

Epidemiology and Transmission of Pathogenic *Escherichia coli*

CO-ORDINATION ACTION
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Pathogenic *Escherichia coli* Network

Editors

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1. Introduction

Epidemiological studies form the basis of public health research and preventative medicine. They provide a method of risk assessment from which precautionary measures can be put into clinical practice. *Escherichia coli* commonly colonises the human gut. While some strains are regarded as being relatively harmless, there are many which are associated with human disease. *Escherichia coli* can be subdivided into pathological groups according to the main features of the induced disease. One of these pathogroups, commonly referred to as Diarrheagenic *E. coli* (DEC) includes *E. coli* strains which cause gastroenteritis in humans and animals. DEC pathotypes, in particular some strains of Verocytotoxic *E. coli* (VTEC), are pathogens of significant public health concern and have been the subject of epidemiological studies for some time. While there is significant data available on the VTEC pathotype there remains a gap in knowledge surrounding the remaining pathotypes; Enteropathogenic *E. coli* (EPEC), Enterotoxinogenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAaggEC) and Diffusely adherent *E. coli* (DAEC).

E. coli strains are also responsible for disease in animals, where some of them, namely VTEC, are important zoonotic agents. It is known that farm animals can act as carriers or reservoirs for some of the main pathotypes of DEC, making them vectors for the spread of these pathogens in the environment, providing a way of entry into the food chain and ultimately transmission to humans.

Studies on the prevalence of VTEC on farms have shown that *E. coli* O157 is present on most farms sporadically. Research has also shown that all ages of cattle are susceptible to colonisation with VTEC and intermittent shedding of the organism. While cattle are known to be one of the most common carriers of VTEC, other food animals such as sheep have also been shown to be significant sources of these bacteria. Moreover, studies aimed at the determination of VTEC presence in sheep have reported that prevalence rates may be higher than those in cattle.

The prevalence of pathogenic *E. coli* at farm level can result in direct and indirect transmission to humans via direct contact with animals or their faecal matter, or indirectly via consumption of VTEC contaminated food or water. Meat products such as ground beef and fermented sausages are well documented sources of *E. coli* O157. Dairy products, in particular those made from raw milk, are also a typical source of VTEC, including pathogenic strains. The objective of this document is to review current data on the epidemiology of pathogenic *E. coli* from farm to fork and highlight the gaps in knowledge that currently exist.

2. Epidemiology of pathogenic *Escherichia coli* on farms

2.1 Verocytotoxin-producing *E. coli* (VTEC)

VTEC can be present in the intestinal tract of a wide range of domestic and wild animals and ruminants (sheep, goats and cattle), especially cattle, are considered to be the major reservoir for *E. coli* O157 and other VTEC. They are usually asymptomatic carriers, with the bacteria surviving in the colon and being passed in the faeces. The reported prevalence of *E. coli* O157 in cattle is shown in Table 1.

While the results are not comparable because of the different laboratory detection methods employed, sample sizes, age of animals, etc., several general features regarding the epidemiology of *E. coli* O157 on farms are notable:

- *E. coli* O157 have been found on virtually all farms at least intermittently
- Strong seasonality with a peak in late Summer to early Autumn
- Transient residence in the gastrointestinal tract of individual animals (2 months or less) that is usually not associated with clinical disease
- All ages of cattle are susceptible to colonisation with VTEC, however, it is rarely detected in pre-weaned calves and is most common in cattle from weaning to 24 months of age
- The prevalence of cattle herds shedding *E. coli* O157 is usually high although at any given point in time relatively few animals within the herd are shedding

- Occasionally shedding may be confined to a few single animals within the herd, known as supershedders
- Broad host range including cattle, sheep, dogs, birds, horses, amongst others
- Faecal shedding is confined to sharp bursts in a high percentage of animals separated by long periods of very low prevalence
- Complex molecular epidemiology with several PFGE subtypes existing on the same farm with periodic additions and turnover even on farms where no new animals are introduced

Table 1. *E. coli* O157 prevalence in cattle.

| Country | Incidence | Comment |
|-----------------|------------|---|
| Denmark | 5% | 82 cattle tested |
| England & Wales | 4% | 2,103 bovine rectal swabs |
| France | 0.2% | 471 faecal samples from cattle with a low sensitivity screening method |
| Ireland | 3% | 750 samples at slaughter |
| Italy | 13% | Highest in feedlot cattle (17%) and dairy cows (16%). Highest in Summer (18%) and lowest in Winter (3%) |
| The Netherlands | 0.8 to 22% | Excretion rates peaked in Summer and were lowest in Winter |
| Norway | 0.3% | 1,970 animals tested using Immunomagnetic separation (IMS) and PCR |
| Spain | 2% | 383 slaughter cattle by IMS |
| Sweden | 14% | 631 animals tested in 6 herds |
| USA | 2% | 11,881 faecal samples from cattle in 100 feedlots |

The strong seasonal pattern of *E. coli* O157 prevalence in cattle has been reported by several research groups. A similar Summer increase has been reported in human infections in temperate climates. Although it was initially speculated that the human Summer peak could be attributed to out-door cooking patterns, it is interesting to note that the contamination rates in retail

meats roughly agree with the seasonal pattern thus establishing a harmony between incidence in cattle, retail meats and human illness.

The effect of diet on *E. coli* O157 prevalence in cattle remains controversial. Higher prevalence of *E. coli* O157 in grain fed cattle and lower prevalence in cattle on a hay only diet has been shown, although the reasons for this difference have not been fully explained. Several studies suggest that levels of *E. coli* O157 in cattle faeces are variable and often very low usually less than 10 organisms per gram. The average concentration of *E. coli* O157 in cattle faeces in Scotland has been estimated to be 1.19×10^3 organisms per gram during the warmer months and 3.30×10^2 organisms per gram in the cooler months. Animals shedding high numbers of *E. coli* O157 (i.e. $>10^4$ organisms per gram) in their faeces have been referred to as “high shedding animals” or “super shedders”. These animals pose a significant risk of contaminating other animals within the herd or the environment. Housing and animal stocking density are other factors that can influence transmission of *E. coli* O157 between animals in a herd.

Other ruminants such as sheep and goats are known carriers of *E. coli* O157 and other VTEC but there have been fewer surveys to determine prevalence rates in these animals compared with cattle. Prevalence rates for *E. coli* O157 and other VTEC in sheep and goats are reported to be higher than those in cattle. In Australia, 88% of faecal samples from sheep grazing on pasture were reported to be positive for VT coding genes (*vtx*).

Sheep and cattle can carry a diverse range of VTEC serotypes with a number of these linked to human disease. One Spanish study reported that 85% of bovine VTEC serotypes identified in one study were human-associated VTEC serotypes and 54% were VTEC serotypes associated with human haemolytic uremic syndrome (HUS). *E. coli* O157 has been isolated sporadically from pigs. Therefore foodstuffs from these species can be a major source of human infection.

Host association with particular VTEC serotypes and certain animals appear to exist although it is true that some serotypes of VTEC isolated from cattle and other animals are rarely or never isolated from humans. Examples include serotype O113:H21 which is commonly isolated from cattle and O91:H⁻ and O5:H⁻ which are found in sheep. Associations between specific animal hosts, toxin type and other virulence genes carried by VTEC have also been reported. Some variants of the Verocytotoxin 2-coding genes are associated with animal hosts such as VT2e, causing oedema and post-weaning diarrhoea syndrome in pigs and the VT2f found in *E. coli* strains isolated from pigeons. In cattle, the VT1-coding gene was reported to be the most common toxin type, present in over 90% of O26 strains together with *eae* and *E-hly* genes. Other studies have found that almost half of the VTEC serotypes isolated from sheep and goats were associated with human disease although some lacked the *eae* gene.

2.2 Survival and persistence of VTEC

The environment is important in the epidemiology of VTEC in terms of persistence outside the main animal reservoir and dissemination from one animal to another. It has long been recognised that *E. coli* are capable of survival and growth in a range of natural environmental conditions and VTEC are well adapted to survive in faeces, soil and water posing a significant risk of transmission to and (re)infection of farm animals. Survival in animal manures and slurry for periods ranging from several weeks to many months has been observed and VTEC will also persist on grass and on pasture land facilitating transmission to other herbivores grazing the contaminated grass.

Survival in a range of different types of water for extended periods of up to several weeks facilitates widespread dissemination to other farms and wild animals over a wide area. In the aqueous environment, virulence and resistance to biocides and antibiotics may be enhanced by intraprotozoal growth. The spreading of manures and slurry may also disseminate these pathogens over fields which may be subsequently grazed by previously uninfected animals.

Bacteriophages play a major role in the transmission of the *vtx* and related genes within strains of *E. coli*. Although little is known about the actual incidence of active gene transfer under natural conditions, transduction among other species increases in aqueous environments. There is also evidence that transfer rates may be even higher under natural conditions. Furthermore, bacteriophages, which are the reservoir of *vtx* in nature, survive longer than VTEC in water. Thus, the farming environment is a critical stage for the development of new VTEC strains as well as the dissemination of existing VTEC.

The prophylactic application of antibiotics in farming selects for more resistant strains. With VTEC there is a further complication as antibiotics induce lysogenic Verocytotoxin encoding bacteriophages increasing the overall horizontal transfer of VT-coding genes within the animal intestine and the excretion of bacteriophages into the environment. Thus, antibiotic based animal husbandry interventions may be an important contributor to the emergence of new strains of VTEC in the future.

2.3 Epidemiology of Non-VTEC pathogenic *E. coli* on farms

Although non-VTEC pathotypes such as EPEC and ETEC have animal reservoirs (the latter represents a significant pathogen in piglets, calves and lambs), there is a dearth of information on epidemiology of these *E. coli* in the farm environment.

ETEC can be isolated from domestic animals and humans and frequently occurs in diarrhoeal disease afflicting domestic animals. Two important determinants of virulence that play a role in the development of ETEC infection are the ability to colonise the small intestine and the production of enterotoxins. Strains of ETEC may synthesize thermolabile (LT-I and LT-II) and/or thermostable (STa and STb) enterotoxins. The ability of ETEC strains to cause diarrhoea is also conditioned by the possession of colonisation factors. Both enterotoxins and colonisation factors are encoded by plasmids.

Owing to the specific recognition between bacterial colonisation factors and epithelial receptors during host-parasite interaction a high level of specificity exists between ETEC strains causing diarrhoea in humans and those found in domestic animals. Human ETEC strains commonly belong to serogroups O6, O8, O15, O20, O25, O27, O63, O77, O78, O114, O115, O126, O128, O139, O148, O153, O159 and O167 and are associated with production of LT-I and/or STa enterotoxins and possession of colonisation factors CFA/I, CFA/II, CFA/III or CFA/IV. ETEC strains from pigs are associated with production of LT-I, STa and/or STb, possession of colonisation factors F4, F5, F6 or F41 and generally belong to serogroups O8, O9, O20, O45, O64, O101, O115, O138, O141, O147, O149 and O157. ETEC in cattle and sheep generally belong to serogroups O8, O9 and O101 and are usually STa producers possessing the bacterial surface F6 or F41 colonisation factors.

Not all *E. coli* that cause diarrhoea in farm animals act by elaborating the classical heat-labile or heat-stable enterotoxins produced by ETEC strains. The virulence mechanisms most commonly associated with Enteropathogenic *E. coli* (EPEC) are those involved in attachment and effacement (A/E) and cytoskeletal rearrangement of intestinal brush borders, which are distinct from ETEC. Animal EPEC attach to and efface the microvilli of the gut epithelium and resemble the human EPEC. However, compared to the adhesion of animal ETEC and human EPEC, the adherence factors important in the pathogenesis of animal EPEC are not so well understood.

3. Epidemiology of pathogenic *Escherichia coli* in food

3.1 Epidemiology of VTEC in food

The hides and fleeces of ruminant animals represent a key source of VTEC contamination of meat at slaughter plants. The prevalence of *E. coli* O157 on cattle hides presented for slaughter has been shown to range from about 7 to 20% and on sheep fleece presented for slaughter, the prevalence of *E. coli* O157 is reported to be about 5%. Unpasteurised (raw) milk may be contaminated with VTEC and the potential for the pathogen to survive in dairy products made from raw milk, particularly in soft and semi-soft cheeses, make these commodities a potential risk. Some strains of *E. coli* O157 and a

number of other VTEC serotypes appear to have a tolerance to acid which allows survival in acidic foods. Such foods have been associated with food poisoning outbreaks and include ready-to-eat fermented meats such as salami and pepperoni type products. Thus, unless intervention steps are included during processing to specifically reduce any potential VTEC on raw meat, these products pose a high risk to consumers. Fresh vegetables, salads and fruits can be contaminated with VTEC from direct contact with faecally contaminated soil, agricultural run-off or irrigation water. All these commodities have been implicated as transmission routes for VTEC to humans, most notably in a recent large spinach related outbreak in the USA. However, prevalence studies have only rarely detected the presence of the pathogen on fresh produce.

There is much published scientific data on the prevalence of VTEC in a range of foods, but mainly on *E. coli* O157, although in recent years, a number of studies on non-O157 VTEC serogroups have been carried out. The Food Safety and Inspection Service (FSIS) in the US reviewed literature data produced between 1997 and 2005 for non-US countries describing the prevalence of non-O157 VTEC in different food items. The FSIS produced a report which serves as a comprehensive reference for interested stakeholders, a synopsis of which is reported in Table 2. (www.fsis.usda.gov/PDF/STEC_101207.pdf).

The prevalence data on VTEC in food in Europe reported by 16 EU Member States (MS) in 2006 have been collated in the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the European Union, published by the European Food Safety Authority (EFSA, 2007) and shown in Table 3. It should be noted, however, that the data presented on the prevalence of non-O157 VTEC are not comparable due to the different sampling strategies adopted and to the non homogeneous methods applied in the different surveys. In fact, while standard methods are available for the detection of *E. coli* O157 in foodstuffs (ISO 16654:2001 and AOAC Official Method 2005.04), as regards other VTEC serogroups, this issue is still under debate. In the

framework of the European Committee for Standardisation activities (CEN WG6 Microbial Contamination, CEN/TC/275 Food analysis-horizontal methods) a technical specification (TS) regarding a horizontal method for the detection of VTEC belonging to the serogroups identified as those being pathogenic to humans (O157, O26, O111, O103, and O145) has been drafted and is now being voted upon. Should the TS be approved and published, it will represent the first standard method available for producing harmonised data to be used in risk profiling and assessment programs and to obtain data suitable for comparisons between MS.

Table 2. Prevalence of VTEC in retail foods in retail meats

| Country | Organism | Reported prevalence | Reference |
|-------------|------------------------------------|---|--|
| Argentina | <i>E.coli</i> O157:H7 | 4.8% of fresh sausages; 3.8% of raw ground beef; 3.3% of dry sausages | Chinen <i>et al.</i> , 2001 |
| Belgium | All VTEC | 4.6% of raw meat samples (beef, mutton and venison) | Pierard <i>et al.</i> , 1997 |
| Botswana | <i>E. coli</i> O157:H7 | 5.2% of meat cubes; 3.8% of raw ground beef; 2.3% of fresh sausages | Magwira <i>et al.</i> , 2005 |
| England | <i>E. coli</i> O157:H7 | 2.9% of lamb products, 1.1% of beef products | Chapman <i>et al.</i> , 2000 |
| France | All VTEC | 11% of beef, 10% of cheese | Pradel <i>et al.</i> , 2000 |
| France | <i>E. coli</i> O157:H7 | 0.1% of raw ground beef | Vernozy-Rozand <i>et al.</i> , 2002 |
| India | Non-O157 VTEC | 3.3% fish samples; 6.25% clam samples | Sanath Kumar <i>et al.</i> , 2001 |
| Italy | <i>E. coli</i> O157:H7 | 0.4% of raw ground beef | Conedera <i>et al.</i> , 2004 |
| New Zealand | All VTEC | 17% of lamb; 12% of beef, 4% of pork, 0% of chicken | Brooks <i>et al.</i> , 2001 |
| Sweden | <i>E. coli</i> O157:H7 All VTEC | 0.06-0.5% of raw ground beef 4% of raw ground beef | Lindqvist <i>et al.</i> , 1998 |

Source: Eblen, DR. (2007). Public Health Importance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (non- O157 STEC) in the US Food Supply. USDA, FSIS, OPHS (www.fsis.usda.gov/PDF/STEC_101207.pdf).

Table 3. VTEC findings in other foodstuffs

| | Description | VTEC | | VTEC O157 | |
|-----------------|-------------------------------|--------------|-----------|------------|----------|
| | | N | Pos | %Pos | Pos |
| Austria | Juice | 118 | 0 | - | - |
| Germany | Vegetables | 179 | 0 | - | - |
| Italy | Eggs | 130 | 0 | - | - |
| | Fishery products, unspecified | 101 | 0 | - | - |
| | Other food | 90 | 0 | - | - |
| Netherlands | Fruits | 816 | 0 | - | - |
| Slovenia | Vegetables (sprouted seeds) | 30 | 0 | - | - |
| | Vegetables | 50 | 0 | - | - |
| Spain | Eggs | 76 | 0 | - | - |
| | Fishery products | 350 | 13 | 3.7 | 0 |
| | Vegetables | 51 | 0 | - | - |
| EU Total | | 1,991 | 13 | 0.7 | 0 |

Source: The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the European Union in 2006. (*EFSA Journal*, December 2007)

The food safety authorities in industrialised countries have thus introduced VTEC into the relevant regulations on microbiological criteria to be applied to foodstuffs. In 1994, following a large food-borne outbreak in the US caused by the consumption of under-cooked contaminated hamburgers (Center for Disease Control and Prevention, CDC, 1993), the FSIS declared that *E. coli* O157 were to be considered as an adulterant in raw ground beef, and established a zero-tolerance policy for this pathogen in this food matrix. FSIS established that any raw ground beef found to contain *E. coli* O157 must be disposed of, or sent for further processing involving a lethality step and instituted routine testing of ground beef for the presence of this pathogen.

The New Zealand Food Safety Authority has adopted several risk profile studies concerning the prevalence of *E. coli* O157 and other VTEC in different food items, such as raw milk and uncooked comminuted fermented meat in order to identify the risk management options.

In European countries, VTEC is included as one of the pathogens to be observed under the EU Directive 2003/99/EC on monitoring of zoonoses and

zoonotic agents. Included are foods with the highest risk of transmitting VTEC infection to humans, namely raw and undercooked meat; minced meat; raw milk and unpasteurised dairy products; fresh produce fruit and vegetables (sprouts, lettuce, spinach, tomatoes); unpasteurised fruit and vegetable juice.

In October 2007 the EFSA adopted an opinion from the Panel on Biological Hazards concerning the monitoring of VTEC and the identification of human pathogenic VTEC types. Recommendations were issued on monitoring of animal populations and foodstuffs. It was proposed that as part of the European food-borne zoonoses monitoring scheme, the scheme should initially focus on *E. coli* O157 and then be extended to other VTEC serogroups (such as O26, O103, O91, O145 and O111) that are identified as pathogenic for humans based on the periodical analysis of human disease and epidemiological data.

In the EFSA opinion it is suggested that data on the prevalence and concentration of VTEC in ruminants at pre-harvest stage (faeces, coat), and post-harvest stage (carcasse after chilling at the abattoir) would assist in the assessment of the level of risk to consumers. It is also recommended that targeted surveys are carried out on a range of other foodstuffs that have been associated with human illness.

3.2 Epidemiology of other pathogenic *E. coli* in food

As VTEC represent the only pathotype with a well-recognised zoonotic origin and foodborne route of transmission, pathogenic *E. coli* belonging to different pathogroups may be occasionally present in foodstuffs and water as secondary contamination.

EPEC infections are common in developing countries where sanitation and water quality may be poor. ETEC is a common cause of travelers' diarrhoea resulting from consumption of contaminated water or food. There is epidemiological evidence of outbreaks of ETEC on cruise ships associated with consuming beverages with contaminated ice cubes on board the ship.

Food and water may be vectors of EIEC and there have been some large outbreaks associated with this group of *E. coli*. In the USA, 227 became ill, in 96 separate outbreaks from the consumption of imported French Camembert or Brie Cheese contaminated with EIEC serogroup O124. Another outbreak occurred on a cruise ship from consumption of potato salad contaminated with EIEC.

EAggEC infections may occur as both sporadic cases or outbreaks, but sources of infections have rarely been identified in these episodes. A study carried out on a large number of farm animals (cattle, sheeps and pigs) in the United Kingdom failed to show the presence of EAggEC in the intestinal content of the animals. Recently, there was a report of two gastroenteritis outbreaks associated with EAggEC in Italy and epidemiological investigation indicated a typical cheese as the source of infection, even though the hypothesis that contamination occurred during food handling could not be overlooked. The role of food in the transmission of this pathotype may be underestimated due to the lack of routine examination of foods for this group of micro-organisms.

4. Epidemiology of pathogenic *Escherichia coli* in humans

4.1 Epidemiology of VTEC infections in humans

In 1982, *E. coli* O157 was recognised as a human pathogen for the first time and, since then, has been increasingly reported as the cause of illness and outbreaks. *E. coli* O157:H7 belongs to the group of VTEC of which > 200 different serotypes of *E. coli* have been reported so far, with many of these associated with human disease. The clinical features of *E. coli* O157 infections include diarrhoea, which is often bloody, and may progress to severe haemorrhagic colitis. About 10% of these patients can go on to develop HUS, a potentially life-threatening complication characterised by acute renal failure, thrombocytopenia, and haemolytic anaemia that is particularly serious in young children and elderly people. Neurological

involvement is also possible especially among elderly patients. On average, the case fatality rate of HUS is about 3–5%.

The most commonly reported VTEC serogroup associated with human disease is O157. Surveillance of *E. coli* O157 infections is now well established in many countries due to the availability of simple screening techniques and sensitive cultural methods for the isolation and characterisation of these strains. In Europe, 25 of 32 member states have compulsory surveillance of VTEC-infections. It is, however, apparent that there are geographical differences in both the incidences of infection and distribution of serotypes. In contrast to *E. coli* O157, much less is known about the real incidences of non-O157 VTEC. Within Europe, data from Enter-net show that in Scotland the rate of VTEC infections in 2005 was 3.26/100,000 of which 95% were O157 compared to, for example, 1.4/100,000 in Germany of which only 10% were O157. The highest VTEC rate in Europe in 2005 was found in Sweden, with an incidence of 4.09/100,000, with about 50% O157. It should be noted that routine clinical laboratories in Scotland did not look for non-O157 VTEC, while the laboratories in Germany and Sweden both used detection of Verocytotoxins (VT) or *vtx* for initial screening. The overall incidences in Europe during 2005 spanned from 0 to 4.09; this difference covers both real differences in incidences and differences due to guidelines for sampling, diagnostic methods and reporting systems. For the years 2000-2005, data from the Enter-net VTEC database show an upward trend in reported cases with numbers rising from 2022 cases to 2660 (31.6%).

Experience from a number of countries, including many of those in continental Europe clearly, indicates that several non-O157 VTEC are capable of producing the full spectrum of disease that we have come to recognise as characteristic of *E. coli* O157 infection. There are numerous reports of sporadic infections with non-O157 but even smaller outbreaks have been described in Germany, Denmark, and Norway. The most commonly implicated serogroups in Europe other than O157 include O26, O103, O91, O145, O121 and O111, with a similar picture worldwide. The attributes, other than VT production, that determine the pathogenicity of any given VTEC isolate are not completely understood, however, it is known that factors which permit

colonisation of the human gut and expression of virulence are involved. There is clearly a need to learn more about the clinical and epidemiological features of infection with serogroups other than O157, and this will require increased standardisation and implementation of existing methods for detection in the routine laboratories.

According to the Enter-Net annual report of 2005 the highest incidence of VTEC infections is seen in children ≤ 4 years of age (9.04 per 100,000), with the incidence rate falling rapidly with increasing age. Data on gender distribution available for 2,074 cases, showed no differences between the incidence in females (0.49 per 100,000) or males (0.46 per 100,000).

VTEC infections show a marked seasonal tendency with more cases being reported as the temperature rises, reaching a peak in September.

Outbreaks of VTEC have been reported worldwide, either as food borne outbreaks due to faecal contamination of foods, or in many cases as a result of direct contact with infected animals and swimming outdoors in contaminated recreational waters.

4.2 Epidemiology of EPEC infections in humans

EPEC were originally defined as specific serogroups of *E. coli* associated with infantile diarrhea. EPEC identification is now based on the presence of specific virulence genes, thus including strains not belonging to classic EPEC serogroups. The presence of both the *eae* (intimin) and *bfpA* (bundle-forming pilus) genes is used for the identification of typical EPEC, while atypical EPEC do not encode *bfp*.

EPEC are among the most important pathogens causing diarrhoea in children under 2 years in developing countries. EPEC prevalence varies according to different study populations, age distribution, diagnostic criteria and diagnostic methods, amongst other variables. Some studies report that when diagnosis was based on intimin-coding gene identification, EPEC was responsible for an average of for 5–10% of paediatric diarrhoeal episodes in the developing world. However, when phenotypic criteria such as HEp-2 adherence pattern or

serotyping were used for diagnosis, the estimated prevalence rates of EPEC were increased by an average of 10–20%, with large variability among studies.

While typical EPEC have been regarded as one of the leading causes of infantile diarrhoea in developing countries, atypical EPEC is the causative agent in industrialised countries. Recent data suggests that atypical EPEC are more prevalent than typical EPEC in both developing and developed countries. Data from several studies has shown overall that atypical EPEC was responsible for 78% (131/169) of all EPEC cases in children younger than 5 years with diarrhoea.

The duration of diarrhoea in patients infected with atypical EPEC has been shown to be significantly longer than that caused by other pathogens. Atypical EPEC was the most common pathogen in two studies from Australia (43% [12/28]) and Norway (22% [20/89]) among children with persistent diarrhoea. Moreover, in contrast to typical EPEC, it has been hypothesised that atypical EPEC may have an animal reservoir which is important for transmission to humans.

4.3 Epidemiology of ETEC infections in humans

ETEC are classified on the basis of their ability to produce the heat stable ST and/or heat labile LT enterotoxins. They represent an important cause of diarrhoea in developing countries in children and are the leading cause of traveller's diarrhoea in high-risk areas in Africa, Asia and Latin America. The incidence of ETEC infections in developing countries decreases after 5 years of age, but then increases again in the age groups over 15 years of age. The explanation of this age distribution may be due to factors related both to the microbe, the host and the environment.

ETEC are not considered zoonotic agents as there are different adherence factors found in human and animal strains. Outbreaks among humans are associated with different food stuffs reported to be contaminated by human faeces. Contaminated drinking water and seafood, notably oysters, appear to be among the most important sources for infection

Little is known about the real incidences of ETEC in the industrialised world. This is mainly due to lack of surveillance systems, lack of mandatory reporting systems and that ETEC is not included in the panel of microbes that are tested for in routine diagnostic investigations for enteropathogens. Incidences are generally regarded as negligible, however, several studies have demonstrated that ETEC prevalence's are higher than expected. A one year study at an infectious disease clinic in Stockholm showed that ETEC were at least as common as *Salmonella* spp.

4.4 Epidemiology of EIEC infections in humans

This group is highly related to *Shigella flexneri* and *Shigella sonnei*. The definition of EIEC differs from country to country and is not clear-cut. Typical serotypes associated with the EIEC pathotype is O159:H2 and O143:H⁻. EIEC is not a zoonotic agent and transmission occurs via the faecal-oral route, with contaminated food and water being the main source of infection. Person-to-person transmission is also common. Outbreaks and sporadic cases of gastroenteritis are reported especially from regions with poor hygiene and sanitary conditions.

4.5 Epidemiology of EAggEC infections in humans

Enteraggregative *E. coli* (EAggEC) cause acute and persistent diarrhoea among children and adults in developing countries, and HIV-infected persons in both developing and developed countries. They are being increasingly recognised as world-wide distributed enteropathogens.

A meta-analysis of published studies suggests that EAggEC was the cause of acute diarrhoeal illness in a median of 15% of children living in developing countries and 4% of children living in industrialised countries. The virulence of EAggEC is still being investigated by several research groups. Data that supports the virulence of EAggEC comes from a volunteer challenge study of the prototype strain O42; outbreaks reported in Europe and Japan; large case-series and cohort studies of children; adults and HIV-infected persons living in developing and developed countries; and cases among international travellers to developing countries.

Risk factors for EAaggEC have been identified to include travel to developing countries, ingestion of contaminated food and water, poor hygiene, host susceptibility, and possibly immunosuppression (HIV infection).

4.6 International Surveillance Systems

Enter-net has been an international dedicated surveillance network for the enteric pathogens *Salmonella* spp., *E. coli* and *Campylobacter* spp. between 1997 and 2007. In 2007, responsibility for the co-ordination of Enter-net was transferred to the European Centre for Disease Prevention and Control under the Programme on Food and Waterborne diseases and Zoonoses.

In the U.S.A., a national molecular sub typing network for food borne disease surveillance; PulseNet, was established by Centre for Disease Control and Prevention in 1996. Shortly after the successful launching of PulseNet, PulseNet International was gradually formed. PulseNet International is a network of regional networks worldwide, and presently includes the USA, Canada, Europe, Asia Pacific, Latin America, and Middle East. These networks have proved to be very beneficial in the investigation of food borne disease outbreaks and in the early identification of food borne disease clusters.

Data on the monitoring of zoonoses and zoonotic agents, including VTEC, are collated and reported by the European Food Safety Authority (EFSA) under the Directive 2003/99/EC of the European Parliament. With the assistance of the Community Reference Laboratory for the Epidemiology of Zoonoses, the Commission collects and compiles the results of monitoring annually from Member States. The reports provide a basis for the evaluation of the current situation concerning zoonoses and zoonotic agents. However, the data collection systems are not harmonised and therefore do not permit comparison between different countries.

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