RISK ASSESSMENT OF VEROCYTOTOXIN-PRODUCING
E. COLI (VTEC) IN DANISH PORK

Sara Monteiro Pires*, Claes Enøe†, Flemming Scheutz‡, Flemming Hansen§, Lis Alban

*The National Committee for Pig Production, Danish Bacon & Meat Council, Axelborg, Axel-Holm 3, DK-1609 Copenhagen V, Denmark. Ph: +45-33-73-25-88, Email: cen@danishmeat.dk; †The International Escherichia and Klebsiella Centre (WHO), Statens Serum Institut, Copenhagen, Denmark; §The Danish Meat Research Institute, Roskilde, Denmark

Abstract Verocytotoxin-producing Escherichia coli (VTEC) is recognized as a food-borne pathogen of major importance. Infection by VTEC is known to cause a wide range of symptoms, from uncomplicated diarrhoea to haemorrhagic colitis, haemolytic uraemic syndrome and death. VTEC has primarily been isolated from cattle. However, in several countries VTEC has also been isolated from pigs. The prevalence in Danish pigs remains unknown but a small-scale study indicated that it was very low (2.1%) (non-published). All isolates were VTEC types that very rarely have been associated with human disease and are considered low pathogenic VTEC.

The present risk assessment aimed at assessing whether human cases of VTEC could be attributed to Danish pigs and pork. A review of recent studies on VTEC gave no indication that VTEC in Danish pork should be considered a risk to the consumers.

Introduction VTEC is an emerging zoonotic pathogen causing severe illness in humans. The main sources of human exposure to VTEC have been considered to be contaminated bovine products but several other sources have been identified. At present, pigs and pork are not considered to be the source of human VTEC infections in Denmark. However, VTEC has been reported in pigs and pork in several countries and therefore, it cannot be ruled out that pork could be a source of VTEC infection in humans.

The present risk assessment was carried out to assess whether human cases of VTEC could be attributed to Danish pigs and pork.

Materials and Methods The structure in the Codex Alimentarius guideline for risk assessment was adopted—including hazard identification, hazard characterization, exposure assessment and risk characterization.

The hazard identification and characterization covered a description of the agent and its virulence and pathogenicity. A description of the range and severity of clinical manifestations of the infection in humans was also included.

In the exposure assessment, both cattle and pigs were considered but the primary focus was on pigs and pork. The exposure assessment covered:

- the origin of the meat consumed in Denmark;
- the prevalence of VTEC in live animals, and in raw meat and meat products in several countries;
- the growth in meat and processed meat and factors influencing it;
- the consumption of meat and meat products in Denmark.

However, the prevalence data was insufficient and incomplete. Especially, information about the prevalence of VTEC in Danish pigs and pork was sparse. Data about the consumption of meat and meat products was also poor, in particular for children below 5 years of age. Considering that children in this age group appear to be highly susceptible, this constituted a serious problem in the risk quantification step of the risk characterization. For these reasons, we decided to abandon the quantitative risk assessment approach and instead carry out a qualitative risk assessment. Expert opinion was used to increase the applicability of the incomplete data.

Results Hazard identification VTEC is a food-borne pathogen of major importance. This was realized for the first time in 1982 in connection with two outbreaks of haemorrhagic colitis (HC) in the states of Oregon and Michigan, USA (Riley et al., 1983). Since then, several human out-breaks have been reported throughout the world.

In Denmark, human cases of VTEC infection have been reported since 1997, when molecular detection methods were introduced. VTEC is now the 5th most frequent cause of gastrointestinal infection in humans and more common than Shigella infection (Scheutz et al., 2005).

Since 1997, there appears to be an increase in the National incidence (3.1 per 100.000 per year in 2004) (Scheutz et al., 2005) but this may partly be explained by an overall increase in sen-
Escherichia coli (DEC). DEC can be grouped according to serotypes are non-pathogenic and an essential part of the normal intestinal flora in humans and animals. However, certain pathotypes are known to cause gastrointestinal symptoms in humans and is referred to as diarrhoeagenic E. coli (DEC). DEC can be grouped according to various virulence factors, including toxin production and adhesion genes. Of these groups only VTEC is considered to be a zoonotic agent and only VTEC is an occasional finding in pigs.

Many serotypes of VTEC have been identified. VTEC O157:H7 has most commonly been associated with disease in man but other VTEC serotypes are known to cause severe human infection. Infection by VTEC causes a spectrum of symptoms in humans: from uncomplicated diarrhoea to haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) an even death.

**Exposure assessment** The main sources of human exposure have been contaminated bovine products such as ground beef, alfalfa sprouts, and raw or improperly pasteurized milk and cheese. Also beef, sausages (beef and pork) and apple cider have been associated with human cases (Cassin et al. 1998). Additional sources of exposure have been cross-contamination of ready-to-eat foods from raw foods or contaminated utensils and by contamination of milk after pasteurization (Anon., 2004a). Infection may also occur following direct contact with infected animals, person-to-person transmission, swimming in or drinking contaminated water or eating contaminated fruits and vegetables (Anon., 2004a). Recent studies have indicated that live animal contact (Jensen et al., 2004) and person-to-person transmission may be more important than previously assumed (Scheutz et al., 2003).

There have been 2 outbreaks of VTEC in humans in Denmark—both in 2004. One was associated with a farm visit (Jensen et al., 2004) and one was associated with contaminated cow’s milk (Mølbak, 2005).

VTEC has been reported in cattle and beef from many countries (Anon., 2004a). The occurrence of VTEC in Danish cattle has been monitored since 1997 by bacteriological examination of faecal samples collected at slaughter. In 2003, VTEC O157 was detected in 7.1% of 212 faecal samples from slaughter calves (Anon., 2004b). Examination of meat and meat products in the retail stage is not part of the routine surveillance but none of the few samples that were examined in 2003 were found positive for VTEC (Anon., 2004b).

VTEC has also been isolated from pigs (faeces samples or carcasses) in several countries. Although the prevalence in most studies is relatively low (<5%) there are also reports of very high prevalences: 74% in Greece (Anon., 2004a), and 69% in Chile (Borie et al., 1997). The prevalence of VTEC in the Danish pig herd remains unknown. VTEC is not considered to be a zoonotic problem in Danish pigs and pork and is therefore not subject to surveillance. However, between 1997 and 1998, the Danish Bacon & Meat Council (DBMC) conducted a small-scale screening (non-published). In this study, 40 of 473 faecal samples (8.5%) were found positive in PCR, and 10 of 473 samples (2.1%) were culture positive (Flemming Hansen, personal communication, 2005). However, it is worth noting that all the culture positive samples were negative for the verocytotoxin 2 (vtx2), E. coli attaching and effacing (eae) gene, the plasmid encoded entero-haemolysin gene ehxA and plasmid marker for enteroaggregative E. coli (CVD432). Lack of these virulence factors is indicative of low pathogenic VTEC.

The prevalence of VTEC O157 in Danish pork was examined in 1996. The 32 regional food control units placed under the Danish Food and Veterinary Administration conducted a screening for VTEC in minced meat. A total of 2,112 samples of ground beef and pork were examined for presence of VTEC O157:H7. The prevalence in ground beef was 0.1% (2/1584), and the prevalence in pork was 0.4% (2/528) (Anonymous, 1997). It should be noted, that the VTEC positive samples of minced pork contained traces of bovine serum protein, which indicated spill-over from grinding of beef. It was concluded that there had likely been cross-contamination of the pork samples with VTEC from beef (Anonymous, 1997).

Denmark imports pork and beef from several countries. The majority of the meat products are imported from Germany, The Netherlands, UK, Sweden and Spain. (Danish Meat and Bacon Council, 2005). In 2002, VTEC was found in pigs in The Netherlands (1/3), and in pork in Spain (5/434=1.2%). VTEC was not detected in pigs in Germany (0/50) nor in Sweden (0/2), and it was not found in pork in The Netherlands (0/89) nor in Ireland (0/494) (Anon. 2004a).

**Risk characterization** The present study indicated that there is a low risk that human cases of VTEC could be attributed to Danish pigs and pork. This is based on the prevalence of culture positive faecal samples in the DBMS study (2.1%) of which all were of the low pathogenic type. Moreover, there was a low prevalence of culture positive samples of VTEC O157 in Danish pork.
(0.4%) and it was probably the result of cross-contamination from beef.

The risk assessment drew attention to the fact that additional or better quality data is needed about the prevalence and virulence patterns of VTEC in pigs and pork, consumption of meat in children below 5 years of age, and additional risk factors that may be equally or even more important than contaminated food.

**Discussion and Conclusions** VTEC can be found in Danish cattle and pigs. However, the prevalence in Danish pigs seems to be low. The risk assessment is primarily based on the fact that the prevalence of VTEC in Danish pigs, as determined in the DBMC study (1997-1998), was low.

The VTEC isolated from pig faeces appear to be low pathogenic. Ethelberg et al. (2004) studied the virulence factors for VTEC's associated with HUS in Denmark, as well as the risk factors for contracting both HC and HUS. HC and HUS are considered to be the most severe complications of VTEC infection. The determinants associated with the development of HUS were the vtx2 gene, the eae gene and young age. Considering HC, the determinants found were, again, the presence of the eae gene and the vtx2 genes, although the vtx2 association was much less pronounced. For this reason, we put much weight on the fact that all the VTEC isolates from pigs in the DBMC study (1997-1998) were found to be low pathogenic, lacking the important virulence factors vtx2, eae gene, ehxA and CVD432.

VTEC is occasionally found in Danish beef and has been found once in Danish pork suspected to be cross-contaminated from ground beef (Anon., 1997). None of the Danish outbreaks were related to pigs or pork.

Apart from the known sources of exposure, infection may also occur following live ruminant animal contact (Jensen et al., 2004). Therefore, direct contact between children below 5 years of age, and cattle and small ruminants should be limited and follow certain restrictions and guidelines.

It is essential to provide hygienic food handling, cleaning of utensils such as knives and grinders and good chilled storage conditions to ensure that other foods do not become contaminated (Anon., 2004a).

We acknowledge the fact that more data is required to develop a quantitative risk assessment model to estimate the risk to humans from VTEC in pigs and pork.

**References**


Anonymous, 2004b. Annual report on zoonosis in Denmark in 2003. Ministry of Food, Agriculture and Fisheries, Denmark, 32pp


