Use of oral fluids to detect pathogen presence in swine

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Introduction

Blood, urine, fecal and tissue samples are often used for the detection of pathogen presence in an animal. However, these sample collection methods are often difficult to perform or even impossible to obtain on live animals. Easier sample collection methods may save producers and researchers time and money when trying to identify infections in their animals. Studies have shown that Porcine Circovirus (PCV), Porcine Reproductive and Respiratory Syndrome Virus (PRRS) and Swine Influenza Virus (SIV) can all be detected in oral fluids. The goal of this study is to determine whether other pathogens, such as *Mycoplasma hyopneumoniae* or Hepatitis E, can be detected in oral fluids if present systemically. A secondary objective of this study is to develop and optimize a real-time PCR assay for the detection of PCV2 using oral fluid samples.

Materials and Methods

Oral fluid sample collection

Two groups (Nursery, Finisher) of pigs from the University of Missouri Swine Teaching Farm were used to obtain samples. Collection of oral fluids was performed using a piece of one foot-long, undyed cotton rope, suspended in the pigs' enclosure. Pigs were allowed to chew on the rope, which was then collected and fluids were wrung into collection cups.

Sample preparation and analysis

Approximately 5 mL of oral fluid suspension was collected and saved, while the rest of the fluid was centrifuged to separate supernatant and pelleted fractions. All three fluid fractions were extracted using established Veterinary Medical Diagnostic Laboratory (VMDL) DNA and RNA extraction procedures (Qiagen Valencia, CA). The samples were then screened for the presence of multiple pathogens, including PCV2, general *Mycoplasma*, *Mycoplasma hyopneumoniae*, and porcine parvovirus using conventional PCR assays.

In order to determine the presence of inhibitors within oral fluids, two separate extractions were conducted in which a sample from each of the three fractions was "spiked" with either a DNA (*Trichomonas foetus*) or RNA (PRRS) virus. If the virus was determined to be present, inhibitors were absent within the sample; however, if the virus was absent from detection, inhibitors were present.

PCV2 Real-Time PCR assay optimization

Porcine samples previously tested for PCV2 were gathered from University of Missouri VMDL records. Multiple PCV2-positive samples, each from a different location, from 2011-13 were sequenced and compared to isolates from a previous study in which PCV2-positive samples from 2000-07 were sequenced (Schlink et al., 2008). All of these sequences, as well as other PCV2 sequences from GenBank including recently identified recombinant isolates, were compared to determine the likely ability of different real-time primer/probe sequences to detect a broad range of PCV2 isolates.

Pathogen	Supernatant	Pellet	Suspension
Porcine Circovirus	-	-	-
General Mycoplasma spp.	+	+	+
Mycoplasma hyopneumoniae	-	-	-
Porcine parvovirus	-	-	-

Table 1. Presence of pathogens in three different fractions of oral fluids obtained from 2 different swine populations.

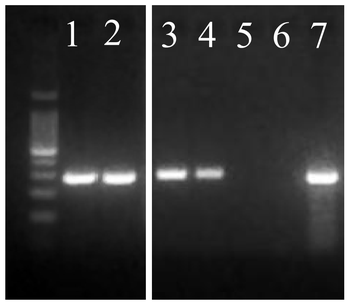

Figure 1. Gel electrophoresis of two different undiluted PCV2-positive samples (lanes 1, 3) and the same two samples at a 1:10 dilution (lanes 2, 4). Lanes 5 and 6 are negative extraction and amplification controls, respectively, and lane 7 is the positive amplification control.

	1	2	3	4	5	6	7
Cycle Threshold (CT)	14.85	15.68	28.21	30.99	-	-	32.30

Table 2. Cycle Thresholds (CT) of the 7 samples shown in Figure 1 on the real-time PCR PCV2 assay.

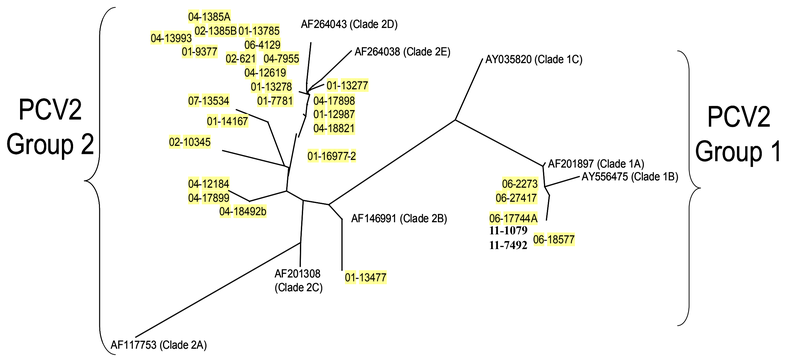

Figure 2. Phylogenetic analysis of PCV2 samples based on ORF2 amino acid sequences. Twenty-nine samples are shown by the year and VMDL case number. The remaining eight sequences are those described by A. Olvera, et. al (2005) and are shown by GenBank accession number. Marker shows the distance which accounts for 0.01% sequence divergence.

Results

Oral fluid fraction analysis

After analysis of the three oral fluid fraction samples, general *Mycoplasma* species, with the exception of *M. hyopneumoniae*, was the only pathogen type detected (Table 1). The samples were negative for all other pathogens. However, detection of *Mycoplasma* species in oral fluids has not been previously described.

PCV Real-Time PCR assay optimization

Extracted samples that were run on a conventional PCR with gel electrophoresis are shown in Figure 1. The bands present in lanes 1 through 4 represent PCV2-positive samples. Lanes 1 and 3 are undiluted samples, while lanes 2 and 4 are samples that were diluted at a 1:10 ratio in order to determine presence of inhibitors in the samples. Lanes 5 and 6 are negative extraction and amplification controls, respectively, while lane 7 is the positive amplification control. PCV2 is was shown to be present, however, the relative amount of PCV2 sample is not indicated on the gel.

These PCV2-positive samples were then run on a candidate real-time PCR assay. The cycle threshold (CT), or the point at which the amount of replicated sample passes a previously determined threshold, of each of the 4 samples is shown in table 2. Samples from 2011-2013 were added to the dendogram (Figure 2) previously presented by Schlink et al. (2008), showing different clades of PCV2 isolates.

Conclusions and Future Implications

Presence of pathogens, such as *Mycoplasma* spp., were detected in the oral fluid samples collected, indicating that oral fluid collection is a viable method for sampling a swine population for pathogen presence. Some inhibition may exist but this must be determined on an individual sample and pathogen basis.

Real-time PCR CT indicates that the relative amount of PCR product can be shown after analysis, whereas this amount cannot be determined using a conventional PCR assay and gel electrophoresis. Therefore, the real-time PCR assay for PCV2 will give a better indication of extent of infection in an individual animal, as well as the herd as a whole.

Using the sequences obtained from the 2011-13 VMDL samples, samples from 2000-07 and the sequences found in GenBank, finding the optimum primer/probe sequences for the real-time PCR assay can be determined. This will ensure that many different isolates of PCV2 can be detected using a single PCR assay.

In the future, we plan to establish and optimize a real-time PCR assay for Hepatits E, similar to the real-time assay for PCV2.

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