ZOONOTIC DISEASES, HUMAN HEALTH AND FARM ANIMAL WELFARE

Escherichia coli

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INTRODUCTION

Escherichia coli (*E. coli*) is recognized as an important zoonotic pathogen, not because of the number of cases of human illness it causes, but by the serious and life threatening disease that a small number of *E. coli* strains may cause. Outbreaks of pathogenic *E. coli* like the German outbreak in 2011 and the outbreak related to Godstone Farm in Surrey in 2009 have a high profile in the media as a result of the serious illness caused. The number of cases of foodborne disease associated with *E. coli* in the UK is around a thousand, dwarfed by an estimated 700,000 cases of *Campylobacter* infection. Although infection with pathogenic *E. coli* is rare, it is the serious and sometimes fatal disease it may cause that give it a high importance[1].

E. coli is a Gram negative bacterium, a member of the family Enterobacteriaciae, and closely related to *Salmonella*. However unlike *Salmonella*, most *E. coli* strains are not associated with disease, but naturally reside in the intestines of animals and humans as a commensal organism. *E. coli* is a key part of our natural microbiota or gut flora. Indeed most *E. coli* within the gut are probably beneficial to health. However a small number of *E. coli* strains or pathotypes can cause disease. These are usually associated with intestinal infection, but some types cause urinary tract and bladder infection and others are associated with meningitis.

E. coli associated with intestinal disease are usually categorized by the disease they cause or the toxins they produce. These include Enteropathogenic *E. coli* (EPEC) that cause infant diarrhoea, Enterotoxogenic *E. coli* (ETEC) that are the main cause of traveller's diarrhoea and Enteroaggregative *E. coli* (EAEC or EAggEC) that cause persistent diarrhoea. However none of these types are considered to be a cause zoonotic infection. The form of *E. coli* most associated with zoonotic infection is Shiga toxin producing *E. coli* (STEC) which are also known as Vero toxin producing *E. coli* (VTEC) and perhaps most frequently as Enterohaemorrhagic *E. coli* (EHEC) and will be referred to as such hereafter. By far the most common and best known EHEC strain or serotype is O157:H7 which is responsible for the majority of UK and North American cases[2]. However a number of other EHEC serotypes may occur notably O111 in Australia and O26 in Japan[3]. EHEC are distributed worldwide with the bacterium isolated in countries as diverse as Argentina, Korea and Ethiopia[4]. The main animal reservoir of EHEC is in wild and more commonly in domestic ruminants, though it may be isolated infrequently in other animals including birds. EHEC is most prevalent in developed nations and is particularly high in North America, UK, Ireland and Sweden[5]. This may be partly explained by better detection and surveillance, though, as discussed later, may also reflect farming practices.

Human disease caused by EHEC

EHEC infections are often severe. Children up to the age of five and the elderly are most at risk. The infectious dose (the numbers of bacteria needed to cause an infection) is low for EHEC. It is thought that a dose as few as 50 bacteria will cause infection, compared with thousands for *Salmonella*. As such exposure to even low numbers is a considerable risk. Infection causes stomach cramps, fever and vomiting around three days after exposure which is frequently

followed by bloody diarrhoea[6]. Up to 90% of children will develop bloody diarrhoea within six days of exposure. Although 85% of children clear infection within a week, around 15% develop a severe complication called haemolytic uremic syndrome (HUS)[7]. In patients with HUS the kidneys may become damaged, largely through damage to the kidney endothelial cells along with damage (lysis) and coagulation of the blood in the vessels supplying the kidney. This can lead to kidney failure leading to a requirement for kidney dialysis and even death. Disease is usually less severe in healthy adults, often limited to diarrhoea.

EHEC employs two distinct mechanisms in causing disease. EHEC is able to bind tightly to the intestinal wall through a bacterial machine called a type III secretion system. This consists of a syringe-like structure that injects proteins and toxins into host cells, altering their structure or function. In EHEC this system is known as the Locus of Enterocyte Effacement (LEE)[8]. The actions of LEE allow EHEC like O157 to bind tightly causing changes to the structure of the cells that make up the cell wall called enterocytes. The cell rises up to form a pedestal-like structure with the bacterium sat on top. These lesions are called attaching:effacing (or AE lesions). It is thought this induces diarrhoea and stomach pains through mechanisms we do not yet understand. LEE is also found in Enteropathogenic *E. coli* (EPEC) and is though to induce the infant diarrhoea caused by these strains. LEE is also important in attaching to the gut of ruminant animals, allowing colonization[9]. The second factor is the action of two toxins called Shiga-like toxins or Stx1 and Stx2. They are so-called because they resemble toxins produced by another bacterium called *Shigella*, the cause of bacterial dysentery. It is the action of these toxins on the kidney that causes HUS, and the action of the toxins on the intestinal blood vessels causes damage and leakage of blood causing bloody diarrhoea. Treatment is difficult, as the use of antibiotics can increase toxin release by breaking open bacteria and lead to more severe disease. As such supportive therapy such as fluid replacement and in more severe HUS cases kidney dialysis and eventually transplantation are the main options[10].

EHEC/STEC-the evolution of a pathogen

E. coli is perhaps the best example we know of a 'bug that turned nasty'. The recent outbreak of O104:H4 STEC was an example of bacterial evolution before our eyes. The Stx toxins were acquired by O104 an enteroaggregative form of *E.coli* that could cause persistent diarrhoea[11]. The presence of the toxins resulted in a new variant of O104 that could cause HUS resulting in an epidemic that unusually affected adults, especially young women[12]. There is no evidence to suggest the infection had a zoonotic source[13] but was likely to be the result of water contamination in bean sprout production. Nevertheless it illustrates the potential of bacteria to evolve new variants that can cause disease. O157:H7 itself is a relatively new bug having emerged in the 1970s. It is considered to developed from an EPEC which has the LEE locus that allows the formation of attaching effacing lesions through acquisition of the Stx toxins from another (unknown) bacterial source (Pennington, 2010). The toxins are carried from one bacterium to another by a type of virus called a bacteriophage. Bacteriophages infect only bacteria. Infection can result in two things. The first is the lytic cycle where the virus causes the bacterium to break up or lyse thereby killing it. The second is that the bacteriophage DNA becomes incorporated into that of the host bacterium, a process called lysogeny. Bacteriophages that undergo lysogeny can transfer DNA from one bug to another. The two Stx toxins are carried on bacteriophages and incorporation of these Stx bacteriophages into the DNA of genome of both O104 and O157 resulted in their increased virulence.

Sources of EHEC

Most EHEC outbreaks have a zoonotic source, though the infection can subsequently be passed from child-to-child such as classmates or siblings through poor hygiene[14]. Food is the most

common source, though direct transmission from animal faecal material on farms or from the environment may occur as *E. coli* survives well in soils, on pasture or in water[15].

The major problem lies at the fact that EHEC can colonize the lower gut of healthy domestic ruminant animals and pigs. This means that food animals carry the infection without disease that may lead to contamination of meat or meat products from faecal material in the lower gut or on the hide of animals at slaughter. Cattle, and as a consequence beef, are without doubt the main source of EHEC[16] though unpasteurized dairy products may also be a foodborne source[17].

Minced or ground beef is a particular source of EHEC, given its large surface area that supports the bacterium and the fact it may be produced from multiple cuts of meat from several animals[18]. As such it became christened 'Hamburger Disease' in the USA after several outbreaks including a major fatal outbreak associated with the 'Jack in the Box' burger chain. As such the UK Food Standards Agency recommends that all mince and burgers are cooked thoroughly, although the practice of serving 'medium rare' burgers is still commonplace in the US. Meat from other animals including sheep, goats and pigs may also carry EHEC, though infrequently. Cross contamination between raw and cooked meat is a particular risk. When this happens in the food industry the consequences can be catastrophic. Two large UK outbreaks In Lanarkshire and South Wales were traced to poor hygiene practice in catering butchers. The Lanarkshire outbreak in 1996/7 was primarily in nursing homes and attendees at a meal for pensioners supplied by the same butchers. The outbreak resulted in more than 500 cases and 22 deaths[19]. The South Wales outbreak in 2005 was mainly in primary school children receiving school meals supplied by the same butcher. In both cases poor practice of storing and cutting cooked and raw meat was found. In both cases vulnerable groups, children and the elderly were infected[20]. Pennington, who conducted the public enquiry into the South Wales outbreak, estimated that the costs associated with this single outbreak were in excess of £3 million[21]. It could be argued this is small change compared to the loss of the 5 year-old boy who died in this outbreak. In large outbreaks there is frequently secondary transmission of the infection from person-to-person either within families, within school or nursery/playgroup classes or within residential nursing homes.

Vegetables, particularly where cattle manure has been used as fertilizer may also be a source of infection[22]. Salad vegetables that are eaten uncooked are a particular risk. Lettuce contaminated by EHEC was responsible for a major outbreak in the Netherlands[23]. Outbreaks have also been traced to contaminated spices and even apple juice[24].

As EHEC are carried and shed by both domestic and wild ruminants then environmental contamination is inevitable. A number of cases have been linked to water such as lakes and ponds used for recreation and a number of cases have been liked to heavy rain washing cattle faeces into water courses, the most high profile of these at the Glastonbury Festival which is held on a dairy farm[25].

Farm visits are another potential source of EHEC and in particular 'open' or petting farms and zoos. The 2009 EHEC outbreak in Godstone Farm in Surrey brought this to the attention of the general public, though the potential risks were identified much earlier[26]. As mentioned earlier, as EHEC have such a low infectious dose, exposure to even low numbers of bacteria may result in disease making direct transmission on the farm relatively easy. In simple terms people, usually children, handle or stroke animals and play in an environment contaminated by ruminant faeces and then put hands or fingers in contact with their mouth without thoroughly washing hands. The Godstone outbreak was the UK's first substantial farm-related outbreak with 93 cases[27],

though similar outbreaks have been recorded on open farms and petting zoos in the USA and Netherlands[28].

EHEC and cattle

Cattle are clearly the main source of EHEC. The bacterium is capable of colonizing the intestines, especially the lower bowel and rectum of cattle without causing disease[29]. It may then be shed in faeces leading to infection of other animals, contamination of hides and of the environment. A particular problem is 'super-shedders', cattle that shed large numbers of EHEC that have considerable impact on the transmission within the herd[30]. The mechanisms by which EHEC colonize the intestines of cattle are not well understood, though it is known that the LEE type III secretion system is important in allowing the bacteria to attach to the gut wall.

The prevalence of EHEC, in particular O157, in cattle may be very high. Studies of beef cattle in the USA have indicated that EHEC may be present in the intestines or on the hides of between 20-28% of cattle at slaughter[31] and in 43% of meat samples after processing[32]. Levels in the UK are lower with 4.7% of cattle, 0.8% of sheep and 0.3% of pigs colonized[33]. Studies in Scotland, where human EHEC cases are more common than the rest of the UK, it has been estimated that 80% of cattle farms will have animals with O157 during the course of a year and that 20% of farms will have infected animals at any one time[34]. In the USA it has been estimated that 73% of beef farms and ranches may have O157 infected cattle[35].

Feedlots, feed and stress

The data given above clearly show that levels of EHEC are very high in the cattle industry in North America. Whilst it is wrong to say that cattle reared 'traditionally' on pasture will not become infected, the management of cattle in pens, frequently indoors in feedlot systems seems to be a contributory factor. Although dairy cattle are frequently raised indoors in the winter in the UK, the risk of zoonotic disease transmission is low unless consuming raw milk. However the use of feedlots as an intensive system to fatten beef cattle prior to slaughter seems to be a particular risk. The system is used both to reduce costs and to meet the United States Department of agriculture (USDA) certification of beef based on extensive fat marbling and softness of the meat[36]. Faecal shedding within a penned group of cattle increases the likelihood of transmission from one animal to another. In such production systems super-shedders are a particular risk[37]. Although relatively few infected animals become super-shedders, in larger housed groups they may easily spread infection. It has been suggested that the 20% of infected animals that become super-shedders are responsible for 80% of transmission[38]. Recent studies in Canada and the USA have shown that super-shedders are a particular risk in hide contamination that can lead to contamination of meat at slaughter[39]. Indeed the large-scale operations used in cattle slaughter and processing can lead to shedding cattle contaminating hides in transport and lairage at the slaughterhouse.

In addition to the more intensive stocking of cattle in feedlots, cattle are fed a diet of grain as a cost-effective means of providing a high-energy diet. A consequence of this is that starch from these grains can pass from the stomachs into the hindgut where it can be broken down (or fermented) and used by *E. coli* as a nutrient source. This promotes the growth of *E. coli* including EHEC in the hindgut. Certain grains such as barley may be fermented quickly and effectively by *E. coli* leading to increased colonization and shedding of EHEC[40]. Furthermore traditional grass and forage diets are higher in plant compounds such as tannins and phenolics that inhibit *E. coli.* Moreover although small amounts of grain supplements are given to grass-fed beef in immediate run-up to slaughter it is considered by most butchers and gourmets that

grass-fed beef is a superior product in terms of flavour and immeasurably more welfare-friendly for an animal that has evolved to eat grass[41].

Cattle fattened in feedlots may also be under considerable environmental stress in hot and relatively crowded conditions. Heat stress in feedlots has been shown to increase faecal shedding[42]. Experimental administration of noradrenaline, a key stress hormone, has also been shown to increase O157 shedding in cattle[43]. Transport and lairage at the slaughterhouse might be considered stressful and would increase shedding in infected animals but there is conflicting evidence as to whether this increases or decreases the level of faecal shedding[44]. However given the clear evidence that stress increases faecal shedding, it would seem likely that long transport times and poor lairage conditions would lead to increased shedding.

Control of EHEC in cattle

As human infection is relatively infrequent most efforts to reduce the burden of EHEC disease have centered on control in cattle or in improvements and better hygiene practice in the food industry. The administration of antibiotics to clear shedders, probiotics, manipulation of feed and the isolation of removal of shedding animals have all been proposed as interventions though their efficacy is questionable[45]. Vaccination has been proposed as potentially the most effective control strategy. Most proposed vaccines have targeted the LEE secretion system due to its role in colonizing the bovine gut. These experimental vaccines clearly induce an immune response, though their efficacy is mixed in trials vaccination would seem the most effective way of reducing levels in cattle[46]. A considerable caveat is that mathematical modeling to predict the effect of interventions on EHEC in cattle in Scotland suggests that elimination of the bacterium is unlikely and that some reduction is the best we can realistically achieve[47].

E. coli and animal disease

EHEC rarely causes disease in livestock, though other strains have considerable impact on pig and poultry production. *E. coli* is a leading cause of post-weaning diarrhoea in pigs leading to poor growth and in severe cases death[48]. Although not exclusively found in intensive production, the disease is related to the presence of certain pathogenic types of *E. coli* accompanied by stresses associated with early weaning in commercial pig production including feed change, mixing of litters and separation from the sow[49]. Vaccines have been developed in an attempt to control the disease[50]. Avian Pathogenic *E. coli* (APEC) is an increasing problem in intensive broiler (meat) chicken productions and is considered to cause the loss of at least 10 million animals per year in the UK.

APEC - future zoonotic disease?

APEC belongs to a group of closely related *E. coli* called extra-intestinal pathogenic *E. coli* or ExPEC that also includes *E. coli* that cause urinary tract infections (UPEC) and meningitis (NMEC) in humans and shares many features that are involved in causing disease in both humans and chickens[51]. As such it is considered that APEC have considerable potential in causing human disease, though as yet this cross-species jump has not happened. However as APEC are capable of causing disease in rat models of human meningitis the possibility of APEC becoming a zoonotic agent are considerable[52].

CONCLUSIONS

EHEC is an uncommon but potentially devastating zoonotic infection that can lead to long-term disease and even death. It is intimately associated with cattle and other ruminants and appears to be part of their normal gut microbiota. As such, control is a difficult prospect. Nevertheless practices employed in cattle production in North America such as the use of grain feed and rearing in feedlots lead to higher levels of EHEC in beef and it is notable that the USA has around 73,000 human EHEC cases a year, compared to less than 1,000 in England and Wales. There is certainly an argument that cattle fed on grass and hay and grown in more extensive systems are less likely to carry EHEC. It could also be argued this produces higher quality meat.

The German *E. coli* outbreak in 2011, though not zoonotic, is a clear illustration of how bacteria may evolve and become more virulent. The zoonotic potential of other *E. coli,* particularly APEC, is a concern given its high prevalence in intensive poultry production. Livestock management practice and disease surveillance needs to take such threats into account to ensure both high levels of animal health and welfare and to maintain public health.

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