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Microbiological Monitoring of Sheep Carcass Contamination in Three Swiss Abattoirs

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ABSTRACT

At three Swiss abattoirs, 580 sheep carcasses were examined at 10 sites by the wet-dry double-swab technique. The aim of this study was to obtain data on microbiological contamination at the abattoirs and to develop a procedure for monitoring slaughter hygiene. Median aerobic plate counts (APCs) (log CFU/cm^2) ranged from 2.5 to 3.8, with the brisket and neck sites showing the most extensive contamination. *Enterobacteriaceae* were detected on 68.1% of the carcasses and in 15.2% of the samples. The proportion of positive results ranged from 2.6% (for the hind leg and the flank at abattoir C) to 42.2% (for the perineal area at abattoir A). The percentage of samples testing positive for *stx* genes by polymerase chain reaction was 36.6%. A significant relationship between APC and the detection of Shiga toxin–producing *Escherichia coli* (STEC) was found for abattoirs A and B (depending on sampling site), whereas a significant relationship between *Enterobacteriaceae* and STEC detection was confirmed only for abattoir A ($P < 0.05$). In 57.1% of the 56 isolated non-O157 strains, *stx*2 genes were detected, and most of them were *stx*2d positive. Additional virulence factors were detected in 50% of the STEC strains, with 8.9% of these strains being *eae* positive, 50% being EHEC-*hlyA* positive, and 3.6% being *astA* positive. For the determination of carcass contamination, the monthly examination of 10 sheep carcasses for APC and *Enterobacteriaceae* counts in the neck, brisket, and perineal areas is recommended. This procedure is a valuable tool for the verification of slaughter hygiene according to hazard analysis critical control point principles.

Strict maintenance of good slaughter hygiene practices in meat production is of crucial importance in the prevention of microbial contamination of the carcass surface in the interest of ensuring both meat quality and health protection. To enable the estimation of the risk involved and the determination of the appropriate measures to be taken, analysis of the slaughtering process must be complemented by the collection of abattoir-specific monitoring data on the microbiological status of the carcasses.

Article 30 of the Swiss meat hygiene regulation outlines the requirements for the systematic monitoring of hygienic conditions by abattoirs *(3)*. In addition to the monitoring of the cooling temperature, this regulation stipulates a visual cleanliness check on each slaughter day and periodical microbiological examinations. The discretionary scope of the regulation leaves it up to the abattoir to define the extent of such examinations and techniques to be used. Various authors and an European Union (EU) commission have suggested that surface microbial counts be determined to assess slaughter hygiene *(2)*. Aerobic plate counts (APCs) have often been used as a general measure of the surface contamination of carcasses, whereas *Enterobacteriaceae* counts (ECs) provide an indicator of fecal contamination *(13, 23, 30)*.

The aim of this study was to obtain data on the microbiological contamination status of sheep carcasses (as indicated by APCs, ECs, and the presence or absence of Shiga toxin–producing *Escherichia coli* [STEC]) in selected Swiss abattoirs and to use the data obtained to develop a reliable routine procedure for the monitoring of slaughter hygiene.

MATERIALS AND METHODS

This study was based on investigations that were carried out within one and a half years in three EU-approved slaughterhouses in Switzerland. Mechanized line slaughtering was used in all abattoirs. Each sampling involved 10–15 sheep carcasses. Ten sampling sites (each 40 cm^2, marked by a sterile disposable paper template) per carcass were examined by the wet-dry double-swab technique *(2)* (Fig. 1). Sampling sites were selected on the basis of abattoir-specific contamination risks and existing recommendations for sheep and beef carcasses *(4, 14, 16, 40)*. In total, 5,800 samples were examined. Sampling was carried out within 3 h after the carcass had left the slaughter line. Microbiological examination was carried out within 2 to 4 h after sampling.

APCs and *Enterobacteriaceae*. Both swabs were homogenized together for 30 s in 20 ml of 0.85% saline in a stomacher. The undiluted sample was plated with a spiral plater (Autoplate 4000, Spiral Biotech, Bethesda, Md.) on plate count agar (CM 325, Oxoid) and violet red bile glucose (VRBG) agar (BBL 4311807, Becton Dickinson). Plate count agar was incubated aerobically for 48 h at 30°C, and VRBG agar was incubated for 48 h at 30°C under anaerobic conditions provided by commercial gas packs (GasPak Anaerobic System, BBL 270304). Manual counting was carried out. The detection limit was 1.0×10^1 CFU/cm^2.

STEC. From each sample, 5 ml was enriched in 15 ml of brilliant green bile broth (BBL) for 24 h at 37°C. For the STEC assay, a pooled sample for each animal was then evaluated by

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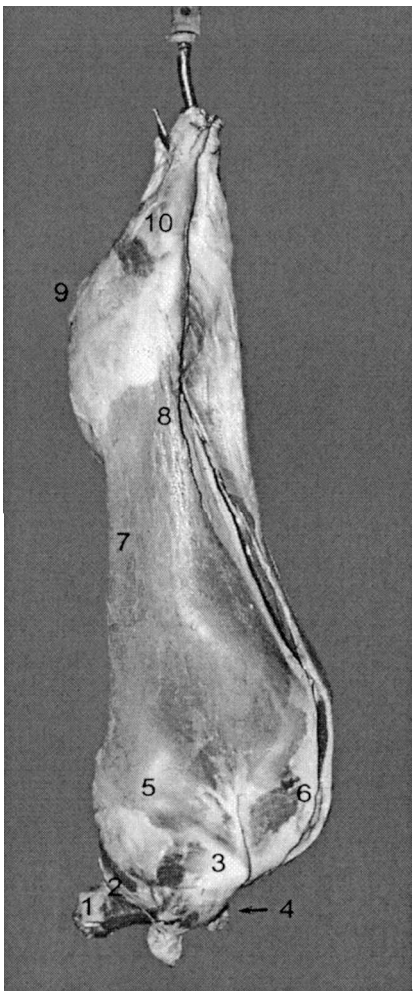

FIGURE 1. *Locations of sampling sites on sheep carcasses in the present investigation. 1, neck; 2, side of neck; 3, outside foreleg; 4, inside foreleg; 5, shoulder; 6, brisket; 7, back; 8, flank; 9, perineal area; 10, hind leg.*

polymerase chain reaction (PCR) with primers based on sequences targeting a region conserved between *stx*1 and *stx*2 genes *(11, 37).* Bacterial DNA was prepared by incubating 2 μl of each pooled sample in 42 μl of double-distilled water for 10 min at 100°C. Amplifications were carried out with a total volume of 50 μl containing 200 μM dNTP, 30 pmol of each primer, 5 μl of 10-fold concentrated polymerase synthesis buffer, and 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wis.) in a Biometra DNA Cycler. The amplified products were visualized by gel electrophoresis on 0.9% agarose agar stained with ethidium bromide. From 90 PCR-positive pooled samples, the enrichment broth was plated on sheep blood agar (Trypticase soy agar [BBL, Cockeysville, Md.] with 5% sheep blood), and single colonies were tested by the same PCR protocol to obtain STEC isolates. The strains were identified with classical biochemical tests (acid production from mannitol, the *o*-nitrophenyl-β-D-galactopyranoside test, H_2S and indole production, and proof of urease and lysine decarboxylase). All strains were examined for sorbitol fermentation, for β-D-glucuronidase activity, and (by PCR) for the presence of *stx*1 and *stx*2 genes *(26, 32).* PCR-restriction fragment length polymorphism was used for the subtyping of *stx*2 variants *(26).* Furthermore, PCR was employed to determine the presence of the *eae, hlyA,* and *astA* genes *(33, 34, 42).*

Statistical analysis. Colony counts were transformed to log values and depicted in box plots. With the results illustrated in such a way, median values, 50, 75, 80, and 90% ranges, and extreme values can be analyzed more conveniently *(15).* Possible relationships between APC and *Enterobacteriaceae* detection, STEC detection, and different slaughter days were analyzed by the Wilcoxon rank-sum test ($P < 0.05$). The chi-square test was used to compare STEC detection and *Enterobacteriaceae* detection ($P < 0.05$).

RESULTS

APC. APC results for 147 (abattoir A), 318 (abattoir B), and 115 (abattoir C) sheep carcasses are shown in Figure 2 as box plots. The highest APC median values were those for the brisket (3.3 log CFU/cm^2 for abattoir A, 3.7 log CFU/cm^2 for abattoir B, and 3.8 log CFU/cm^2 for abattoir C) and the neck (3.5 log CFU/cm^2 for all three abattoirs). The lowest APC median values were those for the shoulder (2.5 log CFU/cm^2) and the outside foreleg (2.6 log CFU/cm^2) at abattoir A, for the back (2.8 log CFU/cm^2) and the flank (3.0 log CFU/cm^2) at abattoir B, and for the hind leg (2.6 log CFU/cm^2) and the back (2.9 log CFU/cm^2) at abattoir C. Differences between the median values for the sampling sites with the highest and lowest levels of microbial contamination were along 1.0 log unit and quartile deviation at 0.7 to 0.8 log unit. The microbial counts for abattoir A were lower than those for the other abattoirs, especially at the outside foreleg, inside foreleg, and shoulder sites, whereas the perineal area proved to be an exception. At abattoirs B and C, carcasses showed significantly higher levels of microbial contamination at the sampling sites in the cranial region than at those in the caudal region. Significant differences in APCs on different slaughtering days were found for abattoir B during the first half of the examination period ($P < 0.05$). In the majority of cases, the APC median values at the examined sites were below 3.0 log CFU/cm^2 on Tuesdays, whereas they exceeded 3.0 log CFU/cm^2 on Wednesdays and Thursdays.

Enterobacteriaceae. The average proportion of *Enterobacteriaceae*-positive carcasses was 68.1% (81.0% for abattoir A, 65.1% for abattoir B, and 60% for abattoir C), and the average proportion of positive samples was 15.2% (20.1% for abattoir A, 14.4% for abattoir B, and 11.2% for abattoir C) (Table 1). Overall, the largest proportion of positive samples was that for the brisket (for the perineal area and the brisket at abattoir A, for the brisket and the neck at abattoir B, and for the neck and the perineal area at abattoir C). The smallest proportion of positive samples was that for the hind leg. *Enterobacteriaceae* were found only in small numbers in most cases. Values of $>1.0 \times 10^3$ CFU/cm^2 were obtained for only four samples from abattoir A (with a maximum count of 9.8×10^3 CFU/cm^2), for seven samples from abattoir B (with a maximum count of 1.9×10^4 CFU/cm^2), and for no samples from abattoir C. The frequent presence of *Enterobacteriaceae* at abattoir A, especially in the perineal area, was striking in comparison with the APC results (Fig. 3). The relationship between APC values and qualitative *Enterobacteriaceae* detection turned out to be statistically significant ($P < 0.05$) for the

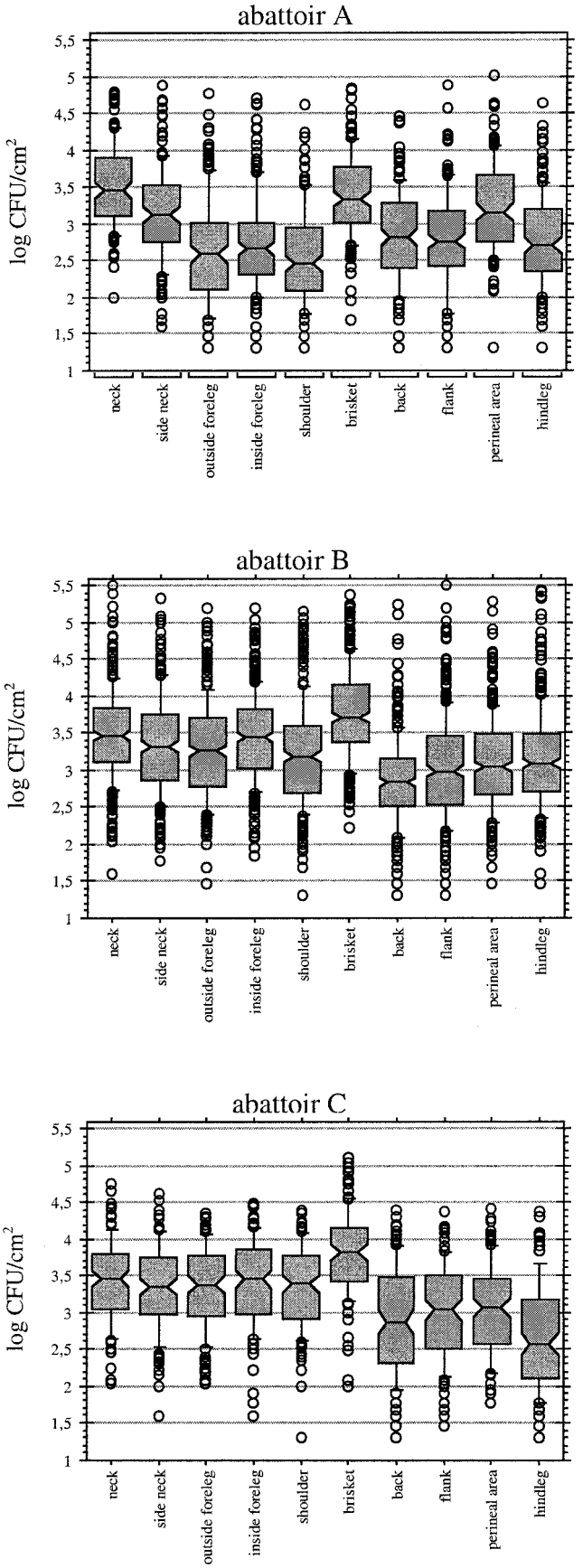

FIGURE 2. *APC results for the abattoirs (A, B, C) at 10 different sampling sites on sheep carcasses (147 carcasses for abattoir A, 318 carcasses for abattoir B, and 115 carcasses for abattoir C).*

brisket at abattoir A, for all sites except the inside foreleg at abattoir B, and for the back at abattoir C. In contrast, no significant relationship between APC and EC could be detected for any of the abattoirs.

STEC. Of the 535 sheep carcasses examined by PCR for *stx* genes, 196 were found to be *stx* positive (36.6%), with the detection rates being 59.9% for abattoir A (n = 147), 20.9% for abattoir B (n = 318), and 44.3% for abattoir C (n = 115). A comparison of APC results for the different sampling sites with the frequency of STEC detection on carcasses showed a significant relationship ($P < 0.05$) for the brisket, flank, and hind leg sites at abattoir A, for the side of neck, outside and inside foreleg, shoulder, brisket, and hind leg sites at abattoir B, and for none of the examined sites at abattoir C. Although there was a significant relationship between *Enterobacteriaceae* and STEC detection for abattoir A, such a relationship was not observed for abattoirs B and C.

A total of 56 STEC strains were isolated from 52 *stx*-positive screening samples. All of these strains belonged to the non-O157 *E. coli* group and tested positive for sorbitol and β-D-glucuronidase. Of the isolated strains, 42.9% tested positive for only the *stx*1 gene, 12.5% tested positive for only the *stx*2 genes, and 44.6% tested positive for both the *stx*1 and the *stx*2 genes. Further characterization of the *stx*2 variants showed 3 *stx*2-positive strains (5.4%), 1 *stx*2c-positive strain (1.8%), 27 *stx*2d-positive strains (48.2%), and 1 *stx*2c- and *stx*2d-positive strain (1.8%) (Table 2). Fifty percent of the 56 STEC strains isolated showed further virulence factors. Five (8.9%) strains tested positive for the *eae* gene, 28 (50%) tested positive for the EHEC-*hlyA* gene, and 2 (3.6%) tested positive for the *astA* gene. The most frequently detected virulence factors were *stx*1 and *stx*2 (21.4%), followed by the combination of the *stx*1 gene and the EHEC-*hlyA* gene (19.6%). Each of the five *eae*-positive isolates showed the EHEC-*hlyA* gene occurring once in combination with the *stx*1 and *stx*2 genes, once in combination with the *stx*2 genes, and three times in combination with the *stx*1 gene.

DISCUSSION

APCs were variously accepted as a criterion for the microbial contamination of carcasses, as a useful hygiene indicator, and, when determined regularly in combination with ECs, as a criterion for the verification of slaughter hygiene *(24, 40).* The swab technique is preferred for routine examinations because of its low cost in terms of time, effort, and money but requires a larger number of samples to compensate for its disadvantages, poor repeatability and standardization *(31).* It should be noted that the microbial yield obtained with the swab technique is subject to fluctuations and is lower than that obtained with destructive methods. In addition, microbial counts from various sites permit useful conclusions with regard to slaughter hygiene only if they are not specified as mean or total values for the entire carcass and if the same technique is used consistently in an abattoir to obtain comparable data *(29, 36, 40).* Despite different contamination profiles for the various ab-

TABLE 1. *Prevalence of* Enterobacteriaceae *at 10 different sampling sites on sheep carcasses at three abattoirs*

Sampling site	No. (%) of *Enterobacteriaceae*-positive samples			
	Abattoir A	Abattoir B	Abattoir C	Total
Neck	44 (29.9)	68 (21.4)	29 (25.2)	141 (24.3)
Side of neck	35 (23.8)	44 (13.8)	13 (11.3)	92 (15.9)
Outside foreleg	17 (11.6)	43 (13.5)	10 (8.7)	70 (12.1)
Inside foreleg	19 (12.9)	53 (16.7)	8 (7.0)	80 (13.8)
Shoulder	16 (10.9)	39 (12.3)	10 (8.7)	65 (11.2)
Brisket	57 (38.8)	81 (25.5)	20 (17.4)	158 (27.2)
Back	16 (10.9)	29 (9.1)	12 (10.4)	57 (9.8)
Flank	21 (14.3)	28 (8.8)	3 (2.6)	52 (9.0)
Perineal area	62 (42.2)	49 (15.4)	21 (18.3)	132 (22.8)
Hind leg	9 (6.1)	24 (7.6)	3 (2.6)	36 (6.2)
Total	296 (20.1)	458 (14.4)	129 (11.2)	883 (15.2)

attoirs, however, certain trends for the contamination levels at the sampling sites did emerge (Fig. 2). Repeated microbiological examinations yielded abattoir-specific result patterns that permitted conclusions with regard to systematic errors in the slaughtering process to be drawn. Significantly larger median values were found for abattoirs B and C for both the brisket and the entire front half of the carcass, which suggests weak points in slaughter hygiene. The significant differences in APCs for different slaughter days at abattoir B are probably due to differences in the hygienic conditions of the animals or carcasses. One possible explanation is that animals slaughtered on Wednesdays and Thursdays, which had been sporadically delivered the day before, either were inserted between other animals during the slaughtering routine or were part of the last batch of the working day, at which point the hygienic conditions of the animals worsened as staff attention slackened.

APC results reported in the literature for sheep carcasses after nondestructive sampling range from 2.1 to 5 log CFU/cm^2, but comparison is difficult because of different sampling sites, different modes of evaluation, some

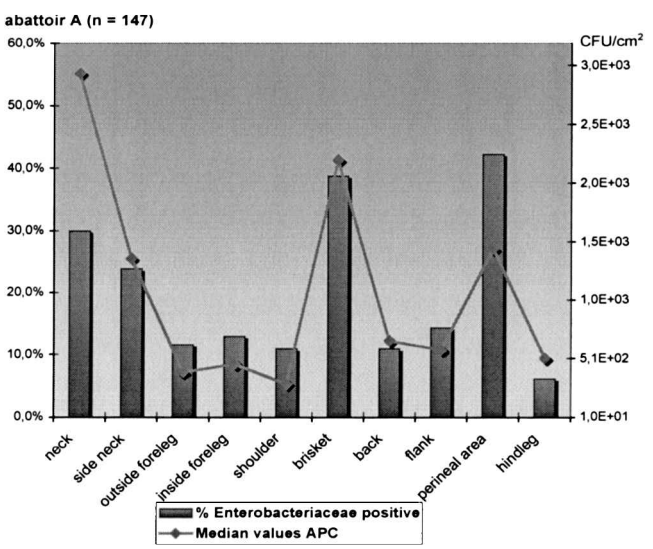

FIGURE 3. *Comparison of the* Enterobacteriaceae *prevalence levels with the APC median values for the different sampling sites at abattoir A.*

treatments being restricted to highly specific problems, and APCs being determined for entire carcasses in some cases *(4, 7, 14, 16, 18, 19, 25, 35).* Furthermore, our study showed a contamination distribution on sheep carcasses that was similar to that on beef carcasses *(40).* In contrast to beef carcasses, the examined sheep carcasses showed lower microbial scatter between the examined sites, which may be due to their smaller body size and the resulting more uniform contamination. APC results for sheep carcasses were also 1 log unit higher than those for beef carcasses *(40).* These results are consistent with the observations of various authors *(18, 35).* It is important to take into account the higher risk of contamination for sheep carcasses in organizing the slaughtering sequence. From the standpoint of meat hygiene, it is inadvisable to slaughter cattle after sheep on the same slaughtering line without intermediate cleaning or to slaughter sheep between sequences of cattle. Our results confirm the suitability of APC determination to aid in the monitoring of sheep slaughter hygiene as well as the observation that regular microbiological examinations of carcasses reliably represent long-term hygienic conditions in a slaughterhouse. In view of the contamination profile obtained, APC determination for the highly contaminated

TABLE 2. *Characterization results for the 56 STEC strains isolated*

No. of strains	Result for gene					
	*stx*1	*stx*2	*rfbE*	*eae*	EHEC-*hlyA*	*astA*
10	+	−	−	−	−	−
11	+	−	−	−	+	−
3	+	−	−	+	+	−
5	−	*stx*2d	−	−	−	−
1	−	*stx*2	−	+	+	−
1	−	*stx*2c	−	−	−	−
12	+	*stx*2d	−	−	−	−
8	+	*stx*2d	−	−	+	−
2	+	*stx*2d	−	−	+	+
1	+	*stx*2	−	+	+	−
1	+	*stx*2	−	−	+	−
1	+	*stx*2d, *stx*2c	−	−	+	−

neck and brisket can be recommended for a routine examination.

The Commission of the European Communities recommends that EC be used as an indicator of fecal contamination in addition to APC *(2).* However, several authors are of the opinion that the determination of the EC yields no additional information or shows no direct correlation with the presence or absence of pathogens *(1, 36).* In the present study, *Enterobacteriaceae* were detected in 883 (15.2%) of the 5,800 samples, with significant differences between the abattoirs being found (with a strikingly high percentage [20.1%] of samples testing positive for *Enterobacteriaceae* at abattoir A) (Table 1). The proportion of *Enterobacteriaceae*-positive samples ranged from 2.6% (for the hind leg and the flank at abattoir C) to 42.2% (for the perineal area at abattoir A). Comparable data from the literature on the prevalence of *Enterobacteriaceae* on the surfaces of sheep carcasses is scarce. Dependent on animal soiling, Hadley et al. *(20)* found *Enterobacteriaceae*-positive-sample percentages of 20 to 100% for the shoulder and 5 to 100% for the abdomen, as well as ECs of 0.19 to 1.84 log CFU/cm^2 for the shoulder and 0.17 to 2.01 log CFU/cm^2 for the abdomen. In a similar examination of beef carcasses, low levels of *Enterobacteriaceae* (5% of the swab samples examined [n = 6,300]) were found *(40).* These results suggest that sheep carcasses cause higher contamination pressure than beef carcasses and that it is thus inadvisable to slaughter sheep between sequences of cattle on the cattle slaughtering line. Compared with the other abattoirs, abattoir A yielded the highest percentages of *Enterobacteriaceae*-positive samples for 5 of the 10 sampling sites (Table 1). The relatively high prevalence of *Enterobacteriaceae*-positive samples in the perineal area (42.2%) compared with the APC was striking (Fig. 3). This finding may suggest that fecal contamination is more frequent at this site in particular and on the whole carcass in general as a result of the slaughtering technique used. The situation for abattoir A indicates that APC results alone (without taking *Enterobacteriaceae* into consideration) would have given a distorted impression of this abattoir in comparison with the other abattoirs. Therefore, the additional inclusion of the perineal area in the monitoring program is recommended.

In view of the importance of STEC as foodborne pathogens, at the insistence of customers, routine examinations aimed at the determination of STEC are increasingly required. The suspicion that ruminants represent a STEC reservoir has been confirmed by worldwide examinations *(5, 8, 9, 21, 41).* Our examination of sheep carcasses showed a significantly higher level of contamination (36.6%, n = 535) than that found for beef carcasses (5.4% *stx*-positive samples, n = 166 *(37)*). This finding is consistent with the increased prevalence of STEC in fecal samples obtained from small ruminants compared with those obtained from cattle *(39).* No statistical relationships that were applicable to all examined abattoirs and sampling sites could be found between APC and the prevalence of *stx*-positive samples or between *Enterobacteriaceae* and STEC detection. However, a significant relationship between *Enterobacteriaceae* and STEC detection was found for abattoir A, for which the percentage of *Enterobacteriaceae*-positive samples was high. In the literature, it was reported that poor hygienic conditions and the associated increase in *Enterobacteriaceae* detection led to an increased percentage of STEC-positive samples collected from beef carcasses *(41).* The lack of a significant relationship between *Enterobacteriaceae* and STEC detection for abattoirs B and C can be interpreted on the basis of the methods applied. A direct plating procedure without enrichment was used for the detection of *Enterobacteriaceae,* whereas an enrichment method was used to detect STEC.

The finding of no O157 *E. coli* among the 56 STEC strains agrees with the assumed low prevalence of the O157 serotype in Central Europe *(12, 43).* The distribution sequence found for the Shiga toxin variants in strains isolated from sheep carcass samples (with 44.6% of samples testing positive for both *stx*1 and *stx*2, 42.9% testing positive for *stx*1 only, and 12.5% testing positive for *stx*2 only) agrees with results for ovine STEC strains isolated from feces as reported by several authors, although at different frequencies *(5, 17, 22).* STEC strains isolated from patients frequently show a typical virulence spectrum, with such strains tending to be *stx*2 and *eae* positive *(10, 27).* In our examinations of sheep carcasses, *stx*2 subtypes were detected in 57.1% of the strains isolated (with 5.4% of samples testing positive for *stx*2, 1.8% testing positive for *stx*2c only, 48.2% testing positive for *stx*2d only, and 1.8% testing positive for both *stx*2c and *stx*2d). The *stx*2d variant was also found to be the principal *stx*2 subtype in ovine isolates in another study *(28).* Furthermore, the *stx*2d variant, frequently found in strains isolated from asymptomatic human carriers *(38),* has so far not been detected in STEC strains isolated from patients. The *stx*2d variant appears to exhibit a reduced pathogenicity. Only five of the 56 STEC strains isolated contained the *eae* gene (8.9%) in addition to the *stx* genes (three of these isolates contained *stx*1 only, one contained both *stx*1 and *stx*2, and one contained *stx*2 only), and these isolates were also EHEC-*hlyA* positive (Table 2). Similar prevalences of the genes for intimin and enterohemolysin have also been found in various studies *(5, 6, 17, 43).* For example, of all of the animal species examined in a German study, only sheep were found to test *eae*-positive (at a rate of 2.5%), and genes for an enterohemolysin (65.8%) were also found most frequently among sheep *(5, 6).* Two of the three *stx*2-positive strains simultaneously harbored the *eae* gene and the EHEC-*hlyA* gene and showed virulence patterns corresponding to those of typical strains isolated from hemolytic-uremic syndrome patients. However, the great majority of the STEC strains isolated from sheep carcasses were low-virulence variants.

From the standpoint of meat hygiene, it must be assumed that levels of STEC contamination of sheep carcasses in slaughterhouses are relatively high. Strains subjected to further genotyping included some with virulence factors that were in principle classified as human pathogens. The maintenance of slaughter hygiene is consequently of central importance in meat production. In view of the high prevalence of STEC, sheep should not be slaughtered until the end of a working day or should be slaughtered on a

separate slaughtering line. Such practices should be carried out to minimize the risk of contamination when other animal species with lower STEC prevalence levels are slaughtered on the same slaughter line and a danger of cross-contamination exists. With highly soiled animals, it is possible that the level of *Enterobacteriaceae*, and perhaps STEC, carcass contamination will increase at slaughter. Therefore, it would then seem to make more sense to use *Enterobacteriaceae* detection for the regular microbiological monitoring of slaughter hygiene than to examine carcass surfaces for STEC contamination by random sampling with the use of complex and expensive methods. In addition, a positive *stx*-PCR screening result or a positive toxin enzyme-linked immunosorbent assay says nothing about the type of Shiga toxin formed or the presence of any additional virulence factors and thus says nothing about the possible pathogenicity of a STEC strain.

Our results show that regular microbiological examinations of sheep carcasses allow reliable conclusions with regard to long-term hygienic conditions in an abattoir to be drawn. The distribution profiles for microbiological contamination on the carcasses were so consistent within an abattoir that a convenient selection of sampling sites could be made. For routine examinations, the selection of sampling sites with the highest abattoir-specific contamination risk and the inclusion of a sufficient number of carcasses are recommended. APCs and ECs are useful for the verification of slaughter hygiene in practice and are therefore appropriate for inclusion in a verification system according to hazard analysis critical control point principles for ovine slaughter and dressing. It is important to conduct regular evaluations, if necessary, in addition to identifying faults and introducing suitable control measures. The complex and expensive STEC detection procedure is less suitable for regular use within the scope of microbiological monitoring of slaughter hygiene. It would make more sense to base such monitoring on the detection of *Enterobacteriaceae*. On the basis of our results, it is recommended that 10 sheep carcasses be examined monthly at the three sites (the neck, the brisket, and the perineal area) with the use of the wet-dry double-swab technique for the determination of APCs and ECs.

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