

## Virulence Factors of Verocytotoxin-Producing *Escherichia coli* Isolated from Raw Meats

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**PCR for verocytotoxin-producing *Escherichia coli* (VTEC) was positive in 4.6% of 2,440 raw meat samples; only beef, sheep, and venison samples were positive. None of the isolated VTEC strains belonged to serogroup O157. Additional virulence factors were detected in only a minority of strains, suggesting that most of these meat VTEC isolates are not pathogenic.**

In humans, verocytotoxin (Shiga-like toxin or Shiga toxin)-producing *Escherichia coli* (VTEC) are associated with watery or bloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) (8, 13). The source of *E. coli* O157:H7 infection was generally found to be foods of bovine origin or other foods cross-contaminated by beef products or cow manure (8, 13). Person-to-person transmission (8) or transmission by contact with infected livestock (23) has also been reported. The epidemiology of non-O157 VTEC infections remains less well known. Non-O157 VTEC is more readily isolated from food, particularly from meat, than *E. coli* O157:H7 (8, 22, 27). However, whether consumption of these meats leads to disease is unknown. Furthermore, although non-O157 VTEC serotypes such as O26:H11 (8, 13), O103:H2 (15), and O111:H- (5) have been associated with HUS, the pathogenicity of non-O157 VTEC remains questionable (25). VTEC strains seem to be pathogenic for humans only if they possess accessory virulence factors associated with the capacity to colonize the gut. The *eaeA* gene is responsible for attachment-effacement lesions similar to those in enteropathogenic *E. coli* strains (7), and the large EHEC virulence plasmid is probably involved in adhesion to enterocytes (10). Production of enterohemolysin has also been described as a virulence factor (2). In a study on human VTEC isolates, we have shown that the presence of these virulence factors was correlated with VTEC-associated symptoms in the infected patients (18). In the present study we investigated virulence factors in VTEC meat isolates.

(Preliminary data were presented during the 2nd International Symposium and Workshop on Verocytotoxin (Shiga-like toxin)-Producing *Escherichia coli* Infections, Bergamo, Italy, 27–30 June 1994 [17]).

The animal species included in this study are shown in Table 1. Two thousand four hundred forty fresh meat samples and carcass swabs obtained from August 1991 to July 1996 were screened by PCR as described before (18, 19). Briefly, approximately 1 ml of a suspension obtained by homogenizing about 10 g of meat in 90 ml of sterile physiological saline supplemented with 1% peptone was inoculated into 10 ml of MacConkey enrichment broth and incubated overnight at 37°C. Carcass swabs were incubated in the same enrichment broth. After subculture of this broth on MacConkey agar, a colony sweep (a loopful of confluent bacterial growth) was suspended

in nutrient broth and directly used in a PCR using consensus primers amplifying the verocytotoxin genes VT1 and VT2 and its variants (11). Enrichment with antibody-coated magnetic beads (Dynabeads anti-*E. coli* O157; Dynal, Oslo, Norway) for optimal detection of O157 VTEC was introduced as well for analysis of the last 1,451 samples. For each PCR-positive sample, at least 10 colonies obtained on the MacConkey agar subculture were tested separately in an attempt to isolate the VTEC strain. Additional virulence factors were detected in the meat VTEC isolates as described previously (18). In brief, the *eaeA* gene was detected by PCR with the specific primers AE 9 and AE 10 developed by Gannon et al. (7), and sequences of the large EHEC virulence plasmid were detected with the MFS1F and MSF1R primers described by Fratamico et al. (6); *E. coli* hemolysin phenotypes were detected and characterized by comparison of hemolysis on CaCl<sub>2</sub>-washed blood agar and that on unwashed blood agar (2).

As shown in Table 1, VT genes were detected in 111 meat samples, but VTEC could be isolated from only 67 of them, probably due to an unfavorable proportion of VTEC versus other *E. coli* strains or to the loss of VT genes in vitro by some VTEC strains (12). Production of VT was confirmed in all isolates by cytotoxicity tests performed on Vero cells (18). VT genes and VTEC were found in beef, sheep, and various venison meats. The percentage of positive samples was very high in the latter, in particular in wild ruminants, followed by wild nonruminant mammals and finally by wild birds. The isolation of VTEC from wild animals has been reported only a few times. An outbreak of *E. coli* O157:H7 in Oregon has recently been traced to black-tailed deer (14). Rice et al. (21) and Milley and Sekla (16) have reported the isolation of O157:H7 from deer droppings and from caribou meat. In fact, it seems that most mammals can harbor VTEC as normal flora in the intestine as shown for domestic animals by Beutin et al. (1). These authors isolated non-O157 VTEC but no O157 strains from fecal samples of six of the seven domestic animal species studied. As in our meat sample study, the percentage of positive stools was higher in ruminants. In the samples we examined, the contamination with fecal flora was often very low: as much as 90% of VTEC-positive beef and sheep meat samples had total *E. coli* counts of  $1.1 \times 10^2$ /g or lower; in venison meat total *E. coli* counts were generally higher (data not shown). Serotyping was performed by H. Lior, Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, Canada, and, for a number of isolates from 1995 and 1996, by F. Scheutz, Statens Serum Institut, Copenhagen, Denmark. None of the VTEC strains belonged to serogroup O157. This

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TABLE 1. Isolation of VTEC from raw meats

Origin	No. of samples		
	Total (no. enriched by IMS <sup>a</sup> )	PCR positive <sup>b</sup> (%)	With isolate (%)
Beef/veal	1,532 (1,100)	27 (1.8)	25 (1.6)
Pork	197 (55)	0	0
Mixed beef/pork	29 (4)	0	0
Sheep/lamb	135 (99)	6 (4)	6 (4)
Horse	12 (3)	0	0
Rabbit	7 (0)	0	0
Poultry <sup>c</sup>	255 (8)	0	0
Wild or exotic ruminants	119 (99)	58 (49)	24 (20)
Red deer/doe	74 (59)	30 (41)	12 (16)
Roe deer/doe	24 (24)	19 (79)	5 (21)
Fallow deer/doe	9 (9)	2 (22)	2 (22)
Reindeer/doe	6 (3)	1 (50)	0
Antelope	6 (4)	6 (100)	5 (83)
Other wild or exotic mammals	64 (40)	15 (23)	7 (11)
Wild rabbit/hare	15 (11)	3 (20)	1 (7)
Kangaroo	4 (4)	0	0
Wild boar	45 (25)	12 (27)	6 (13)
Wild or exotic birds	90 (43)	5 (6)	5 (6)
Wild duck	11 (8)	0	0
Partridge	6 (3)	1 (17)	1 (7)
Ostrich	28 (4)	1 (4)	1 (4)
Quail	4 (0)	0	0
Pigeon	15 (8)	1 (7)	1 (7)
Pheasant	26 (20)	2 (8)	2 (8)
Total	2,440 (1,451)	111 (4.6)	67 (2.8)

<sup>a</sup> IMS, immunomagnetic separation using antibody-coated magnetic beads.

<sup>b</sup> VT restriction primers MK1 and MK2, described by Karch and Meyer (11), were used.

<sup>c</sup> 153 chickens, 88 turkeys, 10 ducks, and 4 guinea fowls.

finding is not surprising, since even in countries reporting high rates of human *E. coli* O157 infections, some surveys failed to find this serogroup in beef (22, 27). In Belgium, only a few O157 VTEC strains have been isolated from bovines (20). As in several studies on VTEC from healthy animals (1, 3, 20), many different serotypes occurred in the non-O157 strains we isolated in this study. At least 44 different O:H serotypes were found comprising 23 O serogroups and 19 H serogroups. Thirteen strains were O nontypeable, and seven were O rough; 8 strains were H nontypeable, and 12 were not motile. When we compared the 29 fully defined serotypes found in this survey with the 44 found in human stools from our hospital (18), the only common serotypes were O15:H27, O76:H19, O91:H-, O91:H14, O91:H21, O128:H2, and O145:H-. Four of the seven most frequently isolated O serogroups in Belgian patients—O157, O26, O103, and O111—which are also those most frequently involved in human disease in general, were not isolated from meats. In contrast, Pohl et al. studying VTEC in diarrheic calves (20) isolated several O26, O103, and O111 strains.

Subtyping of the VT genes of the strains isolated in the present study by using VT1- and VT2-specific primers (9) and the PCR-restriction fragment length polymorphism scheme of Tyler et al. (26) showed that 19 strains possessed VT1 alone, 32 strains possessed one or more VT2 genes (including 9 VT2c, 1 VT2e, and 10 nontypeable variant genes), and 12 strains pos-

sessed both VT1 and VT2 (including 3 VT2c and 8 nontypeable variant genes). The high number of strains with variant toxins probably reflects a lower pathogenicity since most patients developing HUS are infected with strains that harbor the VT2-type gene (4). The *eaeA* gene was present in only two meat VTEC strains (3%), one of serotype O145:H- isolated from a wild boar carcass and one O-rough:H16 isolate from beef. The large EHEC virulence plasmid was detected in 12 strains (18%). *E. coli* hemolysins were present in 21 isolates (31%); 20 were enterohemolytic, and 1 was alpha-hemolytic. The presence of these three virulence factors was correlated: the two *eaeA*-positive strains harbored the EHEC virulence plasmid, and all plasmid-positive strains were hemolytic. The lack of additional virulence factors in most VTEC isolates from meats, and in particular the near absence of the *eaeA* gene, which is best correlated with symptomatic disease in humans, is the most striking observation of this study.

In conclusion, VTEC, exclusively of non-O157 serotypes, was detected in meat, especially in venison, sheep, and beef samples. However, none of the strains belonged to serogroup O157, and the majority of strains lacked additional virulence factors. These non-O157 VTEC isolates from meat are probably not pathogenic for humans. Indeed, the majority of VTEC strains pathogenic for humans harbor all the accessory virulence factors mentioned above. However, some strains lacking the *eaeA* gene such as serotypes O113:H21 and O104:H2 seem to be clearly associated with human disease (24).

Surveys of VTEC in foods should include study of additional virulence factors. Awareness and prevention measures to minimize bacterial contamination of foods, including good hygienic standards in the kitchen, remain essential. Indeed, O157 VTEC outbreaks can occur very suddenly, as recently seen in Sweden, a country with very low O157 VTEC incidence (28).

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