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Detection of swine influenza virus in porcine oral fluid samples

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In pigs, oral fluids have recently been used as a surveillance tool for porcine reproductive and respiratory syndrome virus (PRRSv) and PRRSv antibodies using quantitative reverse transcription polymerase chain reaction, enzyme-linked immunosorbent assay and indirect fluorescent antibody tests[1]. This method used a 3-strand twisted cotton rope that was suspended within a pen of 4 to 6 pigs. The pigs chewed on the rope, moistening it with oral fluids, and then the oral fluids were collected for analysis[1].

The objective of this present study was to determine whether swine influenza virus was present in oral fluids at diagnostic levels. The animals used in this study had been infected with swine influenza virus (SIV), A/Swine/Iowa/00239/2004 H1N1, for *in vivo* evaluation of the efficacy of an antiviral compound. The isolate of swine influenza had previously been used in inoculation and vaccine evaluation studies[2,3].

Forty-eight 3-week-old pigs were purchased from a single, influenza-negative source and brought to the isolation unit at the University of Minnesota where they were randomly divided into 6 different rooms in the isolation unit. These animals were divided into 3 treatment groups: a positive control group to verify infection without treatment, a sham infected group that received the medication and a negative control group. For the purpose of the oral fluid detection, the pigs were grouped as “infected” (groups 1-4) and “non-infected”.

Oral fluids were collected on days 3, 4, 5, and 6 post-infection from each of the 6 groups. Pen-based oral fluid samples were assayed for SIV by real-time reverse transcription polymerase chain reaction (RT-PCR) and virus isolation. The oral fluid samples from the infected groups were positive for SIV by RT-PCR from 3 out of 4 infected groups on day 3, 4 out of 4 infected groups on day 4, 4 out of 4 infected groups on day 5, and 4 out of 4 infected groups on day 6. All oral fluid samples from the non-infected groups were negative for SIV by RT-PCR at all time points. No virus was isolated from the oral fluids in two passages on Madin-Darby Canine Kidney (MDCK) cells.

On days 3 and 7, nasal swabs were collected from the same 36 animals (24 infected and 12 non-infected) that the oral fluids were collected from. Nasal swab samples were assayed for SIV by virus isolation and negative nasal swabs were assayed by RT-PCR. SIV was isolated from 21 out of 36 pigs on day 3 and 12 out of 36 pigs on day 7. RT-PCR analysis of the swabs that were negative by virus isolation revealed a suspect animal on day 3 and 5 additional positive animals on day 7.

The expected and observed results for the RT-PCR of the oral fluid and virus isolation of the nasal swab were compared using Chi-square analysis. The results showed a strong association between infection with SIV and positive test results. Although further evaluation of the sensitivity of this test and validation of field samples needs to be performed, the results of this experiment suggest that pen-based oral fluid sampling could be used for surveillance of influenza viruses in the swine population.

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