Environmental prevention of human disease from verocytotoxin-producing Escherichia coli

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Abstract
Verocytotoxin-producing Escherichia coli (VTEC) haemolytic uraemic syndrome (HUS) is an important cause of mortality and renal failure worldwide. For those patients who need medical attention, no treatment aside from supportive care has proven effective for this disease. This has prompted a broader look at environmental prevention, with a particular emphasis on the transmission of bacteria from animal carriers to human beings. Here, we review animal- and meat-handling strategies to reduce the burden of VTEC human disease.

Introduction
Haemolytic uraemic syndrome (HUS) is a major cause of acute renal failure worldwide. Ninety percent of childhood cases of HUS are associated with diarrhoea and gastroenteritis (D+HUS) due to verocytotoxin-producing Escherichia coli (VTEC) [1]. Many E. coli strains, including O157:H7 and more recently O103, O26:H11 and O145, have been implicated in human illness and large population outbreaks [2–6] leading to extensive morbidity and financial consequences [7].

Unfortunately, with the exception of early recognition and supportive care, no medical treatment has proven effective for the prevention or treatment of HUS [8]. Avoidance of antibiotics and anti-motility agents during gastroenteritis [9,10], corticosteroids, anticoagulants, thrombolytics [11], plasma infusion [12], protective antibodies [13] and oral synthetic verotoxin receptor analogues attached to chromosorb (Synsorb-Pk) [14] are all of absent or questionable benefit. This has prompted a broader look at environmental prevention, in an effort to reduce the transmission of VTEC infections.

The main source of VTEC infection is animals, particularly cattle, to which these bacteria are non-pathogenic. There is evidence that bacteria reside in the gastrointestinal tract of animals, possibly adhering to lymphoid follicles of the anorectal mucosa [15], and shedding in the faeces. Foods such as lettuce [16], alfalfa sprouts [17], unpasteurized milk [18], apple cider [19] and water can all become contaminated. Direct contact with cattle or attendance at agricultural fairs is also a source of human illness [20]. However, contaminated meat available for consumer purchase remains the biggest area of concern. Nineteen percent of retail uncooked beef may be contaminated with E. coli, with 4% being the E. coli O157:H7 isotype. E. coli O157:H7 may also be present in 1.5% of pork, 1.5% of poultry and 2% of lamb samples [21]. Here, we review opportunities in animal farming and the handling of meat to prevent animal-to-human transmission of VTEC (Figure 1).

Animal farming
Cattle are the main livestock reservoir for VTEC, particularly E. coli O157:H7. Its prevalence in the faeces of North American cattle ranges from 10 to 28%, with a recent study finding 96% of feedlots and 52% of pens having at least one positive faecal sample [22] (Figure 1). Other farm animals and pets [23] also shed VTEC; in one study as many as 75% of goats and 32% of sheep were affected [24]. As such, efforts to reduce the survival and transmission of VTEC have focused on farm management and disinfectant practices, and strategies to increase animal resistance to infection, such as the use of antibiotics, vaccines and probiotics.

Unfortunately eradicating all VTEC from the farm remains impossible. Cattle are often housed outdoors, where they interact with other animals and insects that all serve as potential sources of recolonisation [25]. Simply brushing up against a barn wall can be a source of E. coli O157 transfer, as this microbe is able to survive in manure [26], water [27], feed and on barn surfaces [28]. General disinfection has not proven as effective as one would hope. Chlorination of water troughs results in only small, non-sustained reductions of faecal coliforms [29]. Quarantining animals until they test negative is impractical because of the transient...
nature of faecal shedding and the current lack of real-time diagnostic testing [30].

To increase cattle resistance to infection, antibiotics, vaccines and probiotics have all been considered. Many prophylactic antibiotics (particularly neomycin sulphate) [31] reduce the prevalence of E. coli; however, no antibiotics are licensed for use in cattle for the control of VTEC given concerns of antibiotic resistance [32,33]. Vaccinating animals against infection remains promising although its effectiveness still remains to be established and studies with a risk-benefit analysis are lacking. Current vaccines utilise intimin and adhesion molecule of E. coli O157:H7, or LEE-encoded type III secreted proteins [34–36]. Unfortunately given recent announcements by the United States Department of Agriculture, a vaccine is unlikely to be licensed for use in the immediate future. Finally, probiotics, or competitive inhibitor cultures, can reduce the gut colonisation and faecal shedding of pathogenic E. coli including VTEC in animals [36,37,38]. The United States has recently licensed the use of the NP51 strain of Lactobacillus in cattle, which reduced E. coli O157:H7 in faeces by 49 to 77% [39]. Currently, studies are also evaluating the benefits of Enterococcus- and non-pathogenic E. coli [40].

**Animal transportation**

Trucks that transport animals between farms and processing plants can serve as a source of infection for cattle and processing plants. Universal contamination of truck sidewalls and floors with generic E. coli has been reported [41]. With proper washing and sanitisation levels of bacteria were significantly reduced on trucks carrying swine [42], and may also prove effective for trucks carrying cattle. Cleaning trucks with heat and sodium hypochlorite drastically reduces coliform numbers transported by poultry, and is most effective when it is performed after each load to prevent the build-up of pathogens and biofilms [43]. When transport trucks do remain contaminated, it is often because procedures were not followed [41].

**Processing unit**

After transport to the abattoir, animals are kept in holding pens until they enter the processing plant (Figure 1). Extrapolating from other organisms, the holding pen may be a potential source of VTEC infection [44]. Infection with Salmonella has been reported in swine within 30 min of entry into a holding pen contaminated with relatively low levels of the bacteria (10³ CFU/g) [45]. Disinfection has decreased Salmonella contamination [41], and these procedures should be examined for their effects on VTEC.

E. coli O157:H7 is considered as an adulterant of beef in many countries including the United States. Mandatory performance standards for meat processing plants are in place. In Canada and the United States these standards are implemented through Hazard Analysis Critical Control Point (HACCP) systems. Through HACCP, Critical Control Points (CCPs) are identified and preventative procedures, corrective actions and verifiers are applied to manage the process [46]. Since the mandatory implementation of HACCP in the United States, the incidence of E. coli O157 in the USA has decreased by >40% [47]. The specific interventions used in HACCP vary between processing plants.

VTEC can be transferred from the hide onto the meat during hide removal [48]. Methods to reduce carcass contamination in the processing plant include spraying cattle with a powerhouse to reduce visible signs of faeces from hides [49], bagging removed anuses [50], washing with hot water or aqueous ozone [51], steam vacuuming [52] and the use of alkaline spray solutions [53].

The processing unit must be cleaned regularly [54]. E. coli contaminate machinery in food processing plants [55] and this is likely true for meat processing plants. One study demonstrated cross-contamination on the processing line [56]. Operators must also clean their hands, arms, aprons and knives to reduce bacterial transfer [57]. Hand washing removes some organisms [58], and sanitising knives and plastic gloves with 82°C water or chlorine solutions is recommended [49].
Irradiation is another avenue through which bacteria can be eliminated from meat. Its use is controversial, and is not yet approved in many countries. However, irradiation of fresh meat, ground meat and hamburger is now allowed in the United States.

Distributor

Registered meat distributors are required to adhere to appropriate storage and packaging guidelines to ensure product safety. While refrigerated storage of meat at 4°C (40°F) and vacuum packaging do not kill VTEC, these practices do help keep microorganism levels low [59].

Retailer/preparer/consumer

Zhao et al. [21] found that uncooked meat from several supermarket chains tested positive for various pathogens, regardless of the season. They also identified large differences in bacterial contamination rates between the supermarket chains, stating that publishing company names that sell foods with high bacterial loads could increase corporate accountability and influence consumer choice.

Care is needed to avoid transmission of bacteria from meat to other foods being prepared in the kitchen. Pathogenic organisms can often be cultured from domestic and commercial kitchens, including the wash-up water [60]. Organisms persist on kitchen surfaces if there is inadequate cleaning [61].

The hands of individuals in charge of food preparation may also become contaminated with pathogenic organisms. Many food products come into contact with contaminated hands. Proper hand washing remains essential.

Finally, all meat must be cooked to a safe internal temperature. The United States Food and Drug Administration recommends cooking ground beef to an internal temperature of 71°C (160°F).

Conclusions

In conclusion there are several potential environmental targets for the control of human disease due to VTEC. By addressing these steps in animal and meat handling, it is hoped that large outbreaks and individual illness will be prevented, reducing the burden of human disease.

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