



Pig infection studies with influenza A (H1N1) associated with global epidemic in humans

An EU funded study through an EU project consortium coordinated by VLA-Weybridge

Preliminary Summary - Update 1, 29/05/09

Study aims

To investigate infection dynamics, clinical outcome, pathogenesis, host susceptibility and transmissibility of influenza A (H1N1) [associated with global epidemic in humans, hereafter referred to as influenza A (H1N1)] in pigs.

Study design - Overview

Group A

Eleven pigs directly inoculated aerogenically into the nasal passages with influenza A (H1N1).

Group B

Mock-inoculated control pigs (n=2); non-inoculated control pig (n=1).

Groups C-F

Eight non-infected pigs, which were sequentially introduced in pairs for contact transmission (at monitored intervals) with infected pigs (see below).

- Transmission dynamics were initially established by placing the first pair of contact pigs in the same room as the directly infected pigs at dpi 2. The first pair of contact pigs remained in contact until virus shedding in both contacts was established (~72hrs). The first two contact pigs were then removed to a separate room and two more naïve pigs were introduced with the first pair of contact pigs (2+2) until shedding was established. This process has been repeated for up to four cycles of transmission.

Monitoring & Procedures

- Full clinical assessment: Daily, including temperature determination and weighing.
- Sampling: Daily oropharyngeal, nasal, ocular and rectal swabs. Between days -1 and 4, plus day 7, blood samples were collected daily to assess viraemia and acute phase protein production. Twice-weekly blood sampling for humoral antibody response determination.

- Necropsies: Two pigs from Group A were removed and a full post mortem examination (PME) performed on day 1 (one pig only), and days 2, 3, 4 and 7 post-infection. A full range of tissue specimens was collected.

Preliminary Results

NB. Please note these results are preliminary and may be subject to modification once full analyses are complete. Therefore, they should be used as a guide only at this time.

- **Clinical signs:** Included nasal discharge, pyrexia (temperature >39.5°C), respiratory signs (cough mainly, some increased respiratory rate), ocular discharge, lethargy and inappetence. Limited morbidity. Clinical scores peaked at dpi 4-6 with progressive recovery evident (n=2) after dpi 7. For in contact animals, pyrexia was evident from day post-contact (dpc) 5-9. Clinical signs appeared milder than in those pigs directly infected.
- **Virus shedding:** Determinations by real-time RT-PCR; all inoculated animals became infected and shed virus mainly via the naso-pharyngeal route from dpi 1-9, peak shedding occurred between dpi 3-5. Oral and ocular shedding was detected intermittently, but rectal shedding was not detected. Shedding appeared to have ceased by dpi 10. No viral RNA was detected in plasma samples collected between day -1 and dpi 7.
- **Gross pathology:** Necropsy at dpi 2 revealed only a mild/moderate catarrhal rhinitis with no gross pulmonary pathology. At dpi 3 and dpi 4, PME of pigs (n=2 each day) revealed no evidence of rhinitis with only minimal lung pathology (discrete, rare focal areas of lobular consolidation). At dpi 7 more extensive gross lung lesions were present, characterised as an acute lobular bronchopneumonia with associated marked lymphadenopathy in the bronchial and retropharyngeal lymph nodes.
- **Transmission:** The contact animals developed similar profiles to the directly infected animals, particularly after transmission cycles one and two (72 hour contact period). Evidence of successful transmission was apparent between both the direct infected and first pair of contact animals (transmission cycle 1), and between the first and subsequent pairs of contact pigs (transmission cycles 2, 3 and 4), based on RRT-PCR shedding profiles. For transmission cycle 1, shedding peaked at dpc 3, and for transmission cycle 2 at dpc 5-6, with shedding apparently ceased by dpc 6-9. Transmission cycles three and four appeared substantially delayed (third cycle >4 days).

Preliminary analyses: Summary Conclusions

Pigs are susceptible to infection with influenza A (H1N1) virus that results in the induction of detectable levels of clinical disease, virus shedding and pathology in an experimental setting. Importantly, mortality was not a feature and infected animals were able to transmit the virus to naïve contact pigs successively for at least three cycles of transmission, suggesting the virus could become established in susceptible pig populations if introduced.