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# Asymptomatic carriage of verocytotoxin-producing *Escherichia coli* O157 in farm workers in Northern Italy

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## SUMMARY

Faecal samples from 350 farm workers on 276 dairy farms and 50 abattoir employees from seven different operations were examined for the presence of Verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) by an O157-specific enzyme-linked fluorescent assay followed by immunoconcentration. VTEC O157 was isolated from four (1·1%) of the farm workers. A second stool sample was obtained from the positive farm workers as well as from their household contacts. VTEC O157 was isolated from the wife of one of them. The strains from the same household shared the same Verocytotoxin genes profile, phage type and pulsed-field gel electrophoresis pattern. The VTEC O157-positive subjects had neither intestinal symptoms at the moment of sampling nor a history of bloody diarrhoea or renal failure. Our study seems to confirm the hypothesis that farm residents often develop immunity to VTEC O157 infection, possibly due to recurrent exposure to less virulent strains of VTEC.

## INTRODUCTION

*Escherichia coli* O157 is an important cause of foodborne disease. Outbreaks have been reported throughout the industrialized world, with clinical manifestations ranging from asymptomatic carriage to severe illnesses such as haemorrhagic colitis and haemolytic–uraemic syndrome (HUS) [1, 2]. The gastrointestinal tract of ruminants, particularly cattle, represents the main natural reservoir of *E. coli* O157 and other verocytototxin (VT)-producing *E. coli* (VTEC) [2]. Human infections are mainly foodborne: undercooked beef and beef products, raw milk, and vegetables and fruit contaminated with ruminant manure have all been identified as sources of infection [1, 2]. However, epidemic and sporadic cases of VTEC O157 infection have been increasingly associated with direct contact with farm animals, and with different exposures to an environment contaminated with ruminant manure [3–7]. In particular, several outbreaks in visitors to open farms have been reported [8], confirming the circulation of VTEC O157 in the farm environment.

Persons professionally exposed to cattle, for example dairy-farm workers or slaughterhouse employees, can have an increased risk of acquiring VTEC O157 infection. In a study conducted on Canadian dairy farm families [9] 21 out of 335 (6·3%) of the subjects examined were found to be carriers of VTEC, but *E. coli* O157 was isolated from only one of the subjects. Investigations conducted in meat-processing plants in Switzerland [10, 11] showed rates of VTEC carriage ranging from 2·6% among employees involved in cutting to 9·0% in those directly involved in slaughtering; also in that case

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only one out of the 47 isolates obtained was *E. coli* O157. However, both the Canadian and the Swiss studies were based on the detection of VT genes and did not use isolation procedures specific for *E. coli* O157.

The aim of the present study was to investigate the presence of VTEC O157 in stool samples from asymptomatic subjects working in dairy farms or abattoirs in northern Italy, by using a sensitive isolation procedure based on screening with an O157-specific enzyme-linked fluorescent assay (ELFA) followed by immuno-concentration of *E. coli* O157 [12, 13].

## MATERIALS AND METHODS

### Sampling

Sampling was conducted between May and October 2002 in Piemonte, one of the 20 Italian regions located in the North-West of Italy. The subjects were randomly selected among the farm workers and abattoir employees enrolled in the food-handler regional register. Each subject was asked to provide a faecal sample and was questioned regarding episodes of diarrhoea in the preceding week. Stools were placed in sterile containers and stored at 4 °C for up to 3 days until processed. A second stool sample was obtained from the *E. coli* O157-positive subjects, and stools were also collected from their household contacts.

The families of the *E. coli* O157-positive subjects were interviewed about episodes of diarrhoea in the 2-month period preceding and following the positive stool specimen, recent antimicrobial therapy, and long-term histories of bloody diarrhoea or renal disease. Information on potential risk factors for *E. coli* O157 infection was also collected, including consumption of raw milk or beef, use of unchlorinated well water, use of manure in the garden.

### Detection and isolation of *E. coli* O157

Faecal specimens were examined for the presence of the O157 antigen by ELFA, followed by an *E. coli* O157 immuno-concentration procedure [12, 13]. One gram of faeces was suspended in 9 ml of modified TSB added to 20 mg/l of novobiocin. After 6 h incubation at 41 °C, 1 ml of the suspension was further enriched in 9 ml of cefixime–tellurite MacConkey broth (Oxoid Italiana, Garbagnate Milanese, Italy) at 37 °C for 18 h. The O157 ELFA was performed using the VIDAS *E. coli* O157 ECO kit (bioMérieux, Marcy-l'Etoile, France), and the positive samples were subjected to immuno-concentration by using the VIDAS *E. coli* O157 ICE kit (bioMérieux), following the manufacturer's instructions. Aliquots of the immuno-concentrated material were streaked onto Sorbitol MacConkey Agar (SMAC; Oxoid), and cefixime–tellurite SMAC (CT-SMAC; Oxoid) and incubated at 37 °C for 18 h. Sorbitol non-fermenting colonies were tested with an *E. coli* O157 Latex test kit (Oxoid); the agglutinating cultures were biochemically confirmed as *E. coli* (API 20E, bioMérieux) and further characterized as below.

### Characterization of the *E. coli* O157 isolates

Serotyping was confirmed by tube agglutination of heat-treated cultures. VT production was determined by the Vero cell assay and the presence of *vt1* and *vt2*, and intimin-coding *eae* (attaching–effacing) genes was assessed by PCR as previously described [14]. PCR was also used to assess the presence of the plasmid virulence genes *toxB* [15], *E-hly* [16], *katP* [17], *etpD* [18], *espP* [19] and *astA* [20], and of the chromosomal gene *efa1* [21]. Due to the large dimensions of *toxB* and *efa1*, both the 5′ and the 3′ regions of the genes were separately amplified. The sequences of primers were described in the respective papers except for *toxB* (toxB 5′ upper AAAATAATTCATCCCCCAGTTCT; toxB 5′ lower CCGCACCAAAGGCATTAG and toxB 3′ upper TAGCGGAAAGAATATTGGTAGTCA; toxB 3′ lower CTGTAGTGTGGCGGGAAC, this study).

Phage typing was kindly performed by the Laboratory for Enteric Pathogens of the Health Protection Agency, London, UK.

Pulsed-field gel electrophoresis (PFGE) was performed as previously described [22], using 100 units of *Xba*I (New England Biolabs Inc., Beverly, MA, USA) for overnight restriction.

## RESULTS

### Detection and isolation of *E. coli* O157

Stool specimens were collected from 50 abattoir employees working at seven different plants and from 350 farm workers from 276 different dairy farms. The farms were all small to medium premises, with a number of cows ranging from 50 to 300. The median age of persons submitting samples was 43 years

Table. *Characteristics and virulence determinants of the VTEC O157 strains isolated from healthy farm workers*

| | | | PCR for other virulence genes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain | Phage type | *vt* genes | *eae* | *efa1** | *toxB*† | *E-hly* | *katP* | *espP* | *etpD* | *Asta* |
| ED 497 | PT8 | *vt1/vt2* | + | + | + | + | + | − | + | + |
| ED 498‡ | PT34 | *vt2* | + | + | + | + | − | − | + | + |
| ED 499 | PT8 | *vt2* | + | + | + | − | + | − | + | + |
| ED 507 | PT8 | *vt1/vt2* | + | + | + | + | + | − | + | + |
| ED 508‡ | PT34 | *vt2* | + | + | + | + | + | − | + | + |

* All isolates possessed only the 5′ region of the gene.
† All isolates possessed both the 5′ and the 3′ region of the gene.
‡ Strains isolated from members of the same household.

(range 9–76 years) and 92% of them were males. All the subjects denied having had episodes of diarrhoea during the week preceding stool collection.

Fifteen specimens were positive at the O157 ELFA test and further tested with the immuno-concentration assay. *E. coli* O157 was isolated from four (1·1%) of the specimens, obtained from farm workers working at four dairy farms located in different municipalities.

A second stool sample was obtained from the four positive farm workers as well as from 12 of their household contacts. The interval between the collection of the first positive sample and the collection of the other samples from the household ranged between 35 and 84 days. The four farm workers were negative, but *E. coli* O157 was isolated from the wife of one the farm workers, whose stool sample was collected 80 days after the positive specimen from her husband.

### Characterization of the *E. coli* O157 isolates

The characteristics of the *E. coli* O157 isolates are reported in the Table. Both the *E. coli* O157 isolates from the farm worker and his wife carried *vt2* genes and belonged to phage-type (PT) 34. The other three isolates were PT8, two of them carried *vt1* and *vt2* genes, while the remaining isolate harboured the *vt2* gene only. All the strains showed a similar array of virulence-related genes except for strains ED 498 and ED 499, which were negative for *katP* and *E-hly* genes respectively. PFGE analysis showed that the two PT34 strains from the same household had the same profile, while the three PT8 strains showed different restriction patterns.

### Health outcomes and risk factors for VTEC O157

The families of the VTEC O157-positive subjects were interviewed about episodes of VTEC O157-related illness and potential risk factors for acquiring the infection. All the families denied recent episodes of diarrhoea. Bloody diarrhoea or renal failure were not reported in the histories of any family members. Routine consumption of raw milk or beef, or well water was not reported, but all the families used manure in the garden, where vegetables and/or soft fruit for home consumption were grown.

## DISCUSSION

Contact with the farming environment is considered to be a risk factor for acquiring VTEC infections, and it has been associated with both epidemic [4, 8, 23] and sporadic [6, 7] episodes of infection with VTEC O157. Accordingly, this organism was isolated from approximately 1% of the dairy farm workers examined in this study. Dairy farm workers are exposed directly to cattle manure and, more generally to an environment into which VTEC are widespread [2, 24, 25]. In a study conducted on Canadian dairy-farm families [9], more than 40% of the subjects examined had microbiological or serological evidence of current or past infection with VTEC. However, most of these infections appeared to be due to VTEC other than serogroup O157. In that study, young age was the only risk factor associated with VTEC infections, while habitual consumption of raw milk was reported in almost all the families. In this study, the families of the VTEC O157-positive subjects denied routine consumption of raw milk or beef, but all of them used manure in the garden, where vegetables for home consumption were grown. Such a practice represents a well-established risk factor for acquiring VTEC infections [5, 26], and it appeared to be very common among the farms in the area of the study.

The four positive farm workers identified proved negative when a second stool specimen was examined. However, VTEC O157 was isolated from the wife of one the farm workers when their household contacts were examined. VT type, phage type, and PFGE profile of the strain were identical to those of the strain isolated from the husband 80 days before. It is difficult to say if the infection was acquired by person-to-person transmission, which has been frequently reported for VTEC O157 [1, 27], or by exposure to a common source within the farm. In any case, this finding confirms the ability of VTEC O157 clones to persist for long periods within the farm environment [24, 25].

As already reported by Wilson et al. [9] in the Canadian investigation, all the subjects found positive in this study had neither intestinal symptoms at the moment of sampling nor a history of bloody diarrhoea or renal failure. Since all the VTEC O157 strains isolated possessed the main genetic determinants known to be associated with virulence [28], our study seems to confirm the hypothesis that farm residents often develop immunity to VTEC O157 infection, possibly due to recurrent exposure to the less virulent and more frequent strains of VTEC non-O157 [9, 29].

The prevalence of 1·1% of farm workers found positive for VTEC O157 in this study appears to be remarkable, especially if one takes into account that Italy is considered to be a country with a relatively low incidence of VTEC O157 infection [30]: indeed, the incidence of HUS in Italy has been estimated around 0·3 cases/100 000 residents in the age band 0–14 years [14], a figure that appears to be between two and three times lower than those reported in similar studies conducted in other European countries [30]. This prevalence rate appears to be higher than those reported in other studies conducted on persons professionally exposed to cattle. VTEC O157 was rarely isolated from dairy-farm residents in Canada [9] and from employees of meat-processing plants in Switzerland [10, 11], although both studies reported prevalence rates of VTEC non-O157 ranging from 2·6 to 9%. However, it should be considered that the diagnostic procedures adopted in those investigations were based on the detection of VT genes, while a sensitive isolation procedure specific for *E. coli* O157 has been used in this study.

In conclusion, this study confirms that professional exposure to cattle represents a risk factor for acquiring infection with VTEC O157. The isolation of VTEC O157 strains in the absence of disease also supports the view that farm residents may develop immunity to VTEC infection, possibly by exposure to the more common and less virulent VTEC non-O157 [9, 29]. The public health significance of asymptomatic human carriers of VTEC O157 is still unclear [31, 32] but it should not be underestimated.

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