A REVIEW

Verotoxigenic *Escherichia coli* from animals, humans and foods: who's who?

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1. SUMMARY

Verocytotoxigenic (shigatoxigenic) and enterohaemorrhagic *Escherichia coli*, VTEC (STEC) and EHEC, produce a toxin active on Vero cells *in vitro*. VTEC and EHEC have been isolated from humans and different animal species, mainly ruminants and pigs. The verocytotoxins, also named shiga toxins (Stx), are active *in vivo* on the endothelial cells of the blood vessels of the gastro-intestinal mucosa, the kidneys, the brain, and other tissues of humans and piglets, leading to fluid leakage or haemorrhages. Conversely, their role in diseases of young ruminants remains unclear. Adult ruminants can also act as asymptomatic carriers of VTEC and EHEC strains similar to those causing diseases in humans. And they are incriminated as an important source of direct or indirect contamination of humans by the most famous EHEC strain belonging to the O157:H7 serotype, through faecal contamination of either foods of animal origin, or other foodstuffs (fruit, vegetables, etc.), or the environment. But dozens of non-O157 human and ruminant VTEC and EHEC strains with similar general and virulence-associated properties, have been described, whose epidemiology is much less well understood. The purpose of this review manuscript is to describe and compare the properties of human, ruminant and food VTEC and EHEC strains.

2. INTRODUCTION AND SCOPE

Among the numerous classes of pathogenic *Escherichia coli* the verocytotoxigenic (or shigatoxigenic) strains have certainly the widest notoriety. Their names originate from the production of a toxin active on Vero cells in culture, which is also related to the shiga toxin (Stx) of *Shigella dysenteriae* type 1. Such verocytotoxigenic *E. coli* (VTEC) have been known for half a century in the pig industry, as the cause of oedema disease (ED) in recently weaned piglets. But until the early 1980s they were of little significance, if any, in human medicine. Why they have become so notorious in a few years in comparison with the other pathogenic strains of *E. coli* is certainly related to their high pathogenicity in humans, with potentially serious clinical outcomes and to their possible transmission to humans via foods of animal

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origin, especially ruminants. These particular *E. coli* strains have therefore rapidly become a major concern and study subject in human medicine, molecular biology and veterinary medicine.

The purpose of this review is to (i) present the history and definitions of VTEC (ii) describe the current knowledge on their virulence properties and (iii) compare them based on their origin. This review manuscript is based on several review articles and books recently published in the medical, microbiological and veterinary fields (Ludwig and Goebel 1997; Scotland and Smith 1997; Hancock *et al.* 1998; Kaper *et al.* 1998a,b; Melton-Celsa and O'Brien 1998; Meng and Doyle 1998; Nataro and Kaper 1998; O'Brien and Kaper 1998; Paton and Paton 1998; Mainil 1999; Duffy *et al.* 2000; Blanco *et al.* 2001; Brown *et al.* 2001; De Boer and Heuvelink 2001; Gyles 2001; Schmidt *et al.* 2001; Tozzi *et al.* 2001; Koronakis and Hughes 2002; Thorpe *et al.* 2002) and on national and international scientific reports (Anonymous 2003; Vernozy-Rozand and Roze 2003). Other important past or recent research papers are also cited in the text.

3. HISTORY AND DEFINITIONS

The history of VTEC follows two convergent paths. The first pathway began in 1977, when the production of a cytotoxin causing the death of Vero cells in culture (hence the name verocytotoxin) was reported for strains of *E. coli* isolated from humans suffering from diarrhoea and from piglets suffering of postweaning ED (Konowalchuk *et al.* 1977). A few years later, several human *E. coli* strains, including one strain previously studied by the group of Konowalchuk (strain H30) were reported to produce a similar cytotoxic effect on HeLa cells in culture, which could be neutralized by an immune serum produced against the Stx of *S. dysenteriae* type 1 (Stx), hence the name shiga-like toxin (O'Brien *et al.* 1982). During the following years, the identity of these two types of toxins was recognized, as was their heterogeneity. The nomenclature 'verocytotoxin and VTEC' has been used since then by British and Canadian teams, while the nomenclature 'shiga-like toxins and shiga-like toxin-producing *E. coli*' was used by the American teams until 1996, when the names shiga toxins and shigatoxigenic *E. coli* (STEC) were proposed, based on the biological relation to the Stx produced by *S. dysenteriae* type1 (Calderwood *et al.* 1996).

The second pathway starts in 1983, with the report that a rare *E. coli* serotype, O157:H7, was the cause of a distinctive clinical entity, named at the time 'haemorrhagic colitis' (HC) and characterized by bloody diarrhoea (Riley *et al.* 1983). In the following years O157:H7 *E. coli* strains were associated with mild, undifferentiated to severe, bloody diarrhoea, with, in some patients, sequelae such as a haemolytic uraemic syndrome (HUS), characterized by renal failure, and a thrombotic thrombocytopaenic purpura (TTP), possibly with CNS involvement. As this *E. coli* serotype had been initially associated with HC, the strains were named 'enterohaemorrhagic *E. coli*' (EHEC) and for many years EHEC remained a clinical definition, synonym of O157:H7 *E. coli* causing HC. Progressively *E. coli* strains belonging to other serotypes (026:H11, 0103:H2, 0111:H–, 0145:H–, O157:H–, etc.) were associated with HC, HUS and TTP. Although sometimes reluctantly, the original EHEC definition was widened to include those serotypes. Meanwhile, the properties of the EHEC strains of the O157:H7 and other serotypes were progressively uncovered. Among others, production of the histological attaching/effacing (AE) lesion, very close to the AE lesion caused by enteropathogenic *E. coli* (EPEC) was recognized. Today the EHEC include all *E. coli* strains, from humans and animals, producing Stx and AE lesion, or harbouring the genetic information coding for them. However, this definition is not universally accepted as not all of these *E. coli* cause HC in humans. VTEC and STEC are the names for the strains producing only Stx, like the ED-associated *E. coli* in piglets.

4. SPECIFIC PROPERTIES OF EHEC AND VTEC

The EHEC and VTEC possess general (serotypes, biotypes and lysotypes) and specific properties (virulence-associated). They are subdivided into different evolutionary lineages: the EHEC-1 lineage comprises the O157:H7 and closely related strains (e.g. O145:H–) that are highly pathogenic in humans (HC, HUS and TTP) and are derived from the O55 EPEC. The EHEC–2 lineage regroups all other EHEC strains belonging to a wide variety of O serogroups (O5, O26, O103, O111, O118, etc.) and of various pathogenicity in humans (diarrhoea, HC and HUS); the VTEC-1 lineage strains belong to the H21 serogroup (O91, O113, etc.) and are pathogens in humans (HUS and TTP). The VTEC-2 lineage corresponds to all other VTEC of low or no pathogenicity (asymptomatic carriage, diarrhoea and rare HUS) in humans. This section will review the specific virulence-associated properties of EHEC and VTEC, i.e. the Stx, the AE lesion and the enterohaemolysins (Ehly).

4.1. The verocytotoxins

The *E. coli* Stx toxins consist of two groups: Stx1 are antigenically very close to the Stx toxin of *S. dysenteriae* type 1 while Stx2 are more distantly related. Variants of Stx1 have been described which differ only slightly in their gene sequences with no consequence on their antigenicity and cell toxicity. The prototype Stx1 is produced by the *E. coli* strains H19, H30 and EDL933. Several variants of Stx2 have also been described which differ much more from each other in their antigenicity, toxicity and gene sequences. The

classification of the Stx2 variants is confusing. It has been proposed that five main biological variants are recognized (Melton-Celsa and O'Brien 1998; Duffy *et al.* 2001; Thorpe *et al.* 2002): Stx2 (human strains EDL933 and E32511), Stx2c (human strains E32511 and B2F1), Stx2d [used widely in the literature to describe several other (sub)variants] identical to Stx2c but activatable by components associated with the intestinal mucus (human strain B2F1), Sx2e (porcine strains 412 and S1191) and Stx2f (human strain H.I.8; previously named Stx2ev). VTEC strains can produce one Stx toxin or two: Stx1 and Stx2 (strain EDL933), Stx2 and Stx2c (strain E32511), two Stx2 subvariants (strain B2F1).

4.1.1. Structure/activity.

The Stx are two subunit toxins: the A subunit of *c.* 33 kDa is the biologically active part and the B subunit, of *c.* 7·5 kDa is present in five copies and binds to the specific-cell receptor. Following binding, the Stx toxins are internalized by receptor-mediated endocytosis and retrospectively transported into the endoplasmic reticulum after migration through the Golgi apparatus. The A subunits then translocate into the cytoplasm using the Sec61 transmembrane protein complex and are activated after cleavage of a 4 kDa C-terminal A2 peptide. The resulting active A1 peptides have N-glycosidase activity and cleave a purine residue from the 28S rRNA, altering the function of the ribosomes, which are no longer able to interact with elongation factors EF1 and EF2. The protein synthesis is therefore inhibited within the target cells that will finally die.

Not all Stx toxins are equally active on different cell lines. If all Stx are highly toxic for Vero cells, the Stx1 and Stx2 only are fully toxic for HeLa cells, whereas the Stx2c and Stx2d are partially (100 times less) and the Stx2e and Stx2f are not (10 000-fold less). Conversely, only Stx2e and Stx2f are fully toxic for Madin-Darby bovine kidney (MDBK) cells, whereas the Stx2c and Stx2d are partially and Stx1 and Stx2 are not. The cell target specificity of a Stx depends on the amount of specific receptor(s) present on the cell membrane: Gb3 (Galα1-4Gal) for Stx1 and Stx2 is present on Vero and HeLa cells; Gb4 (GalNAcβ1-3Galα1-4Gal) for Stx2e and Stx2f is present on Vero and MDBK cells.

4.1.2. In vivo activity.

In vivo the Stx are produced in the intestines after colonization by the EHEC or VTEC, then cross the intestinal wall (Stx2 more efficiently than Stx1) and enter the blood stream (toxaemia). The main target cells are the endothelial cells of small arteries, in the kidneys (humans), brain (piglets and to a lesser extent humans), gastro-intestinal mucosa (humans and piglets) and other tissues (piglets). The consequences are fluid leakage and/or haemorrhages leading to tissue lesions and clinical syndromes (Table 1).

Table 1 Diseases caused by enterohaemorrhagic and verotoxigenic *Escherichia coli* in humans and animals

Hosts	Enterohaemorrhagic *E. coli*	Verotoxigenic *E. coli*
Humans	Diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome, thrombotic thrombocytopaenic purpura	Haemolytic uraemic syndrome, thrombotic thrombocytopaenic purpura
Cattle	Haemorrhagic diarrhoea in young calves, healthy carriage in adults	Healthy carriage in adults
Sheep/goats	Healthy carriage in adults	Healthy carriage in adults
Piglets	–	Oedema disease

Nephrotoxicity is observed in humans as a sequela after intestinal infection by EHEC or VTEC strains in 10% of patients. The Stx cause chronic kidney damage leading to dialysis and possible need for transplantation (HUS). HUS is also characterized by microthrombus formation, thrombocytopaenia and haemolytic anaemia (Monnens *et al.* 1998; Rose and Chant 1998). Although calves are frequently infected by EHEC, HUS never develops as they lack receptors on the endothelial cells of their blood vessels (Pruimboom-Brees *et al.* 2000).

Neurotoxicity is observed first of all in piglets suffering ED. Fluid leakage causes compression of the brain with loss of coordination, lameness, paralysis and sudden death, as main clinical signs (Gyles 2001). Neurological disorders can also be observed in a few humans after intestinal infection by EHEC or VTEC as part of a sequela known as TTP. TTP is also characterized by haemolysis, thrombocytopaenia, renal failure and a fluctuating fever (Hutchison *et al.* 1998; Rose and Chant 1998; Duffy *et al.* 2000).

A number of workers have reported that the Stx1 and Stx2 are enterotoxic, i.e. cause of fluid accumulation in ligated intestinal loops in rabbits and therefore diarrhoea, but this is still controversial and can vary according to the animal species (Pruimboom-Brees *et al.* 2000). Today, diarrhoea observed during infection by EHEC in humans and calves is considered to be the consequence of formation of the AE lesion (see below) and not the production of any Stx. This is supported by the absence of diarrhoea in piglets killed by ED and in humans infected by VTEC strains. On the contrary, the haemorrhagic aspect of the diarrhoea in humans, when present, would be the consequence of the action of the Stx on the endothelial cells of the vessels present in the intestinal mucosa. This is not the case in calves as the endothelial cells of their blood vessels lack Stx receptors (Pruimboom-Brees *et al.* 2000) and the role of Stx in calves is still unknown.

In piglets fluid extravasation also cause oedema in the eyelids, in the larynx leading to the development of a characteristic hoarse squeal and, during the terminal stages

Table 2 Overall % homology between the genes coding for the A and B subunits of the *stx*, *stx1* and *stx2* toxin variants of *Shigella dysenteriae* and *Escherichia coli* (Part A) and of the different *stx2* gene variants of *E. coli* (Part B)

(a)

% homology to	*stx1* genes (%)	*stx2* genes (%)
stx (A subunit)	>99	57
stx (B subunit)	>99	60

(b)

% homology to	*stx2c/d* genes (%)	*stx2e* genes (%)	*stx2f* genes (%)
stx2 (A subunit)	>95*	94	78
stx2 (B subunit)	>95*	70	79

*Variable according to the subvariant Scotland and Smith (1997), Bastian *et al.* (1998), Piérard *et al.* (1998) and Duffy *et al.* (2001).

of a subacute ED, in the lungs leading to respiratory distress.

4.1.3. Genetic basis. The A and B subunits of the Stx toxins are coded by two different open reading frames organized in one single transcriptional unit. Sequence identity between the *stx1* and *stx2* prototype genes are 57% in *stxA* and 60% in *stxB* genes at the nucleotide level (Scotland and Smith 1997; Bastian *et al.* 1998; Piérard *et al.* 1998; Mainil 1999) (Table 2a). The *stx2* genes coding for the different variants are more closely related to the *stx2* (74–96% overall identity) than to the *stx1* genes (55–60% overall identity). The more distantly related to the *stx2* genes are the *stx2e* variant genes and the *stx2f* variant gene (Table 2b). Within each group the subvariants are highly homologous (>95% sequence identity).

The *stx1*, *stx2*, *stx2c* and *stx2d* operons are present on chromosomally located lambdoid phages. The *stx2e*, *stx2f* and *stx* genes of *S. dysenteriae* are also located on the bacterial chromosome, but not on phages. The transfer of the *stx1* and *stx2* operons to recipient strains by phage transduction has been performed *in vitro* and *in vivo* (Mainil 1999; Toth *et al.* 2003). After synthesis the Stx toxins are exported to the periplasm by a type II signal sequence secretion system. The Stx1 toxins are mainly present in the periplasm, while the Stx2 toxins are more easily excreted into the environment at least *in vitro*. The synthesis of Stx and Stx1, but not of the different Stx2, is repressed at the transcriptional level by high iron concentration via the Fur system and by high temperature.

4.2. The AE lesion

The AE lesion is a specific-histological lesion (Fig. 1) of the gut caused by a class of pathogenical strains, named EPEC.
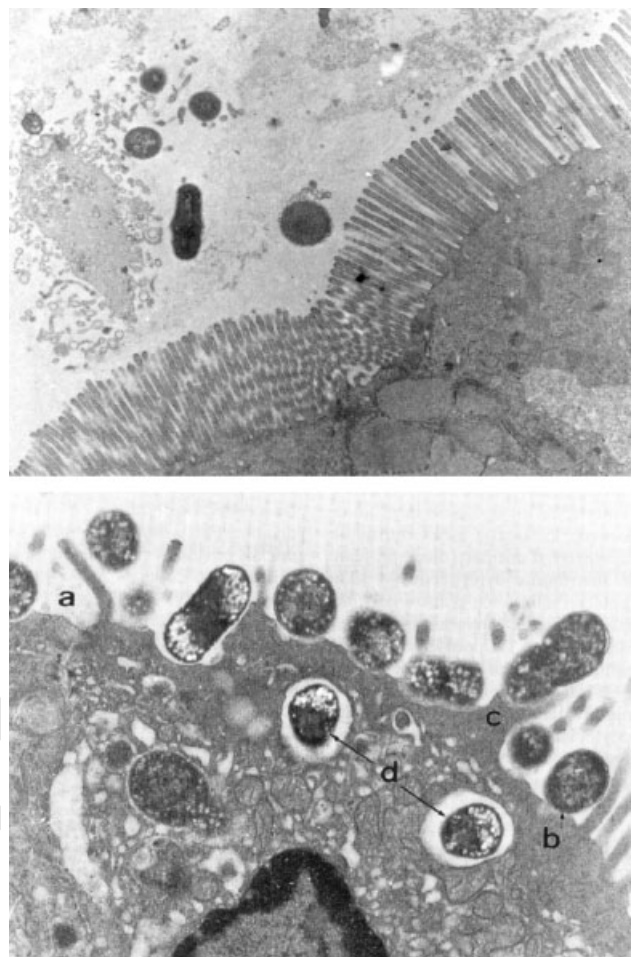

Fig. 1 Intact intestinal microvilli and attaching/effacing lesions at the electron microscope (15 680×). (a) Effacement of the microvilli of the enterocyte; (b) intimate attachment of the bacteria to the enterocyte; (c) pedestal structure; (d) internalization of a few bacteria (author's collection: Ann Méd Vét, 2000, **144**, 121–136; with permission of the publisher)

Most of these strains have been known for decades, but only in the early 1980s was the production of this lesion fully recognized and systematically studied (Moon *et al.* 1983; Clarke *et al.* 2003). Subsequently, it was observed that O157:H7 EHEC could produce a very similar lesion and the definition of EHEC was extended to all *E. coli* strains producing Stx and AE lesions.

4.2.1. Description. The production of AE lesions is the result of a very specific and tightly regulated interaction between the bacteria and the eucaryotic cell, the enterocytes *in vivo*. This multistep event occurs in three stages. The first step is the initial adherence of the bacteria to the surface and microvilli of the host cells. In human EPEC this initial adherence triggers the expression of several genes located on

a pathogenicity island (Pai) on the chromosome, the locus of enterocyte effacement (LEE), via a plasmid located regulator. The identity of the primary adherence factor remains unknown for animal EPEC and all EHEC strains.

The second step consists in sending signals into the eucaryotic cell via a type III secretion system. The type III secretion system and the translocated proteins are coded by genes located on the LEE. Through phosphorylation of several eucaryotic cell proteins the type III secreted proteins cause polymerization of actin, cytoskeleton rearrangements and effacement of the enterocyte microvilli (Fig. 1). Actual modifications of biochemical pathways can differ between EPEC and EHEC strains belonging to different serotypes and to the target cells.

The third and final stage is an intimate adherence of the bacteria to the nude cytoplasmic membrane of the enterocyte (Fig. 1). The bacterial adhesion is a LEE-encoded type II secreted outer membrane protein, the intimin. Surprisingly the cell receptor is also a bacterial protein (Tir for 'translocated intimin receptor'), coded by the LEE, type III secreted and phosphorylated inside the enterocyte, before integrating the eucaryotic cell membrane. At this stage the rearrangements of the cytoskeleton are amplified, a pedestal forms under the adhering bacterial cells (Fig. 1) and bundles of actin filaments can be detected in and under the pedestal.

With some EPEC and EHEC, strains invasion of the eucaryotic cells have been observed *in vitro* and *in vivo* (Fig. 1). Although the actual significance of this observation is unknown it may be related to the fact that the intimin can also use $\beta 1$ integrin as a receptor, very similar to the invasion of *Yersinia enterocolica*.

4.2.2. The locus of enterocyte effacement. The genes necessary and sufficient for the production of the AE lesion

are grouped on a 35–45 kb chromosomal DNA fragment, which represents a type III Pai, originally named LEE. The LEE comprises up to 50 genes and open reading frames, five transcriptional units and three functional regions (Fig. 2).

The genes coding for the type III secretion system (*esc* and *sep* genes) are located by convention in what is arbitrarily called the left part of the LEE. The genes coding for the type III secreted proteins (*esp* genes) are located in the right part of the LEE. In the middle part of the LEE are present the *eae* gene coding for the bacterial intimin adhesin and the *tir* gene coding for its translocated receptor. The LEE of human and animal EHEC and EPEC can differ in size, numbers of genes and ORF, and insertion sites on the chromosome (*selC* locus, *pheU* locus, *pheV* locus, etc.). However, the internal organization of the genes on the LEE is conserved (Kaper *et al.* 1998b; Goffaux *et al.* 2001a).

Variation has also been described within some LEE-located genes. The number of variants of the *eae* gene has dramatically increased as the original descriptions of the prototype EPEC and EHEC LEE (Adu-Bobie *et al.* 1998; Oswald *et al.* 2000; Zhang *et al.* 2002; Blanco *et al.* 2004a). Currently, 14 variants, of relative importance and frequency, have been identified by PCR: α, β, γ, δ, ε, ζ, η, ι, κ, λ, μ, ν, θ, ξ and other variants are still unnamed. In addition, restriction variants of the amplified fragments exist for some PCR variants: $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$ and $\gamma 2$. Most variants differ highly (<60–70% homology) in the 3′-end third of the gene while the 5′-end two-thirds of the gene are highly homologous (>90% homology). Moreover, the variants $\beta 2$ and δ are identical and the variants $\gamma 2$ and θ are very closely related. If the α and $\gamma 1$ variants are typical, but not exclusive, of human EPEC and of O157:H7 EHEC, respectively, the other variants are more or less widely distributed in human and animal EHEC and EPEC.
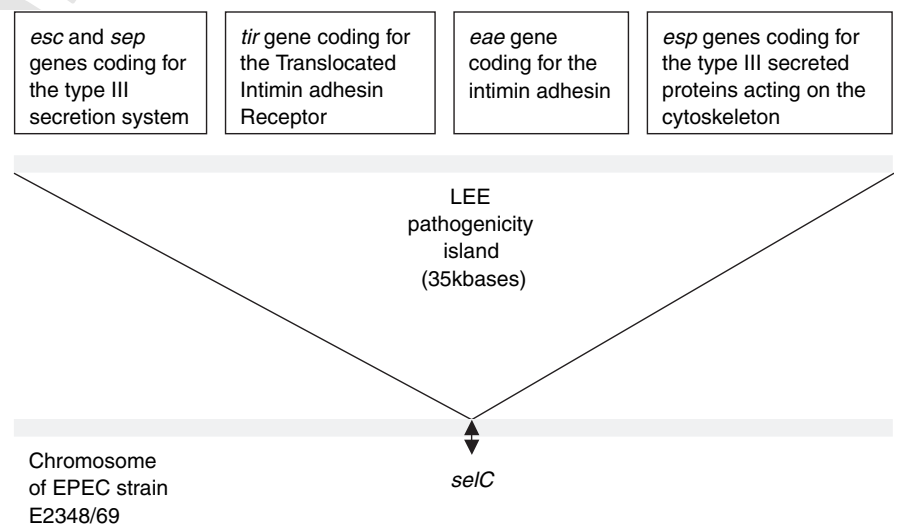

Fig. 2 Schematic description the locus of enterocyte effacement (LEE) pathogenicity island of the human enteropathogenic *Escherichia coli* strain E2348/69 after Nataro and Kaper (1998) and Duffy *et al.* (2000)

Besides the *eae* gene, variants have also been described in the *tir* and *esp* genes (α, β and γ), while the *esc* and *sep* genes are more conserved. The following associations between *eae* (α, β, γ1, γ2 and ε), *tir* and *esp* genes observed so far are: α/α/α, β/β/β,γ1/γ/γ, γ2/α/α,ε/β/β (China *et al.* 1999; Goffaux *et al.* 2001b). Associations between *tir*, *esp* and the other *eae* gene variants have not been studied so far to our knowledge.

4.2.3. The diarrhoea. Although spectacular, the AE lesion is not directly responsible for the diarrhoea observed during infections with EPEC or EHEC. The actual reason for the diarrhoea is still unknown, but several hypothesis have been advanced according to *in vitro* and *in vivo* studies. These include inhibition of sodium and chloride absorption, activation of the major chloride channel in the microvilli, opening of the tight junctions, increased paracellular permeability, production of another toxin, inflammatory response in the mucosa and production of cytokines. The cause of diarrhoea may differ between strains of different serotypes, according to the biochemical modifications initiated within the eucaryotic cells.

4.3. The enterohaemolysins

Several types of haemolysins (α, β, etc.) are produced by invasive, uropathogenical and enterotoxigenic *E. coli*. More recently, haemolysins active only on washed red blood cells have been associated with EHEC strains called Ehly. Different Ehly have been described in *E. coli*, but one is specific of EHEC strains, the so-called EHEC-Ehly or Ehx (Karch *et al.* 1998; Beutin 2000).

4.3.1. Structure/activity. The Ehx belongs to the RTX (repeats in ToXin) family of toxins, like the α-haemolysin (α-Hly). The Ehx, like the α-Hly, is a monomeric pore-forming toxin that inserts into the cytoplasmic membrane of the eucaryotic target cells and creates pores. It is exported by a type I secretion system, but remains attached to the bacteria in contrast to α-Hly, probably as a consequence of a mutation in the genes coding for the transport mechanism.

4.3.2. In vivo *activity.* Despite the observation of a preferential association with the development of HUS in humans, the actual contribution of the Ehx to the pathogenesis of EHEC and VTEC remains unclear.

4.3.3. Genetic basis. The Ehx is encoded by four genes (*ehxC*, *ehxA*, *ehxB* and *ehxD*) located on a plasmid of *c.* 60 Mdal (pEHEC plasmid). The structural gene *ehxA* sows *c.* 60% homology with the *hlyA* gene coding for the α-Hly. The α-Hly (HlyA), as all RTX toxins, is secreted by a type I

secretion system involving the participation of the HlyB and HlyD proteins. HlyB is a membrane-bound ATPase providing energy for the secretion of the HlyA. HlyD forms a channel through the bacterial membranes with the help of the TolC outer membrane protein through which HlyA is secreted. The *ehxB* and *ehxD* genes appears to be deficient in the secretion of EhxA and can be complemented by the *hlyB* and *hlyD* genes. In that case Ehx is excreted into the supernatant and gives an α-Hly like haemolysis.

Other genes are located on the pEHEC plasmid coding for a type II secretion system (*etp* genes), for a catalase-peroxidase (*katP* gene) and for a secreted serine protease (*espP* gene in O157:H7 EHEC or *pssA* gene in O26:H11 EHEC). The pEHEC plasmid carrying the *ehx* and *etp* genes is present in all O157:H7 EHEC strains, in a majority of O26:H11 EHEC strains and in variable proportion of EHEC strains belonging to other serotypes. The *katP*, *espP* and *pssA* genes are, however, present on a majority, but not all, pEHEC plasmids (Karch *et al.* 1998; Beutin 2000).

5. EPIDEMIOLOGY OF EHEC AND VTEC IN HUMANS

Human EHEC and VTEC strains belong to three classes on a clinical basis: (i) those highly infectious in humans, but only exceptionally in animals (essentially EHEC-1 and VTEC-1) (ii) those frequently associated with diseases in humans and animals (essentially EHEC–2) and (iii) those more rarely associated with disease in humans or animals (essentially VTEC-2). The most famous EHEC strains belong to the EHEC-1 lineage and to the O157:H7 serotype.

National surveillance programmes tend to concentrate on the O157:H7 EHEC. Infections of humans with the O157:H7 serotype occur most frequently as food-borne outbreaks in communities, such as families, schools, elderly homes and day care centres. These outbreaks are for reasons unknown primarily restricted to North America and UK. They are rare in continental Europe, South America, Asia (Japan), Oceania and Africa. Mainly individual cases are reported in these other locations. The food vehicles are mainly of ruminant origin such as raw, inadequately cooked meat products or unpasteurized milk. O157:H7 EHEC are carried by healthy cattle and, to a lesser degree, other ruminants in the gastro-intestinal tract. Contamination occurs during milking or slaughter and processing of the carcass. The primary colonization site appears to be the recto-anal junction (Rice *et al.* 2003). The faecal shedding lasts for <2 months in most animals, but it is believed that some cattle are effectively colonized for longer periods of time. Food vehicles can also originate from other ruminants (unpasteurized goats' cheese, venison, etc.).

Increasingly, infections originate from nonruminant sources, which have been contaminated directly or indirectly by ruminant faeces or by infected humans. Thus transmission occurs through consumption of nonruminant meat and meat products (poultry meat sandwiches, cold pork meat, salami, etc.) and of fruit and vegetables (potatoes, cabbages, sprouts, cole slaw and unpasteurized apple cider) or via nonfood-borne routes (water in swimming pools or lakes, mud, direct animal contacts including sporadically nonruminants and person-to-person contacts). Although the sources are numerous, they have one common feature – the foods and food products have been inadequately prepared, stored or cooked, allowing survival and growth of the O157:H7 EHEC.

Infections, outbreaks and individual cases, with other serotypes occur to a lesser extent, although they represent the majority of cases in some areas (continental Europe for instance). The serogroups O26, O103, O111 and O145 are the most frequent. The sources of infection for humans, the rate of carriage in ruminants and the true incidence in humans of these non-O157:H7 cases are impossible to estimate, as surveillance programmes include only the nonsorbitol-fermenting O157:H7 EHEC.

Human infections with VTEC (serogroups O48, O91, O104 and O113) have also been reported, but more recently. They do not seem to find their origin in food consumption, although similar strains can be isolated from ruminant faeces and intestinal content and from foodstuffs. It is important to emphasize that porcine VTEC causing ED in weaned piglets differ by their general and specific properties and that porcine VTEC should be considered as very unlikely zoonotic agents.

6. PROPERTIES OF EHEC AND VTEC FROM DIFFERENT ORIGINS

As porcine VTEC responsible for ED do not represent any important public health concern and as the epidemiological significance of EHEC and VTEC described outside the ruminant animal species is still largely speculative (Mainil 1999; Gyles 2001; Wasteson 2001), this section will focus on EHEC and VTEC isolated from humans, ruminants and foods (Tables 3–5).

6.1. From humans

Humans are infected by EHEC which are responsible for HC, HUS and TTP and by VTEC, which are responsible for HUS and TTP only (Table 1). Asymptomatic carriage also exists, especially of VTEC strains. Human EHEC and VTEC belong to an ever-increasing list of O serogroups and serotypes. The most well-known is, of course, the O157:H7 EHEC and among the VTEC serotypes the most famous ones are O91:H21 and O113:H21. But more than 130 other O serogroups in combination with various H serogroups have been described in the literature and new ones are described each year (Blanco *et al.* 2004a). More than 100 O:H serotypes have been isolated from patients with HUS. The incidence of the serotypes varies geographically.

Infection with O157:H7 EHEC has been directly or indirectly associated with cattle and other ruminants. Nevertheless, direct and indirect human-to-human contamination also occurs frequently, especially during an outbreak. Conversely, the source of human contamination by other serotypes of EHEC and VTEC (O26, O91, O103, O111, O113, O118 and O145) is never identified in most instances, although many human serotypes have also been isolated from ruminants.

The O157:H7 and non-O157 EHEC and VTEC produce Stx1 and/or Stx2, in relatively similar proportions, although differences have been observed, probably because of geographical and population sample variations. The isolates producing Stx2 are more often associated with HUS than those producing only Stx1. The *stx2* variants are mainly *stx2*, *stx2c* and *stx2d*. There is some degree of association between the serotypes and the *stx* gene variants. In only very few cases the variants *stx2e* and *stx2f* have been described.

Most human strains are EHEC and their *eae* genes belong most frequently to the following variants: β, $\gamma1$, $\gamma2$, ε. For

Table 3 Comparison of the properties of O157 enterohaemorrhagic *Escherichia coli* (EHEC) isolated from humans, animals and foodstuffs

Origin (disease)	Stx profiles	Stx2 variants	LEE	Ent-Hly and pEHEC (%)
Humans (HC, HUS, TTP)	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	*eae* $\gamma1$, *tir* $\gamma1$, *esp* $\gamma1$	100
Calves (diarrhoea)	Not associated	Not relevant	Not relevant	Not relevant
Cattle (healthy)	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	*eae* $\gamma1$, *tir* $\gamma1$, *esp* $\gamma1$	100
Sheep/goats (healthy)	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	*eae* $\gamma1$, *tir* $\gamma1$, *esp* $\gamma1$	100
Foods	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	*eae* $\gamma1$, *tir* $\gamma1$, *esp* $\gamma1$	100

HC, haemorrhagic colitis; HUS, haemolytic uraemic syndrome; TTP, thrombotic thrombocytopaenic purpura; Stx, shiga toxins; LEE, locus of enterocyte effacement; Ent-Hly, O157 enterohaemolysin.

Table 4 Comparison of the properties of non-157 enterohaemorrhagic *Escherichia coli* (EHEC) isolated from humans, animals and foodstuffs

Origin (disease)	Most frequent serogroups	Stx profiles	Most frequent Stx variants	Frequent LEE profiles	Ent-Hly and pEHEC (% of different serotypes)
Humans (HC, HUS, TTP)	O26, O49, O103, O111, O118, O145, O156, etc.	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c, Stx2d	*eae β/tir β/esp β*, *eae γ2/tir α/esp α*, *eae γ1/tir γ1/esp γ1*, *eae ε/tir β/esp β*	67 (50–100)
Calves (diarrhoea)	O5, O26, O111, O118, etc.	Stx1, Stx1/STx2, Stx2	Not reported	*eae β/tir β/esp β*, *eae γ2/tir α/esp α*	Variable
Cattle (healthy)	O5, O26, O103, O111, O118, O145, etc.	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	*eae β/tir β/esp β*, *eae γ2/tir α/esp α*, *eae γ1/tir γ1/esp γ1*, *eae ε/tir β/esp β*	50 (25–100)
Sheep/goat (healthy)	O26, O49, O52, O111, O156, O177, etc.	Stx1, Stx1/Stx2, Stx2	Stx1c, Stx2d	*eae β/tir β/esp β*, *eae γ2/tir α/esp α*, *eae γ1/tir γ1/esp γ1*	25 (?)
Foods	Very many	Stx1, Stx1/Stx2, Stx2	Rarely reported, Stx2, Stx2c	Not reported	Not reported

HC, haemorrhagic colitis; HUS, haemolytic uraemic syndrome; TTP, thrombotic thrombocytopaenic purpura; Stx, shiga toxins; LEE, locus of enterocyte effacement; Ent-Hly, O157 enterohaemolysin.

Table 5 Comparison of the properties of verocytotoxigenic *Escherichia coli* isolated from humans, animals and foodstuffs

Origin (disease)	Frequent serogroups (serotypes)	Stx profiles	Stx variants	Ent-Hly and pEHEC
Humans (HUS, TTP)	O91, O113, O128, O146, etc. (H21+/H21−)	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c, Stx2d	Variable
Calves (?)	O8, O20, etc.	Stx1, Stx1/Stx2, Stx2	Not reported	Variable
Cattle (healthy)	O113:H21, etc.	Stx1, Stx1/Stx2, Stx2	Stx2, Stx2c	Variable
Sheep/goats (healthy)	O6, O91, O128, O136, O146, etc.	Stx1, Stx1/Stx2, Stx2	Stx1c, Stx2d	Variable
Foods	Very many	Stx1, Stx1/Stx2, Stx2	Rarely reported	Not reported, Stx2, Stx2c

HUS, haemolytic uraemic syndrome; TTP, thrombotic thrombocytopaenic purpura; Ent-Hly, O157 enterohaemolysin.

instance, all O157 and O145 EHEC harbour the γ1 variant and all O26 EHEC, the β variant of the *eae*, *tir* and *esp* genes. In many others the γ2 variant of the *eae* gene and the α variant of the *tir* and *esp* genes are associated with the O111 serogroup while the ε variant of the *eae* gene and the β variant of the *tir* and *esp* genes are associated with the O103 serogroup (China *et al.* 1999; Goffaux *et al.* 2001b; J. Mainil *et al.*, unpublished data).

All O157:H7 EHEC are positive for the Ehx Hly and the pEHEC plasmid. Two-thirds of the EHEC and VTEC of other serotypes are positive for the Ehx (% variable according to the O serogroup) whose encoding genes are often located on a pEHEC-like plasmid.

6.2. From cattle

Infections in cattle with EHEC/VTEC show two distinct patterns, with some strains producing diarrhoea in young calves of 2 weeks to 2 months of age and other strains being carried asymptomatically by adult animals (Table 1).

6.2.1. From diarrhoeic calves. Strains causing diarrhoea in calves belong almost exclusively to the EHEC pathotype. Like human strains, they belong to an always extending number of serotypes, many of which are also associated with diseases in humans. On the contrary, O157:H7 EHEC have been exceptionally associated with diarrhoea in young calves (Dean-Nyström *et al.* 1998; Brown *et al.* 2001; Kang *et al.* 2004). The most important calf serotypes are O5:H-, O26:H-, O26:H11, O111:H- and O118:H16. Data on VTEC strains causing disease in calves are rare. When they have been reported they belonged to other serotypes (O8:H8, O20:H19; O113:H21) than the EHEC. Some of these VTEC serotype have been isolated from human cases of HUS (O113:H21).

The majority (>85%) of these bovine EHEC are positive for the *stx1* gene only. When *stx2*-positive, bovine EHEC contain the *stx2* or *stx2c* gene variants. Conversely, bovine VTEC are positive for the *stx1* and/or *stx2* genes without any apparent preference. Their *stx2* gene variants have not been identified to our knowledge.

The majority (80%) of bovine EHEC from diarrhoeic calves are positive for the *eaeβ* gene variants of the *eae*, *tir* and *esp* genes. The second most frequent variants (20%) are the *eaeγ2* gene along with the *tirα* and *espα* genes. The typing results are homogeneous in some serogroups (O5:H-, O26:H11; O118:H16), but not in others (O111:H-). Other variants have not yet, or rarely, been recorded. Also important to note is the identity of the *eae*, *tir* and *esp* genes of bovine diarrhoea-associated and human EHEC belonging to the same serotype (China *et al.* 1999; Goffaux *et al.* 2001b; J. Mainil *et al.*, unpublished data).

Several bovine EHEC and VTEC associated with diarrhoea in young calves are also positive for the Ehly phenotype or genetically for the EHEC Ehly, but the actual proportion can vary from region to region.

6.2.2. From healthy cattle. More than 120 O serogroups of EHEC and VTEC, in combination with different H serogroups, have been isolated from healthy cattle. Of these, more than 100 have been described in humans. More than one type of EHEC and VTEC strains can be isolated not only on the same farm, but also from the same animal. Reported prevalence in cattle varies greatly according to the country and to the detection method (PCR-based methods always give much higher results). Animal prevalence varies from 0·1 to 63% and herd prevalence, between 0·3 and 87% in herds. The great majority of isolates are VTEC and only a minority are actually O157:H7 EHEC.

The O157:H7 EHEC prevalence is always higher in the USA than in Europe: 2–20% *vs* 0–3% as herd prevalence, representing nevertheless <1% of the animals. The bovine O157:H7 EHEC are identical to the human strains in the *stx* genes, in the LEE genes and in the pEHEC plasmid. In addition, the same lysotype is dominant amongst O157:H7 EHEC isolated in Belgium from humans, healthy cattle and beef meat (Daube *et al.* 1999). Conversely, bovine O157:H7 EHEC on one farm and even in one animal can belong to multiple pulsotypes as evidenced by PFGE. Comparison of PFGE of human and bovine O157:H7 EHEC lead to the hypothesis that not all bovine O157:H7 EHEC are a major threat and associated with severe diseases in humans (Liebana *et al.* 2003). Isolation of O157 non-EHEC non-VTEC *E. coli* has also been reported (Rogerie *et al.* 2001).

The most frequent non-O157 EHEC isolated from healthy cattle belong to the following O serogroups: O5, O26, O103, O111, O118 and O145. There exist geographical variations in the predominance of the different serotypes. Most surveys indicate that they are also similar to human isolates beyond the serotype. They are *stx1*, *stx2* and/or *stx2c*-positive and the *eae* gene belongs to the same group of variants. As *stx* and *eae* gene profiles are correlated to the O serogroup bias can occur in some surveys if one serogroup predominates. More than half of them are positive for the

production of Ehx and for the presence of the *ehx* genes on a pEHEC-like plasmid.

Some VTEC isolated from healthy cattle are also similar to human VTEC associated with disease (VTEC-1 lineage) including HUS by their serotypes (O91:H21; O113:H21, etc.) and pathotypes (*stx1*, *stx2* and/or *stx2c*), but can differ according to the pulsotype (Pradel *et al.* 2001). However, most of them (VTEC-2 lineage) belong to serotypes that have been rarely reported from humans or are of low pathogenicity in humans.

6.3. From other ruminants

The EHEC and VTEC have also been isolated from other domestic ruminants (sheep and goats) and from wild ruminants, initially from healthy carriers (Table 1).

Although the prevalence of O157:H7 EHEC in faeces or intestinal contents of healthy sheep and goats is lower than in cattle, their profiles do not differ from the profiles of human and bovine strains. Therefore sheep and goats also represent a potential source of contamination for humans. Transmission to humans has been reported essentially via consumption of raw milk or cheese (Mainil 1999; Blanco *et al.* 2001; Brown *et al.* 2001; Blanco *et al.* 2003).

In sheep and goats, non-O157:H7 EHEC and VTEC are detected much more often than O157:H7 EHEC: 43–95% and 7–95% of faecal samples respectively. Even more in cattle, VTEC are more numerous than EHEC and one animal can excrete more than one type of strain. Ovine and caprine non-O157 EHEC and VTEC belong to human disease-associated serotypes (O26:H11, O49:H-, O156:H- for EHEC and O91:H-, O128:H2, O146:H21 for VTEC), or to more sheep/goat-specific serotypes (O52:H12, O156:H25, OX177:H11 for EHEC and O6:H10, O136:H20 for VTEC). The majority of isolates are positive for the *stx1* gene (mainly the *stx1c* variant); a minority for the *stx2* gene (mainly the *stx2d* variant) (Ramachandran *et al.* 2001; Brett *et al.* 2003). Several *eae* gene variants have been identified in a similar way to that in human and cattle EHEC and the *ehx* gene is present, but in only *c.* 25% of cases. The *stx* and *eae* gene profiles are also correlated to the serogroup (Blanco *et al.* 2003). The role of non-157:H7 EHEC, and maybe VTEC, in diseases in lambs and goat kids is unknown.

The O157:H7 and non-O157:H7 EHEC and VTEC are also present in farmed and free wild ruminants, but prevalence varies greatly depending on geography. O157:H7 EHEC from deer has been associated with a limited number of human outbreaks. On the contrary, they do not appear to be an important source of contamination for domestic cattle, as their pulsotypes are different (Brown *et al.* 2001). Moreover, O157 VTEC or non-EHEC–non-VTEC *E. coli* can often be isolated from wild ruminants leading to confusion in reporting. In general, and particularly in Belgium (Pirson *et al.*

2000; Canivet *et al.* 2002), VTEC are more frequent than EHEC. The role of these different strains in the development of pathologies in the wild ruminants is unknown.

6.4. From foods

Raw foods of animal origin are the most likely to be contaminated by EHEC and VTEC. Milk becomes contaminated during milking, carcasses during slaughter and further processing at the abattoir, and cross-contamination may occur in butcher's premises. The level of contamination is linked primarily to the level of intestinal infection, faecal excretion and, of course, general hygiene. Because O157:H7 and other EHEC strains are particularly resistant in the environment (Duffy *et al.* 1999; McDowell and Sheridan 2001; Brown *et al.* 2002), secondary contamination food can occur at many places within the food chain. If 30% of beef carcasses of O157:H7 EHEC excreting animals are contaminated, a tenth of carcasses of nonexcreting animals become, almost certainly by cross-contamination during the slaughter process.

Survey results can be difficult to compare because researchers use different protocols for sampling, screening, isolation, identification and typing procedures. For instance, positive results obtained by PCR tend to be several times higher than those obtained with other methodologies. In addition, enrichment, isolation and identification methods for non-O157:H7 EHEC and VTEC are not as reliable. As a general rule, non-O157:H7 EHEC and VTEC are more frequent than the O157:H7 EHEC. Conversely, ratios between EHEC and VTEC are variable.

Most European countries report isolation rates of O157:H7 EHEC from beef carcasses and from raw beef products between 0 and 4%. Results are similar in the USA, but can occasionally be higher (up to 36%) in less developed countries. Isolation rates from raw milk vary between 0 and 10%. Isolation rates of non-O157 EHEC and VTEC from meat products and milk are higher (between 1 and 40%), even in developed countries.

When reported, the combination of *stx1*, *stx2* and *eae* 4 genes and serotypes are identical to those identified in EHEC and VTEC from humans and from healthy cattle. The most frequent *stx2* gene variants identified are *stx2* and *stx2c* (Guth *et al.* 2003), but most surveys do not type the *stx* and *eae* gene variants.

In other ruminant carcasses and meat products, the situation is even less clear, because official surveillance is not undertaken. General isolation rates of EHEC and VTEC are under 5% and O157:H7 EHEC are very rare as most fial to isolate these strains. Serotypes of non-O157 EHEC and VTEC isolated from lamb and deer meat are similar to those detected in living animals, but no typing results of the *stx* and *eae* genes have been reported to our knowledge.

In nonruminant meat and meat products (pork and poultry for instance) non-O157 EHEC and VTEC can be frequently isolated (1–50%), but O157 EHEC are very rare, even absent, in most of the surveys. Most strains belong to host-specific serotypes and pathotypes. O157 *E. coli* lacking the *stx* and/or the *eae* genes have also been isolated from nonruminant meat products and can lead to confusion in reports.

7. CONCLUSIONS

The role of ruminants as direct and indirect source of contamination of humans by O157:H7 EHEC is today beyond any doubt, although human–to–human contamination also clearly exist. Less data are available for most of the other strains. Very little is known about the dynamics of the carrier state, the epidemiology in cattle and in humans, and the actual host specificity of the non-O157:H7 EHEC and VTEC strains.

Identical strains have been isolated from healthy cattle, young and adult animals, and diseased humans, favouring the hypothesis of animals as sources of contamination for humans. One cannot exclude the possibility that bovine and human strains of EHEC and VTEC strains belonging to the same serotype and pathotype could be grouped differently on the basis of their host specificity (Mainil 1999; Brown *et al.* 2001; Pradel *et al.* 2001; Liebana *et al.* 2003). A first group would include calf-specific strains, while other strains would be human-specific. Finally a third group would comprise strains devoid of host specificity. This hypothesis is supported to some extent by the PFGE comparison of strains. Their host specificity in pathology and in carriage, if any, would not reside in the Stx, in the LEE-located genes, nor in the pEHEC plasmid-located genes. However, it would reside in the production of adhesion acting as specific-colonization factors of the host intestinal tract. Candidates have been described for different EHEC and VTEC, but their role in the colonization of the mucosa is unconfirmed (Paton *et al.* 2001; Szalo *et al.* 2001).

Future research efforts must not only target improved diagnosis, traceability and prevention of O157:H7 EHEC infections in humans, ruminants and nonruminant animals, but also focus on the non-O157 EHEC and VTEC combining microbiological, medical and veterinarian studies, to bring better understanding their epidemiology, aetiology, virulence properties and host specificity.

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