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Short Communication

Feral swine virome is dominated by single-stranded DNA viruses and contains a novel *Orthopneumovirus* which circulates both in feral and domestic swine

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Feral swine are known reservoirs for various pathogens that can adversely affect domestic animals. To assess the viral ecology of feral swine in the USA, metagenomic sequencing was performed on 100 pooled nasal swabs. The virome was dominated by small, ssDNA viruses belonging to the families *Circoviridae*, *Anelloviridae* and *Parvovirinae*. Only four RNA viruses were identified: porcine kobuvirus, porcine sapelovirus, atypical porcine pestivirus and a novel *Orthopneumovirus*, provisionally named swine orthopneumovirus (SOV). SOV shared ~90 % nucleotide identity to murine pneumonia virus (MPV) and canine pneumovirus. A modified, commercially available ELISA for MPV found that approximately 30 % of both feral and domestic swine sera were positive for antibodies cross-reactive with MPV. Quantitative reverse transcription-PCR identified two (2 %) and four (5.0 %) positive nasal swab pools from feral and domestic swine, respectively, confirming that SOV circulates in both herds.

Besides causing damage to the environment, agriculture and wildlife, feral swine harbour a number of pathogens which can infect livestock. Pseudorabies virus (PRV) was eradicated from domestic animals from the USA in 2004; however studies have found evidence of widespread circulation in feral swine (Gaskamp *et al.*, 2016; Pedersen *et al.*, 2013; Müller *et al.*, 2011). While not a concern for human health, PRV transmission from feral swine to other species has been documented (Cramer *et al.*, 2011). Molecular and serological surveys have found that feral swine are variably infected with porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus, vesicular stomatitis virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Lawsonia intracellularis*, *Salmonella*, *Streptococcus suis*, *Brucella suis* and *Brucella abortus* (Gaskamp *et al.*, 2016; McGregor *et al.*, 2015; Baroch *et al.*, 2015; Stephenson *et al.*, 2015; Corn *et al.*, 2009; Rodriguez *et al.*, 2000). For PCV2, feral swine appear to be an important reservoir for genetic diversity (Franzo *et al.*, 2015; Fabisiak *et al.*, 2012). Feral swine are also commonly infected with influenza A virus and the pandemic H1N1 virus of 2009 was identified in feral swine in Texas, representing a potential host and reservoir for domestic swine and zoonotic infections (Feng *et al.*, 2014; Corn *et al.*, 2009; Clavijo *et al.*, 2013).

In order to assess the ecology of viruses infecting feral swine in the USA, we performed viral metagenomic sequencing on 600 nasal swabs assembled into 100 pooled samples. Pools of six nasal swabs were assembled from nasal swabs collected from the same state and county in 2011 and 2012. Samples originated from AL (8 pools), TX (13 pools), OK (63 pools), KS (4 pools), NH (1 pool), NC (10 pools) and MO (1 pool). Metagenomic sequencing was performed as previously described using three separate MiSeq runs (Mitra *et al.*, 2016). Reads were mapped to the host (*Sus scrofa*) and unmapped reads were assembled *de novo* using CLC Genomics. Contigs were analysed by BLASTN to identify viruses using previously described criteria (Mitra *et al.*, 2016).

The genome sequence of swine orthopneumovirus strain 57 was submitted to GenBank under accession number KX364383.

A total of 16 different viruses were identified by BLASTN analysis. The feral swine virome was dominated by small, ssDNA viruses in the families *Circoviridae* and *Anelloviridae*. Torque teno virus (*Anelloviridae*) was the most commonly detected virus and was identified in 73 % of the samples. Reads mapping to other diverse anelloviruses were the second most common group of viruses detected, with 26 % of samples positive. PCV2 was identified in 13 % of the samples. Members of the family *Parvovirinae* were also frequently detected, with porcine parvovirus 1, porcine parvovirus 2, porcine parvovirus 5, porcine parvovirus 6, porcine bocavirus and porcine hokovirus detected in 2, 2, 4, 8, 4 and 1 % of the pools, respectively. Other DNA viruses detected include porcine cytomegalovirus in 5 %, porcine papillomavirus in 1 % and porcine adenovirus in 1 % of pooled samples.

Only four RNA viruses were detected. Porcine sapelovirus and porcine kobuvirus were each detected in a single pool. The recently described atypical porcine pestivirus was also identified in a single pool (Hause *et al.*, 2015). A final virus showing ~90 % sequence similarity to murine pneumonia virus (MPV; formerly pneumonia virus of mice) and canine pneumovirus (CPV) was identified in two pools. No viruses were identified in 20 % of the pooled samples. The high prevalence of DNA viruses here is in contrast to metagenomic surveys of the swine faecal virome which were dominated by RNA viruses (Shan *et al.*, 2011; Zhang *et al.*, 2014). These differences may be due to the type of sample sequenced or due to differences between the viromes of domestic and feral swine. Previously, the same viral metagenomic sequencing methodology was applied to nasal swabs collected from feedlot cattle with bovine respiratory disease where 13 of 21 (62 %) of the viruses identified were RNA viruses (Mitra *et al.*, 2016). We have also applied this methodology to nasal and faecal swabs collected from domestic swine and found that 18 of 27 (67 %) viruses detected were RNA viruses (Hause *et al.*, 2016). Consequently, we do not suspect a detection bias. We hypothesize that increased environmental stability of DNA viruses coupled with lower swine population density in feral swine as compared to domestic swine may account for the dominance of DNA viruses in the feral swine nasal virome.

To our knowledge, members of the genus *Orthopneumovirus* have not been previously identified in swine; however a serological survey of pigs in Ireland using a bovine respiratory syncytial virus (BRSV) antigen found that 41 % of sera contained antibodies that cross-reacted with BRSV (Allan *et al.*, 1998). *De novo* assembly of one of the orthopneumovirus-positive samples generated a 14 885 bp contig comprised of 19 672 reads mapping which was 93 and 91 % identical to MPV and CPV by BLASTN, respectively, suggesting this virus represents a novel swine orthopneumovirus (SOV). ORF analysis determined that the genome was organized similar to members of *Orthopneumovirus*, with genes NS1, NS2, N, P, M, SH, G, F, M2-1, M2-2 and L oriented from the 3′-end of the single, negative sense RNA molecule (Wang *et al.*, 2011). Similar to MPV, ORF overlap was identified for M2-1 and M2-2 only (Krempl *et al.*, 2005). Other members of *Orthopneumovirus*, human respiratory syncytial virus (HRSV) and BRSV, differ in having ORF overlap between M2 and L in addition to M2-1 and M2-2 (Wang *et al.*, 2011). The genome sequence for SOV strain 57 was deposited in Genbank under accession number KX364383.

Table 1. ORF composition and length for swine orthopneumovirus strain 57 (SOV), murine pneumonia virus strain PVM3666 (MPV) and canine pneumovirus strain dog/Bari/100-12/ITA/2012 (CPV). Pairwise amino acid identity between SOV ORFs and counterparts in PVM and CPV are shown

	Swine orthopneumovirus strain 57	Murine pneumonia virus PVM3666		Canine pneumovirus dog/Bari/100-12/ITA/2012		
ORF	aa*	aa	Identity to SOV (%)	aa	Identity to SOV (%)	Identity to MPV (%)
NS1	113	113	86.0	113	83.3	93.0
NS2	156	156	94.3	156	90.5	95.5
N	393	393	97.5	393	97.5	98.2
P	295	295	94.9	295	94.3	97.0
M	257	257	97.7	257	98.5	99.2
SH	92	114	90.2	92	90.2	97.8
G	414	396	88.1	414	84.1	91.9
F	537	537	95.5	537	94.8	98.3
M2-1	176	176	97.2	176	98.3	97.7
M2-2	98	98	95.0	98	93.9	97.0
L	2038	2040	96.9	2040	96.5	98.5

*aa, Amino acids.

BLASTN and BLASTP analysis of the 11 ORF nucleotide and predicted proteins of SOV, respectively, found ≥90 % identity to MPV and CPV with the exception of the attachment glycoprotein (G) which only showed 81–88 % amino acid identity to MPV and CPV. This is lower than the 90.3 ±1.7 % identity between G amino acid sequences of MPV and CPV (Glineur *et al.*, 2013). Pairwise amino acid alignments of the ORFs for SOV, CPV and MPV (Table 1) found greater than 90 % identity between the three viruses except for NS1 (83.3–86.0 % identity) and G (84.1–88.1 % identity). Similar to CPV, the G protein of SOV is 18 aa longer than its counterpart in MPV (Table 1) (Renshaw *et al.*, 2011; Glineur *et al.*, 2013). Variability in protein length has also been observed for the SH protein. Like CPV, the SH protein of SOV is predicted to have 92 aa residues (Renshaw *et al.*, 2011). MPV in contrast has SH proteins of 92, 96 and 114 aa residues depending on the isolate (Thorpe & Easton, 2005; Renshaw *et al.*, 2011). The L protein of SOV contained a 2 aa deletion, S_{1955}–N_{1956}, relative to MPV and CPV. All other protein lengths were conserved between MPV, CPV and SOV.

To explore the evolutionary relationship between SOV and other members of *Orthopneumovirus*, phylogenetic analysis was performed using MEGA 6.0 (Afonso *et al.*, 2016). Complete genome nucleotide sequences were aligned by ClustalW and phylogeny was inferred using the maximum likelihood algorithm using the best fitting General Time Reversible model with data gamma distributed. Tree topology was verified by 1000 bootstrap replicates. SOV was closely related to MPV and CPV (Fig. 1a), and the clade formed by SOV, CPV and MPV was distantly related to BRSV and HRSV. Phylogeny was also explored using a conserved region of the L gene in order to include a sequence from a recently detected bat pneumovirus (Drexler *et al.*, 2012). SOV occupied an ancestral position to MPV and CPV and together formed a sister clade to one composed of BRSV and HRSV along with an ancestral bat pneumovirus (Fig. 1b). Phylogenetic analysis was also performed on the gene encoding the G protein because it is the most variable region of the genome (Glineur *et al.*, 2013). Similar to a previous report, CPV and genetically similar feline pneumoviruses (FPVs) clustered into two distinct clades which were separate from a clade formed by MPV (Fig. 1c) (Glineur *et al.*, 2013). SOV occupied an ancestral position on the tree most closely related to MPV.

To assess the frequency of SOV infection in swine, a commercially available ELISA designed to detect antibodies to MPV (XpressBio, Frederick, MD) was modified by replacement of the secondary anti-mouse peroxidase antibody with anti-swine peroxidase antibody using the same 1 : 5000 dilution. While it is unknown whether anti-SOV antibodies will cross-react with MPV antigen, previous work found cross-reactivity between an mAb to HRSV and CPV (Renshaw *et al.*, 2010). Given the close genetic relationship between MPV and SOV, we hypothesized that cross-reaction was likely. Sera from 42 feral swine collected across the USA in 2010 and 2011 were analysed by ELISA and 13 (31 %) were antibody positive according to the manufacturer's specifications of a sample net absorbance between the positive viral antigen well and negative viral antigen well greater than 0.3, along with positive and negative mouse control serum net absorbance of greater than 0.6 and less than 0.25, respectively. Positive samples' net absorbances ranged from 0.38 to 2.43, with positive and negative mouse control serum net absorbances of 2.80 and 0.00, respectively. Likewise, 46 domestic swine serum samples originally submitted to Iowa State University in 2014 for unrelated diagnostic testing were analysed by ELISA and 15 (33 %) were positive (net absorbances 0.76–2.18). In addition, samples from two different sow farms located in North Carolina were analysed by ELISA. Sera from farm 1 were all negative (n=10), while 13 of 14 (93 %) samples from farm 2 were positive (net absorbances 0.51–1.53). Twenty-one sera collected from high health-specific pathogen free pigs were analysed as negative controls and were negative. These results suggest that SOV circulates in both feral and domestic swine, however further confirmatory serological testing using SOV antigen is needed to verify these results.

To further assess whether MPV-like viruses circulate in swine, quantitative reverse transcription PCR (qRT-PCR) was performed using a previously described assay to detect MPV due to the high genetic similarity between MPV and SOV (Percopo *et al.*, 2014). Screening of the 100 feral swine nasal swab pools by qRT-PCR identified two positives with C_t values of 31.8 and 32.5. The positive pools were the same ones identified as SOV positive by sequencing, validating the ability of the MPV PCR to detect SOV. We next designed a qRT-PCR targeting the SOV G gene (Forw, 5′-CAG AAT GCC ACA ACT CAG AAC-3′; Rev, 5-CAT TTT GAC AGG CTT CGT GG-3′; Probe, 5′-FAM-AAC CAC TAG CCT ACC TCC CAC AGA-3′). The same two pools were again the only positive samples (C_t values 30.2 and 30.3). qRT-PCR was also performed on 80 nasal swab pools (three swabs per pool) collected from domestic pigs in 2015 with acute respiratory disease and submitted to Iowa State University for diagnostic testing. Using the MPV assay, one pool was positive with a C_t value of 33.7. The assay design based on SOV sequence identified the same positive sample as the MPV assay, as well as three additional positives, with C_t values 35.3–36.5. These results, in conjunction with the serological results, suggest that SOV circulates in both feral and domestic swine at low incidence, however more thorough epidemiological testing is needed to confirm this conclusion.

In order to investigate the genetic diversity of SOV, the G gene was amplified by PCR with primers designed to amplify the complete gene (Forw: 5′-CTA TCG GAA CCG AAT GAG AC-3′; Rev: 5′-TGC CAG GAG CCA TAT TTG-3′). RT-PCR was performed for the six positive pools, however amplicons were only obtained from the two feral swine nasal swab pools. This is likely due to the very low amounts of SOV RNA present in four samples from domestic pigs. Sanger sequencing of the PCR products found that they were 99.9 % identical.

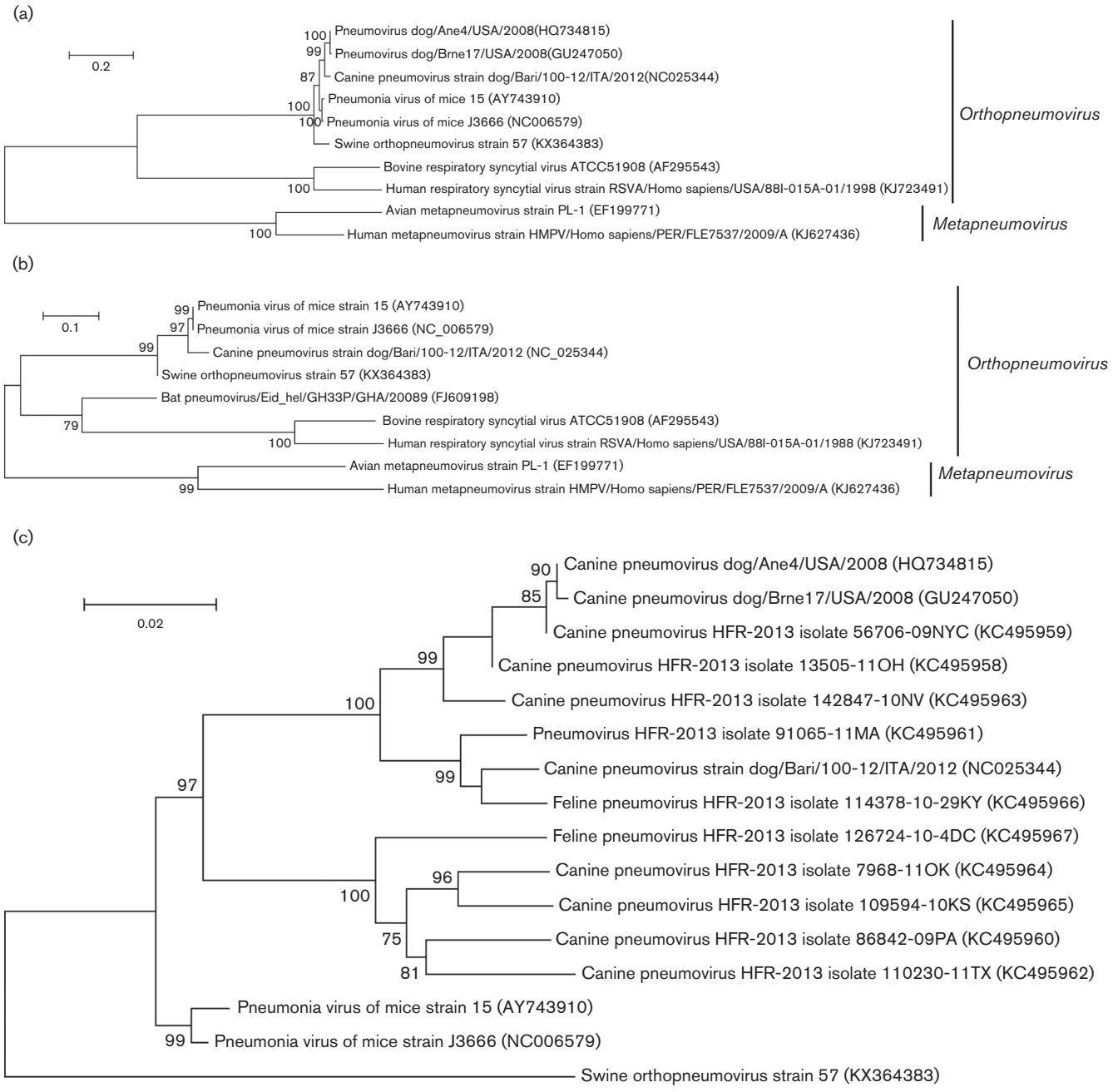

Fig. 1. Phylogenetic analysis of nucleotide sequences of (a) the complete genomes, (b) partial L gene and (c) the attachment glycoprotein (G). Phylogenetic trees were constructed by maximum likelihood analysis using the GTR+G model of nucleotide substitution with tree topology evaluated using 1000 bootstrap replicates. GenBank accession numbers are shown in parentheses. Recognized *Orthopneumovirus* species included murine pneumonia virus (pneumonia virus of mice), bovine respiratory syncytial virus and human respiratory syncytial virus (Afonso *et al.*, 2016). Recognized *Metapneumovirus* species included were avian metapneumovirus and human metapneumovirus. Strain designation in the trees is as listed in GenBank and have not been corrected for changes in taxonomical nomenclature. Bars, 0.2 (a), 0.1 (b) and 0.02 (c) nucleotide substitutions per site.

MPV causes respiratory disease in rodents and has been used as a laboratory model for severe HRSV infections (Glineur *et al.*, 2013). While initially identified in laboratory animals, MPV was recently identified in an African hedgehog with neurological disease (Madarame *et al.*, 2014). Viruses genetically and antigenically similar to MPV have also been characterized from dogs and cats (Glineur *et al.*, 2013). Investigation of an outbreak of respiratory

disease in a dog shelter isolated CPV, which was genetically and antigenically similar to MPV (Renshaw *et al.*, 2010). CPV has subsequently been identified in dogs with respiratory disease in Italy and the UK (Mitchell *et al.*, 2013; Decaro *et al.*, 2014). The finding of viruses closely related to MPV in multiple species with an etiological role in disease demonstrates the propensity of MPV-like viruses for interspecies transmission. Previous work found that another orthopneumovirus, HRSV, could infect and replicate in piglet tracheal organ cultures and caused death of ciliated epithelial cells (Fishaut *et al.*, 1978). However, further research is needed to determine if SOV infection causes disease in swine. Importantly, MPV does not readily infect humans (Brock *et al.*, 2012). The finding of SOV, a close relative of MPV, extends the host range to this group of viruses. Given the ability of MPV and CPV to cause respiratory disease, SOV should be considered for differential diagnostic testing for pigs with respiratory disease.

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References

Afonso, C. L., Amarasinghe, G. K., Bányai, K., Bào, Y., Basler, C. F., Bavari, S., Bejerman, N., Blasdell, K. R., Briand, F. X. & other authors (2016). Taxonomy of the order *Mononegavirales*: update 2016. *Arch Virol* **161**, 2351–2360.

Allan, G. M., McNeilly, F., Walker, I. W., Young, J. A., Fee, S., Douglas, A. J. & Adair, B. M. (1998). Serological evidence for pneumovirus infections in pigs. *Vet Rec* **142**, 8–12.

Baroch, J. A., Gagnon, C. A., Lacouture, S. & Gottschalk, M. (2015). Exposure of feral swine (*Sus scrofa*) in the United States to selected pathogens. *Can J Vet Res* **79**, 74–78.

Brock, L. G., Karron, R. A., Krempl, C. D., Collins, P. L. & Buchholz, U. J. (2012). Evaluation of pneumonia virus of mice as a possible human pathogen. *J Virol* **86**, 5829–5843.

Clavijo, A., Nikooienejad, A., Esfahani, M. S., Metz, R. P., Schwartz, S., Atashpaz-Gargari, E., Deliberto, T. J., Lutman, M. W., Pedersen, K. & other authors (2013). Identification and analysis of the first 2009 pandemic H1N1 influenza virus from U.S. feral swine. *Zoonoses Public Health* **60**, 327–335.

Corn, J. L., Cumbee, J. C., Barfoot, R. & Erickson, G. A. (2009). Pathogen exposure in feral swine populations geographically associated with high densities of transitional swine premises and commercial swine production. *J Wildl Dis* **45**, 713–721.

Cramer, S. D., Campbell, G. A., Njaa, B. L., Morgan, S. E., Smith, S. K., McLin, W. R., Brodersen, B. W., Wise, A. G., Scherba, G. & other authors (2011). Pseudorabies virus infection in Oklahoma hunting dogs. *J Vet Diagn Invest* **23**, 915–923.

Decaro, N., Pinto, P., Mari, V., Elia, G., Larocca, V., Camero, M., Terio, V., Losurdo, M., Martella, V. & Buonavoglia, C. (2014). Full-genome analysis of a canine pneumovirus causing acute respiratory disease in dogs, Italy. *PLoS One* **9**, e85220.

Drexler, J. F., Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., Gloza-Rausch, F., Cottontail, V. M., Rasche, A. & other authors (2012). Bats host major mammalian paramyxoviruses. *Nat Commun* **3**, 796.

Fabisiak, M., Szczotka, A., Podgórska, K. & Stadejek, T. (2012). Prevalence of infection and genetic diversity of porcine circovirus type 2 (PCV2) in wild boar (*Sus scrofa*) in Poland. *J Wildl Dis* **48**, 612–618.

Feng, Z., Baroch, J. A., Long, L.-P., Xu, Y., Cunningham, F. L., Pedersen, K., Lutman, M. W., Schmit, B. S., Bowman, A. S. & other authors (2014). Influenza A subtype H3 viruses in feral swine, United States, 2011–2012. *Emerg Infect Dis* **20**, 839–842.

Fishaut, M., Schwartzman, J. D., McIntosh, K. & Mostow, S. R. (1978). Behaviour of respiratory syncytial virus in piglet tracheal organ culture. *J Infect Dis* **138**, 644–649.

Franzo, G., Cortey, M., de Castro, A. M., Piovezan, U., Szabo, M. P., Drigo, M., Segalés, J. & Richtzenhain, L. J. (2015). Genetic characterisation of porcine circovirus type 2 (PCV2) strains from feral pigs in the Brazilian Pantanal: an opportunity to reconstruct the history of PCV2 evolution. *Vet Microbiol* **178**, 158–162.

Gaskamp, J. A., Gee, K. L., Campbell, T. A., Silvy, N. J. & Webb, S. L. (2016). Pseudorabies virus and *Brucella abortus* from an expanding wild pig (*Sus scrofa*) population in Southern Oklahoma, USA. *J Wildl Dis* **52**, 383–386.

Glineur, S. F., Renshaw, R. W., Percopo, C. M., Dyer, K. D., Dubovi, E. J., Domachowske, J. B. & Rosenberg, H. F. (2013). Novel pneumoviruses (PnVs): evolution and inflammatory pathology. *Virology* **443**, 257–264.

Hause, B. M., Collin, E. A., Peddireddi, L., Yuan, F., Chen, Z., Hesse, R. A., Gauger, P. C., Clement, T., Fang, Y. & Anderson, G. (2015). Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. *J Gen Virol* **96**, 2994–2998.

Hause, B. M., Duff, J. W., Scheidt, A. & Anderson, G. (2016). Virus detection using metagenomic sequencing of swine nasal and rectal swabs. *J Swine Health Prod.* (in press).

Krempl, C. D., Lamirande, E. W. & Collins, P. L. (2005). Complete sequence of the RNA genome of pneumonia virus of mice (PVM). *Virus Genes* **30**, 237–249.

Madarame, H., Ogihara, K., Kimura, M., Nagai, M., Omatsu, T., Ochiai, H. & Mizutani, T. (2014). Detection of a pneumonia virus of mice (PVM) in an African hedgehog (*Atelerix arbiventris*) with suspected wobbly hedgehog syndrome (WHS). *Vet Microbiol* **173**, 136–140.

McGregor, G. F., Gottschalk, M., Godson, D. L., Wilkins, W. & Bollinger, T. K. (2015). Disease risks associated with free-ranging wild boar in Saskatchewan. *Can Vet J* **56**, 839–844.

Mitchell, J. A., Cardwell, J. M., Renshaw, R. W., Dubovi, E. J. & Brownlie, J. (2013). Detection of canine pneumovirus in dogs with canine infectious respiratory disease. *J Clin Microbiol* **51**, 4112–4119.

Mitra, N., Cernicchiaro, N., Torres, S., Li, F. & Hause, B. M. (2016). Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. *J Gen Virol* **97**, 1771–1784.

Müller, T., Hahn, E. C., Tottewitz, F., Kramer, M., Klupp, B. G., Mettenleiter, T. C. & Freuling, C. (2011). Pseudorabies virus in wild swine: a global perspective. *Arch Virol* **156**, 1691–1705.

Pedersen, K., Bevins, S. N., Baroch, J. A., Cumbee, J. C. Jr, Chandler, S. C., Woodruff, B. S., Bigelow, T. T. & Deliberto, T. J. (2013). Pseudorabies in feral swine in the United States 2009–2012. *J Wildl Dis* **49**, 709–713.

Percopo, C. M., Dyer, K. D., Karpe, K. A., Domachowske, J. B. & Rosenberg, H. F. (2014). Eosinophils and respiratory virus infection: a dual-standard curve qRT-PCR-based method for determining virus recovery from mouse lung tissue. *Methods Mol Biol* **1178**, 257–266.

Renshaw, R. W., Zylich, N. C., Laverack, M. A., Glaser, A. L. & Dubovi, E. J. (2010). Pneumovirus in dogs with acute respiratory disease. *Emerg Infect Dis* **16**, 993–995.

Renshaw, R., Laverack, M., Zylich, N., Glaser, A. & Dubovi, E. (2011). Genomic analysis of a pneumovirus isolated from dogs with acute respiratory disease. *Vet Microbiol* **150**, 88–95.

Rodriguez, L. L., Bunch, T. A., Fraire, M. & Llewellyn, Z. N. (2000). Re-emergence of vesicular stomatitis in the western United States is associated with distinct viral genetic lineages. *Virology* **271**, 171–181.

Shan, T., Li, L., Simmonds, P., Wang, C., Moeser, A. & Delwart, E. (2011). The fecal virome of pigs on a high-density farm. *J Virol* **85**, 11697–11708.

Stephenson, R. J., Trible, B. R., Wang, Y., Kerrigan, M. A., Goldstein, S. M. & Rowland, R. R. (2015). Multiplex serology for common viral infections in feral pigs (*Sus scrofa*) in Hawaii between 2007 and 2010. *J Wildl Dis* **51**, 239–243.

Thorpe, L. C. & Easton, A. J. (2005). Genome sequence of the non-pathogenic strain 15 of pneumonia virus of mice and comparison with the genome of the pathogenic strain J3666. *J Gen Virol* **86**, 159–169.

Wang, L.-F., Collins, P. L., Fouchier, R. A. M., Kurath, G., Lamb, R. A., Randall, R. E. & Rima, B. K. (2011). The family *Paramyxoviridae*. In *Virus Taxonomy – Ninth Report of the International Committee on Taxonomy of Viruses*, pp. 672–685. Edited by A. M. Q. King, M. J. Adams, E. B. Carstens & E. J. Lefkowitz. London, UK: Elsevier/Academic Press.

Zhang, B., Tang, C., Yue, H., Ren, Y. & Song, Z. (2014). Viral metagenomics analysis demonstrates the diversity of viral flora in piglet diarrhoeic faeces in China. *J Gen Virol* **95**, 1603–1611.