MINIREVIEW

The ecological habitat and transmission of *Escherichia coli* O157:H7

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Abstract

Since its first description in 1982, the zoonotic life-threatening Shiga toxin-producing *Escherichia coli* O157:H7 has emerged as an important food- and water-borne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome in humans. In the last decade, increases in *E. coli* O157:H7 outbreaks were associated with environmental contamination in water and through fresh produce such as green leaves or vegetables. Both intrinsic (genetic adaptation) and extrinsic factors may contribute and help *E. coli* O157:H7 to survive in adverse environments. This makes it even more difficult to detect and monitor food and water safety for public health surveillance. *E. coli* O157:H7 has evolved in behaviors and strategies to persist in the environment.

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC), in particular serotype O157:H7, is a highly pathogenic subset of Shiga toxin-producing *E. coli* (STEC) that causes gastrointestinal illnesses ranging from aqueous and bloody diarrhea to hemorrhagic colitis in humans (Werner *et al.*, 1990; Karmali, 2009). Hemolytic-uremic syndrome (HUS) is a potentially life-threatening complication that can arise from STEC infection. The production of Shiga toxins (Stx) is a key factor contributing to the development of HUS (Griffin & Tauxe, 1991). In addition to Stx, a type III protein secretion system (T3SS), through which the pathogen translocates effector proteins into host cells, causes attaching and effacing (A/E) lesions (Karmali, 2004). The genes required for A/E lesions are encoded within a chromosomal pathogenicity island named the locus of enterocyte effacement (LEE; McDaniel *et al.*, 1995). The LEE encodes T3SS, an adhesin (the intimin Eae) and its receptor (Tir) required for intimate adherence to epithelial cells, and effector proteins translocated through the T3SS that are injected into the host cell (Naylor *et al.*, 2005). The genome sequences of O157:H7 strains isolated from the major outbreaks share about 75% of a highly conserved sequence backbone of the *E. coli* chromosome (Hayashi *et al.*, 2001; Perna *et al.*, 2001). The remaining O157:H7-specific sequences are named O islands, most of which are horizontally transferred and include other virulence genes in addition to *stx* and LEE genes (Croxen & Finlay, 2010).

Cattle are recognized as the main reservoir for *E. coli* O157:H7 resulting in zoonotic transmission by consumption of undercooked meat or dairy products inadequately pasteurized and contaminated with bovine feces (Jay *et al.*, 2004; Kassenborg *et al.*, 2004). Here, we review the established and putative environmental behaviors of *E. coli* O157:H7 and present potential reservoirs and ecological niches where EHEC may persist in the environment.

Escherichia coli O157:H7: an emerging food- and water-borne pathogen

Escherichia coli O157:H7 and other serotypes of the STEC group are naturally acquired infections that have been

FEMS MICROBIOLOGY LETTERS

detected in a wide spectrum of animal species (cattle, sheep, goat, deer, moose, swine, horse, dog, cat, pigeon, chicken, turkey, gull) sometimes even with considerable prevalence (Beutin *et al.*, 1993; Wieler *et al.*, 1996). In particular, cattle have been identified as major reservoirs of STEC strains that are highly virulent in the human host (e.g. EHEC O157:H7). However, in contrast to the human host, most STEC infections of animals are clinically asymptomatic (Hancock *et al.*, 2001; La Ragione *et al.*, 2009). *Escherichia coli* O157:H7 infections in humans often occur through consumption of contaminated food products derived from cattle. Analysis of 90 confirmed *E. coli* O157:H7 outbreaks that occurred between 1982 and 2006 in Canada, Great Britain, Ireland, Japan, Scandinavia, and USA, indicated that 20% of cases were the result of secondary spread (Snedeker *et al.*, 2009). The authors found that the source of transmission was food and dairy products in 54%, water and environment in *c.* 10%, and animal contact in *c.* 8%. Consumption of any food or beverage contaminated with animal manure/feces can result in disease. For this reason, food sources causing illness secondary to *E. coli* O157:H7 outbreaks have changed in the past several years. Interestingly, fresh greens, fruits, and vegetables have become important sources of human infection. In the USA, *E. coli* O157:H7 infections from contaminated fruits and vegetables increased from 11% to 41% from 1998 to 2007 (Xicohtencatl-Cortes *et al.*, 2009). Contamination of fresh produce was associated with fecal contamination in agricultural irrigation water or runoff.

Along the same line, the recent enterohemorrhagic *E. coli* strain O104:H4 was implicated in the spring 2011 European outbreak, and its possible source was associated with raw vegetables (fenugreek seeds or sprouts) consumed raw or undercooked (King *et al.*, 2012). It was difficult to establish the link between vehicle, source, and cause of this STEC outbreak. This raised the importance of epidemiological and microbiological investigations in food and in the environment.

The global community has been experiencing food-associated outbreaks of non-O157 STEC for nearly two decades. As a result, increasing scientific evidence supports that *E. coli* non-O157 strains have a high prevalence in meat products and are equally capable of causing severe food-borne illness outbreaks (EFSA, 2011). Hussein (2007) reviewed reported levels of non-O157 STEC in whole cattle carcasses, ground beef, retail beef cuts, and sausage and found 1.7–58%, 2.4–30%, 11.4–49.6%, and 17–49.2%, respectively (Hussein, 2007). In Table 1, the reports of non-O157 STEC in foods, associated with human infections from numerous countries, reveal wide variation in prevalence estimates and in the major non-O157 serogroups reported (Hussein & Bollinger, 2005; Hussein & Sakuma, 2005; Erickson & Doyle, 2007; EFSA, 2012).

STEC non-O157 are less likely than O157 STEC to cause outbreaks or severe disease, and because they are more challenging diagnostically, many non-O157 infections may not be investigated fully, and their sources may thus remain undetermined. The incidence of non-O157 STEC declaration has increased as laboratories got involved in testing for those strains (Gould, 2009; Gould *et al.*, 2009; Stigi *et al.*, 2012). It is possible that the occurrence of non-O157 outbreaks is due to environmental persistence (Bolton *et al.*, 2011).

Escherichia coli O157:H7 outbreaks related to consumption of contaminated water or to the use of surface water for recreational purposes have been reported (Licence *et al.*, 2001; Olsen *et al.*, 2002; Bruneau *et al.*, 2004). In 1999, people became sick after drinking contaminated water in Washington County, New York, and swimming in contaminated water in Clark County, Washington. The outbreak in Walkerton, Canada (May 2000), related to consumption of drinking water that was contaminated by

Table 1. Incidence of STEC and the percentage of non-O157 in food related to human infections in different area around the world

Country/Geographic area	EIR of STEC	% of STEC Non-O157	Major non-O157 serogroups*	References
Australia	0.4	*c.* 42	O26, O111	Vally *et al.* (2012)
European Union†	1.1	*c.* 48	O26, O91, O103, O111, O113, O128, O145, O146	EFSA (2011)
Canada	3.0–6.0	< 15	O26, O91, O103, O111, O121	Thompson *et al.* (2005) and Gill & Gill (2010)
Japan	2.0–3.0	*c.* 40	O26, O111, O121, O103, O145	Kudoh *et al.* (1994) and Sakuma *et al.* (2006)
Argentina	10.4–12.2	*c.* 40	O8, O26, O113, O145, O174	Rivas *et al.* (2006) and Masana *et al.* (2011)
USA	1.04–1.2	*c.* 30	O26, O45, O103, O111, O121, O145	Brooks *et al.* (2005) and Scallan *et al.* (2011)

EIR, estimated incidence rate.

*Major non-O157 serogroups.

†Data based on 25 reporting countries from the European Union (EU).

feces caused 2300 disease cases (Hrudey *et al.*, 2003). *Escherichia coli* O157:H7 and *Campylobacter jejuni* were identified as the main pathogens responsible for these disease cases, and *E. coli* O157:H7 was responsible for seven deaths (Hrudey *et al.*, 2003). One factor explaining this contamination is the impact of climate. Indeed, surface water bodies can become contaminated by *E. coli* O157:H7 after a heavy rainfall or snowmelt, causing sewers to overflow or animal feces and manure to mix into surface water (Bruce *et al.*, 2003). Also, some human-to-human contamination cases were reported due to the presence of *E. coli* O157:H7 in water such as public pools or lakes (Bruce *et al.*, 2003; Varma *et al.*, 2003). Persons with diarrhea, especially children after shedding or changing soiled diapers, can contaminate recreational waters, and infection could occur by bathing and/or swallowing (Williams *et al.*, 1997; Bruce *et al.*, 2003; Varma *et al.*, 2003; Lee & Greig, 2008). Outbreaks associated with non-O157 STEC strains through water and produce have been documented (Doyle & Kaspar, 2010). Some strains of non-O157 STEC have been reported to survive in untreated well water for several months (Watterworth *et al.*, 2006). Persistence might be underestimated and could be comparable with O157.

How *E. coli* O157:H7 persists in natural ecological niches

Pathogenic *E. coli* strains can survive in open environments. The ability to use nutrients and to attach to surfaces plays a crucial role in their survival in open environments. *Escherichia coli* O157:H7 is found in soil, manure, and irrigation water or contaminated seeds. Also, it may colonize the interior of plants such as radish, lettuce, and internal plant compartments (Itoh *et al.*, 1998; Solomon *et al.*, 2002a, b). This makes it difficult to remove or kill these germs by washing and/or disinfection. Moreover, *E. coli* O157:H7 may be introduced into the food chain by splashing during rainfall or irrigation (Natvig *et al.*, 2002). Thus, from contaminated consumable products and the transfer to other products during food processing and packaging, the organism can be disseminated in the food production chain.

Escherichia coli from livestock feces is known to survive on grass pasture for at least 5 months, affording opportunity for *E. coli* O157:H7 to be recycled by animals (Avery *et al.*, 2004). Furthermore, the immediate environment of the animal and its feeding and drinking water are important sources of *E. coli* O157:H7 infection of cattle (reviewed in Fairbrother & Nadeau, 2006). The risk factors for carriage and infection of cattle are age, weaning, shipping, season, and feed composition and the bacteria's ability to persist in the farm environment for months (Fairbrother & Nadeau, 2006). Thus, *E. coli* O157:H7 represents an underestimated environmental risk.

Factors involved in environmental persistence of *E. coli* O157:H7

Bacteria constitute the most successful form of life in environmental habitats. This is due to the ability to respond to environmental stimuli by phenotypic plasticity. In their ecosystem cycle, bacteria like *E. coli* O157:H7 are subjected to fluctuations in environmental conditions in soils and water. Viability and growth of bacteria depends primarily on availability of essential nutrients including organic carbon, phosphate (P), and nitrogen (Peterson *et al.*, 2005). However, *E. coli* O157:H7 survives even at low density in oligotrophic environments such as surface water or groundwater that may be used as a raw water source for drinking water. This may be in river water containing low concentrations, 0.1−0.7 mg L^{-1}, of organic carbon (Leclerc, 2003).

The presence of *E. coli* O157:H7 in aquatic environments is the common denominator linking diverse transient habitats and transmission to animals and humans. Therefore, it appears essential to understand the aquatic ecology of *E. coli* O157:H7. This section highlights the adaptation of *E. coli* O157:H7 in aquatic environments and analyzes its survival and growth in sessile biofilm state and unfavorable conditions that support a viable but nonculturable (VBNC) state and free planktonic cells in water that may promote dissemination.

Aquatic *E. coli* O157:H7 – a starvation–survival lifestyle

Various studies have reported that survival times of *E. coli* O157:H7 strains in aquatic environments vary importantly, ranging from 2 weeks to over 10 months (Warburton *et al.*, 1998; McGee *et al.*, 2002). It is thus important to attempt to identify the factors responsible for its survival rate. Many factors, individual or combined, may influence the pathogen's survival such as the temperature, the bacterial cell numbers, strain variation, oxidative stress, nutrient availability, and the substrate type or source.

Unlike some Gram-positive bacteria that respond to starvation by producing spores or cysts, *E. coli* O157:H7, a nonsporulating bacterium, might respond more by an altered physiological or metabolic state instead of developing resistant structural modifications. On the other hand, *E. coli* growing under nutrient-sufficient conditions (i.e. animal feces or manure) accumulates reserve carbon sources that can be stored for use in nutrient-poor environments (Morita, 1997). Indeed, when nutrient

conditions in aquatic habitats are unfavorable, *E. coli* O157:H7 might reduce cell size, thereby increasing its surface/volume ratio and allowing more efficient uptake of poorly available nutrients. This physiological state resulting from an insufficient amount of nutrients is known as the starvation–survival state (Burgess, 1998). In this state, *E. coli* has evolved strategies to acclimate rapidly to surrounding environmental changes. The adaptive response begins by activation of enzymes required to catabolize available nutrients (Tao *et al.*, 1999). After that, *E. coli* may increase its production of toxins or antibiotics, to promote killing or invading of other cells in the environment (Miller *et al.*, 1989; Martin, 2004). Finally, the bacterium switches to a survival state, which enhances its resistance to many stresses and its ability to remain viable during long periods without nutrients (Siegele & Kolter, 1992).

Carbon and phosphate stress responses of aquatic *E. coli* O157:H7

Escherichia coli O157:H7 may survive and even grow in sterile freshwater at low carbon concentrations (Vital *et al.*, 2008). Bacteria respond to specific nutrient stresses by producing transport systems with increased affinities for the nutrients most easily exploited, and then, they express transport and metabolic systems for alternative nutrient sources. Thus, these bacteria may be able to escape starvation by more efficient scavenging of a preferred nutrient or by using another, relatively more abundant source.

When *E. coli* O157:H7 is starved or stressed, cells enter into a general stress response phase (Peterson *et al.*, 2005). At this point, the production of RpoS orchestrates the transcription of a series of overlapping networks of genes responsible for the *E. coli* general stress response (Hengge-Aronis, 2002). RpoS competes with the housekeeping sigma factors to direct a core RNA polymerase in the transcription of specific gene subsets, switching on the stress metabolisms to prepare the bacteria to resist multiple environmental stresses including starvation (Lange & Hengge-Aronis, 1991). High osmolarity, fluctuations in temperature, low pH, and low growth rate also induce the RpoS response in *E. coli* cells (Bearson *et al.*, 1996; Muffler *et al.*, 1996, 1997; Ihssen & Egli, 2004). *Escherichia coli* O157:H7 was found to be more acid resistant than generic *E. coli* strains (Diez-Gonzalez & Russell, 1997). Acid tolerance varies among strains (Lin *et al.*, 1995). Oh *et al.* (2009), reported significantly higher tolerance to acetic acid of *E. coli* O157:H7 strains from environmental sources including water and bovine feces as compared with human outbreak-related strains. In addition, it has been shown that *E. coli* O157:H7 *rpoS* deletion impaired expression of genes responsible for stress response including *gadA* (part of glutamate-dependent acid resistance system 2) and *ler* (LEE-encoded regulator; Dong & Schellhorn, 2009).The importance of *rpoS* in aquatic environments is also supported by the decreased survival of *E. coli rpoS* mutant in stationary phase in sea water (Rozen & Belkin, 2001).

Growth and survival of *E. coli* in open environments is often restricted by the availability of nutrients and energy sources. However, in surface waters, viable *E. coli* O157:H7 was detected over a 2-month period, in spite of a decline in the cell numbers (Avery *et al.*, 2008). Regarding *E. coli* O157:H7, the dynamics of gene expression in a transient habitat such as surface water remain underexplored. Recently, it has been shown that, even as the population of *E. coli* O157:H7 declined, some cells survived in sterile stream water for up to 234 days (Duffitt *et al.*, 2011). In this study, *E. coli* O157:H7 in natural sterile water triggered a stress response metabolism and DNA repair mechanisms indicating that bacteria remained active. However, no variations were reported concerning expression of virulence genes. In contrast, in another study, it was found that the gene expression response of *E. coli* O157:H7 to a growth transition in minimal glucose medium triggered expression of genes located on pathogenicity islands and toxin-converting bacteriophages (Bergholz *et al.*, 2007). It is possible that gene expression could vary considerably between adaptations to growth in minimal glucose medium compared with survival in water.

Phosphate is a highly sought after resource. Once used, it is often a limiting nutrient in environments, and its availability may govern the rate of growth of organisms. This is generally true of freshwater environments (Doering *et al.*, 1995; Correll, 1999; Paytan & McLaughlin, 2007). In most environments, when inorganic phosphate (Pi) availability becomes limiting (< 4 μM), the Pho-regulon is activated (VanBogelen *et al.*, 1996; Lamarche *et al.*, 2008). Such a global regulatory system permits an optimal adaptive response and the efficient use of phosphate under Pi-limited conditions, which may lead to survival of pathogenic *E. coli* under phosphate-limiting conditions. In addition to playing an important role in virulence, the Pho-regulon may therefore also contribute to persistence of pathogenic *E. coli* in the environment (Crepin *et al.*, 2011). More recently, Yoshida *et al.* identified novel Pho-regulon genes within specific O157 islands that are not localized in the backbone region shared with commensal *E. coli*. They showed that some of those genes are not related to Pi metabolism or utilization. This suggests that in response to environmental Pi stress, the Pho-regulon regulates genes involved not only in Pi homeostasis but also in other functions of *E. coli* O157:H7 (Yoshida *et al.*, 2012).

Environmental *E. coli* O157:H7 and biofilm formation

Some strains of *E. coli* O157:H7 form biofilms on both biotic and abiotic surfaces outside the host such as stainless steel, glass, and polystyrene (Dewanti & Wong, 1995; Ryu *et al.*, 2004; Ryu & Beuchat, 2005; Torres *et al.*, 2005; Rivas *et al.*, 2007). The genetic mechanism of *E. coli* O157:H7 biofilm formation is a complex process and is linked to the production of curli, long polar fimbriae, elements encoded by genes carried by O island OI-1, cellulose, and colonic acid (Torres *et al.*, 2002; Ryu & Beuchat, 2005; Uhlich *et al.*, 2006; Lee *et al.*, 2007, 2011; Saldana *et al.*, 2009; Allison *et al.*, 2012). *Escherichia coli* O157:H7 biofilm formation is also linked to the expression of some virulence genes including gene on the virulence plasmid pO157 (Puttamreddy *et al.*, 2010). Additionally, intercellular signal molecules, such as autoinducer-2 and indole, are also involved in *E. coli* O157:H7 biofilm formation (Lee *et al.*, 2007; Bansal *et al.*, 2008; Yoon & Sofos, 2008). *Escherichia coli* O157:H7 uses the T3SS, flagella, and the pilus curli to attach and colonize surfaces (plant stomata and internal tissues), which constitute the first step of biofilm formation (Xicohtencatl-Cortes *et al.*, 2009; Berger *et al.*, 2010; Saldana *et al.*, 2011). Growth of *E. coli* O157:H7 in protected biofilms proved to be a great advantage in open environments. In diverse habitats, bacteria within biofilms are notably resistant to bacteriophages and to free-living amoeboid predators (Costerton *et al.*, 1995).

Escherichia coli O157:H7 represents a persistent contamination from both the industry sector and throughout its ecological cycle (Fig. 1). The shedding of *E. coli* O157:H7 ranges from 10^2 to 10^5 CFU g^{-1} of feces in cattle (Campbell *et al.*, 2001) and where they can persist and be recycled in the farm environment, soil and water (Mead & Griffin, 1998; McGee *et al.*, 2002). Regardless of whether the water habitat is oligotrophic surface water or groundwater, it should be viewed as an environmental source of *E. coli* O157:H7. Considering all, in their planktonic forms, environmental *E. coli* O157:H7 could be present in a variety of ecosystems, and when nutrient conditions become favorable, phenotypic flexibility allows them to form biofilms. Furthermore, it is now established that the biofilm mode of growth is predominant in aquatic ecosystems, as planktonic populations have been shown to constitute $< 0.1\%$ of the total microbial community.

While there is still no clear link between biofilm formation and the presence or survival of *E. coli* O157:H7 in water, some studies showed that *E. coli* O157:H7 persists in water obtained from bottom-shore sediments (Czajkowska *et al.*, 2004). Interestingly, it has been shown

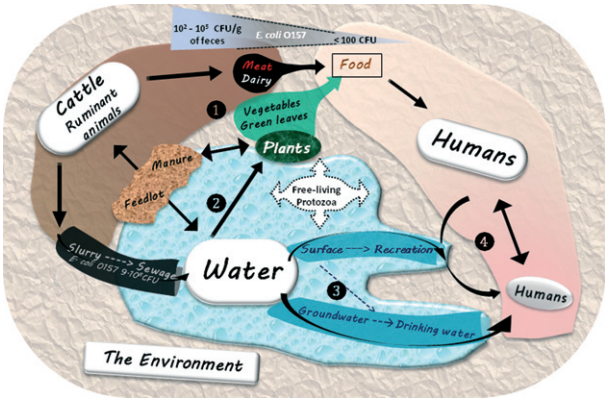

Fig. 1. Illustration depicting the ecological habitat and transmission of *Escherichia coli* O157:H7 in a global ecosystem. (1) On-farm cattle are the main reservoir for *E. coli* O157:H7 and may contaminate its immediate environment (feedlot) and sewage through cattle manure. Consumption of contaminated animal products (meat and dairy) is the main route of transmission to humans. Also, *E. coli* O157:H7 animal feces (10^2–10^5 CFU g^{-1}), through the use of manure, may contaminate plants, allowing the pathogen to also enter the food chain. (2) Water can be contaminated by the animals' immediate environment and manure deposited on lands, sometimes after a heavy rainfall. Furthermore, soils contaminated with sewage waste and overflowing of sewers are causes of contaminated water. This route may then lead to contamination of plants. Also, soils and water containing the free-living protozoa may serve as vectors for *E. coli* O157:H7. (3) Contaminated surface water bodies are a source of human infection through recreational water activities, while groundwater is the raw water source for human consumption. (4) Human-to-human oro-fecal transmission is another potential route of *E. coli* O157:H7 infection. The human-to-human transmission could occur directly or indirectly through contamination of water by infected humans in recreational waters.

that when growing in biofilm, *E. coli* O157:H7 increased its retention and survival in the effluent through a bench-scale sand aquifer system (Wang *et al.*, 2011). Also, in slaughter plants, the biofilm mode increases the persistence and acid tolerance of *E. coli* O157:H7 in liquid meat wastes (Skandamis *et al.*, 2009). Furthermore, *E. coli* O157:H7 persists in soils around farms and livestock production and can resist to fumigation. It has been suggested that chemical fumigation that decreases the microbial diversity would favor *E. coli* O157:H7 (Ibekwe & Ma, 2011). Thus, the microbial species diversity participates in the environmental protection of *E. coli* O157:H7.

In these wet or dry surfaces, biofilms could provide an ideal microenvironment for the establishment of syntrophic relationships in which *E. coli* O157:H7 would depend on other bacterial populations to utilize specific substrates, typically for energy production. In fact, it has been shown that non-biofilm-forming *E. coli* O157:H7 strains are retained on solid surfaces associated with biofilms generated by companion strains (Uhlich *et al.*, 2010).

Cellular quiescence: a possible mechanism of *E. coli* O157:H7 survival in water

There is little information regarding the behavior and metabolic status of *E. coli* O157 in environmental water sources. However, some survival studies have used culture-based methods that rely on sampling of environmental material, followed by plating on selective media, such as cefixime and potassium tellurite containing sorbitol MacConkey agar (Bergholz *et al.*, 2007). *Escherichia coli* O157:H7 cells in a 'dormant' state, also called VBNC, are still alive and demonstrate very low levels of metabolic activity and are not easily recovered on standard laboratory media (Oliver, 2005).

The VBNC state can be triggered by stress conditions in surface water that are imposed by low temperature or toxic metals (Klein & Alexander, 1986). However, the occurrence of the VBNC state in enteric bacteria is highly disputed by some reports, while others suggest it does occur in *E. coli* O157:H7 maintained in water and under saline conditions or in cattle manure and slurry (Bogosian *et al.*, 1998; Wang & Doyle, 1998; Makino *et al.*, 2000; Semenov *et al.*, 2007, 2009). These findings showed significantly higher numbers of the organisms by direct microscopic counts when compared with plating on a selective medium, which indicated the prevalence of dormant cells in the total *E. coli* O157 population. The use of bioluminescence such as a *lux* marker system, which indicates the energy status of the cell, provides an alternative way to assess the viability of bacteria including VBNC cells (Ritchie *et al.*, 2003).

Aquatic detection and isolation of *E. coli* O157:H7: an epidemiological challenge

Escherichia coli O157:H7 in surface waters constitutes a potential threat to human health through either drinking or ingestion during recreational activities. Currently, water contamination detection is based on standard guidelines that rely on microbial indicator concentrations of thermo-tolerant coliforms and enterococci (Wade *et al.*, 2006). Still, there are no established correlations between the prevalence and concentration of these fecal indicators of contamination and the presence of *E. coli* O157:H7 (Sugumar *et al.*, 2008; Duris *et al.*, 2009). For this reason, quantitative PCR of virulence genes such as genes *stx* and *eae* represents a more targeted detection method. However, PCR-assay sensitivity can be limited by low *E. coli* O157:H7 abundance in samples (< 100 cells mL^{-1}; Dharmasiri *et al.*, 2010). Pre-enrichment of samples is used to improve sensitivity and circumvent the limitation of PCR assays. However, further studies are needed to interpret, for example, the presence of *stx* genes in water samples. In fact, *stx* genes are carried by the temperate-like phage. The presence of *stx* cannot be directly correlated with intact or VBNC STEC as *stx* phages can be released from lysed cells in the environment.

Interestingly, new methods are developed to detect *E. coli* O157:H7 from aquatic environments. Using an anti-O157 antibody-modified microfluidic chip permits the specific enrichment of *E. coli* O157 including VBNC (Dharmasiri *et al.*, 2010). Another method involves the concentration of bacterial cells by filtration of water samples on a low-protein-binding membrane (polyvinylidene difluoride hydrophilic membrane) followed by direct extraction of the total RNA and specific RT-PCR amplification for *rfbE* for O157 antigen and *fliC* for H7 flagellin and then electronic microarray detection of *E. coli* O157:H7 (Liu *et al.*, 2008).

Interaction with protozoa: a possible persistence strategy for *E. coli* O157:H7

Over the last several decades, the importance of protozoa in soil, sewage, and water ecosystems and their role in controlling microbial populations has been widely acknowledged (Barker & Brown, 1994). Protozoa are widely distributed in water, soils, and effluents (Rodriguez-Zaragoza, 1994). They are likely to constitute an important environmental reservoir for transmission of *E. coli* O157:H7 and other pathogens. Bacterial pathogens including *Vibrio*, *Legionella*, *Mycobacteria,* enteropathogenic *E. coli*, and the meningitis-causing *E. coli* strain K1 multiply and/or survive within protozoa (King *et al.*, 1988; Werner *et al.*, 1990; Barker & Brown, 1994; Fields, 1996; Steinert *et al.*, 1998; Alsam *et al.*, 2006; Huws *et al.*, 2008). *Escherichia coli* O157 and non-O157 strains have been shown to survive within the environmental protozoa *Acanthamoeba polyphaga* and *Acanthamoeba castellanii* (King *et al.*, 1988; Barker *et al.*, 1999; Carruthers *et al.*, 2010).

In addition to enhanced environmental survival, bacterial co-habitation with protozoa could induce adaptative changes in bacteria (King *et al.*, 1988; Barker *et al.*, 1993). Furthermore, we recently observed that the co-culture of *E. coli* O157:H7 with *A. castellanii* increased bacterial persistence over 3 weeks (Chekabab *et al.*, 2012). In addition, there was a transient internalization and intracellular survival that increased in an isogenic mutant that did not produce Stx. Carruthers *et al.* (2010) showed an increased expression of virulence gene expression such as genes encoding Stx, LEE, and non-LEE T3SS effectors when *E. coli* O157 was co-cultured with *A. castellanii*.

This suggests that EHEC virulence factors may contribute to persistence and survival during interactions with amoebae. The ability of *E. coli* O157:H7 to enter and invade mammalian cells such as bovine mammary cells as well as also human macrophages was also observed (Matthews *et al.*, 1997; Poirier *et al.*, 2008; Etienne-Mesmin *et al.*, 2011). Moreover, it is noticeable that amoebae and human macrophages share morphological and functional similarities, especially in their phagocytic activity and parallel mechanisms in their interactions with many bacterial pathogens (Yan *et al.*, 2004; Siddiqui & Khan, 2012). Consequently, amoebae have been suggested to be a key step in the evolution of environmental bacteria to become human pathogens. Thus, *Acanthamoeba* may provide a useful model to study EHEC pathogenesis and to understand their immune evasion mechanisms.

Lainhart *et al.* (2009), found that Stx-encoding bacteria killed the ciliate protozoa using the holotoxin Stx as an antipredator weapon. Other studies have shown that the presence of Stx-encoding prophage augmented the fitness of *E. coli* in co-culture with the ciliate protozoa (Steinberg & Levin, 2007). These authors found that the ratio of Stx+ to Stx− bacteria increased after 3 days' co-culture with *Tetrahymena thermophila* that belong to ciliates protozoa present in ruminants gut. In contrast, other investigators did not observe any advantage or disadvantage of Stx lysogenic phage with the rumen protozoa (Burow *et al.*, 2005). Thus, the contribution of Stx to bacterial survival when facing a protozoan seems to be variable depending on the conditions of the challenge and the protozoan models used in the co-culture assay.

There is growing concern about the survival of pathogens in sewage and waste effluents because it is known that *E. coli* O157:H7 can survive in cattle manure slurry for at least several weeks (LeJeune *et al.*, 2001; Lee *et al.*, 2009). Protozoa present in these effluents could provide a protective niche for pathogens such as *E. coli* O157:H7 (King *et al.*, 1988). The role of protozoa in the survival of *E. coli* O157:H7 in the natural environment has been less studied. Soils contaminated with organic matter and sewage waste contain greatly increased numbers of protozoa such as *Acanthamoebae* (Rodriguez-Zaragoza, 1994). It is possible that *E. coli* O157:H7 in soil and slurry could be preyed on by free-living protozoa then serving as vectors for the spread of this pathogen. This is especially true if the bacteria are able to survive within cysts (the resistant forms of amoeba) as has been shown for *Legionella pneumophila*, *Vibrio cholerae*, and *Mycobacterium avium* (Steinert *et al.*, 1998; Brown & Barker, 1999). Bacteria within amoeba cysts could be dispersed by aerosol transmission. Grazing cattle would ingest protozoa in silage and grass, and the ingested protozoa containing bacterial pathogens such as *E. coli* O157 could also be a route of transmission to cattle.

Different species belonging to the three protozoan groups (flagellates, ciliates, and amoebae) have been isolated from fresh green products found in the supermarket (i.e. spinach and lettuce). Gourabathini *et al.* (2008), demonstrated that those protozoa can ingest bacterial pathogen including *E. coli* O157:H7 and S. Enterica and then produce vesicles containing intact bacteria. Thus, the presence of protozoa on leafy vegetables and their sequestration of enteric bacteria in vesicles indicate that they may play an important role in the ecology of *E. coli* O157:H7 on fresh green products.

Conclusion

In Fig. 1, we present a global view of the ecology and potential source reservoirs of *E. coli* O157:H7. This pathogen has shown the ability to survive in many adverse conditions. In addition to its capacity to cause infection to humans through consumption of contaminated foods, *E. coli* O157:H7 is able to survive in water, making this pathogenic bacterium an environmental threat to humans. The bacteria may enter a starvation and survival state allowing it to adapt and persist in low-nutrient environments such as water. Furthermore, *E. coli* O157:H7 has been shown to survive within environmental protozoa; this could contribute to its persistence. However, the distribution of this bacterium in the environment and the increasing reports of different routes of transmission make it difficult to set up efficient strategies to prevent contamination. There is a need to include environmental monitoring as a surveillance method for EHEC where such infections are problematic worldwide. Information collection and sampling methods should be standardized to more clearly understand the ecology of this environmental pathogen. This could lead to rational development of preventative measures to limit its presence in environments that may lead to increased risk of contamination of water, soil, and animals and transmission and infection of humans.

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