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Investigation of three outbreaks of Porcine Epidemic Diarrhea in Germany in 2016 demonstrates age dependent differences in the development of humoral immune response

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ABSTRACT

Porcine epidemic diarrhea (PED) has reemerged in Europe since 2014. Characterized by a rapid onset of diarrhea in pigs of all ages, morbidity can reach up to 100% whereas mortality is variable. The virus strains involved in the recent European outbreaks all cluster together with US strains (S INDEL) that lead to less severe clinical signs. In this study, fattening pigs and suckling piglets (n = 105) on farms with no prior PED history were monitored after an acute outbreak of the disease, caused by an S INDEL strain of PED virus (PEDV). For diagnostic investigations in the affected farms, real time RT-PCR was performed to detect PEDV RNA in individually taken fecal samples, and two commercial ELISA kits, both based on the N protein of PEDV, were used to detect IgG in serum samples of pigs experiencing acute signs of the disease. PEDV RNA could be detected in fecal samples up to 14 days after initial sampling. Comparing both ELISAs by Cohens Kappa showed substantial agreement (κ = 0,771). Antibodies were detectable in all fattening pigs (100%) within 10 days after the occurrence of first clinical signs and remained detectable for about two months at least in 20.6% (farm 1) and 45.7% (farm 2) of the animals, respectively. In contrast, only 18 of 34 (52.9%) suckling piglets seroconverted. Although, PEDV RNA was found in fecal samples of all piglets, 13 piglets did not demonstrate antibodies at any sampling day. PCR to detect PEDV RNA in fecal samples seems to be a reliable diagnostic tool during and after the acute outbreak. In the present study, IgG ELISA kits proved to be a feasible diagnostic tool, but age dependent differences in detection rate and persistence of antibodies need to be considered.

1. Introduction

Porcine epidemic diarrhea virus (PEDV), the causative agent of *Porcine epidemic diarrhea* (PED), is an enveloped, positive-sense, single-stranded RNA virus of the family *Coronaviridae*. Seven open reading frames (ORFs) of the PEDV genome encode four structural proteins, N (capsule), S (spike), E (envelope) and M (membrane) and three non-structural proteins (Masters and Perlman, 2013). The infection induces diarrhea in pigs of all ages and is characterized by a rapid onset of mild to severe diarrhea and potentially vomiting (Pensaert and de Bouck, 1978). While morbidity reaches up to 100%, mortality varies and is influenced by the virulence of the virus strain, the age of the pig and the immune status of the herd (Wang et al., 2014; Annamalai et al., 2015; Goede et al., 2015).

After the first detection in Europe and epidemics in several European countries during the 1970s and 1980s (Pensaert and de Bouck, 1978; Song and Park, 2012), porcine epidemic diarrhea (PED) was only sporadically reported from Europe (Martelli et al., 2008). However, PED causes high economic losses in Asian swine-producing countries since the 1980s (Song and Park, 2012). Recently, PEDV was

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introduced to the US and has led to immense losses in the swine population (Stevenson et al., 2013). These prototype PEDV strains caused mortality rates of up to 100% in suckling piglets. Simultaneously, a variant PEDV strain with insertions and deletions in the spike protein gene (S INDEL strain) was identified from US outbreaks (Vlasova et al., 2014; Wang et al., 2014), leading to mild clinical signs and no or low mortality. Experimental infections with S INDEL strains seem to entail less severe clinical signs and variable mortality rates (0–75%) compared to US prototype strains (Lin et al., 2015a; Chen et al., 2016a). According to Goede and Morrison (2016) S INDEL strains influence production data and the time to the return to baseline production less severely compared to US prototype PEDV (Goede et al., 2015). Since 2014, PED reemerged in Germany (Hanke et al., 2015; Stadler et al., 2015) and other European countries (Grasland et al., 2015; Mesquita et al., 2015; Theuns et al., 2015) with high mortality rates in individual herds (up to almost 70%) (Mesquita et al., 2015; Stadler et al., 2015). The identified PEDV strains are closely related to US S INDEL strains, but differ from strains found in Europe in the 1970s and 1980s. So far, Ukraine was the only European country in which a PEDV strain closely related to US prototype isolates has been found (Dastjerdi et al., 2015).

Effective and rapid diagnostic tools are required for PEDV diagnosis of affected pigs to prevent spreading of the disease and to facilitate the implementation of control measures. Showing good sensitivity and specificity, polymerase chain reactions (PCR) are easy to perform and provide fast and reliable PEDV RNA detection (Kim et al., 2007). To determine previous exposure of herds to PEDV as well as their immune status (Bjustrom-Kraft et al., 2016), serological investigations are required. Antibodies of different isotypes (IgA, IgG) can be detected by diverse assays (Carvajal et al., 1995; Gerber et al., 2014; Okda et al., 2015). As no vaccines are licensed in Europe to date, serological investigations provide a feasible tool to monitor the herd status or to perform prevalence studies. Therefore, two indirect ELISAs based on a recombinant N protein of PEDV are available on the European market.

In this study, we surveyed two fattening farms and one farrow-to-finish farm in Germany after an acute outbreak of PED in 2016. The objectives were to describe the detection of PEDV RNA in fecal samples as well as serum anti-PEDV-IgG antibodies in individual fattening pigs and suckling piglets after an acute outbreak of PED under field conditions. The obtained results may help to elucidate the infection dynamic of S INDEL field infections and might lead to a better understanding of diagnostic tools in order to implement PED monitoring programs for swine herds.

2. Material and methods

2.1. Experimental design

The study was approved by the government of Baden-Wuerttemberg (approval number 35-9185.82/0342) and was conducted on two fattening farms and one farrow-to-finish farm in Southern Germany between February 2016 and June 2016. All three farms had no prior exposure to PEDV and were experiencing an acute outbreak of the disease. The distance between farm 1 and 3 was 6 km; farm 2 was 60 km away from farm 1 and 3. The naturally infected pigs showed typical clinical presentation with morbidity reaching up to 100%. PEDV was confirmed by RT-PCR of pooled fecal samples from different age groups. None of the herds was vaccinated against PEDV, as no vaccine is licensed in Germany. Detailed information on the farms is displayed in Table 1. For long term monitoring purposes animals from the youngest age group showing diarrhea were selected for the study. On each farm, 35 pigs from one age group with diarrhea were included in the study. However, the number of investigated animals differs after d0 since one fattening pig died after d14 without association to PED on farm 1 and one suckling piglet was crushed after d0 on farm 3 (Table 2). Clinical signs for individual animals were recorded by the same observer in all three farms according to a scoring system (Suppl. Table I). On farm 3 suckling piglets were housed in one farrowing unit and transferred to one nursery barn after weaning. Subsequently, due to a continuous flow production of the finishing compartment pigs were housed together with other age groups. On both fattening farms study pigs were housed in one barn and were not moved for the duration of the study. Change of boots and equipment between different barns was performed in both fattening farms to avoid on farm spread of PEDV.

Table 1
Production type, farm size, housing, management of the farms.

	farm 1	farm 2	farm 3	
farm type	fattening/ finishing farm	fattening/ finishing farm	farrow-to-finish	
size	780	2500	170 sows	
housing condition	fully slatted floor	fully slatted floor	farrowing	conventional farrowing crates
			nursery	fully slatted floor
			finishing	partially slatted floor with outdoor area
	all-in-all-out	all-in-all-out	farrowing	all-in-all-out
			nursery	all-in-all-out
			finishing	continuous flow production

Table 2
Study animals, number of investigated animals and days between onset of clinical signs and first sampling day (d0).

	study animals	investigated animals (n)	days between first clinical signs and first sampling (d0)
farm 1	35 kg fattening pigs	34 (after d14)	4
farm 2	35 kg fattening pigs	35	3
farm 3	1-week-old suckling piglets	34 (until d42)	2
	from 5 litters	31 (after d42)	
		5 sows (d0)	

2.2. Sample collection

First samples were collected shortly after the onset of clinical signs on the farms (see Table 2), hereafter called d0. All study animals were individually marked. Subsequently, samples were taken over a period of two months on d7, 14, 28, 42, 63; on farm 3 additional samples were collected on d84 and 105. In farm 3, the dams were also sampled once on d0. Blood samples and individual fecal samples (fattening pigs) or rectal swabs (suckling piglets) were collected. Serum was obtained after coagulation and centrifugation (10 min, 2000 × g) and was stored at −20 °C until analysis. Fecal samples were stored at -20 °C until analysis. Approximately 0.5 ml of the thawed fecal sample was suspended with 0.5–1.5 ml phosphate-buffered saline (PBS), depending on the consistency. Rectal swabs were suspended with 1.5 ml of PBS and incubated for 30 min. After vortexing and centrifugation (2 min, 6000 × g), the supernatant was used for testing by PEDV real time RT-PCR.

2.3. Real time RT-PCR for PEDV detection

Viral RNA was isolated from sample supernatants (100 ml) using the NucleoSpin® Virus Core Kit (Macherey-Nagel GmbH Co. KG, Dueren, Germany) and a Microlab® STARLet workstation (Hamilton Robotics GmbH, Martinsried, Germany) according to the manufacturer's instruction (elution volume = 100 ml). A TaqMan-PCR (Kim et al., 2007) modified: primer F1: CGC AAA GAC TGA ACC CAC TAA TTT, 500 nM;