

## NON-O157:H7 VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM CATTLE AT SLAUGHTER IN NORTHERN ITALY

Bonardi S.<sup>1\*</sup>, Chiapponi C.<sup>2</sup>, Bacci C.<sup>1</sup>, Paris A.<sup>1</sup>, Salsi A.<sup>1</sup>

### Introduction

The clinical importance of verocytotoxin-producing *Escherichia coli* (VTEC) was first recognised in the U.S.A. in 1982, when *E. coli* O157:H7 infection was associated to an outbreak of haemorrhagic colitis due to the consumption of ground beef (Riley *et al.*, 1983). Since then, numerous epidemiological studies have been carried out to better understand the role of cattle as reservoir of the microorganism. Nevertheless, *E. coli* O157:H7 is not the only VTEC serotype responsible of human illnesses. Approximately 60 other VTEC serotypes have been implicated in human diseases ranging from mild diarrhoea to severe haemorrhagic colitis (HC) and life-threatening haemolytic uraemic syndrome (HUS). Toxins produced by VTEC are considered the major causes of illnesses; they are referred as verocytotoxin 1 (VT1) and verocytotoxin 2 (VT2).

The involvement of non-O157:H7 VTEC strains in human foodborne outbreaks has increased dramatically in the past decade. In Australia (Goldwater and Bettelheim, 1996) and Argentina (Lopez *et al.*, 1989), non-O157 VTEC infections appear to be more common than O157:H7 infections, and in Germany non-O157 VTEC serotypes have replaced O157:H7 as the VTEC most commonly isolated in HUS cases (Huppertz *et al.*, 1996). The most common non-O157 VTEC serogroups associated with human diseases are O26, O111, O128, and O103 (Bettelheim, 2000). Infections by O111:H- in Italy (Caprioli *et al.*, 1994) and Australia (Cameron *et al.*, 1995) are well documented, as infections by O111:H2 in Germany (Morabito *et al.*, 1998), by O111:H8 in the USA (CDC, 2000), by O103:H2 in France (Mariani-Kurkdjian *et al.*, 1993), in the USA (Tarr *et al.*, 1996) and in Germany (Karch *et al.*, 1997), by O145:H5 in Japan (Kudoh *et al.*, 1994) and by O104:H21 in the USA (CDC, 1995). In December 2005, an outbreak of HUS associated to O26 VTEC contaminated unpasteurized Camembert cheese was recognized in France (<http://www.promedmail.org>). In Italy, in 2005 six children infected with O26 VTEC developed HUS in Salerno Province and one of them died (Scavia *et al.*, 2005).

Previous surveys elucidated the role of cattle as reservoirs of VTEC O157:H7 in Italy (Conedera *et al.*, 1997; Bonardi *et al.*, 1999; Bonardi *et al.*, 2001) but

<sup>1</sup> Dipartimento di Salute Animale, Sezione di Ispezione degli Alimenti di origine animale  
Facoltà di Medicina Veterinaria, Università di Parma

<sup>2</sup> Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sede di Parma

\* corresponding author

e-mail address: [silvia.bonardi@unipr.it](mailto:silvia.bonardi@unipr.it)

little is known about the prevalence of other VTEC serotypes in cattle reared and slaughtered in our country. Therefore, as nowadays it seems unrealistic to investigate cattle for only one serotype as O157:H7, the aim of latter studies was to examine the prevalence of non-O157 VTEC serotypes in cattle at slaughter in Italy (Bonardi *et al.*, 2004).

## **Materials and Methods**

### **Sampling**

Between September 2001 and September 2004, a total of 247 samples of bovine caecal content were collected immediately after slaughter in five abattoirs of Emilia Romagna Region, northern Italy. The samples were collected and analysed in two different periods. In particular, 145 samples were collected between September 2001 and June 2002 (part i sampling), and the remaining 114 samples were collected from October 2003 to September 2004 (part ii sampling). Specimens were from 121 feedlot cattle (intensively reared cattle, not at pasture; live weight 550-650 kg) and 126 dairy cows. The animals were reared in 71 and 87 farms respectively, located in eight Italian provinces (Parma, Reggio Emilia, Piacenza, Modena, Mantova, Cremona, Brescia, and Perugia).

Faecal material was aseptically collected from the caecum of cattle immediately after slaughter, placed in separate sterile containers and examined on the day of collection. During transport to the laboratory, all samples were stored at + 4°C.

### **Immunomagnetic separation - slide agglutination technique**

For non-O157 VTEC strains detection, two different analytical procedures were followed in the two sampling periods.

In part i sampling, a 10-g aliquot of caecal material was suspended in 90 ml Tryptone Soya Broth (TSB, CM 129; Oxoid, Basingstoke, UK) added with vancomycin 8 µg/l (N1404; Sigma-Aldrich, Steinheim, Germany), and incubated at 37° C overnight. After enrichment, the brothcultures were ten-fold diluted to 10<sup>-6</sup> in Phosphate Buffer Solution (PBS, BR 14G; Oxoid) and a 10 µl-aliquot of each dilution was plated onto Enterohaemolysin Agar (EHLY Agar, PB 5105; Oxoid) and onto MacConkey Agar (CM 007; Oxoid). Plates were incubated at 37° C for 24 h. Colonies surrounded by a narrow zone of haemolysis (Beutin *et al.*, 1989) were selected for confirmation. Moreover, as not all VTEC strains produce the enterohaemolysin (Beutin, 2001; Jenkins *et al.*, 2002), non-haemolytic colonies on EHLY agar, or purple colonies on MacConkey agar, were picked up for further testing. Up to 10 colonies per sample were tested for indole production. Indole-positive cultures were confirmed biochemically as *E. coli* by using API 20E system (bioMérieux, Marcy-l'Etoile, France).

In part ii sampling, faecal samples were examined for the presence of non-

O157 VTEC by using an immunomagnetic separation (IMS) technique. A 10-g aliquot of caecal material was suspended in 90 ml of modified Tryptone Soya Broth (m-TSB, CM 989; Oxoid) supplemented with novobiocin 20 mg/l (N1628; Sigma-Aldrich). The enrichment broths were incubated at 37° C overnight. IMS was performed using the Dynabeads® anti-*E. coli* O26, O103, O111, and O145 (DY 71013, DY 1011, DY 1009, DY 1007; Dynal, Oslo, Norway), following the manufacturer's instructions. Two-50 µl-aliquots of serogroup O26, O103, O111, O145 beads were streaked onto Chromocult® Coliform Agar (1.10426; Merck, Darmstadt, Germany) and EHLy agar. For O26 VTEC detection, the chromogenic medium was replaced by Cefixime Tellurite Rhamnose Mac Conkey Agar (CT – RMAC). RMAC was prepared as follows from Mac Conkey agar base (281810; Difco, Detroit, Mich.) with no lactose: MacConkey agar base (40 g) and rhamnose (10 g) were mixed in 1 liter of distilled water, autoclaved at 121°C for 15 m and then supplemented with Cefixime (0.05 mg/l) and Potassium Tellurite (2.5 mg/l) (CT Selective Supplement, SR 172E; Oxoid). In CT-RMAC, lactose of Mac Conkey agar is replaced by rhamnose because VTEC O26 strains do not ferment the latter carbohydrate, generating colourless colonies easier to select (Hiramatsu *et al.*, 2002).

Chromocult® Coliform agar, EHLy agar and CT-RMAC plates were incubated at 37° C for 24 h. Suspect *E. coli* colonies (dark blue-violet on Chromocult® Coliform agar, smooth and surrounded by a narrow halo of haemolysis on EHLy agar, colourless on CT-RMAC plates) were picked up for further testing. Colonies from EHLy agar and CT-RMAC were tested for indole production and, if positive, for slide agglutination with specific antisera. Colonies from Chromocult® Coliform agar were tested for slide agglutination, without performing the indole-production screening test. Slide agglutination test was performed with serogroup O26, O103, O111, and O145 specific antisera (Statens Serum Institut, Copenhagen, Denmark). Agglutinating colonies were plated on Tryptone Soy Agar (TSA), incubated at 37° C overnight, and pure cultures were biochemically identified as *E. coli* by using the API 20E system (bioMérieux).

#### **Identification of VTEC strains by the Vero Cell Assay (VCA) and PCR analysis**

Presumptive O26, O103, O111, and O145 VTEC strains were tested on Vero Cell monolayers (Caprioli *et al.*, 1992) and VCA positive cultures were analysed for the presence of virulence genes. PCR amplification was performed by using the primer pairs KS7/KS8 for *vt1* gene (Rusmann *et al.*, 1995), GK3/GK4 for *vt2* (Rusmann *et al.*, 1995), and SK1/SK2 for *eae* gene (Karch *et al.*, 1993).

#### **Serotyping of non-O157 VTEC**

Serotyping of O-antigens of non-O157 VTEC isolates was performed by the “Robert Koch Institute” of Berlin, Germany, according to Orskov and Orskov

methods (1984). Typing of H-antigens was performed by the “National *Escherichia coli* Reference Laboratory” of Melbourne University, Australia.

## Results

A total of nine non-O157 VTEC strains were isolated from the faecal matter of 247 slaughtered cattle (3.6%) in the two different sampling periods, as shown in Tables 1 and 2.

The isolates were detected from 7 of 126 dairy cows (5.7%) and from 2 of 121 feedlot cattle (1.6%). Eight of nine strains lacked the *eae* gene for intimate adherence to epithelial cells of large bowel and for A/E lesions, while only the O26 *E. coli* isolate possessed the *eae* gene virulence determinant.

The faecal carriers came from nine farms located in different regions of Italy.

Animal	VCA	Toxin genotype		<i>eae</i> gene	Serotype
		<i>Vtx1</i>	<i>Vtx2</i>		
Dairy cow	+	-	+	-	O91:H-
Dairy cow	+	+	+	-	O109:H-
Dairy cow	+	+	-	-	O116:H21
Feedlot cow	+	+	+	-	ONT
Dairy cow	+	+	+	-	O117:H7
Dairy cow	+	+	-	-	O110:H2
Dairy cow	+	+	+	-	O74:HNT

**Table 1:** Non-O157 VTEC strains isolated from the intestinal content of cattle at slaughter (first sampling period: September 2001 – June 2002)

Animal	VCA	Toxin genotype		<i>eae</i> gene	Serogroup/serotype
		<i>Vtx1</i>	<i>Vtx2</i>		
Dairy cow	+	+	-	+	O26
Feedlot cow	+	-	+	-	O91:H-

**Table 2:** Non-O157 VTEC strains isolated from the intestinal content of cattle at slaughter (second sampling period: October 2003 – September 2004)

## Discussion and conclusions

The two studies on non-O157 VTEC carriage by feedlot cattle and dairy cows reared in Italy showed that different VTEC serotypes were shedding by slaughtered animals, although the prevalence was low (3.6%).

The only VTEC strain responsible for severe human disease, isolated from healthy cattle, belonged to serogroup O26. Nowadays in Italy O26 VTEC isolates represent the major cause of HUS in children (Scavia *et al.*, 2005). Since 1988 to 2004, O26 VTEC strains have been responsible for 21.1% of aetiological identified HUS cases in paediatric patients (EnterNet Italia, 2005). In USA, a twenty-year study that confirmed the importance of non-O157 VTEC strains in human infections, pointed out that, among non-O157 serogroups, the most common were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%) (Brooks *et al.*, 2005). As described by Bettlheim (2003), O26 VTEC should be considered as pathogens for both cattle and humans, being isolated from sick and healthy cattle (ratio 4:3) as well as from sick and healthy people (ratio 76:3). The O26 VTEC serogroup probably occurs exclusively in cattle, their foodstuffs, and humans (Bettleheim, 2003).

Among *eae*-negative VTEC strains, two O91:H- isolates were detected in the intestinal content of healthy dairy cows. VTEC O91:H- , VT2 positive, is responsible for human non-bloody diarrhoea (Pierard *et al.*, 1994; Beutin *et al.*, 2001; Caprioli *et al.*, 2001) and has been isolated from healthy people and cattle (Bettelheim, 2003) as well as from healthy pigs (DesRosiers *et al.*, 2002). Another human pathogenic VTEC isolate, *E. coli* O117:H7, VT1 and VT2 positive, shedded by a dairy cow, is responsible for diarrhoeal disease in humans (Pierard *et al.*, 1990; Pierard *et al.*, 1994).

*E. coli* serotype O116: H21, VT1 positive, detected in a dairy cow, was isolated from healthy cattle in previous studies in USA (Wells *et al.*, 1991) and in Canada (Sandhu *et al.*, 1996) and can be considered distributed all over the world.

Two different analytical procedures were employed to detect VTEC strains. In the previous study, the faecal samples enrichment step was followed by serial dilutions and plating onto EHLy Agar and MacConkey Agar. Therefore, phenotypic characters (enterohaemolysin production and lactose fermentation) were employed as markers for the selection of colonies and further characterization of the isolates. Moreover, as some VTEC strains do not produce the enterohaemolysin (Beutin, *et al.*, 2001; Jenkins *et al.*, 2002), for each sample at least two or three not-haemolytic colonies were selected for confirmation. In this investigation, anyway, no isolate lacking the enterohaemolytic phenotype was recognized as VTEC strain. In the second study, after the enrichment step, the faecal cultures were tested with IMS technique by the use of O26, O103, O111, and O145 antibodies coated beads and thereafter the beads-bacteria complexes were plated onto different selective media. The most useful medium for the isolation of O26 VTEC strains resulted to be the CT-RMAC (Hiramatsu *et al.*, 2002). Surprisingly, in the second study one O91:H- VTEC strain was detected because of its not-specifically coating to O103 Dynabeads®.

On our opinion, the present results could be useful for other non-O157 VTEC investigations based on conventional microbiological methods, because the contemporary employ of both analytical procedures seems to be necessary for the detection of a wider spectrum of VTEC serotypes. After a common enrichment step, the cultures should be both processed by IMS technique and plated onto EHLy agar after serial dilutions, in order to recognize VTEC strains on the basis of the serogroup (IMS) and the enterohaemolysin production (EHLy agar medium). The use of CT-

RMAC is strongly recommended for selecting rhamnose not-fermenting O26 VTEC colonies after O26-IMS technique.

The major problem in detecting non-O157 VTEC is, in fact, that apart from VT1 and/or VT2 production, they are very similar to other commensal *E. coli* strains (Bettelheim, 2003). The only characteristics that could be really discriminating for colonies selection is a decreased ability to ferment carbohydrate-like substances (Bettelheim, 1997), as sorbitol for O157 VTEC and rhamnose for O26 VTEC, and the enterohaemolysin production (Beutin *et al.*, 1989). Anyway, VTEC colonies confirmation is necessary linked to virulence determinants identification, mostly performed by PCR technique.

As there are over 60 VTEC serotypes that have been associated with human illnesses (HC and HUS), any attempt should be made to isolate them from human or animal sources (Bettelheim, 2003). Moreover, as VTEC strains may be often harmless to ruminants, in which they are able to spread from animal to animal, while they are so potent human pathogens, surveillance in the natural host niche (ruminants) and in foodstuffs thereof should be never interrupted.

### Acknowledgements

The authors gratefully acknowledge Dr. Lothar Beutin, Robert Koch Institut, Berlin, and Prof. Karl Bettelheim, National *Escherichia coli* Reference Laboratory, Melbourne University, for VTEC strains serotyping.

We are grateful to Mrs. Ida Poli and Mrs. Giuseppina Trentadue for technical assistance.

### References

- Bettelheim K.A. Biochemical studies on enterohaemorrhagic *Escherichia coli* (EHEC). In: Kay D., Fricker C., Eds. Coliforms and *E. coli*. Problem or Solution. Gateshead, Tyne, and Wear, UK: Royal Society of Chemistry. Ateneum Press, pp. 243-248, 1997.
- Bettelheim K.A. Role of non-O157 VTEC. J. Appl. Microbiol. Symp. Suppl. **88**: 38S-50S, 2000.
- Bettelheim K.A. Non-O157 Verotoxin-producing *Escherichia coli*: a problem, paradox, and paradigm. Exp. Biol. Med. **228**:333-344, 2003.
- Beutin L., Montenegro M. A., Orskov I., Orskov, F., Prada, J., Zimmermann, S. Stephan, R. Close association of Verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J. Clin. Microbiol. **27**: 2559-2564, 1989.
- Beutin L., Zimmermann S. Gleier K. Association between serotypes, virulence markers and disease in a group of 679 Verocytotoxin-producing *Escherichia coli* (VTEC) strains isolated from human patients in Germany (1997-1999). A technical booklet produced for an EU Concerted Action (CT98-3935), pp. 5-11,

ISBN 1 84170 147 5, 2001.

- Bonardi S., Maggi E., Bottarelli A., Pacciarini M.L., Ansuini A., Vellini G., Morabito S., Caprioli A. Isolation of Verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. *Vet. Microbiol.* **67**: 203-211, 1999.
- Bonardi S., Maggi E., Pizzin G., Morabito S., Caprioli A. Faecal carriage of Verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. *Int. J. Food Microbiol.* **66**: 47-53, 2001.
- Bonardi S., Foni E., Brindani F., Bacci C., Chiapponi C., Cavallini P. Detection and characterization of verocytotoxin-producing *Escherichia coli* (VTEC) in cattle at slaughter. *New Microbiologica*, **27**, 255-261, 2004.
- Brooks J.T., Sowers E.G., Wells J.G., Greene K.D., Griffin P.M., Hoekstra R.M., Strockbine N.A. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002.
- Cameron S., Walzer C., Beers M., Rose N., Aneer E. Enterohaemorrhagic *Escherichia coli* outbreak in South Australia associated with consumption of mettwurst. *Comm. Dis. Intell.* **19**, 70-71, 1995.
- Caprioli A., Luzzi I., Rosmini F., Pasquini P., Cirrincione R., Gianviti A., Matteucci M.C. Rizzoni G. Hemolytic-uremic syndrome and Vero cytotoxin-producing *Escherichia coli* infection in Italy. The HUS Italian Study Group. *J. Infect. Dis.* **166**: 154-158, 1992.
- Caprioli A., Luzzi I., Rosmini F., Resti C., Edefonti A., Per fumo F., Farina C., Goglio A., Gianviti, Rizzoni G. Community-wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. *J. Infect. Dis.* **169**, 208-211, 1994.
- Caprioli A., Morabito S., Minelli F., Marziano M.L., Goriotti S., Pinchiorri T., Tozzi A. E. VTEC infections, 1988-2000. *Notiziario dell'Istituto Superiore di Sanità*, **14**, Suppl. 1, 2-3, 2001.
- Conedera G., Marangon S., Chapman P.A., Zuin A., Caprioli A. Atypical strains of verocytotoxin-producing *Escherichia coli* O157 in beef cattle at slaughter in Veneto region. *J. Vet. Med. B* **44**: 301-306, 1997.
- Centers for Disease Control and Prevention. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21 – Helena, Montana, 1994. *Centers for Disease Control and Prevention. Morbid. Mortal. Weekly Rep.* **44**:501-503.
- Centers for Disease Control and Prevention. *Escherichia coli* O111:H8 outbreak among teenage campers - Texas, 1999. *Centers for Disease Control and Prevention. Morbid. Mortal. Weekly Rep.* **49**:321-324.
- DesRosiers A., Fairbrother J.M., Johnson R.P., Desautels C., Letellier A., Quessy S. Phenotypic and genotypic characterization of *Escherichia coli* verotoxin-producing isolates from humans and pigs. *J. Food Prot.*, **64**, 1904-1911.
- ENTER-NET Italia. Sorveglianza delle infezioni da *E. coli* produttori di verocitotossina (VTEC) nella popolazione umana: 1988-2004. (Surveillance of human infection by verocytotoxin-producing *E. coli* (VTEC): 1988-2004). Available from: [www.simi.iss.it/enternet/dati](http://www.simi.iss.it/enternet/dati)
- Goldwater P.N., Bettelheim K.A. An outbreak of haemolytic uremic syndrome due

- to *Escherichia coli* O157:H7: or was it? *Emerg. Infect. Dis.* **2**: 153-154, 1996.
- Hiramatsu R., Matsumoto M., Miwa Y., Suzuki Y., Saito M., Miyazaki Y. Characterization of Shiga-toxin producing *Escherichia coli* O26 strains and establishment of a selective isolation media for these strains. *J. Clin. Microbiol.* **40**: 922-925, 2002.
  - Huppertz H., Busch D., Schmidt H., Aleksic S., Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxins. *J. Pediatr.* **128**: 341-346, 1996.
  - Jenkins C., Pearce M.C., Chart H., Cheasty T., Willshaw G.A., Gunn G.J., Dougan G., Smith H.R., Syngé B.A., Frankel G. An eight-month study of a population of verocytotoxigenic *Escherichia coli* (VTEC) in a Scottish cattle herd. *Appl. Microbiol.* **93**: 944-953, 2002.
  - Karch H., Geitz C., Schmidt H. Increased Incidence of Infections with EHEC O103:H2. *Notiziario dell'Istituto Superiore di Sanità* **10** (3):2, 1997.
  - Kudoh Y., Kai A., Obata H., Kusunoki J., Monma C., Shingaki M., Yanagawa Y., Yamada S., Mtushita S., Itoh T., Ohta K. Epidemiological Surveys on Verocytotoxin-Producing *Escherichia coli* Infections in Japan. In: Karmali M.A., Goglio A.G., Eds. *Recent Advances in Verocytotoxin-Producing Escherichia coli Infections* (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp. 53-56, 1994.
  - Lopez E.L., Diaz M., Grinstein S., Devoto S., Mendila-hatzu F., Murray B.E. *et al.* Hemolytic uremic syndrome and diarrhoea in Argentine children: the role of Shiga-like toxins. *J. Infect. Dis.* **160**: 469-475, 1989.
  - Mariani-Kurkdjian P., Denamur E., Milon A., Picard B., Cave H., Lambert-Zechovsky N., Loirat C., Gouillet P., Sansonetti P., Elion J. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of haemolytic uremic syndrome in France. *J. Clin. Microbiol.* **31**: 296-301, 1993.
  - Morabito S., Karch H., Mariani-Kurkdjian P., Schmidt H., Minelli F., Bingen E., Caprioli A. Enterohemorrhagic, Shiga-toxin producing *Escherichia coli* O111:H2 associated with an outbreak of haemolytic-uremic syndrome. *J. Clin. Microbiol.* **34**: 840-842, 1998.
  - Orskov F., Orskov I. (1984). Serotyping of *Escherichia coli*. *Methods in Microbiology* Academic Press, London. **14**, 43-112.
  - Pierard D., Van Etterijck R., Breynaert J., Moriau L., Lauwers S. *Escherichia coli* in faeces in Belgium. *Eur. J. Clin. Microbiol. Infect. Dis.*, **9** (3): 198-201, 1990.
  - Piérard D., Van Damme L., Stevens D., Moriau L., Lauwers S. Three years screening for VTEC in human stools in Brussels. In: Karmali M.A., Goglio A.G., Eds. *Recent Advances in Verocytotoxin-Producing Escherichia coli Infections* (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp. 33-36, 1994.
  - Riley L.W., Remis R.S., Helgerson S.D., McGee H.B., Well J.G., Davis B.R., Hebert R.J., Olcott E.S., Johnson L.M., Hargrett N.T., Blake P.A., Cohen M.L. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Engl. J. Med.* **308**: 681-685, 1983.
  - Sandhu K.S., Clarke R.C., McFadden K., Brouwer A., Looie M., Wilson J., Lior

- H., Gyles C.L.. Prevalence of the *eaeA* gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. *Epidemiol. Infect.*, **116**, 1-7, 1996.
- Scavia G., Botta A., Ciofi degli Atti M.L., Di Fluri G., Ferretti A., Galero G., Marziano M.L., Merla R., Minelli F., Montini G., Pecoraro C., Pizzuti R., Tozzi A.E., Trani A.M., Caprioli A. Episodio epidemico di Sindrome Emolitico Uremica (SEU) associata a infezione da *E. coli* O26, in provincia di Salerno. V Workshop Nazionale Enter-net Italia, Sistema di sorveglianza delle infezioni enteriche. Sorveglianza e prevenzione delle infezioni gastroenteriche. Istituto Superiore di Sanità, Roma, 1-2 Dicembre 2005, pp. 73.
  - Tarr P.I., Fouser L.S., Stapleton A.E., Wilson R.A., Kim H.H., Vary J.C., Clausen C.R. Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2. *N. Engl. J. Med.* **335**: 635-638, 1996.
  - Wells J.G., Shipman L.D., Greene K.D., Sower E.G., Green J.H., Cameron D.N., Downes F.P., Martin M.L., Griffin P.M., Ostroff S.M. *et al.* Isolation of *Escherichia coli* O157:H7 and other Shiga-liketoxin-producing *E. coli* from dairy cattle. *J. Clin. Microbiol.*, **29**, 985-989, 1991.

## Riassunto

Gli stipiti di *Escherichia coli* verocitotossici (VTEC) rappresentano un problema per la salute pubblica, in quanto responsabili di gravi forme cliniche nell'uomo, quali la sindrome emolitico-uremica e la colite emorragica, oltre ad episodi di diarrea non ematica. Oltre ad *E. coli* O157, che vede nella specie bovina un importante reservoir, le infezioni umane sono sostenute da altri sierogruppi VTEC, quali O26, O103, O111, O145. Allo scopo di valutare l'importanza dei bovini quali portatori intestinali di *E. coli* produttori di verocitotossine appartenenti a sierogruppi diversi da O157, 247 animali sono stati esaminati al termine delle operazioni di macellazione. Dal contenuto cecale di 8 soggetti (3,2%) sono stati isolati ceppi di *E. coli* produttori di verocitotossine appartenenti ai sierotipi O91:H-, O109:H-, O116:H21, O117:H7, O110:H2, O74:HNT, ma sprovvisti del gene *eae* per l'adesione alle cellule dell'epitelio intestinale. Da un solo animale (0,4%) è stato isolato un ceppo di *E. coli* O26 provvisto dei geni *vtx1* ed *eae*, quindi uno stipite altamente patogeno per l'uomo.

**Parole chiave:** *Escherichia coli*, verocitotossine, VTEC, bovini

## Summary

Verocytotoxin-producing *Escherichia coli* (VTEC) strains have emerged as pathogens that can cause food poisoning and severe and potentially fatal illnesses. They are a major cause of gastroenteritis that may be complicated by hemorrhagic colitis or the haemolytic-uremic syndrome. Human infections are caused by *E. coli*

serogroup O157, frequently shedded by cattle, and by other VTEC serogroups such as O26, O103, O111, O145. The aim of this study was to evaluate the carriage rate of non-O157 VTEC strains by cattle at slaughter. A total of 247 cattle were randomly selected at slaughter and from the caecal material of 8 animals (3.2%) were detected the following VTEC serotypes lacking the *eae* gene: O91:H-, O109:H-, O116:H21, O117:H7, O110:H2, O74:HNT. *E. coli* O26, positive for *vtx1* and *eae* genes, was isolated from only one slaughtered animal (0.4%).

**Key words:** *Escherichia coli*, verocytotoxins, VTEC, cattle