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SHORT REPORT
A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami

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SUMMARY

A family outbreak of *Escherichia coli* O157 infection was microbiologically associated with consumption of dry-fermented salami made with pork meat only and produced in a local plant. *E. coli* O157 strains isolated from a wife and husband, both hospitalized with bloody diarrhoea, and from the salami carried *vt1*, *vt2* and *eae* genes and shared the same PFGE pattern. The food vehicle implicated in this outbreak is unusual because of both the animal species from which it originates and the fermentation and drying steps of the manufacturing process. This could be the first report of an outbreak associated with a product containing pork meat only. Even though sources of contamination other than pork meat could not be excluded, pork products should not be neglected in *E. coli* O157 outbreak investigations.

Verocytotoxin (VT)-producing *Escherichia coli* O157 (VTEC O157) is a zoonotic pathogen associated with a broad spectrum of human diseases, including haemorrhagic colitis and haemolytic–uraemic syndrome (HUS). The infection is mainly foodborne, but it can also be acquired by person-to-person spread or direct contact with animals or a contaminated environment [1]. Ruminants, and in particular cattle, are considered to be the principal reservoir of VTEC O157, and food of bovine origin such as beef and dairy products has often been associated with outbreaks [1]. Moreover, an increasing number of episodes have been associated with consumption of fruits and vegetables fertilized with ruminants' manure or contaminated during harvesting or processing [2]. This report describes a family outbreak of *E. coli* O157 infection caused by an unusual food vehicle: a dry-fermented traditional salami made with pork meat.

On 24 January 2004, a husband (aged 64) and wife (aged 56) living in a small town in the Veneto region, northeast Italy, attended the emergency room of the local hospital with bloody diarrhoea, severe abdominal pain and nausea and were admitted to the surgery unit. The third family member, their 29-year-old daughter, had mild diarrhoea and did not seek medical attention. Both patients underwent colonoscopy, which revealed in both an oedematous and hyperaemic colonic mucosa. Both patients received oral rehydration, recovered spontaneously and were discharged on 6 February without adverse consequences.

Stool cultures performed on 26 January by direct plating onto sorbitol–MacConkey agar (SMAC;

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Oxoid, Garbagnate Milanese (MI), Italy) yielded sorbitol non-fermenting (SNF) *E. coli* O157 from both patients. The local health authority was informed and an epidemiological investigation began. The family lived in an urban area and did not keep pets. They denied any recent contact with farm animals or a rural environment. Questions on foods eaten in common excluded raw or undercooked beef or unpasteurized milk and indicated that all three family members had consumed a dry-fermented traditional salami on day 6 and day 4 before hospitalization. The salami had been purchased entire and sliced just before consumption. Part of the unsliced salami was kept in the family house fridge; it was collected for laboratory investigations which started on 12 February.

The presence of *E. coli* O157 was investigated by using a sensitive immuno-magnetic separation (IMS) method, according to the ISO 16654:2001 procedure. Briefly, 50-g and 25-g aliquots of the deep parts of the salami were aseptically collected after removing the casing and pre-enriched for 6 h and 24 h at 41·5 °C in modified tryptone soya broth supplemented with 20 mg/l novobiocin (m-TSB + N). The IMS was performed using Dynabeads anti-*E. coli* O157 (Dynal, Oslo, Norway) according to the manufacturer's instructions. After the final step, the magnetic beads were inoculated onto SMAC (Oxoid) and SMAC supplemented with 0·05 mg/l cefixime and 2·5 mg/l tellurite (CT-SMAC, Oxoid). A few colonies of SNF *E. coli* O157 were isolated from the CT-SMAC plate seeded with the 24-h enrichment culture of the 50-g sample, but not from the other cultures and from the 25-g sample, suggesting a very low concentration of the organism in the product.

The human and food isolates of *E. coli* O157 were characterized as previously described [3]. Briefly, the O157 serogroup was tested by latex agglutination with O157 antiserum and confirmed by tube agglutination of heat-treated cultures; H7 antigen was tested by latex agglutination with H7 antiserum and evaluated after up to 10 serial passages through motility agar medium. VT production was assessed by the Vero cell assay and further characterization was performed by PCR amplification of VT and *eae* genes. Finally, strains were compared by pulsed field gel electrophoresis (PFGE) as previously described [3], using 100 U of XbaI for overnight restriction digestion. The two human strains and the isolate from salami were non-motile *E. coli* O157, carried *vt1*, *vt2* and *eae* genes and shared the same PFGE profile, thus confirming the salami as the source of the outbreak.

The meat composition of the product was verified by an agar-immuno-diffusion assay using anti-species sera, which confirmed the presence of pork meat and excluded bovine, ovine and chicken meats. The water activity (a_w) of the salami was 0·90. The product had been manufactured by a non-industrial, local plant which was certified according to European Community requirements. The plant processed only pork meat and produced ~1500 kg of different traditional products per week. The implicated type of salami is usually made from coarsely minced pork meat with addition of salt (2·7%), sodium or potassium nitrate, sucrose, garlic, spices, stuffed into a natural casing (bovine bowel maintained in 100% salt 'upper brine') dried for 6 days at 18–20 °C and then seasoned at 16 °C and 82% humidity for around a further 24 days before distribution. The fat content was 29–30% and pH at time of distribution was ~5·5. The product can be consumed either raw or, less frequently, after cooking.

The contaminated salami was part of a 600-piece lot produced at the beginning of December 2003, using pork meat and trim coming from a cutting operation located in a neighbouring region and obtained from carcasses of different farms. The natural casing was imported from Brazil, with the supplier's information that the bovine gut was washed and scraped immediately after slaughtering, and kept in upper brine for at least 30 days before distribution. Before stuffing the casing was carefully washed inside and outside at the local plant. The salami was purchased by the family at the retail level and consumed raw when it was at around the 50th day of seasoning. An attempt to recall the product was performed in February but all the pieces of the same lot had already been sold. No samples of the spices and the natural casing used to produce the lot of salami were available for testing.

To ascertain the existence of additional cases, the clinical microbiology laboratories in the Veneto region were asked to report any isolate of *E. coli* O157 in the period between December 2003 and February 2004. No other reports of *E. coli* O157 infection were obtained. This could be explained by the very low concentration of the organism in the salami or possibly by a non-homogeneous distribution in the ground pork used to prepare the lot of product. It was also possible that in a few cases the salami could have been cooked. Specific culture for *E. coli* O157 is not routinely performed in Italy, and other small episodes of less severe intestinal illness may have passed unnoticed.

This outbreak is unusual because of the nature of the food item implicated. Indeed, pork dry-fermented salami is usually not included among the potential vehicles of VTEC O157 infection because of the animal species from which it originates and the fermentation and drying steps of the manufacturing processes.

Dry-fermented sausages have traditionally been considered safe due to the low pH, low water activity and high salinity conditions. However, several studies have shown that *E. coli* O157 is highly tolerant to acidic conditions and can survive many of the typical dry fermentation processing conditions [4–6]. In this case, it should be noted that the contaminated product was consumed $\sim$50 days after production and was still positive for VTEC O157 when tested after a further 18 days, having reached a relatively low level of a_w. Due to the very low infective dose and high fat content of the product any remaining *E. coli* in a ready-to-eat product has the potential to cause illness. This episode confirms that concern, as the *E. coli* O157 load was very low and only the use of the sensitive IMS procedure on a 50-g sample allowed us to detect the organism.

Dry-fermented sausages have previously been associated with outbreaks of VTEC O157 infection in other countries [7–9]. However, the implicated products were generally made from a mixture of raw pork and beef, or their composition was not specified. To the best of our knowledge, this is the first report of an outbreak associated with a product containing pork meat only and in which the source of VTEC O157 was most likely to have been the pork meat. Unfortunately we were not able to trace back the pork meat and trim to the farm of origin to verify the hypothesis.

Pigs are not considered to be a major source of VTEC O157 and other VTEC-associated human infections [10]. However, VTEC O157 has been isolated from pigs both at slaughter and at farm level in European countries [11–13], Japan [14] and the United States [15], even though the reported rates of faecal carriage were usually low. On the other hand, we cannot exclude with certainty other possible sources, such as the natural casing and the spices used to prepare the lot of salami, as well as the persons involved in the manufacture.

The casing was of bovine origin and could be considered a risk for VTEC O157. However, its treatment makes the presence of microbial contaminants unlikely. Assuming an occasional contamination due to the casing, this would have happened during the stuffing step, possibly involving just the limited quantity of product in contact with the contaminated casing.

To the best of our knowledge, contamination of spices with *E. coli* O157 has not been reported. However, the presence of other foodborne pathogens such as *Salmonella* [16–18] and enterotoxigenic *Clostridium perfringens* [19] in spices is well described and this possibility cannot be ruled out.

The food handlers were an unlikely source of *E. coli* O157, as the plant followed an HACCP programme and all the workers denied having had recent episodes of gastrointestinal illness.

In conclusion, this episode indicates that pork products, even if dry fermented through a rather long seasoning period, can be implicated as a source of VTEC O157 infections and should not be neglected in outbreak investigations. Studies on the presence of *E. coli* O157 in either pork trim or natural casings of bovine origin would also be worthwhile, as well as the determination of the survival of this organism throughout the steps of processing and seasoning of traditional pork salami.

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DECLARATION OF INTEREST

None.

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