

Isolation of *Escherichia coli* O157 in pigs at slaughter in Northern Italy

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Abstract

A study of VTEC O157 intestinal carriage was performed in pigs at slaughter, carrying out surveys respectively in the Veneto and the Lombardia regions of Italy within a common research project. The study was conducted for 15 months, starting in June 2002. As a minimum, a sample size of 300 samples was defined for each survey, assuming an expected prevalence of 1%, C.I. 95%, accuracy 5%. One gram samples of intestinal content from the distal gut were tested for *E. coli* O157 using an isolation method based on immunomagnetic separation. In the survey performed in the Veneto region, all the 397 samples collected from pigs of 132 farms tested negative for VTEC O157, but one *E. coli* O157 harbouring the *eae* gene only was isolated. In the survey performed in the Lombardia region, VTEC O157 was detected in 3 (0.63%, 95% C.I. 0.12 – 1.81) of the 480 sampled pigs from 3 (2.80%, 95% C.I. 0.58 – 7.97) of the 107 farms of origin. Therefore in the study a total of 877 pigs were tested in 15 slaughters of two regions, with a prevalence of 0.34% (95% C.I. 0.07 – 0.99) of positive pigs from 1.26% (95% C.I. 0.25 – 3.62) of the herds. In one of the positive farms also cattle were reared with pigs, even if housed separately, and in a follow-up investigation VTEC O157 strains sharing more than 96% homology with the pig strain were found in cattle.

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) and in particular *E. coli* O157 has emerged in the past two decades as a significant human pathogen that can cause a broad spectrum of diseases including severe illnesses such as haemorrhagic colitis and haemolytic uraemic syndrome.

Most human cases have been associated with consumption of contaminated food (in particular of bovine origin) or water, although direct contact with animals and person-to-person transmission have also been documented.

Cattle are considered to be the major reservoir of *E. coli* O157 but the organism has also been isolated from other ruminants and more occasionally from other animals. *E. coli* O157 has been reported from swine in different countries (Japan, The Netherlands, Sweden, Norway, Canada, United States), generally at a low prevalence (0.1-2.0%), while in South America a surprisingly high rate of carriage was found (8-10.0%) maybe due to differences in pig husbandry practices.

In Italy in a one-year survey, VTEC O157 was isolated from the intestinal content of one slaughtered pig (0.7%) and from one carcass (0.7%) of 150 randomly selected pigs. Since as only few data are available for swine, the aim of this study was to investigate more extensively the intestinal carriage of VTEC O157 in pigs at slaughter, collecting samples from several abattoirs of two regions of Northern Italy, in which swine productions has particular relevance.

Materials and methods

Sampling scheme

Two surveys (A and B) were carried out respectively in the Veneto and the Lombardia regions in Northern Italy during a 15 months period, from June 2002 to September 2003. Samples of distal intestinal content from fattening pigs were collected at slaughter, equally distributed in the period. One animal was sampled for each batch. A sample size of 300 samples was defined, assuming an expected prevalence of 1%, C.I. 95%, accuracy 5%. Samples were sent to the laboratory in refrigerated boxes and analyzed as individual samples within 48-72 hours after sampling. In both the surveys the sampled pigs originated from farms located in Northern Italy.

In "Survey A" samples were collected in 11 slaughterhouses of the Veneto region, having a capacity ≥ 3000 animals slaughtered per year. Samples were stratified according to the capacity of each slaughterhouse. Samples were sent for analysis to IZS delle Venezie - Laboratory of Pordenone.

"Survey B" was performed in 4 slaughterhouses of the Lombardia region having a capacity ≥ 5000 animals slaughtered per year. Samples were sent for analysis to IZS della Lombardia e dell' Emilia Romagna - Laboratory of Pavia.

A "Follow-up survey" with rectal samples collected from cattle was performed on the farm of origin of one VTEC O157 positive pig, in which both pigs and cattle were kept. This farm was divided in two subunits located at a distance of about 4 Km, both of them rearing pigs as well as cattle at different production stages, housed separately but with farmworkers and veterinarian in common.

Laboratory methods

In both the surveys the same laboratory methods were performed.

Isolation of *E. coli* O157

One gram faecal sample (distal gut contents) was added to 9 ml of Buffered Peptone Water (BPW, Oxoid), and incubated at $37 \pm 1^\circ\text{C}$ for 6 hours. After incubation, Immunomagnetic Separation (IMS) was performed using Dynabeads anti-*E. coli* O157 (Dynal, Oslo); magnetic beads were inoculated onto Sorbitol MacConkey agar (Oxoid) supplemented with cefixime and tellurite (CT-SMAC) and incubated, at $37 \pm 1^\circ\text{C}$ for 24 hours. Sorbitol non-fermenting colonies were tested for agglutination with O157-latex test (Oxoid) and positive isolates were confirmed biochemically as *E. coli* and further characterized.

Characterization of virulence factors

The presence of virulence genes was determined by polymerase chain reaction (PCR) amplification using the primer pairs KS7/KS8 for VT1, GK3/GK4 for VT2 and SK1/SK2 for the intimin-coding *eae* gene.

Pulsed field gel electrophoresis

Plugs were prepared as previously described (Morabito et al. 1999). Restriction endonuclease digestion was performed with 50 Uts of *Xba* I (Takara Biomedicals) at 37°C overnight. Electrophoresis was performed with a 1% agarose gel in the following conditions: 2.2 sec. initial switch time, 48.5 sec. final switch time, 6 V/cm for 20 hours in Tris-Borate-EDTA buffer 0.5 x at 14°C . Gels were stained with ethidium bromide (0.5 $\mu\text{g/ml}$) and analysed under UV light.

For analysis of genetic relatedness, PFGE profiles were analyzed by the BioNumerics Software (Applied Maths Belgium) using the UPGMA algorithm with Dice coefficient.

Results

Survey A

In survey A, 397 samples from pigs originating from 132 farms were tested. No VTEC O157 was isolated. Only one non-sorbitol fermenting *E. coli* O157 harbouring the *eae* gene but not the VT genes was detected.

Survey B

In survey B, VTEC O157 was detected in 3 (0.63%, 95% C.I. 0.12 - 1.81) out of 480 samples collected from pigs originating from 3 (2.80%, 95% C.I. 0.58 - 7.97) out of 107 farms. All the 3 isolates possessed the *eae* gene; two of them carried the VT1 and VT2 genes, one the VT2 gene only. PFGE analysis showed that the 3 strains had different profiles.

Follow-up survey

The follow-up survey showed that the positive pig was kept during the finishing period in one of the two farm subunits in which cows were reared, but had moved there approximately 2 months before from the other subunit where young heifers were kept. 60 rectal samples, 30 for each subunit were collected from cattle; eight of 30 (26.6%) sampled heifers tested positive for *E. coli* O157 carrying VT1 and VT2 genes, while all the 30 cows were negative.

The genetic relatedness among isolates was investigated by performing a cluster analysis on the PFGE profiles obtained from the isolates. All the bovine isolates were about 90-100% homologous and were closely related to the VTEC O157 strain from the positive pig, sharing more than 96% homology.

Discussion and conclusions

In this 15 months study on fattening pigs, VTEC O157 was isolated from 3 (0.63%) of the 480 sampled pigs from 3 (2.80%) of the 107 farms of origin in "survey B" conducted in slaughters of the Lombardia region and it was not detected in any of 397 pigs from 132 farms in "survey A" performed in slaughters of the Veneto region. Considering these data as a whole due to the similar criteria and period of the two surveys and the same laboratory methods used, in this study a total of 877 pig intestinal samples were tested in 15 slaughters of two regions, isolating VTEC O157 from 3 of them (0.34%, 95% C.I. 0.07 – 0.99). The positive pigs originated from 3 of 239 farms (1.26%, 95% C.I. 0.25 – 3.62). Our data confirm the very low prevalences found in studies carried out at slaughter in other European countries: a prevalence of 0.1% was reported in Norway (Johnsen et al., 2001), 0.08% in Sweden (Eriksson et al., 2003), 0.67% in The Netherlands (Heuvelink et al., 1999).

In Italy a survey performed in two slaughterhouses of the Emilia-Romagna region between December 1999 and December 2000 found VTEC O157 in one of 150 randomly sampled pigs with a prevalence of 0.7% (Bonardi et al., 2003); in a survey of the Piemonte region between May and October 2002, the organism was not detected in any of 504 pigs originating from 6 farms and tested at slaughter Decastelli et al., 2004).

In non European countries a prevalence of 2.0% was reported from USA (Feder et al., 2003). About the herd prevalence, a large study in The Netherlands (Schouten et al., 2005) with sampling performed on farms detected VTEC O157 in 0.4% of finishing pig herds.

Even though these prevalences can be considered low, the isolation of VTEC O157 from pig herds has raised concern. Studies in the USA have shown that pigs experimentally infected with *E. coli* O157 can shed the organism in their faeces for at least two months and that from magnitude and duration of shedding it cannot be excluded that swine could serve as a reservoir host under suitable conditions (Cornick et al., 2004).

Considering the risk of introduction in the pig herds, the results of other studies suggest that keeping pigs and cattle together on farms, with possibility of direct or indirect contact with one another, can be a risk factor for establishing VTEC O157 in pigs (Eriksson et al., 2003). An exposure could result from contamination of feedstuff or environment with ruminant manure; dogs, insects, rodents and bird could play a role in transmission of the organism in the farm environment as well as the man itself, in case of improper management or hygienic practices.

A follow-up investigation conducted in this study on the farm of origin of one positive pigs where both pigs and cattle were reared, showed that 26.6% of the heifers were harbouring VTEC O157; the analysis of PFGE profiles demonstrated that all the bovine isolates were 90-100 homologous, indicating that colonization with a prevalent clone had occurred. The VTEC O157 strain isolated from the positive pigs was closely related to the bovine strains, sharing more than 96% homology, suggesting that the same clone may have been spread crosswise to the two species.

The risk of introduction of this foodborne pathogen in the swine food chain should not be neglected and other surveys have shown that the contamination of pig carcasses by *E. coli* O157 at slaughter is possible.

So far pork meat or products containing pork meat only have not been definitely identified as a source of human outbreaks, although sausages containing beef and pork have been implicated in human infection and a family cluster of *E. coli* O157 infection microbiologically associated with consumption of salami that contained pork meat only, but stuffed in a natural casing of bovine origin (Conedera et al., 2007), has recently been described in Italy.

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