ESCHERICHIA COLI 0157: H7 RESERVOIR, TRANSMISSION, DIAGNOSIS AND THE AFRICAN SITUATION: A REVIEW

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ABSTRACT

Objectives: To provide an overview of the current understanding of verotoxigenic *Escherichia coli* 0157:H7 (VTEC) and to describe clinical picture, reservoir, transmission and diagnosis and African situations of VTEC.

Data source: A literature review was performed of major published series between 1980 and 2001 inclusive, using the PUB MED and MEDLINE search. Some earlier published series were also reviewed in instances where they directly led to the understanding of current review.

Study selection: Data from laboratory studies on cultural and isolation, serological and molecular techniques are summarised in this review.

Results: Verotoxigenic *Escherichia coli* 0157:H7 (VTEC) is an important cause of uncomplicated diarrhoea, bloody diarrhoea (BD) and haemolytic uremic syndrome (HUS) in developed countries. The incidence and importance of 0157: H7 (VTEC) infections in most developing countries are not known; however, 0157: H7 (VTEC) cases have been isolated from many sporadic cases of diarrhoea, BD and HUS, while several cases have also been associated with diarrhoeal disease outbreaks in Africa.

Conclusion: The morbidity and mortality associated with several recent outbreaks of VTEC disease have highlighted the threat these organisms pose to public health. For this reason, there is an increasing demand for improved diagnostic procedures for detection of VTEC in clinical specimen and in particular, in foods such as meat and dairy products in developing countries.

INTRODUCTION

Acute diarrhoea is still the leading cause of infantile diarrhoea in less developed countries and the most important symptom associated with poor hygiene, faecal contamination of food, water and environment. The first world wide study of morbidity and mortality from diarrhoea disease, based on population estimates in 1980, showed that there were 744 to 1000 million episodes of diarrhoea and 4 to 6 million deaths from diarrhoea in children below five years in Africa, Asia, and Latin America each year in that decade. A decade later, improved management reduced world-wide mortality to 33 million deaths per year (estimated range: 1.5-5.1 million), but the incidence of diarrhoea (2.6 episodes per child per year) remained virtually unchanged. In Brazil, infants younger than one year of age represent the age group with a higher than average risk of death due to diarrhoea (5.5 deaths per 1000 inhabitants)(1).

The main bacterial enteric pathogen in less-developed countries, and particularly among infants two months to five years of age, is enteropathogenic *Escherichia coli* and other enteric pathogens such as entroinvasive *E. coli* (EIEC), enterohaemorhagic *E.coli*, (EHEC)(1). The first recognised *Escherichia coli* 0157:H7 outbreaks, which occurred in 1982 in Oregon and Michigan and were associated with eating hamburgers from a particular fast-food chain (2). Evidence indicating rare sporadic infection occurred prior to 1982 comes from a retrospective review by the Centres for Disease Control and prevention (CDC) of over 3,000 *Escherichia coli* serotypes identified from 1973-1983, in which 0157:H7 was detected only once in a 1975 isolates from a 50 year old Californian woman(2). The subsequent occurrence of large outbreaks and the widespread distribution of cases has led to the designation of *E. coli* 0157:H7 as a new emerging pathogen.

The disease caused by *E. coli* 0157: H7 is haemorrhagic colitis and is characterised by severe cramping (abdominal pain) and diarrhoea (watery and /or bloody). Other symptoms may include vomiting and /or low-grade fever. The illness lasts for an average of eight days. Treatment for *E. coli* infection is primarily supportive, including management of dehydration and complications such as anaemia and renal failure (3). Antimotilily agents do not appear to diminish the severity of illness or prevent the development of haemolytic uremic syndrome (HUS) (3). Potential explanations for the lack of benefit for antibiotic treatment are first elimination of the competing bowel flora by antibiotic giving a competitive advantage to *E. coli*

0157:H7, and second lyses/death of *E. coli* 0157: H7 leading to increased release of vero toxin (3). The proportion of all cases of diarrhoea estimated to be associated with *E. coli* 0 157: H7 is 0.6% to 2.4%. Of all cases of bloody diarrhoea or haemorrhagic colitis, 15% to 36% are estimated to be caused by *E. coli* 0157: H7 (3). Serious complications of *E. coli* 0157: H7 disease occurs in 0 to 15% of cases and is experienced more frequently by the very young and elderly.

These complications are HUS and thrombotic thrombocytopenic purpura (TTP). HUS primarily affects infants and young children and is characterised by renal failure and haemolytic anaemia. HUS is the most common cause of acute renal failure in children and the mortality rate is 5% to 10%. TTP primarily affects the elderly, and is characterised by HUS plus two other symptoms namely fever and neurological syndromes (3). TTP has a mortality rate as high as 50%(3). Other potential complications are erroneous surgical intervention, coma or seizures, pancreatitis and diabetes mellitus (3).

PREVALENCE OF VTEC IN ANIMALS

The early associations between *E. coli* 0157: H7 and cattle products quickly led to identification of cattle as natural hosts of VTEC 0157: H7 (4). Subsequent investigations have confirmed that a variety of animals, especially ruminants may carry numerous serotypes of VTEC in their intestinal tracts (4,5). Animals and herds prevalence estimates varies with study design, numbers of herds and cattle sampled, type and age of cattle, methodology and season. In three surveys, involving up to nine selected dairy herds in Wisconsin, *E. coli* 0157: H7 was isolated from faeces of 1.2-2.2% of cattle on 27.3-100% of farms (6). Where higher numbers of farms were investigated on a single sampling, the prevalence rates were lower both in individual animals (0-0.7%) and farms (0-16%)(7). Prevalence rates were higher in growing cattle, especially among newly weaned dairy calves, and during the summer(7,8). In a point-prevalence study of 100 feedlots in the USA, *E. coli* 0157: H7 was isolated on 63% of the feedlots and overall, from 1.8% of 11,881 faecal samples (8). Prevalence rates between feedlots and between pens within feedlots were highly variable, with the highest rates for pens holding cattle recently entering the feedlots (32-53%).

In UK the peak rates of shedding ranged from 40-68% (7). Infection was not associated with clinical disease in either study. These findings are generally consistent with the fact that 0157: H7 *E. coli* is not a bovine pathogen in naturally reared cattle (9) and is shed for 1-2 months following natural exposure (9). There are however, herds in which *E. coli* 0157: H7 was isolated more frequently over time (8), possibly due to continuing exposure to the organism in water, feed or other environmental sources or to management factors. Serological studies provide further evidence that *E. coli* 0157: H7 is widespread in cattle.

In a study of cattle on 80 dairy farms (7), over 85% of 885 adult dairy cattle and 49% of 589 calves less than three months old had antibodies reactive with the 0157:H7 lipopolysaccharide (LPS). Calves aged 9-13 weeks had the lowest rate of seropositivity (37%) to this antigen (10). However, the high rates of seropositivity to the 0157: H7 LPS should be interpreted with awareness of the occurrence of non-VTEC of serogroup 0157: H7 in cattle, as well as potential cross-reactions due to antibodies to other organisms (10).

Domestic ruminants other than cattle also harbour VTEC (4). In Germany, sero-prevalence rates of all VTEC were higher in sheep (66%), and goats (56%) than in cattle (21%) (11). Also, 43% out of 400 sheep and 51.1% of 262 goats tested in Italy had antibodies to Verotoxin type 1 (VT1) (12). While the serotypes of VTEC isolated from these species differ somewhat from those in cattle, several are common to cattle, sheep and goats, and include serotypes associated with BD and /or HUS in human (11). Although not isolated from German sheep or goats, *E coli* 0157: H7 was present in 1-4% of sheep surveyed at abattoirs in Australia, UK, The Netherlands and USA (5).

Doyle and Schoeni, (13) isolated *E. coli* 0157: H7 from 1.5% samples, while Abdul-Raouf *et al* (14), isolated the organism from 4% of chicken samples. In both instances, cross-contamination from other meat products may have occurred. Other surveys have failed to isolate the organism from poultry products indicating a prevalence of less than 0.25% (5). Other animals that have been either shown to carry *E. coli* 0157:H7 or epidemiologically implicated in the transmission include dogs (15), deers (16), goats (15), orang-utans (11), and wild birds (17). *E coli* has also been isolated from faeces of apparently healthy pigs and piglets; however, the strains isolated were serotypes other than H7 (5).

Mode of transmission: The earliest reported outbreaks of *E. coli* 0157: H7 infections were associated with consumption of ground beef (2). Carcasses can become contaminated with VTEC during the slaughtering process. Faecal contamination on the hide and leakage of the visceral contents has been shown to be the most likely source of contamination (2). Processing steps such as washing of the carcass can potentially lead to the re-distribution of contaminants on the surface of individual carcass and to cross-contamination of other carcasses. Some of the unexpected food borne vehicles of transmission are acidic foods, salad vegetables, turkey roll, lettuce and venison(18).

The acidic foods confirmed as sources of outbreaks include unpasteurized apple and apple cider, mayonnaise and yoghurt (19). Fresh-pressed, unpasteurized apple cider was first identified as a vehicle for *E. coli* 0157: H7 in an outbreak in Massachusetts in 1991, although HUS was first linked to apple juice in 1982 (18,20). In October 1996, two separate outbreaks associated with drinking unpasteurized apple cider occurred, one in Connecticut and the other in the western USA. The Connecticut outbreak involved 14 cases and was associated with drinking a specific brand of cider (19). The second outbreak involved

66 persons in multiple states in the western USA and was associated with drinking a specific brand of apple juice or brand's juice mixtures containing apple juice (21). Salad vegetables have also been implicated as an outbreak vehicle. Populations of viable *E. coli* 0157: H7 inoculated onto vegetables declined when vegetables were stored at 5°C and increased on vegetables stored at 12°C and 21°C for up to 14 days (14).

Dry cured salami was implicated as the vehicle in an outbreak in the state of Washington (22) and venison jerk was reported as the likely vehicle for an outbreak in Oregon in 1995 (18). Consumption of deer steak, was being investigated as the cause of *E. coli*, 0157: H7 illness in two individuals in Illinois in early 1997 (19). Raw milk can be a vehicle of transmission for *E. coli* 0157: H7 but confirmed outbreaks have been few. The presumed mechanism of contamination was during milking. Two outbreaks associated with raw milk have been documented by the CDC, one in 1992 with nine cases and the other in 1993 with six cases. Both outbreaks occurred in Oregon and were traced to two specific dairies, which were licensed to sell raw milk (23). The estimated number of raw milk drinkers in the USA is only 1 to 2%(23). This small population at risk may partly explain the small number of outbreaks due to raw milk consumption. Drinking water and recreational water have been linked to outbreaks of *E. coli* 0157: H7 infection (24). The only known outbreak in the USA, associated with drinking water occurred in 1989 in Missouri. An unchlorinated municipal water source and deficiencies in the water distribution system were implicated as the probable source of contamination (24). Outbreaks associated with freshwater swimming/ recreational areas have been more frequent. During 1982-1994, only two (2.8%) outbreaks associated with swimming water were identified (24). During 1995-1996, however, there were eight (1.3%) associated with swimming water. The importance of person-to-person spread should not be overlooked. During 1994-1995 in the USA, person-to-person spread was identified as the likely vehicle in seven (11%) outbreaks(23). In 1996 there were nine (31%) outbreaks attributed to person-to-person spread. The most frequent setting for person-to-person spread is a day-care facility, but person-to-person spread has occurred in other institutional settings such as nursing homes and mental health facilities, and is common among family members. A small, recent outbreak involving five cases of *E. coli* 0157: H7 in Florida involved two cousins and three siblings. The two cousins contacted *E. coli* 0157: H7 during international travel, and, upon return to the USA, had contact with the three siblings who became affected (23). Person-to-person transmission from asymptomatic cases also occurs (23).

Diagnosis: In majority of laboratories this will take the form of isolation *E. coli* 0157 from faeces using a selective differential agar, with or without an enrichment phase. Commonly employed media are Sorbitol Mac Conkey agar (SMAC) Cefixime-tellurite SMAC

(CT-SMAC) and CT-SMAC supplemented with rhamnose (CTR-SMAC). These rely on phenotypic characteristics of *E. coli* 0157: H7 such as inability to ferment sorbitol, and rhamnose and tolerance to tellurite. Although inexpensive and technically straightforward this approach has the disadvantage that it is designed to detect only serogroup 0157 strains and not strains of other serotypes which, with a few exceptions(25,26), do not ferment sorbitol. Furthermore, 0157: H7 strains., which ferment sorbitol, have been well-documented(25). In addition, these strains are extremely susceptible to tellurite. The sensitivity of culture can be improved by the use of immunomagnetic, separation (IMS)(27). The major disadvantage of this technique is its serotype specificity, although, in at least one instance, antiserum to another serotype has been used (26). Nevertheless, IMS followed by culture on CT-SMAC was more than twice as sensitive as direct culture method in patients with HUS (27). Presumptive colonies of 0157: H7 are further characterised by agglutination in commercially available 0157 or H7 antisera. Because of cross-reactions between the 0157: H7 antigen and other *E. coli* serotypes, *Escherichia* species and other members of the Enterobacteriaceae, biochemical confirmation of the isolates are mandatory(27). There are several alternatives to traditional culture techniques for identification of VTEC in samples(5,27). Detection of free faecal verocytotoxin (VT) by vero-cell assay has been used with success as a diagnostic method(5,27). However, routine use of cell cultures has a drawback of microbial contamination of cell monolayers and interference in the assay by a number of other microbial toxins, which are active against cultured vero-cells(27). Detection of free faecal VT may also be performed by enzyme immunoassays of different formats(5), but although these are more rapid to perform and less subjective than cell culture assays, they lack sensitivity. Enzyme immunoassays and immunoblot techniques for the detection of *E coli* 0157 antigen have been described as methods for the detection of the organism in enrichment culture of food and environmental samples(13). Although sensitive, these methods may be time-consuming, technically demanding, expensive and prone to give false-positive results that cannot be confirmed by culture(25). Testing for verotoxin can also be done using toxin specific antibodies and genes using DNA probes(25). Testing for verotoxin will also identify verotoxin-producing serotypes other than 0157: H7. Identification of *E.coli* 0157: H7 can also be done rapidly, specifically and sensitively using DNA based polymerase chain reaction (PCR) methods. One multiplex PCR method amplifies simultaneously three different DNA sequences of *E.coli* 0157: H7; a specific fragment of the *eae* gene, conserved sequences of verotoxin1 and II and a fragment 60 Mda plasmid. Since this test detects other virulence markers besides verotoxin, it is more specific than tests, which only identify verotoxin genes. PCR methods however, are affected by many laboratory variables and are less reproducible between laboratories than other methods, and are often less sensitive than direct

culture. Molecular methods for interstrain differentiation of *E. coli* 0157: H7 have been developed. These methods are useful in distinguishing between outbreaks of unrelated isolates. The most commonly used DNA fingerprinting tests are based on restriction fragment length polymorphism (RFLP) methodology where restriction enzymes are used to cut genomic DNA into fragments that are separated by agarose gel electrophoresis. A pattern of fragments "fingerprints" is resolved for particular bacterial strains. Several RFLP methods have been developed; one uses pulsed field gel electrophoresis (PFGE), others use conventional gel electrophoresis (26).

Situation of Verotoxigenic E. Coli 0157:H7 in Africa

South Africa: The first reported *Escherichia coli* 0157: H7 haemorrhagic colitis case was in 1990 (28). Since then many sporadic cases of bloody diarrhoea have been reported in many areas of South Africa. Effer *et al* (29) reported verotoxigenic *E. coli* from South Africa in 1992, a large outbreak of bloody diarrhoea caused by *E. coli* 0157:H7 infections occurred in Swaziland, Southern Africa. As many as 40,912 patients less than five years of age visited physicians for diarrhoea during October through November 1992. This was a seven-fold increase over the same period during 1990-1991. The attack rate was 42% among 778 residents screened. Female gender and consumption of beef and untreated water were significant risks for illness. *E coli* 0157:H7 was recovered from seven affected foci in Swaziland and South Africa, 27 out of 31 patients, and environmental isolates had undistinguished pulsed field gel electrophoresis patterns. Compared with previous years, a five-fold increase in cattle deaths occurred in October 1992 due to verotoxigenic *E. coli*. The first heavy rain of 36mm fell that same month, following three months of dry period. Drought carriage of *E. coli* 0157: H7 by cattle, and heavy rains with contamination of surface water appears to be important factors contributing to verotoxigenic *E. coli* outbreak. Molecular techniques were also used for studying the epidemiology of diarrhoea infections due to *E. coli* in Gauteng region in South Africa. From a total of 151 *E. coli* isolates from stools of patients with diarrhoea and 30 strains isolated from stools of healthy individuals were collected between March 1996 to May 1997. Forty eight (26.5%) strains belonged to enteropathogenic *E. coli* (EPEC) O groups and 14 (7.7%) to verotoxigenic producing *E. coli* (VTEC) 0157: H7 serotype. A high percentage (28.2%) of atypical EPEC strains possessing the *eae* A but not the *bfp* A genes were isolated (30). Muller *et al*(31) also investigated occurrence of *E. coli* 0157: H7 in selected water samples in South Africa using chromogenic Rainbow 0157 agar medium. They selected a total of 204 samples from 15 different sites where water was used for direct or indirect human consumption. They found that none of the suspected colonies contained all the virulence factors necessary to classify them as *E. coli* 0157:H7. None of these organisms agglutinated with antisera against *E. coli* 0157: H7. They concluded that probability of being infected with *E. coli*

0157:H7 from direct or indirect consumption of these river water sources is therefore low. Some samples did, however, contain enterohaemorhagic *E. coli* virulence properties, such as Stx1 and Stx2, and enterohaemolysin, which might impose a health risk if ingested.

East Africa: In Uganda, faecal samples were collected from 237 diarrhoeic infants in Kampala, Uganda and from 159 healthy cattle from a ranch in the Central Region of Uganda. These were investigated for the presence of *E. coli* 0157:H7 and other types of Shiga toxin producing *E. coli* (STEC) by Kuddu *et al* (32). *E. coli* 0157:H7 were not detected in 150 stool samples on SMAC. Eightyseven additional human stool samples were tested with an enzyme-immunoassay for shiga toxins (Premier EHEC) and were also negative. Forty two stool samples from infants were also investigated for enteropathogenic *E. coli* (EPEC) by hybridisation with *eae*-specific gene probe. Compared to STEC, EPEC were frequent and found in six (14.31%) of these 42 randomly selected stool specimens. In the same study by Kuddu *et al* (32) STEC were isolated from 45 out of 159 cattle from a herd in the Central Region of Uganda. STEC strains from cattle belonged to different O- and nine different H types. The nine 0: H types were identical to those found in bovine STEC from other continents. Only one bovine STEC strains was positive for the *eae*-gene, and O groups associated with enterohaemorhagic *E. coli* (EHEC) types (026, 0103, 0111, 0145, and 0157) were not found. Their reports demonstrated that STEC are not frequent in urban children in Uganda, but domestic cattle were identified as an important natural reservoir for these organisms in Uganda. In Tanzania a study was conducted by Gaswn *et al* (33) by matched case - control study in the maternal and Child Health Clinic (MCH) in Ifakara during the rainy season in order to elucidate the risk factors for and aetiology of diarrhoeal diseases in children under five years of age. Enterohaemorhagic, enteropathogenic, enterotoxigenic and entroaggregative strains of *E. coli* were not related with diarrhoea, and neither were *Giardia lamblia* or *Salmonella species*. But studies in beef animals by Hayghaimo *et al* (34), showed that beef carcasses are contaminated with VTEC organisms. The authors concluded that this might pose a health hazard especially with under cooked, meat and meat products in this region.

West Africa: In Nigeria Akinyemi *et al*(35), studied *E. coli* infections for over a period of 12 months. A total of 852 stool samples from patients (both children and adults) with acute diarrhoeal diseases attending some public and some government recognised health institutions in Lagos metropolis, were screened for diarrhoeagenic bacterial agents. Of all 83 isolates for *E coli* group, 49 (59%) were EPEC, 17(20.5%) ETEC, 10(12.1%) EIEC, and seven (8.4%) EHEC. The EPEC strains particularly serotypes 055, were mostly encountered in children aged over five years. On the other hand, EIEC and ETEC strains were found mainly in adults while EHEC 0157:H7 strains

occurred in all the age groups studied. Their study further stresses the important role EIEC and ETEC strains play in acute diarrhoeal diseases and the possible implication of EHEC in acute gastroenteritis especially in children in Lagos, Nigeria. Okeke *et al.* (36) also reported that in a study carried out in small-towns and rural primary health care centres in South-western Nigeria, 330 *Escherichia coli* strains isolated from 180 children with diarrhoea and 144 apparently healthy controls, were examined for virulence traits. On results of colony blot hybridization, strains were categorised as enteropathogenic *E. coli* (1.8%) enterotoxigenic *E. coli* (2.4%), enteroinvasive (1.2%) enterohaemorhagic *E. coli* (0.6%), enteroaggregative *E. coli* (10.3%), diffusely adherent *E. coli* (7.9%), cell-detaching *E. coli* (6.9%) and cytolethal distending toxin-producing *E. coli* (0.9%). *E. coli* strains that hybridized with a shiga toxin gene probe that lacked other characteristics usually present in enterohaemorhagic *E. coli* constituted 8.4%. While Olorunshola *et al* (37) examined the prevalence of sorbitol- non fermenting. *E. coli* 0157:H7 (EHEC) in 100 patients with diarrhoea by stool culture on sorbitol MacConkey agar in Lagos Nigeria. The detection rate of 0157:H7 was 6%. Five of the six patients were from children below five years of age and one was from a teenager. All strains induced cytotoxic effect in the vero-cell assay. All isolates were susceptible to most of the antimicrobials tested. There must therefore be adequate meat and food inspection to improve the general hygiene of local fast food restaurants; so-called "bukkas" that are regarded as likely sources of infection. In Cote d'Ivoire the first report of Shiga-toxin producing *E. coli* was from Dadie *et al* (38), who reported two 0157:H7 strains from children. One (EA 47) 0157:H7 was isolated from a child and the other (EH 144) 0157:NM from human diarrhoea stool specimens. Both 0157 strains carried Stx2, *eae*, and *Uid A* genes but not *e-hly* gene. The author concluded that *E. coli* did not appear to be a public health problem in Cote d' Ivoire.

Central Africa: In Cameroon the first reported *E. coli* 0157: H7 case was in November 1997 and April 20, 1998 when bloody diarrhoea of 298 sick people in Cameroon was investigated in a laboratory (39,40). The epidemic recorded a case fatality rate of 16.4%. Amoebiasis was isolated from one of the three patients where three types of pathogens were also found. The pathogens were multiple drug resistant *Shigella dysenteriae* type 1, *Shigella boydii* and enterohaemorrhagic *E. coli* 0157:H7.

North Africa: The only report was that of survey done in middle Egypt to determine if *E. coli* 0157:H7 was present in 175 samples of raw ground beef, chicken, lamb and unpasteurized milk. The pathogen was detected in three out of 50(6%) beef samples, two out of 50(4%) chicken samples, one out of 25(4%) lamb samples obtained from slaughter houses and three out of 50 (6%) milk samples obtained from supermarkets and farmers' homes (14).

In conclusion, surveillance of VTEC 0157:H7 infection is well established in many developed countries and it is now apparent that there are geographical differences in the incidences of infection. Although cattle and other ruminants are regarded as the main reservoir of VTEC, these bacteria have also been isolated from a number of non-ruminant animal species. This review has also shown that verotoxigenic producing E. *coli* are also present in man and animals in Africa and this may pose a health hazard to the public. There is need for more knowledge of the distribution of VTEC in these reservoirs, and for investigation of the pathogenic potential of the many non-0157 VTEC in animals and foods.

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