Microbial pathogens excreted by livestock and potentially transmitted to humans through water

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Introduction

Protecting surface water quality in the face of a burgeoning human population has become a major challenge for western states such as California. One of the many concerns regarding water quality is to minimize the concentration of pathogens in water and thereby minimize the risk of waterborne disease to humans or to animals. The focus of this article is to review the list of pathogens which can be shed in the excrement of livestock and transmitted to humans through water. The term, waterborne zoonotic disease, is commonly used by public health researchers to refer to pathogens which are transmitted via water from animals to man.

Four primary steps need to occur for waterborne transmission of pathogens from livestock to humans. Eliminate any one of these steps and transmission of the specific pathogen from livestock to humans through water can be significantly reduced or even stopped completely. First, the pathogen must be excreted by livestock. Second, the pathogen must reach a water supply either by the animal defecating in water, by overland flow (runoff from a grazed pasture during rainfall, snowmelt, etc.), by subsurface flow, or by some combination of these three pathways. Third, the pathogen must retain the cellular functions necessary for initiating a new infection in humans during the time it is in the environment. Lastly, given that the pathogen is shed by livestock, reaches a water source, and remains infective until ingested by a human, the concentration of infective pathogens must be sufficiently high in order to initiate an infection. The minimum number of pathogens needed to initiate infection varies from pathogen to pathogen. For example, the protozoal parasites, *Cryptosporidium parvum* and *Giardia lamblia*, have a very low infectious dose (DuPont et al. 1995; Rendtorff, 1954). In contrast, for bacteria such as *Campylobacter jejuni* or *Salmonella typhimurium*, hundreds or thousands of those bacteria need to be ingested in order to initiate an infection (Robinson, 1981; Black et al. 1988).

Protozoa
Primary Concern

Waterborne zoonotic protozoa of primary concern and with a feasible livestock component should include at a minimum *Cryptosporidium parvum* and *Giardia duodenalis*. The principle features of these protozoa are: the infective stages of *Cryptosporidium parvum* (the oocyst) and *Giardia duodenalis* (the cyst) do not reproduce outside the host, the apparent low infectious dose necessary to initiate an infection in humans, and the relative resistance of waterborne cysts and particularly oocysts to chemical disinfectants (Sterling, 1990).

Cryptosporidium parvum

Cryptosporidium parvum (*C. parvum*) is a tiny protozoal parasite that is shed by humans, cattle, sheep, goats, pigs, and horses (Fayer and Ungar, 1986). It is also shed by various wildlife species such as deer, raccoons, opossums, and rabbits. *C. parvum* is not known to replicate within and be shed by chickens or turkeys. The infectious stage of this pathogen is an environmentally-resistant egg, or oocyst, which is infective once shed by the host. These oocysts can be shed for several days by an infected animal, with up to 10 million oocysts per gram (approximately 280 million per ounce) of feces during the peak of shedding (Blewett, 1989; Goodgame et al. 1993; Xiao and Herd, 1994a). Shedding is usually limited to livestock under 6 months of age (Atwill et al. 1998; Atwill et al. 1998a; Xiao and Herd, 1994a; Xiao and Herd, 1994b; Sanford, 1987; Kirkpatrick, 1985), but shedding around lambing has been documented in ewes (Xiao et al. 1994). Therefore, watershed management strategies designed to minimize potential livestock contamination of surface water with *C. parvum* should focus on the young and possibly on animals close to lambing or calving. There are many livestock production systems in which the young are typically excluded from a group of animals, such as feedlots, some slaughterhouses, dairies who do not raise their own calves, and backgrounder/stocker beef cattle operations (calves 6-14 months old) where the risk of shedding is likely to be minimal. Researchers in Europe have reported shedding of *C. parvum* in adult beef cattle (Scott et al. 1995; Lorenzo et al. 1993), but this has not been confirmed by studies in this country (Atwill et al. 1998; Atwill et al. 1998a; Xiao and Herd, 1994a; Xiao and Herd, 1994b; Sanford, 1987; Kirkpatrick, 1985).

Although the occurrence of waterborne outbreaks of human cryptosporidiosis is well documented, the source of the organism is typically unknown (Juranek, 1995; Atwill, 1996; Sterling, 1990; Smith and Rose, 1990). This is somewhat troubling since *Cryptosporidium* oocysts were present in 39-87% of the surface waters (rivers, lakes, etc.) that were tested throughout the United States during 1988-1993 (Rose et al. 1991; LeChevallier et al. 1991; LeChevallier and Norton, 1995).

If the livestock feces are deposited on dry land, the means by which *C. parvum* oocysts travel from the manure to a nearby body of water are not well understood. Factors such as soil type, slope, vegetation, and other factors present on California lands used for livestock production will affect the process. Research using intact soil columns and simulated rainfall indicates that *C. parvum* oocysts are carried up to 30 cm (approximately 12 inches) through clay loam, silty loam, and loamy sandy soil, but the majority of oocysts remained within the upper 2 cm (approximately 3/4 inches) of the soil column (Mawdley et al. 1996). Oocysts appear to die after several hours of being dry, but such results need to be interpreted with caution since infectivity was determined by indirect assay (Robertson et al. 1992).

Temperature has also been shown to influence the viability of the oocyst. Oocysts recovered from calf feces which had been kept inside a barn in summer or inside an unheated shed in winter became non-infective for mice in 1-4 days (Anderson, 1986). *C. parvum* oocysts become non-infectious if frozen for longer than 1 hour at minus 70E C, longer than 24 hours at minus 20E C, and longer than 168 hours at minus 15E C (Fayer and Nerad, 1996). What if fecal material is deposited directly in a stream? One study found that after 33 days in cold river water, an estimated 34-40% of purified oocysts were apparently non-viable, using an indirect assay. After 176 days, 89-99% mortality was reported (Robertson et al. 1992). Although there are severe environmental pressures against oocysts remaining infective when excreted on land, only a few oocysts would need to remain

viable in order to pose a risk to humans. Experimental studies in healthy humans determined that the infectious dose at which 50% of subjects acquired infection (ID_{50}) was 132 bovine-derived oocysts (DuPont et al. 1995). As few as 30 oocysts were shown to induce cryptosporidiosis (DuPont et al. 1995), but since a dose of less than 30 oocysts was not fed to the volunteers, the minimal infectious dose could be lower.

Giardia duodenalis (G. intestinalis, G. lamblia)

Waterborne giardiasis in humans is well documented, with over 100 reported outbreaks since 1965. Contamination of surface water with human sewage was responsible for a majority of these outbreaks, but the source of infection for sporadic cases of waterborne human giardiasis is much less clear (Craun, 1990; Levine et al. 1991; Thompson and Boreham, 1994). Similar to the widespread presence of *Cryptosporidium* oocysts in surface water (LeChevallier et al. 1991), a majority of surface water samples also contained *Giardia* cysts. This creates some concern given the ability of *Giardia* cysts to survive extended periods of time under moist and cool environmental conditions (de Regnier et al. 1989) and the low infectious dose for humans (Rendtorff, 1954). Similar to *C. parvum, G. duodenalis* is commonly shed by a wide variety of livestock species, wildlife, companion animals and humans (Xiao and Herd, 1994a; Xiao and Herd, 1994b; Xiao et al. 1994; Erlandsen, 1994; Wallis, 1994). It has not been determined which of these sources, including humans and their sewage treatment facilities, are the primary source(s) of *G. duodenalis* for surface water in California.

Unlike *C. parvum* in which there is clear evidence for its infectious potential in humans (DuPont et al. 1995), considerable controversy surrounds the issue of whether *G. duodenalis* cysts obtained from livestock can infect humans. Two reviews (Thompson and Boreham, 1994; Erlandsen, 1994) of the scientific literature both concluded that convincing data for the zoonotic potential of *Giardia* still does not exist. Additional review articles debating this issue can be found in an article by Erlandsen (1994). Fayer (1994) also concluded that Αno human infection related to these animals has been reported@ with respect to pigs, cattle, sheep, and goats. Although unequivocal evidence is lacking regarding successful transmission of *G. duodenalis* cysts from livestock to humans via water, finding such evidence remains very difficult given the investigator=s poor ability to identify the vertebrate source of waterborne *G. duodenalis* cysts (Thompson and Boreham, 1994). The seriousness of this debate should not be overlooked as the implementation of the US EPA=s Information Collection Rule and development of the Enhanced Surface Water Treatment Rule approaches. This debate also remains central to the growing national interest in developing watershed management plans that minimize the risk of waterborne giardiasis and other waterborne diseases in humans.

Secondary Concern

This group includes waterborne protozoa of secondary importance whose waterborne transmission to humans do not appear to have a major livestock component, or where a waterborne route is rarely documented. All cats (*felids*) can serve as the definitive host for *Toxoplasma gondii*, hence, livestock are incapable of contaminating water with the infective oocyst (Acha and Szyfres, 1987; Fayer, 1994). *Balantidium coli*, a ciliated protozoan found in the intestines of humans and pigs, is a rarely reported disease. Its potential to be transmitted from pigs to humans remains controversial (Acha and Szyfres, 1987). The reservoir of the intestinal amoeba, *Entamoeba*

histolytica, is thought to be humans, with livestock having no clear role in human infection (Acha and Szyfres, 1987; Fayer, 1994). *Cyclospora cayetanensis* and the opportunistic intestinal microsporidia, *Enterocytocytozoon bieneusi* and *Septata intestinalis*, are not known to have a livestock reservoir at this time (Goodgame, 1996).

Bacteria
Primary Concern
Campylobacter spp

There are several species of *Campylobacter* which can cause infection in humans (e.g., *C. jejuni, C. coli, C. lardis, C. fetus* subspecies *fetus*), with *C. jejuni* accounting for almost all of the diagnosed cases. There are approximately two million cases of human campylobacteriosis per year in the United States, comparable to the estimated annual incidence of human salmonellosis. The majority of human campylobacteriosis occurs as sporadic cases as opposed to outbreaks involving large numbers of people (Tauxe, 1992). *C. jejuni* is common in the environment and shed in the feces of humans, livestock, and wildlife, including birds. It is also found in a wide variety of surface waters, stream sediments, and sewage effluents (Tauxe, 1992; Stern, 1992). The primary routes of transmission appear to be ingestion of contaminated foods (primarily poultry and raw milk), ingestion of untreated surface water, and contact with pets (primarily dogs) suffering from diarrhea. Direct human-to-human transmission occurs only rarely (Tauxe, 1992; Altekruse et al. 1994; Franco and Williams, 1994; Adak et al. 1995). Rough estimates attribute 19% of outbreak-associated and 9% of sporadic human campylobacteriosis to waterborne transmission (Tauxe, 1992). A common vehicle of foodborne transmission is raw milk (primarily cattle) and inadequately cooked poultry presumably contaminated with infective feces. Thus it is feasible that manure from cattle and poultry operations or from the effluent of slaughterhouses or poultry processing plants could contaminate water supplies with strains of *C. jejuni* which are infectious to humans (Stern, 1992). Feces from cattle and poultry and effluent from poultry processing facilities have been shown to contain *C. jejuni* which in some cases are very similar to human isolates (Tauxe, 1992; Stern, 1992; Altekruse et al. 1994; Koenraad et al. 1995). Similarities between pathogens obtained from humans and poultry do not establish causal connection or its direction; they only provide tentative support that cross-transmission is occurring. *C. jejuni* can survive for a limited time in stream water (Terzieva and McFeters, 1991), and as few as 500-800 organisms appear sufficient to cause clinical illness in humans (Robinson, 1981; Black et al. 1988). Despite these findings, documented cases of human campylobacteriosis attributable to water contaminated with livestock manure or livestock effluent are uncommon. This is not the result of other vertebrate sources being identified as the contaminating source instead of livestock since the source remains unidentified (Cowden, 1992). Better DNA fingerprinting techniques and more sensitive and specific methods for identifying *C. jejuni* in water and other environmental sources should help identify the primary source(s) of this common cause of gastroenteritis in humans.

Salmonella spp

Human infection with non-typhoid causing *Salmonella* species remains one of the primary foodborne pathogens for humans. However, a concise review of the role of livestock in the annual incidence of waterborne human salmonellosis is difficult at best. The large number of serotypes and

their highly variable medical ecology across both geography and time, and between vertebrate hosts, have made analysis difficult. The most noticeable observation regarding human salmonellosis is the lack of reported waterborne outbreaks definitively traced to livestock (Acha and Szyfres, 1987; Ziprin, 1994).

Although non-typhoidal *Salmonella* species are widely present in domestic and wild animals, livestock are important sources for several serotypes infectious to humans. For example, *S. dublin* is one of the more frequently isolated serotypes from cattle (Ferris and Miller, 1990) and a serious foodborne pathogen for humans. Cattle have been shown to persistently shed *S. dublin* in their feces following infection with this serotype (Sojka et al. 1974). *S. newport* has been shown to survive extended periods of time in freshwater sediments (Burton et al. 1987). It is plausible that surface water contaminated with bovine-derived *S. dublin* or foods rinsed in contaminated water could serve as vehicles of human infection. *S. typhimurium* is another common isolate from cattle (Graeber et al. 1995). In Germany, isolates of *S. typhimurium* which were obtained from nearby calf-rearing facilities were not the strains contaminating associated coastal waters nor the isolates obtained from nearby human patients (Graeber et al. 1995), but studies such as this provide no assurance regarding the modes of transmission in the United States. *S. enteritidis*, now one of the most commonly isolated serotypes from human cases, is highly associated with ingestion of contaminated shell eggs (Mishu et al. 1994). Whether manure from layer or other poultry operations or the effluent from poultry processing facilities is a significant source of waterborne salmonellosis in humans is unclear at this time.

Various pathogenic strains of *E. coli*
(Enteropathogenic/Enterotoxigenic/Enteroinvasive/Enterohemorrhagic)

Some strains of pathogenic *E. coli* have emerged as leading foodborne pathogens for humans, but their role in waterborne diseases is less clear (Neill et al. 1994). It is important to realize that the *E. coli* referred to during general water quality tests (total coliforms, fecal coliforms, *E. coli*, etc.) are not necessarily pathogenic strains, but instead are an indicator of general fecal contamination. When used as an indicator of water quality, *E. coli* refers to the harmless strain of this facultative anaerobe which helps maintain normal intestinal functions (Draser and Hill, 1974).

Although subclassification of this group of pathogens is still under development, there are four primary categories of pathogenic *E. coli* (enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic), each with their own common and unique features. The reader is referred to Neill (1994) for a more thorough review. Several waterborne outbreaks of pathogenic *E. coli* have been reported, but livestock were not definitively identified as the source of contamination (Lanyai et al. 1959; Schroeder et al. 1968; Swerdlow et al. 1992). With respect to sporadic human cases of pathogenic *E. coli*, whereby livestock are the source of contamination, it is unknown what component is attributable to waterborne transmission. While enteroinvasive *E. coli* have not been isolated from animals, the highly pathogenic (for humans) enterohemorrhagic *E. coli* (O157:H7) have been cultured from a low proportion of cattle (Wells et al. 1991).

Yersinia spp

The estimated number of cases of human infection with *Yersinia* species in the United States is 3,000-20,000 per year, far less than the reported number of cases of campylobacteriosis and salmonellosis (Roberts, 1989; Todd, 1989). Most of these infections are due to *Yersinia*

enterocolitica in the United States; one of the primary routes of transmission being foodborne and waterborne (Feng and Weagant, 1994). The proportion of sporadic cases attributable to food versus water versus other routes of transmission is not well established. The bacteria are widespread in water (streams and lakes), foods (pork products, vegetables, dairy products, tofu, seafood), wild and domestic animals, and humans, but many of the strains obtained from these sources were not pathogenic for man. Relative to *Campylobacter jejuni, Yersinia enterocolitica* is better able to survive in stream water (Terzieva and McFeters, 1991). Despite swine being considered as one of the primary environmental reservoirs of these bacteria, documented waterborne outbreaks of human yersiniosis have not been definitively linked to livestock (Acha and Szyfres, 1987). It could be argued that due to the low number of cases of human yersiniosis this pathogen would be classified as a waterborne disease of secondary importance in the United States.

Secondary Concern

This group includes waterborne bacteria of secondary importance whose waterborne transmission to humans do not appear to have a major livestock component, or where a waterborne route is rarely documented. *Clostridium perfringens* types A and C are found in human and animal feces, soil, green and decaying plant material, sewage, and water (Wrigley, 1994). It is a common cause of foodborne illness in humans, but is not recognized as a primary waterborne disease nor is there a clear role for livestock in the medical ecology of waterborne infection (Acha and Szyfres, 1987). *Listeria monocytogenes*, a very common bacterium, has been associated with large foodborne outbreaks of disease (Fleming et al. 1985; Linnan et al. 1988; Donnelly, 1994), but is not recognized as a primary waterborne disease nor is there a clear role for livestock (Acha and Szyfres, 1987; Skovgaard and Morgen, 1988; Schuchat et al. 1991). Moreover, if the waterborne route comprises a minor part of the annual incidence of sporadic human listeriosis, then the incidence for waterborne listeriosis will be considerably less than the overall total annual incidence of seven cases per million people (Schuchat et al. 1992). It could be argued that if the case fatality rate associated with waterborne infection was similar to that seen with foodborne infection (23%), even small levels of waterborne transmission may pose a serious health risk (Schuchat et al. 1992). The waterborne route of transmission is not known to play a significant role in human infection with *Brucella* spp. (Acha and Szyfres, 1987; Chomel et al. 1994). Human infections with serovars of *Leptospirosis interrogans* are rare and confined mostly to direct contact with infected animals, as might occur among veterinarians and slaughterhouse workers (Acha and Szyfres, 1987; Heath and Johnson, 1994). Although occasional waterborne outbreaks have been reported, the role of livestock is unclear (Shaw, 1992; Jackson et al. 1993).

Viruses

At this time there is little evidence that the group of viruses shed in the excrement of livestock have posed a waterborne threat to human health in the United States (Cliver, 1994). Although interspecies transmission of rotaviruses has been demonstrated experimentally, the role of livestock-derived rotaviruses in the epidemiology of human waterborne infection is not known (Acha and Szyfres, 1987).

Conclusion

Although a variety of protozoa and bacteria can be shed by livestock and transmitted to humans through water, the relative significance of this route of transmission in the overall epidemiology of human infection is not clear. The reason for this lack of clarity is that humans and various wildlife species can also shed these pathogens and thereby serve as a source of infection for humans. For example, both protozoal parasites, *Cryptosporidium* and *Giardia*, and various *Salmonella* and *Campylobacter* species can be shed by humans and various wildlife. Animal agriculture is likely responsible for a percentage of many of the pathogens we find in surface water, but whether that percentage is 5%, 50% or 95% compared to non-agricultural sources such as humans or wildlife is unknown at this time for most watersheds. For example, it is unknown what proportion of the annual incidence of human cryptosporidiosis (infection with the protozoal parasite, *Cryptosporidium parvum*) is attributable to waterborne transmission compared to foodborne, direct human-to-human, or direct animal-to-human transmission. If we spend our public health dollars on controlling waterborne cryptosporidiosis yet the primary route of transmission for humans is direct human-to-human fecal-oral transmission, then we will likely have little impact on reducing the annual incidence of this disease despite having controlled the waterborne route of transmission. Moreover, the annual incidence of waterborne cryptosporidiosis cannot be separated into the proportion attributable to domestic animals, wildlife, and humans (Juranek, 1995; Atwill, 1996). Again, if the primary source of waterborne *C. parvum* is human sewage, then regulatory policies that focus on animal agriculture will likely have little impact on reducing the concentration of waterborne *C. parvum*. Of course, if animal agriculture is the primary source of these pathogens, then minimizing fecal contamination of water with livestock manure will have a dramatic impact on reducing human infection. The advent of new DNA fingerprinting tools and more sensitive and specific procedures for detecting pathogens in water should help identify and better quantify sources of these pathogens in the future. In the meantime, we should proceed carefully and without assumption on how to control these waterborne pathogens and always insist that public policy is grounded in good science.

List of pathogens of primary concern that can be shed in the feces of livestock and transmitted to humans through water. Pathogens of secondary concern whereby livestock have either no role or an unclear role in human waterborne infection have also been listed.

Waterborne protozoa pathogens of primary concern (known livestock component)	Special concerns and comments
Cryptosporidium parvum	Low infectious dose; environmentally resistant oocysts; oocyst 5 x 5 microns
Giardia duodenalis	Low infectious dose; environmentally resistant cysts; zoonotic potential under debate; cysts approximately 12 x 15 microns
Waterborne protozoa pathogens of secondary concern	
Toxoplasma gondii	Felines are the definitive host, not livestock
Balantidium coli	Swine suspected, but no clear role
Entamoeba histolytica	Human reservoir
Cyclospora cayetanensis and microsporidia (*Enterocytocytozoon bieneusi*, *Septata intestinalis*)	Unknown reservoir and livestock not know to shed these protozoa at this time
Waterborne bacterial pathogens of primary concern	size ranges from 0.2 x 1.5 to 1.5 x 6.0 microns
Campylobacter spp.	Common in livestock and wild birds
Salmonella spp.	Common in livestock feces
Pathogenic strains of *E. coli*	Can be highly virulent for humans
Yersinia spp.	Swine are considered a primary reservoir; apparent low annual incidence in humans
Waterborne bacterial pathogens of secondary concern	
Clostridium perfringens types A and C	Waterborne transmission unclear

Listeria monocytogenes	Waterborne transmission unclear; human infection typically foodborne
Brucella spp.	Waterborne transmission unclear
Leptospirosis interrogans	Waterborne transmission unclear; human infection typically by direct contact
Waterborne viral pathogens from livestock	Little scientific evidence that viruses shed in the feces of livestock pose a health threat to human in the U.S.A.

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