Relationship between Respiratory Health Status (ReHS) and PCR Ct values from testing oral fluids and air samples following multiple respiratory disease challenges

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Abstract

An experimental study was conducted to evaluate the relationship between detectable respiratory disease episodes using SoundTalks technology and multiple PCR testing of weekly oral fluid samples (every pen) as well as weekly air samples (every zone of 12 pens). Pigs used for this study were sourced from a PRRS virus negative and *Mycoplasma hyopneumoniae* negative breeding herd. All pigs were vaccinated for PCV2 and *Mycoplasma hyopneumoniae* at weaning. In every pen, three randomly selected seeder pigs were challenged seven days apart with *Mycoplasma hyopneumoniae* (MHP) and PRRS virus (PRRSv). Respiratory health scores were highly correlated with the air sample and oral fluid respiratory disease PCR test results. Using a stochastic model, air samples were shown to detect MHP at least one week earlier than oral fluids at all oral fluid sample sizes modeled.

Keywords: respiratory disease, cough, oral fluids, air sampling, stochastic modeling

Introduction

Respiratory disease outbreaks continue to be a major pig production problem, impacting antibiotic use, welfare, productivity and profitability (Lopes et al, 2019). SoundTalks is an audio-based technology that continuously identifies and quantifies respiratory problems in pigs as a Respiratory Health Score, or ReHS. The ReHS value is continuously derived from raw audio data by a proprietary algorithm developed by SoundTalks (Leuven, Belgium) that is based on more than two million labeled audio events across multiple years representing a wide range of pig and non-pig origin sounds from a wide range of pig production acoustic environments throughout the world.

Raw audio data originating within an approximately 10 meter radius zone is captured by six MEMS microphones built into each SoundTalks Monitor device. These data are continuously edge-processed by on-Monitor software into audio feature data. Using the Monitor devices as a WiFi mesh network, the audio feature data is then transmitted through the SoundTalks Gateway and pig site Modem-Router ISP to the SoundTalks Cloud, where the audio feature data is further processed into ReHS values.

Based on ReHS levels relative to alert trigger thresholds, SoundTalks generates alerts (yellow warnings, red alarms) when the algorithm determines that a respiratory outbreak onset is detected. These alerts have enabled triggering earlier caregiver awareness than caregiver observations alone (Polson et al., 2018). However, further research is needed to determine if and how disease agent levels correspond to objectively measured clinical indicators like the SoundTalks ReHS.

An experimental study was conducted to evaluate the relationship between detectable respiratory disease episodes using SoundTalks technology and multiple PCR testing of weekly oral fluid samples (every pen) as well as weekly air samples (every zone of 12 pens).

Materials and methods

Study animals and research barn

Pigs used for this study were sourced from a PRRS virus negative and *Mycoplasma hyopneumoniae* negative breeding herd. All study pigs were vaccinated with a porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* vaccine at weaning (i.e., three weeks of age). Eleven-week-old pigs (n=1655) were allocated to 72 pens targeting 23 pigs per pen across two rooms. There were three SoundTalks Monitor devices per room (airspace), with one Monitor device covering each of three zones per room. Each Monitor device was placed two meters above slat-level over the middle of the center alleyway in the center of each designated zone. Each designated zone included 12 pens (six pens on each side of the center alleyway). In between each SoundTalks Monitor zone there were two pens intentionally left empty on each side of the alleyway to create an audio buffer "dead-space" between zones to minimize the probability of coughs being detected by multiple SoundTalks monitors in adjacent zones.

Each of the 72 study pens containing 23 pigs. Of the 23 pigs per pen, three randomly selected pigs (13%, 3/23) were designated as seeder pigs and directly challenged at 12 weeks of age (9 weeks post-weaning) for two consecutive days with a virulent wild-type *Mycoplasma hyopneumoniae* (MHPwt) and then challenged seven days later with a virulent wild-type PRRS virus (PRRSwt, RFLP type 1-7-4). The remaining 20 non-directly-challenged pigs per pen were considered contact-exposed pigs over the course of the study period.

Sampling and testing

Beginning just prior to first challenge, both oral fluid samples (one per pen of pigs per week x 72 pens) and air samples (one per SoundTalks zone per week x six zones, each zone covering 12 pens of pigs) were collected weekly throughout the study period, resulting in 13 total samplings during the study. Weekly oral fluid samples (n=72 per week) were collected by attaching a non-bleached cotton rope to the alleyway gate in each pen for 30 minutes. After 30 minutes, liquid was squeezed from each rope by gloved hand (single-use) into a single-use Ziplock bag, then transferring the liquid sample into a plastic snap-cap falcon tube.

Weekly air samples (n=6 per week) were collected using a commercial air sampler and electrostatically charged filter plus elution kit (AirPrep Cub and Filter Elution Kit, Innovaprep, Drexel Missouri) from each SoundTalks zone. Air samplers were placed approximately 1.5 meters above the floor slats of an outside wall pen adjacent to each SoundTalks Monitor device. Each air sampler was set to collect continuously for 60 minutes at a flow rate of 200 liters per minute. After the 60-minute air sample collection period, each filter disk was removed from the air sampler and eluted into a collection cup in the reverse direction of the air collection flow using a wet foam method by attaching a pressurized canister (included with the filter plus elution kit) containing 0.15% Tween and PBS. The resulting liquid air-origin sample was then transferred into a plastic falcon tube.

Immediately after collection, all collected oral fluid and air-origin samples were then transported on ice in a clean insulated cooler to a nearby processing facility. At this processing facility each oral fluid sample and each air-origin sample was transferred into cryovials (three aliquots per sample) and stored in an Ultralow freezer at -70ºC until the study was completed. After study completion, all samples were transported in Styrofoam containers containing dry ice to the testing laboratory, where they were held in an Ultralow freezer at -70ºC until they were tested.

All oral fluid and air-origin sample testing was conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL, Ames, Iowa). For the purposes of this study, air-origin eluted liquid samples were considered to be comparable to "very clean oral fluid samples", thus both the oral fluid samples as well as the air samples were extracted using the same ISU-VDL method designed to be used with oral fluid samples (Rotolo et al, 2017). All extracted samples were then tested by rtPCR for *Mycoplasma hyopneumoniae*, PRRS

virus and swine influenza virus (IAV-S). To confirm challenge agents and document/characterize other agent exposure-infection-circulation, select oral fluid and air samples were sub-typed (IAV-S HN subtyping PCR) and genetically sequenced (MHP P-146, PRRS ORF-5, IAV-S HA).

Descriptive analysis

Following testing of all oral fluid and air-origin samples, Ct (cycle threshold) values were obtained for each of the three PCR tests run on each sample. Also, the Ct values representing the positive-negative (PN) cutoff were obtained for each of the three PCR tests run on samples collected during this study.

Using a commercial spreadsheet software (Excel, Microsoft 365, Microsoft Corporation), PCR Ct delta values (positive-negative cutoff Ct minus sample test result Ct) were calculated for each of the three PCR results for each sample. The resulting Ct delta values for each PCR assay were then used to calculate the mean Ct delta for each sampling date. In turn, the mean Ct delta values for all three PCR assays were summed for each sampling date. These PCR Ct delta data for both oral fluid and air samples were then compared to each other, as well as each to the SoundTalks ReHS data corresponding to the date of oral fluid and air sampling.

Stochastic model analysis

For this part of the analysis, a purpose-written stochastic model was built in a commercial spreadsheet software (Excel, Microsoft 365, Microsoft Corporation) to assess comparability of the two sample types (oral fluid vs air-origin) for tracking the onset, development and progression of infection levels for each of the three agents detected during the study.

The stochastic model was designed to randomly sample the selected number of pens from each zone (containing 12 pens per zone). The oral fluid PCR test results for the selected pen(s) were then obtained for all sampling dates, with each result for each PCR assay being assigned a value of zero (0=negative) or one (1=positive) based on the positive/negative cutoff Ct value for each assay. These steps were repeated for each model iteration for each comparison scenario until the total model iterations met the number of total iterations required for the analytic dataset (n=1,000). Six comparison scenarios were evaluated: 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1 oral fluid(s): air sample(s) per zone per week.

After running 1,000 model iterations for each of the oral fluid:air sample comparison scenarios, the PCR positive-negative results for each set of oral fluid model output data (ranging from 1-6 oral fluids per zone per week, i.e., 6-36 oral fluids per barn per week) were compared to the PCR positive-negative results for the one air-origin sample per zone per week (i.e., six air-origin samples per barn per week).

Results

Descriptive results

The sum of the oral fluid Ct delta values (n=72 per barn per week) for the three respiratory disease tests was highly correlated (r=-0.794, p=0.0012) with the corresponding air sample Ct delta sum values (n=6 per barn per week) (Figure 1).

ReHS was highly correlated with the sum of the air sample Ct delta values (n=6 per week) for the three respiratory disease PCR tests (r=-0.809, p=0.0008) (Figure 2). ReHS was also highly correlated with the sum of the oral fluid sample Ct delta values (n=72 per week) for the three respiratory disease PCR tests (r=-0.909, p<0.0001).
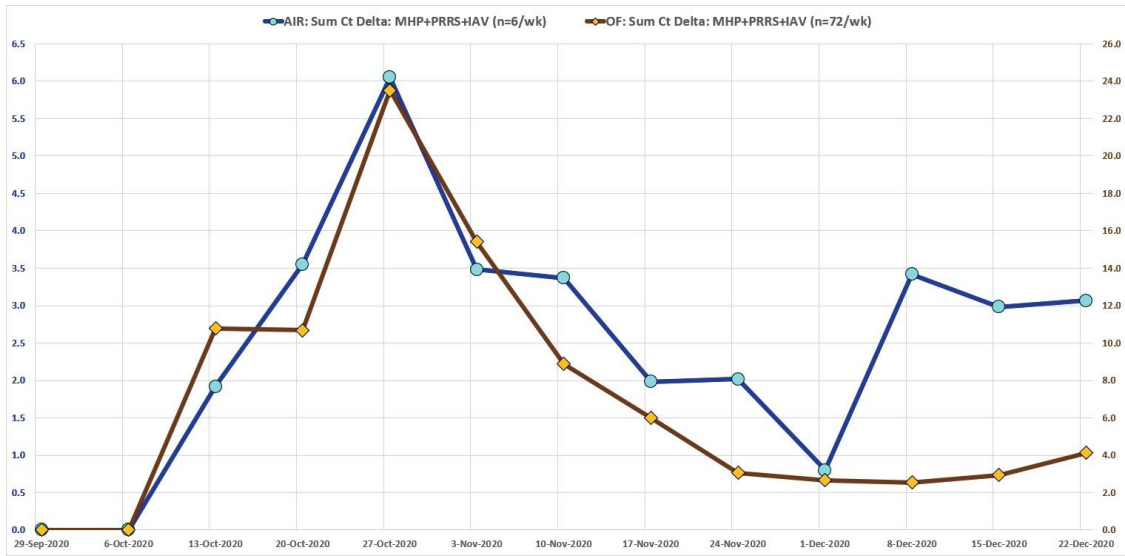
Figure 1: Comparison of summed PCR mean Ct delta values of three PCR tests (MHP, PRRS, IAV-S) for weekly oral fluid samples (n=72 per barn week, Y2 axis) and weekly air-origin samples (n=6 per barn week, Y1 axis).
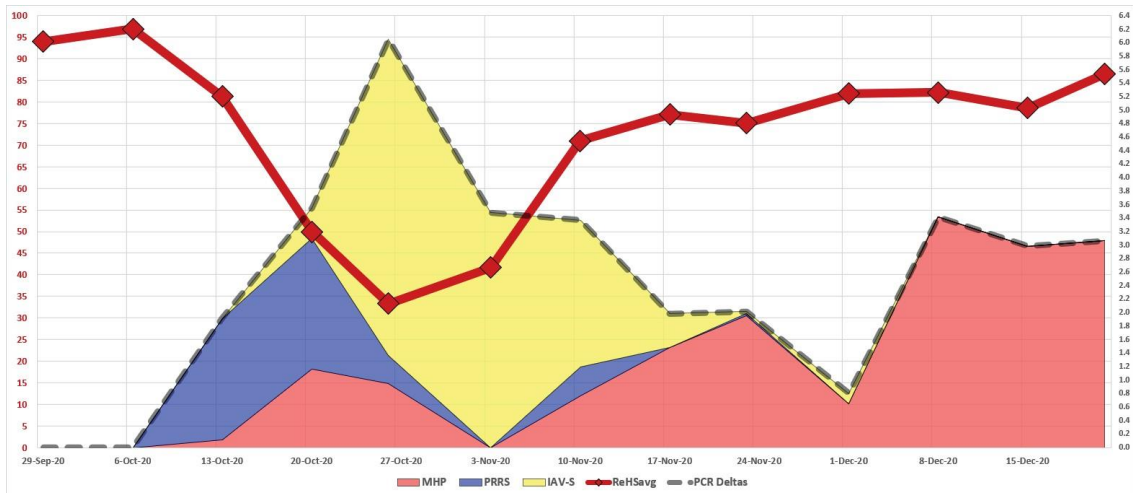

Figure 2: Comparison of mean ReHS (Y1 axis) from six SoundTalks monitors per barn by week and summed PCR mean Ct delta values (Y2 axis) for weekly air-origin samples (n=6 per barn per week).

Stochastic Model Results

The stochastic model showed no difference in early diagnostic detection by PCR between oral fluids and air-origin samples (1 per zone per week) for PRRS or IAV-S across the range of oral fluid samples per zone evaluated (1-6 oral fluids per zone per week). However, air samples were shown to detect MHP one week earlier than oral fluids at all oral fluid sample sizes modeled, with detection probabilities ranging from 21.2% to 87.3% for one (1) to six (6) oral fluids per zone as compared to one (1) air-origin sample per zone in the week that MHP was first detected in air samples, the week of 13October2020.

Figure 3 shows the week-over-week cumulative difference in MHP PCR detection probability between one oral fluid sample per zone per week (six per barn per week) and one air-origin sample per zone per week (six per barn per week). In the first week that MHP was first detected (13October2020) via air-origin samples, 78.8% of 1,000 stochastic model iterations did not detect MHP when sampling an equivalent number of oral fluids (1 per zone, 6 per barn). Across the study period, weekly air-origin samples (1 per zone, 6 per barn) detected MHP PCR positives a weighted average of 6.95 days sooner than oral fluid samples (1 per zone, 6 per barn).
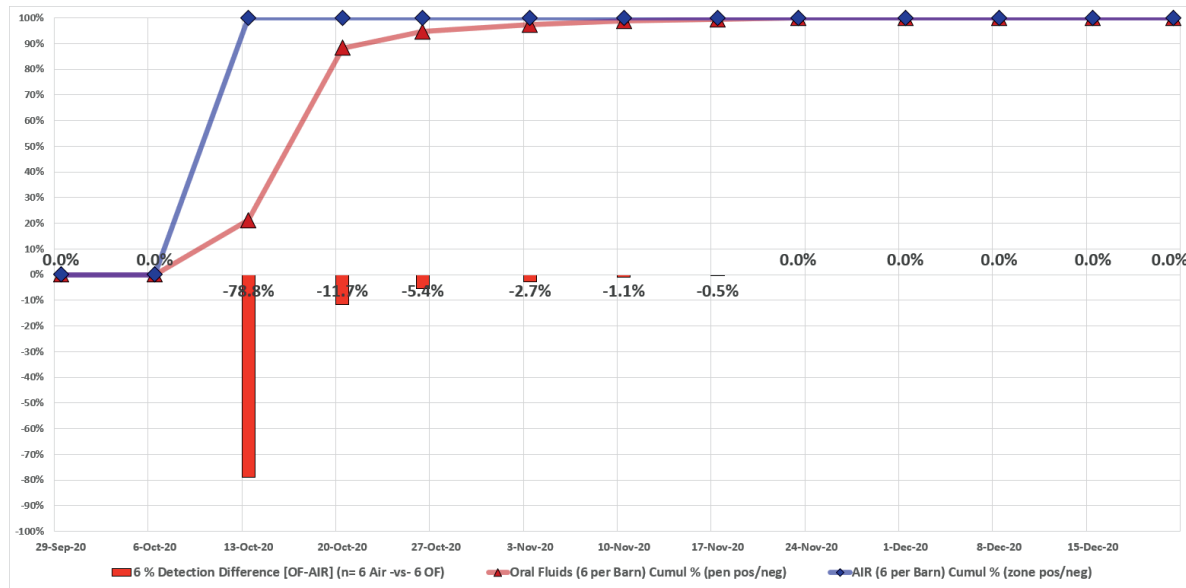

Figure 3: Stochastic model results of the week-over-week cumulative detection probability for oral fluid MHP PCR positive-negative results (1 per zone, 6 per barn per week) versus air-origin MHP PCR positive-negative results (1 per zone, 6 per barn per week).

Discussion

In this study PCR Ct delta values from both oral fluid and air-origin samples were useful to diagnostically characterize the combined clinical effect of multiple respiratory disease agents over time, as continuously measured and graphically represented as the SoundTalks ReHS. A high degree of correlation was observed between ReHS and summed PCR Ct deltas for three assays targeting detection of relatively common respiratory disease agents – *Mycoplasma hyopneumoniae*, PRRS virus and swine influenza virus (IAV-S).

This study was designed as a dual-seeder-staggered challenge study, intending to mimic a scenario of growing pigs being sourced from a PRRS-negative and MHP-positive but relatively low prevalence ("MHP stable") sow farm that subsequently become infected with PRRSwt from a lateral source and/or external virus carrying agent (e.g., people, supplies, feed). While an IAV-S challenge was not designed into the study, experiencing a natural IAV-S wild-type challenge was not unexpected, given the fact that the source sow farm was not a proven IAV-S negative source and also given the proximity of other commercial pig production sites to the research barn location.

These results suggest that both oral fluid and air-origin sample PCR testing are useful for the detection and characterization of specific respiratory disease agents at the onset of clinical disease episodes as quantified by the SoundTalks ReHS. In this study, first diagnostic detection of PRRS and IAV-S was the same. However, air-origin samples may be a marginally better sample type for earliest detection of MHP-related clinical

disease episodes detected by SoundTalks ReHS, particularly where the number of oral fluid samples was the same (1:1) or similar (2:1, 3:1) to the number of air-origin samples collected per barn.

This approach can be useful for characterizing clinical episode patterns driven by one or multiple swine respiratory disease agents.

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