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Manure and Microbes: Public and Animal Health Problem?

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ABSTRACT

Most environmental concerns about waste management either have focused on the effects of nutrients, especially N and P, on water quality or have emphasized odor problems and air quality. Microbes from manure are often low on the priority list for control and remediation, despite the fact that several outbreaks of gastroenteritis have been traced to livestock operations. The pathogens discussed in this paper include protozoans (*Cryptosporidium parvum*, *Giardia* spp.), bacteria (*Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Mycobacterium paratuberculosis*), and some enteric viruses. Clinical symptoms, prospects for zoonotic infection, and control methods other than the use of antimicrobials are considered. Recommendations to avoid disease transmission include taking steps to ensure the provision of clean, unstressful environments to reduce disease susceptibility and the careful handling and spreading of manure from animals at high risk for infection, especially young calves. Composting and drying of manure decrease the number of viable pathogens. Environmental controls, such as filter strips, also reduce the risk of water contamination.

(Key words: manure, pathogen, water quality, waste management)

INTRODUCTION

Maintaining profitability in the dairy industry while protecting water quality and the health of humans and animals is one of the challenges that farmers face in the 1990s. Run-off of sediment, pesticides, and nutrients such as phosphorus and nitrogen have been considered the greatest environmental threats to water quality posed by animal agriculture.

Pathogen contamination of the water and food supplies recently has received national attention because of the presence of *Escherichia coli* O157:H7 in ham-

burgers, *Salmonella* spp. in ice cream and eggs, and *Cryptosporidium parvum* in the Milwaukee water supply (32). In the ice cream and hamburger cases, livestock were not directly linked to the outbreaks, but, as reservoirs of these pathogens, animals were indirectly involved. In Milwaukee, a malfunctioning water treatment plant was the direct cause of the outbreak of diarrhea that made 403,000 people sick (32), and there is still debate about the original source of the oocysts.

Protozoan pathogens (*C. parvum* and *Giardia* spp.); bacteria including *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, and *Mycobacterium paratuberculosis*; and viruses in the food and water supply can cause these headline-grabbing outbreaks. The goal of this paper is to identify which microbes are of most concern, to review the potential sources of these microbes, and to describe farm level approaches to minimize human and animal infection. The discussion is limited to cattle, especially dairy cows. This distinction is important because many swine enteroviruses can infect humans, and organisms such as *Campylobacter* spp., which are major problems for the swine and poultry industries, are of somewhat less concern to the dairy industry. The microbes to be discussed were selected based on the likelihood of transmission from dairy cattle to humans, and the potential threat to human health. In some cases, such as *Yersinia enterocolitica* and *Campylobacter jejuni*, the decision was difficult; these bacteria pose significant risks to human health, and they are found in cattle feces, but they are more frequently found in poultry and swine manure.

When considering the problem of pathogen transmission through manure, it is important to recognize that manure, which consists of animal excreta (feces and urine), bedding, and dilution water, also contains secretions from the nose, throat, blood, vagina, mammary gland, skin, and placenta. Microbes in these secretions, as well as those in the excreta, can potentially accumulate on the barn floor. Unless the manure is handled appropriately, these pathogens may infect other animals or humans. Aside from the problem of disease transmission among animals, more than 150 pathogens can cause zoonotic infec-

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tions (from animals to humans) (52). Slurry, like manure, contains excreta but does not contain significant amounts of bedding. This distinction has important implications for handling strategies because slurry is more difficult to compost than manure is (25). For materials to reach 60°C in 3 d, a target for composting, they should contain at least 30% DM.

Manure or slurry handling may cause organisms to become airborne. A Dutch composting facility significantly exceeded the Dutch standards for total airborne bacteria (42) in an air quality study. However, when air quality was monitored after land application of anaerobically digested sewage sludge, the risk of transmission of *Salmonella* and pathogenic clostridia was low (41).

BACTERIA

The Commission of the European Communities identified reportable bacteria that are of particular concern for animal and human health [(27) as cited in (52)]. Included in their list are *Salmonella* spp., *E. coli*, *Bacillus anthracis*, *Mycobacterium* spp., *Brucella* spp. (especially *Brucella abortus*), *Leptospira* spp., *Chlamydia* spp., and *Rickettsia* spp. In addition to these organisms, other potential bacterial pathogens in manure include *L. monocytogenes*, *Y. enterocolitica*, *Clostridium perfringens*, and *Klebsiella* spp. (34). In this review, the emphasis is on *L. monocytogenes*, *Salmonella* spp., *M. paratuberculosis*, and *E. coli* O157:H7 because outbreaks caused by these organisms have been linked, tenuously in some cases, to cattle. All of these organisms can be transmitted from animals to humans directly or through the food and water supplies. Although anthrax is usually fatal, salmonellosis, listeriosis, and infection with *E. coli* O157:H7 are of greater public health concern currently because the bacteria that cause these infections have multiple hosts, and the prevalence of infection has increased over the past 20 yr (51). *Mycobacterium paratuberculosis* has been included in this review because the issue of whether there is a link between *M. paratuberculosis* and Crohn's disease has been raised. *Mycobacterium paratuberculosis* also is an organism that can have severe economic impacts on infected herds.

To assess the threat posed by different microorganisms in manure, bacterial survival in manure as it is usually handled on farms must be evaluated. Survival is affected by the source, pH, dry matter content, age, and chemical composition of the manure as well as by microbial characteristics. Manure that is well mixed with bedding is more likely to undergo aerobic fermentation with accompanying temperature

increases than is slurry with minimal amounts of bedding. Because of their ability to grow under a wide range of conditions, organisms such as *L. monocytogenes* and *M. paratuberculosis* (2, 46) are widely dispersed in the environment, making their control difficult. The endospores of spore-forming bacteria such as *Cl. perfringens* and *B. anthracis* often withstand much harsher environmental conditions than the bacteria themselves can tolerate.

Sensitive 16S rRNA or DNA probes, which now exist for most of the bacteria mentioned in this paper, have resulted in an improved ability to detect low levels of most of these organisms (13, 33). However, these methods require careful sample handling and the use of sophisticated laboratories to ensure complete DNA extraction without contamination. Without these methods, scientists are still dependent on the ability to culture and enrich for these bacteria. In some cases, such as *Salmonella*, the conventional methods are quite sensitive, but some of the leptospira and mycobacteria are difficult to grow. For example, the conventional culture techniques for *Mycobacterium* spp. may require more than 8 wk (46, 48) and have a sensitivity of 40 to 50% (48).

Manure (excreta plus bedding) typically contains 10^{10} cells/ml. Manure in England typically contains less than 10^2 *Salmonella* cells/g of feces, but this number may be as high as 10^7 cells/g in the feces of apparently healthy animals (25, 51). To bring the problem more clearly into focus, as few as 10 cells of some pathogens may be enough to cause infection. Rapidly changing pathogen numbers, combined with culture problems and changes in infective dose because of the immune status of the host, make quantification of risk difficult.

E. coli O157:H7

Verotoxin-producing strains of *E. coli*, such as strain O157:H7, present problems because the symptoms they can cause are serious, and the bacteria can survive under adverse conditions. The verotoxins produced by *E. coli* O157:H7 can produce three different sets of symptoms, including hemorrhagic colitis (diarrhea that becomes profuse and bloody), hemolytic uremic syndrome (bloody diarrhea followed by renal failure), and thrombocytopenic purpura (symptoms similar to those of hemolytic uremic syndrome with central nervous system involvement in addition). Death often occurs in patients with hemolytic uremic syndrome and thrombocytopenic purpura. This organism is of concern to the dairy industry because many outbreaks have been traced to ground beef and some to raw milk (7, 18) as well as to water,

apple cider (1), and vegetables (56). Dairy farms have been identified as reservoirs of *E. coli* O157:H7 (22, 57, 60). It was suggested, but not proven, that the apples that were the source of the *E. coli* O157:H7 in the Massachusetts cider outbreak had been contaminated by manure that had been deposited under the trees (1).

Research on *E. coli* O157:H7 in dairy cattle from Washington (22), Wisconsin and Washington (57) and two national US studies (20, 60) indicate that the prevalence of this bacterium is less than 5% and usually lower than 1%. It is difficult to compare these studies because different detection methods of varying sensitivities, sample collection strategies, age grouping, and number of samples collected were used. In the Washington study (22), *E. coli* O157:H7 was isolated from 0.28% (10 of 3570) of the fecal grab samples from dairy animals in 60 herds but not from samples of slurry (feces and urine), bulk milk, or milk filters from these farms. In studies that included 1266 samples from 22 farms in Wisconsin and Washington, 2 of which had been linked to an outbreak of gastroenteritis after consumption of raw milk (57), only 1 mature cow of the 662 tested (0.15%) was positive. The heifers and calves under 24 mo of age, however, were more likely to excrete *E. coli* O157:H7 (2.8%). Recovery of *E. coli* O157:H7 was three times more likely on the case farms than on the control farms. Samples were collected from 64 and 50 control herds in 14 states in the national studies, one of which was conducted in 1991 to 1992 (20) and the follow-up study that took place in 1993 to 1994 (60). In addition, 3 case herds were sampled from the first study and 14 case herds were sampled from the second study in which *E. coli* O157:H7 had been identified.

The important conclusions from these studies were that 1) the weaned calves less than 24 mo old were more likely to shed *E. coli* O157:H7 than were the milk-fed calves, 2) very few mature cows shed the pathogen, and 3) neither detection method consistently isolated the pathogen, or shedding was intermittent. Two animals were positive for *E. coli* O157:H7 for more than a year after the initial isolation, but intermediate samples did not always yield positive results. Intermittent shedding does occur with many organisms and must be considered as studies are designed to evaluate prevalence of these organisms. The second study indicated that 22% of the control herds and 50% of the case farms were positive for *E. coli* O157:H7 (60) and that a relatively small number of animals excreted the organisms on each farm.

How cattle are managed affects whether *E. coli* O157:H7 is able to proliferate in the rumen (44). The

gastrointestinal tracts of well-fed cattle were far less conducive to the growth of *E. coli* O157:H7 than the gastrointestinal tracts of cattle that had been deprived of feed, possibly because of the high VFA concentrations and lower pH in the well-fed cattle. Both the ruminal and hindgut fermentations probably affect excretion of *E. coli* O157:H7. This finding has some practical implications: 1) to reduce the likelihood of contamination at the meat processing plant by the pathogen, animals that are shipped to the slaughterhouse should not be deprived of feed, and 2) surveys conducted with well-fed cattle may not reflect the true prevalence of *E. coli* O157:H7 (44).

The fate of *E. coli* O157:H7 after excretion is important in determining whether the organism is introduced into the water or food supply or is transmitted to humans and other animals. That *E. coli* O157:H7 could survive at 8°C and pH <4.0 for up to 31 d in apple cider (59) or in hard salami with its high levels of nitrites, nitrates, and salt (4) shows that *E. coli* O157:H7 is an organism able to grow in adverse environments.

Several trials have been conducted to determine the viability of *E. coli* O157:H7 in manure with various amounts of bedding and under a variety of storage conditions. The survival times of *E. coli* O157:H7 at 5, 22, and 37°C with an initial inoculum of 10⁶ cfu/g were 70, 56, and 49 d, respectively (56). Treatment of manure by mesophilic digestion caused a rapid initial decline in the numbers of viable *E. coli*, but this decrease was followed by a period during which the residual population was maintained for an extended period (26).

L. monocytogenes

Any bacterium that is able to cause severe neurological symptoms and death in humans and that has been isolated from 42 mammals, 22 species of birds, and from fish, crustaceans, and insects (38) is a potentially dangerous organism. *Listeria monocytogenes* is such a bacterium. In addition, *L. monocytogenes* lives naturally in plant and soil environments, and poorly fermented silage often contains high numbers of *L. monocytogenes* (21, 24, 58). Outbreaks from raw vegetables that had been fertilized with sheep manure have been documented, as have infections associated with consumption of unpasteurized dairy products and with ice cream that had been infected during processing (23, 38, 55). Healthy animals can be asymptomatic carriers of *L. monocytogenes*.

In a 2-yr epidemiological study with 3878 fecal samples from 249 dairy herds, more cows excreted *L.*

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monocytogenes during the winter than during the summer, 16.1% of the cows tested positive in December. There was a strong positive correlation between the presence of *L. monocytogenes* and whether silage was fed. The ability of this bacterium to grow at a wide range of temperature (3 to 42°C) and pH (≤ 5.5 to 9.0) and in high (up to 12%) salt concentrations makes its control difficult (2).

Salmonella spp.

The best known member of the *Salmonella* genus is *Salmonella typhi*, the organism responsible for typhoid fever, but many of its near relatives, including *Salmonella typhimurium*, *Salmonella dublin*, and *Salmonella enteritidis*, are also gaining notoriety as formidable pathogens. *Salmonella* spp. have been responsible for 45% of the foodborne disease cases in which the causative agent has been identified (31). Many of these outbreaks of gastroenteritis have been traced to foods of animal origin, including eggs (54), although numerous other sources, including fish, coconut, salad dressing, peanut butter, and chocolate, have been implicated (18). The economic costs of salmonellosis have been estimated at close to \$1 billion/yr (31). In addition to links to foodborne disease, outbreaks from *Salmonella* spp. contamination of water have been documented (5). Symptoms of salmonellosis include nausea, vomiting, cramps, diarrhea, and, in about 2% of the cases, arthritis (18). Immunocompromised patients, especially those with AIDS, are at high risk.

Outbreaks linked to the dairy industry have occurred from contamination of a finished product with raw milk (18) and because a truck used to transport ice cream mix was not properly cleaned after contaminated eggs had been carried in the vehicle (23).

The incidence of *Salmonella* spp. infections has increased substantially since reporting began in 1943, especially since 1970 (54). The problem has been exacerbated by increasing antimicrobial resistance among *Salmonella* spp. serotypes. One of the consequences of antimicrobial resistance is that *Salmonella* spp. become a larger proportion of the microbial population because competing organisms are unable to grow and the risk of infection increases.

M. paratuberculosis

Paratuberculosis, or Johne's disease, is caused by *M. paratuberculosis*. The disease is difficult to control because much of the infection in a herd is invisible for an extended period before clinical symptoms are evident. The bacterium has ample opportunity to become

entrenched in the herd before it is apparent that a problem exists. *Mycobacterium paratuberculosis* may be transmitted prenatally or postnatally, when mast infection occurs through the fecal-oral route (53). The question of prenatal transmission has been controversial, but documentation is abundant that the organism can migrate from the intestinal tract to other organs, including the uterus, the lymphatic system, the udder, and the sex organs of bulls. There is some evidence for sexual transmission of the organism. Young calves are most vulnerable to *M. paratuberculosis*, but the immune resistance of yearlings is comparable with that of mature cattle. Removal of the calf from the dam at birth before nursing is one strategy that helps reduce the incidence of Johne's disease (48).

Although *M. paratuberculosis* is a very costly pathogen to the producer whose herd is infected, it probably would not have been included in this review had not a linkage between *M. paratuberculosis* and Crohn's disease been reported in 1964. Crohn's disease is a hyperresponsiveness of the intestinal immune system, which causes an abnormal inflammatory response (3). Patients suffering from this condition suffer from abdominal pain, weight loss, diarrhea or constipation, vomiting, and malaise. Studies of twins suggest that there is a genetic linkage in Crohn's disease (16).

Chiodini and Rossiter (3) recently wrote an excellent review of the evidence that *M. paratuberculosis* is implicated in the etiology of Crohn's disease. The scientific issues are complex. The available detection methods are inadequate, producing variable results because of the difficulty in growing the organism, and the immunologic responses to *M. paratuberculosis* have been inconsistent. Results using antimycobacterial drugs have varied. Furthermore, knowledge is incomplete concerning the drug susceptibilities of the cell-wall deficient forms of the organism, and *M. paratuberculosis* is resistant to many antimicrobials. Finally, many needed epidemiological studies have not been conducted. Unfortunately, we will not soon have a clear answer to whether *M. paratuberculosis* causes Crohn's disease. Recent advances using the polymerase chain reaction have improved detection methods significantly (14, 16), and progress in this area is continuing. Once the methodological problems have been resolved, experiments are needed to determine whether the DNA insertion element that is specific for *M. paratuberculosis* is found in the tissues of Crohn's patients. Accompanying immunologic experiments would provide additional insight into the relationship between *M. paratuberculosis* and Crohn's disease.

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TABLE 1. List of zoonotic viruses and environmentally stable viruses that are likely to be isolated from dairy cattle (12).

Zoonoses	Environmentally stable
Vaccinia	Adenovirus
Pseudorabies ¹	Parvovirus
Bovine papular stomatitis ¹	Acute respiratory and enteric disease
Bovine rotavirus ¹	Bovine rotavirus
Bovine respiratory virus ²	Astrovirus
Vesicular stomatitis	Bovine enterovirus
Rift Valley Fever virus ³	Rift Valley Fever virus
Rabies	

¹Possible, but not common.²Humans are likely reservoirs of infection.³Primarily found in Africa.

Given this uncertain association between *M. paratuberculosis* and Crohn's disease, how should the dairy industry approach the problem? Decreasing the level of *M. paratuberculosis* infection in dairy herds is a logical goal even without the Crohn's disease problem, because the disease is costly in terms of lost milk and shortened cow life. Rossiter and Burhans (48) have suggested a practical strategy on how to develop programs to control *M. paratuberculosis* on the farm. Their program involves collection of information and history, identification of risks specific to the farm, examination of control options, planning the scope and time frame for the control strategy, and implementation and evaluation of the control program.

VIRUSES

Problems are also posed by viruses, which are obligate intracellular parasites (49) that often have a limited host range. Similar to the protozoans described in the next section, viruses are unable to replicate outside of their host, and, therefore, their numbers never increase once they are released into the environment. Because of the limited host range, relatively few zoonotic viruses infect cattle and humans. Table 1 includes a list of these zoonoses (12) and viruses isolated from cattle that are environmentally stable. Table 2 is a list of viruses likely to be found in manure (51).

In trials in which cattle manure was inoculated with a bovine enterovirus and a bovine parvovirus, inactivation occurred within 30 min during thermophilic anaerobic digestion at 55°C (36). The enterovirus survived for up to 13 d under mesophilic conditions (35°C). Neither of these two environmentally resistant viruses survived aerobic composting for

28 d when the temperature reached 60°C on the 3rd d and was maintained at that temperature for the remainder of the fermentation.

Knowledge is incomplete about the viral attributes and environmental factors that contribute to viral inactivation (40). The stability of different viruses varied, apparently depending on whether the virus particle was adsorbed to or embedded within suspended solids (40). Other factors that affect viral survival include pH, temperature, and the presence of bacteria that can inactivate viruses (10). There is evidence (10) that some bacteria isolated from manure have both proteases and other strategies to inactivate viruses. Laboratory experiments supported previous work that showed that both hepatitis A and polio type 1 viruses were inactivated more rapidly in swine and dairy slurries than when the septic tank effluent containing intentionally added viruses was incubated alone (9, 11, 52).

Viruses were inactivated more rapidly during summer than during winter, but the effect was likely due to a more active microbial fermentation than to direct temperature effects. Samples kept in the laboratory at 4°C without significant fermentation were most likely to survive. The rotaviruses tested remained active for long periods.

Viral groundwater contamination has been a concern, particularly with wastewater irrigation systems. A preliminary study in Texas showed that low levels of bacteria but no viruses in irrigated wastewater inoculated with test organisms reached 1.37 m below the soil surface, although virus particles were present at 0.91 m (37). The distance viral particles can be transported is dependent on many factors, including soil moisture, pH, the adsorptive capacity of the soil,

TABLE 2. List of viruses that might be excreted by cattle.¹

Not enveloped	Enveloped
Bovine enterovirus ²	Infectious bovine rhinotracheitis ²
Bovine adenovirus	Malignant catarrhal fever
Reovirus ²	Bovine mastitis
Bovine parvovirus ²	Bovine virus diarrhea ²
Bovine rhinovirus	Foot-and-mouth
Bovine papillomavirus	Bovine coronavirus ²
	Parainfluenza type ³
	Respiratory syncytial virus
	Rabies ³
	Vesicular stomatitis ³
	Cowpox ³
	Paravaccinia

¹Modified from a report by Strauch and Ballarini (52).²Most likely to be found in feces.³Unlikely to be found in feces.

and ionic concentration. Whether there is danger of groundwater contamination depends on these factors as well as on the depth of the water table.

PROTOZOA

Giardia spp. and *C. parvum* are protozoans that cause severe diarrhea in both animals and humans. In 1993 to 1994, one-third of the outbreaks associated with drinking water for which the causative agent was identified were due to these two pathogens (28). In a large national study, 3.8% of the more than 414,000 stool samples tested were positive for *Giardia*. Age and community affected infection rate significantly; the range varied from 2 to 20% of the population (6). Giardiasis is transmitted by the fecal-oral route, by contact with infected individuals, or by consumption of contaminated drinking water or food (6). Despite the fact that *Giardia* was one of the first "wee animalcules" identified by van Leeuwenhoek in the 17th century, the taxonomy of this genus is not well understood, and more research is needed to determine which species can infect humans.

Cryptosporidium parvum, first identified in 1907, was not considered pathogenic until 1975 (47). In healthy, mature animals (including humans), the infections caused by both *Giardia* and *C. parvum* are usually self-limiting and, although they cause significant discomfort, do not pose serious long-term health risks. In studies with healthy human adult volunteers, the infection dose of *C. parvum* oocysts that was sufficient to cause illness in 50% of the population was 132 (15). Eighty-eight percent of the volunteers became sick when they received more than 300 oocysts. Infected animals may shed as many as 1×10^8 oocysts daily for 1 to 12 d (45). These data, combined with the information from the Milwaukee outbreak in which 403,000 predominantly healthy people became ill (32), show that cryptosporidiosis is not restricted to the old, the infirm, and those with poorly functioning immune systems. Farmers, animal handlers, veterinarians, and others who work with animals are more likely to be infected than the general population (30). The impact of the disease is far more serious for people with AIDS and for those who are receiving chemotherapy or who have had organ transplants than for those who are not immunocompromised. Although many drugs have been tested for activity against *C. parvum*, the only drug that has proved effective is paromomycin (17). Although more drugs are effective against *Giardia*, the treatment options are still limited.

The life cycles of *Giardia* and *C. parvum* are similar in many ways. Both have complicated life

cycles and change forms several times. In both organisms, infection occurs in the gastrointestinal tract of the host animal. When an animal ingests an oocyst (*C. parvum*) or cyst (*Giardia*), these structures excyst in the intestine, releasing an infectious form of the organism. Several changes occur before the organisms are excreted back into the environment as cysts or oocysts. These cysts or oocysts can resist many environmental pressures, which enables them to remain viable in the environment for at least a year (8). Of the two pathogens, *C. parvum* is the most difficult to control because it is not affected by chlorination at levels that are considered safe for water treatment and human consumption and because it can infect a wide variety of mammals (Figure 1) (39). Figure 1 would have more arrows, both uni- and bidirectional, if experiments had been conducted with all of the species or if it had been possible to confirm the source of oocysts in an outbreak.

There are three potential reservoirs of infection for both *Giardia* and *C. parvum*: wildlife, domestic livestock, and humans; outbreaks for both pathogens have been traced to all three sources. If the major concern is safe drinking water, appropriate control measures should be used to minimize risk from all three sources.

Studies to determine prevalence of these two pathogens have been conducted in many domestic species, including cattle, swine, sheep, horses, dogs, and cats. The National Animal Health Survey (19) is a recently completed national study that includes data on *Giardia* and *C. parvum* in beef and dairy animals. More than 7300 dairy calves on 1100 farms were sampled. The data showed that *C. parvum* oocysts were found on 59% of the dairy farms and in 22.4% of the tested heifers. Comparable data were obtained for beef calves. The percentage of dairy calves that were positive for cryptosporidia peaked when the calves were between 1 and 3 wk of age (43). Several studies have shown that few animals shed *C. parvum* oocysts after they are weaned or after they are 3 mo old. Quigley et al. (43) studied how colostrum feeding and housing affected prevalence of *C. parvum* and *Giardia* spp. in Jersey calves. The calves most likely to shed *C. parvum* were those that were housed in a barn rather than in hutches. Differences between nursing and bottle feeding of colostrum were not evident.

In addition to determining the prevalence of these protozoal pathogens, the National Animal Health Survey collected information on the relationship between management and infection (19). The management factor that had the most influence on the infec-

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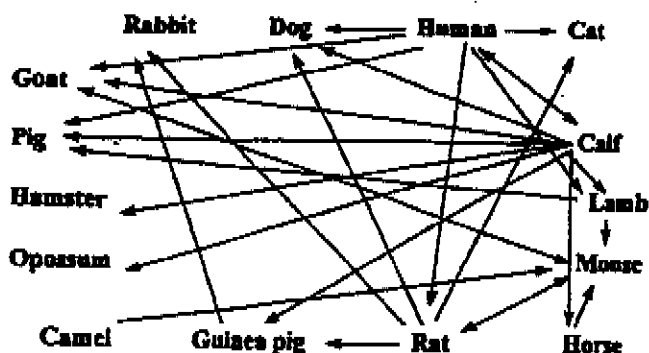


Figure 1. Documented transmission of *Cryptosporidium parvum* among selected mammals. Direction of arrow indicates route of infection. In many cases, especially with humans, the arrows are unidirectional because the relevant studies have not been conducted. Adapted from O'Donoghue (39).

tion rate in calves was whether cows were panned individually or in groups at calving. Calves born in group pens were more likely to become infected than those that were born in individual pens. Surprisingly, no relationship was found between maternity pen cleaning and incidence of infection. However, sanitation of the housing of the unweaned calves was an indicator of whether the calves were likely to have cryptosporidiosis. Those calves housed in pens that were washed were half as likely to become infected as those that were in facilities in which only the bedding was removed during cleaning.

Calves in herds with more than 100 cows were more likely to have *C. parvum* than calves in smaller herds. When results were adjusted for herd size, there were no differences in prevalence by region across the US. More calves were likely to shed oocysts during summer than during the rest of the year. Herds with a history of scours were 50% more likely to have infected calves than those in which health problems were not reported.

Generally, calves that tested positive for *Giardia* spp. were older than those with *C. parvum*, although calves over 6 mo old rarely excreted *Giardia* spp. Careful treatment of the manure from calves that were less than 6 mo old should reduce the risk of infection and water contamination by these pathogens.

IMPLICATIONS

Pathogenic microbes in manure must be considered to improve dairy management. Fortunately, many of the practices that reduce the prevalence of these organisms make economic sense as well: a herd is much better off without Johne's disease. Much of the advice

is not new: the Extension Service has been recommending that calves be kept clean for more than 75 yr. Now, there are new reasons to make and follow these time-tested, but often disregarded, recommendations about calf sanitation.

Other recommendations are common sense: young calves are the most likely animals in the herd to be infected with most of the pathogens included in this discussion; therefore, spreading their manure next to a water course is foolish. Runoff experiments with filter strips of varying widths and soil types have shown that a filter strip as narrow as 0.61 m can significantly reduce the risk of stream contamination (29). Soil type is important in determining microbial transport through soil (35). Identification of hydrologically active areas, where the risk of run-off is high, is a useful strategy in the development of a multiple barrier approach similar to that recommended by the Environmental Protection Agency. In these areas, manure spreading should be restricted both in terms of timing and in the source of the manure. Manure from mature cows can be spread on these areas only when the ground is not frozen and when the risk of run-off is low.

The duration of pathogen survival in different manure handling systems is an area in which additional research is needed. Although some *Salmonella* may survive for 286 d in slurry (52), this statistic is less important than the rate at which the population decreases. In many cases, 90% reduction of *Salmonella* occurs within a month. Describing how storage conditions affect survival is difficult because many variables, including the type of slurry, pH, dry matter content, storage temperature, number and type of pathogens present and storage strategy, all must be considered (52). Spore-forming organisms such as *Cl. perfringens* and *C. parvum* oocysts are likely to be among the long-term survivors. Few pathogens can withstand the heat generated during composting, but ensuring that all parts of a compost pile reach and maintain a temperature of 60°C is important. Drying is an appropriate strategy in parts of the country where the sun shines frequently.

Most important in developing strategies to ensure the prosperity of dairy operations without damage to the environment is to involve farmers throughout the planning and implementation of projects. The suggestions made by Roseiter and Burbans (48) for the control of *M. paratuberculosis* can easily be amended to deal with any of the pathogens found in manure.

As strategies are developed to minimize the threat posed by pathogens, it is important to remember some basic microbiology: microbial survival is limited by

the following (50): sunlight, drying, freezing and thawing cycles, high temperatures, high or low pH, and, for some organisms, exposure to oxygen. Evaluation of the relative risks posed by different pathogens is difficult: farms with poorly fermented silage face a higher likelihood of *L. monocytogenes* infection than farms with well-preserved forages but not if animals are stressed because of inadequate housing. Assuming that a farm is free of one or all of the organisms considered in this paper is risky. A safer approach is to ensure 1) that cattle, particularly young stock, are kept clean and are well nourished; 2) that manure is stored and spread to minimize the risk of disease transmission; and 3) that appropriate environmental safeguards, such as filter strips, are used.

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