

The prevalence of Shiga toxin-producing strains of *Escherichia coli* in cattle and pigs at slaughterhouses in the Czech Republic

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Abstract

Shiga toxin-producing strains of *Escherichia coli* (STEC) are significant agents of alimentary infections in humans. The prevalence of these infections is lower than that of campylobacteriosis and salmonellosis, though there are clinically severe infections that may have lethal consequences in the case of complications. STEC monitoring was performed in cattle and pigs at selected Czech slaughterhouses in June, July and August 2012. A total of 622 cattle and 993 pigs were tested. Samples were collected from a total of 111 slaughter batches. The occurrence of *E. coli* serogroups O26, O103, O104, O111, O145 and O157 was monitored. A PCR method revealed the presence of monitored *E. coli* serogroups in 43.8% of swabs taken from the tested carcasses. Twenty-five strains were subsequently cultivated from the analysed material, of which 11 had the genetic makeup for Shiga toxin production and also carried the *eae* gene.

Alimentary infection, Shiga toxin-producing Escherichia coli, slaughterhouse, zoonoses

Introduction

Escherichia coli is a bacteria commonly occurring in the gastrointestinal tract of healthy humans and animals. There is however, a number of serogroups of these bacteria that may cause serious gastrointestinal and systemic disease and that may be fatal in humans and animals. It includes the group of *E. coli* with the ability to produce toxins extremely similar to *Shigella dysenteriae*. Two types of toxins are described in this group of *E. coli* – Shiga toxin 1 (stx1) and 2 (stx2). Intimins enabling bacteria to adhere to the intestinal mucous membranes are important in the pathogenesis of the disease caused by these bacteria. The production of toxins is coded by the genes *stx1* and *stx2*, the intimins are coded by the gene *eae*. Their detection by molecular genetic methods is used to obtain laboratory proof of Shiga toxin-producing strains of *E. coli* (STEC) in tested material.

More than 70 various serogroups of *E. coli* producing these toxins that are capable of causing disease have been described in man. Shiga toxins may cause a wide range of clinical manifestations of disease in affected people from simple diarrhoea to haemorrhagic colitis, which may progress into haemolytic-uraemic syndrome with microangiopathic haemolytic anaemia, thrombocytopenia and serious acute kidney failure requiring intensive care. Serovar O157:H7 is most frequently associated with the most serious human infections. *Escherichia coli* O157:H7 is one of the most serious bacterial alimentary pathogens that can be isolated from cattle. The skin of cattle is the main source of contamination in beef carcasses at the slaughterhouse, and reduced prevalence of *E. coli* O157:H7 on the skin is directly associated with reduced prevalence on the surface of beef quarters (Arthur et al. 2011).

Many “non-O157” serovars may, however, also be a source of infection. According to the American Centers for Disease Control and Prevention (CDC), the following are the “non-O157” serovars playing the greatest role in human disease and causing as many as 71% of these “non-O157” infections – O26:H11, O45:H2, O103:H2, O103:H11, O103:H25, O111:H8, O121:H19, O121:H7 and O145:NM (nonmotile) (Bosilevac and Koohmaraie 2011).

The importance of faecal contamination of carcass surfaces is shown by the study by the Irish authors Monaghan et al. (2011), who tested faecal samples from cattle and samples

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of agricultural soil for the presence of STEC. Samples were taken during the year on twenty farms throughout Ireland. A total of 1 800 samples were tested. PCR methods demonstrated the genes *stx1* and/or *stx2* in 40% (480 of 1 200) of faecal samples and 27% (162 of 600) of soil samples. STEC strains were cultivated in 1.9% (23 of 1 200) of faecal samples and in 0.7% (4 of 600) of soil samples. Positive occurrence peaked in late summer and at the beginning of autumn.

Although cattle or beef meat is considered as the main source of STEC for man, alimentary infections caused by STEC have also been described after consumption of pork meat. Trotz-Williams et al. (2012), for example, described a family STEC epidemic in Ontario. People attending a family celebration (n = 59) were served various dishes, including dishes made from pork meat. Clinical gastrointestinal symptoms appeared in 29 of those present, some of whom also had bloody diarrhoea, and seven of those affected had to be hospitalised. STEC O157 (which was also confirmed in the pork meat eaten by those affected) was isolated in eleven cases by subsequent laboratory tests of clinical samples taken from the patients. Subsequent comparison of human strains and strains isolated from pork meat performed by the pulsed-field gel electrophoresis method proved positive results. The authors draw attention to the possible risk of alimentary STEC infection from pork meat, particularly at events such as barbecue.

Materials and Methods

Monitoring of the occurrence of STEC in cattle and pigs at designated slaughterhouses was performed in the Czech Republic in June, July and August 2012. The sampled material (abrasive sponges) was tested in laboratories in the State Veterinary Institutes in Olomouc, Jihlava and Prague. A total of 1 615 animals were tested, of which 622 were cattle and 993 pigs. Sampling was performed on a total of 111 slaughter batches.

Sampling and the processing of samples for STEC detection was based on the updated Methodical Guide No. 1/2005 (MG) from the State Veterinary Administration, which defines rules for the regular microbiological testing of zoonoses agents performed at slaughterhouses by state veterinary inspectors in accordance with the Decree No. 356/2004 Coll. on the Monitoring of zoonoses agents. This MG stipulated a procedure for testing swabs from fresh beef and pork meat and detection of STEC belonging to serogroups O26, O103, O104, O111, O145 and O157. Samples for testing of the occurrence of monitored serogroups in cattle and pig carcasses were taken in all cases at designated slaughterhouses once a month in June, July and August 2012. The procedure during sampling with abrasive sponges (Plate IX, Fig. 1) was the same as for sampling of *Salmonella* spp. meaning the same abrasive sponges were used for the STEC detection at the same time. The laboratory analyses themselves were performed in accordance with the methodical procedure coordinated by the EU-NRL for *E. coli*. This procedure was based on CEN/ISO TS 13136 Microbiology of Food and Animal Feed – a horizontal method for detection of STEC belonging to serogroups O26, O103, O111, O145 and O157 – a qualitative method.

The aim of above procedures was, firstly and foremost:

1. To detect the genes *stx1*, *stx2* and *eae*, which indicate the presence of *E. coli* capable of producing Shiga toxins and intimins (*eae*), in the tested material (a sponge in a propagation medium) while using of PCR.
2. To detect the presence of monitored serogroups of *E. coli* (i.e. O26, O103, O104, O111, O145 and O157) in tested material (a sponge in a reproduction medium) when in use of PCR.
3. In the case of positive finding isolate the suspect STEC strain on a solid culture medium as per both points above.
4. To verify the presence of the genes *stx1*, *stx2* and *eae* in the obtained STEC strain and to determine to which serogroup it belongs.

Results

The presence of monitored STEC serogroup was confirmed by PCR method (sponge in a reproduction medium) in 68.5% of slaughter batches in which swabs were taken. The presence of monitored serogroups was confirmed by PCR method in 43.8% of tested swabs taken from carcasses. The most frequent serogroup detected in swabs from cattle and pigs by PCR was *E. coli* O145 (30% from cattle and 32% from pigs). The serogroup *E. coli* O157 was detected by PCR in 14% of cattle swabs and 23% of pig swabs.

Twenty-five strains were cultivated from tested material, of which only eleven had the genetic makeup for the production of toxin while also carrying the *eae* gene (intimin).

Table 1. Concise results of STEC monitoring at slaughterhouses in the Czech Republic

Number of monitored slaughter batches	111
Number of slaughter batches positive for STEC	76
Number of tested samples (cattle + pigs)	1 615
Percentage of positive findings (monitored serogroup)	43.8
Number of isolated bacterial strains	25
Number of strains where genes <i>stx1</i> or <i>stx2</i> and <i>eae</i> were present	11

The findings made are briefly summarised in the Table 1. Individual proportions of detected STEC serogroups in cattle and pigs are given in Plate IX, Fig. 2 and 3.

Discussion and Conclusions

The prevalence of STEC in cattle and pigs at slaughterhouses in Europe varies markedly and any comparison is difficult in view of the differing methods of sampling and detecting STEC strains. According to the report from European Food Safety Authority (EFSA 2012), the prevalence of STEC in cattle carcasses in 2011 was 4.2% in Belgium, 2.3% in Germany and 1% in Ireland. Ramoneda et al. (2013) presented their results of STEC monitoring in 300 cattle for slaughter at slaughterhouses in Catalonia. *E. coli* O157 was proven in 14.7% of carcasses in this study. An older Canadian study documents the occurrence of STEC in swabs taken from cattle and pig carcasses in the Canadian province of Alberta. STEC was proven in 5.4% of the 1 018 swabs taken from cattle. STEC was detected in 4.8% of tested samples of 1 067 swabs taken from the surfaces of pig carcasses (Bohaychuk et al. 2011).

The results of our work show a great difference between a positive finding for the given genes characteristic of the serogroups monitored directly in an extract of swab sponge in a reproduction medium and a positive cultivation finding of these strains that also had the *stx1* and *stx2* (Shiga toxins) and *eae* (intimin) genes. While in the first case, a positive finding was made in 43.8% of tested samples, a cultivation finding of strains with the presence of *stx1*, *stx2* and *eae* was made in only 11 cases which represents merely 0.7% of samples. In the case of tests designed to detect genes of individual virulence factors in an extract of swab sponge, these genes may be dislocated in various strains of *E. coli*, for this reason a positive result should be verified by cultivation proof of the strain to confirm that the strain really carries the genes for both virulence factors – production of toxins and intimins (CEN/ISO TS 13136). It is clear that cattle and pig's carcasses may be a source of STEC strains clinically significant to man, though in view of the frequency of their successful cultivation the risk is not particularly high. Cross contamination between carcasses occurs at slaughterhouses. Knowledge of safe procedures in the handling of raw meat plays an important role in the prevention of alimentary infections. Monitoring of STEC occurrence in animals for slaughter is an essential precondition in the coming period to assure the microbiological safety of meat. For the upcoming testing it would be appreciated to initiate closer cooperation with the National Reference Laboratory for STEC. To confirm all suspect isolates which will be subjected to more detailed molecular genetic analyses to sort out strains with high pathogenic potential for man.

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Fig. 1. Taking of swab with an abrasive sponge (State Veterinary Institute Olomouc)

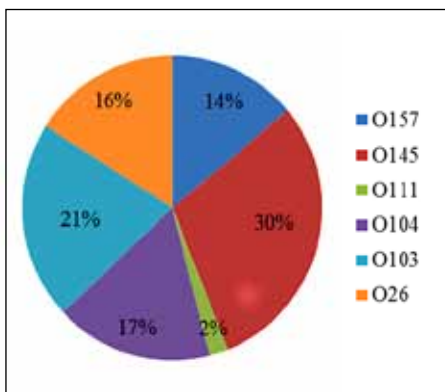


Fig. 2. Proportions of monitored STEC serogroups in samples taken from cattle carcasses

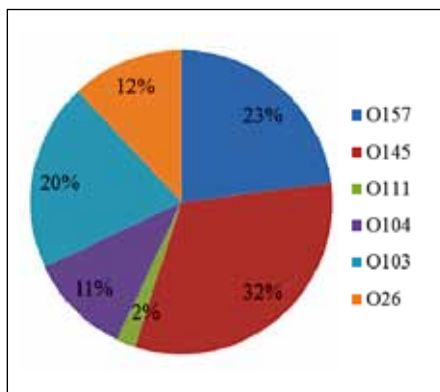


Fig. 3. Proportions of monitored STEC serogroups in samples taken from pig carcasses