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Shedding of food-borne pathogens and microbiological carcass contamination in rabbits at slaughter

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Abstract

To obtain microbiological data from rabbits at slaughter, 500 fecal samples and 500 carcasses samples were examined. All samples tested negative for *Listeria* and *Salmonella*. *Campylobacter* were detected in two fecal samples. Of the 500 fecal samples, 45.8% tested positive for *eae* (intimin), 1.2% for *stx* (Shiga toxin), and 1.8% for both *eae* and *stx*. By colony hybridization, 56 *eae* positive *Escherichia coli* strains were isolated. Among them, 27 strains (48.2%) were of the serotypes O178:H7 and O153:H7, whereas 15 strains (26.8%) belonged to a serogroup that has not yet been described (O(CB10681):H7). All strains possessed intimin β1 and the translocated intimin receptor (*tir*) capable of being tyrosine phosphorylated. None of the strains harbored the genes for Shiga toxins, EAST1 (*astA*), bundlin (*bfpA*), or the EAF plasmid. Slaughter rabbits therefore constitute a reservoir for certain atypical enteropathogenic *Escherichia coli*. On rabbit carcasses, average total bacterial counts accounted for 3.3 log CFU cm^{-2}. *Enterobacteriaceae* and coagulase positive staphylococci (CPS) were detected on 118 (23.6%) and 153 (30.6%) carcasses, respectively. *Enterobacteriaceae* and CPS counts of positive samples were mainly <1.5 log CFU cm^{-2}. Among 153 selected CPS isolates, 98.7% were identified as *Staphylococcus aureus*. None of the 151 isolated strains harbored the gene for methicillin resistance (*mecA*). Genes for staphylococcal enterotoxins (SE) were detected in 102 strains. The combinations of *seg* and *sei* (53 strains) and *sed*, *seg*, *sei*, and *sej* (27 strains) dominated.

Keywords: Rabbit; Fecal shedding; Enteropathogenic *Escherichia coli*; *Staphylococcus aureus*; Carcass contamination

1. Introduction

In Europe and some Asian countries, rabbits (*Oryctolagus cuniculus*) are an important source of meat. Rabbit meat is appreciated for its nutritional and dietary properties (Dalle Zotte, 2002). Especially in Mediterranean countries, rabbit meat is a common item in the diet. According to the Food and Agriculture Organization (FAO) of the United Nations (http://faostat.fao.org), world rabbit meat production is estimated to be over 1.4 million tons annually. In 2006, the major producer was China (500,000 tons),

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followed by Venezuela, Italy, Egypt, Spain, and France.

In view of food-borne diseases, the impact of latent zoonoses has increased in recent years (EFSA, 2007). The healthy animal thereby represents a reservoir for food-borne pathogens. Because fecal carriage is correlated with the probability of carcass contamination, these pathogens may enter the food chain during slaughter. To estimate the risks involved, analysis of the slaughter process should be complemented by the collection of data related to the animals' capability of carrying such pathogens (Zweifel et al., 2004). Beside bacteria originating from the animals (hide, feet and intestine), pathogens may enter the food chain by cross-contamination from various environmental sources during slaughter and processing.

Even though rabbits, rabbit carcasses and rabbit meat have basically the potential to carry pathogenic bacteria and rabbit meat is marketed and consumed worldwide, only limited microbiological data are available for healthy rabbits at slaughter or rabbit carcasses. However, within the implementation of the new food safety legislation of the European Union (EU), rabbit slaughterhouses must also apply compulsory self-checking programs of slaughter hygiene conditions following the HACCP principles. The aim of this study was therefore to assess the spread of food-borne pathogens in healthy rabbits at slaughter, to obtain data on the microbiological contamination of rabbit carcasses, and to further characterize isolated strains of food-borne pathogens.

2. Materials and methods

2.1. Abattoir and slaughter process

This study was based on investigations carried out within 7 months (March to September 2007) in a rabbit slaughterhouse processing 100,000 rabbits annually. Rabbits (ZIKA hybrids) originated from 37 farms distributed throughout Switzerland. Rabbits were raised in groups and slaughtered at an average weight of 2.7 kg. After being stunned (bolt stunner), animals were shackled by the hind legs and the head and forefeet were cut-off. After bleeding, the hide was removed, and carcasses were opened to remove viscera. Liver and kidneys remained with the carcass. Carcasses were then washed with potable water. After removal of the hind feet, carcasses were washed again and transferred to the chiller.

2.2. Sampling

In total, 500 fecal samples and 500 rabbit carcasses were examined. Sampling was performed twice weekly and comprised 10 fecal samples (colon) and 10 carcasses at each visit. Surface samples (40 cm^2) were obtained from the neck and leg by the wet–dry double swab technique at the end of slaughter or within 30 min of chilling. In the laboratory, pooled swabs of each carcass were homogenized in 20 ml of 0.85% saline solution. Fecal samples were analyzed qualitatively for *Campylobacter*, *Listeria*, *Salmonella*, enteropathogenic *Escherichia coli* (EPEC), and STEC. Carcass samples were analyzed for total viable counts (TVC), *Enterobacteriaceae*, coagulase positive staphylococci (CPS), *Campylobacter*, and *Listeria*.

2.3. Campylobacter

From each fecal or carcass sample, 1 g or 1 ml were inoculated in 10 ml of Campylobacter Enrichment Broth containing 28 g l^{-1} Brucella Broth (BBL 211088, Becton Dickinson, Sparks, MD, USA) with 250 mg l^{-1} sodium pyruvate, 250 mg l^{-1} ferrous sulphate, and 250 mg l^{-1} sodium metabisulphite (Enrichment Supplement, Oxoid SR0232E, Oxoid Ltd., Hampshire, UK), and 10 mg l^{-1} vancomycin, 5 mg l^{-1} trimethoprim, and 2500 IU l^{-1} polymyxin (Skirrow Selective Supplement, Oxoid SR69) for 48 h at 42 °C under microaerophilic conditions. Samples were plated onto Campylobacter Selective Agar containing 43 g l^{-1} Brucella Agar (BBL 21086, Becton Dickinson) with 60 ml l^{-1} lyzated horse blood (Oxoid SR48) and 25,000 IU l^{-1} bacitracin, 50 mg l^{-1} cycloheximide, 10,000 IU l^{-1} colistin sulphate, 15 mg l^{-1} cephazolin sodium, and 5 mg l^{-1} 1 novobiocin (Butzler Selective Supplement, Oxoid SR85). Agars were then incubated for 48 h at 42 °C under microaerophilic conditions. By PCR, presumptive colonies were identified as *Campylobacter jejuni* or *Campylobacter coli* (On and Jordan, 2003).

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2.4. Listeria

From each fecal or carcass sample, 1 g or 1 ml were enriched in 10 ml of Fraser Broth (Oxoid CM 895) with Half Fraser Supplement (Oxoid SR166E) for 24 h at 30 °C. From the first enrichment, 0.1 ml were incubated in 10 ml of Fraser Broth with Fraser Supplement (Oxoid SR156E) for 24 h at 37 °C. Samples were then plated onto Palcam Agar and Oxford Agar (Oxoid CM856) and incubated for 48 h at 37 °C. Presumptive colonies were tested for biochemical properties of *Listeria*.

2.5. Salmonella

From each fecal sample, 1 g was pre-enriched in 10 ml of Buffered Peptone Water (Oxoid CM509) for 24 h at 37 °C. From the first enrichment, 0.1 ml were incubated in 10 ml of Selenit-F-Broth (Oxoid CM395) with Sodium Biselenite (Oxoid L121) for 24 h at 37 °C, and in 10 ml of Rappaport Vassiliadis Enrichment Broth (Oxoid CM669) for 24 h at 43 °C. Samples were then plated onto Brilliant-Green Phenol Red Agar (Becton Dickinson) and Mannit-Lysin Crystal Violet Brilliant Green Agar (Brandenberger, Zurich, CH) and incubated for 24 h at 37 °C. Presumptive colonies were tested for biochemical properties of *Salmonella*.

2.6. E. coli harboring eae and stx

From each fecal sample, 1 g was incubated in 10 ml Brilliant-Green Bile Broth (Becton Dickinson) for 24 h at 37 °C. Samples were then plated onto sheep blood agar (5% sheep blood; Oxoid Ltd.). After incubation for 24 h at 37 °C, colonies were washed off with 2 ml of 0.85% saline solution. Each eluate was evaluated by PCR for the presence of *eae* and *stx* genes. All PCR assays were performed in a T3 thermocycler (Biometra, Göttingen, D). PCR mixtures consisted of 2 μl of bacterial suspension boiled in 22 μl of double-distilled water and 25 μl of GoTaq® Green Master Mix (Promega, Madison, WI, USA). PCR primers, target sequences, and product sizes are listed in Table 1.

For strain isolation, 56 randomly selected *eae* positive samples were tested by colony hybridization

Table 1
PCR primers used for the identification and characterization of *eae* and *stx* positive *Escherichia coli*

Target	Primer	Oligonucleotide sequence (5′-3′)	Product size (bp)	Reference
astA	East11a	CCA TCA ACA CAG TAT ATC CGA	111	Kaufmann et al. (2006)
	East11b	GGT CGC GAG TGA CGG CTT TGT		
bfpA	EP1	AAT GGT GCT TGC GCT TGC TGC	326	Beutin et al. (2003)
	EP2	GCC GCT TTA TCC AAC CTG GTA		
eae	SK1	CCC GAA TTC GGC ACA AGC ATA AGC	863	Oswald et al. (2000)
	SK2	CCC GGA TCC GTC TCG CCA GTA TTC G		
eae-α	SK1-LP2	CCC GAA TTC TTA TTT TAC ACA AGT GGC	2807	Zhang et al. (2002)
eae-γ	SK1-LP3	CCC GAA TTC TTC TTT TAC ACA AAC CGC	2792	Zhang et al. (2002)
eae-β	SK1-LP4	CCC GTG ATA CCA GTA CCA ATT ACG GTC	2287	Oswald et al. (2000)
eae-ε	SK1-LP5	AGC TCA CTC GTA GAT GAC GGC AAG CG	2608	Oswald et al. (2000)
eae-ζ	SK1-LP6B	TAG TTG TAC TCC CCT TAT CCC	2430	Zhang et al. (2002)
eae-ι	SK1-LP7	TTT ATC CTG CTC CGT TTG CT	2685	Zhang et al. (2002)
eae-η	SK1-LP8	TAG ATG ACG GTA GAC	2590	Zhang et al. (2002)
eae-k	SK1-LP10	GGC ATT GTT ATC TGT TGT CT	2769	Zhang et al. (2002)
eae-θ	SK1-LP11B	GTT GAT AAC TCC TGA TAT TTT A	2686	Zhang et al. (2002)
EAF	EAF1	CAG GGT AAA AGA AAG ATG ATA A	397	Kaufmann et al. (2006)
	EAF25	TAT GGG GAC CAT GTA TTA TCA		
stx	VT1	ATT GAG CAA AAT AAT TTA TAT GTG	523	Kaufmann et al. (2006)
	VT2	TGA TGA TGG CAA TTC AGT AT	520	
tir	*tir*-R	TAA AAG TTC AGA TCT TGA CAT		Kozub-Witkowski et al. (2008)
tir Y-P	tirY474-F	CAT ATT TAT GAT GAG GTC GCT C		
tir S	tisS478-F	TCT GTT CAG AAT ATG GGG AAT A		

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with an *eae*-specific probe (Kaufmann et al., 2006). Isolated strains were serotyped with O (O1 to O181) and H (H1 to H56) specific antisera produced at the German Federal Institute for Risk Assessment. By PCR, strains were tested for *stx*, *astA*, *bfpA*, and the EAF plasmid (Table 1). Typing of *eae* genes was performed using primer SK1 and specific reverse primers (Table 1). Subtyping of intimins α, β, and γ was done by restriction fragment length polymorphism analysis of PstI digested products. To discriminate between phosphorylated $tir_{E2348/68}$ (EPEC type) and non-phosphorylated tir_{Sakai} (STEC type), specific forward primers were used together with a conserved reverse primer (Table 1). Fifteen selected O178 strains were analyzed by macrorestriction profiling using XbaI and subsequent pulsed-field gel electrophoresis (PFGE) (Avery et al., 2002). Restriction bands were analyzed by GelCompar II (Applied Maths, Sint-Martens-Latem, B).

2.7. TVC, Enterobacteriaceae, CPS, and *Staphylococcus aureus*

Carcass samples were plated with a spiral plater (Eddy Jet, IUL SA, Barcelona, E) onto Plate Count Agar (Oxoid Ltd.; 72 h, 30 °C), Violet Red Bile Glucose Agar (BBL, Cockeysville, MD, USA; 48 h, 30 °C, anaerobic conditions), and Baird Parker plus Rabbit Plasma Fibrinogen Agar (BP-RPF; Oxoid Ltd.; 48 h, 37 °C). The detection limit was at 5 CFU cm^{-2}. Counts were expressed as log CFU cm^{-2}. From 153 BP-RPF agars, coagulase positive colonies were selected. One isolate per sample was taken. For identification of *Staphylococcus* (*S.*) *aureus*, DNA was extracted with QIAGEN DNeasy® Tissue Kit (Qiagen, Basel, CH), and then evaluated for a specific section of the 16S-23S rRNA intergenic spacer region (Table 2). Identified *S. aureus* were examined by PCR assays for *mecA*, *sea*, *seb*, *sec*, *sed*, *seg*, *sei*, and *sej* genes (Table 2).

3. Results

3.1. Listeria, Salmonella, Campylobacter

All samples tested negative for *Listeria* (fecal and carcass samples), and *Salmonella* (fecal samples). *Campylobacter* were not detected on carcasses, but two (0.04%) fecal samples tested positive (*C. jejuni*).

Table 2
PCR primers used for the identification and characterization of *Staphylococcus aureus*

Target	Primer	Oligonucleotide sequence (5′–3′)	Product size (bp)	Reference
23S rRNA	Staur 4 Staur 6	ACG GAG TTA CAA AGG ACG AC AGC TCA GCC TTA ACG AGT AC	1250	Straub et al. (1999)
mecA	GMCAR 1 GMCAR 2	ACT GCT ATC CAC CCT CAA AC CTG GTG AAG TTG TAA TCT GG	163	Mehrotra et al. (2000)
sea	sea-f sea-r	GCA GGG AAC AGC TTT AGG C GTT CTG TAG AAG TAT GAA ACA CG	521	Monday and Bohach (1999)
seb	seb-f seb-r	ACA TGT AAT TTT GAT ATT CGC ACT G TGC AGG CAT CAT GTC ATA CCA	667	Monday and Bohach (1999)
sec	sec-f sec-r	CTT GTA TGT ATG GAG GAA TAA CAA TGC AGG CAT CAT ATC ATA CCA	284	Monday and Bohach (1999)
sed	sed-f sed-r	GTG GTG AAA TAG ATA GGA CTG C ATA TGA AGG TGC TCT GTG G	385	Monday and Bohach (1999)
seg	seg-1 seg-2	TGC TAT CGA CAC ACT A CCA GAT TCA AAT GCA GAA CC	704	McLauchlin et al. (2000)
sei	sei-1 sei-2	GAG AAC AAA ACT GTC G CCA TAT TCT TTG CCT T	630	McLauchlin et al. (2000)
sej	sej-1 sej-2	CAT CAG AAC TGT TGT TCC GCT AG CTG AAT TTT ACC ATC AAA GGT AC	142	Monday and Bohach (1999)

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3.2. E. coli harboring eae and stx

Of the 500 fecal samples, 45.8% tested positive for *eae* (intimin), 1.2% *stx* (Shiga toxin), and 1.8% for both *eae* and *stx*. By colony hybridization, 56 *eae* positive *E. coli* strains were isolated. Thirty strains (53.6%) were attributed to four known O serogroups. These strains comprised the serotypes O15:H1 (2 strains), O49:H2 (1 strain), O153:H7 (12 strains), and O178:H7 (15 strains). Fifteen strains (26.8%) belonged to a serogroup that has not yet been described in literature (O(CB10681):H7). Three strains were non-typeable (ONT:H2 and ONT:H7) and 8 strains had a rough (spontaneously agglutinating) LPS type (Or:H7). The great majority of the 56 strains belonged to the H7 type (91.1%). By means of described PCR and PstI digestion of the products, all strains were identified as harboring intimin β1. Tir capable of being tyrosine phosphorylated (EPEC type) was found in all strains. None of the strains harbored *stx, astA, bfpA*, or the EAF plasmid. PFGE of 15 O178:H7 strains originating from 11 supplying farms formed five pulsotypes with indistinguishable patterns. Some pulsotypes were identical across different farms.

3.3. TVC, Enterobacteriaceae, CPS, and S. aureus from rabbit carcasses

TVC from carcasses ranged from 1.8 to 5.3 log CFU cm^{-2} (Fig. 1). Daily mean TVC lay between 2.6 and 4.2 log CFU cm^{-2}. In total, 367 (73.4%) carcasses yielded results >3.0 log CFU cm^{-2}, and 41 of them exceeded 4.0 log CFU cm^{-2}. Mean log TVC of the different sampling months ranged from 3.1 to 3.6 log CFU cm^{-2} (Table 3). *Enterobacteriaceae* and CPS were detected on 118 (23.6%) and 153 (30.6%) carcasses, respectively (Table 3). The proportion of *Enterobacteriaceae* and CPS positive carcasses from the different months ranged from 10.0 to 48.0% and 12.9 to 57.8%, respectively. Counts of positive samples were mainly <1.5 log CFU cm^{-2}.

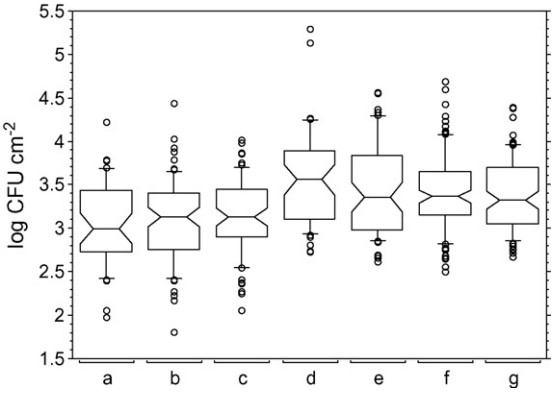

Fig. 1. Total viable counts (log CFU cm^{-2}) from 500 rabbit carcasses sampled during 7 months in the year 2007: (a) March ($n = 40$); (b) April ($n = 80$); (c) May ($n = 70$); (d) June ($n = 50$); (e) July ($n = 60$); (f) August ($n = 110$); (g) September ($n = 90$).

According to the PCR results, 151 (98.7%) isolates selected from BP-RPF agars were identified as *S. aureus*. None of the isolated *S. aureus* harbored the gene for methicillin resistance (*mecA*). Staphylococcal enterotoxins (SE) genes were detected in 102 (67.5%) strains (Table 4). Among them, *seg/sei* (99

Table 3
Total viable count (TVC), *Enterobacteriaceae,* and coagulase positive staphylocci (CPS) results obtained from rabbit carcasses

	No. of strains	TVC[a]				*Enterobacteriaceae*[b]		CPS[b]	
		$\bar{x}$	S.D.	Min	Max	% Pos	Max	% Pos	Max
March	40	3.05	0.50	1.98	4.22	10.0	1.40	15.0	2.18
April	80	3.09	0.48	1.81	4.44	16.3	2.16	16.3	1.54
May	70	3.14	0.43	2.06	4.01	27.1	2.29	12.9	1.18
June	50	3.55	0.56	2.73	5.30	48.0	2.37	34.0	2.67
July	60	3.43	0.52	2.62	4.56	15.0	1.48	25.0	1.74
August	110	3.42	0.45	2.51	4.69	25.5	1.81	37.3	2.57
September	90	3.37	0.41	2.67	4.39	23.3	1.54	57.8	2.91
Total	500	3.30	0.50	1.81	5.30	23.6	2.37	30.6	2.91

[a] $\bar{x}$: mean log CFU cm^{-2}; S.D.: standard deviation; min: minimum (log CFU cm^{-2}); max: maximum (log CFU cm^{-2}).
[b] % Pos: percentage of samples from which *Enterobacteriaceae* or CPS were detected; max: maximum (log CFU cm^{-2}).

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Table 4
Characterization results of *Staphylococcus aureus* isolated from rabbit carcasses ($n = 151$)

No. of strains	*mecA*	Genes for staphylococcal enterotoxins (SE)						
		sea	*seb*	*sec*	*sed*	*seg*	*sei*	*sej*
49	–	–	–	–	–	–	–	–
53	–	–	–	–	–	+	+	–
27	–	–	–	–	+	+	+	+
6	–	–	–	–	+	+	+	–
5	–	–	–	+	–	+	+	–
4	–	–	+	–	–	+	+	–
3	–	+	–	–	–	+	+	+
2	–	–	–	–	–	–	–	+
1	–	–	–	–	–	+	+	+
1	–	–	+	–	–	–	–	–

strains), *sej* (33 strains), *sed* (33 strains), *seb* (5 strains), *sec* (5 strains), and *sea* (3 strains) were found. The combinations of *seg* and *sei* (52.0%) and *sed*, *seg*, *sei*, and *sej* (26.5%) dominated.

4. Discussion

Microbiological data, especially characterization data of food-borne pathogens from rabbits at slaughter are rare. Only recent studies from Spain have addressed this topic comprehensively, although they were based on only 24 carcasses and 27 meat samples (Rodríguez-Calleja et al., 2004, 2006). Most other studies were dedicated to rabbits suffering from disease, specific pathogens, or factors influencing nutritional and sensory traits of rabbit meat.

Beside *Campylobacter*, *Salmonella* are still a major cause of food-borne disease in humans (EFSA, 2007). Comparable to our results, *Salmonella* and *Campylobacter* were not detected or only detected at a low prevalence in healthy rabbits or rabbit meat in most studies (Cerrone et al., 2004; Rodríguez-Calleja et al., 2006; Van Treel, 2006). Thus, compared to other animal species, these two significant bacterial pathogens seem to be rarely found in rabbits and rabbit meat. *Listeria* were not found in the present study. Likewise, intensive farmed rabbits and their meat tested negative in a German study (Van Treel, 2006). Rodríguez-Calleja et al. (2006) found *Listeria* spp. (13.7%) and *Listeria monocytogenes* (5.9%), but the prevalence was higher on rabbit meat at retail than on carcasses.

Genes for Shiga toxins have only been detected in a small minority of the rabbit fecal samples in the present study (3.0%). However, Garcia and Fox (2003) indicated rabbits as a potential reservoir for STEC pathogenic to humans. Moreover, wild rabbits living close to cattle excreting *E. coli* O157 were identified as vector in an outbreak of diarrhea and hemolytic–uremic syndrome involving visitors to a wildlife park (Scaife et al., 2006).

E. coli harboring intimin were found in a high prevalence in Swiss rabbits at slaughter. Such animals represent a source of carcass contamination at slaughter. *Eae* positive *E. coli* that possess the ability to form attaching and effacing lesions on intestinal cells but lack Shiga toxin genes are called EPEC. Of the 56 isolated *eae* positive strains, none belonged to serogroups frequently described as human EPEC (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158), but EPEC of serotype O153:H7 have been associated with diarrhea in humans (Nguyen et al., 2006). All isolated rabbit strains possessed intimin β1, which appears to be the most widespread variant. In that it has been found in EPEC and STEC from humans and animals (Ramachandran et al., 2003; Beutin et al., 2004). Typing of translocated intimin receptor genes revealed that all rabbit strains belonged to the EPEC type. This typing approach may allow recognizing strains that have lost their Shiga toxin genes (Kozub-Witkowski et al., 2008).

Albeit EPEC originated from apparently healthy rabbits, the O15 and O153 strains belong to serogroups associated with diarrhea in weaned rabbits. In rabbit

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farms, EPEC are a major cause of enteritis (Okerman, 1987). These EPEC normally belong to certain serotypes (O2:H6, O15:H−, O20:H7, O26:H11, O103:H2, O109:H2, H7, O128:H2, O132:H2, O153:H7), and especially O15:H-, O26:H11, and O103:H2 strains are considered as highly pathogenic (Blanco et al., 1996; Penteado et al., 2002).

Generally, EPEC are further subdivided into typical and atypical strains. The *bfpA* gene encoding bundlin and the EAF plasmid, which constitute characteristics of typical EPEC, were not detected among the isolated rabbit strains. Thus, slaughter rabbits constitute a reservoir for certain atypical EPEC. The role of atypical EPEC in human infection is probably underestimated and their impact as food-borne pathogens must be evaluated further (Nguyen et al., 2006; Beutin et al., 2003). Such strains might play a role in the emergence of new pathogenic strains because important STEC and EPEC virulence factors are located on mobile genetic elements.

S. aureus, which were detected on almost one-third of the examined rabbit carcasses, are commonly found on the skin and mucous membranes of animals and humans. In does and young rabbits, *S. aureus* are also associated with subcutaneous abscesses, mastitis, exsudative dermatitis, and pododermatitis (Hermans et al., 2003). Even though the emergence of methicillin-resistant *S. aureus* (MRSA) of animal origin in humans and the role of food is controversially discussed (De Neeling et al., 2007; Normanno et al., 2007; Van Loo et al., 2007), the *mecA* gene was not detected in the present study. About two-third of the isolated *S. aureus* harbored SE genes, especially *seg* and *sei*, which coexist on the enterotoxin gene cluster (*egc*). The *egc* locus in absence of classical SE was also detected in *S. aureus* from food-borne outbreaks (Omoe et al., 2005; Cha et al., 2006). Interestingly, the *egc* locus has also been indicated as virulence factor of high virulence *S. aureus* in rabbits (Vancraeynest et al., 2006). Besides, genes for classical SE, especially SED, were detected in more than 30% of the rabbit isolates. In a Spanish study, 14.8% of 27 *S. aureus* isolates from rabbit carcasses and meat harbored SEA and SEB (Rodríguez-Calleja et al., 2006).

In addition, rabbit carcasses were examined for TVC, *Enterobacteriaceae*, and CPS to obtain information on the slaughter hygiene conditions. Overall, mean log TVC accounted for 3.3 log CFU cm^{-2}. In recent Spanish studies, average TVC on rabbit carcasses 24 h post mortem ranged from 4.0 to 5.0 log CFU g^{-1} (Rodríguez-Calleja et al., 2004, 2005). In general higher results were thereby reported for rabbit meat at retail than for carcasses. In an Italian study, more than 87% of carcass swabs yielded results <3.5 log CFU cm^{-2} (Cerrone et al., 2004). Within the EU, regulation (EC) No. 2073/2005 set out performance criteria only for red meat carcasses sampled by the destructive technique (Anonymous, 2005). Such limits have also been evaluated for the wet–dry double swab technique (Zweifel et al., 2005). Although no criteria were determined for rabbits, TVC limits (satisfactory: <3.0; acceptable: 3.0–4.0; unacceptable: >4.0 log CFU cm^{-2}) may be used for orientation purposes. Hence, the majority of the rabbit carcasses (65.2%) were rated as acceptable. Besides, *Enterobacteriaceae*, which are used as indicators for fecal contamination, and CPS, which are used as indicators for *S. aureus*, were found in remarkable frequencies but accounted only for a small subset of the total microbial contamination on the rabbit carcasses.

Consequently, strict maintenance of good practices of slaughter hygiene is of central importance to ensure both public health protection and meat quality. The maintenance of slaughter hygiene can be measured in daily practice by slaughter process analysis. In this context, it must be considered that the water applied during carcass washing may have contributed to carcass contamination because the hind feet remained on rabbit carcasses after skinning. Microbiological data are thereby necessary for the assessment of process performance, the implementation of HACCP-based systems, and may be used for verification of carcass contamination. For these purposes, rather TVC and indicator organisms as *Enterobacteriaceae* or *E. coli* than pathogens should be monitored.

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