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## **Pathogenesis, Epidemiology, and Control of VTEC Infection in the Cattle Industry**

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### **Introduction**

*Escherichia coli* that produce at least one type of verotoxin (VT) are called verotoxigenic *E. coli* (VTEC). There are two major types of VT, VT1 and VT2, but there are at least five antigenic types of VT2. Over 200 serotypes of VTEC have been isolated from cattle and over 60 serotypes have been recovered from humans with disease, but fewer than 10 of these occur with regularity as causes of severe disease in humans. VTEC are of interest to the cattle industry because they are important pathogens of humans, in which they cause watery diarrhea, bloody diarrhea, and the hemolytic uremic syndrome (HUS). Most studies on pathogenesis have involved O157:H7, the serotype most frequently implicated in severe disease in humans. VTEC are carried normally by healthy cattle, but some serotypes have been associated with diarrhea and others have been implicated in bloody diarrhea. Several post-harvest control methods have been adopted but pre-harvest interventions are needed to make an impact on human disease.

### **Pathogenesis of disease in humans**

Disease in humans starts with ingestion of food or water contaminated by VTEC<sup>5, 9</sup>. A low dose (< 100 CFU) of O157:H7 VTEC can initiate disease, likely because of the acid resistance of these organisms. In addition to other acid-resistance mechanisms, O157:H7 VTEC possess urease genes that might provide additional acid resistance. VTEC pass through the small intestine and express intestinal colonization factors when they encounter the large intestine.

Contact with enterocytes, likely through fimbrial adhesins, is probably responsible for activation of genes encoded by the locus for enterocyte effacement (*LEE*). Factors that regulate expression of genes involved in colonization include high osmolarity, bicarbonate ion, and the catecholamines epinephrine and norepinephrine. Several fimbrial and non-fimbrial adhesins have been identified but their specific involvement in colonization of the intestine has not been delineated. O157:H7 expresses intimin- $\gamma$  that mediates adherence initially to follicle-associated epithelium (FAE) in human *in vitro* organ culture<sup>10</sup>, suggesting that these sites are targeted. Following contact, *LEE*-encoded gene products result in assembly of a molecular syringe, whose needle connects the cytoplasm of the bacterium and the cytosol of the host enterocyte, and transfer of specific effector molecules from bacterium to host cell. One of the proteins transferred to the host is Tir, which associates with the host cell membrane and serves as a receptor for the adhesin intimin or Eae on the bacterial surface. The molecules injected into the

host activate signaling pathways that result in remodeling of the host cell cytoskeleton and destruction of microvilli, so that O157 VTEC adhere intimately to depressions in pedestals on enterocytes depleted of their microvilli. This lesion, known as the attaching and effacing (AE) lesion, is characteristic of LEE-positive VTEC and has been demonstrated *in vitro* and *in vivo*. VTEC that lack the LEE and are unable to induce AE lesions and the mechanisms employed for intestinal colonization by these strains appear to involve fimbrial, non-intimate adherence.

VT produced in the intestine by VTEC that colonize is critical in pathogenesis. This toxin can translocate *in vitro* across polarized epithelial cells and a similar mechanism *in vivo* would permit access to the submucosa. VT can induce production of interleukin-8 (IL-8) by enterocytes, thereby attracting neutrophils to the site. The ensuing inflammation and migration of poly-morphonuclear leukocytes (PMNs) into the intestinal lumen may allow VT to also pass between epithelial cells into the submucosa<sup>7</sup>. There is considerable mucosal damage, attributable to the action of PMNs and to VT in some host species<sup>7</sup>. The PMNs may also serve as Trojan horses that allow VT from the intestine to be transported into the circulation and to be released at sites rich in the VT receptor globotriaosyl ceramide (Gb3). These sites include vascular endothelium in the renal glomeruli and brain, and renal proximal tubular epithelium. LPS from the intestine may enter the circulation and stimulate production of Gb3 sites and enhance the action of VT at these sites. Endothelial injury induced by VT leads to production of IL-8 and accumulation of PMNs which become activated and adhere to injured cells leading to production of reactive oxygen intermediates and further endothelial cell injury<sup>11</sup>. Exposure of the subendothelium releases prothrombotic substances and platelet aggregation results in thrombocytopenia.

Damage to vascular endothelium by VT leads to increased permeability and loss of fluids and erythrocytes into the surrounding tissue and to thrombosis of small blood vessels<sup>11</sup>. VT-induced apoptosis may be present in epithelial cells of the renal tubules and the glomeruli and necrosis may be observed in enterocytes as well as in the renal cortex.

Watery diarrhea may be attributed to loss of absorptive intestinal epithelial surface due to effacement of microvilli, the inflammatory response to infection, and signal transduction that results in active chloride secretion<sup>5</sup>. Bloody diarrhea is caused by VT-mediated damage to small blood vessels in the large intestine. Renal damage, a major element in HUS, is the result of vascular and epithelial cell damage caused by VT. CNS signs are due to VT action on blood vessels in the CNS.

### **Epidemiology and infection in cattle**

VTEC are not commonly associated with disease in calves, and O157:H7 has not been implicated in disease in cattle. However, VTEC of a few O groups including O5, O26, O111, and O118 may cause watery diarrhea and bloody diarrhea in calves. These VTEC are typically *vt1* and *eae* positive. Recently, VTEC of O group OX186, were implicated in severe hemorrhagic enteritis in calves and in HUS in humans<sup>2</sup>. O26 VTEC produce intimin- $\alpha$  and, unlike O157 VTEC, extensively colonized the large intestine of experimentally-infected 4-day-old calves<sup>14</sup>. Even in calves that do develop enteric disease due to O5, O26, O111, or O118 VTEC, there are no signs of systemic complications. It is possible that limited intestinal colonization results in insufficient production of VT for systemic effects. Also, most calves

acquire maternal antibodies to VT1, which potentially afford protection against toxemia. Cattle possess the Gb3 vascular endothelial receptors for VT, and the occurrence of bloody diarrhea in calves suggests that blood vessels in the colon are susceptible to VT.

O157 VTEC induce AE lesions in the bovine intestine<sup>3, 12, 13</sup> and this may be important in maintaining the bacteria in the intestine of cattle. However, *eae*-negative mutants are able to colonize some areas of the gastrointestinal tract of cattle<sup>3, 13</sup>. The EHEC factor for adherence (Efa1), which is associated with a wide range of VTEC, has recently been shown to contribute to colonization of the bovine intestine<sup>13</sup>. Why do O157:H7 VTEC not cause disease in cattle under natural circumstances? There is no clear answer, but some possibilities are suggested. O157 VTEC do not appear to colonize the bovine intestine intensively, even in ligated loops which prevent removal of the bacteria<sup>12</sup>. The bovine intestinal environment may lack the signals to fully turn on the genes required for extensive colonization, or may be deficient in accessory factors needed for extensive development of the AE lesion. In this connection, there is evidence that host tissue plays a role in adherence of O157 VTEC<sup>10, 13</sup>. Also, high prevalence rates of VTEC in the bovine environment suggest exposure and stimulation of local and systemic immune responses in cattle early in life.

The prevalence rates for VTEC of all serotypes in individual animals have varied between 6% and 100%<sup>1</sup>. For O157:H7 VTEC, early data on prevalence in cattle ranged from 0.1% to 4% for individual animals<sup>1</sup>. With improved techniques in recent studies, the reported range has now increased to between 2% and 28%. In some groups of animals the rate of excretion may exceed 80% (Gyles, unpublished). There is no way to determine whether the new data reflect real increases as well as improved methodologies, but we have not seen an increase in human infections during this period of dramatic increases in reported prevalences.

Fecal culture and serology have shown that most cattle herds have been exposed to O157 and other VTEC, but we know very little about specific factors that affect shedding of VTEC by cattle. In temperate regions, there is a marked seasonal prevalence in shedding of O157 VTEC, with a peak in the summer and early fall and a marked decline through the winter; and rates of excretion are relatively low in pre-weaned calves, highest in the post-weaning period, and decline subsequently. Shedding follows an intermittent pattern which is consistent with transient infection and periodic re-infection. Typically, the concentration of O157 VTEC in the feces is low, ranging from <50 to 10<sup>3</sup> per gram but high level shedders (animals with >10<sup>5</sup> CFU/g of feces) are likely very important in maintaining infection in herds and in transmission to humans. Omisakin *et al.* found that 44/589 cattle at an abattoir were shedding O157 VTEC and that of 4 of the 44 were high shedders and were responsible for >96% of the total O157 that were shed<sup>8</sup>. Persistent shedders have a narrow strip of the terminal rectum at the recto-anal junction as the main site of colonization<sup>6</sup>; here the bacteria form AE lesions on follicle-associated epithelium. VT are not cytotoxic for bovine primary intestinal epithelial cells, likely due to inactivation in lysosomes<sup>4</sup>. Furthermore, VT1 suppresses the proinflammatory cytokine response of bovine but not of human epithelial cells, suggesting that lack of cytotoxicity and the muted inflammatory response may aid in colonization of cattle by VTEC.

### **Control at the post-harvest stages**

Changes in industry practices and consumer education may have helped in preventing escalation in disease. Post-harvest control measures that have been implemented to reduce contamination of meats and other foods include: i) steps to eliminate fecal contamination of produce and to wash these products with chlorinated water, ii) measures such as pasteurization or freeze-thaw plus organic acids to eliminate or significantly reduce O157 VTEC in products such as apple juice and fruit drinks, iii) thorough cleaning and sanitation of surfaces and instruments that contact foods during processing, iv) institution of HACCP procedures, v) steps to minimize fecal contamination of carcasses, vi) application of antimicrobial carcass treatments, v) irradiation.

### **Abstract.**

Pathogenesis of VTEC infections in humans involves colonization of the large intestine, with attaching and effacing (AE) lesions for some VTEC and less intimate attachment for others. VT (verotoxin) is responsible for severe forms of disease due to vascular endothelial damage in target organs. VTEC are carried by healthy cattle, whose manure may contaminate meats and other foods. Several measures are taken by industry to minimize transfer of O157 and other VTEC to humans.

### **Résumé**

La pathogénèse des *E. coli* producteurs de vérotoxines (VTEC) chez les humains se manifeste par la colonisation des gros intestins ainsi que par des lésions d'attachement et d'effacement (AE) dans la muqueuse intestinale. La vérotoxine est responsable de la forme grave de la maladie suite à l'endommagement endothélial des vaisseaux dans les organes atteints. Le principal porteur des VTEC est le bovin en bonne santé dont les matières fécales contaminent la viande et plusieurs autres aliments. Diverses mesures sont prises par l'industrie alimentaire en vue de prévenir la transmission du VTEC O157 et autres VTEC aux humains.

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