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Citation for published version:

Digital Object Identifier (DOI):
10.1128/AEM.70.12.7456-7465.2004

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Applied and Environmental Microbiology

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Shedding Patterns of Verocytotoxin-Producing *Escherichia coli* Strains in a Cohort of Calves and Their Dams on a Scottish Beef Farm

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Received 26 February 2004/Accepted 6 August 2004

Rectal fecal samples were taken once a week from 49 calves on the same farm. In addition, the dams of the calves were sampled at the time of calf birth and at the end of the study. Strains of verocytotoxin-producing *Escherichia coli* (VTEC) were isolated from these samples by using PCR and DNA probe hybridization tests and were characterized with respect to serotype, verocytotoxin gene (*vtx*) type, and the presence of the intimin (*eae*) and hemolysin (*ehxA*) genes. A total of 170 VTEC strains were isolated during 21 weeks from 130 (20%) of 664 samples from calves and from 40 (47%) of 86 samples from their dams. The characteristics of the calf strains differed from those strains isolated from the dams with respect to verocytotoxin 2 and the presence of the *eae* gene. In addition, no calf shed the same VTEC serogroup (excluding O?) as its dam at birth or at the end of the study. The most frequently detected serogroups in calves were serogroup O26 and provisional serogroup E40874 (VTEC O26 was found in 25 calves), whereas in dams serogroup O91 and provisional serogroup E54071 were the most common serogroups. VTEC O26 shedding appeared to be associated with very young calves and declined as the calves aged, whereas VTEC O2 shedding was associated with housing of the animals. VTEC O26 strains from calves were characterized by the presence of the *vtx2*, *eae*, and *ehxA* genes, whereas *vtx3* was associated with VTEC O2 and provisional serogroup E40874. The high prevalence of VTEC O26 and of VTEC strains harboring the *eae* gene in this calf cohort is notable because of the association of the O26 serogroup and the presence of the *eae* gene with human disease. No association between calf diarrhea and any of the VTEC serogroups was identified.

Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) causes a wide spectrum of diseases in humans ranging from mild diarrhea to severe diseases, such as hemorrhagic colitis and hemolytic-uremic syndrome (17). The production of VT by *E. coli* was first described by Konowalchuk et al. (20). Subsequent studies described two types of VT, VT1 and VT2, and showed that the VT gene (*vtx*) is phage encoded (27, 39). VTEC is also referred to as Shiga toxin-producing *E. coli*. There are seven established *vtx* subtypes, including *vtx1* (subtype), *vtx1c* (53), *vtx2* (subtype), *vtx2c*, *vtx2d*, *vtx2e*, and *vtx2f* (37).

Strains of VTEC belonging to serotype O157:H7 and nonmotile serotype O157 (O157:H?) are most commonly associated with severe human disease in the United Kingdom (47). However, infections with other VTEC strains, including strains belonging to serogroups O26, O103, O111, and O145, have been detected (48), and outbreaks caused by VTEC serogroups other than O157 have been described outside the United Kingdom (4, 24, 31). Some VTEC strains have the ability to cause attaching and effacing lesions in the human intestine and carry the intimin or *E. coli* attaching and effacing (*eae*) gene (16), which is part of a pathogenicity island called the locus of enterocyte effacement and is responsible for intimate attachment of VTEC to the intestinal epithelial cells (10, 16). There are at least 10 different intimin subtypes, including alpha (*α*), beta (*β*), gamma (*γ*), delta (*δ*), epsilon (*ε*), iota (*ι*), kappa (*κ*), theta (*θ*), zeta (*ζ*), and eta (*η*) (54). Some VTEC strains also produce a plasmid-encoded VTEC hemolysin (*ehxA*) (38). Strains of VTEC carrying the *vtx2*, *vtx2c*, and *eae* genes are thought to be associated with more severe human disease (11, 33).

VTEC, including serogroup O157, can be isolated from the feces of cattle, which represent an important source of human infection (1, 19). There is no evidence that VTEC is pathogenic for adult cattle, although some serogroups can cause diarrhea in calves (9, 36, 40, 41). Humans can become infected with VTEC by ingesting contaminated food or water or by transmission from infected animals or humans (47). However, it is not yet clear if all VTEC strains found in cattle and other animals are pathogenic for humans (31).

Previous studies have shown that VTEC strains harboring the *vtx1* and *eae* genes are isolated more frequently from calves than from adult cattle (2, 46). The epidemiology of VTEC in calves is interesting because of the possible association between certain VTEC strains and diarrhea in young animals (9, 36, 40, 41) and because *eae*-positive VTEC strains are isolated more commonly from patients with hemolytic-uremic syn-
drome than from patients with less severe diseases (3, 52). Furthermore, over the first couple of months of calf life the gut physiology changes and the immune system develops (13, 35), yet very little is known about how this alters the presence and characteristics of VTEC in calves.

A large study was carried out on a Scottish farm, on which rectal fecal samples were taken beginning at birth once a week from 49 calves on the same farm over a 5-month period. An analysis of the epidemiology on this farm of E. coli serogroups O26, O103, and O145, as detected by immunomagnetic separation (IMS), has been described previously (32). Further investigations in which PCR and DNA probes were used to detect vtx genes showed that during this study, the calf cohort shed many other VTEC serogroups. In this paper we describe the isolation of all strains of VTEC by a PCR-DNA probe hybridization method (which detects vtx genes) and the shedding patterns of VTEC serogroups in the herd.

MATERIALS AND METHODS

Fecal sampling. Per rectum fecal samples for this study were taken from 49 calves and their dams (n = 44) on a beef cattle farm in northern Scotland. The calves were born between August and November 2001 and were sampled weekly from birth until the end of the study in January 2002, except for a fortnight around Christmas (weeks 18 and 19) (Fig. 1). One calf died shortly after birth and was sampled once; a second calf died 4 weeks after birth and was sampled three times. Most dams were sampled at the time of calving and at the end of the sampling period; the exceptions were one dam which was sampled only at calving, one dam which was sampled only at the end of the sampling period, and a third dam which was sampled neither at calving nor at the end of the sampling period (Fig. 1). A total of 664 samples were taken from calves, and 86 samples were taken from dams. The presence or absence of calf diarrhea was noted in calves at the time of sampling. Sample collection was carried out in accordance with United Kingdom Government Home Office license requirements. From August until 9 November, the study animals were kept together in the same field on pasture. After 9 November the group was housed in a barn on straw bedding.

Identification of samples containing verocytotoxin genes by PCR. Within 48 h of sampling, 1 g of feces from each sample was suspended in 4 ml of phosphate-buffered saline, and 200 µl was added to 10 ml of buffered peptone water and incubated for 18 h at 37°C. The cultures were plated on MacConkey agar and incubated for 18 h at 37°C. Nutrient broths were inoculated with a sweep of mixed colonies from the MacConkey agar plates, incubated at 37°C for 2 to 4 h, and examined for the presence of the vtx2 and vtxc genes by PCR, as described previously (47).

Identification of VTEC by DNA hybridization. Mixed colonies from an original MacConkey agar plate, which were shown to contain verocytotoxin genes by PCR, were transferred by replica plating onto a nylon membrane placed on a nutrient agar plate prior to incubation at 37°C for 4 to 6 h. The membrane was prepared for hybridization by the method of Maniatis et al. (23). Individual VTEC colonies were identified by colony DNA hybridization with a mixture of the vtx2 and vtxc polynucleotide probes (43, 50). Up to five VTEC colonies detected by the probe were marked on the master plate and inoculated onto MacConkey agar. PCR was carried out as described above with pure growth to confirm that VTEC had been isolated.

Serotyping. Each isolate was serotyped by using the scheme described by Kaufman (18), which depends on identification of the heat-stable lipopolysaccharide somatic (O) and flagellar (H) antigens (14).

Strain characterization. Subtyping of vtx was carried out by using digoxigenin-labeled DNA probes for vtx2 and vtxc (50). vtx subtyping was performed by using the PCR-restriction fragment length polymorphism method described by Lin et al. (21). The eae gene and the ecf gene were detected by using PCR (30, 38), and intimin subtypes were determined by using a PCR-restriction fragment length polymorphism method described by Jenkins et al. (15).

Statistical analysis. All analyses were carried out by using S-plus (Insightful Corp, Seattle, Wash.). When we considered the changes with age or time with respect to (i) VTEC-positive samples or (ii) virulence factors and serogroups excreted for VTEC-shedding calves, generalized linear mixed effect models with binomial errors (to account for the binary data) were used, with a logit link between the observed binomial and unobserved normal scales (34). The calf identification number was entered as a random factor (to account for nonindependence of samples). Age and time were then added as linear covariate fixed effects, and whether calves were housed was added as a two-level class fixed effect. For the majority of analyses fixed effects were first added to univariate models singularly. When no fixed effects were significant, then only univariate results were reported. If more than one fixed effect was significant at the 5% level, the effects were added in a multivariate model in order of greatest significance, and the relative contributions of the fixed effects and their interactions were evaluated. In such cases the multivariate results are also reported below.

We were also interested in ascertaining the significance of any association among the presence of virulence factors, serogroups, and whether a sample came from a calf or a dam. Ideally, just the shedding by calves at birth and at the end of the study compared to the shedding by their dams at the same time would have been compared. However, there were very few calves that shed VTEC at these times. Therefore, generalized linear mixed-effect models with binomial errors were used to look at the presence of VTEC or virulence factors in calves and dams. The calf identification number was entered as a random variable to account for the nonindependence of samples. Odds ratios (OR) (with 95% confidence limits [95% CI] [8]) were calculated to determine whether a sample that came from a dam was more or less likely to have particular virulence factors than the samples from its calves.

Generalized linear models with Poisson errors were used to look at changes in the number of different VTEC serogroups excreted in a particular week (either sampling or age) or to see whether more serogroups were excreted during the housed period than during the nonhoused period (7). Data were square root transformed prior to analysis to obtain normalized residuals. Different numbers of samples were examined for a given week or age class, so the analysis was weighted by the number of samples examined per week. In addition, there was nonindependence of samples when we looked at the number of different serogroups observed in a week. Therefore, a dummy variable of the number of serogroups observed in the previous week was created as described by Syngc et al. (42). This information was added to the statistical model prior to age or sampling time. In all cases a Pvalue of <0.05 was taken to indicate significance.

RESULTS

Summary of strains isolated. A total of 170 VTEC strains were isolated during 21 weeks from 47% of the dam samples (40 of 86 samples) and 20% of the calf samples (130 of 664 samples) (Tables 1 and 2 and Fig. 1). The proportion of VTEC-positive samples isolated from calves was significantly lower than the proportion of VTEC-positive samples isolated dams (P < 0.001, regression coefficient [•r] = −0.649), and the OR indicated that samples from dams were 3.63 (95% confidence limit, 2.27 to 5.80) times more likely to be VTEC positive than from samples from calves.

The most commonly isolated VTEC serogroup obtained from dams was serogroup E54071 (Table 1 and Fig. 1) (the prefix E indicates a provisional new serogroup that has not yet been designated a formal O group), whereas the most frequently detected VTEC serogroup in calves was serogroup O26 (Table 2). Sixteen VTEC strains from dams and 35 VTEC strains from calves that could not be typed by the current serotyping scheme were designated O? VTEC serogroup O157 was not detected by the PCR-DNA probe method on this farm during this study. Of the five typeable serogroups that were detected in the dams, four were also detected in the calves (O26, O91, O113, and E54071). However, no calf excreted the same VTEC (excluding O?) as its dam at the time of calf birth (Fig. 1). In addition, 9 of 13 typeable VTEC serogroups obtained from the calves were not detected in any of the dams.

Shedding patterns in calves. During the course of the study there was no age-related change in the percentage of calves excreting VTEC (P = 0.514, •r = 0.002), and the prevalence was around 25% (Fig. 2a). While the percentage of calves
FIG. 1. Summary, by sampling week, of VTEC serogroups isolated from the 49 calves during the course of the study. Also included are the VTEC serogroups detected in the dams at the time of birth of the calves (Dam cb) and at the end of the study (Dam 21). Open boxes indicate weeks when samples were taken but no isolates were obtained. No sampling occurred during weeks 18 and 19. The cross-hatched areas show when all cattle on the farm were housed (9 November onward). Calf ID, calf identification number.
TABLE 1. Characteristics of verocytotoxin-producing E. coli isolated from dams on a Scotland beef farm between August 2001 and January 2002

<table>
<thead>
<tr>
<th>Serogroup&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Week isolated</th>
<th>vtx type</th>
<th>vtx&lt;sub&gt;2&lt;/sub&gt; subtype</th>
<th>eae (subtype)</th>
<th>ehec distribution&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>O8 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O26 (1)</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O91 (1)</td>
<td>3</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O91 (4)</td>
<td>21</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O113 (1)</td>
<td>8</td>
<td>2</td>
<td>2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O113 (2)</td>
<td>21</td>
<td>2</td>
<td>U&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E40874 (1)</td>
<td>8</td>
<td>1 + 2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E40871 (10)</td>
<td>21</td>
<td>1 + 2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O7 (1)</td>
<td>1</td>
<td>2</td>
<td>2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O113 (1)</td>
<td>8</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O113 (1)</td>
<td>10</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O113 (2)</td>
<td>21</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O84 (4)</td>
<td>1</td>
<td>2</td>
<td>2c (2), 2c (2), 2 + 2c (7)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>O20 (1)</td>
<td>7</td>
<td>1 + 2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O26 (1)</td>
<td>21</td>
<td>2</td>
<td>2c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Samples were collected either at the time of calf birth (weeks 1 to 8) or at the end of the study (week 21).

<sup>b</sup> The prefix E indicates a provisional new serotype that has not yet been designated a formal O group. Strains that could not be typed by the current serotyping scheme were designated O?.

<sup>c</sup> The numbers in parentheses are the numbers of strains isolated.

<sup>d</sup> U, PCR-restriction fragment length polymorphism pattern does not conform to any known vtx subtype.

Excreting VTEC slowly increased to around 20% by January 2002 (Fig. 2b), the increase over time was not significant (P = 0.732, rc = <0.001). In addition, being housed did not make a difference in the percentage of calves excreting VTEC strains.

TABLE 2. Characteristics of verocytotoxin-producing E. coli strains isolated from a cohort of calves on a Scotland beef farm between August 2001 and January 2002

<table>
<thead>
<tr>
<th>Serogroup&lt;sup&gt;a&lt;/sup&gt;</th>
<th>vtx&lt;sub&gt;2&lt;/sub&gt; type</th>
<th>vtx&lt;sub&gt;2&lt;/sub&gt; subtype</th>
<th>eae</th>
<th>ehec distribution&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 (10)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>2 (8), 2c (2)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O2 (2)</td>
<td>2</td>
<td>2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O15 (1)</td>
<td>2</td>
<td>2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>O20 (1)</td>
<td>2</td>
<td>2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O26 (35)</td>
<td>1</td>
<td>2</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>O26 (1)</td>
<td>2</td>
<td>U&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>O26 (4)</td>
<td>1</td>
<td>2 (3), 2c (1)</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>O84 (4)</td>
<td>1</td>
<td>2</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>O91 (1)</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O103 (1)</td>
<td>2</td>
<td>2c</td>
<td></td>
<td>+ (β)</td>
</tr>
<tr>
<td>O103 (1)</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O113 (2)</td>
<td>2</td>
<td>2c (1), U (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O128ab (1)</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O162 (1)</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O168 (2)</td>
<td>2</td>
<td>2 + 2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E40874 (17)</td>
<td>2</td>
<td>2c (10), 2 + 2c (1)</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>E40871 (10)</td>
<td>1</td>
<td>2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>O2 (2)</td>
<td>2c</td>
<td>2c</td>
<td>+</td>
<td>(β)</td>
</tr>
<tr>
<td>O11 (2)</td>
<td>2</td>
<td>2c (2), 2c (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O21 (2)</td>
<td>2</td>
<td>2 (10), 2c (10), U (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1 (1)</td>
<td>2</td>
<td>2c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The prefix E indicates a provisional new serotype that has not yet been designated a formal O group. Strains that could not be typed by the current serotyping scheme were designated O?.

<sup>b</sup> The numbers in parentheses are the numbers of strains isolated.

<sup>c</sup> U, PCR-restriction fragment length polymorphism pattern does not conform to any known vtx subtype.

(S = 0.302, rc = 0.105). Significantly fewer serogroups were isolated as calves aged (Fig. 2c) (P = 0.037, rc = -0.024), but multivariate analyses revealed that there was a significant interaction between housing status and age (P < 0.001, rc = -0.099); there was no change with age during the nonhoused period (P = 0.684, rc = 0.009), but there was a significant decrease with age when calves were housed (P < 0.001, rc = -0.191). While there was no significant change in the number of serogroups (excluding O7) with sampling time (Fig. 2d) (P = 0.289, rc = 0.015), calves excreted significantly more serogroups during the housed period (P = 0.032, rc = 0.833).

Furthermore, multivariate results revealed that there was an association between housing status and time of sampling (P = 0.004, rc = -0.066); there was a significant increase in the number of serogroups excreted during the nonhoused period (P = 0.008, rc = 0.147), and there was a nonsignificant decrease during the housed period (P = 0.101, rc = -0.026).

Altogether, VTEC strains were detected in 45 of the 49 calves by the PCR-DNA hybridization technique (Fig. 1). Four main serogroups were detected, O26 (24 isolates), O2 (8 isolates), E40874 (13 isolates), and E54071 (7 isolates), but other serogroups also were detected in more than one calf, including O84 (4 isolates), O103 (3 isolates), O113 (2 isolates), and O168 (2 isolates) (Fig. 1). VTEC O26 was the serogroup that was most frequently isolated from calves born during the first 7 weeks of the study, whereas in the calves born between week 7 and week 10, serogroups E40874 and E54071 were dominant.

Shedding of VTEC O26 decreased both as the age of the calves increased (P < 0.001, rc = -0.029) (Fig. 3a) and with time of sampling (P < 0.001, rc = -0.043) (Fig. 3b). There was also a significant difference in VTEC O26 shedding between non-housed and housed calves (P = 0.005, rc = 1.081) (Fig. 3b), and most of the O26 was shed prior to housing. Multivariate results revealed no interaction between time and age (P < 0.001), and housing status did not alter the age-related decline in O26 shedding (P = 0.146, rc = 0.018). There was an overall increase in the number of calves shedding VTEC O2 with age (P < 0.001, rc = 0.056) (Fig. 3c) and time (P < 0.001, rc = 0.052) (Fig. 3d). However, no O2 strains were detected before the calves were housed (Fig. 3d), and if only the data for the housed period were included, then O2 shedding was not age dependent (P = 0.499, rc = 0.008) or time dependent (P = 0.521, rc = -0.008). There was also an apparent decline in VTEC E40874 shedding with increasing calf age (P = 0.039, rc = -0.17) (Fig. 3e). No effect of housing status was observed (P = 0.956, rc = -0.014). In addition, no linear time-dependent relationship was observed (P = 0.192, rc = -0.010) (Fig. 3f); however, 88% of the E40874 shedding occurred in the middle of the study. Therefore, if a quadratic function of time was fitted first to capture this shedding pattern (P < 0.001, rc = -0.001), then E40874 shedding was not age dependent (P = 0.148, rc = -0.032). The shedding pattern of VTEC E54071 was not significantly associated with age, time, or housing status (P > 0.270, rc < -0.012) (Fig. 3g and h).

Presence of vtx genes. The vtx<sub>2</sub> genes were detected in 13 of 40 (33%) strains from dams and in 55 of 130 (42%) calf strains, and there was no significant difference between calves and dams (P = 0.240, rc = -0.226, OR = 0.64 [OR 95% CI, 0.30 to 1.35]). In contrast, vtx<sub>2</sub> genes were found in 39 (98%) and 90 (69%) of the strains from dams and calves, respectively, and...
VTEC strains from dams were significantly more likely to harbor \(\text{vtx}_2\) genes than VTEC strains from calves were (\(P = 0.006, \text{OR} = 1.444, \text{CI} = 1.235\) to 1.673). VTEC O26 strains from calves were characterized by the presence of \(\text{vtx}_1, \text{eae}, \text{and} \text{ehxA}\) genes (\(P < 0.001\)), whereas \(\text{vtx}_2\) was associated with VTEC O2 and provisional serogroups E40874 and E54071 (\(P < 0.032\)). There was a significant decline in the percentage of VTEC strains from calves harboring the \(\text{vtx}_1\) gene with age (\(P < 0.010, \text{OR} = 0.026\) (Fig. 4a), from a peak when calves were between 3 and 6 weeks old to almost zero (except for two 19-week-old calves) in calves older than 12 weeks. There were also fewer VTEC strains harboring \(\text{vtx}_1\) genes over the sampling time (\(P < 0.001, \text{OR} = 0.031\) (Fig. 4b) and during the housed period (\(P < 0.002\)) (Fig. 4b). The multivariate results revealed that there was no interaction between the rate of decline with age and housing status (\(P = 0.229, \text{OR} = 0.012\) or between age and sampling time (\(P = 0.625, \text{OR} = 0.001\)). In animals older than 7 weeks, \(\text{vtx}_2\) was the dominant gene type, and there was a significant increase in the percentage of VTEC strains harboring \(\text{vtx}_2\) genes with calf age (\(P = 0.002\)) (Fig. 4c); however, once the higher percentage of VTEC strains harboring \(\text{vtx}_2\) genes during the housed period (\(P < 0.001\)) (Fig. 4d) was taken into account, there was no significant change in \(\text{vtx}_2\) detection and calf age (\(P = 0.139\)). If only VTEC strains harboring \(\text{vtx}_2\) gene subtype \(\text{vtx}_2\) were considered, then again there was an increase with calf age (\(P = 0.003\)), but this increase with age was significantly steeper during the housed period (\(P = 0.002\)). No age-, time-, or housing status-related patterns were observed for \(\text{vtx}_2c\) (\(P = 0.052\)).

Presence of \(\text{eae}\) and \(\text{ehxA}\) genes. The \(\text{eae}\) genes were detected in one strain (serogroup O26) from dams and in 64 (50%) of the strains (serogroups O26, O84, and O103 and provisional serogroup E40874) from calves; i.e., VTEC strains carrying the \(\text{eae}\) genes were significantly associated with calves (\(P < 0.001, \text{OR} = 0.031, \text{CI} = 0.01\) to 0.21). There was no significant association between dams and the presence of \(\text{ehxA}\) genes (\(P = 0.080, \text{OR} = 0.453, \text{CI} = 0.253\) to 0.791) (Tables 1 and 2).

The percentage of VTEC strains harboring \(\text{eae}\) significantly declined with calf age (\(P = 0.008, \text{OR} = 0.033\) (Fig. 4e) and time (\(P < 0.001, \text{OR} = 0.038\) (Fig. 4f). However, significantly fewer VTEC strains harboring \(\text{eae}\) were detected during the
FIG. 3. Changes in the percentage of VTEC-shedding calves that shed O26 (a and b), O2 (c and d), E40874 (e and f), and E54071 (g and h) by calf age (a, c, e, and g) and sampling time (b, d, f, and h). The vertical dashed lines indicate 95% confidence intervals for the percentages, and the numbers in parentheses are the numbers of VTEC strains (of a total of 130 strains) that were detected in calves that were sampled for each week.
FIG. 4. Changes in the percentage of VTEC-shedding calves that shed vtx₁ (a and b), vtx₂ (c and d), eae (e and f), and ehxA (g and h) by calf age (a, c, e, and g) and sampling time (b, d, f, and h). The vertical dashed lines indicate 95% confidence intervals for the percentages, and the numbers in parentheses are the numbers of VTEC strains (of a total of 130 strains) that were detected in calves that were sampled for each week. Shaded areas are as for Fig. 2.
housed period ($P = 0.012, \text{rc} = -1.83$) (Fig. 4f), and multivariate analyses revealed an interaction between housing status and age of the calf, with a steeper age-related decline during the housed period ($P = 0.007, \text{rc} = -0.115$). Sampling time was no longer significant if housing status was considered first ($P = 0.510, \text{rc} = -0.021$). The percentage of VTEC strains harboring eahA genes significantly declined with calf age ($P < 0.034, \text{rc} = -0.012$) (Fig. 4g) and with sampling time ($P = 0.048, \text{rc} = -0.012$) (Fig. 4h) but not with housing status ($P = 0.092$) (Fig. 4h). There was no interaction between calf age and sampling time ($P = 0.552, \text{rc} < 0.001$). The most common intimin subtype was β-intimin (serogroup O26 and provisional serogroup E40874), which was present in 92% of the eae-positive calf strains. There were also four strains (all serogroup O84) which carried the ζ-intimin gene, and one O103 strain carrying θ-intimin was detected (Table 2).

In dams, there were no significant associations between the detection of vtx1 and the detection of either eae or eahA ($P > 0.325$) or between the detection of vtx1 and the detection of eahA. However, there was a negative association between the detection of vtx1 and the detection of eae ($P = 0.025$), and no vtx2* strains were eae+. The detection of vtx1 was positively associated with the detection of eae and eahA in VTEC strains from calves ($P < 0.001$), whereas the detection of vtx2 was negatively associated with the detection of eae and eahA ($P < 0.003$). In addition, the detection of eahA was positively associated with the detection of the eae gene in the VTEC strains ($P < 0.001$).

**Calf diarrhea.** Throughout the study, 14 calves showed evidence of diarrhea. However, VTEC (serogroup O26) was detected in only one calf at the same time as diarrhea. One week before diarrhea was observed, two calves had VTEC in their fecal samples (one had serogroup O26, and the other had provisional serogroup E54071).

**DISCUSSION**

The results presented here are results for one cohort of calves and dams on a single farm and should therefore be interpreted with some caution. Furthermore, the calves were born throughout the first half of the study, and all animals were housed in the second half of the study, leading to possible confounding among time of sampling, age of the calf, and housing status. Nevertheless, such studies are rare, and interesting patterns were observed. While samples were collected from dams only at the time of calf birth and at the end of the study, the proportion of samples containing VTEC was higher for dams (46%) than for calves (20%); this is in contrast to the results of other studies, in which the incidence of VTEC in calves was usually higher than the incidence of VTEC in adult cattle (2, 5, 45, 51). Cobbold and Desmarchelier (5) suggested that dams shed more VTEC during and after calving, because pregnancy and calving would be expected to be a stressor for the cows. However, they did not find any obvious correlation between calving and the number of animals shedding VTEC (5).

Thirteen different, typeable VTEC serogroups were detected in the calves (Fig. 1), whereas only five typeable VTEC serogroups were detected in the dams sampled at the time of calf birth and at the end of the study. The most frequently isolated VTEC serogroup in calves was VTEC O26. In the study of Pearce et al. (32) with the same cohort, in which IMS rather than PCR was used to detect E. coli serogroups O26, O103, O111, O145, and O157, VTEC O26 was also the most frequently isolated serogroup. However, IMS was shown to be 2.5 times more sensitive (for this serogroup) than PCR. Calves were kept together (either in the same area of pasture or, once housed, in the same area of the shed), which could have facilitated the spread of strains of VTEC from calf to calf. However, it is interesting that other serogroups did not appear to be distributed in the calf population in the same way as VTEC O26. This serogroup has been isolated from healthy adult cattle and calves in many studies (1, 2, 5, 32), although it has also been associated with diarrheal disease in calves (9, 40, 41). In a study of diarrheic calves, Orden et al. (28) found that the most common VTEC serogroup in calves was VTEC O26 (30%). Cobbold and Desmarchelier (6) looked for VTEC on three farms in Australia and found that 7.8% of the isolates were VTEC O26:H11.

The apparent association of VTEC O26 with calves that were between 1 and 7 weeks old in this study may simply reflect the serogroups present in the environment at the time of birth, although calves born when other serogroups predominated also shed VTEC O26. While this finding may be specific to this cohort, the replacement of one serogroup with another as the calves' ages increased may have been due to changes in the calf gut and the availability of niches for particular VTEC populations. For example, Naylor et al. (26) found recently that VTEC O157 preferentially occupied a niche at the recto-anal junction of the calf gut in calves that were 8 to 14 weeks old.

It has been suggested that VTEC O26 and VTEC harboring vtx1, and eae are associated with diarrheagenic calves (9, 22, 25, 29, 46). The serotypes reported to be associated with diarrhea in calves include O5:H-, O8:H8, O20:H19, O26:H11, O103:H2, O111:H-, O118:H16, O128:H-, and O145:H- (22, 28). In this study, there was no association between calf diarrhea and any of the VTEC serotypes or their characteristics.

The analysis of the associations between serogroups and virulence factors also revealed that there appeared to be a number of distinct serogroup-virulence factor combinations that were observed in many calves in the calf cohort at certain times in the study. The decline in strains harboring vtx1 genes with calf age and the increase in vtx2* positive strains were related to the decline in VTEC O26 strains, which in this study and characteristically have the vtx1 genes, whereas the increase in VTEC O2 strains later in the study was associated with an increase in detection of the vtx2 genes. There was also a population of VTEC E40874 strains which were vtx2*, eahA*, and eae that was present in young calves and then declined. Finally, a population of VTEC E54071 strains (vtx1* vtx2* eahA*) was detected in older calves.

By using the methods described in this study, all VTEC serogroups could be detected if bacteria representing the serogroups were present in sufficient numbers, and therefore, fecal samples containing more than one VTEC serogroup could be identified. Despite this, different VTEC serogroups were isolated from only one sample (Fig. 1). Pearce et al. (32) also found that concurrent shedding of more than one of the five serogroups considered was uncommon. These data and the present data suggest that cattle shed a single predominant...
VTEC serogroup (and associated virulence factors) at any one point. Previous studies have shown that calves as young as 48 to 72 h old excrete VTEC (5) and that a calf is more than twice as likely to shed VTEC if it is born to a VTEC-positive cow, although a VTEC-negative cow has an equal risk of producing either a VTEC-positive calf or a VTEC-negative calf (12). This suggests that calves may acquire VTEC from sources other than their dams (5, 32). In our study, the VTEC serogroup was isolated from the calf and its dam on only four occasions, but the serogroups were all O? and in three of the four cases the concurrent shedding occurred at the end of the study (Fig. 1). VTEC serogroups O26, O91, O113, and E54071 detected in fecal samples from dams at the time of calf birth were found in other calves, perhaps indicating that calves may acquire strains of VTEC from adult cattle other than their dams. In this cohort, some VTEC serogroups were detected in calves but not isolated from any of the dams, suggesting VTEC acquisition from other sources. Alternatively, serogroups that are dominant in adult cattle may be different from the dominant serogroups in calves (e.g., O26 and E40874). The DNA hybridization method can detect between 0.1 and 760 organisms per g of tissue in calves. The DNA hybridization method can detect between 0.1 and 760 organisms per g of tissue in calves.

VTEC serogroups O26, O103, O111 and O145 from calves aged 72 h old excrete VTEC (5) and that a calf is more than twice as likely to shed VTEC if it is born to a VTEC-positive cow, although a VTEC-negative cow has an equal risk of producing either a VTEC-positive calf or a VTEC-negative calf (12). This suggests that calves may acquire VTEC from sources other than their dams (5, 32). In our study, the VTEC serogroup was isolated from the calf and its dam on only four occasions, but the serogroups were all O? and in three of the four cases the concurrent shedding occurred at the end of the study (Fig. 1). VTEC serogroups O26, O91, O113, and E54071 detected in fecal samples from dams at the time of calf birth were found in other calves, perhaps indicating that calves may acquire strains of VTEC from adult cattle other than their dams. In this cohort, some VTEC serogroups were detected in calves but not isolated from any of the dams, suggesting VTEC acquisition from other sources. Alternatively, serogroups that are dominant in adult cattle may be different from the dominant serogroups in calves (e.g., O26 and E40874). The DNA hybridization method can detect between 0.1 and 760 organisms per g of tissue in calves. The DNA hybridization method can detect between 0.1 and 760 organisms per g of tissue in calves.


