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Epidemiological Relatedness and Clonal Types of Natural Populations of *Escherichia coli* Strains Producing Shiga Toxins in Separate Populations of Cattle and Sheep

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Two separate animal populations consisting of a herd of cattle (19 animals) and a flock of sheep (25 animals) were investigated for strains of *Escherichia coli* producing Shiga toxins (STEC) over a time period of 6 months. Thirty-three STEC were isolated from 63.2% of cattle and grouped into 11 serotypes and eight electrophoretic types (ETs) by multilocus enzyme analysis. In sheep, 88% of the animals excreted STEC ($n = 67$ isolates) belonging to 17 different serotypes and 12 different ETs. STEC from cattle and sheep differed with respect to serotype, and only 4 of the 16 ETs occurred in both animal populations. In cattle, ET14 (O116:H21) strains predominated, whereas other STEC serotypes occurred only sporadically. The predominating STEC types in sheep were ET4 (O125 strains), ET11 (O128:H2 and others), and ET14 (O146:H21). In contrast to their diversity, STEC originating from the same animal population were similar with respect to Shiga toxin (*stx*) genes. Almost all STEC isolated from cattle were positive for stx_2 and stx_{2c}; only one was positive for stx_1. In sheep, almost all STEC isolated were positive for stx_1 and stx_2, whereas stx_{2c} was not found. *Xba*I-digested DNAs of genetically closely related O146:H21 strains have different restriction profiles which were associated with size alterations in *Xba*I fragments hybridizing with stx_1- and stx_2-specific DNA probes. Our results indicate that *stx*-encoding bacteriophages might be the origin of the genetic heterogeneity in STEC from animals.

Strains of *Escherichia coli* producing Shiga toxin (STEC) carry genes encoding one or more genetically related types of cytotoxins, called verotoxins or Shiga toxins (Stx) (7, 10). In *E. coli*, the Shiga toxin genes stx_1, stx_2, and stx_{2c} are encoded by lysogenic bacteriophages (10). A major source of STEC in nature are domestic animals, especially ruminants like cattle, sheep, and goats (4, 28, 29). Animal carriers of STEC shed these organisms into the environment by fecal excretion. In adult animals, STEC usually are not pathogenic, although some strains cause diarrhea in calves and others cause edema disease in pigs (9, 20). STEC are normally not present in the feces of healthy humans. However, some STEC originating from healthy animals can behave as human pathogens and cause serious illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (1, 10). Humans can become infected with STEC by consumption of contaminated foodstuffs or by direct transmission of STEC from infected humans or animals (10). It is not yet clear if all STEC occurring in animals are pathogenic for humans (2, 5, 15).

The proportion of STEC carriers among cattle was found to be important. Investigations carried out in geographically different places in Europe, Asia, and North America revealed that 10 to 80% of cattle were infected with STEC (4, 28, 29). Even higher proportions of STEC-carrying animals were found among adult healthy sheep and goats (4). Serological analysis of O:H types of STEC isolated from different sources revealed that STEC are present in a high number of different *E. coli* serotypes. More than 100 different O:H serotypes of STEC have been isolated from cattle, and the number of STEC serotypes associated with human disease exceeds 150 (8, 10, 32). Certain serotypes of STEC are more frequently isolated than others, and some STEC serotypes seem to be associated with their animal host species (4, 9, 17, 20, 26).

Little is known about the ecology of STEC and the epizootiology in their animal hosts. Most work has been focused on STEC O157:H7, which is considered the most virulent type for humans (1, 10). Shedding of *E. coli* O157:H7 by cattle was found to be affected by factors such as age, diet and feeding, various sources of stress, and possibly seasonal effects. Although cattle are regarded as a natural host for STEC O157:H7, the prevalence of these organisms in cattle is generally very low (1, 4, 8, 17, 29). Types of STEC other than O157:H7 were found to occur more frequently in cattle and could be detected in relatively high numbers in fecal samples (17, 28). Similar observations were made for sheep and goats which harbor other types of STEC than cattle (4, 5). These findings suggest that some STEC types are adapted for colonizing the intestine of their animal hosts well and become residents over long time periods (28).

The aim of this study was to investigate the relationship between the animal hosts and their natural colonizing STEC more closely. To do so, we examined a herd of cattle and a flock of sheep for fecal STEC over a period of 6 months and analyzed the STEC isolated for serological and genetic relationships and for typical virulence markers. Our results indicate that STEC occurring in single populations of cattle and sheep are epidemiologically related despite their serological diversity. A serotype specificity for their animal host species was found.

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MATERIALS AND METHODS

Animals. A herd of cattle (19 animals) and a flock of sheep (25 animals) were investigated for carriage of STEC during a time period of 6 months. The cattle and sheep populations were housed at separate localities in the Berlin, Germany, area and were not in contact with each other. Only animals without any signs of disease during the time of investigation were taken into the study. The herd of cattle was housed in a single free stall, and stall feeding was performed automatically by a computerized feeder. Individual cattle could be in contact with each other. The cattle investigated were all adult cows between 2.5 and 7 years of age (mean age, 4 years 5 months).

The flock of sheep was housed in a single large stall with boxes harboring three to four animals each. The sheep were fed in the stall and grazed on an enclosed pasture situated directly next to their stall. During the period of investigation, all sheep of this flock could be in contact with each other but were not in contact with other sheep. All sheep were between 1 and 2 years old. Except for one ram, all sheep were female.

The animal feed was free of antibiotics or food additives which are used as growth promotors in agriculture. Fecal samples were taken once a month beginning in February 1992 until July 1992 (Tables 1 and 3). Thus, a total of six fecal samples were obtained from each of the animals investigated.

Isolation of STEC from animals and characterization of virulence traits. Collection of fecal samples from animals and isolation of STEC were performed as described previously (4). Aliquots of fecal samples were inoculated in 5 ml of tryptic soy broth, on Endo agar (Merck, Darmstadt, Germany), and on blood agar plates (Difco Laboratories, Detroit, Mich.) containing 5% washed sheep blood (Unipath, Wesel, Germany). The inoculated liquid cultures and solid media were incubated for 24 h at 37°C. Coliform colonies grown on washed sheep blood and Endo agar were examined for hemolysis, fermentation of lactose, and morphological differences. Representatives of different coliform grown colonies obtained from each fecal sample were purified and stored at −70°C for further examination as described previously (4). Enrichment cultures of fecal bacteria previously grown in tryptic soy broth were used for plating when no growth was obtained by direct inoculation of fecal material on solid media. *E. coli* isolates were identified by biochemical reactions as described previously (4). *E. coli* strains were detected in 110 of 114 examined fecal samples from cattle and in 138 of 150 fecal samples from sheep. In total, 498 *E. coli* strains (one to four morphologically different single-colony isolates per fecal sample) originating from 238 fecal samples of animals were investigated. Verotoxigenic *E. coli* strains were detected by the Vero cell test and were further investigated for the presence of stx_1 and stx_2 DNA sequences by colony blot hybridization with digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany)-labeled specific gene probes NTP705 and NTP707 and by PCR with stx_1- and stx_2-specific primer pairs KS7-KS8 and GK5-GK6 as previously described (4, 5, 6). stx_2 and stx_{2c} were distinguished by separate digestions of stx_2 PCR products with *Fok*I and *Hae*III (5). Stx-producing strains were additionally examined for the enterohemolytic (Ehly) phenotype as described previously (4) and for the presence of *eae* genes by colony blot hybridization using gene probe pCVD434 (5, 12).

Serotyping of *E. coli*. *E. coli* O and H antigens were examined according to standard methods (19).

Multilocus enzyme electrophoresis. To analyze enzyme electrophoretic variation, bacterial cell lysates were prepared and subjected to multilocus enzyme electrophoresis (25). Twenty enzymes were examined for allelic variation as described previously (30). For each enzyme, electromorphs were determined through comparisons to standard mobility variants and assigned numbers by their rate of anodal migration. Each isolate that lacked detectable enzyme activity was designated as having a null allele at the locus in question.

To estimate the genetic relationship among isolates, electromorphs were equated with alleles at the corresponding enzyme locus, so that each bacterial strain was characterized by its multilocus genotype (allele combination) for the enzyme-encoding loci assayed. Distinctive multilocus genotypes were designated electrophoretic types (ETs) and were numbered by their inferred relationships from phylogenetic analysis. Genetic distance between ETs was calculated from the proportion of mismatched alleles, under the assumption that a mobility difference results from at least one codon change at the nucleotide level. The genetic distances were used to generate a dendrogram by the average linkage algorithm and tested by bootstrapping as described previously (30).

Preparation of total genomic DNA, *Xba*I digestion, and pulsed-field gel electrophoresis (PFGE). Bacteria were grown at 37°C overnight from single colonies in 10 ml of 1% Bacto Tryptone (Difco). Grown cultures were harvested by centrifugation and resuspended in the same volume of SE buffer (10 mM Tris-Cl, 25 mM EDTA, and 75 mM NaCl [pH 7.5]). After a second centrifugation, the bacterial pellet was resuspended in 1 ml of SE buffer. A 300-μl aliquot of concentrated bacteria was mixed rapidly with the same amount of molten 2% Rapid-Agarose (Gibco-BRL, Eggenstein, Germany) followed immediately by casting mixtures in sample inserts in a Bio-Rad sample mold (Bio-Rad Laboratories, Richmond, Calif.) as instructed by the supplier. Lysis of bacteria embedded in agarose was performed by 15 h of incubation at 56°C in lysis buffer (10 mM Tris-Cl, 1 mM EDTA, 1% *N*-laurylsarcosine sodium salt [pH 9.5]) containing 0.5 mg of proteinase K (Boehringer Mannheim) per ml. After lysis, agarose plugs were washed five times in sterile TE buffer (10 mM Tris-Cl, 1 mM EDTA [pH 8.0]) followed by a 1-h incubation at 37°C in TE buffer containing RNase (final concentration, 20 μg/ml).

For *Xba*I restriction endonuclease cleavage of genomic DNA, the agarose plugs were equilibrated for 1 h at 4°C in *Xba*I restriction enzyme buffer (Reac 2; Gibco-BRL) followed by an overnight incubation in Reac 2 containing 50 U of *Xba*I (Gibco-BRL) at 37°C. After enzymatic digestion, the agarose plugs were washed in 10 mM Tris–50 mM EDTA (pH 8.0) and were kept at 4°C in the same type of buffer for long-term storage.

PFGE was performed with the clamped homogeneous electric field (CHEF-DR II) system from Bio-Rad Laboratories as instructed by the supplier. A slice of agarose plug was sealed into a well of 1% horizontal agarose gel as described in the Bio-Rad instruction manual. Lambda concatemers (Bio-Rad) were used as size markers. Gels were run at 200 V for 24 h at 14°C with a pulse time increasing from 5 to 50 s. After electrophoresis, gels were stained with ethidium bromide for visualization of single bands and photographed under UV light as described previously (23).

Preparation of plasmid DNA from STEC. Plasmid DNA of STEC was prepared from 5-ml overnight cultures of bacteria grown in Luria broth by the method described by Kado and Liu (13). Plasmid DNA patterns were obtained on vertical 0.7% agarose gels as described previously (23).

Preparations of stx_1- and stx_2-specific DNA probes and Southern hybridizations. Specific DNA probes were prepared from PCR-amplified DNA of strains C600(H19J) for stx_1 and C600(933W) for stx_2. PCR conditions and primer pairs KS7-KS8 for amplification of stx_1 sequences GK5-GK6 for stx_2 were as previously described (5). Probe-specific DNA fragments were isolated from agarose gels with a JETSORB gel extraction kit as instructed by the supplier (Genomed GmbH, Bad Oeynhausen, Germany). Labeling of DNA probes with digoxigenin-11-dUTP and DNA hybridization were performed as described previously (5).

Agarose gels from PFGE were blotted on nylon membranes (Boehringer Mannheim) by capillary transfer as described previously (24). Southern hybridizations with digoxigenin-11-dUTP-labeled *stx* probes were performed as described previously (14).

RESULTS

Characteristics of STEC excretion and STEC types in the herd of cattle. The pattern of STEC excretion of 19 cattle between February and July 1992 is shown in Table 1. Fecal samples from seven cows (36.8%) were negative for STEC during this time period. The remaining 12 cows (63.2%) excreted STEC one or more times during the period of investigation. In total, 30 (26.3%) of the 114 fecal samples from cattle were found to be positive for STEC, and from three samples (2.6%), more than one type of STEC was isolated. The 33 bovine STEC strains could be grouped into 11 different serotypes and eight different ETs (Table 2). The average number of different ETs found in STEC excreting cows was 1.8. Nine cows excreted STEC belonging to the same ET more than once during the 6-month period of investigation (Table 1). ET14 STEC comprising all O116:H21 strains predominated, with 18 single isolates obtained from 9 (47.4%) cows. STEC with ET14 and ET15 were continuously present in this herd over the 6-month period of investigation. All other STEC types occurred only sporadically and only in a few animals (Tables 1 and 2).

The 33 bovine STEC were very similar in regard to *stx* genotype. stx_1 was found only in one strain (rough:H25, ET2) and was absent in all others. All 33 STEC were positive for stx_2 and with the exception of three strains also for the stx_{2c} variant (Table 2). All but one strain (Ont:H−, ET15) were negative for *eae*-specific DNA sequences. The Ehly$^+$ phenotype was found in all STEC from cattle except in group O87:H16,H31 strains (ET7 and ET8).

Characteristics of STEC excretion and STEC types in the flock of sheep. Excretion of STEC by sheep is shown in Table 3. A total of 150 fecal samples were examined. STEC were isolated from 56 fecal samples originating from 22 (88.0%) of 25 sheep examined. Multiple types of STEC were found in 11 (7.3%) of the 150 fecal samples. The 67 STEC isolates from sheep could be grouped in 12 different ETs and 17 different serotypes (Table 4). The average number of different ETs found in STEC excreting sheep was 2.2. Twelve sheep excreted

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TABLE 1. Excretion of STEC in a herd of cattle over a 6-month period

Cattle no.[a]	STEC isolated[b] in:					
	February	March	April	May	June	July
12	—	—	—	—	O116:H21, ET14	O116:H21, ET14
14	—	O116:H21, ET14	—	—	—	—
18	O91:H49, ET11	—	Ont:H49, ET6; O90:H24, ET15	O116:H21, ET14	—	O116:H21, ET14
53	O91:H21, ET15	Ont:H33, ET2	—	—	—	Ont:H24, ET15
61	O116:H21, ET14	O116:H21, ET14	O90:H24, ET15	—	O116:H21, ET14	—
65	—	Rough:H25, ET2	—	O87:H16, ET7; O87:H16, ET8	O87:H31, ET7	—
76	—	O116:H21, ET14	—	—	O87:H31, ET8; O116:H21, ET14	O116:H21, ET14
81	—	O116:H21, ET14	O116:H21, ET14	—	Ont:H24, ET15	—
88	O116:H21, ET14	—	O116:H21, ET14	—	—	—
116	—	—	—	—	O116:H21, ET14	O116:H21, ET14
122	O74:Hnt, ET16	—	—	—	—	O116:H21, ET14
128	—	Ont:H−, ET15[c]	—	—	—	—

[a] No STEC were isolated from cows 11, 43, 49, 67, 73, 80, and 126) (not shown).
[b] STEC isolates are indicated by O:H serotype and ET. Rough, O antigen rough; Ont, O antigen not typeable; Hnt, H antigen not typeable. —, no STEC isolated.
[c] The only STEC isolate which was *eae* positive.

STEC of the same ET more than once during the 6-month period (Table 3). Interestingly, three different ETs (ET4, ET11, and ET14) and O serogroups (O125, O128, and O146) predominated in STEC from sheep (Tables 3 and 4). Of the 22 sheep excreting STEC, 9 (40.9%) carried ET4 (n = 13), 11 (50.0%) carried ET11 (n = 13), and 9 (40.9%) carried ET14 (n = 14) STEC. ET4 was mainly associated with O125:H−, O125:H14, and O125:H26 strains, ET14 was associated with O146:H21 strains, and ET11 was associated with most of the O128:H2 strains and with some other serotypes. The prevalence of these major ETs and serogroups in sheep changed with time. ET4 STEC were only found between February and May, showing a peak in March. ET11 and ET14 strains were both present in the flock for the whole period of investigation. Interestingly, the latter types became more prevalent in June and July, when the ET4 STEC were no longer detectable (Table 3).

Almost all ovine STEC were very similar with respect to *stx* genes (Table 4). stx_1 was present in 66 (98.5%) of these strains and was absent in only one strain (Ont:H−, ET15). stx_2 was present in 61 (91.0%) strains and absent only in 6 STEC strains belonging to rare ETs (ET12, ET15, and ET16) and serotypes. *eae*-specific DNA sequences were not found in any of the ovine STEC, and 59 (88.1%) were Ehly$^+$. The three major ETs and serotypes of ovine STEC were all positive for stx_1, stx_2, and Ehly.

Relationships in strains representing the major groups of bovine and ovine STEC. The serotypes found in ovine and bovine STEC were different from each other with the exception of O90:H24 strains, which were sporadically found in sheep and in cattle. Only 4 (ET11, ET14, ET15, and ET16) of the 16 different ETs occurred both in sheep and cattle. Of these, ET14 STEC were most frequent as O116:H21 in cattle and as O146:H21 in sheep.

Interestingly, bovine and ovine ET14 STEC were not similar with respect to *Xba*I restriction patterns and plasmid profiles. All bovine ET14 O116:H21 STEC strains had homogeneous *Xba*I and plasmid profiles (data not shown). The ovine ET14 O146:H21 strains exhibited four different *Xba*I patterns (A, B, G, and H) (Fig. 1). *Xba*I pattern A was most frequent among these strains and was also found in two single O146:H21 strains which belonged to ET1 and ET9 (Table 4). Pattern H and G strains were rare and occurred in only one sheep each. *Xba*I patterns A, B, and H were found to be very similar to each other, differing only in a few bands. Pattern G was less similar to patterns A, B, and H, showing differences in seven or more bands (Fig. 1A).

Association of *Xba*I restriction fragment length polymorphism in ovine STEC O146:H21 strains with altered chromosomal locations of stx_1 and stx_2 DNA sequences. To analyze the association of *stx* genes with the restriction fragment length polymorphism found in O146:H21 strains, *Xba*I-cleaved total DNA of representative O146:H21 strains with different PFGE patterns was Southern blotted and hybridized with stx_1- and stx_2-specific DNA probes. The results are shown in Fig. 1B. By DNA hybridization, the O146:H21 strains each showed one stx_1-hybridizing fragment and two stx_2-hybridizing fragments, indicating the presence of three different *stx* genes. This was different from what was found in O128:H2 STEC, each of which hybridized with only one stx_1 and one stx_2 fragment (Fig. 1B and data not shown). The differences in *Xba*I patterns found in O146:H21 strains were associated with alterations in sizes of the stx_1- and stx_2-hybridizing fragments (Fig. 1; Table

TABLE 2. Serotypes, ETs, and *stx* genotypes of STEC isolated from the herd of cattle

Serotype[a]	ET	*stx* genotype	Hemolytic phenotype	Total no. of isolates	Total no. of animals
Rough:H25	2	stx_1 stx_2	Ehly	1	1
Ont:H33	2	stx_2 stx_{2c}	Ehly	1	1
Ont:H49	6	stx_2 stx_{2c}	Ehly	1	1
O87:H16,H31	7	stx_{2c}	Negative	2	1
O87:H16,H31	8	stx_{2c}	Negative	2	2
O91:H49	11	stx_2 stx_{2c}	Ehly	1	1
O116:H21	14	stx_2 stx_{2c}	Ehly	18	9
O90:H24	15	stx_2	Ehly	2	2
Ont:H24	15	stx_2 stx_{2c}	Ehly	2	2
Ont:H− (*eae*$^+$)	15	stx_2 stx_{2c}	Ehly	1	1
O91:H21	15	stx_{2c}	Ehly	1	1
O74:Hnt	16	stx_2 stx_{2c}	Ehly	1	1

[a] Rough, O antigen rough; Ont, O antigen not typeable; Hnt, H antigen not typeable.

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TABLE 3. Excretion of STEC in a flock of sheep over a 6-month period

Sheep no.[a]	STEC isolated[b] in:					
	February	March	April	May	June	July
3	—	O125:H14, ET4; Ont:H10, ET12	O125:H14, ET4; O91:H−, ET12	O125:H−, ET5	—	O125:H14, ET4; Ont:H− ET11
8	—	O125:H14, ET4	O90:H24, ET15	—	—	O128:H2, ET3
11	—	—	—	—	—	O146:H21, ET14
13	O125:H26, ET5			O125:H−, ET4	—	Rough:H−, ET5
15	—	—	—	O128:H−, ET10	Rough:H10, ET10	Ont:H−, ET11
20	O128:H2, ET3	—	—	—	O128:H2, ET11	—
26	—	O125:H14, ET4	—	—	O146:H21, ET1	—
31	—	O125:H−, ET4	—	—	—	—
50	—	—	—	O128:H2, ET11	O128:H2, ET11	O128:H2, ET3
89	—	O128:H2, ET4	O128:H2, ET10	O90:H24, ET11	—	Ont:H−, ET11
92	O125:H−, ET4	O125:H14, ET4	—	—	—	—
138	—	—	—	—	—	O146:H21, ET14
179	—	—	—	O146:H21, ET14	—	O146:H21, ET14; O30:H12, ET16
197		O146:H21, ET14; Ont:H10, ET16	O146:H21, ET14; Rough:H−, ET12	O146:H21, ET14; O91:H− ET12	O91:H−, ET12; O90:H21, ET13	O146:H21, ET14
198	—	O6:H10, ET16; Ont:H−, ET15	O128:H2, ET9	Ont:H8, ET15	O90:H−, ET11	—
495	—	O128:H2, ET11	O128:H2, ET9	—	O146:H21, ET14	—
501	—	O128:H2, ET9	O128:H2, ET11	—	—	—
543	O125:H26, ET4	—	—	O125:H−, ET4	—	—
829	—	—	O125:H14, ET4	O125:H−, ET4	—	—
A2	O128:H2, ET11; O146:H2, ET14	—	—	—	—	—
D5	—	—	O146:H21, ET14	—	O128:H2, ET11; O146:H21, ET9	—
R003	O128:H2, ET11	O146:H21, ET14	O128:H2, ET10	—	O146:H21, ET14	—

[a] No STEC were isolated from sheep 24, 29, and 90 (not shown).
[b] STEC isolates are indicated by O:H serotype and ET. Rough, O antigen rough; Ont, O antigen not typeable. —, no STEC isolated.

5). *Xba*I patterns A, B, and H were identical for the stx_1-hybridizing fragment (306 kb) but were different from each other in their stx_2-hybridizing bands. The less related O146: H21 pattern G differed from the others with respect to the positions of both the stx_1-hybridizing band (325 kb) and the second stx_2-hybridizing fragment (170 kb). Interestingly, pattern G occurred in only one animal, which was the ram. The hybridization profiles of O146:H21 strains were more similar to each other than to those of O128:H2 strains (ET3), which are genetically unrelated and show fully different *stx*-specific hybridization profiles.

DISCUSSION

Prevalence and type specifity in natural colonizing STEC in a herd of cattle and a flock of sheep. Previous studies have shown that healthy domestic animals constitute important natural reservoirs of STEC of different serotypes (4, 10, 17, 28, 29, 32). Most epidemiological data are from studies with cattle where more than 100 different O:H types of STEC were detected by examining bovine herds at geographically different places (10, 32). Similar results were obtained with STEC isolated from small populations of sheep and goats (4). Apart from studies performed on *E. coli* O157:H7, little is known about the natural colonization of domestic animals with STEC over longer time periods (1, 28).

In this work, we investigated the prevalence and types of naturally colonizing STEC in a herd of cattle and a flock of sheep over a period of 6 months. The possible origin and the relationship between ovine and bovine STEC were investigated by comparing STEC isolates with respect to genetic similarity, serotypes, and *stx* genes. STEC were more frequent

TABLE 4. Serotypes, ETs, and *stx* genotypes of STEC isolated from the flock of sheep

Serotype[a]	ET	*stx* genotype	Hemolytic phenotype	Total no. of isolates	Total no. of animals
O146:H21	1	stx_1 stx_2	Ehly	1	1
O128:H2	3	stx_1 stx_2	Ehly	3	3
O125:H[b]	4	stx_1 stx_2	Ehly	12	8
O128:H2	4	stx_1 stx_2	Ehly	1	1
O125:H[c]	5	stx_1 stx_2	Ehly	2	2
Rough:H−	5	stx_1 stx_2	Ehly	1	1
O146:H21	9	stx_1 stx_2	Ehly	1	1
O128:H2	9	stx_1 stx_2	Ehly	3	3
O128:H2	10	stx_1 stx_2	Ehly	3	3
Rough:H10	10	stx_1 stx_2	Ehly	1	1
O128:H2	11	stx_1 stx_2	Ehly	8	7
Ont:H−	11	stx_1 stx_2	Ehly	3	3
O90:H[d]	11	stx_1 stx_2	Ehly	2	2
Ont:H10	12	stx_1 stx_2	Negative	1	1
Rough:H−	12	stx_1	Negative	1	1
O91:H−	12	stx_1 stx_2	Negative	3	2
O90:H21	13	stx_1 stx_2	Ehly	1	1
O146:H21	14	stx_1 stx_2	Ehly	14	9
Ont:H8	15	stx_1	Ehly	1	1
O90:H24	15	stx_1	Negative	1	1
Ont:H−	15	stx_2	Ehly	1	1
Ont:H10	16	stx_1	Negative	1	1
O30:H12	16	stx_1	Ehly	1	1
O6:H10	16	stx_1	negative	1	1

[a] Rough, O antigen rough; Ont, O antigen not typeable.
[b] O125:H− (five strains), O125:H14 (six strains), and O125:H26 (one strain).
[c] One O125:H− strain and one O125:H26 strain.
[d] One O90:H− strain and one O90:H24 strain.

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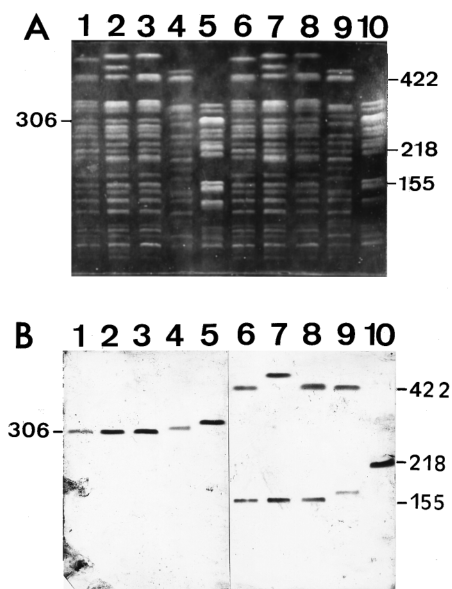

FIG. 1. (A) *Xba*I-digested total DNA of STEC from sheep separated by PFGE. Lanes: 1 and 6, O146:H21 (*Xba*I pattern H); 2 and 7, O146:H21 (pattern B); 3 and 8, O146:H21 (pattern A); 4 and 9, O146:H21 (pattern G); 5 and 10, O128:H2, (pattern E). (B) Southern blot of the agarose gel shown in panel A. Lanes: 1 to 5, hybridization with the stx_1-specific DNA probe; 6 to 10, hybridization with the stx_2-specific DNA probe. The positions and sizes of some of the stx_1-hybridizing (left) and stx_2-hybridizing (right) *Xba*I DNA fragments are indicated in kilobases; all are listed in Table 5.

in the flock of sheep than in the herd of cattle. It is likely that even more fecal samples were positive for STEC than were detected since only small numbers of coliform colonies were examined per fecal specimen.

Except for O90:H24 strains, cattle and sheep were completely different with respect to the O:H types of their STEC flora, indicating a host serotype specificity. Similar findings were reported for porcine STEC, showing serotypes which are generally not found in other animals or in humans (9, 26, 32). In our study, STEC of serotype O116:H21 were most frequent in cattle, and three other serotypes (O125:H diverse, O128:H2, and O146:H21) dominated in sheep. All other serotypes of STEC occurred only sporadically in a few animals each. It was shown previously that *E. coli* of animal origin can spread rapidly and can colonize the intestinal tracts of animals in a farm environment in the absence of antibiotic selection (16). This might also be the case with the clonally related predominant STEC types which were found in the herd of cattle and in the flock of sheep over longer time periods.

The occurrence of predominant and transient STEC types was also found in a longitudinal study on bovine STEC in Sri Lanka; however, other serotypes among these STEC O153:H12 were reported to predominate (28). According to many reports, STEC of serotypes O113:H21, O116:H21, and O153 are more frequently found worldwide in cattle and in beef products than others and might thus be characteristic for resident STEC types in cattle (4, 17, 20, 21, 22, 27, 28, 33).

The finding of resident and transient STEC types in cattle herds and sheep flocks could be explained in two ways. On one hand, both types of STEC might be present in about equal numbers in fecal specimens differing mainly in their occurrence within individual animals. On the other hand, both types may occur equally frequently in animals but differ mainly in their quantities in feces. Since we have not quantified the STEC in fecal specimens, we cannot discern between these two possibilities.

Association of *stx* genes and other virulence markers with origin and types of STEC in cattle and sheep. Previous studies of STEC originating from cattle, sheep, and goats or from infected humans have shown that the genes coding for Stx_1, Stx_2, or both toxins are present in similar proportions in many serotypes of strains (3, 4, 26, 28, 31). By comparing STEC of different geographical origin and sources, most serotypes, including O157:H7, O113:H21, and O116:H21, were not linked to a specific *stx* genotype, and different combinations of *stx* genes were found in strains belonging to the same serotype (4, 6, 11, 17, 27, 33). In contrast, the serotype was closely associated with a given *stx* genotype when distinct populations of cattle or food-borne single-source outbreaks of human disease were examined (17, 18, 28). In our study, all STEC strains belonging to one serotype also had the same *stx* genotype. The only exceptions were O90:H24 strains with stx_2 in bovine isolates and stx_1 and stx_2 in ovine isolates. Moreover, when bovine STEC were compared with ovine STEC, two major different *stx* genotypes were found. In cattle, 25 of the 33 STEC were positive for stx_2 and stx_{2c} and only one strain was positive for stx_1. In sheep, 60 of 67 strains were positive for stx_1 and stx_2 and only one strain was negative for stx_1.

The absence of *eae* genes and the almost uniform *stx* genotype found in single populations of cattle and sheep could indicate that most STEC within an animal population are epidemiologically related. However, this would be in contrast with the genetic and serological diversity found in STEC from single animal populations. It therefore appears possible that the serologically and genetically heterogeneous types of transient STEC found in populations of cattle and sheep are generated by spread of *stx*-encoding bacteriophages to *stx*-negative *E. coli* colonizing the intestine of the animals. The resident STEC type in an animal population might thus serve as the major source for *stx*-encoding bacteriophages since it is harbored by many animals and shows the same *stx* genotype which is present in practically all STEC arising from this animal population.

The differences found between genetically closely related STEC ET14 strains from cattle and sheep indicate that some STEC types such as O116:H21 are genetically very stable, showing homogeneous *Xba*I and plasmid patterns, whereas others, such as O146:H21, are more variable. The different *Xba*I and PFGE patterns in O146:H21 strains corresponded to individual animals, whereas the same PFGE pattern was found in animals excreting O146:H21 over several months (data not shown). It appears possible that transmission of O146:H21 from one sheep to another can be accompanied by genetic

TABLE 5. Size distributions of stx_1- and stx_2-hybridizing *Xba*I-cleaved DNA fragments in ovine O146:H21 and O128:H2 strains

Serotype (*Xba*I pattern)	ET	From sheep no.	DNA hybridization of *Xba*I-cleaved genomic DNA fragment with the indicated stx_1- or stx_2-specific DNA probe (fragment size [kb])		
			stx_1	stx_2 (I)	stx_2 (II)
O146:H21 (H)	14	11	306	422	155
O146:H21 (B)	14	495	306	534	155
O146:H21 (A)	14	D5	306	437	155
O146:H21 (G)	14	R003	325	422	170
O128:H2 (E)	3	50	345	218	None

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rearrangements which become visible as altered *Xba*I restriction patterns. A major cause of such rearrangements could be *stx*-encoding bacteriophages, which might excise and reintegrate at different sites on the bacterial chromosome. This could also explain why O146:H21 strains with different *Xba*I restriction patterns were all different with respect to sizes of *Xba*I-generated DNA fragments carrying stx_1 and stx_2 DNA sequences. The role of *stx*-encoding bacteriophages in the generation of new STEC types and as a source of genetic diversity in closely related STEC needs to be further investigated by in vitro and in vivo *stx*-encoding phage infection studies.

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