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# Indirect transmission of influenza A virus in two different biosecurity settings

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## Introduction

Influenza A virus is a common cause of respiratory disease in pig populations; however, little information is available regarding rates of transmission via different routes within and between farms. While it is known that direct transmission of influenza viruses is an important means of transmission in pig populations, indirect routes (e.g. fomites) have not been studied in detail. Indirect transmission has been suggested following influenza virus outbreaks in swine herds with no obvious link to infection via the introduction of infected pigs (Tofts, 1986; Desrosiers, et al., 2004). In addition, transmission of influenza virus via fomites has been documented in guinea pigs and chickens (Mubareka, et al., 2009; Yee, et al., 2009). The objective of this study was to evaluate the role of fomites in influenza virus transmission between pig populations separated by two different biosecurity settings.

## Materials and methods

Thirty-five influenza virus negative pigs were assigned to one of four experimental groups. Ten pigs (1 replicate) were assigned to the infected group (I); 10 pigs (2 replicates of 5 pigs) were assigned to sentinel group A (SA); 10 pigs (2 replicates of 5 pigs) were assigned to sentinel group B (SB); and 5 pigs (1 replicate) were assigned to the negative control group (NC). Eight of 10 pigs in the infected group were challenged intra-tracheally and intra-nasally with 1 ml of viral inoculum (A/Sw/MN/07002083/07, H1N1) in each location. Two pigs in the infected group served as direct contact controls. Thirty-six hours following inoculation of pigs in the infected group, personnel movement events took place in order to move potentially infectious clothing and personal protective equipment (PPE) to sentinel pig rooms.

A movement event was defined as direct contact for 45 minutes with the infected group which was followed by movement of personnel to the sentinel groups. Personnel then had contact with the sentinel groups for an additional 45 minutes. Personnel moved directly to sentinel group A, without changing clothing/PPE or washing hands and face (low biosecurity). Personnel moved to sentinel group B after changing clothing/PPE in addition to washing their hands and face (high biosecurity). Nine movement

events from infected pigs to sentinel pigs in each group occurred during the course of this study over a 5 day period. Influenza virus infection status of pigs was determined daily via nasal swabs tested by RRT-PCR. Clothing and PPE (fomites) were also swabbed and tested via RRT-PCR following contact with infected pigs and following biosecurity measures. Air samples were also collected and tested via RRT-PCR from the infected group room during days in which movement events took place.

## Results

All experimentally inoculated pigs (8/8) in the infected group were infected with influenza virus and all direct contact controls were infected (2/2) following direct exposure to infected pigs. During the 5 days in which the 9 movement events took place, 5, 8, 9, 10, and 10 pigs were influenza virus positive in the infected group. In addition, air samples were positive for influenza virus on three of these 5 days.

Influenza virus RNA was detected on a low proportion of fomite samples. Of the 144 samples collected following contact with infected pigs, 11 (8%) were low level positives (Ct value > 35 and < 40) via RRT-PCR. All samples collected from gloves and hairnet/mask were negative, while 7/36 (19%) and 4/36 (11%) samples collected from boots and coveralls were low level positives, respectively.

One replicate of each sentinel groups A and B were infected with influenza virus. In each of these replicates, all five pigs were infected over time and confirmed positive by RRT-PCR. All pigs in the negative control and the remaining replicates of sentinel groups A and B remained negative throughout the study.

## Discussion

This study provides evidence of indirect transmission of influenza A virus from an infected population of pigs to sentinel pigs. Influenza virus RNA was detected from fomites following contact with infected pigs and just prior to contact with sentinel pigs in the low biosecurity groups. Transmission occurred in two replicates under differing biosecurity settings, while transmission did not occur in the additional two replicates.

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