ORIGINAL RESEARCH PEER REVIEWED

Comparison of postmortem airway swabs and lung tissue for detection of common porcine respiratory pathogens by bacterial culture and polymerase chain reaction assays

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Summary

Objective: To compare pathogen detection from tracheobronchial swabs with lung tissue in diagnostic submissions from pigs with reported respiratory disease.

Materials and methods: Individual lung samples (n = 153) from 133 laboratory submissions were included in this study. Inclusion criteria were a lung sample where the tracheal bifurcation or major bronchus was readily identifiable and a clinical report of respiratory disease symptoms. Sterile, nylon-flocked swabs were used to sample the largest available airway before the lung tissue was routinely processed for diagnostic testing. Swabs were placed in Amies transport medium and tested in blinded parallel with the lung tissue by bacterial culture and polymerase chain reaction (PCR) for common swine respiratory pathogens.

Results: There was excellent agreement between PCR detection from lung and bronchial swab samples for porcine reproductive and respiratory syndrome virus, influenza A virus, *Mycoplasma hyopneumoniae*, and porcine circovirus 2 (kappa > 0.8, all assays). Agreement between bacterial culture from lung and swabs was substantial for *Pasteurella multocida* and *Salmonella* spp. and fair for *Streptococcus suis*. Lung tissue was culture positive more often than swabs for *Haemophilus parasuis* and *Actinobacillus* spp.; however, in these cases, PCR for the respective pathogen was 100% positive on swab samples regardless of culture status of the swab.

Implications: Tracheobronchial swabs are a single, uniform sample that can be easily collected at postmortem and transported to the laboratory for detection of swine respiratory pathogens by culture and PCR. Such swabs may serve as a rapid screening tool for unexpected mortalities in a population.

Keywords: swine, respiratory disease, diagnostic sensitivity, airway swabs

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Resumen – Comparación de tejido pulmonar y muestras de vía respiratoria post mórtem para la detección de patógenos respiratorios porcinos comunes mediante cultivo bacteriano y pruebas de reacción en cadena de polimerasa

Objetivo: Comparar la detección de patógenos de muestras traqueobronquiales con tejido pulmonar en muestras diagnósticas de cerdos con reporte de enfermedad respiratoria.

Materiales y métodos: En este estudio se incluyeron (n = 153) muestras de pulmón individuales de 133 entregas de laboratorio. El criterio de inclusión fue una muestra de pulmón en la que la bifurcación traqueal o bronquio principal fuera fácilmente identificable y con reporte clínico de síntomas de enfermedad respiratoria. Se utilizaron hisopos estériles de nylon agrupado para tomar muestras de las vías respiratorias más grandes disponibles antes de que el tejido pulmonar fuera procesado de forma rutinaria para pruebas de diagnóstico. Los hisopos se colocaron en un medio de transporte Amies y se probaron a ciegas y en paralelo con el tejido pulmonar mediante cultivo bacteriano y reacción en cadena de la polimerasa (PCR por sus siglas en inglés) para detectar patógenos respiratorios porcinos comunes.

Resultados: Hubo una concordancia excelente entre la detección del PCR del pulmón y los hisopos de muestra bronquial para el virus del síndrome reproductivo y respiratorio porcino, virus de la influenza A, *Mycoplasma hyopneumoniae*, y circovirus porcino 2 (kappa > 0.8, todas las pruebas). La concordancia entre el cultivo bacteriano del pulmón y los hisopos fue sustancial en la detección de *Pasteurella multocida* y *Salmonella* spp. y media para *Streptococcus suis*. El tejido de pulmón resultó positivo en cultivo más frecuentemente que en hisopos en la detección de *Haemophilus parasuis* y *Actinobacillus* spp.; sin embargo, en estos casos, el PCR para los patógenos respectivos fue 100% positivo en muestras de hisopos independientemente del estado del cultivo o del hisopos.

Implicaciones: Los hisopos traquobronquiales son una muestra única y uniforme que puede recolectarse fácilmente post mórtem y transportarse al laboratorio para detección de patógenos respiratorios porcinos mediante

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cultivo y PCR. Esos cotonetes pueden servir como una herramienta rápida de revisión para mortalidades inesperadas en la población.

Résumé – Comparaison d'un écouvillonnage post-mortem des voies respiratoires et de tissu pulmonaire pour la détection d'agents pathogènes communs du système respiratoire porcin par culture bactérienne et réaction d'amplification en chaîne par la polymérase

Objectif: Comparer la détection d'agents pathogènes à partir d'écouvillons trachéo-bronchiaux à du tissu pulmonaire provenant de porcs avec des problèmes respiratoires et soumis pour diagnostic.

Matériels et méthodes: Des échantillons individuels de poumon (n = 153) provenant de 133 soumissions au laboratoire étaient inclus dans l'étude. Les critères d'inclusion étaient un échantillon de poumon où la bifurcation avec la trachée ou une bronche majeure était facilement identifiable et un rapport clinique de symptômes de maladie respiratoire. Des écouvillons stériles en nylon étaient utilisés pour échantillonner la voie respiratoire la plus grosse qui était disponible avant que le tissu pulmonaire ne soit utilisé pour les tests diagnostiques de routine. Les écouvillons étaient placés dans le milieu de transport Amies et testés à l'aveugle en parallèle avec le tissu pulmonaire par culture bactérienne et réaction d'amplification en chaîne par la polymérase (PCR) pour les agents pathogènes respiratoires fréquents chez le porc.

Résultats: Il y avait un excellent accord entre la détection par PCR à partir du tissu pulmonaire et les écouvillons bronchiaux pour le virus du syndrome reproducteur et respiratoire porcins, le virus de l'influenza A, *Mycoplasma hyopneumoniae* et le circovirus porcin de type 2 (kappa > 0.8 pour tous les tests). L'accord entre la culture bactérienne du tissu pulmonaire et les écouvillons était substantiel pour *Pasteurella multocida* et *Salmonella* spp. et raisonnable pour *Streptococcus suis*. Le tissu pulmonaire était positif pour la culture plus souvent que les écouvillons pour *Haemophilus parasuis* et *Actinobacillus* spp.; toutefois, pour ces cas, l'analyse par PCR pour les agents respectifs était 100% positive sur les écouvillons indépendamment du résultat de la culture à partir de l'écouvillon.

Implications: Les écouvillons trachéo-bronchiaux sont un échantillon unique uniforme qui peut être facilement prélevé en post-mortem et transporté au laboratoire pour la détection d'agents pathogènes du système respiratoire porcin par culture et PCR. De tels écouvillons peuvent servir d'outils rapides de tamisage lors de mortalités inattendues dans une population.

There is considerable variation in sample collection, handling, preservation, and shipping of specimens from swine populations to diagnostic laboratories. Each of these factors can affect the results of diagnostic tests for the multitude of infectious agents of disease. For detection of swine respiratory pathogens, lung tissue has been the sample type of choice for bacterial culture and molecular testing by polymerase chain reaction (PCR) assays. However, lung tissue has potential disadvantages for detection of pathogens because of variation in sample size, variation in location from which the sample is selected within the organ (sampling bias), uneven pathogen distribution within the lung, packaging selected to contain the sample, and speed of tissue cooling post-collection based on size and packaging. Moreover, swine diagnostic samples are commonly collected in the field by lay personnel (animal owners, farm operators, and farm managers) not formally trained in nuances of agent pathogenesis, pathology, and intricacies of diagnostic testing.

When respiratory disease is present, offending pathogens are usually in high concentrations but may not be uniformly distributed throughout the lung, nor are all contributors to the porcine respiratory disease complex all found at a single location or in a single sample of lung. Detection of offending pathogens in high numbers, along with compatible gross and microscopic lesions, is a core concept for disease diagnosis. Since the mucociliary system continuously moves material up from the deeper lung, airways theoretically contain any pathogens that may be contributing to respiratory disease distal to where collection occurs. Collection of conducting-airway exudates from primary bronchi at the tracheal bifurcation should thereby reflect the entire associated lung lobe and may serve as an alternative to individual lung lobe samples for diagnostic testing. Such sampling could serve to better standardize the collection process and reduce sampling bias inherent in individual lung lobe samples without substantially influencing diagnostic sensitivity.

The objective of this study was to determine the correlation between results of parallel testing of lung tissue and bronchial swabs for the detection of common porcine respiratory pathogens by bacterial culture and PCR. Pathogens included in this analysis were porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), porcine circovirus 2 (PCV2), *Actinobacillus* spp. (ACT), *Bordetella bronchiseptica* (BB), *Haemophilus parasuis* (HPS), *Mycoplasma hyopneumoniae* (MHP), *Pasteurella multocida* (PM), *Salmonella* spp. (SAL), and *Streptococcus suis* (SS).

Materials and methods

No animal use approvals were required for this study as all samples used in this investigation were derived from routine diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) between May 10, 2017 and June 30, 2017. The samples used were limited to those cases in which respiratory disease was reported and at least a majority of one lung lobe was submitted such that the main conducting airway could be easily visualized. One hundred fifty-three individual lung samples from 133 unique diagnostic submissions were utilized for this investigation. At the time of initial case processing, a sterile nylon-flocked swab (ESwab, Copan Diagnostics, Inc, Murrieta, California) was introduced into the largest available conducting airway (tracheal bifurcation, primary bronchus, or secondary bronchus) and swabbed 3 to 5 times before removal and placement into the polypropylene screw-cap tube containing 1 mL of Amies transport medium that is provided with each swab. The swab samples were individually labeled, processed, and tested in parallel, yet independently, thus blinded from the corresponding lung tissue from which they were obtained. The lung tissue was then processed routinely by ISU VDL staff for bacterial culture and PCR. Each swab sample was subjected to the same PCR testing and bacterial culture as was requested on the corresponding lung tissue and this testing varied in the context of the specific diagnostic question for each submission. At the completion of the study, PCR for ACT, HPS, or both was performed on the swab samples from all cases where ACT or HPS was recovered by culture of either lung or swab samples.

Lung and swab samples were processed routinely for the detection of PRRSV, IAV, PCV2, MHP, ACT, and HPS nucleic acid

Table 1: Primers and probes used for rPCR reactions for *Mycoplasma hyopneumoniae*, porcine circovirus type 2, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, and *Actinobacillus suis* at the ISU VDL

	Primer, nM	Probe, nM	Internal control	Forward primer	Reverse primer	Probe
MHP	*	*	Xeno	†	†	†
PCV2	400	200	XIPC	TGGCCCGCAGTATTCTGATT	CAGCTGGGACAG-CAGTTGAG	CCAGCAAT-CAGACCCCGTTG-GAATG
HPS	400	200	XIPC	TTACGAGTAGGGCTACAC	CTTCATGGAGTC-GAGTTG	CGCGATTGCATA-CAGAGGGYGAC-GAAGCATCGCG
APP	200	200	Xeno	GGGGACGTAACTCGGTGATT	GCTCACCAACGTTT-GCTCAT	CGGTGCGGA-CACCTATATCT
A suis	400	350	none	GAGCTGGGAAGCTCGACTAT	CCCCCATCTTCAAA-CAGGAT	AGCTAACGACAAG-TAGGGCG

* For each reaction, 0.08 mL VetMAX Primer-probe mix was added.
† Sequence data not supplied.
rPCR = real-time polymerase chain reaction; ISU VDL = Iowa State University Veterinary Diagnostic Laboratory; MHP = *Mycoplasma hyopneumoniae*; PCV2 = porcine circovirus type 2; XIPC = exogenous internal positive control; HPS = *Haemophilus parasuis*; APP = *Actinobacillus pleuropneumoniae*; *A suis* = *Actinobacillus suis*.

by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for RNA viruses or real-time polymerase chain reaction (rPCR) for DNA virus and bacteria. To extract RNA, the MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific, Waltham, Massachusetts) and a Kingfisher 96 or Flex instrument (Thermo Fisher Scientific, Waltham, Massachusetts) were used according to manufacturer's instructions. Lung tissue homogenate and processed bronchial swabs were extracted using the standard lysis (SL) procedure with 50 µL of sample added to 130 µL of lysis-binding solution and carrier RNA mixture prepared according to the kit insert, 20 µL magnetic bead mix, and 90 µL of elution buffer. The SL protocol used 150 µL of wash solution I and II provided with the kit. The SL extractions were conducted using the Kingfisher program *AM1836 DW 50 v3* (supplied by Thermo Fisher) with a 5 min pause added at the end of the program to allow the eluate to reach room temperature.

Real-time reverse transcriptase PCR or rPCR was performed on nucleic acid extracts using commercially available reagents for PRRSV (Applied Biosystems TaqMan NA and EU PRRSV Real-Time PCR assay, Thermo Fisher Scientific, Waltham, Massachusetts), IAV (VetMAX-Gold SIV Detection Kit, Thermo Fisher Scientific, Waltham, Massachusetts), and MHP (VetMAX *M hyopneumoniae*, Thermo Fisher Scientific, Waltham, Massachusetts) with proprietary primer and probe information. For both PRRSV and IAV, rRT-PCR setup and thermal cycling conditions were performed according to manufacturer's recommendations. For MHP rPCR, the primer-probe mix was used with the TaqMan Fast 1-Step Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts). Real-time PCR was performed using previously published primers and probes for PCV2[1], *Actinobacillus suis*[2], and *Actinobacillus pleuropneumoniae* (APP).[3] For HPS, an in-house rPCR assay was utilized. See Table 1 for primer and probe sequences.

An exogenous internal positive control (XIPC, based on Schroeder et al[4]) or Xeno RNA (Thermo Fisher Scientific, Waltham, Massachusetts) was included in the extraction (50,000 or 20,000 copies per sample for XIPC or Xeno, respectively) and appropriate primers and probe included in each master mix to monitor PCR amplification and inhibition. Two positive extraction controls, one negative extraction control, and a negative amplification control are also included with each extraction and PCR run.

Each rRT-PCR or rPCR reaction for PCV2, MHP, and HPS was set up using TaqMan Fast 1-Step Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts). The APP PCR utilized the Quanta ToughMix and *A suis* PCR was set up with VetMAX-Plus qPCR Mix (Thermo Fisher Scientific, Waltham, Massachusetts). All these reactions were set up according to manufacturer's recommendations, using 5 µL extracted nucleic acid per reaction. See Table 1 for agent-specific details.

Real-time reverse transcriptase PCR or rPCR for PRRSV, PCV2, MHP, and HPS was performed using an AB 7500 fast thermocycler (Thermo Fisher Scientific, Waltham, Massachusetts) in fast mode with the following cycling conditions: 1 cycle of 50°C for 5 min, 1 cycle of 95°C for 20 sec, and 40 cycles of 95°C for 3 sec and 60°C for 30 sec. Amplification curves were analyzed with commercial thermal cycler system software. Cycling conditions for IAV include 1 cycle of 48°C for 10 min, 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 45 sec. The APP PCR was conducted on a Qiagen RGQ (Qiagen, Germantown, Maryland) with the following cycling conditions: 1 cycle of 95°C for 2 min followed by 40 cycles of 95°C for 10 sec and 56°C for 1 min. For *A suis*, the following thermal cycling profile was utilized: 1 cycle of 95°C for 15 min followed by 45 cycles of 94°C for 15 sec and 60°C for 1 min.

Assays conducted on the AB 7500 Fast instrument used the auto baseline to determine fluorescence baselines and cycle thresholds (Ct) set at 0.1 for all agents except type 1

PRRSV, which was set at 0.05, and IAV, which was set according to the manufacturer's kit insert. For APP samples run on the RGQ instrument, the threshold was set at 0.02. Internal control Xeno or XIPC RNA Ct values were set at 10% of maximum. Paired lung and swab samples were tested separately as previously described under different accession numbers to keep the molecular diagnostics staff blinded to the pairing and to prevent any potential reporting bias. The number of pairs tested for each assay was as follows: PRRSV (111 pairs), IAV (118 pairs), MHP (49 pairs), and PCV2 (24 pairs).

For bacterial culture, all lung and swab samples were plated onto 5 different agar plates and atmospheric conditions for isolation of pathogens associated with respiratory disease including the 6 bacteria of interest in the study (ACT, BB, HPS, PM, SAL, and SS). The 5 plates included (1) blood agar (2% agar) with a *Staphylococcus* nurse, incubated with 5% CO_2, (2) blood agar (4% agar) with a *Staphylococcus* nurse, incubated with 5% CO_2, (3) blood agar incubated anaerobically, (4) Hektoen enteric agar incubated at normal atmosphere, and (5) Tergitol-7 agar incubated at normal atmosphere (all media, Thermo Fisher Scientific, Lenexa, Kansas). All plates were incubated at 35°C for a minimum of 48 hours. Identification of pathogens was done via matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry following standard laboratory protocol. A minimum MALDI-TOF confidence score of 2.10 was required for a confirmatory identification. The 153 paired lung and swab samples were cultured separately under different accession numbers to keep the bacteriology staff blinded to the pairing and to prevent any potential interpretation bias.

Statistical analyses were performed using a commercial statistical software package (JMP Pro 11, SAS Institute, Cary, North Carolina). A kappa coefficient was calculated to determine the degree of agreement for detection of each pathogen between sample types and coefficients are reported and interpreted as follows: values > 0.8 indicate excellent agreement, values ≤ 0.8 and > 0.6 indicate substantial agreement, values ≤ 0.6 and > 0.4 indicate moderate agreement, and values ≤ 0.4 indicate no better than fair agreement.[5] A McNemar test was used to determine if sample type specifically contributed to disagreement. A Wilcoxon signed rank test was used to compare differences in PCR Ct values between sample types. For all tests, $P < .05$ was considered significant.

Results

Contingency tables summarizing the results of PCR testing for PRRSV, IAV, MHP, and PCV2 are presented in Table 2. For all four assays, there was excellent agreement between the results of detection from lung tissue and swab samples (kappa > 0.8, all assays). While neither sample type was statistically associated with any observed disagreement, the results of disagreement analysis approached significance ($P = .08$) for PRRSV and MHP with swab samples being occasionally positive when tissue samples were negative while the reverse did not occur.

Mean PCR Ct values for PRRSV, IAV, MHP, and PCV2 and associated differences between results from lung tissue and swab samples are summarized in Table 3. For PRRSV, Ct values were lower in lung samples compared to swabs ($P = .02$) while MHP Ct values were lower in swabs than in lung samples ($P = .002$). Differences in Ct values were not detected for IAV or PCV2 PCR ($P > .05$).

Contingency tables reflecting the results of bacterial culture are presented in Table 4. For the six species of bacteria included in this analysis, there was substantial agreement between detection from lung tissue and swab samples for three (ACT, PM, and SAL), fair agreement for one (BB), and poor

Table 2: Contingency tables for results of PCR assays applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Swab, positive	Swab, negative	Kappa statistic (SE; 95% CI)*	McNemar test†
PRRSV				
Lung, positive	40	0	0.94 (0.03; 0.87-1)	0.08
Lung, negative	3	68		
IAV				
Lung, positive	27	3	0.85 (0.06; 0.73-0.96)	0.71
Lung, negative	4	84		
PCV2				
Lung, positive	7	1	0.90 (0.09; 0.71; 0.71-1)	0.32
Lung, negative	0	16		
MHP				
Lung, positive	18	0	0.87 (0.07; 0.73-1)	0.08
Lung, negative	3	28		

* Values > .8 indicate excellent agreement.
† Values < .05 were considered significant.
PCR = polymerase chain reaction; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; MHP = *Mycoplasma hyopneumoniae*.

Table 3: Comparison of differences in PCR Ct values for assays applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Mean Ct from lung	Mean Ct from swab	Mean difference (SE)	Correlation	*P* value*
PRRSV	32.11	32.64	-0.53 (0.22)	0.95	.02
IAV	34.8	34.56	0.24 (0.26)	0.90	.36
PCV2	32.54	33.66	-1.12 (0.52)	0.95	.11
MHP	33.78	32.59	1.19 (0.39)	0.88	.002

* Wilcoxon signed rank test for matched pairs; $P < .05$ are considered significant.
PCR = polymerase chain reaction; Ct = cycle threshold; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; MHP = *Mycoplasma hyopneumoniae*.

agreement for the remaining two (HPS and SS). Additionally, only fair agreement was observed between sample types for the reporting of 'no significant growth' by the laboratory. Disagreement analysis revealed a statistically significant association between sample type and detection for ACT and HPS ($P = .046$ and .03, respectively) with lung tissue being culture positive while swabs were culture negative more often than the reverse. A similar trend was observed for PM with results approaching significance ($P = .08$). For BB, there was also an association between sample type and detection ($P = .046$) but with swabs being culture positive while lung tissue was negative more often than the reverse.

For all cases where ACT or HPS was recovered by culture of either lung or swab samples, PCR was positive for the cultured organism (10 ACT and 17 HPS) even when the swab had been culture negative. For HPS there was no difference in mean PCR Ct by culture status with culture positive swabs having a mean Ct of 16.88 ± 3.99 and culture negative swabs a mean Ct of 16.58 ± 2.29. For ACT, mean PCR Ct values also did not differ ($P > .05$) with culture positive swabs having a mean Ct of 21.70 ± 6.42 and negative swabs a mean Ct of 23.88 ± 7.25.

Discussion

Overall there was excellent agreement between lung tissue and swab samples for the PCR assays tested indicating that swabs can be a reliable alternative sample for routine PCR detection of these agents in swine. This sample type should also be effective for use in multiplex PCR assays targeting PRRSV, IAV, MHP, and PCV2 and there is a critical need for the development and implementation of such assays in routine veterinary diagnostics to reduce turnaround time and costs. In sufficiently large-sized animals, collection of postmortem swab samples from the tracheal bifurcation or large conducting airways from affected lobes can easily be standardized and incorporated into field personnel training such that a consistent sample is provided to the laboratory and can reduce shipping costs associated with large volumes of tissue. Submission of formalin-fixed lung sections from any abnormal lung tissue in tandem with bronchial swabs would differentiate mere agent detection from agent-associated disease, that is, associate what may otherwise be an endemic agent with a lesion to have greater confidence that the agents detected are truly causing disease.

Interestingly, for both PRRSV and MHP PCR, there were a few cases where swab samples were positive while tissue homogenates were negative. Moreover, there were no instances where swabs were negative while tissues were positive suggesting swabs from conducting airways may be a more sensitive sample for detection of these pathogens. This is consistent with previous reports for MHP, where tracheobronchial samples were preferred for detection[6,7]; however, additional testing of a larger sample set is warranted to further explore this observation for PRRSV. It is worth emphasizing that the focus of this study was for pathogen detection in diseased tissue and not merely agent detection. The PCR Ct values were also significantly lower in swab samples for MHP which further supports that swabs collected from the primary bronchi are of higher diagnostic sensitivity than lung tissue samples for the diagnosis of enzootic pneumonia. This aligns with historic recommendations to include large conducting airways in fresh tissue sections submitted for MHP testing.[8]

For bacterial isolation, there was lower agreement between lung tissue culture and culture of swabs collected from primary bronchi. This is not entirely unexpected given that microbial culture requires organism viability and there are likely differences between maintenance in a transport medium such as Amies medium and lung parenchyma. This was particularly an issue for the more fastidious organisms ACT and HPS, which were recovered more frequently from lung tissue. This is consistent with a recent study where culture for HPS was a more sensitive assay for detection than direct PCR and lung was a preferred sample type.[9] In the present study, PCR of bronchial swabs for ACT and HPS was 100% sensitive for detecting cases where either organism was recovered by culture of lung tissue or swab samples suggesting PCR testing for these agents should be performed in parallel with culture when lesions suggest these agents are involved and only swab samples are tested. Common colonizing bacteria (BB and SS) were readily recovered from both sample types and with fair to poor agreement indicating that culture results from either sample type should be interpreted in the context of any observed gross and microscopic lesions. As with any endemic pathobiont, while the organism may not be active in the individual animal sampled, its presence remains a risk factor for the population and increases the likelihood that other animals may have clinical infections with these agents.

Limitations of this study include the use of routine diagnostic samples, which can vary in their preservation and handling prior to analysis and potential biases impacting the kappa statistic when determining agreement. The use of diagnostic samples replicates field conditions and thereby reflects the applicability of results to practitioners; however,

Table 4: Contingency tables for results of bacterial culture applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Swab, positive	Swab, negative	Kappa statistic (SE; 95% CI)*	McNemar test†
***Actinobacillus* spp.**				
Lung, positive	6	4	0.74 (0.13; 0.49 to 0.98)	0.046
Lung, negative	0	143		
Bordetella bronchiseptica				
Lung, positive	9	4	0.47 (0.11; 0.26 to 0.69)	0.046
Lung, negative	12	128		
Haemophilus parasuis				
Lung, positive	0	13	-0.04 (0.02; -0.07 to -0.01)	0.03
Lung, negative	4	137		
Pasteurella multocida				
Lung, positive	19	9	0.71 (0.08; 0.56 to 0.87)	0.08
Lung, negative	3	122		
***Salmonella* spp.**				
Lung, positive	5	1	0.76 (0.13; 0.5 to 1)	0.56
Lung, negative	2	145		
Streptococcus suis				
Lung, positive	26	19	0.33 (0.08; 0.17 to 0.49)	0.37
Lung, negative	25	83		
No significant growth				
Lung, positive	44	23	0.46 (0.07; 0.32 to 0.61)	0.34
Lung, negative	17	69		

* Kappa values > 0.8 indicate excellent agreement, values ≤ 0.8 and > 0.6 indicate substantial agreement, values ≤ 0.6 and > 0.4 indicate moderate agreement, and values ≤ 0.4 indicate no better than fair agreement.

† Values < .05 are considered significant.

this also increases potential for variation in PCR Ct values from testing on different PCR plates and on different days. Variation between plates is continuously monitored at the ISU VDL through quality management software and by maintaining statistical process control charts that require Ct values of positive controls to remain within specified limits. For the kappa statistic, results are not interpretable when there is significant disagreement detected by the McNemar test as with ACT, BB, and HPS in this report. Additionally, the kappa statistic becomes unstable when prevalence is below 20% or above 80%.[5] Low prevalence may have impacted the agreement analysis of several bacteria in this study (ACT, BB, HPS, and SAL).

Taken together the results of this study show that swabs with Amies transport medium provide a single uniform sample that can be easily collected at postmortem and transported to the laboratory for detection of common swine respiratory pathogens. This dual-use sample has enough fluid for multiple PCR assays and the swab itself is used for culture of bacterial agents. Swabs are also easily adapted to automated bacterial culturing systems.[10] For detection of fastidious bacteria such as HPS and ACT, either PCR from tracheobronchial swabs or culture from lung tissue are appropriate and similar in sensitivity; however, lung tissue remains a preferred sample for cultural confirmation of those agents. Submission of formalin-fixed tissue in parallel with tracheobronchial swabs remains an important practice to differentiate the presence of an agent from an actual causal role in disease.

Implications

- Tracheobronchial swabs are an effective sample for detecting PRRSV, IAV, PCV2, MHP, ACT and HPS by PCR.
- For MHP, tracheobronchial swab samples are more sensitive than lung tissue.
- For common colonizing bacteria such as BB and SS, tracheobronchial swabs are often positive when lung tissue is negative.
- Fastidious bacteria, such as ACT and HPS, are more reliably cultured from lung tissue than from tracheobronchial swabs, therefore parallel application of both culture and PCR is recommended to detect these pathogens from swab samples.

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Conflict of interest

None reported.

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