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Porcine epidemic diarrhea virus (PEDV) introduction into a naive Dutch pig population in 2014

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is the highly contagious, causative agent of an economically important acute enteric disease in pigs of all ages. The disease is characterized by diarrhea and dehydration causing mortality and growth retardation. In the last few decades, only classical PEDV was reported sporadically in Europe, but in 2014 outbreaks of PEDV were described in Germany. Phylogenetic analysis showed a very high nucleotide similarity with a variant of PEDV that was isolated in the US in January 2014. The epidemiological situation of PEDV infections in the Netherlands in 2014 was unknown and a seroprevalence study in swine was performed. In total, 838 blood samples from sows from 267 farms and 101 samples from wild boars were collected from May till November 2014 and tested for antibodies against PEDV by ELISA. The apparent herd prevalence of 0.75% suggests that PEDV was not circulating on a large scale in the Netherlands at this time. However, in November 2014 a clinical outbreak of PEDV was diagnosed in a fattener farm by PCR testing. This was the first confirmed PEDV outbreak since the early nineties. Sequence analyses showed that the viruses isolated in 2014 and 2015 in the Netherlands cluster with recently found European G1b strains. This suggests a one event introduction of PEDV G1b strains in Europe in 2014, which made the Netherlands and other European countries endemic for this type of strains since then.

1. Introduction

Porcine epidemic diarrhea (PED) is an economically important acute enteric disease in pigs of all ages. The disease is characterized by diarrhea and dehydration causing mortality - particularly in neonatal piglets – and growth retardation. The causative agent is porcine epidemic diarrhea virus (PEDV), which is an enveloped, positive single-stranded RNA virus, belonging to the family of *Coronaviridae* (Pensaert and de Bouck, 1978). The genome of PEDV is approximately 28 kb long and about two-third encodes for non-structural proteins and one-third of structural proteins (Kocherhans et al., 2001). Among these proteins, the main research interest is focused on the Spike (S) gene and its glycoprotein product S, mediating receptor binding and membrane fusion (Li et al., 2016). Although only one serotype has been described, phylogenetic studies of the S gene showed that PEDV can be genetically separated into two groups: genogroup 1 (G1) and genogroup 2 (G2). Each genogroup can be further divided into subgroups 1a and 1b, and 2a and 2b, respectively (Lee, 2015).

Classical PED, now grouped G1a, was first recognized as a severe swine enteric disease separate from Transmissible Gastro Enteritis (TGE) in the United Kingdom in 1971 and first described in Belgium in 1978 (Pensaert and de Bouck, 1978). In the eighties and nineties, the virus was detected in many countries in Europe including the Netherlands and from Europe PEDV spread to Asia, where it caused large outbreaks with considerable losses in the pig industry (Song and Park, 2012). Until 2013, North America was considered to be free of PEDV infections (Cima, 2013), but in that same year highly virulent strains of PEDV emerged in the United States of America (US), causing diarrhea, vomiting and loss of appetite in pigs of all age groups and up to 100% of mortality in suckling piglets (Chen et al., 2014; Huang et al., 2013; Stevenson et al., 2013). This strain, typed as G2b, rapidly spread across the US, Canada, Mexico and several countries in South America (Vlasova et al., 2014).

In the last few decades, only classical PEDV (or G1a) was reported sporadically in Europe (Alborali et al., 2014; Martelli et al., 2008). In 2014, outbreaks of PEDV were described in Germany and phylogenetic

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analysis showed a very high nucleotide similarity with a variant of PEDV (OH851) containing nucleotide insertions and deletions in the S gene (S-INDEL) that was isolated in the US in January 2014 (Hanke et al., 2015; Stadler et al., 2015). This variant, typed as G1b, caused mild clinical signs and lower mortality rates in suckling piglets compared to other circulating PEDV G2b strains in the US (Wang et al., 2014). Since the reports of outbreaks in Germany, more reports about outbreaks of this particular S-INDEL virus in several European countries have been published, among which France, Belgium, Spain, Portugal and Austria (EFSA, 2016; Grasland et al., 2015; Mesquita et al., 2015; Steinrigl et al., 2015). This suggests that this mild PEDV variant is circulating in Europe since the beginning of 2014.

The aim of this study was to determine the status of PEDV in the Netherlands with a serological survey and to investigate the first PEDV outbreak in the Netherlands since the early nineties.

2. Materials and methods: serological survey

2.1. Calculation of number of required samples and farms

The number of required blood samples from animals and farms to estimate the seroprevalence of PEDV in Dutch sow herds was calculated based on the following assumptions: PEDV is highly contagious and no vaccination against this virus was carried out in the Netherlands. As a result, it was expected that, if PEDV was present in a sow herd, the within herd prevalence would be very high (Bertasio et al., 2016; Goede et al., 2015). The required number of blood samples per herd was calculated using WinEpiscope 2.0 (Thrusfield et al., 2001). To detect infection in a herd with 95% probability, an estimated within herd prevalence of 70%, and an average herd size of 464 sows per farm (WUR, 2016), three blood samples per farm were required. In 2014, there were approximately 2061 sow farms in the Netherlands (WUR, 2016). In order to show with a high probability (95%), that less than 1% of the Dutch farms (N = 20) were infected, 286 farms would need to be tested (Thrusfield et al., 2001). Herds were randomly selected stratified by pig density per province to represent the total Dutch sow herd population (Fig. 1A and B). Statistical analyses were performed using STATA/SE version 14.1 software (Stata Corporation, 2017).

2.2. Collection and storage of samples

For the serological survey, 410 blood samples were selected from samples collected for the obligatory monitoring of Aujeszky's disease (pseudorabies), swine vesicular disease (SVD) and classical swine fever (CSFV). Additionally, 428 blood samples were collected from sows at slaughter (VION, Groenlo, the Netherlands). Herds were identified based on their Unique Herd Number (UBN). Samples were randomly selected given the availability of enough serum to perform all assays. Samples were collected from May till up to and including November 2014.

Commissioned by the Dutch government, GD Animal Health was also monitoring wild boars for Aujeszky's disease, SVD, foot and mouth disease, CSFV, Trichinellosis and African swine fever. Because it is suggested that wild boars may play an important role as a PEDV reservoir (Lee et al., 2016), blood samples of wild boar collected as part of this monitor were also tested. These blood samples were collected from regions close to the borders with Germany and Belgium, from April till August 2014.

Blood samples collected were stored at −70 °C, before dispatched to the Virology Division of the Faculty of Veterinary Medicine, at Utrecht University (UU).

2.3. Indirect ELISA

For the detection of PEDV antibodies in serum samples an in-house indirect ELISA based on the viral spike (S) protein S1-part of the G2b strain GDU (Non-S-INDEL, GenBank KU985230.1) was used, similar as the ELISA previously described (Gerber et al., 2014). The S1 antigen used in this study is produced in HEK293 T cells, a mammalian expression system. To facilitate the purification from the cell culture supernatant, the protein is associated with the Fc part of murine IgG. Per well 4 ng of protein was used to coat the plates.

The in-house ELISA was optimized by UU with sera from PEDV-infected (G2b) pigs from the US and with hyperimmune sera of animals vaccinated with the PEDV S1 protein. Sera were tested in duplicate at a 1:100 dilution, the absorbance was measured with an ELISA plate reader at 450 nm. The mean OD value of PEDV-negative sera was 0.11. Sera with OD values of > 0.33 (3× OD value PEDV negative sera) were considered positive. The aforementioned PEDV-positive sera from infected animals were high positive (> 0.8 OD) in this ELISA (Supplementary Fig. 1). The calculated sensitivity and specificity of the ELISA was 100% (CI 95%: 90–100%; N = 43) and 100% (CI 95%: 92–100%; N = 57), respectively.

2.4. Virus neutralization test

For the detection of virus-specific antibodies in serum samples the virus neutralization test (VNT) was used as an alternative sero-diagnostic assay. A previously described VNT was used (Li et al., 2013) to test all samples positive by ELISA. The validation of this test is shown in

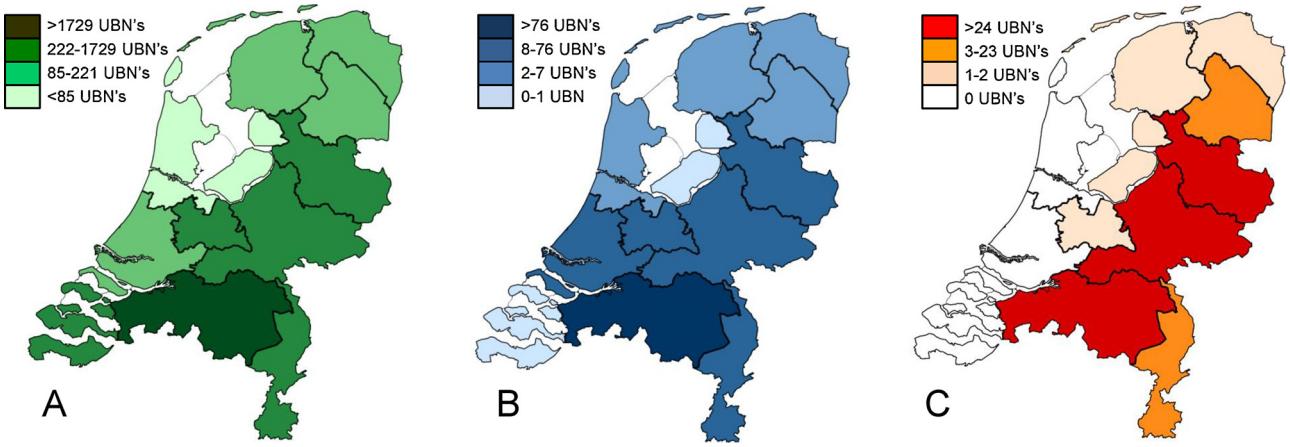

Fig. 1. Map of the 12 provinces of the Netherlands. A) Pig herd density in 2014. B) Herd sampled to determine the seroprevalence for infection with PEDV from May till December 2014. C) Areas with PCR positive PEDV herds registered by GD Animal Health from November 2014 till January 2016. The darker the color, the more farms were positive. UBN: Unique Herd Number.