Prevalence and Virulence Factors of *Escherichia coli* Serogroups O26, O103, O111, and O145 Shed by Cattle in Scotland

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A national survey was conducted to determine the prevalence of *Escherichia coli* O26, O103, O111, and O145 in feces of Scottish cattle. In total, 6,086 fecal pats from 338 farms were tested. The weighted mean percentages of farms on which shedding was detected were 23% for *E. coli* O26, 22% for *E. coli* O103, and 10% for *E. coli* O145. The weighted mean prevalences in fecal pats were 4.6% for *E. coli* O26, 2.7% for *E. coli* O103, and 0.7% for *E. coli* O145. No *E. coli* O111 was detected. Farms with cattle shedding *E. coli* serogroup O26, O103, or O145 were widely dispersed across Scotland and were identified most often in summer and autumn. However, on individual farms, fecal shedding of *E. coli* O26, O103, or O145 was frequently undetectable or the numbers of pats testing positive were small. For serogroup O26 or O103 there was clustering of positive pats within management groups, and the presence of an animal shedding one of these serogroups was a positive predictor for shedding by others, suggesting local transmission of infection. Carriage of *vtx* was rare in *E. coli* O103 and O145 isolates, but 49.0% of *E. coli* O26 isolates possessed *vtx*, invariably *vtx₁* alone or *vtx₁* and *vtx₂* together. The carriage of *eae* and *ehxA* genes was highly associated in all three serogroups. Among *E. coli* serogroup O26 isolates, 28.9% carried *vtx*, *eae*, and *ehxA*—a profile consistent with *E. coli* O26 strains known to cause human disease.

Verocytotoxigenic *Escherichia coli* (VTEC) strains are important animal and human pathogens (18, 23, 24, 29, 31, 39, 50). In humans, VTEC strains are associated with illnesses ranging from uncomplicated watery diarrhea to hemorrhagic colitis and potentially fatal hemolytic uremic syndrome (50). In North America, Japan, and much of Europe, the VTEC serogroup usually associated with human disease is serogroup O157 (3, 22). However, in parts of the southern hemisphere and continental Europe, other VTEC serogroups are significant causes of human disease (5, 10, 16, 28, 36, 46), especially VTEC O26, O103, O111, and O145, which are highly associated with serious human infections (4, 8, 43, 49, 50). To date, isolation of non-O157 VTEC strains from human infections has been uncommon in Scotland (3) though VTEC serogroups O26, O103, O111, and O145 are known to be carried by cattle (47, 48, 50). This study was therefore undertaken to improve our understanding of why non-O157 VTEC human infections are uncommon in Scotland, and it had two major objectives. The first was to conduct a national survey of the feces of Scottish cattle to determine the prevalence of *E. coli* serogroups O26, O103, O111, and O145, which are potential VTEC strains. The second objective was to screen bacterial isolates for genes encoding enterohemolysin (*ehxA*) and the

virulence factors verocytotoxin 1 (*vtx₁*), verocytotoxin 2 (*vtx₂*), and intimin (*eae*), which are associated with strains causing human disease (7, 14, 23, 27, 38, 50, 51).

MATERIALS AND METHODS

The farms used as the sampling frame for the study were chosen from the 1997 Scottish Agricultural and Horticultural Census data and randomly selected across all Scottish State Veterinary Service animal health divisions (AHDs). A five-stage stratified sampling plan was used to select farms to ensure that similar numbers were included from each stratum and that strata were sampled evenly over time between March 2002 and February 2004. In each individual management group, sufficient fecal pats from the sampled farms were tested to ensure an 80% chance of identifying at least one positive pat when there was at least one shedding animal within the group, assuming a mean within-farm shedding prevalence of 7.8% on farms with positive-testing animals.

In total, tests were performed on 6,086 fecal pats collected from 338 farms, representing 3.5% of Scottish farms with cattle of older than 1 year of age that were store (i.e., young over-wintered animals) or were being finished for human consumption (2003 Scottish Agricultural and Horticultural Census). In the individual AHDs, from 51 to 59 farms and 912 to 1,142 fecal pats were sampled. In each season, with winter defined as December, January, and February, spring as March, April, and May, summer as June, July, and August, and autumn as September, October, and November, from 79 to 90 farms and 1,291 to 1,748 fecal pats were sampled. Fecal pat sampling was used because it was more practicable and ensured higher farmer compliance than sampling individual animals, and the results are thus representative of the Scottish cattle population and may be regarded as a proxy for animal level prevalence.

Bacterial isolation was conducted within 48 h of sampling with 1 g of feces from each sample preenriched in buffered peptone water and tested for the presence of *E. coli* serogroup O26, O103, O111, or O145 by use of immunomagnetic separation (IMS) and slide agglutination (35). All isolates provisionally identified as belonging to serogroup O26, O103, O111, or O145 were further tested by tube agglutination (32). All isolates with a positive tube agglutination

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result were submitted to The Laboratory of Enteric Pathogens, Health Protection Agency, Colindale, United Kingdom, and each isolate was biochemically identified as *E. coli* and the somatic O antigens were confirmed (2, 25).

RESULTS AND DISCUSSION

Farm-level data. The mean percentages of farms with shedding cattle were estimated using generalized linear mixed models (GLMMs) (9, 34), with binomial response terms and a logit link function. Data were binary in form, so dispersion was set equal to 1. Farm cluster and farm were fitted as random effects. Including AHD and season as fixed effects, we used GLMMs to determine the impact of AHD and season on the percentage of farms with shedding cattle and to estimate the mean percentage of farms with shedding cattle in each AHD and season. The GLMM parameter estimations were converted into mean prevalences using both the transformed means and random effects (12). National farm-level prevalence figures were derived from these estimates using data defining the number of cattle farms with cattle over 1 year of age in each AHD. A confidence interval for the farm-level *E. coli* O111 mean prevalence estimate was derived numerically, assuming that the log likelihood ratio associated with an absence of positive pats in each AHD would be well approximated by a χ^2 distribution. Confidence intervals for serogroup O26, O103, and O145 means were derived by reweighing output from the appropriate GLMM. The effect of AHD on the mean prevalence of farms with cattle shedding *E. coli* O145 was investigated further using Fisher's exact test, and log-linear generalized linear models were used to examine associations between *E. coli* O26, O103, and O145 shedding results on farms (44).

The observed number and percentage of farms positive for each serogroup are given in Table 1. The slight bias in the raw figure arises from the imbalance in the proportions of farms sampled from different AHDs relative to their full populations. The unbiased estimate of the mean percentage of farms in Scotland with cattle shedding *E. coli* serogroup O26 was 23% and was 22% for serogroup O103, which are results similar to the mean percentage of farms with cattle shedding *E. coli* O157 (22.8%) (45). In contrast, the mean percentage of farms with cattle shedding *E. coli* O145 (10%) was much lower.

Farms with cattle shedding *E. coli* serogroup O26, O103, or O145 were widely dispersed across Scotland, with no statistical evidence of regional differences in distribution, though shedding of *E. coli* O145 was not detected on any farms in northeast AHDs (Fig. 1). Season had a statistically significant effect (Fig. 2) on the mean percentages of farms with cattle shedding *E. coli* serogroups O26 ($\chi^2_{df=3} = 13.04$; $P = 0.005$) and O103 ($\chi^2_{df=3} = 9.86$; $P = 0.021$), with positive results detected most frequently in summer and autumn. A similar trend, but with no detectable significant seasonal effect ($P = 0.44$), was seen for the percentage of farms with cattle shedding *E. coli* O145.

Pat-level data, all farms. For the whole of Scotland and within each AHD and season, the distribution of the mean percentages of positive pats was approximated using bootstrapping procedures (13), as the skewed and potentially bimodal distribution of the data resulted in poorly fitted GLMMs. Each bootstrap exercise comprised 10,000 iterations of a two-stage bootstrapping procedure. Within each iteration, farm clusters were sampled with replacement, all farms were sampled within

TABLE 1. Numbers, observed percentages, and weighted mean percentages of farms with one or more store or finishing cattle shedding *E. coli* O26, O103, O111, or O145

Serogroup	No. of farms with positive results	Observed %	Weighted mean		
			%	95% Confidence limits	
				Lower	Upper
O26	68	20	23	18	29
O103	75	22	22	17	27
O111	0	0	0	0	2
O145	26	8	10	4	21

each cluster, all management groups were sampled within each farm, and pats were sampled with replacement in each management group. The contribution of each sampled pat was weighted by the inverse of the probability of selection from its management group. Differences in the observed prevalences between AHDs and seasons were each assessed by generating bootstrap samples under the null hypothesis that the distributional properties were the same over the entire data set (13). The coexistence of *E. coli* O26, O103, or O145 strains in pats was analyzed using log-linear models (44).

The observed numbers and percentages of positive pats for each serogroup are given in Table 2 with a weighted, unbiased prevalence estimate and confidence interval. The bias in the raw figure arises because a larger percentage of pats were sampled from small management groups. The unbiased estimates of the pat-level prevalences of *E. coli* O26, O103, and O145 shedding by cattle over 1 year old across Scotland are 4.6%, 2.7%, and 0.7%, respectively. The mean pat-level prevalence of *E. coli* O26 detected by IMS is slightly more than one-half the prevalence of *E. coli* O157 (7.9%) previously described using similar methods (45). For all three serogroups the observed pat-level shedding was highest in summer and autumn, but across all farms, there was a statistically significant effect of season on only the mean percentage of pats positive for *E. coli* serogroup O103 ($P = 0.04$).

E. coli of serogroup O111 was not found in any samples, and no upper confidence boundary is calculated for its prevalence because any meaningful estimate would require information about the clustering of positive samples. The absence of *E. coli* O111 is consistent with the results of Scottish studies that used IMS and also PCR with DNA hybridization techniques (19, 20, 35), though *E. coli* O111 has been previously isolated from cattle (26, 39, 47, 48) and there is a report of the isolation of this serogroup from United Kingdom cattle in the early 1980s (41).

On individual farms, fecal shedding of *E. coli* O26, O103, or O145 was usually undetectable or the number of positive pats was small (Fig. 3). However, the observed pat-level prevalences of shedding ranged widely between management groups on different farms. Within sampled groups, 0% to 83% of samples were positive for *E. coli* serogroup O26, 0% to 55% for serogroup O103, and 0% to 25% for serogroup O145. Clustering of shedding is thus evident within management groups, and the presence of an animal shedding serogroup O26 or O103 is a positive predictor for shedding by other cattle in the group. There is no statistical evidence that within a group the mean percentage of pats positive for serogroups O26,
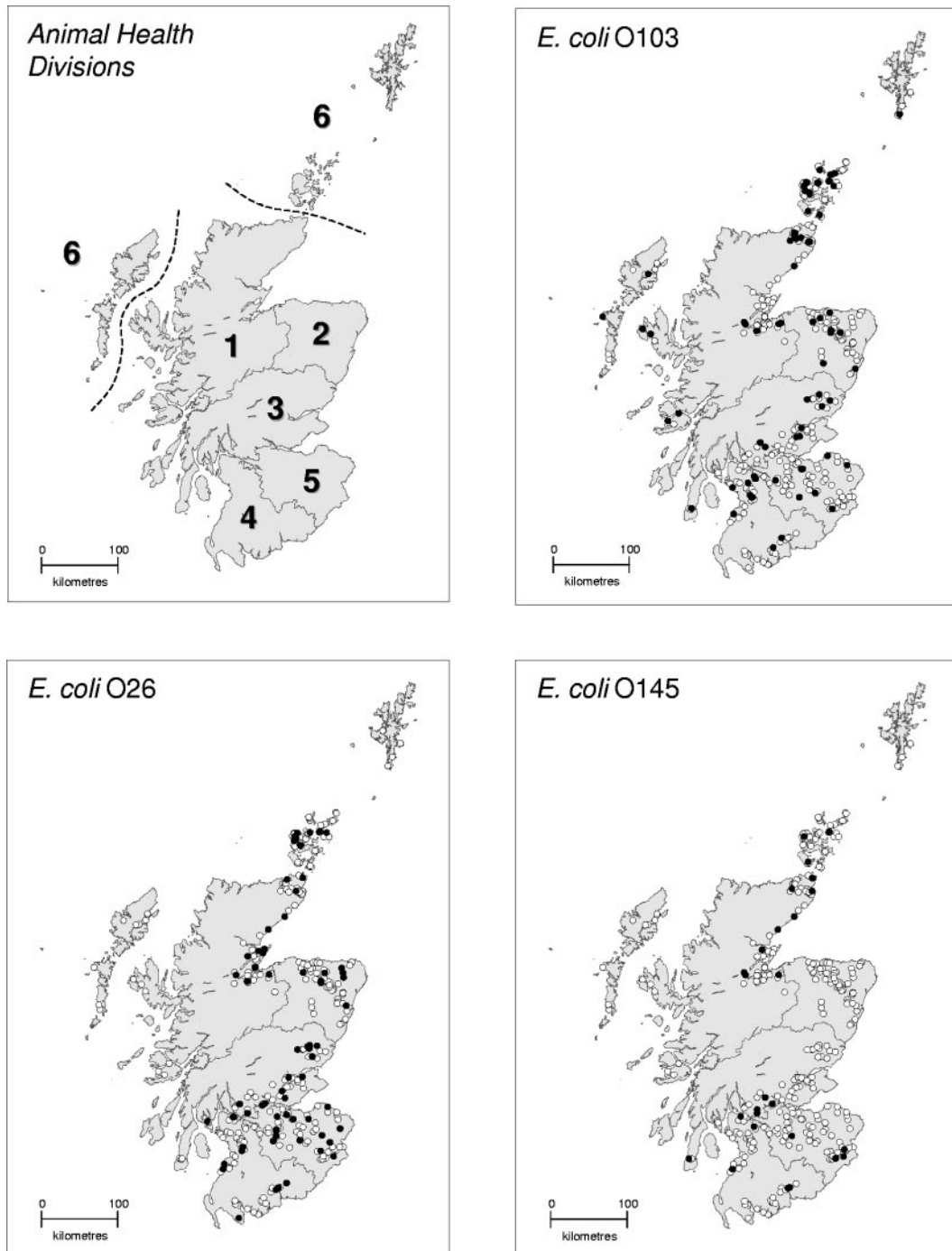
FIG. 1. Location of Scottish State Veterinary Service animal health divisions and sampled farms with store and finishing cattle shedding *E. coli* serogroups O26 and O103 or O145. Animal health divisions: 1, Highlands; 2, North East; 3, Central; 4, South West; 5, South East; 6, Islands. ○, no shedding detected; ●, shedding detected. (Map reprinted with the permission of the ESRC.)

O103, or O145 is correlated with group size, and there is no strong evidence for a correlation between results showing the presence of the different *E. coli* serogroups O26, O103, and O145. These findings most likely suggest local transmission of infection, though an unknown highly localized risk factor cannot be excluded.

Pat-level data, farms with shedding cattle. GLMMs with a binomial response distribution and a logit link function were used to estimate the mean percentage of pats positive for the subsets of positive farms, fitting farm cluster, farm, and sample group as random effects. Incorporating AHD and season as fixed effects, the models were used to determine
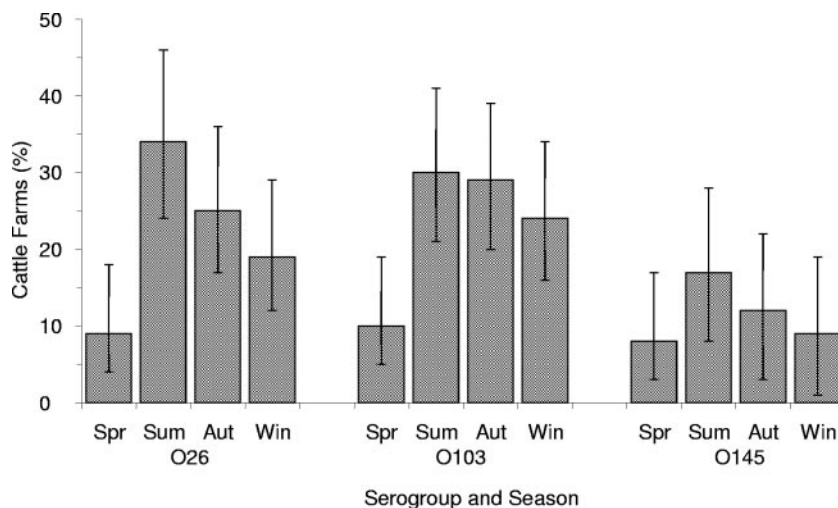
FIG. 2. Mean percentage of farms with fecal pats from store and finishing cattle positive for *E. coli* O26, O103, or O145 by season. Error bars define 95% confidence limits.

the impact of these factors on the mean percentage of positive pats.

In the farms with positive-testing animals, for each of serogroups O26, O103, and O145, there was no statistical evidence of any association between season or AHD and the mean percentage of positive pats. Intriguingly, our results thus indicated an increase in the number of farms with shedding cattle rather than a rise in the number of cattle shedding on farms where shedding is occurring. Other studies have reported that the percentage of farms with cattle shedding *E. coli* O157 is highest in summer and autumn (15, 40) or that the mean level of *E. coli* O157 shedding among cattle is highest in summer and autumn (17). The reasons for the apparent seasonality in shedding are not known. However, the pattern is reflected in the reported number of human cases of *E. coli* O157 infection, which also rise in summer (3, 42), though, so far as we are aware, no seasonal pattern of human infection with serogroup O26, O103, or O145 has been reported.

Molecular characterization of bacterial isolates. The second study objective was to screen by multiplex PCR (33) all bacterial isolates for genes encoding enterohemolysin (*ehxA*) and the virulence factors verocytotoxin 1 (*vtx*$_1$), verocytotoxin 2 (*vtx*$_2$), and intimin (*eae*) that are associated with strains causing human disease (7, 14, 23, 27, 38, 50, 51). Carriage of the genes

vtx$_1$, *vtx*$_2$, *eae*, and *ehxA* by *E. coli* serogroup O26, O103, and O145 isolates is shown in Table 3. A total of 122 (49.0%) *E. coli* O26 isolates carried *vtx*$_1$, and 31 (12.5% of the total) of these also had *vtx*$_2$. No *E. coli* O26 isolates carried *vtx*$_2$ alone. A high percentage (83.9%) of serogroup O26 isolates possessed the *eae* gene, while approximately half (51.8%) carried *ehxA*. For *E. coli* serogroup O26 isolates there were 72 (28.9%) that carried *vtx*$_1$, *eae*, and *ehxA* genes; these results are consistent with strains pathogenic to humans (7, 38, 51). Carriage of *vtx* genes was rare in serogroup O103 and O145 isolates. Only 2 of 168 (1.2%) serogroup O103 isolates were *vtx* positive: one carried *vtx*$_1$, while the other possessed both *vtx*$_1$ and *vtx*$_2$. Among serogroup O145 isolates, 2 of 43 (4.7%) carried *vtx* genes: one carried *vtx*$_1$ and the other *vtx*$_2$.

Associations between isolate virulence factors were assessed using contingency tables and Fisher's exact test (44). For serogroup O26 the percentage of *vtx*-negative isolates with *eae* (88.2%) was not significantly different from the percentage of *vtx*-positive isolates with *eae* (79.5%), but isolates with *eae* were 12 times more likely to carry *ehxA* than isolates without *eae* ($P < 0.001$; relative risk, 12.2; 95% confidence interval, 3.1 to 47.1). In serogroups O103 and O145, carriage of *eae* and *ehxA* was also common, and as has been previously noted (6, 7), these genes were highly associated ($P < 0.001$ and $P = 0.001$, respectively). While both *eae* and *ehxA* encode putative virulence determinants, the isolates from cattle that are *eae* positive but *vtx* negative are generally regarded as atypical enteropathogenic *E. coli* and may be less likely than VTEC to cause human disease (37).

Most studies of non-O157 *E. coli* shedding by cattle detected strains by screening for verocytotoxin production or the presence of *vtx* genes and therefore present results biased to VTEC alone. The IMS technique is advantageous in being directed toward isolation of target serogroup strains irrespective of *vtx* gene carriage. Previous work has shown that, for the detection of VTEC O26, the IMS technique used is 2.5 times more sensitive than replica colony plating and DNA hybridization with *vtx* probes (21). IMS is also more sensitive for detection of

TABLE 2. Numbers, observed percentages, and weighted mean percentages of fecal pats shed by store or finishing cattle positive for *E. coli* O26, O103, O111, or O145
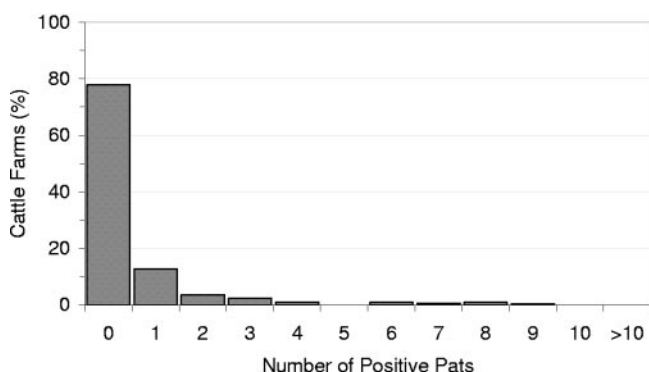
Serogroup	No. of positive pats	Observed %	Weighted mean		
			%	95% Confidence limits	
				Lower	Upper
O26	249	4.1	4.6	3.0	6.6
O103	168	2.8	2.7	1.7	3.8
O111	0	0.0	0.0	0.0	NC[a]
O145	43	0.7	0.7	0.3	1.2

[a] NC, not calculated.

E. coli O26
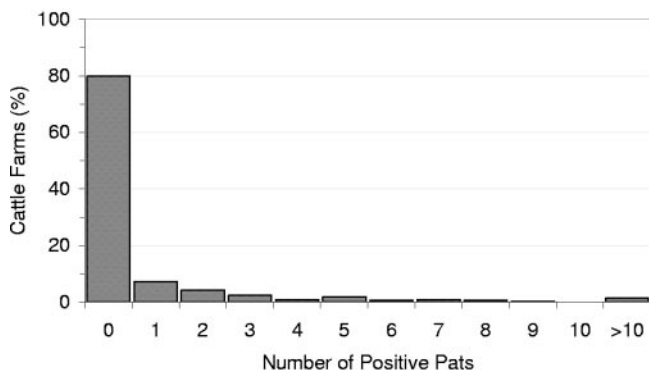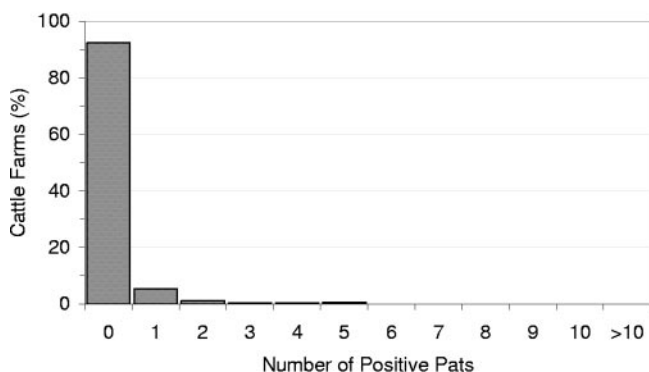

E. coli O103

E. coli O145

FIG. 3. Number of pats positive for *E. coli* O26, O103, or O145 on sampled farms.

E. coli O157 (11). We thus believe that the IMS technique is a valuable tool in the epidemiological study of VTEC and that our prevalence data with identification of low prevalences of VTEC O103, O111, and O145 in Scottish cattle more than 1 year old are more accurate than data from other estimates (1, 26, 35).

The scale of this study is larger than any described in the literature and establishes a benchmark for the prevalence of VTEC O26, O103, O111, and O145 carriage by cattle in Scotland and establishes that VTEC O26 strains are common and widely dispersed. However, the prevalence figures for each *E.*

coli serogroup in this study should be regarded as minimum prevalence estimates as our sampling procedure is designed to give an 80% chance of detecting at least one positive pat from a group if one or more animals were shedding in that group. Additionally, the development and validation of indicator media could increase the sensitivity of detection and, therefore, the accuracy of prevalence estimates, as has happened with *E. coli* O157 studies.

VTEC serogroup O26 is important as a cause of human infection in central and southern Europe (46, 51), and in Italy this serogroup has surpassed *E. coli* O157 as the major cause of hemorrhagic colitis and hemolytic uremic syndrome (46). Though human disease associated with *E. coli* O26 has occurred in England (42), Scotland (3), and the Republic of Ireland (30), the number of serogroup O26 infections reported is very much smaller than for *E. coli* O157. There could be many reasons for the marked differences in the occurrence in United Kingdom of human infections caused by VTEC of *E. coli* O157 and O26 serogroups. However, the common finding in Scottish cattle of *E. coli* serogroup O26 isolates that are consistent with strains pathogenic to humans (7, 38, 51) is of significance. The results raise the issue of whether VTEC O26 strains from cattle pose a human health hazard and whether this serogroup may emerge to become a cause of human infections in Scotland. To investigate this further, our preliminary studies are focusing on a comparison of *E. coli* O26

TABLE 3. *E. coli* serogroup O26, O103, and O145 isolates and carriage of genes encoding verocytotoxin 1 (vtx_1), verocytotoxin 2 (vtx_2), intimin (*eae*), and enterohemolysin (*ehxA*)

Serogroup	Virulence factor gene result[a]				Total no. of isolates
	vtx_1	vtx_2	*eae*	*ehxA*	
O26	−	−	−	−	15
	−	−	+	−	57
	−	−	+	+	55
	+	−	−	−	16
	+	−	−	+	2
	+	−	+	−	20
	+	−	+	+	53
	+	+	−	−	7
	+	+	+	−	5
	+	+	+	+	19
Total	122	31	209	129	249
O103	−	−	−	−	103
	−	−	+	−	2
	−	−	+	+	61
	+	−	−	−	1
	+	+	+	+	1
Total	2	1	64	62	168
O145	−	−	−	−	7
	−	−	+	−	8
	−	−	+	+	26
	+	−	+	+	1
	−	+	+	+	1
Total	1	1	36	28	43

[a] Totals in columns represent numbers of isolates tested.

isolates from humans and animals that have been collected from across Europe.

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