First detection of porcine respirovirus 1 in Germany and in the Netherlands

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Abstract

PRV1 was first detected in deceased pigs from Hong Kong in 2013. It has since been detected in the USA, Chile and most recently in Hungary. Information on the pathogenicity and global spread is sparse, however it has been speculated to play a role in the porcine respiratory disease complex. In an effort to investigate the porcine virome, we screened 53 pig samples from 29 farms using SMg within the Dutch/German border region. In five farms we detected PRV1. qPCR confirmed the presence of the virus in 2 of these farms and found an additional 6 positive farms. Phylogenetic analysis found the closest match to the first detected PRV1 strain in Hong Kong. The Dutch/German region represents a major area of pig farming within Europe and could provide important information on the characterization and circulation of porcine viruses, such as PRV1. Together with the recent detection of PRV1 in Hungary, these findings suggest widespread of PRV1 in Central Europe, highlighting the need for further research on persistence, pathogenicity and transmission in Europe.

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Running title: PRV1 in Central Europe

Summary

PRV1 was first detected in deceased pigs from Hong Kong in 2013. It has since been detected in the USA, Chile and most recently in Hungary. Information on the pathogenicity and global spread is sparse, however it has been speculated to play a role in the porcine respiratory disease complex.

In an effort to investigate the porcine virome, we screened 53 pig samples from 29 farms using SMg within the Dutch/German border region. In five farms we detected PRV1. qPCR confirmed the presence of the virus in 2 of these farms and found an additional 6 positive farms. Phylogenetic analysis found the closest match to the first detected PRV1 strain in Hong Kong. The Dutch/German region represents a major area of pig farming within Europe and could provide important information on the characterization and circulation of porcine viruses, such as PRV1. Together with the recent detection of PRV1 in Hungary, these findings suggest widespread of PRV1 in Central Europe, highlighting the need for further research on persistence, pathogenicity and transmission in Europe.

Keywords : Porcine parainfluenza 1, Porcine respirovirus 1, Europe, Germany, the Netherlands, Shotgun metagenomics sequencing

Introduction

Viruses that belong to the Paramyxoviridae family have been associated with respiratory symptoms in farm animals and may cross host species barriers (Welch et al., 2017). Porcine respirovirus 1 (PRV1), also referred to as porcine parainfluenza 1, belongs to the Respirovirus genus within the Paramyxoviridae family. While the pathogenicity of PRV1 in pigs is still unclear, it has been isolated from pigs with respiratory symptoms (Lau et al., 2013). Moreover, it has been detected in co-infections with swine influenza virus (SIV) and porcine reproductive and respiratory syndrome virus (PRRSV), which suggests it could also play a role within the porcine respiratory disease complex (Welch et al., 2017). However, information on the global spread and epidemiology is sparse. Apart from China (Lau et al., 2013), PRV1 was only detected in the United States of America (USA) in 2016 (Palinski et al., 2016), in Chile in 2020 (Aguero et al., 2020) and very recently in Hungary also in 2020 (Denes et al., 2020).

Here we report the first detection of PRV1 in Germany and in the Netherlands using shotgun metagenomics sequencing (SMg) and qPCR in blood serum (BS), nasal swab (NS), and oral fluid (OF) samples. In an effort to investigate the porcine virome, we screened 53 pig samples from 29 farms using SMg within the Dutch/German border region. In five farms we detected PRV1. qPCR confirmed the presence of the virus in 2 of these farms and found an additional 6 positive farms. Phylogenetic analysis found the closest match to the first detected PRV1 strain in Hong Kong (Lau et al., 2013). The Dutch/German region represents a major area of pig farming within Europe and could provide important information on the characterization and circulation of porcine viruses, such as PRV1.

Materials and Methods

Samples were first pre-selected based on a positive qPCR result for SIV (VetMAX-Gold SIV Detection Kit, Life Technologies) and PRRSV (Virotype, Hilden, Qiagen), which are clinically relevant porcine pathogens. Overall, 34 BS from 10 farms, 4 NS from 4 farms and 15 OF samples from 15 farms (total n=53 samples) from pigs within the Dutch/German border region were selected and screened using SMg to gain an insight into the porcine virome. Next, nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), including in-column DNase digestion.

Complementary DNA for SMg analysis was synthesized, as described previously (Kafetzopoulou et al., 2018). Short-read sequencing (SRS) libraries were generated using the KAPA HyperPlus Kit (Roche, Basel, Switzerland). Additionally, viral enrichment with oligonucleotide bait probes was performed on a selected sample using the SeqCap ViroCap share developer panel (Roche, Basel, Switzerland). SRS libraries were

sequenced on a NextSeq 500, generating 76 base pair-reads (Illumina, San Diego, CA, USA). Furthermore, long-read sequencing (LRS) libraries were generated using the Ligation Sequencing Kit (SQK-LSK109) (Oxford Nanopore Technologies (ONT)) and sequenced on a MinION device (ONT). Lysis buffer served as a negative control.

To obtain rapid viral detection and taxonomic identification, raw-reads were uploaded onto Taxonomer (IDbyDNA, San Francisco, CA, USA). Following the detection of PRV1, sequenced reads were first trimmed, then mapped against a PRV1 database derived from available complete or near complete genomes on GenBank (n=10, 27/08/2020) using CLC Genomics Workbench v.20.0.4 (CLC) (Qiagen, Hilden, Germany). Resulting consensus sequences had to cover >300 bp of the respective reference genome to be considered valid and were subsequently confirmed and characterized by NCBI BLAST. Assembly was only performed on the BS sample as it had a high abundance of PRV1 reads, using the best PRV1 hit as guidance on CLC. To create the PRV1 phylogenetic tree, the near-complete assembled PRV1 genome from this study and PRV1 genomes from GenBank (n=10) were aligned with MAFFT (Katoh et al., 2002). Phylogenetic trees were inferred from the alignments with the Maximum Likelihood approach implemented in RAxML v8.2.10 (Stamatakis, 2014) under the General Time Reversible (GTR-G) substitution model (Stamatakis, 2014) and bootstrap of 1000 replicates. A phylogenetic analysis of the L (RdRp Polymerase) and the F (Fusion protein) genes was also performed.

For a rapid follow-up analysis, a PRV1 specific qPCR was designed to test the 53 samples, where sufficient sample material was remaining (n=50) along with an additional 17 OF samples from 17 farms in the same region. Briefly, qPCR primers were designed by aligning the available PRV1 sequences on GenBank (n=21, 01/10/2020) using Geneious Prime software v2020.0.5. Primers targeting the nucleocapsid gene were designed: 5'-GCACCACCACCTCCTCTATT-3', 3'-GCCAAAATGGCAGGGTTRTT-5', probe: TGCTCTCACTCCTTTTAGAACTAAATGTG. qPCR was performed using the Brilliant II qRT-PCR Master Mix 1-Step, (Agilent Technologies, Texas, USA) with a total volume of 25 µL with the following conditions: 50°C for 45 min, 95°C for 10 min, followed by 45 cycles of 95°C for 20 s, 55°C for 60 s, and 72°C for 30 s.

Results and Discussion

In total, PRV1 was detected in 5/53 samples (9.43%) from 5 different German farms using SMg directly from the sample (Table 1). PRV1 was not detected in the negative control. Four out of the 5 PRV1 sequences (Figure 1) were closely related to the Chinese PRV1 strain S033N (GenBank accession: JX857410.1), which was first isolated from deceased pigs in Hong Kong in 2013 (Lau et al., 2013). Interestingly, phylogenetic analysis from the 3 available PRV1 strains from Hungary (Dénes et al., 2020) also showed the closest similarity to strain S033N, from partial F and L sequences (Figure S1). This could indicate the S033N strain could be the most prominent strain in Central Europe. In comparison, sample NS-2 showed the closest identity to another Chinese PRV1 strain, gd2018 (GenBank accession: MK395271.1), isolated in 2018.

The untargeted nature of SMg allows the detection of all viruses within one single assay, including unexpected viruses such as PRV1. However, challenges still exist with sensitivity (Greninger, 2018). This becomes apparent even in samples with a high quantity of PRV1 sequence reads, such as in sample BS-1. As we could not recover the complete-genome in our five samples by using a direct shotgun sequencing approach, we selected the sample with the highest PRV1 reads (BS-1) to apply viral enrichment using ViroCap, in an effort to obtain the complete-genome. ViroCap has been reported to increase sensitivity of virus detection in human samples (Wylie et al., 2018). ViroCap was subsequently applied to BS-1, which resulted in a 22.8-fold increase in PRV1 sequence reads and enabled the generation of a near-complete genome sequence (15,345 bp) (GenBank accession: MT995732) and, subsequent, phylogenetic analysis.

Following the detection of PRV1 using SMg, a rapid follow-up analysis was performed using qPCR. After performing a PRV1-qPCR on 4 of the 5 samples which had a PRV1 detection using SMg, only 1 had a Ct value lower than 30 (Table 1). A Ct value of 44 was found for sample BS-1, however this was considered negative. This could be due to primer or probe – template mismatches. Divergence of the template sequence in targeted PCR can result in limited sensitivity due to primer-binding kinetics (Quick et al., 2017). Indeed,

a primer mismatch was detected for the near-complete genome (sample BS-1) in the 3'-5' primer, as well as 3 mismatches in the probe. As few sequences are available for PRV1, viral diversity is difficult to predict leading to problems in finding a sufficient conserved region to design primers and probes.

On the other hand, 3 OF samples which were negative for PRV1 using SMg, had a positive Ct value following qPCR (Table S1) highlighting a potential increased sensitivity of qPCR. Screening of the remaining 17 OF samples from 17 farms that were solely analyzed by qPCR, revealed a further 3 PRV1 positive farms (Table S1). These findings highlight the need to obtain further PRV1 complete-genome sequences in order to refine PRV1 qPCR primers for future surveillance. As demonstrated in this study, oligonucleotide capture probes can be used to help generate near-complete genomes.

SMg was unable to detect several co-detected viral pathogens which were previously detected by qPCR prior to initial sample selection. Indeed, the PRRSV (Ct 30 and 32) and SIV (Ct 36 and 37) co-detections were unable to be recovered from the OF samples. However, SMg was able to recover part of the genome for other co-detections; PRRSV (Ct 26) was recovered from the BS sample and SIV (Ct 19 and 21) was recovered from the NS samples (Table 1). Co-infections with PRRSV and/or SIV have been reported previously (Lau et al., 2013; Welch et al., 2017) but the role of these co-infections in the porcine respiratory disease complex remains to be ascertained (Welch et al., 2017; Park et al., 2019). It must be noted that samples in this study were pooled, and as such, viruses/co-infections cannot necessarily be linked to an individual animal. Nevertheless, information gathered from pooled samples can provide data on what is circulating within a farm.

Previously detected PRV1 were obtained from OF, NS and lung samples, with the upper respiratory tract being suggested to be the most suitable sampling site for detection (Lau et al., 2013; Park et al., 2019; Agüero et al., 2020). We detected the highest PRV1 read count in a BS sample, which suggests that BS could be evaluated as a possible suitable sample matrix to screen for PRV1 in the future.

In conclusion, to the best of our knowledge, we report the first detection of PRV1 in Germany and in the Netherlands, as well as the first near-complete genome in Europe. Moreover, this is the first detection of PRV1 using an untargeted and targeted metagenomic sequencing approach directly from the sample. As the PRV1 sequences from Hungary and Germany were closely related to strains previously found in China, it suggests that there may have been a PRV1 transmission between Europe and China. Furthermore, as PRV1 was detected in pigs from 11 different farms (5 using SMg and 6 using qPCR), it could confirm its circulation in Central Europe. Additional research is required to determine the extent of dissemination of PRV1 in Europe, to determine the relevance of this virus in the porcine respiratory disease complex and its ability to cross host species barriers.

Data availability

The near-complete PRV1 sequence has been deposited under the GenBank accession: MT995732.

Conflicts of interest

John W. A. Rossen is employed by IDbyDNA. Silke Peter consults for IDbyDNA. This did not influence the interpretation of reviewed data and conclusions drawn nor on the drafting of the manuscript, and no support was obtained from them. All other authors declare no conflict of interest.

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Ethical Approval

The animal samples used for this study were collected within the Food Pro-tec-ts project which has been classified as an animal study and was approved on the 22.09.2017 by the respective state office for nature, environment and consumer protection (file reference: 84.02.05.40.17.079).

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Table 1. Characterization of SMg-PRV1 positive samples.

Sample & platform	Best BLAST reference (length)	Genome coverage (%)	Identity (%)	Average sequence coverage	PRV1 qPCR	Co-detection (qPCR)	Date of sampling	Countr of Orig
BS[1]-1-Illumina	SN0330 (15,396 bp)	99.7	96.0	9,793	Ct 44	PRRSV[4] (Ct 26)	10/2018	Germar
OF[2]-1-ONT	SN0330 (15,396 bp)	33.0	96.2	3	- [6]	SIV[5] (Ct 36) PRRSV[4] (Ct 30)	06/2017	Netherl
OF[2]-2-Illumina	SN0330 (15,396 bp)	5.5	89.5	1	Ct 29	PRRSV[4] (Ct 32) SIV[5] (Ct 37)	06/2017	Netherl
NS[3]-1-ONT	SN0330 (15,396 bp)	7.6	95.1	2	Negative	SIV[5] (Ct 21)	10/2018	Germar
NS[3]-2-Illumina	Gd2018 (15,396 bp)	2.4	97.0	1	Negative	SIV[5] (Ct 19)	10/2018	Germar

[1]BS, blood serum; [2]OF, oral fluid;[3]NS, nasal swab; [4]Insufficient nucleic acids left for qPCR; [5]PRRSV, porcine reproductive and respiratory syndrome virus; [6]SIV, swine influenza virus.

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