

# Role of livestock in microbiological contamination of water: Commonly the blame, but not always the source



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## Implications

- Since the 1940s, livestock production practices in North America have evolved from extensive to intensive systems, concentrating animals, nutrients, and their associated microorganisms within limited geographical areas.
- Livestock wastes can harbor both bacterial and protozoal pathogens, and surface and groundwater contamination has been, but is not always, linked to extensive and intensive livestock operations.
- In mixed-activity watersheds, fecal contamination can be of livestock, human, or wildlife origin.
- Fecal indicator microorganisms are not always indicative of the disease risk of water, a limitation that is being overcome by the development of molecular identification methods that specifically target pathogens.
- Best management manure handling, storage, and application practices can substantially reduce the risk of microbial contamination of surface and groundwater.
- Livestock management practices can reduce the release of pathogens into the environment.
- The purity of water can never be fully guaranteed; consequently, a multiple-barrier approach is most efficacious in ensuring the production of pathogen-free drinking water.

though rarely reported, was most often associated with humans (Scheutz et al., 2011). The strain responsible for the European outbreak gained notoriety because of its blended virulence profile, making it particularly adept at causing disease in humans (Bielaszewska et al., 2011). On further investigation, it was theorized that the outbreak occurred as a result of contaminated fenugreek seeds imported from Egypt, although *E. coli* O104:H4 was not isolated from imported seed lots. As is the case in many outbreaks, the true origin of this virulent strain will likely never be known, but this example does illustrate how livestock are often the prime suspect of foodborne disease outbreaks.

Perhaps this is not surprising given that approximately 243 of the 616 pathogens (39%) currently known to infect livestock are also infectious to humans (Cleaveland et al., 2001). These zoonotic pathogens are also twice as likely to be associated with emerging human diseases. Outbreaks that have been linked to contamination of water with livestock manure, such as Canada's largest waterborne disease outbreak in Walkerton, Ontario, in 2000 (Hrudey et al., 2002) or the adulteration of spinach in the United States in 2006 (Jay et al., 2007), do not fade from the public's memory, and they leave a long-term negative perception of the impact of livestock on water quality. As the human population approaches 9 billion people in 2050, global meat and milk production are predicted to nearly double to 465 and 1,043 million tonnes, respectively (Food and Agriculture Organization of the United Nations, 2006). This expansion will mainly occur through the intensification of livestock production in developing countries, regions that are already prone to microbial contamination of water. Implementation of proper stewardship practices will be pivotal in minimizing the microbiological impact of an expanded livestock industry on water quality.

**Key words:** bacteria, extensive livestock, intensive livestock, manure, microorganism, outbreak, protozoa waste

## Introduction

In the spring of 2011, Europe experienced its largest foodborne outbreak in recent history, with *Escherichia coli* O104:H4 causing illness in 3,000 people across 14 countries and at least 39 deaths. At first, the source of the outbreak was not apparent, and a recommendation to cease the consumption of raw vegetables cost the European produce industry millions of euros. Eventually, sprouts were identified as the source of the bacterium, and it was initially assumed that irrigation with water contaminated with livestock manure was the cause of the outbreak. However, *E. coli* O104:H4 had not been previously isolated from livestock, and al-

## Defining the Scope of the Problem

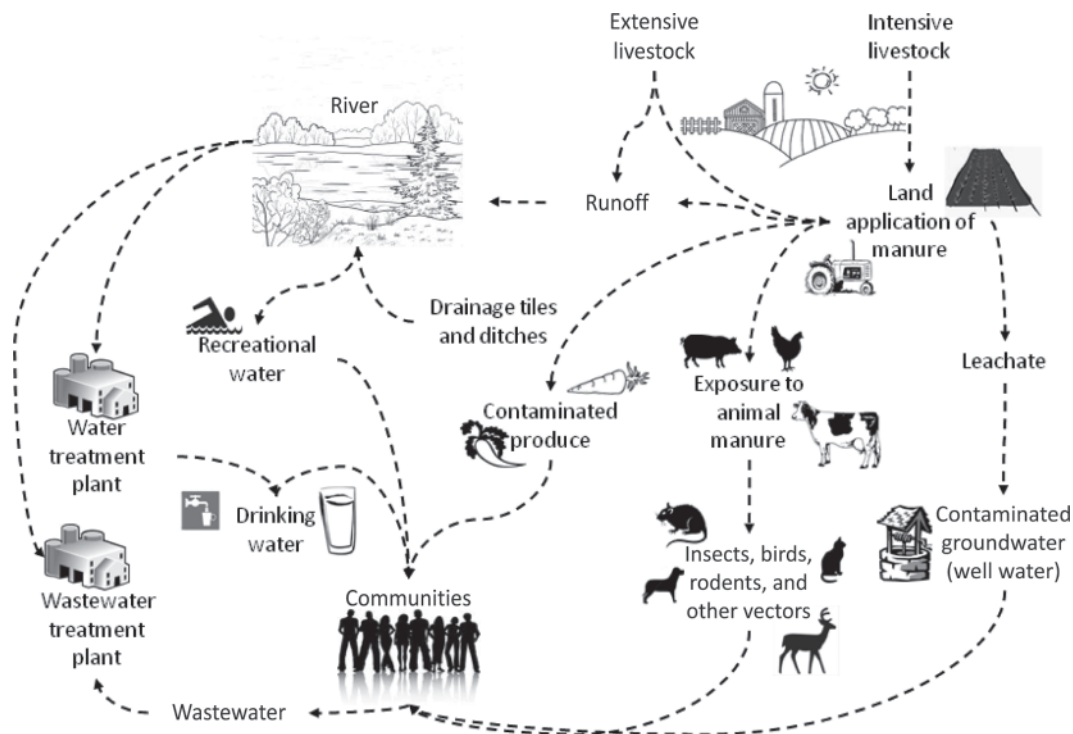
Globally, an estimated 3.2% of human deaths are attributable to unsafe water, a problem particularly acute in rural areas in the developing world (World Health Organization, 2009). However, only a small fraction of these deaths are directly attributable to zoonotic diseases from livestock. Livestock manure can harbor a wide range of bacterial, viral, and parasitic pathogens (Table 1). These microbial pathogens can be mobilized from land-applied manure to surface water, leach into groundwater, or contaminate vegetable crops via irrigation (Figure 1). Pathogen mobility from intensive and extensive livestock operations is complex, and exposure of adjacent water sources occurs through multiple pathways.

Microbial contamination of vegetables (e.g., spinach, tomatoes, lettuce, bean sprouts, cucumbers, onions, celery, broccoli, and asparagus)

Table 1. Selected pathogenic organisms associated with waterborne illness and livestock operations

Pathogen	Infectious dose	Incubation period	Pathogen survivability <sup>1</sup>	Host range/Reservoir	Mortality in humans
<b>Bacteria</b>					
<i>Bacillus anthracis</i>	8,000 to 50,000 cells	2 to 5 days	S: >10 years	Humans, cattle, swine, goats, sheep, horses/Soil	High
<i>Brucella</i> spp.	Unknown	5 to 60 days	S: 4 to 180 days; M: 20 to 240 days; W: 1 to 80 days	Humans, cattle, swine, goats, sheep, deer, caribou, elk, dogs, coyotes/Cattle	Rare
<i>Campylobacter jejuni</i>	≤500 cells	1 to 10 days	W: 120 days; S: 56 days; M: >112 days; C: 7 days	Humans, cattle, swine, goats, sheep, poultry, rodents, birds, household pets/Cattle, swine, sheep, poultry, household pets, rodents, birds	Rare
<i>Clostridium</i> spp.	Toxin is potent	0.5 to 3 days	S: 60 to 120 days; M: indefinite; W: >100 days	Humans, animals/Intestine of animals and contaminated soil	Rare
<i>Coxiella burnetii</i>	10 cells	2 to 3 weeks	NA <sup>2</sup>	Humans, cattle, sheep, goats/Sheep, cattle, goats, especially at parturition	Rare
Enterohemorrhagic <i>Escherichia coli</i>	5 to 10 cells	2 to 8 days	W: >300 days; S: >300 days; M: >365 days; C: 7 days	Humans, cattle, swine, goats, sheep, poultry/Cattle	Rare
<i>Leptospira</i> spp.	Unknown	4 to 19 days	NA	Humans, cattle, swine, horses, dogs, rats, wild animals/Livestock, rodents	High
<i>Listeria monocytogenes</i>	Unknown	3 to 70 days	W: 93 days; S: 128 days; M: 8 days	Mammals, birds, fish, crustaceans, insects/Domestic and wild mammals, fowl, aborted fetuses of livestock	High
<i>Shigella dysenteriae</i>	10 to 1,000 cells	1 to 7 days	NA	Humans, cattle, swine, other animals/Diseased cattle, swine, other mammals	Rare
<i>Salmonella</i>	10 to 1,000 cells	0.25 to 14 days	W: >182 days; S: >84 days; M: >196 days; C: 7 to 14 days	Humans, cattle, swine, other animals/Diseased cattle, swine, other mammals	Low
<i>Vibrio cholerae</i>	100,000 cells	2 to 3 days	NA	Humans, fish/Humans	Rare
<b>Protozoa</b>					
<i>Balantidium coli</i>	Unknown	4 to 5 days	NA	Humans, swine/Primarily swine, also rodents	Rare
<i>Cryptosporidium parvum</i>	130 cells	1 to 12 days	W: >84 days; S: >84 days; M: >84 days; C: 28 days	Humans, small and large mammals, poultry, fish, reptiles/Cattle, other domestic animals	Rare
<i>Giardia lamblia</i>	1 to 10 cells	3 to 25 days	W: 77 days; S: 49 days; M: 365 days; C: 14 days	Humans, wild and domestic animals, household pets/Wild and domestic animals	Rare
<i>Toxoplasma gondii</i>	Unknown	10 to 23 days	NA	Humans, felines, most warm-blooded animals, birds/Cats, cattle, swine, chickens, sheep, goats, rodents, birds	Rare
<b>Virus</b>					
Hepatitis A virus	10 to 1000 cells	30 days	W: >48 days; S: >91 days	Humans, swine, rodents, chickens/Possibly swine	Low
Hepatitis E virus	Unknown	14 to 63 days	S: >70 days	Humans, swine, rodents, chickens/Possibly swine	Low
Influenza A virus	2 to 790 cells	1 to 4 days	NA	Humans, swine, horses, domestic and wild avian species/Humans, livestock	Med
Lymphocytic choriomeningitis virus	Unknown	8 to 21 days	NA	Humans, swine, household pets, rodents/Rodents, swine, household pets	Low
SARS <sup>3</sup> coronavirus	Unknown	6.4 (mean) days	NA	Humans, swine, chicken, ferrets, cats, macaques/Unknown	Low
West Nile Virus	Unknown	3 to 14 days	NA	Mammals, reptiles, birds/Birds	Low

<sup>1</sup>W = water; S = soil; M = manure; C = compost.<sup>2</sup>NA = not assessed.<sup>3</sup>SARS = severe acute respiratory syndrome.



**Figure 1.** Avenues of flow of microbial contaminants from intensive and extensive livestock operations to surface and groundwater. Primary sources include 1) runoff from snowmelt or rainfall events, 2) land application of manure, and 3) leachate from livestock operations into groundwater sources. Note that proper treatment of wastewater and drinking water plays a critical role in preventing community infections. Pathogens acquired from recreational water, contaminated produce, or contact with livestock or wild vectors are not precluded by these controls.

has increasingly been associated with infectious disease outbreaks (Sivapalasingam et al., 2004). Fresh produce is frequently consumed raw, increasing the likelihood that viable pathogens will be consumed. Bacteria, including *Salmonella*, *Shigella*, *Listeria*, *Aeromonas*, *Campylobacter*, *Yersinia*, *E. coli* O157:H7, and *Staphylococcus*, as well as viruses, including hepatitis A and Norwalk, have been isolated from fresh produce linked to outbreaks. Some bacteria are capable of multiplying in fresh produce (Samish et al., 1963), increasing the likelihood that people will receive an infective dose of the pathogen. Where livestock are the source of produce contamination, it is most frequently as a result of contact of vegetables with manure or manure-contaminated water. However, microbial contamination of produce can occur throughout the supply chain, and instances of adulteration of produce during harvesting, postharvest handling, distribution, and serving are well documented (Beuchat and Ryu, 1997; Schwaiger et al., 2011). This requires that steps be taken to reduce the risk of produce contamination throughout the production and distribution process. Thus, proper manure handling in livestock operations is just one of the critical control points required to avoid foodborne illness.

Livestock-related pathogens can also be transferred through manure or water to other hosts, including pets, rodents, insects, and wildlife. Many pathogens (approximately 77%) that infect livestock also infect wildlife, which distribute them over a broader geographical area. In many instances, zoonotic diseases may originate in wildlife (Decker et al., 2010) and inadvertently be transmitted to livestock populations, where they are amplified and more likely to come in contact with humans. In extensive livestock production, wildlife and domestic livestock frequently

occupy the same geographical location, share a common water source, and come into frequent contact, increasing the likelihood of transmission. In some instances, transmission can be reduced by controlling the vector, as is practiced in rodent control programs. However, in many instances, vector control is impractical or considered unethical. In these instances, enhanced biosecurity through controlled confinement of livestock may reduce contact with potential vectors. However, even with the high levels of biosecurity practiced in confined poultry operations, transmission of the avian flu virus has been attributed to wild birds serving as vectors (Otte et al., 2007).

Contamination of groundwater with pathogens originating from livestock is also a concern (Figure 1). Groundwater contamination by pathogenic bacteria and viruses has been responsible for large outbreaks of waterborne disease (Stevik et al., 2004). Numerous factors can influence the downward migration of microbial cells within the soil column, including size, shape, hydrophobicity, and electrostatic charge. Bacteria (1 to 6  $\mu\text{m}$ ) and viruses (20 to 120 nm) are smaller and more amenable to downward movement in the soil column than are larger protozoa (10 to 15  $\mu\text{m}$ ) because they are less likely to be strained or filtered from porosity channels. Surface charge can also influence absorption or desorption of microbial cells to the soil matrix, with cells with negative surface charges exhibiting greater downward migration. Actively mobile microbes may also move through soil subsurface pores via chemotaxis.

The degree of growth and the duration of survival of microbes in the subsoil can also influence their likelihood of reaching groundwater in a viable state. Several factors, including oxygen concentration, salinity, pH,



**Figure 2.** Pathogens could flow with the current over long distances (source: K. Munns; used with permission).

nutrient availability, temperature, and moisture, can influence growth and viability. The properties of the soil itself, including porosity, chemical composition, and degree of saturation, can affect pathogen migration. Microbial movement is generally much greater in saturated than unsaturated soils because pathogens move with groundwater flow. Microbial cells are more likely to bind to soil particles in unsaturated soils and may accumulate in this zone above the groundwater level. However, during significant rainfall events, groundwater tables may rise and downward movement of surface water may cause detachment of viable pathogens from subsurface soils, resulting in their migration into groundwater.

Pathogens do not enter groundwater sources only through soil infiltration because unsealed well heads provide them with direct access to groundwater through flowing surface water, a factor in the Walkerton outbreak. Contamination of wells with surface water is of particular concern during high rainfall events, when wells may become flooded or barriers preventing the flow of surface water into the well are breached. In areas where this occurs, household water treatment is often the last line of defense in preventing waterborne outbreaks in rural areas.

Microbial contamination of surface water can originate from both extensive and intensive livestock production systems. In extensive grazing systems, livestock frequently have direct access to streams and rivers, and defecation into water courses is not uncommon (Davies-Colley et al., 2004). This has obvious negative implications for water quality because pathogens flow with the current, travel long distances, and come in direct contact with humans using water for recreational purposes (Figures 1 and 2) In intensive production systems, pathogens most often enter surface water during intensive rainfall events that exceed the capacity of manure catchment basins, or that promote the overland flow of pathogens from

land-applied manure. Proper water treatment measures are critical to ensuring that infection levels of viable pathogens do not enter the drinking water supply. Heavy rainfall events often affect more than just livestock operations that reside within water catchments. Other sources of pathogens, including urban wastewater, sewage sludge, septic tank discharge, and feces from indigenous wildlife, can also act as a source of pathogens during overland flow or flooding events. There have been examples in which livestock were initially equated with waterborne infectious disease outbreaks that, on further investigation, were found to originate from wildlife or urban sources (Stirling et al., 2001). When outbreaks have been linked to livestock, it has frequently been a result of the failure of multiple components within the water quality assurance continuum. For example, the Walkerton outbreak occurred as a result of a heavy rainfall event that allowed livestock manure to flow directly into an unsealed well connected to the town's water supply. Inadequate chlorination allowed viable pathogens to be distributed throughout the town's waterworks, leading to widespread infection in the community. When such unfortunate events occur, rapid identification of the point-source of the pathogens involved in the disease is integral to stemming the tide of infection. Consequently, source tracking of microbial pathogens is one of the most rapidly evolving areas of water quality assurance.

### Source Tracking of Pathogens in Water

In mixed-activity watersheds, the deployment of effective water-protection strategies are predicated on distinguishing the multiple potential sources of fecal pollution. Source tracking techniques are advancing most rapidly in the field of molecular biology, where the toolbox of "microbial



**Figure 3.** For the same watershed, livestock, wildlife, and humans could be sources of fecal pollution. (source: K. Munns; used with permission).

source tracking” methods is being combined with geostatistical methods to shed new light on the role of livestock as a source of pathogens both spatially and seasonally. The correlation between “traditional” indicators of water quality (e.g., coliforms, fecal coliforms, *E. coli*, enterococci) and the occurrence of waterborne pathogens that negatively affect human health is often weak (Wilkes et al., 2009). Consequently, there is a need to develop new metrics to assess the potential risk that livestock operations impose on water quality.

Of the molecular techniques, perhaps the quantitative polymerase chain reaction (PCR) procedure is the most popular because it has been used to quantify various bacteria (Khan et al., 2009), viruses (Fong and Lipp, 2005), protozoa, and toxic phytoplankton (Humbert et al., 2010). This approach has the advantage that it can specifically quantify the infectious agent even when not amenable to culturing in the laboratory. Extraction of representative DNA from the aquatic environment is paramount to the quantification process, and care must be taken to ensure that matrix components, such as humic acids, do not interfere with the PCR reaction (Girones et al., 2010). One of the limitations of PCR is that without the isolation of individual cells and extensive staining procedures, the technique is unable to differentiate viable from nonviable cells (Rudi et al., 2005). This may result in an overestimation of risk, particularly if the water treatment procedures kill the pathogen without disrupting the nucleic acid structure.

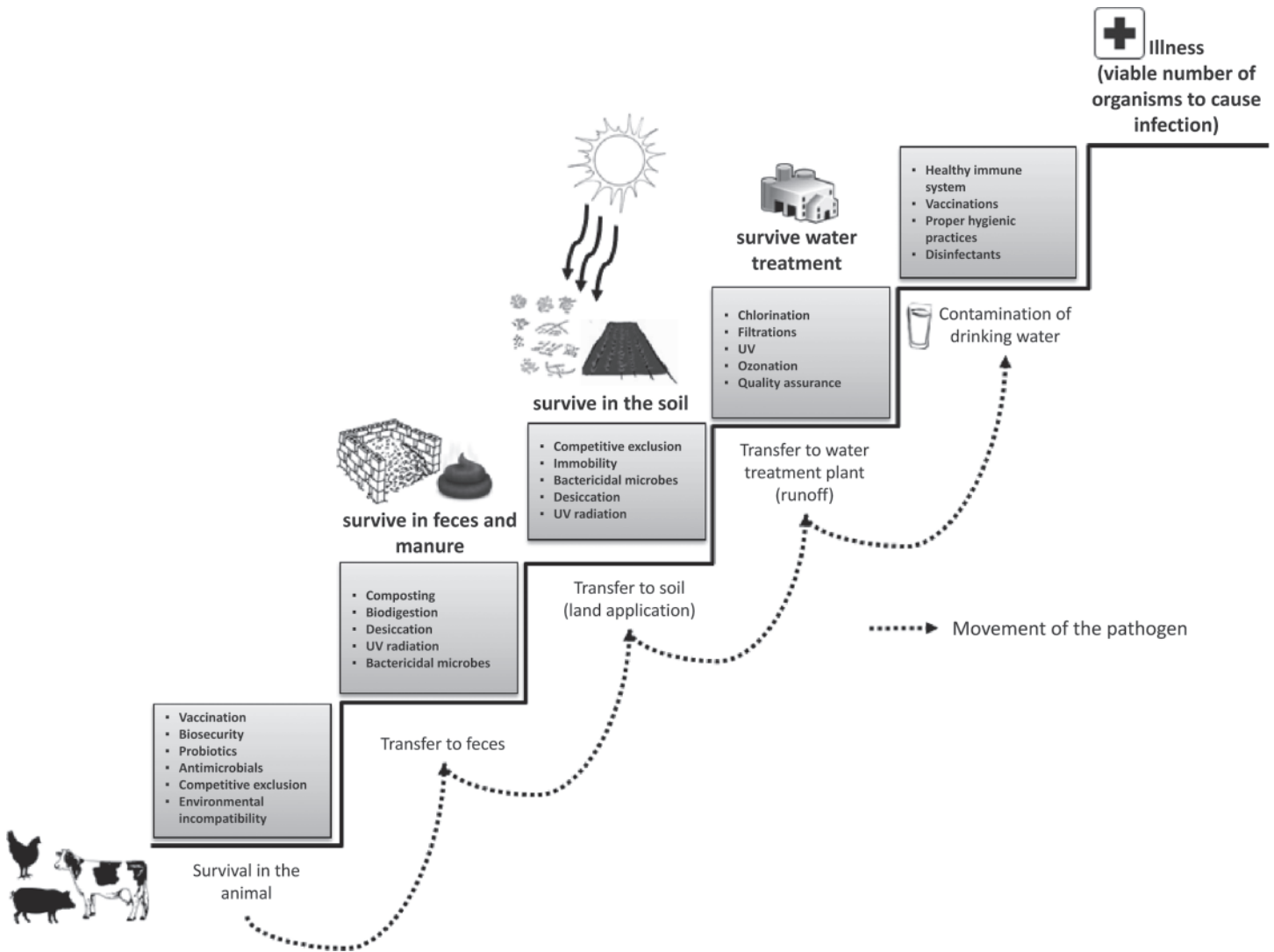
Methods to distinguish and identify the varied point and nonpoint sources of microbial contamination could help guide the implementation of targeted mitigation measures. Source-specific attributes of the enteric flora that distinguish the origin of feces in contaminated water could prove useful in identifying undesirable management practices. For example,

source-specific variation in the sequence of the 16S rRNA gene of *Bacteroidales* has been used to develop markers to detect fecal contamination originating from ruminants, humans (Bernhard and Field, 2000), and geese (Fremaux et al., 2010). Host mitochondrial DNA in fecal material is another useful marker for identifying point-sources of fecal contamination (Baker-Austin et al., 2010).

A variety of molecular and phenotypic methods have been used to compare microbial isolates (*E. coli*, enterococci) collected from feces and water and thereby infer the source (Pillai and Vega, 2007). However, the robustness of this approach depends on the establishment of a comprehensive library of microbial-source information through the collection of a representative set of fecal samples from the environment of interest (Lyautey et al., 2010). Consequently, the reference library is relevant only to the area from which it has been derived and cannot be readily extrapolated to a broader geographical area.

In contrast, library-independent methods that detect source-specific signatures in DNA extracted directly from water are not limited by the above constraints. Source assignment studies in watersheds with abundant and varied avian and mammalian wildlife will, in particular, benefit from an increased availability of diverse markers for microbial source tracking. These could prove useful in differentiating fecal pollution from livestock, wildlife, and humans. Microbial source tracking methods have been used to elucidate fecal pollution sources in studies that vary in size and setting, including surface water affected by agricultural effluent and sewage (Fremaux et al., 2009).

In many watersheds, different potential fecal sources (livestock, wildlife, humans) are in close proximity, and defining land use is a key com-



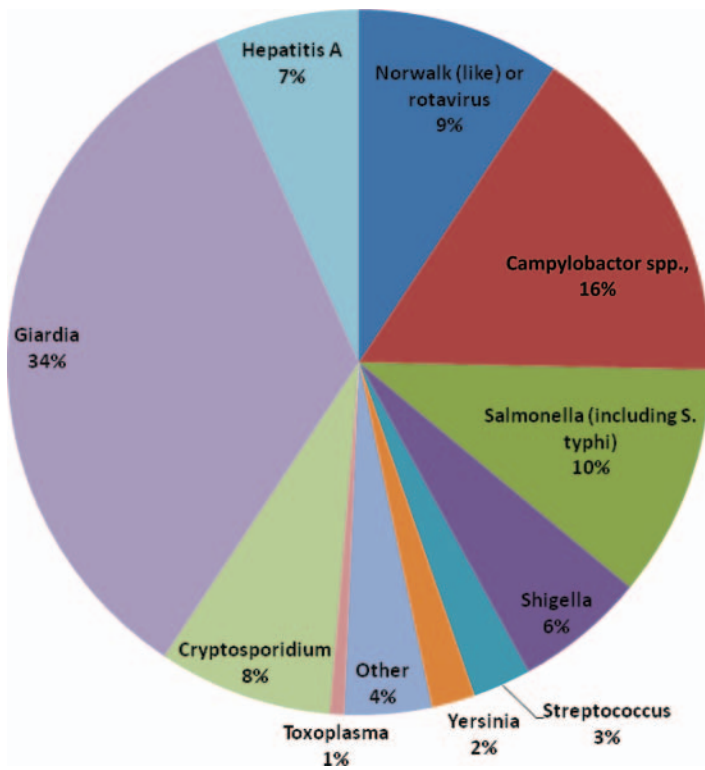
**Figure 4.** Influence of key factors on the infectivity of waterborne pathogens during potential transfer from livestock to humans. Note that a number of factors can influence the likelihood of survival of a pathogen along the livestock farm-to-consumer continuum. Management practices are used throughout the continuum to minimize the risk of humans contracting an infectious pathogen. Properties of the microbe and status of the infectious host ultimately influence the likelihood of an infection occurring.

ponent of any microbial source tracking initiative (Figure 3). This can be done by combining various methodologies, including discrete (point observations from a land use survey) and continuous (raster satellite imagery land use) Geographic Information System data. Satellite imagery can be used to produce land use data sets, and discrete land use observation techniques can be used to delineate locations of specific farming operations and other point data. These types of data can be combined with a digital elevation model to define distances upstream to a particular land use, percent coverage of specific land uses, and their densities within watersheds (Lyautey et al., 2010).

### Factors Influencing Pathogen Proliferation and Survival

The survival and proliferation of pathogens in the primary host depends on a number of factors, including host immune status, the degree of

microbial competition in the digestive tract, host-pathogen compatibility, dietary composition, and the use of additives, such as probiotics or antimicrobials (Figure 4). Phenotypic, as opposed to genotypic, characteristics are usually used to identify pathogens in clinical settings. For example, from 1974 to 2001, *Giardia* was identified as the most common pathogen associated with waterborne outbreaks in Canada (Figure 5). *Giardia* was linked to these outbreaks through microscopic examination of *Giardia* cysts in fecal samples from symptomatic patients. More recently, source tracking sequencing has shown that at least 8 distinct genetic lineages of *Giardia* exist, with each lineage being most commonly associated with a specific host, whether it is humans, cattle, rodents, or seals (Feng and Xiao, 2011). Consequently, although these lineages are indistinguishable by light microscopy, they may be genetically host specific, raising the possibility that strains that reside in livestock may not be those that readily infect humans. Similar observations have been made for bacterial pathogens in livestock, including *E. coli* O157:H7 (Kim et al., 1999) and *Salmonella* (van Duijkeren et al., 2002), suggesting that the pathogen-host relation-



**Figure 5.** Types of pathogens identified in outbreaks in which a single pathogen was identified from drinking water in Canada from 1974 to 2001. (Other bacteria include *Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter hafniae*, pathogenic *Escherichia coli*, *Pseudomonas* spp., and *Staphylococcus aureus*.) Adapted from Schuster et al. (2005). Permission granted by the Canadian Public Health Association for publication of adapted figure.

ship is far more intimate than previously supposed. In many instances, it may be the types of virulence factors the pathogen has acquired that determine the severity of disease, as was the case with the *E. coli* O104:H4 outbreak in Germany.

Assuming that the pathogen persists in the host, it must survive in the manure if it is eventually to enter a watercourse. Although the populations of most pathogens decline in manure, a number of pathogens can persist for prolonged periods (Table 1). Our laboratory has measured increases in *E. coli* in cattle manure shortly after defecation. Pathogens that have resilient components within their life cycle, such as endospores, oocysts, or cysts, may persist in manure for even longer periods. However, conditions in manure are not always favorable for microbial survival. Manure is often highly alkaline (pH  $\geq$  8) owing to its greater ammonia content and is not amendable to the growth or survival of many pathogens. Temperatures also often exceed levels that are optimal for microbial survival as manure heats. Desiccation of manure can induce water stress in pathogens, whereas water saturation can create anoxic conditions, placing some anaerobic pathogens at a competitive disadvantage to other anaerobic microorganisms. Microbial densities in manure are high, and competition for nutrients and resources between pathogen and nonpathogen communities could further contribute to the temporal decline in pathogens frequently observed.

If the manure does not come in immediate contact with a water source, pathogens must survive in the soil after land application of manure if they are to contaminate water (Figure 4). As manure is dispersed, a lack of nu-

trient availability may be a factor that limits pathogen persistence. Surface dispersion of manure can also reduce pathogen survival through increased desiccation, exposure to UV radiation, and unfavorable temperatures.

Once in water, pathogens must remain viable upon consumption by humans to elicit a disease response. Nutrients become increasingly diluted with increasing volumes of water. Predatory protozoa may further reduce pathogen numbers in aquatic environments, although in some instances, they may serve as a refuge for internalized pathogens. Pathogens may also become associated with stream sediment, where they pose little risk unless the sediments are disturbed. Technologies, including filtration and treatment with chlorine, ozone, and ultraviolet light, are used to reduce pathogens in drinking water (Figure 4), with techniques often applied sequentially to minimize viable pathogens from entering drinking water.

Finally, in the event that water contaminated with pathogens is consumed, the impending host must be susceptible to infection for disease to develop (Figure 4). Pathogens are frequently consumed without eliciting a disease response, and it is well known that asymptomatic carriers in the human population can shed pathogens and spread disease. For disease to develop, individuals must receive a minimal infective dose, a value that differs widely among pathogen types (Table 1). Individuals that are immunocompromised are particularly susceptible to waterborne infections. A lack of sanitation and hygienic practices can also increase the spread of waterborne disease. In many instances, waterborne diseases that have been linked to pathogens from livestock have been amplified as a result of person-to-person transmission.

## Reducing the Viability and Mobility of Zoonotic Pathogens in Livestock Waste

In many production systems, waste is stored before being applied to fields. There is an opportunity during manure storage to reduce pathogens, making the material more benign before release into the broader environment (Topp et al., 2009).

### Stockpiling or Composting of Manure

Stockpiling or composting of manure can be an effective method of lowering pathogen viability in manure (Figure 6). Temperatures in the interior of stockpiles or compost rows commonly exceed 55°C for prolonged periods and reach as high as 70°C for short periods. Most of the pathogens listed in Table 1 are killed because of heat exposure, with the exception of spore-forming bacteria, although we have even measured a reduction in the viability of spores under optimal composting conditions. Active turning during composting increases the likelihood that pathogens will encounter temperatures that are not conducive to survival. However, compost can be amorphous, and even with mixing, regions may not reach a temperature sufficient to kill all pathogens. Consequently, although composting will reduce the number of pathogens in manure, it is unlikely to eliminate them.

### Lagoon Storage

Waste from confined livestock operations is often stored as slurry in lagoons. Storage periods may be for several months, notably over winter. Unless the material is mechanically agitated under static conditions, it rapidly becomes anaerobic. Under these conditions, products of anaerobic fermentation, such as volatile fatty acids that are toxic to bacteria, accumulate (Conn et al., 2007). It is interesting that during slurry storage, the



**Figure 6.** Manure composting decreases pathogen viability before application to fields (source: B. Lee; used with permission).

abundance of genes associated with virulence rapidly declines in porcine *E. coli*, suggesting that storage is associated with decreased zoonotic risk (Duriez et al., 2008).

### **Anaerobic Digestion**

Anaerobic digestion of manure as a means of biogas production is becoming an increasingly common practice in livestock operations. Anaerobic digesters may operate under mesophilic (30 to 38°C) or thermophilic (50 to 55°C) conditions, with thermophilic operation eliminating most pathogens (Table 1). Anaerobic digesters that are run in a batch, as opposed to a continuous mode, also tend to be more effective at eliminating pathogens because they prolong the time the pathogens are exposed to adverse conditions before flow from the reactor. Production of volatile fatty acids and low pH can also limit the survival of pathogens, but these conditions may also be less favorable for biogas production. Although biodigester sludge typically contains fewer pathogens than manure, it must still be land applied, with the understanding that residual levels of pathogens may be present.

### **Timing of Land Application**

Prolonging the retention of pathogens within the soil environment can reduce their viability; consequently, the timing of manure application relative to precipitation events influences the likelihood of viable pathogens entering surface or groundwater. Manure should be applied to the land during the dry season, when major precipitation events are unlikely. It should not be applied to frozen ground or during the winter because higher numbers of viable pathogens are likely to enter waterways during the spring snowmelt. Incorporation of manure shortly after application reduces not only odors, but also the likelihood of pathogens coming in contact with vectors that could result in further dissemination.

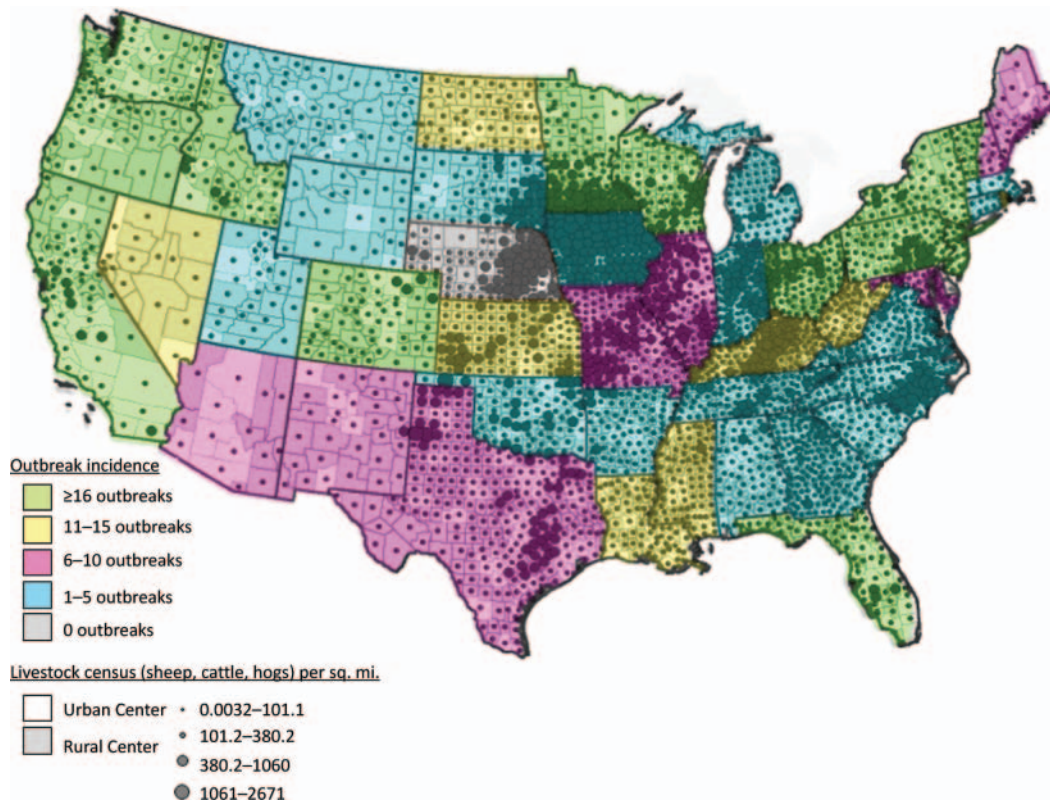
### **Surface Versus Injection Application**

Surface runoff from manured fields into adjacent water is a key high-risk exposure pathway that must be managed. Critical management factors to be considered include the slope of the land, antecedent moisture at the time of application, the risk of precipitation following application, and the method of soil application. The risk of runoff following application of slurry is minimized if the material is injected or deposited a few inches beneath the soil surface. The rate of application must not exceed the moisture-holding capacity of the soil because the risk of preferential flow to shallow groundwater or drainage tiles increases. In the case of solid manure, surface application is the norm, followed by some degree of soil incorporation to reduce the risk of runoff.

### **Establishment of Riparian Areas and Constructed Wetlands**

Riparian zones and constructed wetlands act as filters to the movement of microorganisms and nutrients into adjacent watercourses. Riparian zones also provide natural habitat and foster biodiversity, an important ecological service. Pathogens are retained in this environment for extended periods and are exposed to conditions less suitable for survival. Microbial competition in this environment is high, and pathogens originating from the intestinal environment may lack the competitiveness of the epiphytic microbes that reside in wetlands. Protozoal predation may also reduce pathogens in the water column, and pathogens may not readily integrate into the microbial biofilms associated with persistence. Constructed wetlands have been shown to have a pathogen removal efficiency that is comparable with that of conventional biological wastewater treatment systems.





**Figure 7.** Graphical overlay of the occurrence of infectious disease outbreaks and the density of livestock in the United States. Note that livestock densities are not clearly associated with the occurrence of infectious outbreaks, attesting to the multifaceted nature and complexity of food- and waterborne disease. Adapted from Craun et al. (2010; *Clinical Microbiology Reviews*, vol. 23, pp. 507–528, doi: 10.1128/CMR.00077-09, with permission from the American Society for Microbiology) and United States Department of Agriculture (2002).

## Reducing the Occurrence of Pathogens in Livestock

### Vaccination Programs

Vaccination programs are routinely used to prevent bacterial and viral infections in livestock. However, many of the pathogens carried by livestock that contaminate water do not cause performance-limiting disease. Consequently, there is little financial incentive for pharmaceutical companies to produce, or for producers to purchase, vaccines that limit the occurrence of many intestinal pathogens. Efforts to develop vaccines against microbes that reside in the intestinal tract have proven to be notoriously difficult because eliciting a specific immune response in this environment presents a significant challenge. Increasing concerns over food safety have led to the development of commercial vaccines against *E. coli* O157:H7, *Salmonella*, and *Leptospira*, but uptake of these vaccines has been limited by a lack of financial return to the producer. Introduction of financial incentives for producers to implement sound food safety practices could overcome this adoption barrier.

### Biosecurity

Biosecurity measures have been used to limit the spread of pathogens in livestock production since the beginning of confined livestock production. These measures are most amenable to poultry and swine operations and less suitable for extensive systems used in ruminant production. In these sys-

tems, livestock imports are closely monitored for pathogens and often subject to quarantine, or they must be certified as disease free before entrance into the herd or flock. Feed must be monitored for pathogen contamination, and biosecure programs must be accompanied by vector control measures that limit the introduction of pathogens by insects or rodents. Compared with free-range production, biosecure production of poultry and swine has been shown to reduce pathogen loads of bacteria such as *Campylobacter*, likely as a result of differences in vector contact. However, even with biosecurity measures, absolute elimination of pathogens is not ensured, necessitating the multiple-barrier approach (Figure 3).

### Competitive Exclusion and Probiotics

Considerable effort has been devoted to the development of microbial probiotics that exclude or reduce microbial pathogens within the digestive tract of livestock. This approach has been broadly used to limit the occurrence of *Salmonella* and *Campylobacter* in poultry, enterotoxigenic *E. coli* in swine, and *E. coli* O157:H7 in cattle. However, the impact of this approach on many of the pathogens listed in Table 1 is unknown. *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Enterococcus*, and *Bacillus* are bacteria that are commonly used as probiotics for livestock. Several of these bacteria produce antimicrobial peptides known as bacteriocins that are toxic to bacterial pathogens. Others may compete with pathogens for nutrients within the intestinal tract or block adhesion sites on the intestinal epithelium, thereby limiting the persistence of patho-

gens in the intestinal tract. However, there is little evidence that probiotics have a detrimental effect on pathogens that enter manure in a viable state, still necessitating the proper manure handling practices described above.

## Antimicrobials

In North America, antimicrobials are often included in the water or diet of poultry, swine, and beef cattle at subtherapeutic levels. Several of these antimicrobials have been shown to reduce the occurrence of pathogens in livestock, such as *Salmonella* in poultry and swine and *E. coli* O157:H7 in beef cattle. However, there is a growing concern over the subtherapeutic use of antimicrobials in livestock because it may contribute to the development of resistance to antimicrobials used to treat infection in humans. As a result, it is unlikely that the subtherapeutic use of antimicrobials will be viable for pathogen control in livestock over the long term. Other forms of biological control, such as the use of pathogen-specific bacteriophages, may gain popularity in a post-antimicrobial era. Bacteriophages have the advantage that they specifically target pathogens within the microbial community while leaving commensal bacteria unharmed. Broad-spectrum antimicrobials often remove both pathogenic and commensal bacteria from the intestinal tract, thereby leaving livestock more susceptible to posttreatment infection by pathogens.

## Conclusions

It is clear that many waterborne infections in humans that arise from livestock pathogens result from a series of unfortunate events or failures in the water-quality protection continuum. Given that pathogens contributing to the contamination of water may arise from a variety of point or diffuse sources (i.e., wildlife, livestock, humans), it is perhaps not surprising that the relationship between livestock densities and the occurrence of water- and foodborne outbreaks in the United States is not immediately obvious (Figure 7). That being said, many pathogens that reside in livestock are infectious to humans and can cause serious disease if they enter drinking water systems unabated. Adherence to best management practices that limit the survival and persistence of pathogens in livestock, manure, and the greater agricultural environment are essential to reducing the impact of livestock on water quality. Only through practicing due diligence will livestock production cease to be the first focal point examined in the event of waterborne infectious disease outbreaks.

## Acknowledgments

Our research has been partially funded by Agriculture and Agri-Food Canada through the Agricultural Policy Framework and the Growing Forward programs. We thank the many technologists, students, and postdoctoral fellows who have made invaluable contributions to our research. The editorial assistance and photography of Krysty Munns (Agriculture and Agri-Food Canada, Lethbridge Research Centre) is also gratefully appreciated.

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