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SHORT REPORT
Prevalence of *Escherichia coli* O157 in lambs at slaughter in Rome, central Italy

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SUMMARY

A study on the prevalence of the faecal carriage of *Escherichia coli* O157 in lambs was performed in the major slaughterhouse in Rome, central Italy, during 2002. A total of 643 animals, consisting of 378 weaned and 265 suckling lambs, were assayed for the presence of *E. coli* O157. Five O157-agglutinating *E. coli* strains were isolated (0·8%, 95% CI 0·3–1·9). Only one was positive to PCR specific for the *eae* gene and produced verocytotoxin VT2, with a VTEC O157 overall prevalence of 0·2% (95% CI 0·0–1·0), whereas one strain possessed the *eae* gene only. All the other isolates were negative for the presence of all the virulence genes considered. The animals were either from local farms or imported from Eastern Europe. The results suggest an age-specific difference since the microorganism was isolated only from 0·3% (95% CI 0·0–1·7) of weaned lambs, while all samples from suckling lambs tested negative. From this study, the overall risk of human exposure to pathogenic *E. coli* O157 from lamb meat consumption derived from the major slaughterhouse in Rome can be considered reasonably low, particularly when suckling lamb meat is considered.

Escherichia coli O157 is a human pathogen whose infection can cause life-threatening diseases such as haemorrhagic colitis and haemolytic–uraemic syndrome [1]. The pathogenicity of *E. coli* O157 mainly relies upon the production of verocytotoxins (VT) and the capability of colonizing the intestinal mucosa of the host with a characteristic attaching and effacing (A/E) mechanism of adhesion [2]. Due to VT production, this organism is also referred to as verocytotoxin-producing *E. coli* O157 (VTEC O157).

Outbreaks involving large numbers of cases have often been linked to the consumption of undercooked minced beef [3, 4] or cross-contaminated cooked meats [5].

In Italy, between 1988 and 2000, 250 cases of VTEC infection have been reported. Most of these were sporadic cases, but two outbreaks have been recognized. Microbiological results, showed that the most frequent VTEC serogroup was O157, although it was confirmed that other serogroups such as O26, O111, O145, O103 and O? also played an important role in the epidemiology of VTEC infection in Italy [6].

Many studies have indicated that cattle represent the main reservoir of VTEC O157 [7, 8]. Other ruminants and domestic animals harbour these bacteria, in particular VTEC O157 has been detected both in meat and milk from sheep and goats [9, 10] and sheep

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is now regarded as a possible reservoir for human infection [11].

In Italy 70–80 000 tons of sheep and goat meat is consumed every year, of which 55–65% comes from national flocks [12]. Lamb is one of the most popular types of sheep meat and can be divided in two categories: suckling lambs, 20- to 30-day-old animals weighing between 7 and 10 kg and exclusively milk-fed, and weaned lambs, usually <6 months of age and with a polygastric digestive physiology.

The aim of this study was to estimate the prevalence of faecal carriage of *E. coli* O157 by lambs intended for slaughter in the principal public slaughterhouse of Rome, Italy, and locally distributed for consumption.

Between March and December 2002, a total of 643 faecal samples were collected at the major abattoir of Rome, Lazio region, central Italy. Two hundred and sixty-five samples were collected from suckling lambs and 378 from weaned lambs. For prevalence estimates, a simple random sampling technique was used, with a desired sample size calculated based on a 4% expected prevalence, a 95% confidence level and 1·5% desired absolute precision [13], stratified by age class (suckling or weaned lambs) and origin (local or foreign origin). Samples from weaned lambs were distributed in the four seasons (March, July, October, December), while samples from suckling lambs were collected in March and December. Weaned lambs were from a different origin: 252 animals (67%) came from Eastern Europe (Romania, Slovakia, Hungary and Poland) and 126 (33%) were from central Italy. Suckling lambs were exclusively of local origin (central Italy). The abattoir had throughput ranging from 120 000 to 150 000 lambs slaughtered per year. Faeces were collected from the rectum immediately after slaughter. All specimens were cooled at 4 °C and transported to the laboratory within the day of collection. Most samples were processed within 2 days of collection or stored in liquid nitrogen vapour and processed within 1 week. All samples were examined for the presence of *E. coli* O157 using an immunomagnetic separation (IMS) technique. Specimens were screened in pools of five portions each made from 1 g of faeces and enriched in 45 ml of buffered peptone water (BPW), pre-warmed at 37 °C and incubated at 41·5 °C for 18 h. After enrichment, IMS was performed by the use of the Dynabeads® anti-*E. coli* O157 (Dynal, Oslo, Norway) and the detection of *E. coli* O157 was performed with sorbitol MacConkey agar plates supplemented with 0·05 mg/l cefixime and 2·5 mg/l tellurite, following IMS procedures. Presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were subjected to slide agglutination with latex particles sensitized with *E. coli* O157 antibodies. The O157-agglutinating cultures were confirmed biochemically as *E. coli* using the API 20E system (bioMérieux, Marcy l'Etoile, France).

VT production was determined by the Vero cell assay as described by Caprioli et al. [14]. Polymerase chain reaction (PCR) was used to assess the presence of virulence genes. Primer pairs KS7–KS8 and GK3–GK4 were used for for *vt1* and *vt2* respectively [15]. The intimin-coding *eae* gene was detected according to Oswald et al. [16]. The enterohaemolysin coding gene *E-hly*, was detected as described elsewhere [17]. To assess the genetic relatedness among isolates, pulsed-field gel electrophoresis (PFGE) analysis of *Xba*I restriction patterns of chromosomal DNA was performed as previously described [18].

The PFGE profiles were analysed by the BioNumerics Software (Applied Maths, Sint-Martens-Latem, Belgium) using the UPGMA algorithm with Dice coefficient.

E. coli O157-agglutinating strains have been isolated from the intestinal contents of five out of 643 animals (0·8%, 95% CI 0·3–1·9); however, only one isolate (no. 10140) showed a cytopathic effect (CPE) on Vero cells and possessed the genes coding for the intimin (*eae*), the VT2 and enterohaemolysin (*E-hly*) as assessed by PCR analysis (0·2%, 95% CI 0·0–1·0). Another strain was positive for the presence of the *eae* gene, but did not produce CPE on Vero cells and tested negative to PCR for the VT coding genes (no. 10550/16). The remaining three (nos. 10550/1, 15830, 18526) were negative for all the virulence genes assayed. The age-specific prevalence is shown in the Table. All the positive animals came from Eastern Europe. With the exception of the VTEC O157 strain (no. 10140), all the *E. coli* O157-agglutinating isolates were motile but negative in slide agglutination assay with the H7 antiserum.

In this study, the overall prevalence of VTEC O157 was 0·2% (95% CI 0·0–1·0). This figure is consistent with those described in other surveys conducted in several European countries, in fact, prevalence of VTEC O157 ranging from 0 to 4% have been described in both lambs and adult sheep. In the United Kingdom, VTEC O157:H7 has been detected in 1·7% (71/4171) of the faecal samples [19]. In The Netherlands, VTEC O157 has been isolated from 4% of ewes and 4% of lambs [20], while in

Table. Escherichia coli *O157 estimated age-specific prevalence in lambs from a slaughterhouse in Rome, 2002*

	Overall prevalence (95% CI)	Prevalence VT +ve, eae +ve	Prevalence eae +ve, VT −ve	Prevalence eae −ve, VT −ve
Suckling lambs ($n=265$)	0 (Upper limit 1·4%)	0 (Upper limit 1·4%)	0 (Upper limit 1·4%)	0 (Upper limit 1·4%)
Weaned lambs ($n=378$)	1·3% (0·5–3·2%)	0·3% (0·0–1·7%)	0·3% (0·0–1·7%)	0·8 (0·2–2·5%)

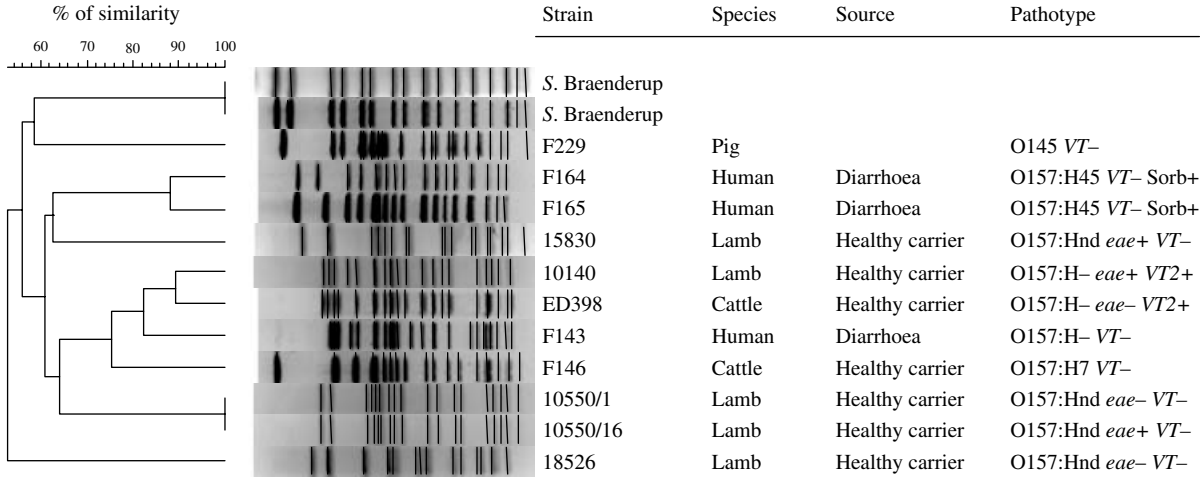

Fig. PFGE analysis of the *E. coli* O157 lamb isolates obtained from this study, compared with isolates from other sources. Two *Salmonella* Braenderup and one *E. coli* O145 strains have been included as standards for normalization of runs. The analysis has been performed using the UPGMA algorithm corrected by the Dice coefficient. Hnd indicates that the strain is motile but negative in the H7 slide agglutination assay.

Norway no VTEC O157 has been isolated from 364 sheep [21]. On the contrary, in the United States the presence of VTEC O157 in 31% of 35 healthy ewes sampled in a flock has been described [11, 22]. These marked differences between the prevalence of VTEC O157 faecal carriage in ovine species in European *vs.* North American countries may be due to differences in the sheep husbandry practices.

In our survey, the isolates were obtained from samples collected between March and June, with the only VTEC O157 strain isolated from a sample collected in March, but the low number of isolates did not allow assessment of any seasonality in the carriage of the microorganism. We did not isolate *E. coli* O157 from suckling lambs, although it should be noted that the sampling period for this age class excluded summer as in this season suckling lambs are unavailable for slaughter.

The PFGE profiles of the *E. coli* O157 isolates are shown in the Figure. Comparison of the restriction fragment length polymorphism patterns showed that the five ovine isolates belonged to four different profiles with a 65% mean homology. The VTEC O157:H^- strain 10140 has the potential to be pathogenic to humans, as it is closely related (about 90% homology) to another VTEC O157:H^- isolated from cattle faeces in a previous study (strain ED 398) and also shares ~80% homology with a VT-negative O157:H^- strain isolated from a human case of diarrhoea.

Interestingly, the *E. coli* O157 isolates described in this study showed different patterns of virulence determinants, in particular most of them were negative for the presence of the *eae* gene which encodes the key factor for the A/E mechanism of adhesion to the enterocyte [23], and only one strain possessed both the VT [24] and the *E-hly* encoding genes which represent the main virulence determinants of enterohaemorrhagic (EHEC) *E. coli* O157 strains [2].

In the typical EHEC O157 isolates, these virulence genes are all located on mobile genetic elements (MGEs). In fact, the *eae* gene is harboured by a pathogenicity island (PAI) termed locus for enterocyte effacement (LEE) [23], the VT-coding genes are transduced by lambdoid phages [24] and *E-hly* is part of an operon on a large virulence plasmid

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[17]. All these elements are unstable and undergo a selective pressure that keeps them to the bacterial cell [25]. The presence of *E. coli* O157 isolates lacking all or part of the virulence gene set, may indicate that in the lamb gut they are subjected to negative selection that forces them to lose these MGEs. This hypothesis is strengthened by the observation that two VT-negative strains isolated in this survey shared indistinguishable PFGE profiles but one of them was negative in PCR specific for the *eae* gene (Fig.), although it cannot be excluded that the negative result of the eae-specific PCR could be due to minor variations in the gene sequence leading to a mis-priming.

In conclusion, the estimated risk of *E. coli* VTEC O157 carriage in lamb categories included in this study may be considered low. Therefore, the risk of human exposure to VTEC O157 due to the consumption of the lamb meat produced in the slaughterhouse in Rome, should be reasonably low, particularly when suckling lamb meat is considered. The lack of virulence determinants in ovine *E. coli* O157 and the small amount of products containing minced lamb/mutton consumed, both in Italy and in Europe, may explain the lack of human *E. coli* O157 foodborne cases attributed to sheep, although in some countries sheep proved to be associated with outbreaks of *E. coli* O157 through direct contact and environmental contamination. This may be also because of the differences in foodborne infection pathways, resulting in lower risk for sheep to contaminate food products: slaughtering techniques and the solid nature of sheep faeces may result in a lower probability of carcass contamination [26]. Further investigations are needed to clarify this matter.

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DECLARATION OF INTEREST

None.

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