# EVIDENCE OF AIRBORNE TRANSMISSION OF SWINE INFLUENZA A VIRUS IN EXPERIMENTAL CONDITIONS

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## / CONTEXT

Swine Influenza A virus (swIAV) may transmit through aerosols. The virus has been detected in air samples collected in affected pig farms [1] and a relationship between airborne swIAV detection and the number of infected pigs was shown [2]. Here, we report swIAV detection in air samples collected in experimental rooms housing specific-pathogen-free (SPF) pigs without or with maternally-derived antibodies (MDA). Thirty-three MDA- piglets were assigned to 3 independent rooms (rooms 1 to 3) and 33 MDA+ piglets to 3 others (rooms 4 to 6) [3]. In each room there were 2 seeder pigs (intra-tracheally inoculated with A/Sw/France/Cotes d'Armor/0388/09 (H1N1) ($10^6$ $EID_{50}$ in 5 mL) and 4 pen-mates in direct-contact, as well as 5 indirect-contact pigs in a neighboring pen, 30 cm apart. (Figure 1).

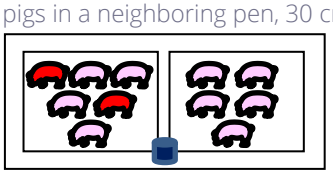

Figure 1. An experimental room was composed of two pens. The air sampler collector was placed in-between. The inoculated pigs are colored in red.

## / MATERIALS

- Coriolis® µ, sterile cones, 15mL of collection liquid (Bertin Technologies)
- Amicon® Ultra-15 Centrifugal Filter 30K Device (Merck Millipore Ltd)
- Nasal swabs Virocult® MW 951 sent (Medical Wire)
- RNeasy Mini Kit© (Qiagen GmbH)
- LSI VetMAX™ Swine Influenza A kit (Life Technologies)

## / PROTOCOL

The Coriolis® µ was put down in the room between the 2 pens, 70 cm away from the ground, at the height of piglets but without direct contact with them (Figure 1).

Air samples were taken 3 times a week for 25 days post-infection (DPI). At each collection time, the collector ran during 10 min to collect 3000 L of aerosols in 15 mL of 0.005% Triton solution.

The air samples were then concentrated thanks to an ultrafiltration step using Amicon® Filter Device and centrifugation for 30 min at 3900 × g.

Viral RNA was purified from 150 µL eluate and 5 µL RNA extract were tested by real-time M gene RT-PCR to detect the swIAV genome.

Nasal swabs were taken on a daily basis until DPI 14, then every 2 days. RNA was extracted from 200 µL supernatants and 5 µL submitted to RT-PCR.

## / RESULTS

The swIAV genome was detected in aerosols from all rooms, from DPI 2 or DPI 4, until the end of the experiment (Figure 2). In each room the viral genome load peaked at DPI 9.

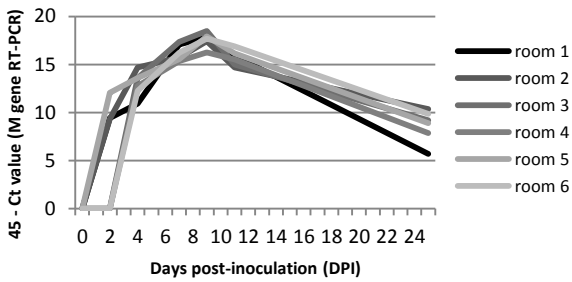


Figure 2. SwIAV genome load in air samples deduced from RT-PCR analyses (45-Ct value)

All indirect-contact (IC) pigs were shown to have been infected. They shed the virus from DPI 4 or DPI 6 depending on their serological status, and until DPI 14 for the last one (Table 1).

| | Days post-inoculation | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 16 | 17 | 18 | 21 | 23 | 25 |
| IC_room 1 | | | | | | | | | | | | | | | | | | | | | |
| IC_room 2 | | | | | | | | | | | | | | | | | | | | | |
| IC_room 3 | | | | | | | | | | | | | | | | | | | | | |
| IC_room 4 | | | | | | | | | | | | | | | | | | | | | |
| IC_room 5 | | | | | | | | | | | | | | | | | | | | | |
| IC_room 6 | | | | | | | | | | | | | | | | | | | | | |

Table 1. SwIAV genome detection in nasal secretions of IC pigs. The room was considered positive (in red) when at least 1 out the 5 IC pigs was found RT-PCR positive.

Thus, the swIAV genome was detected in the air 2 days before the first IC piglet shed the virus and still up to 15 days after the last one did (room 2).

[1] Corzo, C. A. Culhane, M. Dee, S. Morrison, R. B. Torremorell, M. (2013). Airborne detection and quantification of swine influenza a virus in air samples collected inside, outside and downwind from swine barns. PLoS One, 8, e71444.

[2] Corzo, C. A. Romagosa, A. Dee, S. A. Gramer, M. R. Morrison, R. B. Torremorell, M. (2013). Relationship between airborne detection of influenza A virus and the number of infected pigs. The Veterinary Journal, 196 (171-175).

[3] Cador, C. Hervé, S. Andraud, M. Gorin, S. Paboeuf, F. Barbier, N. Quéguiner, S. Deblanc, C. Simon, G. Rose, N. (2016). Maternally-derived antibodies do not prevent transmission of swine influenza A virus between pigs. Veterinary Research 47:86.

## / CONCLUSION

Thanks to the Coriolis® µ instrument, genome of swIAV was detected in air samples collected in experimental rooms housing inoculated and contact SPF piglets, in addition to detection in nasal swabs taken from animals and estimation of indirect transmission rate that revealed that 1.41 piglets became infected per day via the air [3]. All together, these results demonstrate that aerosols are a key point in swIAV spread and persistence in pig herds. They confirm that detection of swIAV in air samples collected within commercial farms would give information on airborne transmission within confinement building environments. Such investigation would help monitor ventilation systems and airflows accurately, in order to reduce the infectious process.

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