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Survey of specific serogroups of *E. coli* in sheep in Norway

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Introduction

The survey detected a low occurrence of stx*-positive and* eae*-positive* E. coli *O26, O103:H2 and O157:H7, and no* stx*-positive and* eae*-positive* E. coli *O103:H25. For* E. coli *O26, O103:H2 and O103:H25 the survey showed higher numbers of* stx*-negative and* eae*-positive strains than of* stx*-positive and* eae*-positive strains.*

Escherichia coli are bacteria normally present in the intestinal flora of both humans and animals. Some *E. coli* may be pathogenic for humans. Of these, only Shiga toxin-producing *E. coli* (STEC), also known as Verotoxin-producing *E. coli* (VTEC), has a defined zoonotic origin with domestic ruminants regarded as the major reservoir.

The Shiga toxins are encoded by the genes stx_1 and stx_2. The toxins are the major virulence factors of STEC and the cause of haemorrhagic uremic syndrome (HUS) in humans.

STEC attach in the human gastrointestinal tract through a complicated mechanism involving among others the protein intimin encoded by the gene *eae* and this attachment induces the (hemorrhagic) diarrhoea seen in human patients. This virulence characteristic is also seen among the Enteropathogenic *E. coli* (EPEC). A subgroup of these; the atypical EPEC appear to be more closely related to STEC in their serotype profiles and genetic characteristics and in recent years it has become clear that atypical EPEC not only has a human reservoir, but also an animal reservoir.

The most well known human pathogenic serotypes of STEC are O26:H11, O111:H8, O103:H2, O145:H21, and O157:H7. However, other serotypes may also cause human infections as observed in the Norwegian outbreak in 2006 with 17 human cases caused by STEC O103:H25 (1). The source of this outbreak was dry-cured sausages with the bacteria originating from contaminated sheep meat.

With the exception of 2006, the annual reported incidence of human STEC infections in Norway has been low (0-17 cases per year) with approximately half of the cases domestically acquired (1). The last ten years, three outbreaks have been registered with 4, 4, and 17 human cases, respectively.

The animal reservoir

There is limited knowledge of the prevalence of STEC in the Norwegian ruminant populations. Studies performed in Norway from 1995 to 1999 reported cattle herd prevalences of STEC O157 of 0.5 % to 1 % (2, 3). Only one study has focused on estimating herd prevalence of STEC O157 in sheep. The study did not detect any STEC O157 (2).

In a surveillance programme for STEC O157 in cattle, sheep, and goat carcasses running in the period 1998-2004, the crude carcass prevalence was 0.06 % for cattle and 0.03 % for sheep. None of the 510 goat carcasses tested were positive (4).

There are less data on the other serogroups. Two studies in cattle have focused on detecting serogroups O26, O103, O111, and O145. The detection of eae-negative STEC O103 was reported from 3.2 % of the herds in one of the studies. In both studies *stx*-negative *E. coli* of the serogroups O26, O103, O145, and O111 were detected (4). In a study of one sheep flock conducted in 2000, 2 lambs (1.6 %) were positive for STEC O103 (5). The isolates were not H-typed, but carried stx_1 and *eae*. In addition, *stx*-negative isolates were detected from 62 of the 96 samples tested.

International studies also report *stx*-negative and *eae*-positive *E. coli*, and *stx*- and *eae*-negative *E. coli* isolates of these serogroups (O26, O103, O111, O145 and O157), indicating that these are relatively common in the microbial flora of animals. During the 2006 outbreak in Norway (1), *stx*-negative and *eae*-positive *E. coli* O103:H25 was detected from several products of sheep origin with no clear epidemiological link, indicating that this serotype is common among sheep in Norway. However, the relationship and ratio between true *stx*- and *eae*-negative *E. coli*, true *stx*-negative and *eae*-positive *E. coli* (possible atypical EPEC), and *stx*-positive and *eae*-negative *E. coli* (STEC), and *stx*- and *eae*-positive *E. coli* (STEC) of a serotype, is unknown and there is a need for more data for assessing these relationships.

The 2006 outbreak (1) emphasised the need for more knowledge regarding *E. coli* of serogroups O26, O103, O111, O145 and O157 in the sheep population. The Norwegian Food Safety Authority therefore decided to initiate a national surveillance programme with sampling in November 2006 and autumn 2007. The National Veterinary Institute was asked to design the programme, perform the analyses, and the

reporting of the results. The samples would be collected by inspectors from the Norwegian Food Safety Authority.

Aims

The aims of the survey were to gather knowledge on the occurrence of Escherichia coli *O26, O103 and O157)* and their virulence factors in sheep, and to investigate geographical distribution and risk factors.

Materials and methods

In November 2006 faecal samples were to be collected from 100 randomly selected sheep flocks (farms). Only sheep flocks with at least 50 sheep of more than 1 year old were eligible. During autumn 2007 faecal samples were to be collected from 520 randomly selected sheep flocks (farms). Only sheep flocks with at least 30 winter-fed sheep at 1st January 2007 were eligible. From each of these flocks, 50 single faecal samples should be taken from the youngest animals (lamb first, then one-year olds etc.).

Autumn was chosen as sampling period to give representative data from the time of year when most sheep is slaughtered and thereby indications of possible contamination risks to sheep products. Lambs were chosen as young ruminants shed more of these bacteria and are also proportionally slaughtered most.

From each farm, a questionnaire addressing potential risk factors for the occurrence of STEC was filled in.

From each farm, pools of 10 individual samples were analyzed for the various *E. coli* serogroups. In the cases where number of samples were not divisible by 10, the last pooled sample consisted of less than 10 individual samples.

The samples from 2006 were enriched using enrichment with antibiotics. However, the bacteria did not survive being frozen in this enrichment, and since the samples later on should be analyzed for other serogroups/-types, choice of enrichment had to be changed for the 2007 samples. Both used enrichment are internationally used for analyzing *E. coli*.

A modified method of NMKL 164 where the Immunomagnetic separation (IMS) method has been further modified by inclusion of an ELISA step was used for detection of *E. coli* O26, O103 and O157. ELISA positive samples were plated onto selective agar for colony isolation. Chromagar O157 and Sorbitol-MacConkey agar with cefixime and telluritt (CT-SMAC) for ELISA positives for serogroup O157, and MacConkey-agar with 4 % CT-supplement and washed sheepbloodagar for ELISA positives for the serogroups O26 or O103. Thereafter, *E. coli* isolates was serogrouped/serotyped and further characterized for virulence factors stx_1, stx_2 and *eae* was performed by PCR.

Information on potential risk factors was gathered from a questionnaire that was filled out at sampling. Association between potential risk factors and the occurrence of *E. coli* O26, *E. coli* O103 and *E. coli* O157 with their respective virulence factors (*stx* and/or *eae*) were performed in STATA version 9.2 by univariat analyse (Fisher's exact test and/or kji-square test). Factors concerning feeding (concentrate, hay, silage, graze), stalling last two weeks (outdoors, different floor types), other animals on the farm (cattle, goat, pig, horse), sharing pasture with other animals (cattle, goat, sheep, horse), purchase of animals, member of ram circles, disease problems, flock size and geography was considered.

In the report numbers of positive flocks are given in percent with 95 % confidence intervals based on a binomial distribution.

Results and discussion

Samples

In total, 592 sheep flocks were sampled; 94 flocks in 2006 and 498 flocks in 2007. Samples from seven flocks were discarded as not suited for analyzes. From most of the farms (449), 50 single samples were collected, but number of samples varied between nine and 51 samples with a mean of 48 (Table 1). The sheep flocks were randomly selected and distributed on the various counties as shown in Table 2.

Table 1. Number of sheep flock sampled and analysed

Number of samples collected per flock	Number of sheep flocks
<10	1
11-20	12
21-30	14
31-40	28
41-50*	537
Total	**585**

*From one farm there were 51 collected samples

Occurrence of *E. coli* O26, O103 and O157

The samples from both 2006 and 2007 (585 sheep flocks) were analyzed for *E. coli* O103 and O157 while only samples from 2007 (491 sheep flocks) were analyzed for *E. coli* O26 (Table 2). *stx*- and *eae*-positive *E. coli* O26 was detected in four sheep flocks (0.8 % (0.2 – 2.1 %)), stx_1- and *eae*-positive *E. coli* O103:H2 was detected from four sheep flocks (0.7 % (0.2 – 1.7 %)) and *stx*- and *eae*-positive *E. coli* O157:H7 from five sheep flocks (0.9 % (0.3 – 2.0 %)). The survey did not detect any *stx*-positive *E. coli* O103:H25 (0 % (0 – 0.6 %)). The survey documented a very low occurrence of *stx*- and *eae*-positive strains of *E. coli* O26, O103:H2 and O157:H7 in sheep in Norway with < 1 % of the sheep flocks positive for these serogroups/-types. These results correspond well with the low number of reported human cases in Norway.

The results from 2007 for *stx*- and *eae*-positive *E. coli* O157:H7 (0.4 % (0.05 – 1.5 %)) showed a lower occurrence compared to the results from 2006 where 3.2 % (0.7 – 9.0 %) of the sheep flocks were positive. This is probably due to the change in enrichment and probably not a true decline in prevalence.

No sorbitol fermenting (SF) *E. coli* O157 (0 % (0-0.6 %)) was detected in this survey. It is more complicated to detect SF *E. coli* O157, but if these had been present in a sample this would have given a positive ELISA-reaction for O157 and thereby an indication to look for SF *E. coli* O157 in the cases where no typical *E. coli* O157 could be detected.

For *E. coli* O26, O103:H2 and O103:H25 the survey showed higher numbers of *stx*-negative and *eae*-positive strains than of *stx*-positive and *eae*-positive strains. *stx*-negative and *eae*-positive *E. coli* O26 was detected from 79 sheep flocks (16.1 % (13.0 – 19.6 %)), *stx*-negative and *eae*-positive *E. coli* O103:H2 from 18 sheep flocks (3.1 % (1.8 – 4.8 %)) and *stx*-negative and *eae*-positive *E. coli* O103:H25 from 34 sheep flocks (5.8 % (4.1 – 8.0 %) (Table 2).

Up to five pooled samples and several isolates per pooled sample were investigated without detecting any *stx*-positives of *E. coli* O103:H25. It is unlikely that all the *stx*-negative and *eae*-positive *E. coli* O103:H25 have lost their *stx* phages during cultivation. This show that there is a reservoir of *stx*-negative and *eae*-positive *E. coli* O103:H25 in Norwegian sheep. However, it may also be a small reservoir of *stx*-positive and *eae*-positive *E. coli* O103:H25 (0 % (0-0.6 %)) in sheep although these were not detected in this survey.

In addition to the results shown in table 2, *stx*-negative and *eae*-negative *E. coli* O26 was detected from 20 sheep flocks and *stx*-negative and *eae*-negative *E. coli* O103 from 147 sheep flocks. These were not H-typed.

A risk factor analyzes for occurrence of *stx*- and *eae*-positive *E. coli* O26, *stx*- and *eae*-positive *E. coli* O103:H2, and *stx*- and *eae*-positive *E. coli* O157 could not be performed due to the low number of farms in which these serotypes were found.

An association was found between occurrence of *stx*-negative and *eae*-positive *E. coli* O26 and factors representing feeding and geographic area (p-value < 0.1), between *stx*-negative and *eae*-positive *E. coli* O103:H2 and factors representing stalling last two weeks (p-value < 0.1), and between *stx*-negative and *eae*-positive *E. coli* O103:H25 and factors representing stalling last two weeks and sharing pasture with other animals (p-value < 0.1). Multivariate analyzes will be performed to further explore the various factor's importance for the occurrence.

There were in addition several farmers with sheep positive for *stx*-negative and *eae*-positive *E. coli* O26 and with *stx*-negative and *eae*-positive *E. coli* O103:H25 that reported having problems with lamb diarrhoea during the previous spring (p-value < 0.1).

Though *eae*-positive *E. coli* O26 internationally have been associated with diarrhoea in lamb and calves, their importance as cause of diarrhoea is still unclear. *E. coli* O103:H25 have not previously been associated with diarrhoea in animals.

Table 2. Number of sheep flocks per county positive for *E. coli* O26, *E. coli* O103:H2, *E. coli* O103:H25 and *E. coli* O157:H7, and their virulence factors (*stx* and *eae*)

	Number of sheep flocks planned being sampled		Number of sheep flocks examined		Number of positive sheep flocks						
					E. coli **O26***			*E. coli* **O103:H2**		*E. coli* **O103:H25**	*E. coli* **O157:H7**
County	**2006**	**2007**	**2006**	**2007**	*stx_1*- **and** *eae*-**positive**	*stx_2*- **and** *eae*-**positive**	*stx*-**negative and** *eae*-**positive**	*stx_1*- **and** *eae*-**positive**	*stx*-**negative and** *eae*-**positive**	*stx*-**negative and** *eae*-**positive**	*stx*- **and** *eae*-**positive**
Østfold	0	3	0	2	-	-	-	-	-	-	-
Akershus	1	10	1	11	-	-	4	-	1	-	-
Hedmark	4	22	3	17	-	-	4	-	2	-	-
Oppland	10	51	10	50	-	-	6	-	1	4	2[#]
Buskerud	5	27	5	25	-	-	2	-	1	4	-
Vestfold	1	3	1	3	-	-	-	-	-	-	-
Telemark	5	13	5	7	-	-	1	1	-	-	-
Aust-Agder	3	5	3	0	-	-	-	-	-	-	-
Vest-Agder	2	13	2	12	-	-	1	-	1	-	-
Rogaland	17	95	17	97	-	-	11	-	1	5	1
Hordaland	9	66	9	62	-	-	11	1	2	4	-
Sogn og Fjordane	9	56	8	56	-	1	3	-	4	2	-
Møre og Romsdal	9	37	9	36	-	-	7	-	-	7	-
Sør-Trøndelag	7	28	5	27	-	2	8	-	1	3	1
Nord-Trøndelag	5	27	5	28	-	-	8	-	1	2	1
Nordland	5	40	5	36	-	-	6	2	3	1	-
Troms	7	18	5	16	-	-	4	-	-	2	-
Finnmark	1	6	1	5	1	-	2	-	-	-	-
Ukjent	-	-	0	1	-	-	1	-	-	-	-
Total	**100**	**520**	**94**	**491**	**1**	**3**	**79**	**4**	**18**	**34**	**5**
Prevalence (%)					**0,8**		**16,1**	**0,7**	**3,1**	**5,8**	**0,9**
95 % Confidence interval					**0,2 - 2,1**		**13 - 19,6**	**0,2 - 1,7**	**1,8 - 4,8**	**4,1 - 8,0**	**0,3 - 2,0**

*only the samples from 2007 were analyzed for *E. coli* O26 [#]one of these was both *stx_1*- and *stx_2*-positive, the other four were *stx_2*-positive

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The Norwegian Food Safety Authority (NFSA) is a governmental body whose aim is to ensure through regulations and controls that food and drinking water are as safe and healthy as possible for consumers and to promote plant, fish and animal health and ethical farming of fish and animals. We encourage environmentally friendly production and we also regulate and control cosmetics, veterinary medicines and animal health personnel. The NFSA drafts and provides information on legislation, performs risk-based inspections, monitors food safety, plant, fish and animal health, draws up contingency plans and provides updates on developments in our field of competence.

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