Assessment of Shiga Toxin-Producing *Escherichia coli* Isolates from Wildlife Meat as Potential Pathogens for Humans[▽]

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A total of 140 Shiga toxin-producing *Escherichia coli* (STEC) strains from wildlife meat (deer, wild boar, and hare) isolated in Germany between 1998 and 2006 were characterized with respect to their serotypes and virulence markers associated with human pathogenicity. The strains grouped into 38 serotypes, but eight O groups (21, 146, 128, 113, 22, 88, 6, and 91) and four H types (21, 28, 2, and 8) accounted for 71.4% and 75.7% of all STEC strains from game, respectively. Eighteen of the serotypes, including enterohemorrhagic *E. coli* (EHEC) O26:H11 and O103:H2, were previously found to be associated with human illness. Genes linked to high-level virulence for humans (*stx*₂, *stx*_{2a}, and *eae*) were present in 46 (32.8%) STEC strains from game. Fifty-four STEC isolates from game belonged to serotypes which are frequently found in human patients (O103:H2, O26:H11, O113:H21, O91:H21, O128:H2, O146:H21, and O146:H28). These 54 STEC isolates were compared with 101 STEC isolates belonging to the same serotypes isolated from farm animals, from their food products, and from human patients. Within a given serotype, most STEC strains were similar with respect to their *stx* genotypes and other virulence attributes, regardless of origin. The 155 STEC strains were analyzed for genetic similarity by XbaI pulsed-field gel electrophoresis. O103:H2, O26:H11, O113:H21, O128:H2, and O146:H28 STEC isolates from game were 85 to 100% similar to STEC isolates of the same strains from human patients. By multilocus sequence typing, game EHEC O103:H2 strains were attributed to a clonal lineage associated with hemorrhagic diseases in humans. The results from our study indicate that game animals represent a reservoir for and a potential source of human pathogenic STEC and EHEC strains.

Shiga toxin-producing *Escherichia coli* (STEC) strains represent an important emerging group of food-borne zoonotic pathogens causing diarrhea, hemorrhagic colitis (HC), and the life-threatening hemolytic uremic syndrome (HUS) in humans (30). Production of potent cytotoxins, which are called Shiga toxins (Stx) or Vero toxins (VT) and are encoded on the genomes of temperate lambdoid bacteriophages, is the major virulence determinant of STEC strains. Additional virulence factors, such as genes encoding the attaching and effacing function and virulence plasmid-encoding genes, contribute to the pathogenicity of STEC strains. These virulence genes are closely associated with a subgroup of STEC strains that are frequently isolated from patients with hemorrhagic diseases (HC and HUS) and were therefore designated enterohemorrhagic *E. coli* (EHEC) strains. Strains belonging to serogroups O157, O26, O103, O111, and O145 are the EHEC types most frequently isolated from humans with HC and HUS (33).

STEC strains are part of the gut flora of different animal species, and ruminants, particularly cattle, have been identified as a major reservoir of STEC strains that are highly virulent to humans (27). Today, it is evident that STEC strains can be transmitted from their animal reservoirs to humans via inges-

tion of contaminated food and water or by contact with STEC-excreting animals or the environment (9).

Recent reports indicate that wildlife animals play an important role as carriers and transmitters of STEC strains in nature. EHEC O157 strains (13, 32, 36, 40, 46) and other STEC strains were isolated from feces of different ruminant deer species at different geographic locations (2, 20, 28, 34, 36, 42). Deer have been suggested to play a role as transmitters of EHEC O157 strains to cattle by fecal contamination of farmland (43). Wild migrating birds have been identified as STEC excretors and participate in the spread of EHEC O157 and other STEC strains over long distances (17, 37, 47). To date, only a few reports have been published on the contamination of raw game meat and other game products with STEC strains. A study conducted in Belgium indicated that about half of meat samples from wildlife ruminants contained STEC strains (38). Deer meat and jerky were identified as sources of EHEC O157 infections in humans in the United States (31, 39). In Germany, different types of STEC strains were isolated from venison samples (34), and surveys performed in the Federal Institute for Risk Assessment revealed a contamination rate of wild meat samples with STEC strains of 9.0% to 14.8% between 2005 and 2006. In this time period, the proportion of STEC-contaminated samples from game was considerably higher than that found with beef samples (1.3% to 4.5% STEC positives) (23, 24).

Current data suggest that wild-living animals and their meat products are underestimated as natural reservoirs for STEC strains and as possible sources for human infections. Game

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TABLE 1. Serotypes and virulence genes of EHEC and STEC strains from wildlife meat

Serotype ^a	Total no. of strains	No. of strains from:			No. of strains of indicated genotype		
		Deer	Wild boar	Hares	Stx1 ^b	Stx2 ^b	<i>e-hlyA</i> ^c
O21:H21	29	27	2	— ^d	<i>stx</i> ₁ (1)	<i>stx</i> ₂ (1), <i>stx</i> _{2d} (2), <i>stx</i> _{2-O118} (27)	11
O146:H28	20	18	2	—	—	<i>stx</i> _{2-O118} (20)	4
O128:H2	12	11	—	1	<i>stx</i> _{1c} (11)	<i>stx</i> _{2-O118} (10)	11
O113:H21	9	7	1	1	<i>stx</i> ₁ (1)	<i>stx</i> _{2d} (7), <i>stx</i> ₂ + <i>stx</i> _{2d} (2)	9
O22:H8	8	8	—	—	<i>stx</i> ₁ (1), <i>stx</i> _{1c} (6)	<i>stx</i> _{2d} (2), <i>stx</i> _{2-O118} (7)	7
O88:H8	7 ^e	5	1	—	<i>stx</i> ₁ (2), <i>stx</i> _{1c} (3)	<i>stx</i> _{2-O118} (2)	4
O103:H2^f	4 ^g	1	1	1	<i>stx</i> ₁ (4)	—	4
O110:H31	4	4	—	—	—	<i>stx</i> _{2-O118} (4)	—
O6:H10	4	3	—	1	<i>stx</i> ₁ (3)	<i>stx</i> _{2d} (4)	—
O91:H21	4	3	1	—	<i>stx</i> ₁ (1)	<i>stx</i> _{2d} (2), <i>stx</i> ₂ + <i>stx</i> _{2d} (2)	3
O174:H8	3	2	1	—	<i>stx</i> _{1c} (2)	<i>stx</i> ₂ (1), <i>stx</i> _{2-O118} (3)	3
O179:H8	3 ^g	2	—	—	—	<i>stx</i> ₂ (2), <i>stx</i> ₂ + <i>stx</i> _{2d} (1)	3
O26:H11^h	3	1	—	2	<i>stx</i> ₁ (3)	—	3
O146:H21	2	2	—	—	—	<i>stx</i> _{2-O118} (2)	1
O6:H34	2	2	—	—	<i>stx</i> _{1c} (2)	—	—
O8:H19	2	—	1	1	—	<i>stx</i> _{2d} (1), <i>stx</i> _{2c} (1)	—
O8:H21	2	1	1	—	<i>stx</i> ₁ (1)	<i>stx</i> ₂ (2)	2
O91:H14	2	2	—	—	<i>stx</i> ₁ (2)	<i>stx</i> _{2d} (2)	2

^a STEC serotypes known to cause human infections (44) are marked in boldface characters. Serotypes represented by a single isolate each were as follows: STEC strains from deer (O101:H7, O110:H45, O153:H4, O177:H18, **O178:HNT**, O2:H6, O27:H30, O36:H14, **O5:H-** (*eae*-beta), **O88:25**, and **ONT:HNT**); from wild boar (O153:H1, O153:H21, O36:H19, O46:H38, O59:H21, O74:H8, and O8:H9), from hares (O15:H16), and from mixed game meat assortments (**O179:H31**).

^b Numbers of strains are shown in parentheses.

^c Data represent numbers of strains positive for the *e-hlyA* gene.

^d —, none of the strains showed the indicated trait.

^e One strain was isolated from mouflon meat.

^f All strains were positive for *eae*-epsilon.

^g One strain was isolated from a mixed game meat assortment.

^h All strains were positive for *eae*-beta.

meat is popular in Germany, since it is considered to be a high-quality product, and per capita consumption is rising steadily (report from the Federal Institute for Risk Assessment [http://www.bfr.bund.de/cd/7134]). To meet the demand for game meat, a total of 36,126 tons of wild animals were hunted from 2005 to 2006. These were divided into 19,000 tons of wild boar ($n = 461,881$ animals), 11,300 tons of roe deer ($n = 905,387$), and about 4,000 tons of red deer ($n = 60,664$) (Deutscher Jagdschutz-Verband [http://www.jagd-online.de]). Taking these data as a basis for estimation, the average amount of annual wild meat consumption is about 0.45 kg/person and accounts for 0.8% of the total meat consumption in Germany (22).

About 62% of retail game meat originates from animals hunted in the wild in Germany. Only 3% of the meat is from animals that are grown in captivity, with fallow deer the most frequently grown captive game animal. Imported game accounts for 35% of retail game meat (26). In compliance with the legal regulations, hunters are educated in meat inspection, and hygiene rules request evisceration of hunted game immediately after killing (C. Commichau [http://www.tiho-hannover.de/einricht/Immikro/wild1.doc]). Inspected and acceptable carcasses are allowed to proceed to immediate sale to individuals, restaurants, and food handlers. For safety reasons, processing of game meat must occur separately from processing of other meat; when processing of game meat is conducted on a larger scale, it is performed in special meat-processing plants. Only a small portion of hunted game meat is inspected by official meat inspection authorities (26).

At present, little is known about the characteristics of STEC

strains other than O157 strains from wildlife meat. In order to provide data for estimating the impact of game as a potential source of human pathogenic STEC types, we characterized 140 STEC strains found in meat isolates from deer, wild boar, and hare. The strains were examined for their serotypes, for properties related to virulence of *E. coli* for humans, and for their genetic relationship to STEC isolates from farm animals, from their food products, and from human patients. The aim was to determine the similarities between STEC strains from wildlife meat and those from other sources, including humans. Our data indicate that game is a natural reservoir for and a potential source of human pathogenic EHEC and STEC types.

MATERIALS AND METHODS

Bacteria. A total of 140 STEC strains isolated from game meat were investigated. Eighteen of these originated from wild boar, 110 from deer (70 roe deer, 36 red deer, and 4 fallow deer), 8 from hare, and 1 from a mouflon, and 3 strains were from mixed assortments of game meat samples. Game meat samples were not differentiated with respect to origin, i.e., whether they were from domestic or imported sources or whether they were from hunted wildlife animals or from animals grown in captivity for meat. The strains were isolated from retail meat in food inspection laboratories in different parts of Germany between 1998 and 2006 during routine diagnostic procedures and were sent to our laboratory at the Federal Institute for Risk Assessment for confirmation and typing. The STEC strains were investigated for their O:H serotypes and for their virulence markers as previously described (7). Nonmotile strains were investigated with respect to their flagellar (*flhC*) genotypes by PCR and HhaI digestion of PCR products as described previously (7). A total of 101 STEC strains from other sources showing serotypes and virulence markers similar to those found in the game isolates were chosen for comparison. Forty-four of these were from farm animals, i.e., cattle ($n = 21$), sheep ($n = 22$), and a goat. Twenty-three STEC isolates were from food; of these, 17 were of bovine origin (meat, milk, and cheese), 3 were from lambs, 2 from pigs, and 1 from a mixed beef-pork sample (7). Thirty-four STEC

TABLE 2. *stx* genotypes of STEC isolates from game meat

<i>stx</i> gene(s) ^a	No. of strains	% of strains
<i>stx</i> ₁	11	7.9
<i>stx</i> ₁ + <i>stx</i> ₂	2	1.4
<i>stx</i> ₁ + <i>stx</i> ₂ + <i>stx</i> _{2d}	2	1.4
<i>stx</i> ₁ + <i>stx</i> _{2d}	8	5.7
<i>stx</i> ₁ + <i>stx</i> _{2d} + <i>stx</i> _{2-O118}	1	0.7
<i>stx</i> _{1c}	9	6.4
<i>stx</i> _{1c} + <i>stx</i> _{2d} + <i>stx</i> _{2-O118}	2	1.4
<i>stx</i> _{1c} + <i>stx</i> _{2-O118}	17	12.2
<i>stx</i> ₂	4	2.9
<i>stx</i> ₂ + <i>stx</i> _{2d}	3	2.1
<i>stx</i> _{2d}	15	10.7
<i>stx</i> _{2d} + <i>stx</i> _{2-O118}	1	0.7
<i>stx</i> _{2-O118}	58	41.4
<i>stx</i> _{2e}	4	2.9
<i>stx</i> _{2g}	3	2.1

^a *stx* types associated with increased virulence for humans (18, 29) are marked in boldface characters.

isolates were from human patients in Germany suffering from diarrhea, HC, or HUS (6).

Detection of EHEC- and STEC-associated virulence markers. All strains isolated from wildlife meat were investigated for the production of Shiga toxins with the Vero cytotoxicity assay and the Gb3 enzyme immunoassay as described earlier (3). The previously described nomenclature for Shiga toxins was used (7). To avoid confusion with older nomenclatures, the *stx*₂ variant that was previously given the designation *stx*_{2d} (or *stx*_{2d-unt}) (GenBank AF043627) but that is functionally and genetically different from the mucus elastase-activatable toxin-encoding genes *stx*_{2d1} (AF479828) and *stx*_{2d2} (AF479829) is referred to as *stx*_{2-O118} in this report (44).

The presence of genes encoding Shiga toxins of the Stx1 and Stx2 family was investigated with common primers for the Stx1, Stx2, and Stx2f family toxins as described previously (7). Detection of genetic variants of both the Stx1 family (*stx*₁, *stx*_{1c}, and *stx*_{1d}) and the Stx2 family (*stx*₂, *stx*_{2-O118}, *stx*_{2d}, *stx*_{2e}, *stx*_{2f}, and *stx*_{2g}) was performed by PCR and analysis of restriction fragment length polymorphisms of endonuclease-digested PCR products (7).

The presence of the *eae* gene encoding intimin was examined by PCR with the common *eae* primers SK1 and SK2, and subtyping of *eae* genes was performed by specific PCRs as previously described (6). Production of EHEC plasmid-associated hemolysin (EHEC-hemolysin) was analyzed using washed sheep blood agar plates and genetically confirmed by PCR with *e-hlyA* (EHEC-hemolysin)-specific primers (6).

PFGE of total DNA of STEC strains. Pulsed-field gel electrophoresis (PFGE) was performed using a standardized PulseNet protocol published previously (21). Briefly, agarose-embedded DNA was digested with 50 U of XbaI (Roche Diagnostics GmbH, Mannheim, Germany) for 4 h at 37°C. The restriction fragments were separated by electrophoresis in 0.5× Tris-borate-EDTA buffer containing 50 μM thiourea at 14°C for 19 h by the use of a CHEF DR-III system (Bio-Rad, Munich, Germany), with pulse times of 2.16 to 54.17 s. XbaI-digested DNA of *Salmonella enterica* serovar Braenderup strain H9812 (Centers for Disease Control and Prevention, Atlanta, GA) was used as a molecular size marker. After staining with ethidium bromide was performed, gel images were captured using a Bio-Imaging system (biostep GmbH, Jahnsdorf, Germany), converted to a Tiff file, and analyzed using BioNumerics software (version 5.10; Applied Maths, Ghent, Belgium). Percentages of similarity between fingerprints were determined using the band-based Dice coefficient and a 1.50% band position tolerance. The unweighted-pair group method using average linkages was used for generating dendrograms.

MLST of *E. coli* housekeeping genes. A previously established multilocus sequence typing (MLST) protocol was applied to EHEC O103:H2 strains which were isolated from wild meat, farm animals, foods, and patients (5). Seven housekeeping genes (*adhA*, *arcA*, *fumC*, *icdA*, *mdh*, *mdh*, and *pgi*) that are evenly spread over the chromosome were chosen in order to minimize the influence of potential chromosomal hot spots of mutation and recombination. PCR product lengths ranged from 556 to 817 bp. Corresponding gene sequences derived from previously investigated *E. coli* O103 strains were used for comparisons (5).

TABLE 3. Serotypes of STEC isolates from different sources taken for analysis of virulence genes and genotyping

Serotype	No. of isolates from indicated source				
	Total	Wildlife meat	Farm animal	Food ^a	Human patient
O103:H2	22	4	6	3	9
O26:H11	11	3	3	1	4
O113:H21	24	9	7	5	3
O91:H21	17	4	4	5	4
O128:H2	34	12	13	2	7
O146:H21	21	2	10	4	5
O146:H28	26	20	1	3	2
Total	155	54	44	23	34

^a Data represent isolates from meat, milk, and cheese prepared from farm animal sources.

Sequencing of PCR products and analysis of gene sequences were performed as previously described (5).

RESULTS

Type diversity of STEC strains from wildlife meat. The 140 STEC strains from game divided into 26 O serogroups and 23 H types. Remarkably, only eight serogroups (O21, O146, O128, O113, O22, O88, O6, and O91) accounted for 71.4% (100 strains) and only four H types (H21, H28, H2, and H8) for 75.7% (106 strains) of all STEC isolates (Table 1). The 140 STEC isolates were assigned to 38 O:H serotypes, including 18 serotypes that have been already implicated as human pathogens (Table 1) (44). Seven (5.0%) of the STEC strains belonged to the classical EHEC types O103:H2 (four strains) and O26:[H11] (three strains).

All 140 strains harbored one to three *stx* genes. These divided into two subtypes of the Stx1 family, namely, *stx*₁ (17.1% of the strains) and *stx*_{1c} (20.0%), and five subtypes of the Stx2 family, namely, *stx*₂ (7.9%), *stx*_{2d} (22.9%), *stx*_{2-O118} (56.4%), *stx*_{2e} (2.9%), and *stx*_{2g} (2.1%) (Table 2). Cytotoxic activity on Vero cells and production of Stx as examined with the Gb3 enzyme immunoassay was found with 138 (98.6%) of the strains. Two strains belonging to serotypes O179:H31 and O36:H14 (Table 1) that each carried an *stx*_{2g} gene did not produce detectable amounts of toxins in the Vero cell and Gb3 assays. Other STEC-related virulence markers, such as the *eae* (in-

TABLE 4. *stx* genotypes and *eae* and *e-hlyA* genes of EHEC and STEC listed in Table 3

Serotype	Total no. of strains	No. of strains of indicated genotype ^c			
		<i>stx</i> ₁ ^a	<i>stx</i> ₂ ^a	<i>eae</i> ^a	<i>e-hlyA</i> ^b
O103:H2	22	<i>stx</i> ₁ (21)	<i>stx</i> ₂ (1)	Epsilon (22)	21
O26:H11	11	<i>stx</i> ₁ (9)	<i>stx</i> _{2d} (1), <i>stx</i> ₂ (2)	Beta (11)	11
O113:H21	24	—	<i>stx</i> ₂ (12), <i>stx</i> _{2d} (22)	—	23
O91:H21	17	—	<i>stx</i> ₂ (6), <i>stx</i> _{2d} (16)	—	16
O128:H2	34	<i>stx</i> _{1c} (33)	<i>stx</i> _{2-O118} (31)	—	32
O146:H21	21	<i>stx</i> _{1c} (17)	<i>stx</i> _{2-O118} (16)	—	18
O146:H28	26	—	<i>stx</i> _{2-O118} (26)	—	6

^a Numbers of strains are shown in parentheses.

^b Data represent numbers of strains positive for the *e-hlyA* gene.

^c —, negative test result.

FIG. 1. Dendrogram based on PFGE patterns of EHEC O103:H2 strains (Tables 3 and 4). Game isolates are marked in boldface characters. The 22 strains fall in three major clusters, namely, clusters A, B, and C (>68% similarity), and form subclusters in clusters A (A1 and A2) and B (B1 and B2). Strain 327/98 from a mixed game meat assortment grouped in subcluster B1 (88% similarity) together with four strains from cattle, milk, and two human patients. Strain 327/98 shows 95% similarity to a bovine strain (GSO740-1) and to a strain from a HUS case (CB5500). Two EHEC O103:H2 strains from wild boar (351/04) and roe deer (105/03) grouped in subcluster B2 (90% similarity) together with three strains from cattle and humans. Strain 351/04 is 100% similar in its PFGE profile to the human strains CB08223 and D242/02-3. In cluster C, strain RL0523/05 from hare meat shares 79% similarity with a bovine isolate (RW2198) and a human isolate (CB8198).

timin) and *e-hlyA* genes, were present in 8 (5.7%) and 77 (55.0%) of the STEC strains, respectively (Table 1).

Comparison of virulence genes of STEC isolates from game with those from serologically related STEC isolates from farm animals, from their products, and from human patients. For comparisons of STEC isolates from game to those from other sources, we chose representative strains belonging to the classical EHEC serotypes O103:H2 and O26:H11 as well as strains belonging to STEC types that are frequently isolated from different sources, including human patients (O113:H21, O91:H21, O128:H2, O146:H28, and O146:H21) (Table 3). Although STEC O21:

H21 strains are frequently isolated from game (Table 1), this serotype was not found among the isolates from human patients (44). A total of 155 STEC strains originating from game, farm animals, their food products, and human patients were compared (Table 3). Within a given serotype, EHEC and STEC strains were similar in their virulence attributes, regardless of the source (Table 4). Genes *stx*₂ and *stx*_{2d} were found to be most frequently associated with STEC O113:H21 and O91:H21 strains. Both *stx*_{1c} and *stx*_{2-O118} genes were present in most STEC O128:H2 and O146:H21 strains, whereas O146:H28 strains carried only the *stx*_{2-O118} gene. The combination of *stx*₁, *e-hlyA*, and *eae*-epsilon or

FIG. 2. Dendrogram based on PFGE patterns of EHEC O26:H11 strains (Tables 3 and 4). Game isolates are marked in boldface characters. Ten strains subdivided into major clusters A and B (82% similarity). Strain D618/98 from cheese was different with respect to its *stx*_{2d} gene and showed a nonclustering PFGE pattern. Cluster A (84% similarity) consists of five strains originating from deer meat, cattle meat, and a human patient. The PFGE patterns of strain 331/02 (deer meat) and bovine strains D316/04 and CB07505 were 92% similar. Cluster B (85% similarity) consists of two strains from hare meat and two human strains. The strains from hare meat were 88% similar to the human RL 06/0524 strain.

FIG. 3. Dendrogram based on PFGE patterns of STEC O113:H21 strains (Tables 3 and 4). Game isolates are marked in boldface characters. Twenty-two of the 24 strains grouped in major clusters A and B (82% similarity). Subcluster A1 is composed of seven strains from deer meat, beef, and cattle. The strain from deer meat (001/05) shows 91% similarity in its PFGE profile to the strains from cattle. Subcluster A2 harbors three strains with 93% similarity: one each from deer meat (140/04), a beef-pork isolate (RL0464/05), and a human strain (CB08578). Subcluster A3 (89% similarity) was composed of a strain each from wild boar meat, from cattle, and from beef. Five strains from red deer meat (206/99, 539/99, RL 06/0377, 641/99, and 014/04) grouped in subcluster B1 together with strain D366/99 from lamb meat (90% similarity).

ee-beta genes was highly associated with EHEC O103:H2 or O26:H11 strains, respectively (Table 4).

Similarity of STEC isolates from game to those from other sources as determined by PFGE genotyping. All 155 STEC isolates that were analyzed for their virulence markers (Table 4) were compared with respect to their XbaI macrorestriction patterns by PFGE. PFGE typing was performed to determine the genetic relatedness of STEC strains from game to those

from the other sources. Dendrograms based on similarities of PFGE patterns of strains of each serotype were established using BioNumerics software (Materials and Methods), and the results are shown in Fig. 1, 2, 3, 4, 5, 6, and 7. The genetic relatedness of strains from game to those from other sources differed for the different serotypes. Within serotype O26:H11, O113:H21, O128:H8, and O146:H28 strains, STEC strains from game were found distributed over most of the PFGE clusters

FIG. 4. Dendrogram based on PFGE patterns of STEC O91:H21 strains (Tables 3 and 4). Game isolates are marked in boldface characters. Fourteen of the 17 strains grouped into four clusters designated A (82% similarity), B (85%), C (89%), and D (94%). Three strains (170/00 from wild boar meat, RL06/0393 from deer meat, and RL06/0468 from pork) presented individual PFGE patterns. Strains 092/02 and RL 06/0303 from deer meat grouped in cluster A together with a human strain (CB07753) and a bovine strain (D392/99) (84 and 82% similarity). No strain from game was present in clusters B, C, and D.

FIG. 5. Dendrogram based on PFGE patterns of STEC O128:H8 strains (Tables 3 and 4). Game isolates are marked in boldface characters. All but 1 (RL0529/05) of the 34 STEC O128:H8 strains were arranged in clusters A and B. Strain RL0529/05 represents the only isolate lacking the *stx*_{1c} gene and shows a nonclustering PFGE pattern. Cluster A (79% similarity) was formed exclusively by strains from deer meat isolated between 1999 and 2006. Subcluster B1 (84% similarity) was composed of a strain from deer meat (RL 06/0367) and three STEC strains from sheep. Subcluster B2 (85% similarity) was formed by 21 strains; 10 of these, including 2 deer, 2 lamb, 1 sheep, and 5 human strains, showed 91% similarity in their PFGE profiles. One strain from game (467/01) showed 95% similarity to all of the nongame strains grouped in subcluster B2.

(Fig. 2, 3, 5, and 7), indicating that they were genetically similar to STEC strains from farm animals, food, and patients.

By cluster analysis, isolates from game and human patients were shown to share 90 to 100% similarity among EHEC O103:H2 strains (Fig. 1, clusters B1 and B2), 88% similarity among EHEC O26:[H11] strains (Fig. 2, cluster B), 93% similarity among STEC O113:H21 strains (Fig. 3, cluster A2), 91 to 95% similarity among STEC O128:H8 strains (Fig. 5, cluster B2), and 92% similarity among STEC O146:H28 strains (Fig. 7, clusters B2 and B4).

Strains from game and cattle showed 90 to 95% similarity among EHEC O103:H2 strains (Fig. 1, cluster B1 and B2), 92% similarity among O26:[H11] strains (Fig. 2, cluster A), and 89 to 91% similarity among STEC O113:H21 strains (Fig. 3, cluster A1).

Within the STEC O128:H8 group, strains from game, lamb, and sheep shared 91 to 95% similarity (Fig. 5, cluster B2). A lower degree of similarity was found among serotype O91:H21 strains from game, humans, and cattle (82 to 84% similarity; Fig. 4, cluster A) and among serotype O146:H21 strains from game, sheep, food, and human patients (less than 69% similarity; Fig. 6).

With some serotypes, single strains were detected that did not cluster with the other representatives. Their different PFGE profiles corresponded to altered numbers of *stx* genes or *stx* genotypes present in the divergent strains (Fig. 2, 5, and 6).

Comparison of EHEC O103:H2 strains from different sources by MLST. It was previously reported that most EHEC O103:H2 strains from human patients, farm animals, and food belong to a distinct clonal lineage (MLST I), as determined by MLST analysis (5). We used the previously published MLST typing protocol for investigation of the four EHEC O103:H2 strains from wild game (327/98, 105/03, 351/04, and RL 0523/05) as well as one O103:H2 strain from cattle (D332/04), one from beef (D469/02), and one from milk (D286/03). An EHEC O103:H2 CB5500 strain from a HUS patient which was previously investigated by MLST was used as a control (5). All 10 EHEC O103:H2 strains examined in this study were assigned to the MLST I profile, which was previously found to be associated with the majority of EHEC O103:H2 strains from humans and farm animals (reference 5 and data not shown). The MLST results indicate that EHEC O103:H2 strains from different types of game (deer, hare, and wild boar) belong to the clonal lineage of EHEC O103:H2 strains involved in diarrheal and hemorrhagic diseases in humans.

DISCUSSION

Compared to those from studies of the farm animal reservoir, few reports are available on the role of wildlife animals and their products as sources of STEC strains. Most of the

FIG. 6. Dendrogram based on PFGE patterns of STEC O146:H21 strains (Tables 3 and 4). Game isolates are marked in boldface characters. Two strains from game (264/04 and RL06/0466) present in this group formed PFGE cluster A (73% similarity). All other STEC O146:H21 strains except DG074/2 grouped into cluster B (>74% similarity) and were from sheep, beef, and humans. The bovine DG074/2 strain was different from all other STEC O146:H21 strains with respect to its *stx*_{1d} gene and showed a nonclustering PFGE profile. The two STEC strains from game were less than 69% related to the other STEC O146:H21 strains and lacked the *stx*_{1c} gene which was present in all but one (CB07743) cluster B strains.

reports that are available deal with wildlife animals such as deer and have focused on the O157:H7 serotype (11, 13, 16, 20, 36, 40, 41, 43). Only a few cases of human EHEC O157 infection associated with consumption of game have been reported,

including a family outbreak linked to jerky from black-tailed deer meat in 1995 and a sporadic case linked to consumption of white-tailed deer meat in 2002 (31, 39).

A few reports indicate that non-O157 STEC strains are

FIG. 7. Dendrogram based on PFGE patterns of STEC O146:H28 strains (Tables 3 and 4). Game isolates are marked in boldface characters. The 26 O146:H28 strains divided into major clusters A, B, and C (>72% similarity). High-level similarities between strains from game and strains from patients were found in clusters B2 (RL0510/05 and CB08026 [92% similarity]) and B4 (RL0160/05 and CB07787 [90% similarity]). In cluster B3, one strain from roe deer (RL06/0123) and one from beef (D166/00) showed 92% similarity. Lower levels of similarity between strains from game and other sources were found in cluster A (75%) and cluster C (81%).

frequently isolated from wildlife animals in different parts of the world. Prevalence rates of 10.5 to 16.3% were reported for non-O157 STEC strains in two studies of wild deer from Japan (2, 19). A report from the United States indicates a prevalence rate for non-O157 STEC strains of about 5% in fecal specimens from deer (28). A study conducted in Argentina revealed 38.5% of investigated wildlife ruminant species to be STEC excretors (35). High rates of STEC-excreting wildlife ruminants were also reported from studies conducted in Europe. In Germany, 51.8% of wildlife ruminants were identified as carriers of STEC (34); similar high rates of carriage were reported for STEC-excreting animals from a Spanish study performed using different deer species (42).

Few data have been published on carriage rates of non-O157 STEC strains in wildlife meat. In a Belgian study, STEC strains were found in 16% of meat samples from red deer, 21% from roe deer, and 22% from fallow deer (38). In Germany, 7.5% of roe deer meat samples were found to test positive for STEC (45), and reports from the Federal Institute for Risk Assessment indicate that 9.9 to 14.8% of game sampled in 2005 and 2006 yielded STEC isolates (23, 24).

Strains of STEC other than O157 isolated from game have not yet been identified as causative agents of food-borne infections in humans. On the other hand, 80 (57.1%) of the 140 STEC strains examined in our study belonged to 18 serotypes previously associated with human pathogenicity (44). STEC strains belonging to the prevalent serogroups found in game in our study (O26, O91, O103, O113, O128, and O146) are frequently isolated from human patients in Europe (15, 49). Virulence genes associated with severe clinical outcome (*stx*₂, *stx*_{2d}, and *eae*) were present in 46 (32.8%) of the 140 STEC strains from game. Strains belonging to the classical EHEC types O26:H11 and O103:H2, which rank among the five STEC serotypes most frequently isolated from human patients in Europe (15), were isolated from deer, hare, and wild boar. To our knowledge, this is the first report of the detection of EHEC O26 and O103 strains in wildlife meat.

STEC strains investigated in this study showed distinct types of virulence genes which were related to serotypes but were not related to strain origin or source. With a few exceptions, STEC strains from game were similar with respect to their virulence genotypes to STEC strains from farm animals, food, and human patients. Cluster analysis of PFGE patterns revealed that certain STEC isolates from game were genetically highly similar to STEC isolates from farm animals, food, and human patients. Similarities of >90% between STEC isolates from different sources were found for O103:H2, O26:H11, O113:H21, O128:H2, and O146:H28 strains, and these serotypes were previously reported as the cause of human infections (15, 49). EHEC O103:H2 strains of PFGE clusters A and B (75% similarity; Fig. 1) were shown by MLST analysis to belong to one clonal lineage (MLST I). This clonal type was previously found to be associated with most EHEC O103:H2 strains from human patients, cattle, and food (5). Our findings show that the predominant type of EHEC O103:H2 strains responsible for HC and HUS in humans is also present among isolates from game.

These and other published data indicate that wildlife animals serve as natural reservoirs for major types of STEC strains that are known to cause disease in humans. We cannot

exclude the possibility that a portion of the STEC-positive meat samples were from animals grown in captivity for meat; however, meat from this group constitutes only 3% of the game meat retailed in Germany, in contrast to the 62% representing hunted, free-living game (26). We were also unable to distinguish between domestic and imported meat; the latter constitutes 35% of the retailed game in Germany (26). It is likely, however, that most of the STEC strains found in game animal meat originate from fecal contamination of the animals during slaughter and processing. Fecal contamination of carcasses by the producer animal was found to be the most important source of STEC strains in meat products from farm animals (8, 10, 14, 25, 27). Slaughterhouses were identified as the primary source of meat contamination by STEC strains. Contamination rates were shown to be reduced by employment of high standards of hygiene and by safe separation of the intestine from the carcass after slaughter (1, 25, 27, 28). Game is even more likely to be contaminated with fecal STEC strains, since killing and evisceration of animals and further processing of meat is performed under hygiene conditions that are less controlled than those employed in abattoirs.

The finding that similar types of STEC strains are found in game animals and farm animals and in their products indicates that transmission between the wildlife reservoir and the domestic animal reservoir occurs. STEC strains were shown to be spread to different animal species on farms (4, 12, 37, 48), and transmission of STEC strains from wildlife to farm animals and vice versa may occur through fecal pollution of farmland (43). Migrating birds serve as vectors of transmission of STEC strains over long distances (17, 37, 47). Taken together, these data indicate that there is a flow of STEC strains between the wildlife reservoir and the domestic animal reservoir. Accordingly, the natural reservoir for human pathogenic types of STEC strains is much larger than had previously been thought.

The results of our study demonstrate that STEC strains in meat from wildlife animals such as deer, hare, and wild boar are potential human pathogens and should be recognized as a public health problem. Further studies are needed to clarify whether the high incidence of EHEC and STEC contamination of wildlife meat is linked to a lack of hygiene during meat processing or to a higher proportion of wildlife animals colonized with STEC strains. Hunters who handle game in the field might be unaware of the contamination risk with STEC strains due to improper handling and processing. Specific hygiene recommendations should be formulated for hunters, manufacturers, food control agencies, and private households in order to minimize the risk of EHEC and STEC food-borne infection in humans.

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