Transboundary and Emerging Diseases

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RAPID COMMUNICATION

Outbreak of Porcine Epidemic Diarrhea Virus in Portugal, 2015

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Summary

An outbreak of porcine epidemic diarrhea virus (PEDV) in the South of Portugal in January 2015 and the spread of PEDV northwards in the territory are described. Comparative analysis of the amplified sequences showed a very high (99.0%) identity with the PEDV variant most recently reported in the United States and also show complete (100%) identity to the strains recently reported in Germany, supporting the hypothesis that a unique strain is currently circulating in Europe. The origin of this PEDV variant still needs to be elucidated and further studies in the remaining European countries may contribute to the knowledge.

Introduction

Porcine epidemic diarrhea (PED) is an acute and highly con-tagious enteric disease of pigs. Typical clinical symptoms of PED include watery diarrhea, vomiting, dehydration, and Porcine epidemic diarrhea (PED) is an acute and highly con-tagious enteric disease of pigs. Typical clinical symptoms of PED include watery diarrhea, vomiting, dehydration, and Porcine epidemic diarrhea (PED) is an acute and highly con-tagious enteric disease of pigs. Typical clinical symptoms of PED include watery diarrhea, vomiting, dehydration, and Porcine epidemic diarrhea virus (PEDV; family *Coronaviridae*, subfamily *Coronavirinae*, genus *Alphacoronavirus*) is a highly contagious virus responsible for enteric disease in swine characterized by an acute onset of symptoms including severe watery diarrhea, vomiting, dehydration, and high mortality in suckling piglets (ICTV, 2012; Song and Park, 2012).

Upon its first description in 1971 in the United Kingdom, this disease was initially termed "epidemic viral diarrhea" due to the quick spread across Europe (Wood, 1977; Song and Park, 2012). Since then PEDV has caused substantial economic losses, predominantly in Asia, but in May 2013 a new PEDV variant has emerged and rapidly spread in the US (Stevenson et al., 2013). From this time to early June 2014, PEDV outbreaks had been reported in 33 states of the United States causing approximately 7 million piglet deaths, and resulting in severe economic losses to the swine industry (US Department of Agriculture, 2015). In early 2014, a novel variant of PEDV (OH 851) was identified in Ohio, containing insertions and deletions in the S gene (S INDEL), causing mild clinical signs and lower mortality rates in suckling piglets (Wang et al., 2014). More recently, an epidemic of severe watery diarrhea in Southern Germany with typical clinical signs of PEDV was reported and found to be caused by a novel PEDV, closely related to the US strain OH851, causing concern regarding on the possible circulation of a novel more virulent strain (Hanke et al., 2015). In the present work we describe an outbreak of severe watery diarrhea in swine caused by PEDV in Portugal, early 2015, and the spread northwards in the country.

Materials and Methods

Source and collection of samples

In January 2015 a pig farm in the South region of Portugal reported diarrhea in all animals 2 days after introducing new animals from a different producer. Diarrhea lasted for 1 week and a high mortality in piglets was observed. From January to April 2015 another 43 pig farms (4 farms from the South and 39 farms from the Center of Portugal) have reported similar epidemic diarrheas. A total of 84 fecal samples were collected from all 44 farms and submitted to analysis.

Detection and characterization of PEDV

Stools were diluted (10% phosphate buffered saline), and viral nucleic acid was extracted from centrifuged stool suspensions using High Pure RNA isolation kit (Roche Applied Sciences, Mannheim, Germany) and NucleoSpin RNA Virus (Machery Nagel, Haerdt, France). Nucleic acids were tested for the presence of PEDV using a commercial real-time RT-PCR kit according to the manufacturer's instructions that targets the nucleocapsid protein gene (N gene) (ViroReal® Kit PEDV; Ingenetix, Vienna, Austria).

A total of four samples positive for PEDV RNA by real-time RT-PCR (two from a farm in the South and two from a farm in the Center of Portugal) were further tested by conventional PCR using primers S1-univ-F (5′- TAC TTA CAA CTC CAC TG TTT -3′) and S1-univ-R (5′- CCA TTG ATA GTA GTG TCA GA -3′) that amplify a 440 bp region of the S protein. For the cDNA synthesis, 0.2 μl of the S1-univ-R primer (10 μM), 1 μl of dNTP (10 mM), 6.8 μl of H_2O and 5 μl of RNA were incubated for 5 min at 65°C and placed on ice before being added 1 μl of SuperScript® II Reverse Transcriptase (200 U/μl) (Invitrogen), 1 μl of RNasin (40 U/μl) (Promega), 1 μl 0.1 M dithiothreitol and 4 μl first strand buffer (5×) (Invitrogen). The RT mix was incubated for 60 min at 50°C. For the PCR, 2 μl of cDNA were added to 2 μl of Clontech buffer (10×), 0.4 μl 50× dNTP, 0.4 μl 50× polymerase mix, 1 μl of both S1-univ-F (10 μM) and S1-univ-R(10 μM) primers and 13.2 μl of H20. The PCR reactions were carried out under the following program: 5 min at 94°C followed by 40 cycles of 30 s at 94°C, 30 s at 54°C, 30 s at 68°C and a final extension of 10 min at 68°C. After electrophoresis appropriately sized bands (440 bp) were excised and purified using Gel DNA Recovery kit (Zymo Reserch, CA, USA), and sequenced in both directions. Sequence editing and multiple alignments were performed using Bionumerics version 6.6 (Applied Maths, Kortrijk, Belgium). The PEDV positive samples selected for genetic characterization were also screened for rotavirus group A (ViroReal® rotavirus (A); Ingenetix, Vienna, Austria) and transmissible gastroenteritis virus (TGEV) (ViroReal® TGEV; Ingenetix) accoding to the manufacturers instructions.

Results and Discussion

In this study we describe an outbreak of severe watery diarrhea in swine in Portugal, early 2015, with a rapid spread northwards in the territory. From the 44 pig farms that reported severe watery diarrheas, PEDV RNA was detected in 32 (72.7%) by real-time RT-PCR. All 5 farms from the South were positive, as well as 27 from the 39

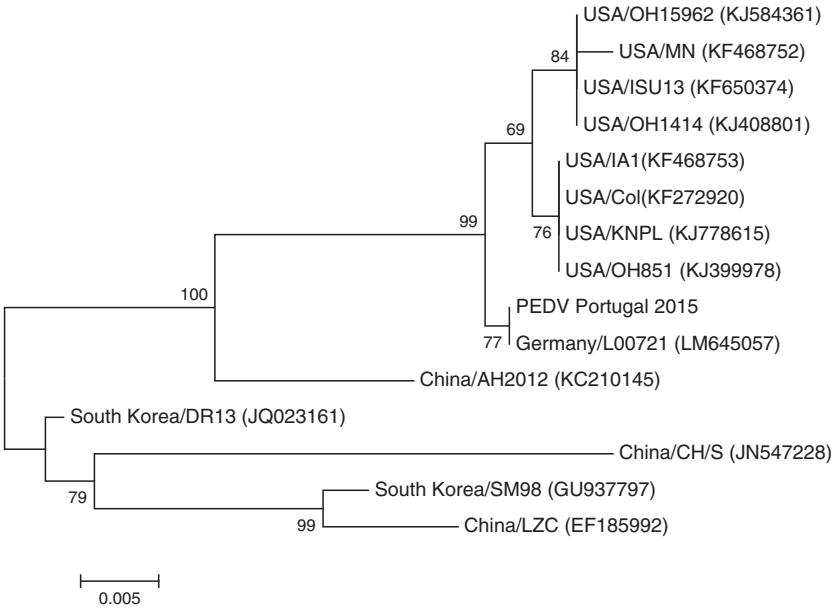

Fig. 1. A neighbor-joining tree (Jukes-Cantor model) of 440 nucleotide (nt) of the spike protein gene of the porcine epidemic diarrhea virus (PEDV) isolates obtained in this study (PEDV Portugal 2015). Phylogenetic analysis was performed using MEGA version 6.0 software (Tamura et al., 2013).

farms from the Center. From the total 84 studied diarrheic stools, PEDV was found in 55. Four of these samples (two were from a farm in the South and two were from a farm in the Center of Portugal) were tested by conventional RT-PCR and amplified products (440 bp) were subjected to sequencing in order to obtain information about their genetic relatedness with PEDV reference strains (Fig. 1). Comparative analyses of these amplicons showed that all amplified sequences were identical (100%), showing that a single strain was responsible for this outbreak. Analysis also showed that the amplified sequences share a very high (99.0%) identity with the new PEDV variant OH851 (GenBank accession no. KJ399978) of the United States that affects sows (Wang et al., 2014). Interestingly, the amplified sequences showed to be identical (100%) to the strains recently reported in Germany PEDV/GER/L00719/2014 (GenBank accession no. LM645058) and PEDV/GER/L00721/2014 (GenBank accession no. LM645057). The PEDV positive samples selected for genetic characterization showed to be negative for rotavirus group A and TGEV.

In conclusion, PEDV infection was confirmed in a pig herd in the south of Portugal in January 2015 and found to be spreading northwards affecting a total of 32 farms. Comparative analyses of a 440 bp region of the spike protein gene showed that the isolates were identical to the ones reported in 2015 in Germany. The findings of an identical PEDV strain in the South of Europe, substantially distant from Germany where the novel strains have been reported seem to indicate that a single strain (different from the American) is circulating in Europe. As with Germany, in Portugal there is no active surveillance scheme for PEDV, hence we cannot state with confidence that this strain has not been circulating in Portugal for a longer time. Also, the origin of this PEDV variant still needs to be elucidated and further studies in the remaining European countries may contribute to the knowledge. The re-emergence of PEDV in Europe with altered virulence seems to be a relevant issue in swine health and may justify active surveillance by the official entities.

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