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ENTEROHAEMORRHAGIC E. COLI (EHEC) - A NATION-WIDE SWEDISH SURVEY OF BOVINE FAECES

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A l'automne 1995, le premier foyer de maladie due à E. coli entérohémorragique touchant 102 personnes a été signalé en Suède. D'après les foyers d'autres pays, on sait que du bétail infecté de façon sub-clinique est souvent la source infectieuse. Par suite, une étude de prévalence a été commencée en avril 1996 et sera terminée en août 1997. Son but est de préciser la fréquence d'E. coli entérohémorragique ou producteurs de vérotoxines dans la population bovine suédoise. Des fèces doivent être prélevées chez 3.000 bovins dans les 16 principaux abattoirs. Le nombre d'échantillons dans chaque abattoir est proportionnel au nombre de bovins abattus. Les résultats seront utilisés pour définir si des mesures spécifiques doivent être prises pour lutter contre ces colibacilles chez les bovins.

En avril 1995, 2.195 échantillons individuels ont été analysés et les résultats préliminaires montrent que 25 (1,1 p. cent) contenaient E. coli sérotype O_{157} produisant des vérotoxines (VT_1 et/ou VT_2), positifs pour le gène eaeA. Deux cent cinquante autres échantillons (11,4 p. cent), non O_{157}, ont été identifiés comme contenant des souches de coliformes produisant une vérotoxine. Parmi elles, environ 13 p. cent possédaient également le gène eaeA. Les sérotypes de ces souches seront déterminés. Les résultats montrent que E. coli entérohémorragique existe dans la population bovine suédoise et que la distribution des bovins hébergeant des souches d'E. coli entérohémorragiques est corrélée à celle de la distribution générale des bovins en Suède.

INTRODUCTION

In the second half of 1995, 114 human cases of infection caused by EHEC were reported in Sweden. In 1996, 118 humans got infected. Before this outbreak, since 1988 only up to three annual cases of the infection have been reported. Experience from outbreaks in other countries shows that subclinically infected beef and dairy cattle in many cases are the source of infection (Rasmussen et al., 1993). A study was initiated in April 1996 to survey the Swedish bovine population for the presence of EHEC and other verotoxin (VT) producing E.coli.

MATERIAL AND METHODS

Faeces samples are collected at the 16 main abattoirs, producing 90% of all Swedish beef. Three thousand cattle are to be individually sampled, the total population of cattle in Sweden being about 1,8 millions. This study was designed so that a prevalence in the population of 0,1% gives 90% confidence to detect one positive animal. The study will run from April 1996 to August 1997. Each month the abattoirs send samples at about two occasions each, the number of samples are proportional to the number of cattle passing through the abattoir. Samples are collected from ampulla recti after the digestive tract has been separated from the body. Samples are sent refrigerated to our laboratory and analysed for the presence of VT-producing E.coli serotyp O157 and other VT producing coliform bacterial strains.

Isolation of E.coli O157 strains are made after pre-enrichment in buffered peptone water followed by immuno-magnetic separation (Karch et al., 1996) and cultivation on sorbitol Mac Conkey agar with cefixime and tellurit. Positive E. Coli O157-strains are then analysed by PCR to identify genes coding for VT-production and attaching and effacing activity (*eae*A). The same PCR-analyse is also used to identify VT- and eaeA-genes in all isolated coliform (non-O157). Positive strains will be serotyped.

RESULTS

In April 1997, 2195 samples have been analysed and preliminary results show that 25 (1,1%) have been identified as VT-producing (VT1 and/or VT2) E.coli serotype O157, positive for the eaeA gene. These 2195 individual samples were to be collected from 1695 different premises, however, this figure probably includes some faults. Both the number of the place of production, were the animals were born and the farm number of the animal owner at the time of slaughter are used and may in some cases be mixed up.

Out of these 25 EHEC-positive samples 17 were VT2+ and 2 were VT1+, 6 samples were both VT1+ and VT2+. Beside these 25 EHEC-positive samples, 3 more samples were found to be E.coli of serotyp O157 but not producing VT. In another 250 (11,4%) samples, coliform bacterial strains other than O 157 (to be serotyped) producing VT1 and/or VT2) have been found. The distribution according to age of the animals shows that young animals (older calves 3,6%) were more often found to be positive than adult cattle (0,4%) (Tab.I).

The distribution according to breed of the animals shows that dairy cattle tend more often to be positive than beef cattle, 1,2% versus 0,6% (Tab.II). However, fewer beef cattle were sampled. Also surplus dairy calves may be raised as beef cattle, these animals are in this study most often recorded as dairy breed.

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During summer and early autumn, i.e. the grazing period (Tab.III), more positive samples were found compared to other seasons. In February, five of six young stock from the same farm were found to be positive when sampled at one occasion. However, this study is still incomplete concerning information about the period April to August 1997.
The geographical distribution of positive animals shows a concentration to the middle and south parts of Sweden and indicate that the findings of EHEC-positive cattle are correlated to the geographical distribution of the cattle population.

Table I
EHEC-positive cattle distributed according to age group

Age group	No. positive	No. sampled	%
Older calves	5	138	3.6
Young stock	17	1281	1.3
Adult cattle	3	771	0.4
Not recorded	0	5	0.0
Total	**25**	**2195**	**1.1**

Table II
EHEC-positive cattle distributed according to breed

Breed	No. positive	No. sampled	%
Dairy breed	16	1299	3.6
Beef breed	3	477	0.6
Crossbreed	0	41	0.0
Not recorded	0	378	1.6
Total	**25**	**2195**	**1.1**

Table III
EHEC-positive cattle distributed according to sampling season.

Month	No. positive	No. sampled	%
April (96+97)	1	136	0.7
May	2	169	1.2
June	0	132	0
July	0	0	0,0
August	0	175	0
September	7	252	2.8
October	3	310	1.0
November	4	289	1.4
December	1	148	0.7
January	0	195	0
February	0	223	0
March	7	166	4.2
Total	**25**	**2195**	**1.1**

DISCUSSION

Before 1996 VT-producing E.coli O157 have never been diagnosed in Swedish cattle. However, only about 200 calves have been investigated (Franklin, personal communication.). The preliminary results from the present study show that EHEC is present in the Swedish cattle population at a prevalence of 1,1%. The prevalence of EHEC-positive healthy cattle seems to be about the same (0,3-1,8%) as in other western countries where extended studies have been performed (Blanco et al., 1996; Faith et al., 1996). Even though this prevalence study is not ended, the Swedish Board of Agriculture has already decided that special restrictions will be stated for EHEC-infected farms, also the prevalence of EHEC in the bovine population will be continually screened. In Sweden EHEC in animals are compulsorily notifiable since October 16, 1996, for this notification EHEC is defined as E.coli of serotyp O157.
Among EHEC infected humans in Sweden about 10% are caused by other serotypes than O157. Infections with EHEC in humans are compulsorily notifiable since January 1, 1996. Up to now EHEC infection in humans in Sweden has caused no deaths. At only one occasion EHEC-infection in humans in Sweden has been correlated to the presence of EHEC in a cattle herd.
In other studies is also noted that young animals are more often found to be positive (Mechie et al., 1997). In one study Cray and Moon (1995) found that after experimental infection with E.coli O157 to calves and adult cattle, calves remained positive for longer times.

Different forms of management of a herd is often discussed and associated with herd status of E.coli O157 (Hancock et al., 1994). Possibly different management regimes can to some part explain the difference in incidence, often seen between dairy and beef cattle.
Dietary changes together with other forms of stress, are often pointed out as reasons to the increased numbers of animals as then are identified as positive for VT-producing E. coli O157 (Mechie et al., 1997). In the beginning as well as in the end of the grazing season dietary changes is taking place.
An interesting consideration concerning EHEC-infected farms is if it is possible to eliminate VT-producing E.coli. from infected premises. Experimental studies have shown that previous infection does not prevent reinfection by the same strain of E.coli O157 (Cray and Moon, 1995). *Escherichia coli* O157 can survive in bovine faeces for extended periods and retain its ability to produce verotoxins (Wang et al., 1996). Also it seems like this serotype of E.coli is unusually tolerant against some environmental stresses, such as acidic and dry conditions, compared with most other serotypes of E.coli (Arnold & Kaspar, 1995; Glass et al., 1992).

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