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PANDEMIC INFLUENZA A H1N1V CIRCULATES IN DANISH PIGS

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Introduction

Influenza subtypes H1N1, H3N2 and H1N2 are circulating in pig populations worldwide. In March-April 2009, a novel pandemic Influenza A H1N1 virus (H1N1v) of swine origin emerged in the human population globally. The first case in pigs was reported from Canada in May 2009 and since then most pig-producing countries have reported cases. To distinguish this new influenza subtype from already circulating strains was a challenge for the veterinary diagnostic laboratories.

We report here the validation of a diagnostic strategy for specific diagnosis of the pandemic H1N1v in Danish pigs and results indicating that this virus has become established in the Danish pig population.

Materials and methods

Routinely, detection of swine influenza virus in clinical specimens is performed by real-time reverse transcriptase PCR assays (rRT-PCR) targeting the M and the NP genes. Alignment of the probe and primer sequences to H1N1v gene sequences from GenBank revealed that these assays most likely would recognize the H1N1v virus, thus not discriminating between the circulating strains and the H1N1v subtype. For specific detection of the H1N1v subtype, an rRT-PCR assay targeting the HA gene developed at the Statens Serum Institute for the diagnosis of H1N1v in humans, was validated for use on pig specimens.

Results

In silico analysis showed that the probe and primers of the H1N1v specific assay had 100% identity to published H1N1v strains and 80-95% identity to classical-swine H1N1 which do not circulate in Denmark. In contrast, there was only up to 60-70% match to the subtypes circulating in Denmark (avian-like H1N1, H3N2, and avian-like H1N2), indicating that these subtypes would not be detected by this assay. The negative outcome by this H1N1v specific assay when testing 76 Danish swine influenza virus positive samples confirmed the specificity of this assay for H1N1v. Test of dilution series of cell culture adapted strains revealed a sensitivity of 1-2 TCID50/ml.

All influenza positive samples from swine submitted to the National Veterinary Institute (NVI) in Denmark during 2009 and 2010 have been tested for H1N1v (table 1). H1N1v was not detected in any of the 2009 samples, whereas samples from 9 different herds in 2010 were positive for H1N1v.

The first two positive H1N1v cases were found in January 2010 and the diagnoses were confirmed on nasal swabs from 60 piglets taken 4 days after the initial sampling. Testing was performed with the H1N1v specific rRT-PCR assay and partial sequencing of the HA gene. In two herds diagnosed in June and August 2010, respectively, follow-up nasal swab samples from piglets taken 5 and 3 weeks after the initial diagnosis, respectively, were positive for H1N1v.

Table 1. Swine influenza virus detected in Danish pigs.

	Submissions (% positive)	H1N1 Avian-like	H3N2	H1N2dk Avian-like	Unknown*	H1N1v
2003	122/16 (13%)	8	5	2	0	
2004	95/20 (21%)	8	5	6	1	
2005	141/29 (21%)	16	4	6	4	
2006	146/27 (19%)	8	4	6	9	
2007	117/36 (31%)	11	5	6	11	
2008	293/90 (30%)	18	3	11	58	
2009	299/81 (27%)	4	3	4	68	0
2010	279/96 (34%)	1	0	1	85	9

*Subtyping unsuccessfull or not attempted beyond H1N1v subtyping.

Discussion

Human H1N1v has been shown under experimental conditions to be able to infect swine and transmit among swine (1). Human exposure is the suggested cause of many first-case country reports of H1N1v in swine around the world. The detection of H1N1v positive herds in Denmark throughout 2010, suggests that H1N1v is now being established in the Danish pig population.

Surveillance of influenza in swine in Denmark has relied exclusively on test of samples submitted to NVI with influenza diagnosis requisitioned. Due to lack of a formal surveillance programme in place, we have currently no overview of the number of H1N1v positive swine in Denmark, but the occurrence of cases even beyond the human influenza season suggests an ongoing swine to swine transmission, and is supported by prolonged detection in two follow-up investigations.

References

1. Brookes et al. (2009). Vet Rec 164, 760-1.