Assessment of Influenza D virus in swine: first serological evidence for exposure of breeding sows in France

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Recent studies have identified a new genus within the *Orthomyxoviridae* family, named Influenzavirus D. During the last years, influenza D virus (IDV) has been isolated from cattle diagnosed with bovine respiratory disease complex in the USA, France, China, Italy and Japan, and bovine are hypothesized to represent the reservoir to IDV. However, IDV was also isolated from pigs exhibiting influenza-like symptoms in the USA and in Italy, and it was shown to replicate efficiently and transmit by direct contact in pigs, guinea pigs and ferrets, suggesting that humans could also be infected. In order to assess the emergence threat associated with IDV and to provide additional information about host range and diversity of the emerging pathogen, we started to investigate IDV circulation among pigs in France.

SPF pigs were inoculated with a bovine IDV in order to isolate an IDV strain that replicated in swine and to provide antisera. After propagation on ST cells, a swine IDV was further inoculated to SPF pigs which were hosted in direct contact to naive pigs. Whereas no clinical signs were observed neither in inoculated nor in contact pigs, IDV was isolated in nasal secretions taken from all animals. The isolates (obtained after one, two or three passages in pigs, respectively) were submitted to whole genome sequencing to assess genomic modifications that could relate to virus adaptation to the species. A specific hemagglutination inhibition assay was developed using swine IDV as an antigen and antiserum as a positive control. Sera from naïve SPF pigs were used as negative controls. The positivity threshold has been set to HI titer of 20. A duplex real-time RT-PCR amplifying the IDV PB1 gene as the target and the β-actin gene as an internal control was developed for virological diagnosis in clinical samples.

First, serological tests were conducted on 1627 archived sera collected from 76 different herds with respiratory disorders, located in Brittany, the highest pig populated area in France. Among them, 1048 sera were obtained from breeding sows sampled from January 2014 to June 2015 in 35 farrow-to-finish herds (30 sows per herd). Within this bank, 31 sera (2,9%) originating from six herds (17.2%) contained IDV-specific antibodies. In four of them, only 1 or 2 sows tested positive, with HI titers of 20. However, in two herds (A and B), 22/30 (73.3%) and 4/30 (13.3%) sera tested positive, respectively, with HI titers ranging from 20 to 160. A second bank comprised 300 sera taken from November 2013 to February 2014 on fattening pigs 16 or 22 week old (15 pigs per batch) and bred in 10 herds. All of them tested negative. In a third bank, 279 sera were obtained between 2012 and 2016 from growing pigs of different ages (from post-weaning to slaughtering) and reared in 31 farrow-to-finish herds. These animals, negative towards influenza A virus (IAV), also tested negative in IDV HI test. In a second step, a new sampling plan was implemented in herds A and B where sows were clearly detected IDV seropositive in 2014-2015. In March 2017, 2/15 sows from herd A and 1/30 sow from herd B tested positive with HI titers of 20 to 80. The 30 fattening pigs sampled in each herd were seronegative. Finally, 112 nasal swabs taken from 2015 to April 2017 on growing pigs with acute respiratory syndrome (26 herds, 3 to 10 samples per herd) and previously found IAV M gene negative, tested negative in IDV RT-PCR.

Altogether, these investigations provide the first serological evidence that breeding sows have been exposed to the virus in Brittany. Whereas IDV circulation was not highlighted among growing pigs to date, these preliminary results ask questions about IDV prevalence in this population and its potential contribution to the porcine respiratory disease complex.