RESEARCH REPORT
SWINE HEALTH

Title: African swine fever surveillance using oral fluids for rapid detection and disease control" - **NPB #19-194**

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Industry Summary:

The main objective of this project was to evaluate the feasibility of utilizing swine oral fluids as a reliable aggregate sample type for early detection of African swine fever (ASF). At present, the recommended diagnostic samples for ASF diagnosis are individually collected whole blood, tonsils, spleen, hemorrhagic lymph nodes, etc. However, for the surveillance of North American swine herds for rapid detection of ASF such individual sampling methods are not feasible. Oral fluid is a promising alternative sample type that has been used effectively to detect and monitor the spread of common swine viral pathogens such as porcine reproductive and respiratory virus and swine influenza virus. Previously, it has been shown that African swine fever virus (ASFV) genomic material can be detected in swine oral fluids collected from small groups of pigs when multiple pigs in the pen are infected with ASFV. The objective of this project was to explore the potential of detecting ASFV genomic material in oral fluids collected from pens similar in size to commercial hog farms in North America when the disease prevalence is at its lowest.

The two specific objectives of the proposal were to:

1. Estimate the likelihood of detection of ASFV as a function of within pen prevalence
2. Compare the timeline of clinical disease progression via observation with the timeline of ASFV detection in oral fluids and individual samples

In order to achieve these objectives, we infected four groups of pigs (each 20-25 pigs)—two with the ASFV Georgia 2007/1 (group 1 & 2) strain and two (group 3 & 4) with the ASFV Malta'78 strain. In each experiment, one randomly selected pig was infected

with the virus and released into the pen as the seeder pig. Aggregate oral fluid samples were collected daily, and individual oropharyngeal swabs and whole blood samples were collected every other day. In addition, clinical parameters such as rectal temperatures, appetite and behavior were observed and recorded.

In experiment #1, the seeder pig remained active and alert until 4 days post-infection (dpi), depressed on 5 dpi and found dead on 6 dpi. In experiment #2, the seeder pig was alert until 5 dpi and found dead on 7 dpi. In experiment #3 and #4, seeder pigs died on 6 and 10 dpi respectively. Whole blood samples are the gold standard for ASFV, and the seeder pig tested positive in all cases by 1-3 dpi. ASFV genome was detected in oral fluids collected from pens as early as 3-5 days post infection (dpi) in all experiments, when the ASF pen prevalence was is at the lowest (4% for ASFV Georgia 2007/1 and 5% for ASFV Malta'78). At this time, no evidence of ASFV infection in contact pigs were observed. It is important to note that oral fluid contains significantly less virus than whole blood. At times, the viral load in oral fluid approaches the assay's limit of detection, raising concerns about the potential for false negatives under field conditions.

Using oral fluid, it was possible to detect the presence of ASFV in the pen at least 3 days prior to the death of seeder pigs. Upon the seeder pig's death, there were at least 2 days before contact pigs started to show mild clinical signs. The disease slowly spread through the pig pens, and it took from 10-18 days before significant mortality, indicating that possible ASF infection was observed in the pens. On the farm, this means that at the time of detection, the infected pig may not be clinically sick or only mildly affected and could be easily missed by the farm staff.

These results show that oral fluids can serve as a very effective sample type that can be easily and passively collected in the commercial swine industry. We were able to detect the presence of ASF virus in a pen containing up to 25 pigs when only one of the pigs in the pen was infected. In an outbreak situation, oral fluids when used with other recommended sample types, could greatly strengthen early detection methods.

Key Findings:

- ASFV genome was detected in oral fluids as early as 3-5 days post introduction of an ASF infected pig into a pen containing 19-24 pigs.
- At the time of the first detection in oral fluid, only the seeder pig had developed mild fever, while the other contact pigs were clinically normal.
- Upon death of the seeder pig, it took at least 2 days for the contact pigs to develop fever and clinical signs.
- Whole blood samples remain the gold standard for ASFV detection, as virus can be detected 1-3 days earlier in blood than oral fluid and blood contains significantly more virus, ensuring detection by PCR.
- Oral fluid contains significantly less virus than whole blood. At times, the viral load in oral fluid approaches the assay's limit of detection, raising concerns about the potential for false negatives under field conditions. Evaluation of oral fluid samples from an ASFV outbreak are critical to fully understand risk.
- Upon death of the seeder pig, it took at least 2 days for the contact pigs to develop fever and clinical signs.

Keywords: African swine fever, oral fluid, early detection, surveillance, aggregate samples

Scientific Abstract:

African swine fever is considered a global animal health priority. Introduction of African swine fever virus (ASFV) into North America would cause severe economic loss both directly and indirectly, as the USA is the world's third largest pig producer. To rapidly identify an ASFV incursion, a robust and effective surveillance program is critical.
Surveillance using individual pig sampling is labor-intensive and costly and therefore, impractical. Pen-based oral fluid (rope sampling) is a non-invasive alternative that requires significantly lower financial and human resources. In order to explore the practicality and efficacy of detecting ASFV in oral fluids in commercial settings, we have conducted four independent animal experiments. Experiments #1 and #2 had 24 and 25 pigs respectively and used the highly virulent ASFV Georgia 2007/1 strain. Experiments #3 and #4 had 20 pigs per pen and used the moderately virulent ASFV Malta' 78 strain. A randomly selected seeder pig from each group was infected intramuscularly with the respective ASFV strain and allowed back into the group. Aggregate oral fluid samples and oropharyngeal swabs were collected daily, and blood samples were collected every other day. ASFV genomic material was detected 3-5 days post introduction of the seeder pig into each pen. During this initial ASFV detection, the seeder was febrile, but this would be difficult to visually recognize in a pen by farm staff in an industry setting. ASFV genomic detection in oral fluids was possible at least 2 days prior to the seeder pigs being found dead. Following the death of the seeder pigs, it took about 3 days until mortality of contact pigs was observed. Although clinically un-apparent, ASFV genome was continuously detected in the oral fluids collected from these pens. Oropharyngeal swabs of contact pigs indicated the presence of ASFV around 3-5 dpi, prior to the contact pigs becoming viremic. By the time viremia was detected at 6-10 dpi, the contact pigs started showing fever, clinical signs and mortality. Oral fluid detection could be utilized for early detection so that affected pens could be identified, and infection would be controlled prior to spreading throughout the farm. According to the results of these experiments, it was evident that oral fluids could serve as an effective sample type to identify the introduction of ASF into commercial pig farms.

Introduction:

ASFV continues to spread in Europe, China, South East Asia and Africa. It is an incurable contagious hemorrhagic disease of pigs, similar to the Ebola infection in humans. ASF causes mortality approaching 100% and there are no available treatments or preventative vaccines for this disease. The USA is the world's third largest pig producer (more than 11 million tons in 2016) and the world's second largest exporter of pork ($4.2 billion in 2016). The US pork industry contributes around $20 billion to the US economy. Introduction of ASFV into the US would cost more than US $4.25 billion with social ramifications affecting farmers and thousands of US workers directly and indirectly due to trade restrictions applied after a disease notification.

If ASFV were to enter the US swine population, zoning and or compartmentalization combined with active surveillance would be critical to facilitate progressive elimination and eradication efforts. Active surveillance based on individual pig sampling is labor-intensive and costly and therefore impractical during a large disease outbreak. Pen-based oral fluid (rope) testing is a non-invasive alternative that requires significantly lower financial and human resources. Collection of oral fluid samples is simple, straightforward and can be completed within 30 minutes.

Briefly, cotton ropes are hung in the pen at pig shoulder height allowing the pigs to chew on the rope, soaking the rope with oral fluids. After 30 minutes, the ropes are collected, placed in a single-use plastic bag and the fluid is wrung from the rope. The oral fluid is then transferred into a sterile screw-cap tube and is submitted to the diagnostic laboratory, or tested on-site using a portable detection system. Oral fluid samples are already being used in the USA as an effective ample type to assess the health status of swine herds for endemic diseases such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV) etc.

In order to evaluate the feasibility of using rope samples for detection of ASF, a preliminary experiment was conducted at the National Centre for Foreign Animal Disease (NCFAD) Winnipeg as a part of previous US National Pork Board Funded research Project NPB# 14-286 (Use of oral fluid samples to monitor virus shedding and antibody responses in pigs experimentally infected with high consequence swine viruses foot and mouth disease, African swine fever, swine vesicular disease and classical swine fever viruses). ASFV genomic material was detected in oral fluid samples one day before the animals showed clinical signs (fever) suggesting that the oral fluid samples could be used in ASF surveillance for early detection of ASF-positive animals. Subsequent testing of these samples, performed collaboratively between the Animal and Plant Health Inspection Service-Foreign Animal Disease Diagnostic Laboratory (APHIS-FADDL) and NCFAD, indicated that both PCR assays, as well a commercially produced PCR assay, all consistently detected ASF virus genome in oral fluid samples.

In this study, we proposed to further evaluate the utility of oral fluid testing in commercial pig barns where larger groups of pigs are housed and only a small number of pigs are infected with ASFV. This is a collaborative project between the USDA Animal and Plant Health Inspection Service at the Plum Island Animal Disease Center (PIADC) and the Canadian Food inspection Agency, National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, Canada. Results from the study will provide experimental data required to determine the suitability of oral fluid samples for the detection of ASFV in commercial pens. The PIADC and the NCFAD are two of the few laboratories in North America that can work with live ASF virus.

The USA and Canada are two of the largest pork exporters in the world with a high volume of bidirectional movement of pigs across the border. Foreign animal diseases such as ASF can have a severe consequence on the swine industry on both countries and therefore establishment of surveillance tools such as oral fluid testing and zoning and/or compartmentalization are critical to maintaining the trade between the US and Canada during a potential ASF outbreak.

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Objectives:

1. Estimate the likelihood of detection of ASFV as a function of within pen prevalence.
2. Compare the timeline of clinical disease progression via observation with the timeline of ASFV detection in oral fluids and individual samples

Materials & Methods:

Experiment #1 and #2 conducted at the NCFAD biosafety level-3 (BSL-3) animal facility contained 24 and 25 pigs per pen, respectively. Experiments #3 and #4 conducted at FADDL BSL-3 animal facility had 20 pigs in each experiment. In each experiment, a randomly selected seeder pig was infected with either highly virulent ASF Georgia 2007/1 strain or moderately virulent ASF Malta' 78 strain by intramuscular injection under anesthesia. After recovery, the seeder was introduced back to its pen mates. Oral fluids were collected daily by allowing pigs to chew two cotton ropes hung for 30 minutes in each pen. In addition, oropharyngeal (OP) swabs were collected from individual pigs daily. Whole blood samples were collected from each pig every other day. Rectal temperatures were taken daily, and clinical condition was monitored daily using a clinical scoring system. When pigs reached the humane end points, they were euthanized, necropsies were performed, and tissue samples were collected.

Results:

1. Estimate the likelihood of detection of ASFV as a function of within pen prevalence.

ASFV genomic material was detected in aggregate oral fluids, 3-5 days post infection (dpi)/post-introduction of the seeder pig (Table 1). When the first oral fluids detection happened, the within pen prevalence for viremia was at its lowest at 4-5%. ASFV genomic material was continuously detected in whole blood of seeder pigs 1-3 dpi while chewing on the rope until 12-24 hours before they were found dead.

Exp.	# of pigs	ASFV strain	Seeder pig death (dpi)	50% mortality (dpi)	First detection in OF (dpi)	Detections in seeder pig (dpi)		Detections in contact pigs (dpi)	
						Blood	OP Swab	Blood	OP Swab
1	24	Georgia	6	14	3	2	3	8	4
2	25	Georgia	7	18	5	2	3	10	5
3	20	Malta'78	6	17	3	1	3	6-7	3
4	20	Malta'78	10	17	3	3	3	8-9	5

Table 1: Summary of ASFV genomic material detection in aggregate oral fluids, and in individual samples in comparison to the seeder pig's death in the experiments 1, 2, 3 and 4 conducted in the NCFAD and FADDL using ASFV Georgia 2007/1 strain and Malta'78 strain.

2. Compare the timeline of clinical disease progression via observation with the timeline of ASFV detection in oral fluids and individual samples

ASFV genomic material was detected in oral fluid around 3-5 dpi, and at this time only the seeder pigs developed viremia and mild to moderate fever. Despite fever, they remained alert and playful and were interested in feed and chewed on the ropes. Seeder pigs started getting febrile and clinically lethargic from 2-3 dpi and were found dead 6-10 dpi. None of the contact pigs developed viremia, fever or other clinical signs at this stage. It took 2-3 days from the death of the seeder pigs until contact pigs started showing clinical signs in a wave of clinical ASF. Although there was an interval with un-apparent clinical disease progression, during this period oral fluids continuously indicated the presence of ASFV genomic material. After the contact pigs started getting clinically sick between 8-10 days post introduction of the seeder pig to the pen, mortality increased and the interest in rope chewing reduced. However, this did not negatively affect the oral fluid detection and the detection continued until the experiment was concluded at 18, 21 and or 25 dpi.

Discussion:

We have conducted four independent animal experiments using two different ASFV strains to evaluate the potential use of oral fluid for early detection of ASFV in commercial farms.

Our results indicate that using oral fluid, ASFV infected pig pens can be identified as early as 3-5 days post introduction of an infected pig into the pens when the ASFV prevalence in the pen is only 4-5% (Table 1). At this stage, the seeder pigs, although infected, were not showing obvious clinical signs. They had elevated rectal temperatures which would not be noticed unless the rectal temperature was monitored daily in the seeder pigs. They were playful, had normal appetite, and chewed on the ropes. At this stage none of the contact pigs were infected or clinically sick. In some animals, individual pharyngeal swabs showed weak positive results for ASFV genomic material, but this was inconsistent.

In conclusion, using four independent experimental inoculation studies, we have shown that oral fluid can be used as an effective, industry feasible, aggregate sample type for early detection of ASFV in commercial pig farms. Further optimization of ASFV genome detection in oral fluid, additional intra and inter-laboratory validation and a field validation of ASF detection in oral fluid are ongoing.