First detection of porcine parainfluenza virus type 1 in Europe

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July 22, 2020

Abstract

A new Respirovirus, Porcine parainfluenzavirus type 1 (PPIV-1) was first identified in 2013 in Hong Kong and later in the USA. Here, we report the first detection of PPIV-1 outside these two regions. Our research group has analyzed 15–15 (3–3 piglets from five litters) nasal swab samples obtained from three-week-old piglets originating from 22 Hungarian farms altogether from which only one farm was found to be positive. Subsequently, 20–20 nasal swab samples were obtained from 2, 4, 6 and 8-week-old piglets of this farm. Virus detection by qRT-PCR showed that although all investigated age groups were positive to PPIV-1, higher number of infected animals and higher viral loads were found among 4-year-old animals. Based on the phylogenetic analyses of partial F and L genes, the 3 Hungarian strains are almost identical and are highly similar to the very first PPIV-1 genome submitted from Hong Kong in 2013, whereas the overall genetic difference compared to the recently described North American isolates was around 10%.

Keywords

genome sequencing, Respirovirus, phylogenetic analysis

Introduction

The family *Paramyxoviridae* consists of viruses that are known to affect a wide range of species including humans, pigs, cattle, poultry and companion animals. Porcine parainfluenzavirus type 1, PPIV-1 (species *Porcine respirovirus 1*) was first detected in rectal and nasopharyngeal swabs obtained from pigs in a slaughterhouse in Hong Kong (Lau et al., 2013). Upon full genome sequence analysis, the authors proposed a novel paramyxovirus. PPIV-1 is a member of the*Respirovirus* genus within the *Paramyxoviridae* family. It has a negative sense, single-stranded RNA genome of approximately 15 kilobases in length consisting of six genes (3'-N-P-M-F-HN-L-5'). These genes encode for major proteins: nucleocapsid, phosphoprotein, matrix, fusion, hemagglutinin-neuraminidase and large proteins, respectively, and two accessory proteins that are associated with the phosphoprotein (Henrickson, 2003; Lau et al., 2013; Palinski et al., 2016; Park et al., 2017, 2019). The paramyxoviral hemagglutinin-neuraminidase (HN) protein is responsible for the attachment of the virus to the target cells, whereas the fusion (F) protein directly mediates the fusion of the membranes (Morrison, 2003).

Full genome sequence analyses have revealed that the Human parainfluenzavirus 1 and the Sendai virus of mice and other rodents are the most similar viruses to PPIV-1 genetically (Palinski et al., 2016). Challenge trials in the United States revealed that inoculation of conventional and CD/CD piglets showed no mortality and minimal morbidity despite significant viral replication (Welch et al., 2018). In a recent study, the same research group found a significant reduction in viral genome copies in BALF, tracheal swab and turbinate in PPIV-challenged animals, that were previously vaccinated with an RNA particle vaccine (Welch et al., 2020).

1. **Materials and Methods,**
2. **Sample collection and viral nucleic acid extraction**

The presence of PPIV-1 has only been reported in Hong Kong and the United States so far, and the aim of our work was to screen large scale pig herd in Hungary for the presence of the virus. We have analyzed 15–15 (3–3 piglets from five litters) nasal swab samples obtained from three-week-old piglets originating from 22 farms altogether. The study was conducted in compliance with the provisions of Directive 2010/63/EU, Hungarian Act XXVIII/1998 and the Hungarian Ministerial Decree No. 40/2013. (II.14.).

The nasal swabs were vortexed in PBS, and RNA was isolated in a QIAcube automatic instrument using QIAmp cador Pathogen Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

Detection and phylogenetic analyses of PPIV-1 in clinical samples

Endpoint PCR for the first round detection of respiroviruses was performed by amplifying a 555 bp fragment of the L gene using degenerate primers (5'-GACTCATCTACTAACGGNTAYGARA-3' and 5'-CACAAACATCTTGCTACTWATDATNGT-3') described in Lau et al. (2013). Real-time quantitative RT-PCR to detect the N gene of PPIV-1 and subsequent melting point analysis was performed in a Rotor-Gene Q instrument (Qiagen) using QuantiNova SYBR Green RT-PCR Kit (Qiagen), with specific primers (5'-TACAATATATGTGGGTGATCCTTACT-3' and 5'-GCCTGAATCTTCATGATCTTCTAAA-3') as described previously in Lau et al. 2013. Positive control RNA obtained from a PPIV-1 isolate was kindly provided by Phillip C. Gauger (Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University). The L gene product used for diagnostic PCR and a partial F gene segment was sequenced by the use of primers and conditions described in Park et al., 2019.

Briefly, PCR products were subjected to gel electrophoresis and amplicons with suitable length were cut out of the gel and purified by the Qiagen Gel Extraction Kit (Qiagen). Bidirectional Sanger sequencing reaction was performed with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Ljubljana, Slovenia) with the corresponding primers, and the capillary electrophoresis was carried out by a commercial provider (Hungarian Natural History Museum).

The obtained sequences were proofread, assembled using E-INS-i method of the online software mafft version 7 (Katoh & Toh, 2008) and aligned against available PPIV-1 genomes, and reference respirovirus sequences downloaded from the GenBank. Maximum Likelihood (ML) analyses were conducted using MEGA version X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) .

Results and Discussion

The initial respirovirus screening of the 22 herds revealed the presence of positive samples in the case of one herd. In order to gather more information on the within-herd infection dynamics we performed cross-sectional nasal swab sampling directly from that herd: 20–20 samples were obtained from 2, 4, 6 and 8-week-old piglets. The samples were analyzed by real-time quantitative RT-PCR. Results of the second-round PCR are shown in Figure 1. Out of 20 samples obtained from the 4-week-old age group, we found 13 positives, 8 in the 6-week-old group and 5 in the 8-week-old group. Only one positive sample was found among the 2-week-old animals. The highest viral burden was observed among the 4-week-old animals, where the mean Cq value was 28,39 (±5,09), but the differences between the groups were not statistically significant (Figure 1).

Melting curve analysis has been conducted after every real-time run and surprisingly there was a 2.5 °C difference between the melting point of the positive control (76 °C) and the positive samples (73.5 °C), suggesting multiple nucleotide differences between the two amplicons.

After sequencing three positive samples (GenBank accession numbers: MT765271–3) with low Cq values, initial BLAST analysis of the partial L and F gene sequences confirmed the presence of PPIV-1 in our samples. The sequences obtained were almost identical to each other with less than 5 nucleotide differences among each other. Maximum Likelihood trees constructed with a 1636 nucleotide long part of the F gene and the

555 nucleotide long part of the L gene revealed almost identical topology (Figure 2). Our sequences grouped together with other PPIV-1 sequences supported by relative high bootstrap values. Pairwise similarities were calculated with every available PPIV-1 sequence and the highest values were found in the case of two sequences originating from Hong Kong submitted by Lau et al. (2013): 95.6% (acc. no.: JX857410) and 92.2% (acc. no.: JX857409). All other PPIV-1 sequences, including the recently submitted ones from the USA, were less than 92% similar.

Interestingly, the partial F and L sequences obtained from our samples showed the highest similarity values to the very first PPIV-1 genomes submitted from Hong Kong in 2013, whereas the overall genetic difference compared to the recently described North American isolates was around 10%. The genetic difference was already visible during the real-time PCR investigations where the melting curve analyses revealed 2.5 °C difference between our samples and the positive control originating from the USA. The pairwise alignment of the amplicons' sequences revealed 6 mismatches 5 of which resulted in smaller GC content of our samples, explaining the decreased melting point.

To the authors' knowledge, this is the first report of porcine parainfluenzavirus 1 outside Hong Kong and the United States of America. Out of the 22 herds screened we only found positive animals in one herd, where the second-round sampling revealed significant virus circulation among the young animals. Both the number of positive nasal swabs and the viral copy numbers were the highest among the 4-week-old animals. Elder animals had fewer positive samples and lower viral amounts.

These results are in harmony with previous data where 37.2% of all the PPIV-1positive cases found at the Veterinary Diagnostic Laboratory of the Iowa State University originated from nursery units (Gauger et al., 2018). On the contrary, however, among the 22 screened, we only found a single herd infected with the virus whereas it seems to be widespread in the USA (Gauger et al., 2018; Palinski et al., 2016). We could not, however, successfully identify the source of infection in our case, as the farm had no prior contact to pigs of personnel coming from either USA or Hong Kong, China.

Acknowledgments

Gyula Balka was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the 17896-4/2018/FEKUTSTRAT grant of the Hungarian Ministry of Human Capacities. The funding sources had no role in study design, data collection and analysis or the decision to publish the work.

Conflicts of interest

The authors have no conflicts of interest regarding the research, authorship, and/or publication of this article.

Availability of data

The data that support the findings of this study are openly available in GenBank at*https://www.ncbi.nlm.nih.gov/genbank/*.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The study was conducted in compliance with the provisions of Directive 2010/63/EU, Hungarian Act XXVIII/1998 and the Hungarian Ministerial Decree No. 40/2013. (II.14.).

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Figure legends

Figure 1

Boxplot showing the mean qPCR Cq values of the nasal swab samples obtained from the different age groups. The numbers above the boxes indicate the number of positives among the 20 sampled animals.

Figure 2

Phylogenetic tree displaying relatedness of the Fusion polyprotein coding gene of representative Respirovirus and PPIV-1 sequences from GenBank and Hungarian strains (marked with a black rectangle). ML bootstrap support values ([?] 70) are shown as percentages above branches. The scale bar indicates 0.50 expected changes per site per branch. Strains displayed on the phylogenetic tree are coded like accession number/name of strain.