

Prevalence of *Escherichia coli* O157:H7 in industrial minced beef

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Aims: The lack of baseline data on the prevalence of *Escherichia coli* O157:H7 in retail minced beef in France prompted this survey of industrial minced beef production.

Methods and Results: An automated enzyme-linked fluorescence immunoassay (ELFA), the VIDAS *E. coli* O157 method, was used to detect *E. coli* O157 in industrial minced beef samples. Confirmation of samples positive according to the ELFA was performed using an automated immunoconcentration (ICE) system, VIDAS ICE, which allows the selective capture and release of target organisms. The ICE was followed by culture on cefixime tellurite sorbitol MacConkey agar and a chromogenic medium, O157:H7 ID. Of the 3450 minced beef samples tested, 175 samples were positive with the ELFA method and, of these, four were confirmed by the ICE method. They were identified as sorbitol-negative, O157-positive, H7-positive, mobile, verotoxin-producing *E. coli*.

Conclusions: The prevalence of *E. coli* O157:H7 in industrial French minced beef was 0.12%, consistent with many other reports.

Significance and Impact of the Study: The low infective dose of *E. coli* O157:H7 presents a major threat. The main means of combating this organism are thermal destruction and good food hygiene covering activities on-farm, in the abattoir and in minced beef industries.

INTRODUCTION

Enterohaemorrhagic strains of *Escherichia coli* are recognized as the primary cause of haemorrhagic diarrhoea and haemolytic uraemic syndrome. Important determinants of disease are two toxins referred to as Shiga-like toxins or verotoxins (Stx1 and Stx2; Karmali 1989). Several serotypes of *E. coli* have been shown to produce one or both of these toxins, but bloody diarrhoeal disease caused by serotypes of verotoxin (VT)-producing *E. coli* other than O157:H7 is not uncommon and outbreaks or cases of haemolytic uraemic syndrome due to these other serotypes have been reported in the USA (Park *et al.* 1996), Italy (Caprioli *et al.* 1994) and Australia (Paton *et al.* 1996). Most outbreaks caused by *E. coli* O157:H7 have been food or water related (Griffin and Tauxe 1991; Swerdlow *et al.* 1992). A common vehicle of

infection is undercooked ground beef burgers (Paton and Paton 1998). Raw milk, cold sandwiches, vegetables and water have also been implicated as sources of some outbreaks. Cattle are consistently found to be a reservoir for this organism in the environment. However, isolation from ground beef is uncommon (Borczyk *et al.* 1987; Chapman *et al.* 1993; Okrend *et al.* 1990). These organisms may be present in low numbers in implicated foods containing high levels of competing microflora (Willshaw *et al.* 1993). Two outbreaks were unusual in that they were both linked to the consumption of low pH foods, which have traditionally been considered safe: an outbreak in Massachusetts was associated with drinking one brand of apple cider (Besser *et al.* 1993) and the other outbreak with ingestion of mayonnaise-containing food from a restaurant chain in Oregon (Keene *et al.* 1993). In both cases, laboratory experiments showed that *E. coli* O157:H7, while dying rapidly in these acid foods at room temperature, survived for weeks at refrigeration temperature (Weagant *et al.* 1994).

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Food industry and public health microbiologists need reliable methods to screen high-risk foods for *E. coli* O157:H7. Immunomagnetic separation (IMS) techniques result in greater sensitivity, specificity and rapidity of screening (Okrend *et al.* 1992; Weagant *et al.* 1995). This method has been used in conjunction with the sorbitol MacConkey agar (SMAC) developed by March and Ratman (1989) and a modification of this medium containing cefixime and potassium tellurite (CT-SMAC), which is more selective and facilitates the recognition of *E. coli* O157 colonies (Zadik *et al.* 1993).

The authors have previously evaluated the VIDAS methodology for the detection of *E. coli* O157 in food samples (Vernozy-Rozand *et al.* 1998).

Although VIDAS seemed reliable for screening naturally contaminated food samples for the presence of *E. coli* O157, consistently detecting contamination at 8 cfu 25 g⁻¹ in 496 retail food samples, none were positive for *E. coli* O157:H7.

The present study is a much larger epidemiological study covering more than 3000 French minced beef samples.

MATERIALS AND METHODS

Collection of minced beef samples

A total of 3450 minced beef samples (200 g) were obtained from three different French minced beef industries. Upon receipt at the laboratory, the samples were analysed immediately or held at 4°C for no longer than 48 h before analysis.

The method used for the detection of *E. coli* O157:H7 is validated Association Françoise de Normalisation (AFNOR) in France (bio 12:08–07/00).

Culture media

The enrichment medium was modified tryptone soy broth (MTSB) containing novobiocin (20 mg l⁻¹). The second enrichment broth used in the VIDAS detection procedure was MacConkey broth (51015; bioMérieux, Marcy l'Etoile, France) containing cefixime (0.05 mg l⁻¹) and potassium tellurite (2.5 mg l⁻¹; CT-MCB). The subculturing medium was sorbitol MacConkey agar (CM813; Oxoid, Basingstoke, UK) containing cefixime (0.05 mg l⁻¹) and potassium tellurite (2.5 mg l⁻¹; CT-SMAC) and a chromogenic medium, O157:H7 ID (bioMérieux).

VIDAS methodology

VIDAS ECO. VIDAS (bioMérieux) uses two disposable units: a pipette-like solid phase receptacle (SPR) for the immunocapture of O157 bacteria from enriched food samples and a plastic strip containing wells for samples and preloaded with wash solutions, conjugate (antibody conju-

gated to alkaline phosphatase) and substrate (4-methylumbelliferyl phosphate) solutions.

In a sandwich reaction, the bound anti-*E. coli* O157 antibody in the SPR captures the bacterial antigen, which is detected by a second antibacterial antibody conjugated to alkaline phosphatase. The bound enzyme catalyses cleavage of the substrate to yield a fluorescent product, which is measured and expressed as a relative fluorescence value. The VIDAS instrument performs reactions and takes readings automatically.

According to the AFNOR-validated method, a 25-g portion of the 200-g food sample was added to 225 ml MTSB containing novobiocin in a stomacher bag with a nylon filter and homogenized using a stomacher. These homogenates were placed in an incubator at 42°C for 6 h; 1 ml of the 6-h enrichment broth was added to a tube containing 9 ml CT-MCB and the tube then incubated at 37°C for 18–24 h and tested by the VIDAS *E. coli* O157 method.

Boiled samples (15 min at 100°C), negative and positive controls were loaded into the sample wells of the strips provided. The strips were placed in the instrument and results were available in 45 min.

VIDAS immunoconcentration. Confirmation of the samples which were positive according to the enzyme-linked fluorescence immunoassay (ELFA) was performed by an automated immunoconcentration (ICE) system (VIDAS ICE) which allows the selective capture and release of target organisms and uses antibodies different from that employed in the screening assay. A 0.5-ml sample of the CT-MCB enrichment broth was used for the ICE, which takes about 40 min to complete. At the end of the ICE, 50 µl of the released suspension were spread on CT-SMAC and O157:H7 ID for incubation at 37°C for 18–24 h. Four to six non-sorbitol-fermenting and sorbitol-fermenting colonies or green colonies on O157:H7 were taken through the confirmation procedures.

Confirmation, characterization and pulsed-field gel electrophoresis of isolates

Confirmation, characterization and pulsed-field gel electrophoresis of isolates were performed according to the method described by Vernozy-Rozand *et al.* (1998) and Bouvet *et al.* (2001).

RESULTS AND DISCUSSION

Screening for *Escherichia coli* O157:H7

Of the 3450 minced beef samples examined, 175 (5.07%) gave positive values with the VIDAS ECO. Of these, only

four samples were confirmed after ICE and proved to contain *E. coli* O157:H7 strains. The other 171 samples contained *Citrobacter freundii* (32 of 171) or sorbitol-fermenting *E. coli* O157 non-H7 strains (139 of 171). It is noteworthy that the ELFA screening method used in this study was only directed at the O157 antigen. Drawbacks of such an ELISA method, like many other commercialized methods, are the false-positive results due to bacteria that exhibit cross reactivity with antibodies against the O157 antigen, e.g. *Cit. freundii*, *Salmonella* group N, *Brucella abortus*, *Br. melitensis*, *Yersinia enterocolitica* serogroup O:9 and *Pseudomonas maltophilia* 555 (Di Fabio *et al.* 1987; Caroff *et al.* 1984). False-positive results due to these bacteria can be suppressed by the use of subculture media such as very specific chromogenic media or those containing cefixime and tellurite because the minimal inhibitory concentrations have been shown to be higher for *E. coli* O157:H7 than for other *E. coli* strains and for many non-sorbitol-fermenting enteric bacteria, including *Aeromonas* spp., *Plesiomonas* spp., *Morganella morganii*, *Providencia* spp., and *Hafnia alvei* (Weagant *et al.* 1995).

Silveira *et al.* (1999) have noted a high level of false presumptive results (positive agglutination with the O157 antiserum) using the 3M immunoassay kit (HEC Test Kit 6477, Minneapolis, MN, USA). The most frequently isolated enterobacteria were *Enterobacter* sp. (33%, especially *Enterobacter cloacae*), *Klebsiella* sp. (22.5%, especially *Klebsiella pneumoniae* and *Kl. oxytoca*), *Citrobacter* sp. (5%), *Pantoea agglomerans* (3.7%), *Kluyvera ascorbata* (2.5%), *E. hermannii*, *E. fergusonii* (2.5%) and unidentified enterobacteria (6.3%).

The prevalence of *E. coli* O157:H7 in industrial French minced beef was 0.12% (four of 3450). This low percentage is consistent with other reports in which *E. coli* O157:H7 occurred at low frequency in meat (Lindqvist *et al.* 1998; Little and de Louvois 1998). Tarr *et al.* (1999) tested for *E. coli* O157:H7 systematically in 1400 samples of retail ground beef in Seattle (USA) in a 1-year prospective study. Non-sorbitol-fermenting, lactose-fermenting, indole-positive colonies isolated after enrichment culture were probed for the presence of Shiga toxin genes. Totals of 67 040 non-sorbitol-fermenting and 66 705 sorbitol-fermenting colonies were characterized but *E. coli* O157:H7 was not identified. These data demonstrated that retail ground beef in Seattle was neither frequently nor heavily contaminated with *E. coli* O157:H7.

Although beef products have been widely implicated as vehicles of *E. coli* O157:H7 infection, studies world-wide have either failed to find the organism (Atalla *et al.* 2000; Brooks *et al.* 2001; Fantelli and Stephan 2001; Silveira *et al.* 1999; Tarr *et al.* 1999) or have reported a very low prevalence in minced beef.

However, Chapman *et al.* (2001) found *E. coli* O157 in 0.44% of all beef and lamb samples tested, a higher

prevalence than that reported in the above studies. Several factors may have influenced this. Firstly, a large number of samples (4983) was examined, collected from a wide range of retail outlets over the period of a full year. Secondly, the IMS technique was used throughout the study. These authors have previously shown this to be 10–100-fold more sensitive than enrichment and subculture for the isolation of *E. coli* O157 from minced beef (Wright *et al.* 1994). Thirdly, the samples were purchased from small butcher's shops within South Yorkshire, all of which obtained meat habitually, although not exclusively, from an abattoir where the authors had previously isolated *E. coli* O157 from the cattle and sheep.

Like Chapman *et al.* (2001), Heuvelink *et al.* (1999) have also reported a higher prevalence of *E. coli* O157:H7 in beef meat. In 1996, they isolated *E. coli* O157:H7 strains from four (1.2%) of 325 samples of raw beef and one (0.4%) of 255 samples of raw minced beef and pork. In 1997, O157 VTEC (verocytotoxin-producing *Escherichia coli*) strains were isolated from two (0.4%) of 469 samples of raw beef and one (0.7%) of 147 samples of raw minced mixed beef and pork. The 11 positive meat samples came from seven different retail outlets and were collected on nine different dates.

The present authors have examined, over the period of a full year, 3450 samples of minced beef samples from three industries and not from a wide range of retail outlets. This difference might explain the low prevalence (0.12%) obtained in the present work, compared with those noted by Heuvelink *et al.* (1999) and Chapman *et al.* (2001).

No seasonality was noted because the four positive samples were collected in February, May, September and October. It was not possible to detect any *E. coli* O157:H7 strain from the remains of the four contaminated minced beef samples, suggesting a low infective dose.

Characteristics of *Escherichia coli* O157:H7 strains isolated from minced beef

The four strains were sorbitol negative, β -glucuronidase (–), *uidA* (+) and *flic* (+). As they had the ability to produce both *stx1* and *stx2* and possessed *eaeA* and *ehx* genes, they can be considered as pathogenic strains. The four strains were isolated from four different minced beef samples which came from three different mixes.

The two strains isolated from the same mix had two different pulsotypes (Fig. 1), suggesting that they originated from different sources.

In summary, the data demonstrate that industrial French minced beef is rarely contaminated with *E. coli* O157:H7. However, it must be kept in mind that Good Manufacturing Practices and the implementation of a Hazard Analysis Critical Control Point programme in food manufactur-

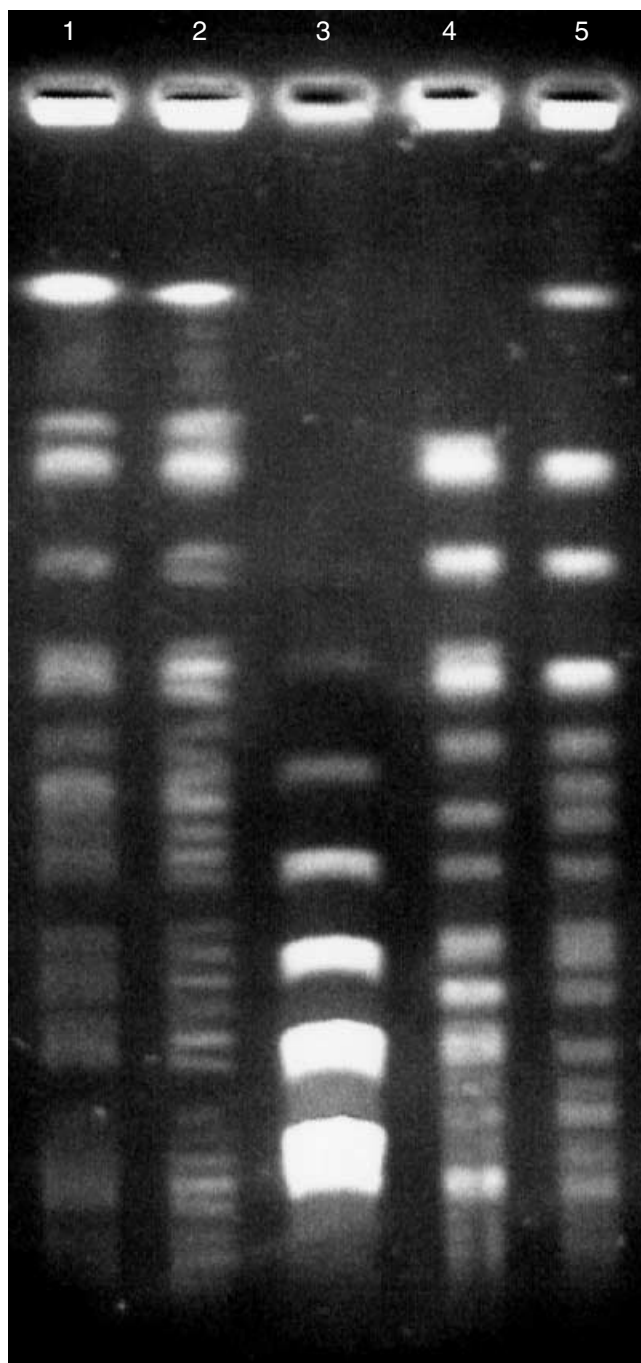


Fig. 1 Pulsotypes of the four *Escherichia coli* O157:H7 strains isolated from minced beef. Lanes: 1, strain 914 (industrial no. 3); 2, strain 56 (industrial no. 3); 3, ladder; 4, strain 435 (industrial no. 2) and 5, strain 448 (industrial no. 2, the same mangle as strain 435)

ing and preparation can help to control O157 VTEC (Attenborough and Matthews 2000) and that, despite the historic association between *E. coli* O157:H7 infection and the consumption of inadequately cooked ground beef, other

routes exist for the acquisition of *E. coli* O157:H7, such as municipal (Swerdlow *et al.* 1992) or swimming water (Keene *et al.* 1994), salami (Tilden *et al.* 1996), unpasteurized apple juice (Besser *et al.* 1993), lettuce (Ackers *et al.* 1998) and radish sprouts (Watanabe and Ozasa 1997).

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