

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 (VI), and the VetScan® (Abaxis Inc., Union City, CA) rapid antigen detection kit ("rapid") 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. Individual nasal swabs (NS) (Figure 2) were collected daily on DPI 0-6, then DPI 8, 10, 12, 14, 16. Pen-based oral fluid (OF) (Figure 3) samples were collected daily on days post inoculation (DPI) 0-16. Samples were randomized and test by RT-PCR (2 laboratories) and VI (ISU-VDL). Samples collected on DPIs 0-10 were tested using a 15 minute antigen detection assay "rapid" test (ISU-VDL).

Figure 1. Detection of influenza A virus in oral fluid¹ and nasal swab² specimens over time in unvaccinated pigs

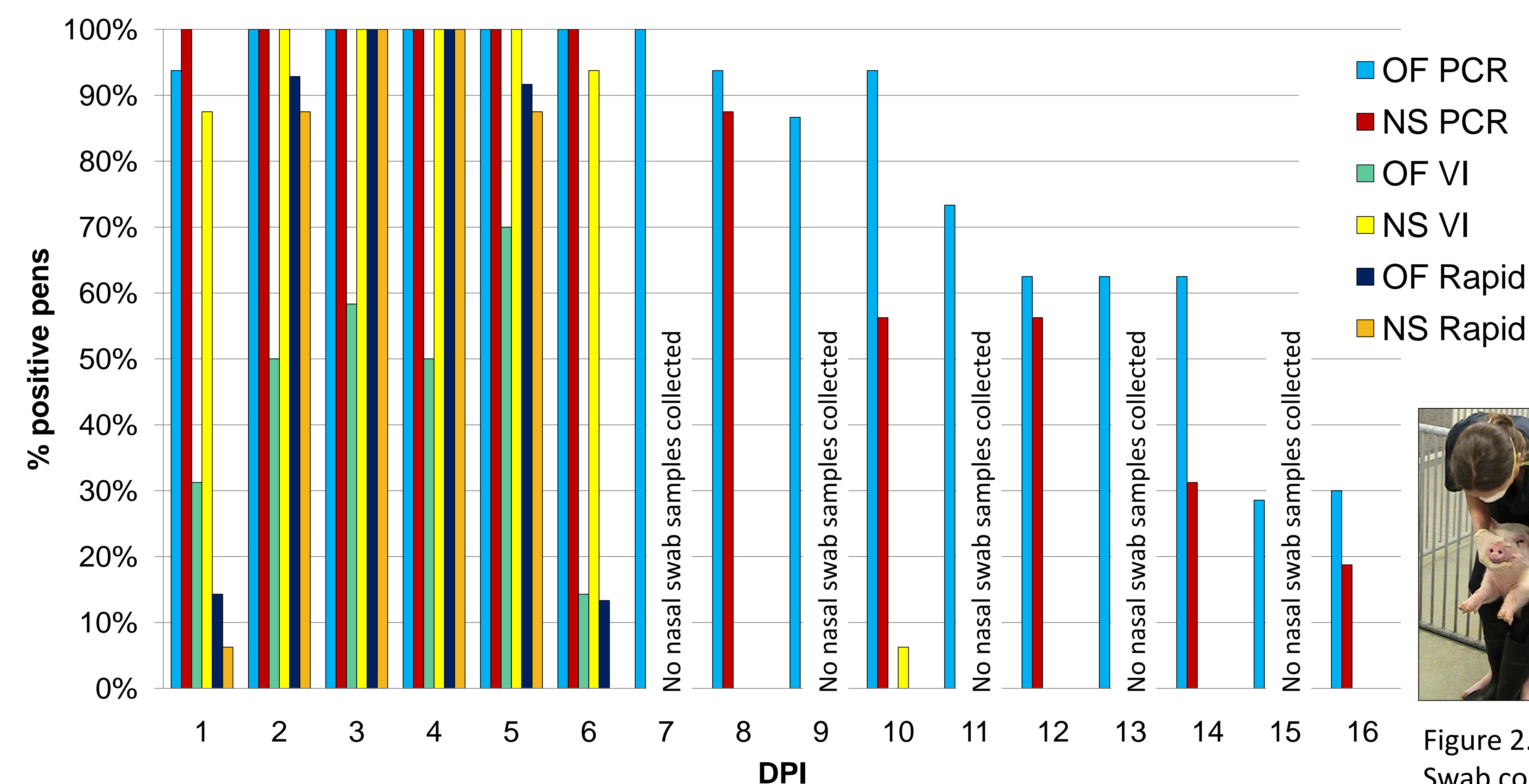


Figure 2. Nasal Swab collection

¹Oral fluids: n= 12-16 pens/day ²Nasal swab: n=16 pens/day

Data not shown:

Rapid: No false positives in OF or NS samples from negative control pigs
 VI: One false positive NS from a negative control pig
 RT-PCR: 2 false positives in OF and 5 in NS from negative control pigs

Analysis and Results

False positive PCRs were reported in both OF (n = 2) and NS (n = 5) samples. One false positive VI was reported in a NS sample. For the "rapid" test, sensitivity of IAV detection in OF DPI 0-5 improved if the assay was read at 30 rather than 15 minutes and was equivalent to NS ($p = 0.74$) (Figure 4). No false positives were observed with the "rapid" test.

A pen was classified NS positive if ≥ 1 pig in the pen was NS RT-PCR, VI, or "rapid" positive. Using this convention:

- **RT-PCR results** NS and OF PCR results were equivalent through DPI 8, with more OF-positive pens thereafter (Figure 1).
- **VI and "rapid" results** In unvaccinated pigs, there was no difference in the duration of detection by VI between NS and OF through DPI 6. However, a higher proportion of NS samples were positive. There was no difference in detection between IAV subtypes (H1N1 vs. H3N2) by VI or "rapid" test.
- **Effect of vaccination** Vaccination reduced the detection of IAV in OF and NS by both VI and "rapid" test, although RT-PCR positive NS and OF specimens were detected through DPI 14.

Conclusions and Discussion

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 "rapid" test could be a useful pen-side test for the detection of IAV antigen in unvaccinated pigs during acute infection using either OF or NS.

References

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Acknowledgements

The study was supported by Pork Checkoff® funds through the National Pork Board (#09-193). VetScan® AIV Rapid Test kits were kindly provided by Abaxis, Inc. FluSure XP™ was kindly provided by Pfizer Animal Health. RT-PCR testing was kindly provided by Tetracore Inc. and Life Technologies Corp.



Figure 3. Oral Fluid collection

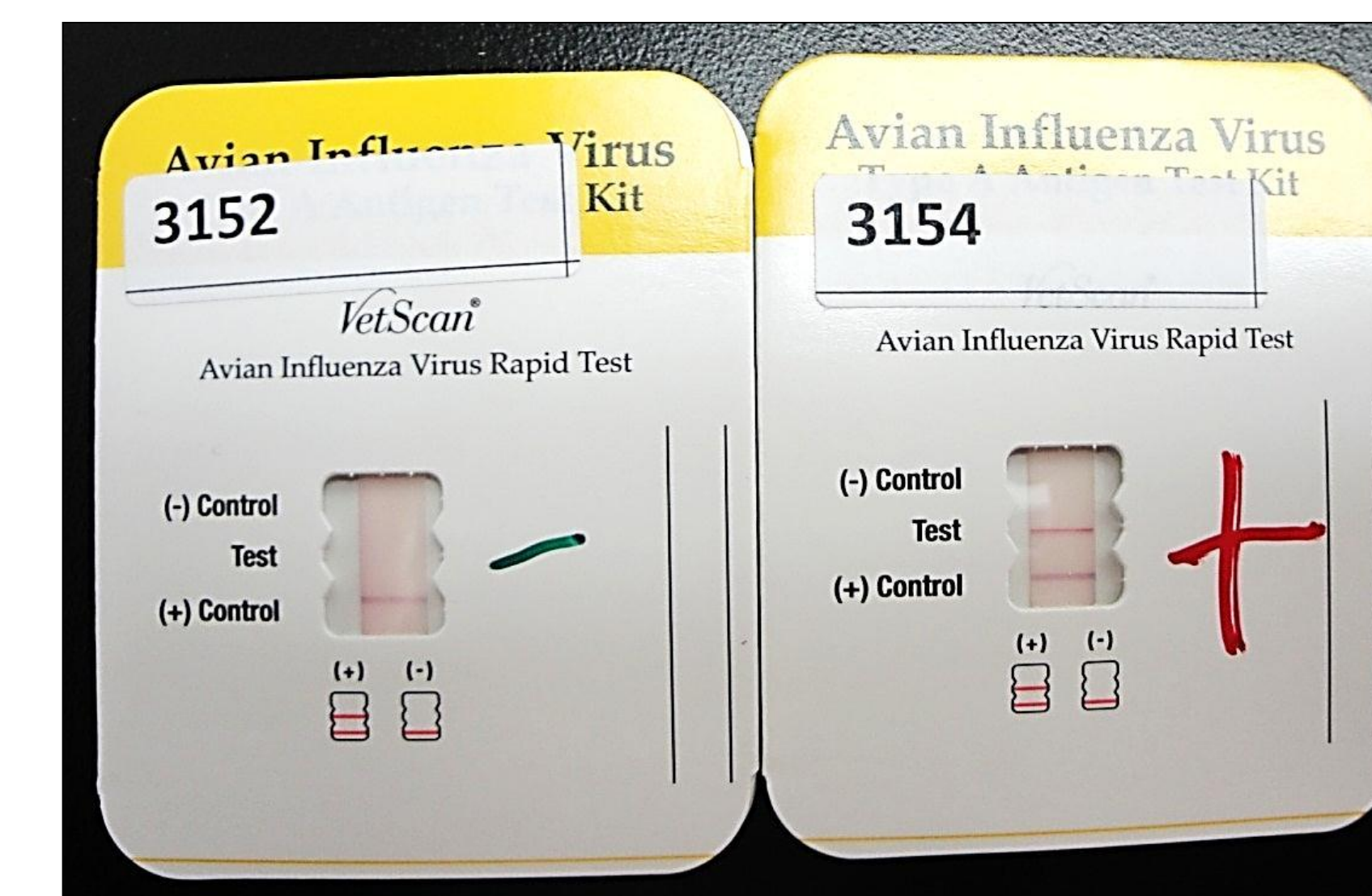


Figure 4. VetScan® Rapid Test