Occurrence of potentially human-pathogenic *Escherichia coli* O103 in Norwegian sheep

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The investigation of an outbreak of hemorrhagic uremic syndrome in Norway in 2006 indicated that the outbreak strain *Escherichia coli* O103:H25 could originate from sheep. A national survey of the Norwegian sheep population was performed, with the aim of identifying and describing a possible reservoir of potentially human-pathogenic *E. coli* O103, in particular of the H-types H2 and H25. The investigation of fecal samples from 585 sheep flocks resulted in 1222 *E. coli* O103 isolates that were analyzed for the presence of *eae* and *stx* genes, while a subset of 369 isolates was further examined for flagellar antigens (H-typing), *stx* subtypes, *bfpA* and *astA*, and molecular typing by pulsed-field gel electrophoresis (PFGE).

The total ovine *E. coli* O103 serogroup was genetically diverse by number of H-types, virulotypes and PFGE banding patterns identified, although a tendency of clustering towards serotypes was seen. The flocks positive for potentially human-pathogenic *E. coli* O103 were geographically widely distributed and no association could be found to county or geographical region.

The survey showed that *eae* *stx* *E. coli* O103, probably non-pathogenic to humans, is very common in sheep with 27.5% positive flocks. Moreover, the study documented a low prevalence (0.7%) of potentially human-pathogenic Shiga toxin-producing *E. coli* O103:H2, while STEC O103:H25 was not detected. However, 3.1% and 5.8% of the flocks were positive for Enteropathogenic *E. coli* O103 belonging to H-type H2 and H25, respectively. These are of concern as potentially human pathogens by themselves, but more important as possible precursors for human-pathogenic STEC.
INTRODUCTION

Escherichia coli is mainly found as an intestinal commensal, although several groups of E. coli may cause disease such as diarrhea in humans and animals (1, 2). Shiga toxin-producing (STEC) and atypical enteropathogenic E. coli (aEPEC) are both zoonotic E. coli pathogroups with animal reservoirs. An array of virulence characteristics have been described for both STEC and aEPEC, and some, such as the adherence factor intimin (eae), is common in both groups (2, 3). In addition to intimin, STEC also possess one or more variants of Shiga toxin (Stx) that are encoded by stx1 and/or stx2 or variants thereof, and which are the major determinants of STEC pathogenicity (2).

E. coli O103:H2 is one of the most common non-O157 STEC serotypes isolated from human cases in Europe. Sporadic cases of human infections caused by E. coli O103 belonging to other H-types than H2 have also been reported, such as E. coli O103:H11 in Japan (4) and in Canada (5). A severe human outbreak was reported in Norway in 2006, caused by an stx2+E. coli O103 with the rare H-type; H25 (6). Human cases of STEC O103:H25 have sporadically been reported (7-12), but in contrast to the Norwegian O103:H25 outbreak strain, these have been reported as stx1+ (when stx properties have been given).

Although several studies of E. coli O103 in animal have been conducted (reviewed in (13), most of these have been limited, either by using small sample sizes or by focusing only on the isolation of STEC O103 and not E. coli O103 in general. Also, most studies have been performed on material from cattle, and consequently the knowledge of aEPEC O103 in other potential animal reservoirs, such as sheep, is limited. Furthermore, possible occurrence of STEC O103 of other H-types than H2 is unknown as H-typing has not always been conducted. In the previously mentioned Norwegian outbreak, sheep were incriminated as the original reservoir for the outbreak strain of STEC O103:H25. This was because fermented sausages mainly consisting of mutton were shown to be the outbreak food source (6).
addition, *stx* negative strains of *E. coli* O103:H25 were isolated both from fermented sausages, ovine meat and ovine fecal samples during the outbreak investigation. The aim of the present study was to describe the occurrence of *E. coli* O103 in Norwegian sheep, and to identify a possible reservoir of potentially human-pathogenic *E. coli* O103 such as STEC and EPEC. In addition, risk factors for flocks being positive for potentially human-pathogenic *E. coli* O103 were to be investigated.

**MATERIAL & METHODS**

**Study design**

The study was designed as a cross sectional study with sheep flock as the unit. A total of 620 flocks that had at least 30 sheep older than 1 year were randomly selected from the Register of Production Subsidies (RPS). This register includes more than 95% of all commercial sheep flocks in Norway. One hundred flocks were selected from flocks registered in the RPS as of 01.01.2006 and 520 were selected from flocks registered as of 01.01.2007 as previously described (14). Sampling was conducted during the autumns of 2006 and 2007, respectively. From each flock, 50 single fecal samples from the youngest animals (lambs first, then one-year olds etc.) were requested. A two-page questionnaire on flock characteristics and management factors was filled in at the time of sampling.

**Fecal samples**

Fecal samples were collected by digital rectal retrieval and transported in coolers to the laboratory where they arrived the following day. Approximately 2.5 to 5 g fecal material from 10 animals per flock were pooled to give a maximum of five samples per flock. If the number of samples from a single flock was not a multiple of 10, the last pooled sample consisted of samples from the remaining one to nine individual samples. The following analyses were...
either started immediately upon arrival at the laboratory or the samples were frozen at -80°C until analyzed.

Detection and isolation of *E. coli* O103

The pooled samples were diluted 1:10 with 37°C pre-warmed buffered peptone water (Difco, Detroit, MI) and pre-enriched for 18 to 24 h at 41.5 ± 1°C. Detection of *E. coli* O103 was performed by automated immunomagnetic separation-enzyme-linked immunosorbent assay (AIMS-ELISA) and plating of ELISA positive samples onto MacConkey agar (Difco, Detroit, MI) with 4% cefixime-tellurite supplement (Dynal Invitrogen, Oslo, Norway) and washed sheep blood agar containing 10 mM CaCl$_2$ as previously described (14). Up to 10 colonies showing a typical *E. coli* appearance were tested by slide agglutination with *E. coli* O103 antiserum (Sifin, Berlin, Germany). Up to five of the positive colonies were subcultured onto blood agar plates before being tested further by slide agglutination to rule out autoagglutination. For species identification, the indole reaction, oxidase test and Rapid One test kit (Remel Inc., Atlanta, GA) were used.

Serotyping and virulence characterization

A conventional serotyping method with O103 antisera (Statens Serum Institut, Copenhagen, Denmark) was used to confirm presumptive *E. coli* O103 isolates. Confirmed *E. coli* O103 isolates were screened for the virulence genes *stx*$_1$, *stx*$_2$ and *eae* with a multiplex polymerase chain reaction (PCR) assay using 16S rRNA gene as a control (15). Subtyping of *stx* was performed as described by Scheutz et al. (16).

A selection of isolates was further examined for the virulence genes *astA* (17) and *bfpA* (18) using conventional PCR. The selection was based on the following criteria: one isolate from each pooled sample was selected (a maximum of five possible from each flock).
If isolates from the same pooled sample had different virulence patterns as defined by PCR for eae and stx, then more than one isolate was further included, so that all virulotypes from a sample should be represented.

Flagellar antigens were investigated by amplifying part of the fliC gene as described by Machado et al. (19). Flagellar antigens H2 and H25 were identified by evaluation of CfoI restriction pattern of the amplicons as previously described (20). Other H-types were investigated by sequencing the PCR product, and designing specific primers for fliC_{H16}, fliC_{H21} and fliC_{H56} for use in PCR. One fliC amplicon from each of the H-types H16, H21 and H56 were sequenced and the sequence confirmed by Basic local alignment search tool before used as positive controls in the PCRs for fliC_{H16}, fliC_{H21} and fliC_{H56}. Boiled bacterial lysates were used as DNA template in all PCRs. Primers and positive controls for each PCR are listed in the supplemental material (Table S1).

**Pulsed-field gel electrophoresis**

PFGE was carried out using XbaI (Sigma, St. Louis, MO) restriction endonuclease as described in Sekse et al. (14). PFGE banding patterns were compared using a combination of visual inspection and the Bionumerics software program, version 6.1 (Applied Math NV, Ghent, Belgium). Dendrograms were generated using band-based Dice similarity coefficient and the unweighted pair group method using geometric average (UPGMA) with 1.1% position tolerance and 0.8% optimization. A cut-off level of 97% similarity was used to define a PFGE profile. Multi-dimensional scaling (MDS) was used to show a three-dimensional representation of the PFGE similarity matrix. The algorithm used calculating MDS in BioNumerics is a Principal Coordinates Analyses.

**Statistical analyses**
The crude county prevalences of *E. coli* O103 with various H-types and virulence profiles, and the corresponding 95% confidence interval (CI), were estimated assuming a binomial distribution by using the function `binom.test` in R software (R-2.15.3 for Windows; R Development Core Team [http://www.r-project.org/]).

The relationship between the occurrence of *eae*⁺ *E. coli* of serotypes O103:H2 and O103:H25 in a sheep flock and potential risk factors was analyzed using flock as the statistical unit and flocks having at least one positive sample for *eae*⁺ *E. coli* O103:H2 or O103:H25, respectively, regarded as positive. Potential risk factors considered were; feed the last two weeks (concentrates, hey, silage, pasture), housing the last two weeks (outdoors, slatted floor, straw bedding, and non-slatted concrete or wooden floor), other animal species on the farm (cattle, goat, pig or poultry), sharing pasture with other animals (cattle, goat, other sheep flocks), purchase of animals within the last four months, being a member of a ram circle, flock size, and geographical region. All variables were initially run by univariable unconditional logistic regression analysis. Variables with Wald $\chi^2$ p-values < 0.20 were selected for multivariable analysis. The multivariable logistic regression was performed by backward stepwise deletion of variables with $p$-values > 0.05. For each step the single least significant term was removed until there was no significant difference between the full and the reduced models. Adjusted odds ratio estimate was used as a measure of association between the response variable and the explanatory variable. The statistical analysis was performed using proc logistic in SAS® 9.1.3 for Windows (SAS Institute Inc., Cary, NC).
RESULTS

Received samples

Fecal samples from 592 (95.5%) of the selected 620 sheep flocks were received by the laboratory; 94 in 2006 and 498 in 2007. Samples from seven flocks were regarded unsuited for analysis, leaving samples from 585 flocks for further investigation. The exact number of 50 individual samples was received from 449 flocks (76.8%). For the remaining flocks, the number of samples varied from 9 to 51 with a mean of 47. There was a deviation of number of flocks examined from a few counties compared to what was planned, and although this may have created a bias, we do not expect it to have had any major effect on the final conclusions.

E. coli O103 isolation, H-typing and virulence characteristics

Of the 585 flocks, 185 (31.6%, 95% CI [27.9 - 35.6%]) were identified as positive for E. coli O103. A total of 1222 E. coli O103 were isolated from these flocks and subjected to PCR for eae and stx. The occurrence of positive ELISA results without a following isolation of E. coli O103 was only seen in a few cases and the freezing of samples was therefore not regarded to have had any major effect on the overall conclusions.

Only five of the 1222 isolates were both eae+ and stx+, and all contained stx1 identified as subtype stx1a. These originated from four flocks (0.7% [0.2 - 1.7 %]). Isolates from 52 flocks were characterized as eae+ stx- E. coli O103 (8.9% [6.7 - 11.5%]). In addition, eae- stx- E. coli O103 was detected from 143 flocks (24.4% [21.0 - 28.1%]), in which eae+ stx- O103 isolates and eae- stx+ E. coli O103 isolates had been detected in twelve and one of these flocks, respectively.

A subset of 369 E. coli O103 isolates were further investigated for H-type, astA and bfpA, and subjected to PFGE. The eae+ stx+ isolates were all identified as E. coli O103:H2,
while the eae+ stx- E. coli O103 isolates possessed either the H2 or the H25 antigen. The eae-
stx- E. coli O103 isolates belonged to the following H-types: H2, H6, H8, H14, H16, H19, H21, H38, H45 and H56. None of the isolates harbored the bfpA gene, while 30 of them possessed the astA gene. Only one of the astA+ isolates also possessed eae, and this was an E. coli O103:H2. The remaining astA+ isolates were negative for the other tested virulence genes and were of different H-types; H2, H38, H45 and H56. Table 1 shows an overview of serotypes and virulence genes identified among the 369 isolates.

Occurrence of E. coli O103:H2 and E. coli O103:H25

The five eae+ stx+ E. coli O103:H2 isolates originated from four flocks (0.7% [0.2 - 1.7 %]) from different parts of Norway (Figure 1). From 18 flocks (3.1% [1.8 - 4.8%]), eae+ stx+E. coli O103:H2 isolates were detected, i.e. 27 of the subset of 369 selected isolates. These positive flocks positive were scattered around in Norway (Figure 1). The majority of the E. coli O103:H2 (170/369) were eae+ stx+ and originated from 84 flocks (14.4% [11.6 - 17.5%]), giving a total of 103 flocks positive for E. coli O103:H2 (i.e. independent of detected virulence genes). Table 2 shows the number of positive flocks per region and county.

As shown in Table 2, no stx+E. coli O103:H25 were detected in the survey (0% [0 - 0.6 %]). However, all the E. coli O103:H25 isolates detected were identified as eae+ and originated from 34 flocks giving a flock level prevalence of 5.8% [4.1 - 8.0 %]. The 34 flocks were located in ten different counties (Table 2 and Figure 1).

Molecular typing of E. coli O103 by PFGE

Of the 369 E. coli O103 isolates, 349 were genotyped by PFGE, while the remaining 20 isolates (one of serotype O103:H38, one of O103:H45 and 18 of O103:H2) were non-typeable using XbaI restriction endonuclease due to DNA degradation. A total of 202 PFGE
profiles were detected among the 349 E. coli O103 isolates. With a few exceptions, isolates of the same H-type and virulence profile from the same flock usually produced identical banding patterns. For instance, PFGE comparison of E. coli O103:H2 from 16 flocks showed minor differences (one to three band differences) between the isolates in ten of these flocks, while the six remaining flocks had isolates with more distinct banding patterns. PFGE comparison of E. coli O103:H25 from one flock showed two band differences in two isolates, while a third isolate produced a more distinct banding pattern compared to the other two isolates. On the other hand, identical PFGE profiles were identified for isolates from different flocks; nine occasions for E. coli O103:H2, one occasion for E. coli O103:H25, five occasions for E. coli O103:H16, two occasions for E. coli O103:H21 and one for E. coli O103:H14. The number of PFGE profiles per serotype is shown in Table 1, and a dendrogram showing PFGE profiles of all 349 isolates is included as a supplemental file. Figure 2 shows a multi-dimensional scaling of the similarity matrix of the 349 PFGE profiles.

**Risk factors for eae⁺ E. coli O103**

No risk factors were found to be associated with the occurrence of sheep flocks with animals positive for eae⁺ E. coli O103:H2 (stx⁺ and stx⁻) in the final multivariable assessment. When considering eae⁺ E. coli O103:H25, "Sharing of pasture with own cattle" was found to decrease the occurrence of eae⁺ E. coli O103:H25 (Odds ratio 9.3 [1.2 - 68.8], p-value = 0.03), while "Sharing of pasture with goats owned by others" was found to increase the occurrence (Odds ratio 0.29 [0.09 - 0.93], p-value = 0.04). The number of flocks positive for eae⁺ stx⁻ E. coli O103:H2 was not sufficient to perform statistical analyses on possible risk factors for the occurrence of this virulotype.

**DISCUSSION**
The present survey documented that there is a low prevalence of eae+ stx+ E. coli O103:H2 in the Norwegian sheep population. This O103 virulotype is well known to be a human pathogen. Moreover, the study did not detect any eae+ stx+ E. coli O103:H25. However, the relatively high prevalence of eae+ stx E. coli O103 (H2 and H25) is of concern as these may be precursors for STEC. Also, the survey shows that eae- stx- E. coli O103 is a very common and diverse E. coli serogroup in sheep with as much as 27.5% positive flocks.

As previously described (14), this study consists of a representative sampling of a high number of sheep flocks from all parts of Norway and, to the authors’ knowledge, no other countries have conducted comparable national surveys in sheep. One recent Scottish study has, however, investigated sheep presented for slaughter in four Scottish abattoirs (21), giving individual sheep prevalences. Neither this study, nor two previous Norwegian studies that examined for all possible O-groups of stx+ E. coli among sheep (22, 23), managed to detect any stx+ E. coli O103. In contrast, four eae+ stx+ E. coli O103:H2/H- were detected among 60 ovine STEC investigated strains from Switzerland (24). However, other studies have also failed in detecting eae+ stx+ E. coli O103 from sheep (25-32), supporting the results of the present study suggesting that O103 may be an uncommon or low prevalent eae+ STEC serogroup in this species.

Two studies has been performed in cattle farms in Norway without detecting any eae+ stx+ E. coli O103 (33, 34), although one of them detected eae- stx+ E. coli O103 in 3.2% of the herds. These studies were performed more than 10 years ago and new studies should be performed to see whether the eae+ stx+ E. coli O103 is low prevalent in Norwegian cattle herds as well.

The finding of eae+ stx+ E. coli O103 in ruminants has occasionally been reported (25, 35, 36), but knowledge of the prevalence of eae+ stx+ E. coli O103 in the ruminant reservoir is sparse. Evans et al. (21) found only 0.6% of the tested sheep positive for eae+ stx+ E. coli
The present study, however, documents a relatively high flock occurrence of \( eae^{+} stx^{-} \) *E. coli* O103 in Norwegian sheep, i.e. of the two serotypes *E. coli* O103:H2 and *E. coli* O103:H25. Similar to the occurrence of *E. coli* O26:H11 (14), the occurrence of \( eae^{+} stx^{-} \) *E. coli* O103:H25 was higher in middle Norway (10.9%) than in the other regions (south east: 5.1%, west: 4.4% and north: 4.4%). However, in contrast to the occurrence of *E. coli* O26:H11, the factor “region” was not significantly associated to the occurrence of neither \( eae^{+} stx^{-} \) *E. coli* O103:H25 nor \( eae^{+} \) O103:H2 (\( stx^{+} \) and \( stx^{-} \)) in the statistical analyses. In contrast to the results in sheep in Norway, the two previous studies in cattle detected only a few \( eae^{+} stx^{-} \) *E. coli* O103 isolates (33, 34), indicating that \( eae^{+} stx^{-} \) *E. coli* O103 may be less common in Norwegian cattle than in sheep. However, these studies were not as extensive as the current study and caution should be taken making comparisons to these results.

The \( eae^{+} stx^{-} \) *E. coli* O103 sheep reservoir is genetically diverse as demonstrated by the number of PFGE profiles found. Identical PFGE profiles were identified from a few flocks, both from contiguous counties and from counties not sharing any border. The authors have no explanation on how a common isolate could have been spread as there is no known epidemiological link between these farms. Feed concentrates used in Norway is heat treated and not considered a risk for spreading *E. coli*. Also, transport of sheep is not allowed between counties, though contacts between sheep of neighboring counties cannot be excluded as it is common to have the sheep free grazing on common pasture areas. This was, however, not a risk factor found to be associated in the statistical analyses. In the final multivariable assessment, "Sharing of pasture with own cattle" was found to decrease the occurrence and "Sharing of pasture with goats owned by others" was found to increase the occurrence, of \( eae^{+} stx^{-} \) *E. coli* O103:H25. We find it, however, difficult to explain these factors biologically.
regardless of owner as possible risk factors, did not result in any statistical association (data not shown). Further investigation would be needed to investigate this aspect thoroughly.

During the 2006-outbreak of *E. coli* O103:H25 in Norway, only *eae*<sup>+</sup> *stx*<sup>-</sup> *E. coli* O103:H25 was isolated from food and sheep (6, 37), and it was argued that the detected *eae*<sup>+</sup> *stx*<sup>-</sup> *E. coli* O103:H25 probably had lost their *stx* genes during cultivation. This has also been reported for other STEC (38-40). In the present study, up to five pooled samples per flock of a total of 585 flocks, and several isolates per positive pooled sample were investigated without detecting any *stx*<sup>+</sup> of *E. coli* O103:H25. We consider it unlikely that all the isolated *eae*<sup>+</sup> *stx*<sup>-</sup> *E. coli* O103:H25 should have lost their *stx* genes during enrichment. The findings of *eae*<sup>+</sup> *stx*<sup>-</sup> *E. coli* O103:H25 in sheep have also been reported by others (41, 42), while there to the authors’ knowledge have not been reported any *stx*<sup>+</sup> of *E. coli* O103:H25 from the animal reservoir. However, although not detected in the present nationwide study, it cannot be excluded that a small reservoir of *stx*<sup>+</sup> *E. coli* O103:H25 do exist among sheep in Norway.

Whether the detected sheep reservoir of *eae*<sup>+</sup> *stx*<sup>-</sup> *E. coli* O103:H2 and O103:H25 have a potential as human pathogens by themselves, is not clear. A previous study from the county Sør-Trøndelag investigating EPEC in children under the age of five (with and without diarrhea), did not report any findings of EPEC O103:H2 or O103:H25 (43). Although in the present study there was a tendency of clustering towards serotypes, the PFGE patterns of both the *E. coli* O103:H2 and the *E. coli* O103:H25 were relatively diverse. Beutin et al. (44) compared EPEC and STEC O103:H2 from humans, animals and meat originating from several countries, and found the PFGE patterns of *E. coli* O103:H2 to be diverse as well. The number of isolates from animals and meat were, however, limited. Also, they report STEC O103:H2, including two EPEC isolates which they conclude to be precursors of STEC, to carry the subvariant *eae*-□, while the ones they call EPEC O103:H2 usually carried the *eae*-β subvariant. This has also been reported in a previous study by Oswald et al. (45). In the
present study, subtyping of eae has not been performed. However, 51 of the isolates have 
been subtyped as part of another study in our laboratory (L. N. Nesse, C. Sekse, K. Berg, K. 
C. S. Johannesen, H. Solheim, L. Vestby and A. M. Urdahl, unpublished data), indicating that 
ovine stx\(^+\) eae\(^+\) O103:H2 carry the eae-\(\square\) subtype. This is in contrast to the findings of Beutin 
et al. (44) and Oswald et al. (45), but their studies included only a limited numbers of EPEC 
O103 and not any ovine isolates. Whether this finding of the eae-\(\square\) subtypes in stx- O103:H2 
is of importance for the isolates’ human pathogenicity, is unknown. 
The previously mentioned study by Nesse and co-workers’, indicate that the ovine E. 
coli O103:H25 in the present study have the eae-0 subtype, as also reported by others for both 
ovine and human isolates (7, 41, 42). However, preliminary comparison of PFGE patterns of 
the ovine E. coli O103:H25 isolates to human E. coli O103:H25 isolates from the Norwegian 
2006 outbreak indicates that only a few of the ovine isolates cluster closely with the human 
isolates (data not shown). A more thorough genomic comparison, including PFGE data, to 
investigate the association between ovine and human strains of E. coli O103:H25 is in 
progress. The detected reservoir of eae\(^+\) stx\(^-\) E. coli O103:H25 may, however, be a 
concern by itself in the context of being precursors for stx positives. Sekse et al. (46) showed 
that isolates of eae\(^+\) stx\(^-\) E. coli O103:H25 may have potential to be infected by stx 
bacteriophages and become stx\(^+\). Recently, L’Abée-Lund et al. (47) found that stx\(^-\) and eae\(^+\) E. 
coli O103:H25 isolates from both patients and food carried an stx negative bacteriophage in 
the same insertion site as the Stx2-encoding bacteriophage of the human eae\(^+\) stx\(^+\) E. coli 
O103:H25 2006 outbreak strain. Whether this is the case for the eae\(^+\) stx\(^-\) E. coli O103:H25 
from the sheep reservoir as well and whether this would influence the ability to acquire stx 
bacteriophages, needs to be further investigated. 
The present survey focused on E. coli O103 in general, giving results on eae\(^-\) stx\(^-\) 
isolates in addition to aEPEC and STEC. Although there was a tendency of clustering towards
serotypes as visualized by the multi-dimensional scaling, the *E. coli* O103 serogroup was genetically diverse as demonstrated by H-type, virulotype and PFGE banding pattern. The high prevalence of eae⁺ stx⁻ *E. coli* O103 including both H2 and other H-types, in addition to 3.1% and 5.8% of eae⁻ stx⁻ *E. coli* O103:H2 and *E. coli* O103:H25 positive flocks, respectively, emphasizes the importance of conducting both virulence characteristics and H-typing, and not concluding from detection of O-group by itself. This is in concurrence with the findings of Beutin et al. (44) that from virulence characterization, PFGE analyses and molecular typing of housekeeping genes conclude that the O serogroup as such cannot be taken as an indicator of genetic relationship between *E. coli* isolates.

MDS is not commonly used to visualize the similarity among PFGE profiles, but is popular for analyzing other fingerprinting methods. The authors find MDS especially useful and illustrative for presenting a rather large and diverse PFGE dataset as in the present study. Compared to the traditional dendrogram that expands over several pages, MDS can be presented in one small figure making it easier to see the whole picture.

Previous to our survey, there was sparse knowledge on the occurrence and distribution of *E. coli* O103 in Norwegian sheep flocks. Documentation of the prevalence of potentially human-pathogenic *E. coli* O103 is of importance for risk assessments. The finding of such relatively high occurrence of eae⁺ stx⁻ *E. coli* O103 in sheep flocks all over Norway was surprising and the pathogenic potential of these isolates needs to be further investigated by comparison with human isolates.
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Figure legends:

**FIG 1** Distribution of flocks positive for A) *eae*+ *E. coli* O103:H2 and B) *eae*+ *E. coli* O103:H2. Red dots marks flocks with *str*+ isolates. Geographical locations of the flocks are set to the centroid of the municipality. The regions, as used in Table 2, are shown by thick lines, and the letters designates the counties: O=Oslo, Ø=Østfold, A=Akershus, HE=Hedmark, OP=Oppland, BD=Buskerud, V=Vestfold, TK=Telemark, AA=Aust-Agder, VA=Vest-Agder, R=Rogaland, H=Hordaland, SF=Sogn og Fjordane, M=Møre og Romsdal, ST=Sør-Trøndelag, NT=Nord-Trøndelag, N=Nordland, T=Troms, F=Finmark.

**FIG 2** Multi-dimensional scaling of 349 pulsed-field gel electrophoresis profiles of ovine *E. coli* isolates found in a study of Norwegian sheep flocks in 2006 and 2007. The distance between two isolates reflects the similarity between them. The various serotypes are shown in different colours.
<table>
<thead>
<tr>
<th>Serotype</th>
<th>N° of isolates</th>
<th>N° of flocks</th>
<th>stx₁⁺</th>
<th>stx₂⁺</th>
<th>eae⁺</th>
<th>bfpA⁺</th>
<th>astA⁺</th>
<th>PFGE profiles⁹</th>
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<td>185ᵈ</td>
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⁹PFGE profiles among 349 isolates, 20 isolates (one of serotype O103:H38, one of O103:H45 and 18 of O103:H2) were nontypeable using XbaI restriction endonuclease

ᵇBoth eae⁺ and eae⁻ isolates from three flocks

ᶜOne eae⁻ stx⁺ isolate

ᵈN° of flocks *E. coli* O103 was detected from. Some flocks were positive for more than one serotype/virulotype, so this column adds up to 219
TABLE 2 Number of sheep flocks positive for *E. coli* O103 per Norwegian region and county. SE = South-Eastern, W = Western, M = Middle and N = Northern Norway.

<table>
<thead>
<tr>
<th>Region County</th>
<th>Other H-types, eae- and stx-</th>
<th>H2</th>
<th>H25, eae- and stx-</th>
<th>Other H- types, eae- and stx-</th>
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<tr>
<td></td>
<td>No. of flocks</td>
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<td>stx1+</td>
<td>stx-</td>
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<td>0.2 - 1.7</td>
<td>1.8 - 4.8</td>
<td>11.6 - 17.5</td>
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