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Influenza A virus detection from oral fluid and nasal swabs in IAV inoculated pigs

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Introduction: The detection of IAV in swine populations using nasal swab specimens is labor intensive and relatively insensitive in non-febrile pigs (1). As an alternative, oral fluid samples have been shown to be an excellent surveillance sample for several swine respiratory viruses (2, 3, 4). The objective of this study was to compare the rate of detection of IAV by RT-PCR, virus isolation, and a rapid antigen detection kit (VetScan® Rapid Test) in nasal swabs and pen-based oral fluid from experimentally inoculated swine over time.

Materials and methods: 82 piglets were isolated for 30 days and confirmed negative for PRRSV, *Mycoplasma hyopneumoniae*, and IAV infections. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). Thereafter, pigs were intratracheally inoculated with one of two IAV viruses (A/Swine/OH/511445/2007 γ H1N1 or A/Swine/Illinois/02907/2009 Cluster IV H3N2) or remained negative controls. The virus isolates were kindly provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN), respectively. Pen-based oral fluid (OF) samples were collected daily on days post inoculation (DPI) 0-16. Individual nasal swabs (NS) were collected daily on DPI 0-6, then DPI 8, 10, 12, 14, 16. Samples were randomized and submitted for IAV detection at the Iowa State University Veterinary Diagnostic Laboratory by RT-PCR, Virus Isolation (VI) and a 15 minute antigen detection assay (VetScan® Rapid Test, Abaxis Inc.). Only samples collected on DPIs 0 to 10 were tested by the Rapid Test.

Results: A pen was classified NS positive if ≥ 1 pig in a pen was NS RT-PCR, VI or VetScan® positive. Using this convention, NS and OF RT-PCR testing results were equivalent through DPI 8, with more OF-positive pens thereafter. False positive PCRs were reported in both OF (n = 1) and NS (n = 3) samples. Differences were noted between the number of VI positive pens (NS vs OF) detected for each serotype by DPI, but over time, statistical differences were seen due to vaccination status and sample matrix. One false positive VI was reported in a NS sample. No false positives were observed with the Rapid Test. In unvaccinated pigs, there was no difference in the duration of detection by VI between NS and OF (DPI 6) regardless of serotype. Similarly, there was no difference in detection between serotypes by the VetScan® Rapid Test. There were minimal differences in rate and duration of detection between NS and OF in unvaccinated pigs. For the VetScan® Rapid Test, sensitivity of IAV detection in OF DPI 0-5 improved if the assay was read at 30 rather than 15 minutes (AUC= 0.752 vs. AUC = 0.701) and was equivalent to NS (p = 0.74).

Vaccination reduced the duration of detection of IAV in both OF and NS, although RT-PCR positive NS and OF were detected through DPI 14. Detection of IAV by VI and VetScan® Rapid Test was inhibited by vaccination.

Conclusions: RT-PCR testing of pen-based OF was equivalent to, or better than, detection using NS at the pen level. Oral fluid is a valid and useful sample type for the detection of IAV by RT-PCR in both unvaccinated and vaccinated pigs for at least 14 days post infection. Pen-based OF is a valid and useful sample type for VI in unvaccinated pigs for at least 6 days post infection. The VetScan® Rapid Test could be a useful pen-side test for the detection of IAV antigen during acute infection using either OF or NS.

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