Arch Lebensmittelhyg 63,
11–15 (2012)
DOI 10.2376/0003-925X-63-11

ISSN 0003-925X

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Occurrence of *Erysipelothrix* spp., *Salmonella* spp., and *Listeria* spp. in tonsils of healthy Swiss pigs at slaughter

Vorkommen von Erysipelothrix, Salmonellen und Listerien in den Tonsillen von Schlachtschweinen in der Schweiz

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Summary

Healthy food producing animals were recognized in recent years as important carriers of bacterial pathogens causing human illness. To obtain microbiological data from Swiss pigs at slaughter, tonsils samples were examined in the present study for the occurrence of *Erysipelothrix, Salmonella,* and *Listeria* over a 3-month period. No *Erysipelothrix* spp. were found in 250 tonsil samples by culture after an enrichment step. *Salmonella* spp. were detected (ISO 6579:09.2006) in only 0.8 % (2/250) of the tonsils from the tested animals, corresponding to a batch prevalence of 1.4 %. The two isolates were identified as *Salmonella* Bredeney and *Salmonella* Kedougou, which are only rarely reported as cause of human illness. *Listeria* spp. were detected (ISO 11290-1:2004) in 5.6 % (14/250) of the tonsils from the tested animals, corresponding to a batch prevalence of 11.1 %. Of the 14 isolates, nine were identified as *Listeria monocytogenes,* four as *Listeria ivanovii,* and one as *Listeria innocua.* The *Listeria monocytogenes* strains belonged to the serotypes 1/2a, 1/2b, and 4b. Though numbers were low compared to the data from some other European countries, the present study shows that *Salmonella* and *Listeria monocytogenes* could be detected in the tonsils from healthy pigs at slaughter and these pathogens might pose a threat for the contamination of carcasses and the slaughterhouse environment.

Keywords: Zoonotic and foodborne pathogens, prevalence, serotyping, tonsils, slaughtered pigs

Zusammenfassung

Die Bedeutung gesunder Nutztiere als Reservoir humanpathogener Keime hat in den letzten Jahren zugenommen. In der vorliegenden Studie wurden über einen Zeitraum von 3 Monaten Tonsillenproben von Schlachtschweinen aus der Schweiz auf *Erysipelothrix,* Salmonellen und Listerien untersucht. *Erysipelothrix* spp. wurden nach einstufiger Anreicherung kulturell-bakteriologisch in keiner der 250 Tonsillenproben gefunden. Salmonellen wurden bei lediglich 0.8 % (2/250) der untersuchten Schweine in den Tonsillen nachgewiesen (ISO 6579:09.2006). Dies entspricht einer Herdenprävalenz von 1.4 %. Die zwei Isolate wurden als *Salmonella* Bredeney und *Salmonella* Kedougou identifiziert, wobei diese Serovare nur eine untergeordnete Rolle bei humanen Erkrankungen spielen. Listerien wurden bei 5.6 % (14/250) der untersuchten Schweine in den Tonsillen nachgewiesen (ISO 11290-1:2004). Dies entspricht einer Herdenprävalenz von 11.1 %. Von den 14 Stämmen wurden neun als *Listeria monocytogenes,* vier als *Listeria ivanovii* und einer als *Listeria innocua* identifiziert. Die *Listeria monocytogenes*-Stämme gehörten zu den Serotypen 1/2a, 1/2b und 4b. Obwohl im Vergleich zu den Daten einiger anderer europäischer Länder nur geringe Nachweisraten vorlagen, zeigen die vorliegenden Ergebnisse, dass Salmonellen und *Listeria monocytogenes* bei gesunden Schlachtschweinen gefunden wurden und daher ein Risiko der Kontamination von Schlachttierkörpern und der Schlachthof-Umgebung besteht.

Schlüsselwörter: Zoonoseerreger, Lebensmittel-assoziierte Erkrankungen, Prävalenz, Serotypisierung, Tonsillen, Schlachtschweine

Journal of Food Safety and Food Quality 63, Heft 1 (2012), Seiten 1–24

Introduction

Zoonotic and foodborne diseases are widespread, thus affecting lives, business, and economies worldwide. They have a major health impact in industrialized countries and remain responsible for high levels of morbidity and mortality in the general population but particularly for at-risk-groups such as infants, young children, pregnant women, elderly, or immunocompromized people. With regard to meat production, healthy food animals including pigs were recognized in recent years as carriers of pathogens causing human illness. Such pathogens harbored by healthy animals may enter the food chain during slaughter (Nørrung and Buncic, 2008; Fosse et al., 2009). Thereby, it must be considered that pork is today the most frequently consumed meat in Europe. To estimate the risk involved, baseline data on the animals' probability of carrying such pathogens are required. For this purpose, *Erysipelothrix* spp. *(E. rhusiopathiae), Salmonella* spp., and *Listeria* spp. *(L. monocytogenes)* were selected as target organisms in the present study.

Erysipelothrix spp. are pathogens or commensals in a wide variety of animals. The disease of greatest prevalence and economic importance is swine erysipelas, which is caused by *E. rhusiopathiae* and occurs in acute (septicemia), subacute (diamond-shaped skin lesions), and chronic forms (arthritis, endocarditis) (Wang et al., 2010). In addition, *E. rhusiopathiae* causes various forms of human disease: a localized cutaneous form (erysipeloid), a generalized cutaneous form, and a septicemic form often associated with endocarditis (Brooke and Riley, 1999). Human infections are often occupationally related (farmers, veterinarians, abattoir employees) and occur via skin lesions. The domestic pig is an important reservoir of *E. rhusiopathiae* and it has been estimated that 30 to 50 % of healthy pigs harbor the organism in their tonsils and lymphoid tissues (Wood, 1999). Carriers can shed *Erysipelothrix* in feces, urine, saliva, and nasal secretions, creating sources of infection. In view of foodborne pathogens, *Salmonella* spp. are worldwide a major cause of acute bacterial gastroenteritis. In the European Union (EU), a total of 108'614 confirmed human cases of salmonellosis (23.7/100'000 inhabitants) have been reported in 2009 (EFSA/ECDC, 2011). *Salmonella* may colonize the intestinal tract of a large number of mammals and birds. A baseline survey on the prevalence of *Salmonella* in European holdings with breeding pigs reported an average *Salmonella* prevalence of 28.7 % (range between member states: 0–64.0 %) for breeding holdings and 33.3 % (range between member states: 0–55.7 %) for production holdings (EFSA, 2009). Healthy pigs therefore constitute an important reservoir for *Salmonella* and a considerable part of the human cases are attributed to the consumption of pork (Berends et al., 1998; EFSA, 2010). Besides, *L. monocytogenes* has significant public health and economic impacts as a foodborne pathogen. Human infections primarily result from eating contaminated food and may lead to serious and potentially life-threatening listeriosis (Doganay, 2003). Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness. In the EU, a total of 1'645 confirmed human cases of listeriosis (0.4/100'000 inhabitants) have been reported in 2009 (EFSA/ECDC, 2011). In several EU member states, the annual incidence rate has increased over the last few years, especially in the elderly population. *Listeria* spp. are widely distributed in the environment and certain strains may become established and persist in the processing environment (Thévenot et al., 2006; Wulff et al., 2006; Blatter et al., 2010). Other reservoirs include domestic and wild animals, but their significance in view of foodborne diseases and potential transmission routes (during slaughter) remains to be elucidated.

Only limited data are available in literature for the occurrence of *Erysipelothrix* spp., *Salmonella* spp., and *Listeria* spp. in healthy slaughtered pigs and such data were so far lacking in Switzerland. The aim of the present study was therefore to assess the occurrence of these pathogens in the tonsils from Swiss pigs at slaughter and to further characterize isolated strains.

Materials and methods

Abattoir and sampling

This study was based on investigations carried out within three months (March to May 2011) in a Swiss abattoir processing 250 pig carcasses per hour. To investigate the occurrence of *Erysipelothrix* spp., *Salmonella* spp. and *Listeria* spp., tonsil samples were collected from healthy slaughtered pigs. Palatine tonsils are known as a portal of entry and a site of multiplication and persistence for several microorganisms in animals including pigs (Salles and Middleton, 2000). Sampled pigs were about six month old and the average weight of each carcass was about 80 kg. On each sampling day, 22 to 49 samples were collected and not more than two samples originated from the same batch/producer. The majority of the producers were distributed over the north and central part of Switzerland. Sampling comprised two phases. In the first phase, 250 samples were collected during eight sampling days and they were examined for *Erysipelothrix* spp. and *Salmonella* spp. These samples were obtained from carcasses originating from 138 different batches and 108 producers. In the second phase, 250 samples were collected during seven sampling days and they were examined for *Listeria* spp. These samples were obtained from carcasses originating from 126 different batches and 108 producers.

Tonsil samples (tonsilla veli palatini) were obtained at the end of the slaughter process when the head had been cut off. For this purpose, forceps and scissors previously sterilized in 96 % ethanol solution were used. After leaning the head with its frontal region on a support, the velum palatinum was stretched and cut with two parallel lines on both sides of the median furrow of the soft palate. Tonsil samples were then excised and placed into sterile stomacher bags. Samples were transported cooled to the laboratory and bacteriological examinations were carried out within 3 h after sampling.

Erysipelothrix spp.

Examination for *Erysipelothrix* spp. was done by culture after an enrichment step. Briefly, half of each tonsil sample was homogenized for 60 s in 10 ml of Brain Heart Infusion Broth (BHI, CM1135, Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 41.5 °C. Subsequently, subsets (one loopful) of the enrichment broth were streaked onto a modified Packers' selective medium (pH=7.58) containing per liter 39 g of blood agar base (Dehydrated Columbia Blood Agar Base EH, Difco™ Laboratories, Detroit, MI, USA), 0.01 g of crystal violet (Sigma-Aldrich, St Louis,

MO, USA), 0.7 g of sodium azide (Sigma-Aldrich), and 5 % of horse blood (SR048, Oxoid Ltd.). Plates were incubated for 48 h at 41.5 °C. Suspicious colonies (very small size, bluish colour, surrounded by a narrow zone of hemolysis) were sub-cultured onto sheep blood agar (Difco™ Laboratories; 5 % sheep blood, SB055, Oxoid Ltd.) for 24 h at 37 °C. After Gram staining, Gram-positive rods were tested for catalase reaction and H_2S production. For confirmation and species identification, presumptive *Erysipelothrix*-positive colonies were verified (i) by appraisal of their biochemical properties using the Api Coryne System in accordance with the manufacturers' instructions (bioMérieux SA, Marcy l'Etoile, F) and (ii) by the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Mabritec AG, Riehen, CH).

Salmonella spp.

Examination for *Salmonella* spp. was done in accordance with ISO 6579:09.2006 using a two-step enrichment procedure. Briefly, the other half of each tonsil sample was pre-enriched in 10 ml of Buffered Peptone Water (BPW, CM1049, Oxoid Ltd.) for 24 h at 37 °C. From the first enrichment, 1 ml was incubated for 24 h at 37 °C in 10 ml of Kauffmann Tetrathionate-Novobiocin Broth (CM1048, Oxoid Ltd.) supplemented with Novobiocin Sodium Salt (74675, Sigma-Aldrich) in accordance with the manufacturers' instructions and 0.1 ml was incubated for 24 h at 41.5 °C in 10 ml of Rappaport-Vassiliadis Soya Pepton Broth (CM0866, Oxoid Ltd.). After plating onto Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB, CM0783, Oxoid Ltd.) and Xylose-Lysine-Desoxycholate Agar (XLD, CM0469, Oxoid Ltd.), plates were incubated for 24 h at 37 °C. Suspicious colonies were tested for biochemical properties of *Salmonella* by using the following tests: oxidase reaction, acid production from mannitol, o-nitrophenyl-ß-D-galactopyranoside (ONPG) test, H_2S and indole production, and proof of urease and lysine decarboxylase. Isolated *Salmonella* strains were affirmatively identified and serotyped at the Swiss National Reference Centre for Enteropathogenic Bacteria and Listeria (Institute for Food Safety and Hygiene, University of Zurich, CH).

Listeria spp.

Examination for *Listeria* spp. and in particular *L. monocytogenes* was done in accordance with ISO 11290-1:2004 using a two-step enrichment procedure. Briefly, each sample was incubated in 10 ml of Fraser Broth (CM0895, Oxoid Ltd.) with Half Fraser Supplement (SR0166, Oxoid Ltd.) for 24 h at 30 °C. From the first enrichment, 0.1 ml were incubated in 10 ml of Fraser Broth (CM0895, Oxoid Ltd.) with Fraser Supplement (SR0156, Oxoid Ltd.) for 24 h at 37 °C. Subsequently, subsets (one loopful) were streaked onto Palcam Agar (Merck Eurolab GmbH, Darmstadt, D) and onto Chromogenic Listeria Agar (CM1084, Oxoid Ltd.) supplemented with Listeria Selective Supplement (SR0226, Oxoid Ltd.) and Listeria Differential Supplement (SR0244, Oxoid Ltd.). Both plates were incubated for 48 h at 37 °C. On the chromogenic agar, colonies of *Listeria* spp. grew with a green-blue colour, whereas colonies of *L. monocytogenes* and *L. ivanovii* grew with a green-blue colour surrounded by an opaque halo. Presumptive *L. monocytogenes* and *L. ivanovii* colonies on the chromogenic agar were streaked onto sheep blood agar for appraisal of hemolysis (CAMP test with *Staphylococcus aureus* and *Rhodococcus equi*). To identify other *Listeria* species, the API Listeria identification Kit was used (bioMérieux SA). Isolated *L. monocytogenes* strains were affirmatively identified and serotyped at the Swiss National Reference Centre for Enteropathogenic Bacteria and Listeria (Institute for Food Safety and Hygiene, University of Zurich, CH).

Results and Discussion

Using the described method for detection of *Erysipelothrix* spp., all 250 tonsil samples obtained from healthy Swiss pigs at slaughter tested negative (Tab. 1). Based on these results, *Erysipelothrix* spp. seem to occur only in low numbers in the Swiss pig population. Our findings are in contrast to the assumption that 30 to 50 % of healthy pigs might harbor the organism (Wood, 1999). As a limitation of the present study, the use of solely the described cultural detection method must be mentioned. In our study, agar plates were often overgrown with different bacteria so that potentially present *Erysipelothrix* spp. may have been competitively suppressed in their growth or missed. Using a cultural method with addition of selected antibiotics, Takahashi et al. (1987) isolated *E. rhusiopathiae* from the tonsils of 10.5 % of 600 healthy slaughter pigs. Nevertheless, none of the available media is considered ideal (Wang et al., 2010). To get a clearer and more accurate picture on the prevalence of *Erysipelothrix* spp. in the pig population, PCR-based methods have been proposed and evaluated (Takeshi et al., 1999; Wang et al., 2002; Yamazaki, 2006; Pal et al., 2010). In view of pig colonization, such methods are also of interest for differentiation of *Erysipelothrix* species, in particular as the role of *E. tonsillarum,* which is considered non pathogenic for pigs, needs to be further elucidated. In a survey from Australia, which investigated 109 abattoir samples (collected from various parts of pig and sheep carcasses as well as from different sections of the slaughter line, pen, soil, and effluent), genus-specific PCR yielded 32.1 % *Erysipelothrix*-positive samples, whereas culture yielded only 15 (13.8 %) *Erysipelothrix* isolates (Wang et al., 2002). However, *Erysipelothrix* spp. were thereby not detected in tonsil samples with either method.

Salmonella spp. were detected in only 0.8 % of the 250 tonsil samples obtained from healthy Swiss pigs at slaugh-

TABLE 1: *Occurrence of Erysipelothrix spp., Salmonella spp., and Listeria spp. in tonsils of healthy pigs at slaughter.*

	Erysipelothrix spp.	*Salmonella* spp.	*Listeria* spp.
No. of sampled animals/ batches	250/138	250/138	250/126
No. (%) of positive animals	nd[a]	2 (0.8 %)	14 (5.6 %)
No. (%) of positive batches	nd	2 (1.4 %)	14 (11.1 %)
Isolates (No.)	nd	*S.* Bredeney (1) *S.* Kedougou (1)	*L. monocytogenes* (9)[b] *L. ivanovii* (4) *L. innocua* (1)

[a]: nd, not detected; [b]: *L. monocytogenes* isolates belonging to serotypes 1/2a, 1/2b, and 4b.

ter (Tab. 1). The two *Salmonella*-positive animals originated from two farms located in the central part of Switzerland. With regard to the batch level, *Salmonella* spp. were detected in 1.4 % of the 138 examined batches. Reported detection rates of *Salmonella* spp. in pig tonsils at slaughter vary between different surveys. From tonsils of Bavarian fattening pigs, no *Salmonella* were isolated (Fredriksson-Ahomaa et al., 2009), whereas Bonardi et al. (2003) detected *Salmonella* in 5.3 % of 150 tonsil samples from slaughter pigs in northern Italy, and a high *Salmonella* prevalence of 19.6 % has been reported in tonsils of slaughter pigs in the Netherlands (Swanenburg et al., 2001). In a European baseline survey investigating slaughter pigs, average *Salmonella* prevalence was 10.3 % in lymph nodes and 8.3 % on carcasses (EFSA, 2008). Prevalence thereby ranged from 0 % in Finland to 29.0 % in Spain for lymph nodes and from 0 % in Slovenia and Sweden to 20.0 % in Ireland for carcasses.

The two *Salmonella* isolates found in the present study belonged to serovars Bredeney and Kedougou. In the study of Bonardi et al. (2003), five *Salmonella (S.)* Bredeney strains and three *S.* Derby strains were isolated out of eight positive tonsil samples. *S.* Bredeney and *S.* Kedougou were also identified in the EU survey on *Salmonella* in holdings with breeding pigs, but these serovars represented in each case less than 5.0 % of the *Salmonella*-positive holdings (EFSA, 2009). The most frequently detected serovars were thereby *S.* Derby and *S.* Typhimurium, which were isolated in 20.1 to 29.6 % of the positive holdings. With regard to human illness associated with *Salmonella, S.* Bredeney and *S.* Kedougou are only rarely reported as cause of human cases or foodborne outbreaks (EFSA/ECDC, 2011). In the year 2009, only one verified outbreak (three cases) caused by *S.* Bredeney has been observed in the EU. The majority of human *Salmonella* cases were caused by *S.* Enteritidis and *S.* Typhimurium. Of all 324 *Salmonella*-associated verified foodborne outbreaks reported in 2009, *S.* Enteritidis and *S.* Typhimurium accounted for 59.6 % and 15.7 %, respectively (EFSA/ECDC, 2011).

Listeria spp. were detected in 5.6 % of the 250 tonsil samples obtained from healthy Swiss pigs at slaughter (Tab. 1). The 14 *Listeria*-positive animals originated from 11 farms located in the north-central part of Switzerland. With regard to batch level, *Listeria* spp. were detected in 11.1 % of the 126 examined batches. Of the 14 *Listeria* isolates, nine, four, and one were identified as *L. monocytogenes, L. ivanovii,* and *L. innocua,* respectively. Overall, *L. monocytogenes* were therefore detected in 3.6 % of the examined animals and 7.2 % of the batches. Reported detection rates of *L. monocytogenes* in pig tonsils at slaughter vary between different surveys. Amongst Bavarian fattening pigs, *L. monocytogenes* was isolated from 32.0 % of 50 tonsil samples (Fredriksson-Ahomaa et al., 2009). In other European studies examining tonsils of pigs, the prevalence of *L. monocytogenes* ranged from 12.0 to 44.6 % (Buncic, 1991; Autio et al., 2000; Autio et al., 2004). Autio et al. (2004) thereby reported that the prevalence of *L. monocytogenes* in tonsils of fattening pigs (22.0 %) was significantly higher than in sows (6.5 %) and that the prevalence among pigs from five abattoirs varied from 3.3 to 25.0 %. In the U.S., Wesley et al. (2008) recently detected *L. monocytogenes* in only 0.6 % of 181 tonsil samples from cull sows, whereas Kanuganti et al. (2002) found *L. monocytogenes* in 7.1 % of 252 slaughter pigs. Interestingly, several studies reported that *L. monocytogenes* was isolated more frequently from tonsils than from fecal samples (Buncic, 1991; Wesley et al., 2008; Fredriksson-Ahomaa et al., 2009). Thus, it was hypothesized that tonsils might be a more accurate predictor of *L. monocytogenes* carrier status in pigs than fecal samples. Of the nine *L. monocytogenes* isolates, three belonged to serotype 1/2a, two to serotype 1/2b, and four to serotype 4b. The majority of human cases are also associated with *L. monocytogenes* of serotypes 1/2a, 1/2b, and 4b and the proportion associated with isolates of serotype 1/2a has increased in recent years (Lukinmaa et al., 2003; Parihar et al., 2008; Allerberger and Wagner, 2010).

In conclusion, although *Erysipelothrix* spp. were not isolated, this study demonstrates that *Salmonella* spp. and *L. monocytogenes* could be detected in tonsils from healthy Swiss pigs at slaughter. Compared to the data from some other European countries, detected prevalence of *Salmonella* spp. and *L. monocytogenes* was low. However, it must be considered that tonsils colonized with pathogens might play a role in the contamination of pluck sets, carcasses, and the slaughterhouse environment during slaughter (Fredriksson-Ahomaa et al., 2009). To encounter this threat, prevention of contamination during slaughter is of major importance, in particular adherence to good hygiene practices and application of effective cleaning and disinfection procedures to prevent equipment contamination.

Acknowledgment

We thank the staff of the slaughterhouse involved in this study for facilitating access and for assistance with the collection of data.

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