About verocytotoxin-producing *Escherichia coli* (VTEC)

Escherichia coli is part of the normal micro flora of the gastrointestinal tract of mammals and birds, but certain strains have been associated with gastrointestinal diseases in both humans and animals. These *E. coli* strains have been categorised into pathogenicity groups, based on their virulence properties [18]. One of these groups is characterised by the production of potent cytotoxins that inhibit the protein synthesis within eukaryotic cells. These toxins are either termed verocytotoxins (VT), because of their activity on Vero cells, or Shiga toxins (Stx), because of their similarity with the toxin produced by *Shigella dysenteriae* [15]. Therefore, these strains are either termed VT-producing *E. coli* (VTEC) or Stx-producing *E. coli* (STEC).

Enterohaemorrhagic *E. coli* (EHEC) constitute a subset of serotypes of VTEC that has been firmly associated with bloody diarrhoea and haemolytic uraemic syndrome (HUS) in industrialised countries [5, 18]. The majority of the cases of severe disease are caused by strains of serotype O157:H7, but infections sustained by strains belonging to serogoups other than O157, like O26, O111, O103, and O145 have been increasingly reported [18, 25]. These strains are now usually referred to as non–O157 EHEC.

Virulence factors

Shiga toxins

Stxs are considered to be the major virulence factor of VTEC and comprise a family of structurally related cytotoxins with similar biological activity. The two main groups consist of Stx1, which is nearly identical to the toxin of *S. dysenteriae* type 1, and Stx2, which shares less than 60 % amino acid sequence with Stx1 [15]. The genetic information for the production of Stx1 and Stx2 is located in the genome of lambdoid prophages integrated in the VTEC chromosome [15]. Whereas Stx1shows only little sequence variations, several variants of Stx2 with altered antigenic or biological characteristics have been described. Epidemiological studies have revealed that Stx2 is more associated with severe human disease than Stx1 [1]. A certain number of variants are produced by strains of animal

origin and are rarely observed in human isolates: Stx2e is mainly found in VTEC causing oedema disease in pigs and Stx2f appears to be closely associated with VTEC of avian origin [21].

Attaching and effacing adhesion

Most VTEC included in the EHEC group colonise the intestinal mucosa with a mechanism that subverts the epithelial cell function [8] and induce a characteristic histopathologic lesion, defined as "attaching and effacing"(A/E). The A/E lesion is due to marked cytoskeletal changes and is characterised by effacement of microvilli and intimate adherence between the bacteria and the epithelial cell membrane, with accumulation of polymerised actin directly beneath the adherent bacteria [18].

The complex mechanism of A/E adhesion is genetically governed by a large pathogenicity island (PAI) defined as Locus of Enterocyte Effacement (LEE) [8, 18].

Other virulence factors

Genetic analysis of the complete DNA sequence of VTEC O157:H7 [19] showed that almost 20% of its chromosome is constituted by foreign DNA not present in the chromosome of *E. coli* K-12 and that has been probably acquired from other bacterial species through horizontal gene transfer. Similarly to the LEE, other regions of this foreign DNA can be considered as putative PAIs since they carry virulence-associated genes, show a lower GC content, and are inserted in tRNA loci. In particular, a PAI termed O#122 is present in most EHEC and enteropathogenic *E. coli* (EPEC), but not in other groups of *E. coli* [17].

VTEC O157 possess a large virulence plasmid of approximately 90 Kb termed pO157. The nucleotide sequence of this plasmid showed that it encodes 35 proteins, some of which are presumably involved in the pathogenesis of EHEC infections [3]. The enterohaemolysin (*hly*) operon is considered the best marker of the presence of pO157 and is also present in the large plasmids that can be detected in most non-O157 EHEC strains [4] Other putative virulence factors harboured by this plasmid comprise a katalase-peroxyidase and a serine protease, encoded by *katP* and *espP* genes, respectively [22]. Another virulence gene, termed *toxB,* has been

recently described in pO157 [23] and it appears to be present in all the VTEC O157 isolates [26]

VTEC are zoonotic pathogens

VTEC can be found in the gut of numerous animal species, but ruminants have been identified as a major reservoir of VTEC that are highly virulent to humans, in particular VTEC O157.

Cattle are considered to be the most important source of human infections with VTEC O157, being asymptomatic excretors of the organism, which is a transient member of their normal gut micro flora [5]. VTEC O157 have also been frequently isolated from the intestinal content of sheep that is now considered a reservoir for human infection [10]. VTEC O157 has also been isolated from goats [20] and water buffalo [9].

VTEC have been sporadically isolated from mammals other than ruminants, like pigs [2], horses [6], dogs [27] and farmed rabbits [12] but these species are not considered as actual hosts but rather as vectors transiently colonised after a contact with ruminant dejections.

Epidemiology of VTEC infections

During the 1980s, most of the outbreaks of VTEC O157 infection were food-borne and the food vehicles implicated were mostly inadequately cooked beef products and unpasteurised milk [18]. In the past ten years, several outbreaks have been associated with low pH products like fermented salami, mayonnaise and yogurt [16]. This has highlighted the tolerance of *E.coli* O157 to acidic pH and its ability to survive the processes of fermentation and drying. In addition, waterborne outbreaks and outbreaks associated with other types of environment-related exposures have been increasingly reported [14, 24]. The dispersion of untreated manure in the environment can cause the contamination of different items, which can then act as secondary vehicles of human infections [5, 7, 14].

An increasing spectrum of fruits and vegetables fertilised with ruminants' manure or contaminated during harvesting or processing has been involved in outbreaks [5, 14, 24].

Control strategies

Having animals and raw products that are free from VTEC is not feasible in practice. However, their occurrence can be minimised by applying high standards of hygiene in all the steps of the food production chain.

At the farm level, good hygiene and management practices remains at the present the best way to reduce the spread and persistence of VTEC O157 in the farm.

VTEC can survive in bovine faeces for a considerable time [14], therefore the handling of the animal dejections represents an important issue and manure and slurries should be properly composted to ensure the reduction of the microbial load [11, 13].

Farmers and people visiting farms should apply hygiene practices. In particular, farms receiving school visits must ensure that adults always control children, facilities for hand washing are easily available, and areas for food consume are clearly separated from those where the animals are kept.

At the abattoir level, good hygiene and manufacturing practices as well as implementation of HACCP will contribute in reducing faecal contamination of carcasses.

The general principles of food hygiene will be effective in preventing VTEC infections also at the processing and retail levels of the food chain.

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