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Zoonotic Agents in Small Ruminants Kept on City Farms in Southern Germany

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Sheep and goats are popular examples of livestock kept on city farms. In these settings, close contacts between humans and animals frequently occur. Although it is widely accepted that small ruminants can carry numerous zoonotic agents, it is unknown which of these agents actually occur in sheep and goats on city farms in Germany. We sampled feces and nasal liquid of 48 animals (28 goats, 20 sheep) distributed in 7 city farms and on one activity playground in southern Germany. We found that 100% of the sampled sheep and 89.3% of the goats carried Shiga toxin-producing *Escherichia coli* (STEC). The presence of *Staphylococcus* spp. in 75% of both sheep and goats could be demonstrated. *Campylobacter* spp. were detected in 25% and 14.3% of the sheep and goats, respectively. Neither *Salmonella* spp. nor *Coxiella burnetii* was found. On the basis of these data, we propose a reasonable hygiene scheme to prevent transmission of zoonotic agents during city farm visits.

City farms provide animal-assisted activities and room for free playing and movement and for experiencing nature as well as contacts without cultural, social, or financial barriers for children and teenagers growing up in an urban environment. They are supervised by pedagogical professionals and additional staff. Many of these farms keep sheep and goats as examples of livestock. Close child-animal contacts occur while the children are cleaning pastures and stables or feeding, currycombing, or petting the animals. Some farms breed their own sheep and goats and use their milk either for direct consumption or to make cheese, and most use the sheep wool for handicrafts (5).

It is widely accepted that sheep and goats play a role in the transmission of several zoonotic diseases (7, 28). These include the most regularly reported zoonoses in Germany and Europe, such as salmonellosis and campylobacteriosis and infections with Shiga toxin-producing *Escherichia coli* (STEC), as well as Q fever, and infections with *Staphylococcus* and many other pathogens (15, 21).

The relevance of visits to petting zoos, city farms, or comparable institutions to the transmission of zoonotic agents has been previously reported (6, 9, 23, 32, 52, 54). Animals living on city farms are closely monitored for general signs of illness, but members of the farm staff should also be aware of the fact that some of the animals might be asymptomatic carriers of zoonotic agents.

The idea of this study was to evaluate which of the most commonly isolated zoonotic agents occur in sheep and goats kept on city farms in Germany, to assess any existing health risks from the agents, and to propose suitable hygiene measures.

MATERIALS AND METHODS

Sampling. Samples were collected from 48 apparently healthy small ruminants (28 goats, 20 sheep) in January and February 2011 on seven city farms and in one activity playground in Bavaria and Baden-Wuerttemberg.

Fecal samples were collected by rectal retrieval for the subsequent cultivation of *E. coli*, *Salmonella* spp., and *Campylobacter* spp. The samples were sent to the laboratories in (semisterile) plastic bags immediately. Samples for *Campylobacter* cultivation were transferred to Campygen compact (Oxoid, Wesel, Germany) atmosphere generation systems to provide a microaerophile atmosphere. Rectal swabs for subsequent *Coxiella burnetii* DNA analysis were immediately frozen. DNA was extracted by using a QIAamp DNA stool minikit (Qiagen, Heiden, Germany), following the manufacturer's instructions. Extraction products were frozen and send to the laboratory.

For the cultivation of *Staphylococcus* spp., nasal swabs were taken from 43 (26 goats, 17 sheep) of the 48 small ruminants in the study. The swabs were sent to the laboratory in sterile tubes immediately.

Methods for isolation and characterization of STEC. (i) Microbiological procedure and isolation of single colonies. Aliquots of 0.5 g of each sample of feces were homogenized after adding 4.5 ml of 0.9% NaCl solution. A 10-fold series of dilutions of each sample were produced from the undiluted specimen up to a dilution of 10^{-5}. For each dilution step, 100 μl was plated directly onto McConkey agar (SIFIN, Berlin, Germany) and incubated overnight at 37°C (5 agar plates per sample).

Agar plates with 30 to 300 pink colonies were chosen for the enumeration of colonies, and the number of CFU per gram of sample was calculated. Plates were precooled at 4°C for 30 min. A nylon membrane disc (Nylon Membranes for Colony and Plaque Hybridization; Roche Diagnostics, Mannheim, Germany) was placed onto the surface of the agar plate with separate colonies and the orientation marked. Subsequently, the membrane disc was removed and placed on filter paper soaked with denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 15 min, transferred to filter paper soaked with neutralization solution (1.5 M NaCl, 1.0 M Tris-HCl, pH 7.4) for 15 min, and air dried. The dry membrane was heated at 80°C for 60 min, equilibrated with 2× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]) for 10 min, and placed on filter paper soaked with dilute proteinase K (Roche Diagnostics, Mannheim, Germany) (14 to 22 mg/ml) at a 1:10 dilution in 2× SSC to remove cell debris.

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The DNA probes for *stx* were labeled using digoxigenin (DIG), MP3 and MP4 primers (38), and a PCR DIG probe synthesis kit (Roche Diagnostics, Mannheim, Germany) as recommended by the manufacturer. DIG Easy Hyb solution (Roche Diagnostics, Mannheim, Germany) was used for prehybridization and hybridization of nylon membranes. The *stx*-positive colonies were detected by the use of a DIG nucleic acid detection kit (Roche Diagnostics, Mannheim, Germany) as recommended by the manufacturer. The *stx*-positive colonies were enumerated, and the proportion of *stx*-positive colonies in the total number of CFU was calculated. A total of up to 20 *stx*-positive colonies per sample were selected. A number of 193 *stx*-positive colonies (up to five per sample) were characterized by genotyping.

(ii) Template preparation. Genomic DNA of the *stx*-positive colonies was prepared from 4 ml of overnight cultures in Luria Bertani broth (LBB; SIFIN, Berlin, Germany). A 1-ml volume of LBB culture was harvested by centrifugation (13,000 × *g*, 3 min) and washed three times in distilled water. Each pellet was resuspended in 200 μl of distilled water, boiled at 95°C for 10 min, and immediately cooled on ice. After centrifugation (13,000 × *g*, 3 min), the supernatants were transferred into clean tubes and stored at −20°C.

(iii) Block cycler PCRs. Differential identification of specific *E. coli* pathotypes was performed by multiplex PCR as described by Müller et al. (38). A 5-μl aliquot of the template in 25-μl reaction mixtures consisting of components of a multiplex PCR Master Mix system (New England BioLabs, Frankfurt am Main, Germany) was used. Primers, thermocycling conditions, and the expected size of the amplicons were as described previously (38).

The stx_1 and stx_2 genes were subtyped by PCR assays according to the methods presented in EQA nomenclature 2011 (WHO Centre *E. coli*, Statens Serum Institut [SSI], Copenhagen, Denmark). Details regarding primers, thermocycling conditions, and the expected size of the amplicons can be found at the website of WHO Centre *E. coli*, SSI, Copenhagen, Denmark (http://www.ssi.dk/English/HealthdataandICT/National%20Reference%20Laboratories/WHO%20Collaborating%20Centre%20for%20Reference%20and%20Research%20on%20Escherichia%20and%20Klebsiella.aspx). The identification of the *eae* subtypes was performed as described previously (44, 56).

In addition to the reactions performed with template DNA from known positive- and negative-control strains, a reaction mixture containing water instead of template was included in each experiment to detect possible reagent contaminations. Amplified fragments were separated by agarose gel electrophoresis and visualized after ethidium bromide staining under conditions of UV illumination.

(iv) Genotype characterization by microarrays. Miniaturized *E. coli* oligonucleotide arrays in the ArrayStrip format (Alere Technologies, Jena, Germany) containing gene targets for the identification of virulence genes and antimicrobial resistance genes and DNA-based serotyping were used for the genetic characterization of the STEC isolates. A complete list of primers and probes and a description of the layout of the array are available in Table SA1 in the supplemental material.

For labeling and biotinylation of the genomic DNA, a site-specific labeling approach was used (44). The primer elongation and the hybridization, washing, and straining of ArrayStrips were described previously (17). The ArrayStrips were photographed using an ArrayMate instrument (Alere Technologies, Jena, Germany) and automatically analyzed. After automated spot detection, mean signal intensity (mean) and local background (lbg) were measured for each probe position and values calculated by the formula value = 1 − mean/lbg. Resulting values below 0.1 were considered to represent negative results, and those above 0.3 were considered to represent positive results. Values between 0.1 and 0.3 were regarded as ambiguous. Validation was performed using a collection of sequenced control strains (GenBank accession numbers AE005174 [*E. coli* EDL933 O157:H7], FM180568 [*E. coli* E2348/69 O127:H6], U00096 [*E. coli* K-12 MG1655], AP009048 [*E. coli* K-12 W3110], CP000247 [*E. coli* O6:K15:H31], CP001509 [*E. coli* BL21], AE014075 [*E. coli* CFT073], and CP000946 [*E. coli* ATCC 8739]). These control strains were tested with the method described above. Theoretically expected hybridization patterns were compared with experimental results. In addition, an enterohemorrhagic *E. coli* (EHEC) O104:H4 outbreak strain (DD-23137) was included as a reference strain.

Methods for isolation and characterization of *Salmonella*. All samples were examined bacteriologically for the presence of *Salmonella* organisms according to ISO 6579 annex D. Sample material was weighted and diluted 1:10 in buffered peptone water (BPW; SIFIN, Berlin, Germany) followed by incubation at 37°C for 18 to 20 h. Afterward, 100 μl of the preenrichment culture was spotted onto modified semisolid Rappaport-Vassiliadis agar (MSRV; Merck, Darmstadt, Germany) and incubated twice for 24 h each time at 42°C and read at 24 h and 48 h. The MSRV plates were examined for the presence of migration zones. A loopful was taken from the edge of the migration zone and streaked onto xylose lysine deoxycholate agar (XLD; Merck, Darmstadt, Germany) and onto xylose lysine tergitol 4 agar (XLT4; Merck, Darmstadt, Germany) and incubated for 18 to 24 h at 37°C. *Salmonella* presumptive colonies from these selective media were streaked onto nutrient agar (SIFIN, Berlin, Germany). The identification of the isolates was carried out by serotyping. For the determination of the presence of species of the genus *Salmonella*, suspected colonies were suspended in polyvalent anti-*Salmonella* A-E and/or A-67 serum (SIFIN, Berlin, Germany) and examined by agglutination. For the identification of the serovar, the somatic and flagellar antigens were determined using anti-*Salmonella* monovalent O and H sera (SIFIN, Berlin, Germany) according to the Kauffmann-White scheme (19).

Methods for isolation and identification of *Campylobacter*, *Staphylococcus*, and other bacterial species. *Campylobacter* strains were isolated from fecal samples according to an ISO-approved method (25). Briefly, 5 g of fecal matter was placed in a plastic bag (Oxoid, Wesel, Germany), and then 5 ml of phosphate-buffered saline (pH 7.4) was added and well mixed in a stomacher (Seward Stomacher 80 Biomaster; Seward Laboratory Systems, Inc., West Sussex, United Kingdom) at normal speed for 1 min. A loopful of a diluted sample (1:100) was streaked directly onto modified CCDA (mCCDA) plates (Oxoid, Wesel, Germany) and incubated at 42°C for 24 h under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) in a gas pack jar (Trilab system; Jenny Medical, Beromünster, Switzerland). In addition, 3 ml of diluted samples was added to 7 ml of Bolton broth (Oxoid, Wesel, Germany) as selective enrichment media and incubated at 42°C for 24 h under microaerophilic conditions. A loopful of broth was plated on two different *Campylobacter* selective media, mCCDA and Brilliance CampyCount agar (Oxoid, Wesel, Germany). Incubation at 37°C for 48 h under microaerophilic conditions was followed by subcultivation of suspected colonies on Mueller-Hinton (MH) blood agar (10% bovine citrated blood) for a further identification process.

For the molecular biological identification of isolates, genomic DNA was extracted from a 48-h bacterial culture by the use of MH blood agar plates and a High Pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA was eluted in 200 μl of elution buffer.

A modified version (14) of a multiplex PCR assay (11) was used to identify thermophilic *Campylobacter* species (*C. jejuni*, *C. coli*, and *C. lari*). DNA samples of *C. jejuni* DSM 4688, *C. coli* DSM 4689, and *C. lari* DSM 11375 were used as positive controls.

16S rRNA genes of non-*Campylobacter* isolates growing on mCCDA agar were partially amplified by PCR with 16SUNI-L (5′-AGA GTT TGA TCA TGG CTC AG-3′) as the forward primer and 16SUNI-R (5′-GTG TGA CGG GCG GTG TGT AC-3′) as the reverse primer (Jena Bioscience GmbH, Jena, Germany) to generate an approximately 1,400-bp fragment in an amplification reaction performed according to the method of Kuhnert et al. (30). PCR products were analyzed by agarose gel electrophoresis, ethidium bromide staining, and visualization under UV light. Bands were cut out, and DNA was purified using a gel extraction kit (Qiagen) according to the manufacturer's recommendations.

Cycle sequencing of partial 16S rRNA genes was done in both directions by using forward and reverse amplification primers with a BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Darmstadt, Germany) according to the recommendations of the manufacturer. Sequencing products were analyzed with an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Darmstadt, Germany).

Identification of *Ochrobactrum* and other isolates was done by a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using 16S rRNA gene sequences.

Cultivation of staphylococci from nasal swabs on Baird Parker agar that included egg yolk tellurite emulsion (SIFIN, Berlin, Germany) was performed by overnight incubation at 37°C. Black colonies were picked and subcultivated on blood agar plates. A loopful of bacteria was suspended in 300 μl of phosphate-buffered saline. Genomic DNA was isolated after enzymatic cell lysis was performed using lysis buffer and lysis enhancer (both from a StaphyType kit; Alere Technologies GmbH, Jena, Germany) under conditions of shaking (1 h, 37°C, 550 rpm in an Eppendorf thermomixer/MTP [Eppendorf, Hamburg, Germany]). Isolation of DNA was carried out with a High Pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA microarray analysis of isolates was done according to the method of Monecke et al. (36). Briefly, DNA was labeled by incorporation of biotin-16–dUTP within an iterated linear primer elongation. Denatured amplicons were hybridized to the array followed by washing and blocking steps. After horseradish peroxidase-streptavidin conjugate addition, incubation, and washing steps, hybridization results were visualized using a precipitation dye. Automated analysis of results was done using a designated reader and software package (Alere Technologies, Jena, Germany).

Real-time PCR for the detection of *Coxiella burnetii* DNA. Detection of *C. burnetii* was performed with a TaqMan-based real-time PCR assay targeting the IS*1111* transposase element as described by Klee et al. (27). Real-time PCR was conducted using 96-well microplates (Applied Biosystems, Foster City, CA) and a Stratagene Mx3000P Thermocycler (Agilent Technologies, Santa Clara, CA). The final 20-μl reaction mixture was made up of 10 μl of Maxima Probe qPCR Master Mix (Fermentas, St. Leon-Rot, Germany), primers (Cox-F 5′-GTCTTAAGGTGGGCTGCGTG-3′ and Cox-R 5′-CCCCGAATCTCATTGATCAGC-3′) (0.3 μM), a probe (Cox-TM 6-carboxyfluorescein [FAM]-AGCGAACCATTGGTATCGGACGTT-6-carboxytetramethylrhodamine [TAMRA]-TATGG) (0.1 μM), and 2 μl of sample DNA. The cycling parameters were 2 min at 50°C, 10 min at 95°C, 40 cycles at 95°C for 15 s, and 30 s at 60°C (annealing and extension). The cycle threshold value (C_T) was calculated using the software (MxPro3000P version 4.01) supplied with the instrument. A result was considered negative when no amplification occurred or when the cycle threshold value was ≥40.

RESULTS

STEC results. (i) Samples and isolates. A total of 48 fecal samples from 20 sheep and 28 goats were tested for STEC. The animals were kept in seven city farms and one activity playground. STEC strains were detected in 45 of the 48 fecal samples (93.75%), all samples from sheep (100%), and 25 of 28 samples from goats (89.3%). The percentage of STEC in the total number of *E. coli* per sample ranged between under 1% and 100%. All colonies were *stx* positive for 12 animals (6 sheep, 6 goats) (Table 1). STEC-positive animals were found in all 8 farms.

The isolation of single STEC colonies by colony blot hybridization was accomplished for all STEC-positive samples. A total of 193 *stx*-positive colonies (up to five per sample) were characterized by genetic typing.

(ii) Analyses of EHEC virulence-associated factors. Numerous EHEC-associated and other *E. coli* virulence markers were tested using oligonucleotide microarrays and PCRs. The stx_1 genes characterized as stx_{1a} (11 isolates) and stx_{1c} (148 isolates) subtypes (EQA nomenclature 2011; WHO Centre *E. coli*, SSI, Copenhagen, Denmark) were found in 159 STEC isolates. An additional stx_2 gene was detected in 103 stx_1-positive isolates. In total, stx_2 genes were found in 107 isolates. All stx_2 genes were subtyped as stx_{2b} (EQA nomenclature 2011; WHO Centre *E. coli*, SSI, Copenhagen, Denmark). No *stx* genes were found in 30 of the 193 isolates (15.5%), although they had previously been screened for *stx* genes by colony hybridization. An EHEC *hlyA* gene was detected often. This gene was found in 142 of the 193 tested isolates. EHEC *hlyA* genes were demonstrated in 135 *stx*-positive isolates. However, intimin genes were found very rarely. Only five isolates contained an *eae* gene; one of these isolates was also positive for stx_{1c} and stx_{2b} genes. No *stx* genes were detected in the other four *eae*-positive isolates. The five *eae*-positive isolates were also positive for an EHEC *hlyA* gene. For the subtyping of the intimin genes, a PCR product of 2,287 bp was amplified in the four *stx*- and *eae*-positive isolates by the use of SK1 and LP4 primers (56). The *eae* genes were therefore considered members of the β-*eae* subgroup. All results from subtyping of the *eae* gene of the single *stx*- and *eae*-positive isolate were negative, as determined by a subtyping PCR method described previously (44, 56). We detected both the locus of enterocyte effacement (LEE) and non-LEE genes of the type III secretion system (T3SS) in the *eae*-positive isolates, but the compositions of the genes differed. The *espA*, *espC*, and intimin receptor (*tir*) genes were found in the single *stx*- and *eae*-positive isolate. Furthermore, genes *espF* and *espI* and *nleA*, *nleB*, and *nleC* were detected in the same isolate. In contrast, the other *eae*-positive isolates contained the *espB* gene, but detection of the *espA*, *espC*, and *tir* genes failed, while the isolates harbored the *nleA* and *espI*, *espF*, and *espJ* genes (Table 1; see also Table SA2 in the supplemental material).

We also frequently detected the gene for a heat-stabile enterotoxin (*astA* gene; 57 isolates), the gene for enterobactin siderophore receptor/adhesin (*iha* gene; 127 isolates), the iron-regulated virulence gene (*ireA* gene; 102 isolates), and the gene for fimbria adhesion (*lpfA* gene; 117 isolates) in the 193 tested isolates. The plasmid-carried virulence gene *espP* was demonstrated in five isolates only, whereas the *katP* gene was found only once. The STEC autoagglutinating adhesion gene (*saa*; 5 isolates) and the *toxB* toxin gene (6 isolates) were only rarely amplified. The *cba* gene (encoding a bacteriocin) was found in 89 isolates (Table 1; see also Table SA2 in the supplemental material).

A complete DNA-based serotype assignment was determined for more than half of the tested isolates by using the oligonucleotide microarrays (1). O113:H4 was the most frequently observed serotype (found in 39/193 isolates), whereas O91:H14 was detected in 8 of 193 isolates. A total of 27 isolates were grouped in serogroup O157. In these isolates, the *fliC* genes for H4 (21 isolates), H8 (2 isolates), H10 (2 isolates), H49, and H56 were detected. The single *stx*- and *eae*-positive isolate was typed as 103:H34 (Table 1; see also Table SA2 in the supplemental material). The number of O antigens detectable by this method is currently limited. Therefore, we could not detect O antigens in 92 isolates. In these isolates, the *fliC* genes for H19 (30 isolates), H21 (25 isolates), and H8 (15 isolates) were found frequently. Additional *fliC* genes such as H4, H7, H16, H28, H30, H38, and H49 were obtained. It is noteworthy that isolates from the same fecal sample were often identical in serotype and virulence marker pattern (Table 1; see also Table SA2 in the supplemental material).

TABLE 1 Phylogenetic characteristics of tested STEC isolates[a]

Farm or reference strain	Sample no.	Animal	% of STEC in the total no. of *E. coli* strains	DNA-based serotype[b]	Virulence marker(s)[c]	No. of isolates
Farm						
1	1	Goat 1	19.1	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	All
	2	Goat 2	100.0	O91:H14	stx_{1a}, *hlyA*, *espP*, *espI*, *saa*, *iha*, *ireA*, *lpfA*	All
	3	Goat 3	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*, (*lpfA*)	All
	4	Goat 4	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	All
	5	Goat 5	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, (*iha*), *ireA*	All
2	6	Goat 1	36.1	NT:H19	stx_{1c}, *hlyA*, *ireA*, *lpfA*, *pic*	All
	7	Goat 2	6.6	O157:H8	stx_{1c}, stx_{2b}, *hlyA*, *katP*, *espI*, *iha*, *ireA*, *lpfA*	1
				NT:H8	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *iha*, (*ireA*), *lpfA*	4
	8	Sheep 3	24.8	NT:H21	stx_{1c}, stx_{2b}, *hlyA*, (*astA*), (*efa1*), *lpfA*, (*hlyE*), (*bfpA*)	All
	9	Sheep 4	31.1	O55:H21	stx_{1c}, stx_{2b}, *hlyA*, *lpfA*	1
				NT:H21	stx_{1c}, stx_{2b}, *hlyA*, *lpfA*	4
	10	Sheep 5	0.9	O104:H16	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *iha*, *lpfA*	1
3	11	Sheep 1	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	All
	12	Sheep 2	8.9	O128:H2	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *iha*, *ireA*, *lpfA*	4
				NT:H19	stx_{1c}, *hlyA*, *espI*, *iha*, *ireA*, *lpfA*	1
	13	Sheep 3	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	All
	14	Sheep 4	100.0	O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	All
	15	Sheep 5	100.0	O91:H14	stx_{1a}, stx_{2b}, *espI*, *iha*, *ireA*, *lpfA*	3
				O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, (*astA*), *iha*, (*ireA*), (*lpfA*)	2
	16	Sheep 6	3.9	O103:H21	stx_{1a}, stx_{2b}, (*espI*), (*iha*), (*ireA*), *lpfA*	3
				O103:H21	stx_{1c}, stx_{2b}, (*hlyA*), (*espI*), *astA*, (*ireA*), *lpfA*	2
	17	Goat 7	2.7	O145:H6	stx_{1c}, stx_{2b}, (*hlyA*), (*espI*), *astA*, (*iha*)	All
	18	Goat 8	100.0	O103:H34	stx_{1c}, stx_{2b}, *eae*, *tir*, *hlyA*, *espA*, *espC*, *espF*, *espI*, *nleB*, *nleC*, *astA*, *iha*, *toxB*, *hlyE*	1
				O113:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*, (*toxB*), (*lpfA*)	3
				O9:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *astA*, *iha*, *ireA*	1
4	19	Goat 1	10.8	NT:H19	stx_{1c}, *hlyA*, *astA*, *ireA*, *lpfA*, *pic*	All
	20	Goat 2	2.1	O145:NT	(*astA*), *iha*, *ireA*	All
	21	Sheep 3	49.6	NT:H19	stx_{1c}, *hlyA*, (*astA*), (*ireA*), *lpfA*, *pic*	4
				O157:H56	*astA*	1
5	22	Goat 1	4.5	O157:H4	stx_{1c}, *hlyA*, *espI*, (*iha*)	2
				NT:H38	*astA*, *lpfA*	2
	23	Goat 2	52.5	O157:H4	stx_{1c}, *hlyA*, *espI*, *iha*, *ireA*, *toxB*	All
	24	Goat 3	33.3	O157:H4	stx_{1c}, (stx_{2b}), *hlyA*, *espI*, *iha*, *ireA*, (*toxB*), (*K88*)	All
	25	Goat 4	32.3	O157:H4	(stx_{1c}), *hlyA*, *espI*, *iha*, *ireA*, (*toxB*), *K88*	All
	26	Goat 5	39.7	O157:H4	stx_{1c}, (stx_{2b}), *hlyA*, *espI*, *iha*, *ireA*, *K88*	All
	27	Goat 6	100.0	NT:H19	stx_{1c}, stx_{2b}, *ireA*, *lpfA*, *pic*	All
	28	Sheep 7	47.3	O157:H4	stx_{1c}, *hlyA*, *espI*, *iha*, *ireA*, *K88*	All
	29	Sheep 8	1.8	NT:H7	(stx_{2b}), *lpfA*	All
6	30	Goat 1	0.0			
	31	Goat 2	0.0			
	32	Goat 3	0.7	O157:H10	(stx_{1c}), (*hlyA*), (*espI*), (*iha*), (*ireA*), *lpfA*	2
				NT:H21	*hlyA*, *espI*, *iha*, *ireA*, *lpfA*	1
	33	Goat 4	1.7	O7:H37	stx_{2b}, *espI*, *iha*, *ireA*	1
				NT:H8	stx_{2b}, *hlyA*, *espI*, *iha*, *lpfA*	1
	34	Sheep 5	0.9	O26:H21	stx_{2b}, *lpfA*	1
				NT:H21	*lpfA*	3
	35	Sheep 6	0.4	O157:H49	*lpfA*	1
				NT:H28	*lpfA*	2
				NT:H49	*lpfA*	1
	36	Sheep 7	100.0	O157:H8	stx_{1c}, stx_{2b}, *espI*, *iha*, *lpfA*	1
				NT:H8	stx_{1c}, stx_{2b}, *espI*, *iha*, *lpfA*	3
	37	Sheep 8	100.0	NT:H8	stx_{1c}, stx_{2b}, *espI*, *iha*, (*ireA*), *lpfA*, (*K88*)	All
	38	Sheep 9	0.1	O79:H8	(stx_{1c}), (stx_{2b}), (*lpfA*)	All
7	39	Goat 1	24.0	NT:H4	stx_{1c}, stx_{2b}, *hlyA*, *espI*, (*espP*), *astA*, *iha*	All
	40	Goat 2	11.3	NT:H19	stx_{1c}, stx_{2b}, *hlyA*, (*espI*), (*iha*), *lpfA*, (*pic*)	All
	41	Goat 3	2.7	NT:H4	stx_{1c}, (stx_{2b}), *hlyA*, (*espI*), (*espP*), (*iha*), *lpfA*, (*pic*)	All
	42	Goat 4	12.3	O26:H19	stx_{1c}, *hlyA*, *lpfA*, *pic*	2
				NT:H19	stx_{1c}, *hlyA*, *lpfA*, *pic*	2

(Continued on following page)

TABLE 1 (Continued)

Farm or reference strain	Sample no.	Animal	% of STEC in the total no. of *E. coli* strains	DNA-based serotype[b]	Virulence marker(s)[c]	No. of isolates
8	43	Sheep 1	1.0	NT:H21	stx_{1c}, *hlyA*, *espI*, *iha*, *lpfA*	3
				NT:H8	stx_{1c}, *lpfA*	1
	44	Sheep 2	4.6	NT:H21	stx_{1c}, *hlyA*, *espI*, *iha*, *lpfA*	2
				NT:H8	stx_{1c}, *hlyA*, *espI*, *iha*, *lpfA*	2
	45	Sheep 3	3.9	NT:H21	stx_{1c}, *hlyA*, *espI*, *iha*, *lpfA*	3
				NT:H8	stx_{1c}, *hlyA*, *espI*, *iha*, *lpfA*	1
	46	Goat 4	0.0			
	47	Goat 5	12.3	NT:H21	β-*eae*, *hlyA*, *espB*, *espF*, *espI*, *espJ*, *nleA*, *iha*, (*lpfA*)	All
	48	Goat 6	25.9	NT:H19	stx_{1c}, stx_{2b}, *hlyA*, *espI*, *iha*, *lpfA*, *pic*	All
Reference strains[d]						
EHEC EDL 933				O157:H7	stx_{1a}, stx_{2a}, γ-*eae*, *tir*, *hlyA*, *espA*, *espF*, *espJ*, *espP*, *katP*, *nleA*, *nleB*, *nleC*, *tccP*, *etpD*, *astA*, *iha*, *toxB*	
EPEC E2348/69				NT:H6	α-*eae*, *tir*, *espA*, *espC*, *espF*, *espJ*, *nleA*, *nleB*, *nleC*, *efa1*, *cif*, *astA*, *toxB*, *bfpA*	
EHEC DD-23137 (outbreak strain)				O104:H4	stx_{2a}, *iha*, *pic*, *ctxM1*, *tem1*	

[a] A table with data presented isolate by isolate is available in Table SA2 in the supplemental material.
[b] NT, not detectable by microarray.
[c] Virulence markers in parentheses were not found in all isolates of the corresponding serotype.
[d] Virulence markers of selected reference strains. The complete lists of the results for all reference strains are available in Table SA1 in the supplemental material.

***Salmonella* and *Coxiella* results.** *Salmonella* organisms and *Coxiella burnetti* DNA were not detected in any of the 48 fecal samples collected from sheep and goats from the 8 farms.

***Campylobacter* results.** Thermophilic *Campylobacter* strains were detected in 9 of 48 fecal samples of sheep ($n = 5$) and goats ($n = 4$) collected from 8 investigated farms. Two goats of farm 1 harbored *C. coli*, and seven *Campylobacter jejuni* isolates came from sheep and goat feces of 3 other farms (farms 2, 5, and 6; Table 2).

***Staphylococcus* results.** A broad range of staphylococcal strains were identified in nasal swabs from sheep and goats (Table 2). The following *Staphylococcus* species were found: *S. aureus* ($n = 8$), *S. equorum* ($n = 4$), *S. sciuri* ($n = 2$), *S. succinus* ($n = 3$), *S. vitulinus* ($n = 4$), *S. warneri* ($n = 1$), and *S. xylosus* ($n = 17$). The samples from farm 2 were the only samples in which staphylococci were not found. In the samples from farms 4 and 7, *S. xylosus* was the only staphylococcal species identified. In other farms, sheep and goats harbored several staphylococcal species. *S. aureus* was detected in 3 farms (farms 3, 5, and 8; Table 2).

S. aureus isolates were characterized in more detail using DNA microarrays. Isolates 14, 15, 16, 25, and 28 (Table 2), which originated from 4 sheep and 1 goat, belonged to clonal complex 133 (CC133) of methicillin-sensitive *S. aureus* (MSSA). All five isolates were typed as *agr* group III and harbored the *lukM-lukF* P83 gene encoding a leukocidin (37). Furthermore, the presence of the epidermal cell differentiation inhibitor B gene *ediNB* was recognized as a virulence marker. No known antibiotic resistance genes were detected in isolates 14, 16, and 28; isolates 15 and 25 harbored the *mpbBM* gene that encodes resistance of the bacteria to macrolides. Isolates 45, 46, and 47 from farm 8 were completely identical and belonged to CC133 and *agr* group I. All of them harbored the *lukM-lukF* P83 gene and the toxic shock syndrome toxin gene *tst* RF 122 in its bovine allelic form. Enterotoxin genes *entA*, *entC*, and *entL* were also detected. The enterotoxin A gene was identified as the rare allele *entA* 320E, which was previously exclusively found in sheep, goat, or camel (37). *fosB*, which is connected with resistance to fosfomycin and bleomycin, was present in those strains.

Results concerning other bacteria. *Ochrobactrum* spp. were identified in samples from three farms. Sheep and goats of farm 3 harbored *Ochrobactrum intermedium*, whereas *Ochrobactrum anthropi* was found in samples from farms 4 and 5 (Table 2). Other bacteria detected in the fecal material maintained on mCCDA agar were *Enterococcus* spp., *Acinetobacter* sp., and *Lactobacillus plantarum* (Table 2).

DISCUSSION

The proportions of STEC-positive samples detected (100% of sheep and 89.3% of goat isolates) were very high compared to other studies. Reported prevalences in sheep ranged between 29.9% and 66.6%, whereas STEC had been detected in goats in from 17.0% to 75.3% of the investigated animals in different studies (2, 10, 13, 39, 58, 57). This difference might be due to the fact that our samples came from small groups of animals that were kept together for extended periods in restricted areas, where the probability of intraherd transmission was very high, as well as to the high number of *cba*-positive strains (46.1%). The fact that there were STEC-positive animals on all farms might emphasize the importance of small ruminants as a reservoir for these bacteria.

The serotypes we found using the genotyping approach described by Ballmer et al. (1) were most often O113:H4, O157:H4, and O91:H4, with high numbers of affected goats as well as sheep. Due to the limitations in the number of O genes detectable in the method used in this study, we could not detect O antigens in 92 (47.7%) of our isolates. In these isolates, the *fliC* genes for H19, H21, and H8 were the ones most frequently appearing (32.6%, 27.2%, and 16.3% of the On.t. [O nontypeable] isolates). Comparing our results to those of Cortés et al. (10), who found O5:H-, O76:H19, O126:H8, O146:H21, On.t.:H-, and On.t.:H21 to be the

TABLE 2 *Campylobacter*, *Staphylococcus*, and other zoonotic pathogens identified

Farm	Sample no.	Animal	*Campylobacter* species identified	Species identified by 16S-UNI-L/R sequencing	*Staphylococcus* species identified
1	1	Goat 1	*C. coli*		*S. succinus*
	2	Goat 2			*S. warneri*
	3	Goat 3	*C. coli*		*S. equorum*
	4	Goat 4			*S. equorum*, *S. xylosus*
	5	Goat 5			
2	6	Goat 1			
	7	Goat 2	*C. jejuni*		
	8	Sheep 3	*C. jejuni*		
	9	Sheep 4			
	10	Sheep 5			
3	11	Sheep 1		*Enterococcus* spp.	*S. xylosus*
	12	Sheep 2			*S. xylosus*
	13	Sheep 3			*S. xylosus*
	14	Sheep 4			*S. aureus*
	15	Sheep 5		*Ochrobactrum intermedium*	*S. aureus*
	16	Sheep 6		*Ochrobactrum intermedium*	*S. aureus*
	17	Goat 7		*Ochrobactrum intermedium*	*S. succinus*, *S. xylosus*
	18	Goat 8		*Ochrobactrum intermedium*	*S. xylosus*
4	19	Goat 1		*Acinetobacter* spp.	
	20	Goat 2		*Ochrobactrum anthropi*	*S. xylosus*
	21	Sheep 3		*Ochrobactrum anthropi*	
5	22	Goat 1		*Acinetobacter* spp.	*S. sciuri*
	23	Goat 2		*Acinetobacter* spp.	*S. equorum*
	24	Goat 3			*S. sciuri*, *S. vitulinus*
	25	Goat 4		*Ochrobactrum anthropi*	*S. aureus*
	26	Goat 5			*S. xylosus*
	27	Goat 6			
	28	Sheep 7		*Ochrobactrum anthropi*	*S. aureus*
	29	Sheep 8	*C. jejuni*		*S. xylosus*
6	30	Goat 1	*C. jejuni*		*S. equorum*
	31	Goat 2			*S. xylosus*
	32	Goat 3			*S. xylosus*
	33	Goat 4			*S. xylosus*
	34	Sheep 5	*C. jejuni*		*S. vitulinus*
	35	Sheep 6			*S. xylosus*
	36	Sheep 7	*C. jejuni*		*S. vitulinus*
	37	Sheep 8	*C. jejuni*		*S. xylosus*
	38	Sheep 9			*S. vitulinus*
7	39	Goat 1			
	40	Goat 2			*S. xylosus*
	41	Goat 3			*S. xylosus*
	42	Goat 4			*S. xylosus*
8	43	Sheep 1			
	44	Sheep 2			*S. succinus*
	45	Sheep 3		Mixed culture (including *L. plantarum*)	*S. aureus*
	46	Goat 4			*S. aureus*
	47	Goat 5		*Acinetobacter* sp.	*S. aureus*
	48	Goat 6		*Lactobacillus plantarum*	

most frequently appearing *E. coli* serotypes in goats (10), On.t.: H21 was the only one of these serotypes that we found in the goats we had examined. This might imply that the goats carry serotypes of STEC in our study region that are different from those found in other countries and that farm-specific associations of serotypes do occur. As there are few data available on serotypes of STEC in goats, it is too early to come to any general conclusions with respect to this issue at this stage. The serotypes that have been most frequently reported to have been found in sheep are O5, O91, O117, O128, and O146. Several serotypes found in sheep, such as O5, O26, O91, O128/O128:H2, and O146:H8, have also been isolated from humans in clinical cases (2, 3, 4, 13, 29, 47, 48, 50, 51). Our findings in sheep also differ from those in previous studies, but the one similarity we detected, represented by O91:H14, is closely related to serotypes that were reported in cases of human disease. Several of the other O groups we found, such as O26, O103, O104, O113, O145, and O157, have also been reported from studies of cases of infections of humans (42). However, O157:H7, the most important EHEC serotype, could not be isolated during this study. Also, other important non-O157 EHEC

serotypes such as O26:H11, O103:H2, O145:H25/H28, and O111:H8 could not be detected.

We did not find any examples of O104:H4, the serotype found in the large EHEC outbreak that occurred in Germany between May and July 2011, even though our method would have been sufficient to detect such strains, including their most important virulence factors such as stx_{2a} and *iha* (33). We found the antigen O104 only once, combined with the *fliC* gene for H16. This strain was *eae* negative and *iha* positive, like the O104:H4 strain, but it was stx_{1c}, stx_{2b}, and *hlyA* positive.

stx genes were found in only 84.5% of the isolates, although they had previously been screened for the presence of *stx* genes by colony hybridization. The loss of phage-encoding *stx* genes during the first cultivation step may be one reason for this phenomenon. Picking wrong colonies could also explain why in many cases no *stx* genes were recovered from cultures shown to be *stx* positive by prescreening.

Almost two-thirds (63.2%) of the *stx*-positive isolates carried both stx_1 and stx_2; 34.4% carried stx_1 only, and the smallest fraction of 2.4% carried stx_2 only. This is in accord with other studies, which mainly focused on sheep and which found that 74% to 88% of their STEC strains carried *stx* genes, with the majority of strains having both stx_1 and stx_2 (53% to 64%), followed by a smaller proportion carrying stx_1 (19% to 43%) and the smallest proportion of the strains carrying only stx_2 (6% to 10%) (13, 16, 47). However, all detected stx_2 genes were subtyped as stx_{2b}. This subtype is not identified as high risk, in contrast to the subtypes stx_{2a} and stx_{2c}. Other toxin-encoding genes were present in fewer isolates (*astA*, 29.5%; *toxB*, 3.1%). We found EHEC *hlyA* genes at a higher percentage than other studies did. Whereas earlier studies reported 9% to 47% of their STEC isolates to carry *hlyA* (*ehxA*) (13, 16, 47), we found 73.6% of our STEC to be *hlyA* positive. Of these, 95% were also *stx* positive. This implies that most of the STEC strains colonizing the intestinal tract of small ruminants have to be considered potential toxin producers. We detected *eae* genes in 2.59% of the strains, all of which were also *hlyA* positive, but the most virulent combination detected included stx_1 and stx_2, whereas the combination of *eae* and *hlyA* was found in one isolate only. This is in concordance with the results of other studies, which found 0% to 12.2% of detected strains to be *eae* positive (13, 16, 47).

Blanco et al. (3) suggested that the low incidence of *eae* genes in STEC isolated from sheep implies that there is less risk of human infection originating from sheep than from cattle (3). We found *iha* genes occurring in 65.8% of our STEC strains. *iha* encodes an enterobactin siderophore receptor and has been found in O157:H7 strains (53). Another factor for adherence that we found frequently was *lpfA* (60.6%). Therefore, the idea has to be taken into consideration that that the factors for adherence found in STEC strains in small ruminants are not fewer than but are different from those found in cattle.

The *ireA* gene, which was present in 52.8% of our isolates, is often present and is an important iron-related virulence marker in uropathogenic *E. coli* (EPEC) (45). Plasmid-carried virulence genes, such as *espP* and *katP*, which have been frequently isolated from O157:H7 strains, rarely occurred in our strains (2.6% and 0.5%, respectively).

In contrast to the findings of Orden et al. (43), we found 2.6% of our STEC strains to be positive for *saa* and *hlyA* in one of the goats in our study (43). This shows that STEC in small ruminants can be associated with these genes.

The possibility of highly virulent strains originating from sheep and goats should not be underestimated, as we found some strains carrying several virulence factors that might enable them to cause severe infections in humans, with, for example, O103:H34 being positive for *stx*, *eae*, *tir*, and *hlyA*. Taking our results into account, it is important to inform farm staff about the fact that small ruminants can host strains of *E. coli* that might be capable of causing illness in humans. Even though it is assumed that strict hygiene management, including hand washing after contact with carrier animals, maintaining careful kitchen hygiene, and pasteurizing milk before consumption, should be sufficient to prevent human infections with EHEC (28), it has recently been proposed that the risk of human infection with STEC can be further reduced by reducing colonization in the main reservoir (41).

Sheep and goats can be carriers of different *Salmonella* serovars, including *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium, the most important serovars for human infections (21, 58). In 2008, 2.25% of the sheep flocks and 1.97% of the individually tested sheep in Germany carried *Salmonella* spp. (21). The prevalences in goat herds were even lower, at 0.51% of the herds and 0.78% of the individually tested goats (21). In another study, 1.3% of the sheep carried *Salmonella* spp., with slightly higher ratios in sheep that were kept on limited pastures and had contact with wild birds (24). The fact that no *Salmonella* organisms were detected in this study might have been due to the generally low prevalence of these agents in small ruminants and also to the rather low number of animals tested. As small ruminants play only a minor role in the transmission of *Salmonella* to human beings, a general surveillance of these animal species for the presence of *Salmonella* spp. does not seem to be necessary. General hygiene management should be adequate for limiting the risk of acquiring *Salmonella* infections from small ruminants.

Our results concerning *Campylobacter* spp. are in line with the results of other studies. *Campylobacter* spp. were found in 14.5% of the investigated sheep flocks in one study, with rates from 9.5% to 17.4% in individually tested sheep and *Campylobacter jejuni* being found to be the predominant species (21, 58), whereas we found *C. jejuni* in 25% of the sheep in our study. Little is known about *Campylobacter* spp. in goats, but the prevalence of those species seems to be lower than in sheep (21). We found *Campylobacter* spp. in 14.3% of goats, with equal proportions of *C. jejuni* and *C. coli* in the samples. There have been no effective vaccines against any of these species available to date. Our animals proved to be silent carriers, as has to be assumed from the data available, of the *Campylobacter* spp. that most likely cause human infections (40). Especially if farms use sheep and goat milk for direct consumption or to make raw milk cheese, farm staff members should be aware of that fact. It would be preferable to pasteurize the milk before consumption. If this is not an option, the occurrence of *Campylobacter* spp. in the flock or herd should be monitored.

Different species of staphylococci can cause infections of greater or lesser severity in humans (26, 28, 40). *S. xylosus* and *S. warneri* might be important in opportunistic infections but should not be a threat to healthy children. *S. aureus*, on the other hand, is more likely to cause mild infections, being a regular cause of infections of skin lesions (28). Frequencies of detection of *S. aureus* of up to 96% in sheep and 80% in goats, along with the

isolation of different other *Staphylococcus* spp., imply that small livestock might be an important reservoir for these bacteria (12, 21, 55). We found *S. aureus* at lower prevalences of 29.4% in sheep and 11.5% in goats. These strains all belonged to the same clonal complex (CC133), which has been predominant in isolates from small ruminants in other studies (17, 20). They carry the leukocidin *lukM-lukF* P83, a toxin host specific for ruminants (35). Although we found few *Staphylococcus* spp. likely to cause infections in healthy, uninjured humans, these bacteria should not be left out of hygiene considerations, as these bacteria have been frequently detected in small ruminants in general.

Of the other bacteria found, *Ochrobactrum* spp. have been reported to cause opportunistic infections in hospitalized individuals (34). As the farms are usually attended by healthy school children, these bacteria are not likely to be a major threat to their health. The risk posed by the other detected species is negligible.

Small ruminants are considered to be the main reservoir for *C. burnetii* (48). Recent Q fever outbreaks have been linked to sheep in Germany and goats in the Netherlands (18, 46, 49). Although all the animals tested in our study were negative for *C. burnetii* DNA, it is generally recommended to immunize animals in close contact with humans—such as animals kept in city farms—with protective vaccines. According to the educational concept of these facilities, children and youths are intended to gain insights into the facts of life, including the birth of offspring. In terms of *C. burnetii* epizootiology, this is a very critical time point, as the agent is shed in very large amounts during parturition (31). It is therefore advisable to separate pregnant animals in the last trimester from the other animals in the flock or facility and to keep those animals away from the public until the end of the puerperium. It also remains a question of debate whether sheep and goats are indeed the ideal animals for breeding on these facilities. As a general rule, a strict hygiene management scheme should be applied when handling animals kept in close contact with humans.

No confirmed case of any human zoonotic disease associated with the sheep and goats has so far been reported from any of the seven city farms or the activity playground investigated in this study. No cases of diarrheic diseases of unknown origin or wound infections originating from bruises acquired during animal contact with members of farm staffs or visitors have been noticed. This implies either that the existing hygiene measures (each farm offers hand-washing facilities with hot and cold tap water, soap, and paper towels [not in or near the animal contact area but in the main buildings of the farm or close to them]; staff members on three farms ask people to wash their hands after leaving the animal contact zone whereas one farm provides a printed notification advising hand washing) are sufficient to guarantee very good hygiene or that the STEC strains found are not highly virulent.

When staff members at the farms were asked which zoonotic diseases they were familiar with, salmonellosis was named only once. Infections with EHEC, *Staphylococcus* spp., or *Campylobacter* spp. and Q fever were not mentioned at all.

Considering our results and the existing literature, salmonellosis is the zoonotic disease least likely to be acquired during close contact with small ruminants. Therefore, better education of the farm staff is surely one of the most important factors in preventing zoonoses. Other studies found that, even when the general hygiene in petting zoos was good, infections with STEC O157 could occur (8), and this is the case for city farms and other zoonotic diseases as well. But there is little evidence that visits to city farms represent a major public health threat (22).

In Great Britain, the Health and Safety Executive (22), a nondepartmental public body for the encouragement, regulation, and enforcement of workplace health, safety, and welfare, suggested the following measures to prevent and control ill health arising from animal contact at visitor attractions: maintaining good general cleanliness of facilities and animals, keeping paths free of animal feces, mandating careful storage of manure, separating animal contact and noncontact areas as well as food areas, providing adequate hand-washing facilities with proper supervision by informed staff, and implementing individual farm health plans (22). We agree with those suggestions and also propose vaccination against *Coxiella burnetii* for animals kept in petting zoos and city farms. We also suggest that milk from small ruminants should always be pasteurized prior to consumption.

It is impossible to have animals and their environments completely free of zoonotic agents. The positive effects that arise from visits to city farms may outweigh the risks. Yet good hygiene and animal health management and well-educated staff members can make those visits even more pleasurable and help to keep the risks at a minimum.

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REFERENCES

1. **Ballmer K, et al.** 2007. Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. J. Clin. Microbiol. **45**:370–379.
2. **Beutin L, Geier D, Steinrück H, Zimmermann S, Scheutz F.** 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. **31**:2483–2488.
3. **Blanco J, et al.** 2003. Verotoxin-producing *Escherichia coli* in Spain: prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp. Biol. Med. (Maywood) **228**:345–351.
4. **Blanco M, et al.** 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. J. Clin. Microbiol. **41**:1351–1356.
5. **Bund der Jugendfarmen und Aktivspielplätze e.V.** 2010. Playing for life— educational work on pedagogically supervised playgrounds, 1st ed. Bund der Jugendfarmen und Aktivspielplätze e.V., Stuttgart, Germany. http://www.bdja.org/files/bdja_englisch.pdf.
6. **Bütikofer B, et al.** 2005. Incidence of zoonoses in petting zoos and evaluation of hygiene measures to prevent the transmission to humans. Schweiz. Arch. Tierheilkd. **147**:532–540. (In German.)
7. **Campbell KLI, et al.** 2006. Smallstock in development [CD-ROM]. DFID Livestock Production Programme, Natural Resources International Ltd, Aylesford, Kent, United Kingdom. ISBN: 0-9546452-8-6.
8. **Centers for Disease Control and Prevention.** 2009. Outbreak of Shiga toxin-producing Escherichia coli O157 infection associated with a day camp petting zoo—Pinellas County, Florida, May–June 2007. MMWR Morb. Mortal. Wkly. Rep. **58**:426–428. http://www.cdc.gov/mmwr/pdf/wk/mm5816.pdf.
9. **Chapman PA, Cornell J, Green C.** 2000. Infection with verocytotoxin-producing *Escherichia coli* O157 during a visit to an inner city open farm. Epidemiol. Infect. **125**:531–536.
10. **Cortés C, et al.** 2005. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. Vet. Microbiol. **110**:67–76.
11. **Denis M, et al.** 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett. Appl. Microbiol. **29**:406–410.

12. **Dimitracopoulos G, Sakellariou C, Papavassiliou J.** 1976. Staphylococci from the feces of different animal species: biotypes of *Staphylococcus aureus* strains of sheep and goat origin. Appl. Environ. Microbiol. **32**:53–55.
13. **Djordjevic SP, et al.** 2001. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. J. Clin. Microbiol. **39**:2017–2021.
14. **El-Adawy H, Hotzel H, Tomaso H, Neubauer H, Hafez HM.** 2012. Elucidation of colonization time and prevalence of thermophilic Campylobacter species during turkey rearing using multiplex polymerase chain reaction. Poult. Sci. **91**:454–459.
15. **European Centre for Disease Prevention and Control.** 2010. Annual epidemiological report on communicable diseases in Europe 2010. European Centre for Disease Prevention and Control, Stockholm, Sweden. http://ecdc.europa.eu/en/publications/publications/1011_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf.
16. **Fegan N, Desmarchelier P.** 1999. Shiga toxin-producing *Escherichia coli* in sheep and preslaughter lambs in eastern Australia. Lett. Appl. Microbiol. **28**:335–339.
17. **Geue L, et al.** 2010. Rapid microarray-based genotyping of enterohemorrhagic *Escherichia coli* serotype O156:H25/H-/Hnt isolates from cattle and clonal relationship analysis. Appl. Environ. Microbiol. **76**:5510–5519.
18. **Gilsdorf A, et al.** 2008. Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. Epidemiol. Infect. **136**:1084–1087.
19. **Grimont PAD, Weill FX.** 2007. Antigenic formulae of the *Salmonella serovars*, 9th ed. WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France.
20. **Guinane CM, et al.** 2010. Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol. Evol. **2**:454–466.
21. **Hartung M.** 2010. Erreger von Zoonosen in Deutschland im Jahr 2008. Mitteilungen der Länder zu Lebensmitteln, Tieren, Futtermitteln und Umweltproben. Berlin, Germany. http://www.bfr.bund.de/cm/350/erreger_von_zoonosen_in_deutschland_im_jahr_2008.pdf.
22. **Health and Safety Executive.** 2011. Preventing or controlling ill health from animal contact at visitor attractions. Agriculture information sheet no 23 (rev2). Health and Safety Executive, London, United Kingdom. http://www.hse.gov.uk/pubns/ais23.pdf.
23. **Heuvelink AE, et al.** 2007. Public farms: hygiene and zoonotic agents. Epidemiol. Infect. **135**:1174–1183.
24. **Hjartardóttir S, Gunnarsson E, Sigvaldadóttir J.** 2002. *Salmonella* in sheep in Iceland. Acta Vet. Scand. **43**:43–48.
25. **International Standards Organization.** 2006. ISO 10272-1: microbiology of food and animal feeding stuffs—horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: detection method. International Standards Organization, Geneva, Switzerland. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=37091.
26. **Kamath U, Singer C, Isenberg HD.** 1992. Clinical significance of *Staphylococcus warneri* bacteremia. J. Clin. Microbiol. **30**:261–264.
27. **Klee SR, et al.** 2006. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. BMC Microbiol. **6**:2.
28. **Krauss, H, et al.** 2004. Zoonosen. Von Tier zu Mensch übertragbare Infektionskrankheiten. 3. Vollständig überarbeitete Auflage. Deutscher Ärzte-Verlag, Cologne, Germany.
29. **Kudva IT, Hatfield PG, Hovde CJ.** 1997. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. J. Clin. Microbiol. **35**:892–899.
30. **Kuhnert P, Capaul SE, Nicolet J, Frey J.** 1996. Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. **46**:1174–1176.
31. **Maurin M, Raoult D.** 1999. Q fever. Clin. Microbiol. Rev. **12**:518–553.
32. **McMillian M, Dunn JR, Keen JE, Brady KL, Jones TF.** 2007. Risk behaviors for disease transmission among petting zoo attendees. J. Am. Vet. Med. Assoc. **231**:1036–1038.
33. **Mellmann A, et al.** 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. PLoS One **6**:e22751.
34. **Möller LVM, et al.** 1999. *Ochrobactrum intermedium* infection after liver transplantation. J. Clin. Microbiol. **37**:241–244.
35. **Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R.** 2007. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. Vet. Microbiol. **125**:128–140.
36. **Monecke S, Jarzwauk L, Weber S, Slickers P, Ehricht R.** 2008. DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Clin. Microbiol. Infect. **14**:534–545.
37. **Monecke S, et al.** 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One **6**:e17936.
38. **Müller D, et al.** 2007. Identification of unconventional intestinal pathogenic *Escherichia coli* isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. Appl. Environ. Microbiol. **73**:3380–3390.
39. **Muñoz M, Álvarez M, Lanza I, Cármenes P.** 1996. Role of enteric pathogens in the etiology of neonatal diarrhea in lambs and goat kids in Spain. Epidemiol. Infect. **117**:203–211.
40. **Nováková D, et al.** 2006. *Staphylococcus equorum* and *Staphylococcus succinus* isolated from human clinical specimens. J. Med. Microbiol. **55**:523–528.
41. **OIE—World Organisation for Animal Health.** 2008. *Campylobacter jejuni* and *Campylobacter coli*. Manual of diagnostic tests and vaccines for terrestrial animals, chapter 2.9.3. OIE—World Organisation for Animal Health, Paris, France. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.09.03_CAMPYLO.pdf.
42. **OIE—World Organisation for Animal Health.** 2008. Verocytotoxigenic *Escherichia coli*. Manual of diagnostic tests and vaccines for terrestrial animals, chapter 2.9.11. OIE—World Organisation for Animal Health, Paris, France. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.09.11_VERO_E_COLI.pdf.
43. **Orden JA, Cortés C, Ruiz-Santa-Quiteria JA, Martinez S, de la Fuente R.** 2005. Detection of the *saa* gene in verotoxin-producing *Escherichia coli* from ruminants. J. Vet. Diagn. Invest. **17**:65–67.
44. **Oswald E, et al.** 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. Infect. Immun. **68**:64–71.
45. **Park HK, et al.** 2009. Comparison of *Escherichia coli* uropathogenic genes (*kps*, *usp* and *ireA*) and enteroaggregative genes (*agr* and *aap*) via multiplex polymerase chain reaction from suprapubic urine specimens of young children with fever. Scand. J. Urol. Nephrol. **43**:51–57.
46. **Porten K, et al.** 2006. A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. BMC Infect. Dis. **6**:147.
47. **Rey J, et al.** 2003. Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep in Spain. Vet. Microbiol. **94**:47–56.
48. **Rodolakis A.** 2009. Q-fever in dairy animals. Ann. N. Y. Acad. Sci. **1166**:90–93.
49. **Roest HI, et al.** 2011. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol. Infect. **139**:1–12.
50. **Sánchez S, et al.** 2009. Longitudinal study of Shiga toxin-producing *Escherichia coli* shedding in sheep feces: persistence of specific clones in sheep flocks. Appl. Environ. Microbiol. **75**:1769–1773.
51. **Sekse C, et al.** 2011. Potentially human-pathogenic *Escherichia coli* O26 in Norwegian sheep flocks. Appl. Environ. Microbiol. **77**:4949–4958.
52. **Stirling J, et al.** 2008. Zoonoses associated with petting farms and open zoos. Vector Borne Zoonotic Dis. **8**:85–92.
53. **Tarr PI, et al.** 2000. *Iha*: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect. Immun. **68**:1400–1407.
54. **Valkenburgh SM, Heuvelink AE.** 2006. Children's farms in the Netherlands: hygiene and zoonotic pathogens. Tijdschr. Diergeneeskd. **131**:224–227.
55. **Valle J, et al.** 1990. Enterotoxin production by staphylococci isolated from healthy goats. Appl. Environ. Microbiol. **56**:1323–1326.
56. **Zhang WL, et al.** 2002. Genetic diversity of intimin genes of attaching and effacing Escherichia coli strains. J. Clin. Microbiol. **40**:4486–5592.
57. **Zschöck M, Hamann HP, Kloppert B, Wolter W.** 2000. Shigatoxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Lett. Appl. Microbiol. **31**:203–208.
58. **Zweifel C, Zychowska MA, Stephan R.** 2004. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. isolated from slaughtered sheep in Switzerland. Int. J. Food Microbiol. **92**:45–53.