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(Article begins on next page)

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## **Phenotypic characterization of a novel HP Italian PRRSV-1 isolate in experimentally infected pigs**

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Highly pathogenic (HP) PRRSV isolates are characterized by high viral loads, severe general clinical signs and high mortality. Their genomes share a discontinuous aa deletion in the non-structural protein 2 (nsp2).

This investigation is aimed at characterizing clinical, virological, pathological and serological outcomes in conventional pigs experimentally infected with a potential highly pathogenic, Italian PRRSV-1 subtype 1 isolate.

The isolate (PR-402014) was obtained from nursery pigs in the course of an outbreak of high post-weaning mortality (up to 50%) associated with severe systemic and respiratory disease. The nucleotide sequence of the ORF5 showed 85.9% homology to LV. The full genome sequence revealed a discontinuous deletion of 42nt and 366nt in the nsp2 region, and a 6nt deletion in the ORF4. To confirm the high pathogenicity of the virus, five conventional pigs of six weeks of age from a PRRSV negative herd were intranasally inoculated with  $10^6$  TCID<sub>50</sub>/pig PR-402014 (HP) in 2 ml of PBS, 1 ml/nostril (HP group). Moreover, five pigs from the same origin were inoculated with the same dose of a recent Italian PRRSV (PR-012014) isolate. This group (NP group) served as a reference group for the comparison of the obtained findings. At day 3 p.i., two more pigs were added to each group to act as “in contact” pigs. In total 7 pigs/group were considered. Thus, a group of 3 pigs intranasally inoculated with 2 ml of PBS served as negative control. Body temperature and clinical signs (respiratory disorders scored from 0 to 6, appetite, level of consciousness) were monitored daily. Blood and nasal swabs were collected at 0, 3, 7, 10, 14, 21, 28, 35, 42 days p.i. in all experimental pigs.

Clinical and virological differences were observed among animals inoculated with the HP and the NP isolates. In particular, high fever (the average temperature was  $>40^{\circ}\text{C}$  from day 2 to 24 p.i. peaking up to  $41,5^{\circ}\text{C}$  in some animals), anorexia and depression of the level of consciousness were the prominent signs in pigs inoculated with the HP from day 3 to 28 p.i.

Fever in NP animals was constantly lower. Four out of 7 (57%) pigs died in the HP group. The ADWG was 264 g/day for the survivors (3 pigs) in group HP, 345 g/day for those of NP group and 497 in the negative control group. Viremia in HP infected pigs was higher and longer in duration as compared to the NP infected animals. Dead animals from HP group showed severe lymphocyte depletion in the lymphoid tissues and organs and severe interstitial pneumonia.

Taken together a) the clinical outcomes of the PRRSV isolate in the field, b) the genome deletions and 3) the experimentally induced severe clinical signs associated with high viremia and pathological lesions in challenged pigs, we can assume that the isolate PR-402014 could be defined as a highly pathogenic PRRSV-1, subtype 1.