Microbiological identification and analysis of swine tonsils collected from carcasses at slaughter

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Abstract

The primary objective of this 7-month study was to determine the prevalence of porcine pathogens of the tonsil of the soft palate of swine at slaughter. Additional objectives were to determine if sampling the carcasses of normal or abnormal hogs provided different microbiological profiles and if the slaughter plant provides a feasible sampling frame and environment for detecting and monitoring important pathogens in tonsils that have health implications for both swine and humans. A total of 395 samples were collected from 264 farms. Of these, 180 tonsils were collected from normal carcasses and 215 tonsils were collected from carcasses that were diverted to the hold rail. Laboratory testing included bacteriological culture and identification as well as real time-polymerase chain reaction (PCR) testing for porcine reproductive and respiratory syndrome virus (PPRSV) and immunohistochemistry (IHC) for porcine circovirus-2 (PCV-2). The most commonly isolated bacteria included: *Streptococcus suis* (53.7%), *Arcanobacterium pyogenes* (29.9%), *Pasteurella multocida* (27.3%), and *Streptococcus porcinus* (19.5%). Virus screening revealed evidence of PRRSV and PCV-2 in 22.0% and 11.9% of the samples, respectively. *Salmonella* Typhimurium and *Yersinia enterocolitica* were isolated in 0.5% and 1.8% of the samples, respectively. Tonsils collected from the hold rail were more likely to be positive for *Staphylococcus hyicus* [odds ratio (OR) = 7.51, confidence interval (CI) = 2.89 to 19.54], *Streptococcus porcinus* (OR = 9.93, CI = 4.27 to 23.10), and *Streptococcus suis* (OR = 2.16, CI = 1.45 to 3.24). Tonsils collected from abnormal carcasses were less likely to be positive for *Staphylococcus aureus* (OR = 0.05, CI = 0.005 to 0.482).

Résumé

L'objectif primaire de cette étude d'une durée de 7 mois était de déterminer la prévalence d'agents pathogènes porcins dans les amygdales du palais mou de porcs au moment de l'abattage. Les objectifs additionnels étaient de déterminer si l'échantillonnage des carcasses des porcs normaux ou anormaux fournissait des profils microbiologiques différents et si l'abattoir fournissait un environnement et une structure d'échantillonnage adéquat pour détecter et surveiller dans les amygdales des agents pathogènes importants ayant des implications sur l'aspect santé des porcs et des humains. Au total, 395 échantillons ont été prélevés à partir de 264 fermes. Parmi ceux-ci, 180 amygdales ont été prélevées de carcasses normales et 215 amygdales ont été prélevées à partir de carcasses qui avaient été placées sur le rail de retenu. Les analyses de laboratoire incluaient une culture bactérienne et l'identification, une épreuve d'amplification en chaîne par la polymérase (PCR) pour le virus reproducteur et respiratoire porcin (PRRSV) et l'analyse par immuno-histochimie (IHC) pour le circovirus porcin de type 2 (PCV-2). Les bactéries les plus fréquemment isolées étaient : Streptococcus suis *(53,7 %),* Arcanobacterium pyogenes *(29,9 %),* Pasteurella multocida *(27,3 %) et* Streptococcus porcinus *(19,5 %). La vérification pour les virus a révélé des évidences de présence de PRRSV et PCR-2 dans, respectivement, 22,0 % et 11,9 % des échantillons.* Salmonella *Typhimurium et* Yersinia enterocolitica *ont été isolées, respectivement, dans 0,5 % et 1,8 % des échantillons. Les amygdales prélevées des porcs sur le rail de retenu étaient plus susceptibles d'être positifs pour la présence de* Staphylococcus hyicus *[rapport de cotes (OR) = 7,51, intervalle de confiance (IC) = 2,89 à 19,54),* Streptococcus porcinus *(OR = 9,93, CI = 4,27 à 23,10) et* Streptococcus suis *(OR = 2,16, CI = 1,45 à 3,24). Les amygdales prélevées des carcasses anormales étaient moins susceptibles d'être positives pour la présence de* Staphylococcus aureus *(OR = 0,05, CI = 0,005 à 0,482).*

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Introduction

Tonsils are aggregates of lymphoid tissue that act as a protective immune barrier to the respiratory and gastrointestinal tracts of mammals against the bombardment of pathogens (1). The principle tonsil of the pig is the tonsil of the soft palate (1,2). Commensal bacteria, such as *Streptococcus porcinus*, *Streptococcus dysgalactiae*, as well as opportunistic pathogens, such as *Staphylococcus aureus*, *Staphylococcus hyicus*, and *Streptococcus suis*, can be isolated from swine tonsillar crypts (3,4). Additionally, some pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), *Salmonella* spp., and classical swine fever virus can persistently colonize tonsil tissue, which may serve as a reservoir of infection (4). Certain foodborne pathogens, such as *Yersinia enterocolitica*, *Salmonella* spp., and *Listeria monocytogenes*, which are associated with swine production and pork consumption, can also be identified and isolated from swine tonsils (5,6).

While tonsils have provided a historical sampling site for detecting normal flora and aided in elucidating clinical disease (1), tonsil sampling should be considered as a monitoring tool for identifying emerging and re-emerging swine pathogens including those with foodborne and zoonotic implications. The emergence of PRRSV in the United States in the 1980s and in Europe and Asia in 1991 was documented when an unknown arterivirus was isolated from the tonsils of affected pigs (7). More recently, an outbreak of *S. suis* infection causing mortality in humans was caused by an emergent, highly virulent strain of *S. suis*, a pathogen that persists in the tonsils of clinically healthy pigs (8,9). Further documentation and surveillance of the normal flora of the swine tonsil is pertinent to the continued understanding of disease pathogenesis including zoonotic pathogens associated with swine and pork production. Due to the difficulty of sampling swine tonsils antemortem, collection at the time of slaughter is an obvious alternative for monitoring purposes (10). In addition to simplifying the process of collecting tonsils at slaughter, the congregation of large numbers of animals from multiple sources at slaughter provides an opportunity to sample pigs representing a large and geographically varied population in a simple and practical manner.

The objectives of this study were to gather data to determine the prevalence of microbiological populations of the tonsil of the soft palate of swine at slaughter, to determine if sampling the carcasses of normal or abnormal hogs provides different microbiological profiles, and to determine if the slaughter plant provides a feasible sampling frame and environment for collecting tonsils to detect and monitor important pathogens that have health implications for both swine and humans.

Materials and methods

Sample collection

Tonsils of the soft palate were collected from swine carcasses at a federally inspected abattoir in southern Ontario, Canada, from June to December 2008. The slaughter plant processed approximately 6000 hogs per day (30 000 to 32 000 per week). Tonsils were sampled as different producer batches (farms) were processed throughout an entire operating day once a week on Wednesdays. The carcasses and tonsils were identified and recorded by their unique, producer-specific slap tattoo. Sampling was done by experienced abattoir staff during normal slaughter operations. Samples were obtained before the carcasses were chilled, but after they had already passed through the scald tank, evisceration stage, and splitting stage. For the first 9 wk of the study, tonsils were collected from normal carcasses that passed veterinarian inspection. For the subsequent 11 wk, tonsils were sampled from carcasses sent to the hold rail, an area of the slaughter plant where carcasses with identified concerns or visible defects are sent for closer inspection by veterinary officials.

Tonsils were collected with a postmortem knife that was cleaned in boiling water between samples. The same abattoir staff member collected all the samples by following routine plant hygiene procedures. The proximal attachments of the tonsil connecting it to the soft palate were dissected. The dimpled appearance of the tonsil was used to distinguish it from the salivary glands. Each tonsil was placed in a sterile plastic bag, sealed, and labelled with the slap tattoo identification of the carcass. The samples were stored at 4°C post-collection and submitted to the Animal Health Laboratory (AHL) at the University of Guelph, Guelph, Ontario for processing within 24 h.

Microbiological methods

Microbiological analysis of the tonsils was conducted by the AHL at the University of Guelph. Upon receipt of the samples, the AHL divided the samples into sections for separate submission and processing to the bacteriology and virology laboratories. Bacteriology was quantified by culture and all bacteriological work, including setup, culture, identification, and typing, was done following standard operating procedures outlined in the Manual of Clinical Microbiology (11). The surface of each individual tonsil was seared and the tonsil tissue was then cut into with a sterile blade and sampled and plated for bacteriology. A different blade was used for each tonsil after being sterilized by dipping in 90% ethanol and flaming to prevent potential cross-contamination of the tonsils. All *Salmonella* spp. isolates were further forwarded for Salmonella serotyping to the Laboratory for Foodborne Zoonosis, Public Health Agency of Canada, Guelph, Ontario for final identification. Testing for PRRSV was done using the Tetracore PRRSV NA/EU RT-PCR kit (Tetracore, Rockville, Maryland, USA) (12), with modifications to adapt to the ABI-7500 thermocycler specific to the AHL. The test, which is a real time-polymerase chain reaction (PCR) using primer sets directed to the 3′ untranslated region of the PRRSV genome, identifies both North American and European strains of PRRSV. Immunohistochemistry (IHC) for porcine circovirus-2 (PCV-2) antigen was performed on histological sections of tissue from cryostat cut sections of frozen tissue and then subsequently formalin-fixed. The method is fully described by Carman et al (13).

Statistical methods

Quantitative bacterial culture data were converted to dichotomous outcomes of positive or negative with ≥ 1+ being classified as a positive culture. Univariable analyses were conducted using multilevel logistic regression models with farm as a random intercept to determine if there were statistically different associations between

pathogens being identified from normal carcasses versus carcasses sampled from the hold rail. Statistical significance was set at $P \leq 0.05$. For variables when the prevalence was low (< 1.0%), exact logistic regression was used. Subsequently, a multivariable, multi-level logistic regression model using farm as a random intercept was built using the variables that had a significance of $P \leq 0.2$ on univariable analyses. Statistical analyses were performed using a statistical software program (Stata 10; StataCorp; College Station, Texas, USA).

Results

A total of 395 tonsil specimens were collected. Over 99.8% (395/396) of the samples collected were tonsil tissue, with the incorrect sample being a salivary gland. Twenty tonsils were collected every week for 20 consecutive weeks with the exception of week 16 when only 15 samples were collected. A total of 180 tonsils were collected from normal carcasses and 215 tonsils were collected from carcasses sent to the hold rail. A total of 264 unique slap tattoo identification numbers (farms) were represented. The identities and frequency of each pathogen isolated are shown in Table I. The bacterium that was most commonly isolated was *Streptococcus suis*, with 53.7% of the samples being positive. Ninety tonsils (22.9%) were positive for PRRSV, while 47 tonsils (11.9%) were positive for PCV-2.

Tonsils were approximately 2, 10, and 8 times more likely to be from the hold rail if they were positive for *S. suis*, *S. porcinus*, or *S. hyicus*, respectively ($P < 0.001$) (Table I). Tonsils positive for *Staphylococcus aureus*, however, were less likely to be from the hold rail (OR = 0.05, CI = 0.005 to 0.482, $P = 0.010$). Tonsils that were positive for PRRSV or PCV-2 were no more likely to be from the hold rail than from the normal carcasses. The odds for being positive for *S. equisimilis* were 2 times greater for tonsils that were PRRSV-positive (OR = 2.08, CI = 1.22 to 3.56, $P = 0.007$). Being positive for PRRSV, however, did not increase the odds of the sample being positive for any other bacteria or for PCV-2. Tonsils that were positive for PVC-2 had approximately 2 times higher odds of being positive for *S. porcinus* (OR = 2.40, CI = 1.04 to 5.55, $P = 0.041$). Being positive for PCV-2, however, did not increase the odds of a sample being positive for any other bacteria or for PRRSV. In the multivariable, multi-level logistic regression model, the odds ratio (OR) for *S. suis*, *S. porcinus*, *S. aureus*, and *S. hyicus* were 2.5 (CI = 1.357 to 4.681, $P = 0.03$), 14.5 (CI = 4.860 to 44.205, $P < 0.001$), 0.06 (CI = .005 to 0.827), and 11.9 (CI = 2.719 to 51.690), respectively and the overall intra-class correlation coefficient (ICC) for farm was 0.38.

Two samples (0.5%) were positive for *Salmonella typhimurium* and then further typed. One bacterium was Phage Type 104a and the other was Copenhagen Phage Type UT1. Bio- and serotyping were undertaken for all the samples that were positive for *Y. enterocolitica* (1.8%). Bio-serotype 4/0:3 was the most common type found in 5 out of 7 of the positive samples (71.4%). The remaining 2 positive samples were bio-serotype 2/0:5,27.

Discussion

The sampling frame and sampling method proved to be an efficacious way of collecting swine tonsil tissue. Accurate tissue recovery occurred (99.7%), the sampling protocol was not technically challenging, and the personnel at the plant were willing to collect the tonsils during normal plant operations. Tissue collection during the slaughter process was a superior method of collecting tonsil tissue compared to reports of antemortem techniques where only 48.9% of samples were correctly obtained by tonsil biopsy methods (10). In live pigs, it has been shown that swabs may be more sensitive than tonsil biopsy for detecting *S. suis* (14). The surface of the tonsil was not swabbed before its removal from the carcass in the present study, but this is worth examining in future work and comparing to the direct collection of tonsil tissue.

Many commensal organisms and opportunistic bacterial pathogens found in the tonsils of swine, such as *S. porcinus*, *S. dysgalactiae*, *S. aureus*, *Pasteurella multocida*, and *Staphylococcus hyicus*, were identified in this study. Other studies show comparable results in the frequency of the commensal organisms identified (3,6). Interestingly, *S. porcinus* was found to be more prevalent in tonsils from pigs from the hold rail than from normal carcasses. The emergence of this bacterium as well as novel strains of *Streptococcus pseudoporcinus*, a potential human pathogen, has been attributed to advancements in diagnostic capabilities and not to the emergence of a new pathogenic strain (15–17).

Streptococcus suis, the most frequently identified bacteria (53.7%) in this study, has been reported as an important swine pathogen and as a pathogen with zoonotic potential (18,19). A previous Canadian study using PCR testing methods reported that 98% of swine farms were positive for *S. suis* (3). The clinical and potential zoonotic significance of the findings in this study cannot be commented on further as typing was not performed. It should be kept in mind, however, that even though only a few human cases have been reported in Canada and the United States (20,21), *S. suis* type 2 has recently been reported as an emerging zoonotic disease in other parts of the world (8,9,19). It has been proposed that the emergence of serious human illness caused by *S. suis* is the result of the emergence of a new highly virulent strain (9). The high recovery rate of *S. suis* in this and previous studies coupled with the reported changes in virulence patterns seen in other parts of the world (9) warrants the continued surveillance of this pathogen with appropriate subtyping.

The prevalence of *Salmonella* spp. in this study was lower than that of other reports. Carlson and Blaha (22) reported an in-herd prevalence of 64% from ileocecal lymph node culture (collected at slaughter) and a pig-level prevalence of 3.69%. Another study found an intestinal carriage prevalence of 23% at slaughter (23). Different microbiological methods, different sample types, and different study designs play a role in the variations between this study and other reports. For this study, cross-contamination among the tonsils was unlikely as bacterial cultures were taken from a freshly prepared surface of the tonsil and IHC samples were taken from freshly prepared cyrostat cut sections of frozen tissue at the laboratory.

Little information is available in the literature about the usefulness of collecting tissue samples from hold rail carcasses versus normal carcasses for surveillance or research purposes. Most studies have focused on the risks of microbiological contamination of the carcass during the slaughter process (5,6). In this study, tonsils collected from carcasses at the hold rail did yield some discordant information about certain pathogens when compared to tonsils from normal carcasses. This study did not target those carcasses on the

Table I. The frequency of individual pathogens isolated from 395 swine tonsils and the univariable[a] associations between tonsil positivity for porcine circovirus type-2, porcine reproductive and respiratory syndrome virus, and bacterial pathogens isolated from swine tonsils collected from abnormal carcasses versus normal carcasses at slaughter

Pathogen isolated	Total number of positive tonsils	Freq (%)	Number of tonsils positive		Odds ratio[a]	*P*-value	Confidence interval (CI)	ICC[a,b]
			Abnormal carcass	Normal carcass				
Porcine circovirus type-2	47	11.9	29/47 (61.7%)	18/47 (38.3%)	1.39	0.297	0.747 to 2.605	NS
Porcine reproductive and respiratory syndrome virus	90	22.9	41/90 (45.6%)	49/90 (54.4%)	0.63	0.055	0.393 to 1.011	NS
Actinobacillus pleuropneumoniae	14	3.5	7/14 (50.0%)	7/14 (50.0%)	0.83	0.735	0.286 to 2.417	NS
Actinomyces sp.	2	0.5	0/2 (0%)	2/2 (100%)	0.35	0.414	0.000 to 4.452	NS
Arcanobacterium pyogenes	118	29.9	65/118 (55.1%)	53/118 (44.9%)	1.04	0.866	0.672 to 1.604	NS
Erysipelothris spp.	3	0.8	1/3 (33.3%)	2/3 (66.6%)	0.42	0.475	0.037 to 4.624	NS
Haemophilus parasuis	4	1.0	0/4 (0%)	4/4 (100%)	0.16	0.085	0.000 to 1.259	NS
Listeria monocytogenes	2	0.5	2/2 (100%)	0/2 (0%)	2.03	0.591	0.157 to Inf	NS
Pasteurella multocida	108	27.3	59/108 (54.6%)	49/108 (45.4%)	1.01	0.961	0.648 to 1.577	NS
Salmonella spp.	2	0.5	1/2 (50.0%)	1/2 (50.0%)	0.836	0.900	0.052 to 13.466	NS
Staphylococcus aureus	**14**	**3.5**	**29/47 (61.7%)**	**18/47 (38.3%)**	**0.05**	**0.010**	**0.005 to 0.482**	**0.3678**
Staphylococcus hyicus	**43**	**10.9**	**41/90 (45.6%)**	**49/90 (54.4%)**	**7.51**	**< 0.001**	**2.890 to 19.537**	**< 0.0001**
Staphylococcus pseudintermedius	1	0.3	7/14 (50.0%)	7/14 (50.0%)	0.84	0.911	0.000 to 32.651	NS
Streptococcus agalactiae	5	1.3	0/2 (0%)	2/2 (100%)	0.55	0.520	0.093 to 3.351	NS
Streptococcus equisimilis	116	29.4	65/118 (55.1%)	53/118 (44.9%)	1.41	0.163	0.869 to 2.301	NS
Streptococcus porcinus	**77**	**19.5**	**1/3 (33.3%)**	**2/3 (66.6%)**	**9.93**	**< 0.001**	**4.272 to 23.096**	**0.1389**
Streptococcus suis	**212**	**53.7**	**0/4 (0%)**	**4/4 (100%)**	**2.16**	**< 0.001**	**1.445 to 3.239**	**< 0.0001**
Streptococcus zooepidemicus	5	1.3	2/2 (100%)	0/2 (0%)	0.16	0.286	.006 to 4.568	NS
Yersinia enterocolitica	7	1.8	71.4% (5/7)	28.6% (2/7)	2.12	0.373	0.406 to 11.055	NS

[a] Univariable logistic regression using farm as a random intercept.

[b] Intra-class correlation coefficient.

NS — not significant.

Bold text — significant.

hold rail with evidence of specific disease processes. For example, carcasses sampled at the hold rail may have had a retained testicle or a non-disease related defect. With the exception of *S. porcinus* and *S. suis*, carcasses diverted to the hold rail did not have an increased risk of harboring zoonotic pathogens. Interestingly, tonsils that were positive for PRRSV and/or PCV-2 also did not have increased odds of being positive for any of the zoonotic pathogens. It has been proposed that these 2 viruses may cause a reduction in the immune function of swine, making them more susceptible to other pathogens (24). In the multivariable, multi-level logistic regression model, the data clustered moderately by farm as indicated by the overall ICC of 0.38. The nature of the processing of the tonsils precludes cross-contamination as an alternative hypothesis for this clustering. The isolation of *Yersinia enterocolitica bioserotype* 4/0:3 as the most common bioserotype is comparable to other studies, although it was found that the overall prevalence of *Y. enterocolitica* was considerably lower than other studies examining live pigs and using fecal samples to assess the presence of this pathogen (25,26,27).

This study provides information on the prevalence of microbiological populations of swine tonsils collected at slaughter. The collection technique used in this study illustrates an effective method for tonsil sampling that could be useful for monitoring swine pathogens. Tonsil collection at slaughter for microbiological examination may be of value in certain disease surveillance strategies, particularly if the sampling process targets disease processes of interest on the hold rail.

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