrhea, fever and muscle aches. The incidence of cryptosporidiosis in the population has been reported to range from 0.6 to 20%, depending on the geographic locale. Incidences of cryptosporidiosis are much higher in developing countries while cryptosporidiosis is associated with 0.4 to 1% of cases of diarrhea in the United States (Rose & Slifko, 1999).

Cryptosporidium is of particular concern for several reasons; the oocysts are extremely resistant to disinfection and cannot be killed with routine water-disinfection procedures, there is no treatment for the disease, the risk of mortality ranges from 50 to 60% in the immunocompromised population, and animal fecal wastes are associated with the transmission of the disease to humans (Rose & Slifko, 1999; Rose, 1997).

The median infectious dose of *Cryptosporidium* in healthy human volunteers is 132 oocysts; however one oocyst carries the probability of causing an infection (Dupont et al., 1995; Haas et al., 1996). Subgroups in the human population with different susceptibilities to cryptosporidiosis include the young, elderly, malnourished, disease impaired (i.e. those with diabetes), and a broad category of immunocompromised individuals (AIDS patients, transplant recipients, and those on chemotherapy) could become infected with lower dose of oocysts (Rose, 1997). *Cryptosporidium* appears to be much more severe in the immunocompromised population than in the other groups, probably because of impaired T-cell functions. The duration and the severity of the disease are significant: whereas 1% of the immunocompetent population may be hospitalized with very little risk of mortality (<0.001%), *Cryptosporidium* infections are associated with a high rate of mortality in the immunocompromised (50%) (Rose, 1997).

Cryptosporidium oocysts are commonly found in surface waters with a range of 0.1 to 10000 oocysts per 100 liters in 4 to 100% of samples examined, depending on the impact of sewage works and domestic livestock (Rose & Slifko, 1999). *Cryptosporidium* oocysts can be found in 17 to 27% of treated drinking water samples at densities of 0.005 to 0.017 oocysts per liter (Smith & Rose, 1998). Currently, between 9.5 and 22% of groundwater samples in the United States are positive for *Cryptosporidium* (Hancock et al., 1998). In the United States, an average of 19 *Cryptosporidium* oocysts per 100 liters were found in irrigational water used for crops traditionally eaten raw, suggesting that there may be a risk of infection to consumers who come in contact with or eat these products (Beauchat & Ryu, 1997). The number of cases of human cryptosporidiosis associated with foodborne or waterborne transmission from cattle manure is unknown as the molecular epidemiology tools to address this question have only been recently developed and applied.

4.2.2 *Giardia lamblia*

Similar to *Cryptosporidium, Giardia* can be both foodborne and waterborne; however, foodborne transmission is not as well documented as waterborne giardiasis. It is estimated that 60% of all *Giardia* cases are associated with fecal-contaminated water (Bennett et al., 1987). The most common symptoms of giardiasis are diarrhea followed by flatulence, foul-smelling stools and cramps. An acute stage can last from a few days to a week or on rare occasions, months. A chronic stage may exhibit itself with milder symptoms, sporadic episodes and weight loss and may last 10 days or more, rarely lasting up to a year (Rose & Slifko, 1999). Giardiasis infects approximately 2% of the adults and 6 to 8% of the children in developed countries worldwide, as with *Cryptosporidium*, infection levels are higher in developing nations (Gardner & Hill, 2001; Upcroft & Upcroft, 2001). Unlike cryptosporidiosis, giardiasis is treatable with as few as 10 cysts establishing an infection (Gardner & Hill, 2001). Like cryptosporidiosis, giardiasis is more of a health risk in the young, elderly and immunocompromised individuals (Gardner & Hill, 2001; Nash, 2001; Mank & Zaat, 2001).

Average levels of *Giardia* cyst contamination in surface waters and pristine watersheds ranges from 0.33 to 104 cysts per liter and 0.6 to 5 cysts per liter, respectively (Rose et al., 1991). Similar levels of *Giardia* cysts as *Cryptosporidium* oocysts can be found in groundwater (Rose & Slifko, 1999; Hancock et al.,

1998). *Giardia* has been isolated in as many as 18% of treated drinking water samples in Canada (Wallis et al., 1996). In the United States, an average of 25 *Giardia* cysts per 100 liters irrigation water was detected (Thurston-Enriquez et al., 2002) but the source of contamination was not identified. Agricultural products like raw fruits and vegetables have the potential to be contaminated through the application of contaminated irrigation waters or manure (Rose & Slifko, 1999).

Certain genotypes are clearly restricted to livestock while others are found in both humans and livestock. The molecular method needed to trace human infection to manure contaminated food or water has not yet been developed.

4.2.3 *Ascaris suum*

Ascaris suum has not been associated with waterborne or foodborne outbreaks of ascariasis. Transmission of *Ascaris suum* is depends on the ingestion of eggs containing infective larvae while human contact with these eggs depends on their degree of accessibility in a contaminated environment (Crompton, 2001). It is currently believed that there are relatively few infections of pig *Ascaris* occurring even where the pig populations are high. The prevalence of ascariasisin pig farming communities and non pig farm communities is similar (Peng & Zhou, 2001). Possible risks for acquiring ascariasis are contact with pig manure, soil contaminated by pig manure and the consumption of improperly cleaned produce grown in soil amended with pig manure (Crompton, 2001).

5 Antimicrobial Resistant Bacteria in Manure

5.1 Introduction

Exposure of bacteria to antimicrobial drugs may lead to the development of antimicrobial resistance. Antimicrobial resistance may also result through the interaction of resistant microorganisms with sensitive bacteria. This is accomplished through the transfer of genetic material that encodes for antibiotic resistance. Antibiotics are used in food animals primarily to treat or prevent diseases but they may also be used to enhance growth or milk production. The gastrointestinal tract of animals is composed of complex microbial communities that may or may not contain animal or human pathogens. The use of antibiotics can influence the microbial ecology by selection of resistance strains or elimination of natural microbial population and allowing fecal pathogens to predominate. Scientists, government regulatory bodies, and the general public are now closely examining the use of antibiotics in animal agriculture. The major issue is the possibility that antibiotic-resistant bacteria may be transferred from livestock to humans. This transfer may be accomplished through exposure to manure or contaminated water. It may also result from food products fertilized with manure (e.g. sprouts).

5.2 Mechanisms of Development of Antibiotic Resistance

Bacteria possess a number of known resistance mechanisms that enable them to avoid the lethal effects of antibiotics and even biocides. Common mechanisms include 1) the production of enzymes that degrade the antibiotic (e.g. Beta lactamase hydrolysis and penicillin resistance), 2) antibiotic efflux pumps which pump the antibiotic from the cell before it can interfere with the cellular processes (e.g. S*almonella* resistance to florfenicol), 3) production of enzymes which inactivate the antibiotic through alterations in chemical structure (e.g. phosphorylation of erythromycin), 4) development of alternative metabolic reactions to bypass the lethal effects of the antibiotic (e.g. trimethoprim resistance), and 5) overproduction of target metabolic products to overwhelm the antimicrobial that has been administered (e.g. sulfonamide resistance).

Bacteria may also develop resistance to antibiotics or biocides simply by forming biofilms. Microbial biofilms are microorganisms attached to a surface encased in a secreted exopolysaccharide. Most bacteria have inherent resistance as biofilms by production of exopolysaccharide coatings and by alteration in gene expression (phenotype change) when they attach to a surface.

5.3 Presence of Antimicrobial Resistant Bacteria in the Environment

Bacteria are continuously evolving in the environment. They have very short life cycles and reach exceedingly high concentrations in manure and soil (10 billion per gram). Soil microorganisms have evolved in an environment where antibiotics are naturally produced by bacteria and fungi. As a result many environmental bacterial isolates have multiple antibiotic resistance. Fortunately these isolates are usually not pathogenic to food animals or humans. However, transmission of antibiotic resistance from environmental organisms to pathogens in manure is potentially feasible. Transmission of antibiotic resistance between different microbial species has been accomplished in the laboratory but has not been demonstrated between soil and manure pathogens.

5.4 Transmission of Antibiotic Resistance in Manure

Bacteria have developed several mechanisms of exchanging genetic material that codes for an antimicrobial resistance trait (Levy 1992). Resistance genes are frequently carried on plasmids that are loops of DNA. Plasmids under some circumstances readily undergo intra- and inter-species transfer thereby permitting antibiotic resistance to be transmitted among microorganisms (Licht et al., 1999). Bacteria can also become infected with viruses (bacteriophages) that may incorporate resistance genes from the host bacteria and transfer these resistance genes to other bacteria. Bacteria can integrate portions of DNA from the environment. Environmental DNA may result from dead bacterial cells or viruses. These segments of genetic material may have specialized properties that promote chromosomal integration into the bacteria leading to families of resistant genes (Bass et al. 1999). However antibiotic resistant bacteria have been identified in liquid and solid manure from cattle and swine (Whitehead and Cotta, 2001). These authors suggest that these environments may serve as reservoirs of antibiotic resistant genes. Further work needs to be done in this area as it probably will be the most important human health issue in the future.

5.5 Effect of Antibiotics on Pathogen Load in Manure

There are numerous reports of isolation of antibiotic resistant bacteria from livestock but most of the reports and controlled studies have been focused on poultry and swine. The influence of the use antibiotics on excretion of antibiotic resistant and antibiotic sensitive pathogens in manure has been reviewed by the Center for Veterinary Medicine (Anonymous, 2000). They reviewed both challenge studies as well as observational studies in poultry, swine and calves.

The pathogens examined were those that are potentially infective to humans. Challenge studies in swine and calves did not demonstrate any evidence that antibiotic use could increase pathogen load in swine. However, the use of avoparcin was demonstrated in several poultry challenge studies to enhance the levels of *Salmonella* shedding. The use of other antibiotics such as virginiamycin, nosheptide, flavophospholipol and salinomycin demonstrated no increase in pathogen load in poultry (Anonymous, 2000).

With the exception of penicillin, observational studies found little evidence to support the hypothesis that antibiotics added to animal feeds affected pathogen load. Penicillin and possibly erythromycin added to feed increased *Salmonella* and total coliform shedding in poultry.

5.6 Effect of Antibiotics on Antibiotic Resistant Pathogens in Manure

There are studies that indicate that short-term and long-term antibiotic use in animals contributes to the development of antibiotic resistant bacteria in manure. This is not surprising, as it is well known that antibiotics exert selective pressure on bacteria wherever they are used. The use of antibiotics will select for antimicrobial resistant microorganisms in

the feces of animals but the microbial ecology of fecal microorganisms is restored once the antibiotic use is terminated. Several studies have recently been completed to determine the role of antibiotic use and antibiotic resistance in pig, poultry and hog manure. One study is looking at antibiotic resistance in humans working in feedlots where short-term and long-term antibiotic use is routinely performed.

5.7 Antibiotic Resistance and Human Health

There is evidence that antibiotic use on the farm can lead to colonization of humans with antimicrobial resistant pathogens (Simonsen et al. 1998). Antimicrobial resistant *Salmonella* in humans has been traced to farms, and contaminated manure is potentially the source of infection (McAllister et al. 2001). Vancomycin-resistant enterococci (VRE) are emerging as a global threat to public health, and VRE's have been isolated from poultry and swine (Aarestrup et al. 2000, Borgen et al. 2000). VRE has not been isolated from cattle. Indeed, the transmission of antibiotic resistant pathogens from animal manure to humans is a pubic heath concern. There is yet little information to support or refute these concerns. The use of molecular epidemiology would assist in resolving some issues. The careful selection of antibiotics for use in food animals will reduce the potential for humans to become infected with a pathogen with resistance to last resort antibiotics used in humans.

6 Gaps and Future Research

6.1 Alberta Information

Although there has been considerable research in pathogens in manure, there is a need for information on human pathogens that can be found in manure. Most information described in this report is derived from studies conducted outside of the province. The survival and transmission of pathogens in manure are influenced by the environment (e.g. temperature, pH, light). Published information on pathogen survival may not be transferable to the Alberta situation. Studies need to be conducted in laboratory simulated or natural situations using Alberta environmental conditions.

6.2 Human Infections

Although many human infections are reportable to public health officials (e.g. *Salmonella, Listeria, Giardia, Cryptosporidium*) some are not reported (*Ascaris, Campylobacter*). When attempting to interpret the impact of animal manure on human health it is necessary to identify pathogens in human infections and the direct association with exposure to animal pathogens in manure. This information is not yet available. Available information exists within the medical records of the province and municipalities. Comparing high-risk populations (farmers) with low risk populations (urban dwellers) is another way for determining infection pathways. By using fingerprinting techniques like pulse field electrophoresis infectious organisms can be followed from animals to humans.

6.3 Animal Infections

Surveys of human pathogens in Alberta livestock and livestock manure have been conducted using largely traditional methods of fecal analysis. Some of the analytical methods may not have been sensitive enough to detect pathogens of low abundance. Fecal pathogens in wildlife are poorly studied. Waterborne infections from wildlife sources have been documented, and food animal manure may be falsely associated with such infections. Wildlife fecal pathogens need to be identified and fingerprinted to identify wildlife infection pathways.

6.4 Antibiotic Resistance

Antibiotic resistance in humans and domestic animals has become a serious public health concern. The transmission of antibiotic resistant pathogens from animal manure to humans has yet to be investigated. In addition genetic material from antibiotic resistant organisms in animal manure can potentially be transferred to human pathogens. This threat is unknown and needs to be investigated thoroughly. The transmission of antibiotic resistance in solid and liquid manure should be a major research focus. The development of antibiotic resistance following the use of antibiotics in poultry and swine should be addressed in a similar fashion that is currently being investigated in feedlot cattle.

6.5 Water Runoff

Manure has been implicated in waterborne infections of humans. The source of waterborne pathogens is often unclear, and farm animals are often implicated. A detailed analysis of human pathogens in agricultural runoff and irrigation canals needs to be conducted.

6.6 Biofilms

When bacteria adhere to surfaces they become inherently more resistant to antibiotics and biocides. These adherent microorganisms are called biofilms and are responsible for persistent infections in humans and animals. Certainly manure contains biofilm microbes attached to fiber and other inert matter. Pathogens in manure are eliminated by chemical and thermal treatment. The efficacy of these treatment methods has not been evaluated on biofilms. Human parasitic, viral and bacterial pathogens can persist within non-pathogenic bacterial biofilms and thereby resist biocide elimination. Conditions to eliminate biofilms and pathogens within biofilms need to be determined.

6.7 Advanced Detection Systems

Molecular diagnostic tests and biosensors are being developed to detect pathogens from humans, animals and the environment. These have not been applied to manure and there is an opportunity to pursue such research directions.

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