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VARIATIONS IN THE DETECTION OF ANTI-PEDV ANTIBODIES IN SERUM SAMPLES USING THREE DIAGNOSTIC TESTS – SHORT COMMUNICATION

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Over the last few years several porcine epidemic diarrhoea (PED) outbreaks have been discovered in Europe including the first PED case in Slovenia in January 2015. The aim of this study was to determine when PED virus (PEDV) infection started in Slovenia. Serum samples collected between 2012 and 2016 were tested. Three hundred and seventy-five serum samples were collected from 132 Slovenian small, one-site pig farms. Samples were tested for PEDV antibodies utilising three different serological methods: commercially-available indirect ELISA, in-house blocking ELISA test and Immunoperoxidase Monolayer Assay (IPMA) test. One hundred and seventy (45.33%) tested samples were found positive by the commercially-available ELISA test kit, and 10 (5.68%) of these 170 samples found positive were positive by the in-house blocking ELISA. Only these 10 samples were collected from a farm where clinical signs of PED infection had been observed and PEDV was confirmed by RT-PCR methodology; the other 160 samples were collected randomly. Thirty-two samples with the highest S/P value obtained with the commercial ELISA were all negative with IPMA. Reasons for the high variance in the results obtained remain unclear; more research is required to ensure higher sensitivity and specificity in terms of PEDV antibody tests and other PED diagnostic methods.

Key words: Swine; porcine epidemic diarrhoea; serology

PEDV is a member of the *Coronaviridae* family. Based on genetic and antigenic criteria, PEDV belongs to the *Alphacoronavirus* genus together with two other pig coronaviruses: transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) (Jung and Saif, 2015).

PED first appeared in the early 1970s in the United Kingdom (Wood, 1977) and Belgium (Pensaert and de Bouck, 1978), and was first isolated in Bel-

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gium in 1977. Serological surveys in Europe during the 1980s and 1990s show a low to moderate prevalence of PEDV (Carvajal et al., 1995), with only sporadic outbreaks in The Netherlands (Pijpers et al., 1993), Hungary (Nagy et al., 1996) and England (Pritchard et al., 1999). The first laboratory-confirmed PED outbreak in Slovenia was diagnosed in January 2015 by means of the commercially-available real-time reverse transcriptase polymerase chain reaction (RT-qPCR) on a fattening pig farm (Toplak et al., 2015). PED appears in epidemic and endemic forms, and is clinically dependent on the infecting PEDV strain. The clinical signs for both PED forms are diarrhoea, vomiting, anorexia and appetite loss (Saif et al., 2012; Jung and Saif, 2015). In the case of epidemic PED, the severity of clinical signs and mortality appear to be inversely related to the pigs' age (Jung and Saif, 2015), and the incubation period ranges from one to seven days (Stevenson et al., 2013). The clinical signs of endemic PED are mild and mostly limited to seronegative animals (Nagy et al., 1996; Jung and Saif, 2015).

Few studies are available on anti-PEDV immune response and the importance of serum antibody detection. The in-house, indirect, enzyme-linked immunosorbent assay (ELISA) is seen as a useful tool for detecting PEDV antibodies in sera and colostrum (Gerber et al., 2014).

The aim of this study was to determine when PEDV infection had started in Slovenia. For this purpose, serum samples collected between 2012 and 2016 were tested utilising three different serological methods.

Three hundred and seventy-five serum samples were collected from boars, breeding sows and older fattening pigs from 132 small, one-site pig farms between 2012 and 2016. Three hundred and sixty-five of the 375 samples were collected at random from pig farms across Slovenia between 2012 and 2015, and 10 samples were collected in April 2016 from a farm where clinical signs of PED had been observed. On this farm, PEDV was confirmed in the faeces with the commercially-available RT-PCR method (Virotype® PEDV/TGEV, Qiagen, Germany). All animals were manipulated solely for diagnostic purposes. Serological testing was performed on all of the 375 collected samples using the commercially available ELISA Test Kit (Swinecheck® PED indirect ELISA Test Kit, Biovet, Canada). Testing was performed according to the producer's manual (Biovet, 2015). One hundred and seventy samples determined to be PEDV antibody positive using the commercially available ELISA test were further tested by means of the in-house blocking ELISA test (DTUVet, Denmark). The testing procedure was analogous to that used in a study published in 1997 (Sørensen et al., 1997). The 32 highest-ranking positive samples determined utilising the commercially available ELISA test kit were further tested by means of Immunoperoxidase Monolayer Assay (IPMA) (Bøtner et al., 1994).

One hundred and seventy (45.33%) of the 375 samples tested were PEDV antibody positive with the commercially available ELISA test kit (Table 1), with the mean S/P value for the 170 positive samples being 1.076. None of the sam-

ples tested by the in-house blocking ELISA test were determined positive. Ten positive samples collected from a PEDV-infected herd in 2016 were all found positive by means of the commercially available ELISA test kit and by in-house blocking ELISA testing. The 32 strongest positive samples obtained by means of the commercially available ELISA test kit, collected between 2012 and 2015 and with S/P ratios higher than 1.362, were all found negative by the in-house ELISA kit and IPMA.

Table 1

Number of tested samples and results for three tests used in this study

PEDV antibody detection method	Year (months) of sample collection	Number of tested pigs	Number of PEDV antibody positive pigs	% of positive pigs
Commercial ELISA test kit	2012 (Jan–Sep)	92	44	47.83
	2013 (Feb–Oct)	91	51	56.04
	2014 (Sep–Dec)	151	47	31.13
	2015 (Jan–Jun)	31	18	58.06
	2016 (Apr)*	10	10	100
	Total	375	170	45.33
In-house blocking ELISA	2012 (Jan–Sep)	44	0	0.00
	2013 (Feb–Oct)	51	0	0.00
	2014 (Sep–Dec)	47	0	0.00
	2015 (Jan–Jun)	18	0	0.00
	2016 (Apr)*	10	10	100.00
	Total	375	170	45.33
IPMA#	2012 (Jan–Sep)	10	0	0.00
	2013 (Feb–Oct)	16	0	0.00
	2014 (Sep–Dec)	6	0	0.00
	2015 (Jan–Jun)	0	0	0.00
	2016 (Apr)*	NT	NT	NT
	Total	32	0	0

*Samples collected from PEDV-positive herd; #only those samples with S/P ratios higher than 1.362 using the commercially available indirect ELISA test kit were tested using IPMA; NT – not tested

Using the commercially available ELISA test kit, almost half of the tested sera samples (45.33%) collected before 2016 were interpreted as positive. At the time of testing, this was the only commercially available ELISA kit for the detection of PEDV antibodies on the market. As PED had never been suspected in Slovenia before 2015, this number seemed very high; we chose an in-house blocking ELISA test and IPMA for confirmatory testing based on the results of a ring trial from 2015 (Bøtner et al., 2015). All 160 samples previously found posi-

tive with the commercially available ELISA test kit were negative by the in-house ELISA testing. In addition, all 32 samples were also deemed negative pursuant to IPMA, suggesting that all results may be false positive when using the commercially available ELISA test kit. In April 2016, a clinical outbreak of PEDV infection occurred on a Slovenian pig fattening farm. All ten serum samples from this herd were found PEDV antibody positive by the commercially available ELISA test kit; these 10 samples were also sent to Denmark for retesting by an in-house blocking ELISA test; this time, all 10 sera samples were found PEDV antibody positive. Samples for in-house blocking ELISA testing and IPMA were tested abroad and the high cost of these tests is the reason why only a limited number of primary samples were retested using both methods. As all of the 10 samples from the farm with PEDV clinical signs were found positive by the commercially available and the in-house ELISA tests, respectively, IPMA testing was not chosen for further confirmation.

The reasons for the discrepancy in the obtained results are difficult to determine. One reason may be that the vast majority of samples in our study (365 out of 375; 97.33%) were randomly collected from the field between 2012 and 2015 for the purpose of monitoring Aujeszky's disease and classical swine fever (CSF). These animals were mainly breeding sows and boars, and a few older fatteners. As the producer of the commercially available ELISA test kit states (Biovet, 2015), this commercially available test successfully detects PEDV antibodies up to 42 days post infection; after that, testing is not clear. As PED mostly affects suckling piglets, weaners and young fatteners (Shibata et al., 2000; Puranaveja et al., 2009; Saif et al., 2012), these 365 pigs, even if affected when young and had recovered from PEDV, were past this 42-day limit, so we believe that it is possible that non-specific reactions may be evidenced when using this commercially available kit on sera from these animals, and that results could be false positive. When the commercially available kit was used on younger fatteners with distinct clinical PED signs in 2016, the results seemed to be accurate and in accordance with those of the in-house ELISA testing. Therefore, we believe that the commercially available kit is, in fact, useful for the detection of antibodies at certain stages, such as after infection, vaccination or natural exposure. In our case, where we wanted to determine seroprevalence in Slovenia, the usefulness of this commercially available ELISA test kit was questionable. A more exact evaluation of result dissimilarity in relation to the three different methods utilised and the implementation of gold standard tests would be of great value. More complete studies on antibody response to PED are needed to enhance the performance of new PEDV antibody detection methods with higher specificity. Since the beginning of this study, new commercial ELISA kits have become available for the detection of PEDV antibodies and their specificities and sensitivities have been compared (Leidenberger et al., 2017). Although the results of this study also showed some variability, more commercial diagnostic tools for

the detection of PEDV antibodies are becoming available, meaning that in the case of uncertainty, there are some commercial tools available for comparison. If positive results are obtained from pigs, especially older pigs with no clinical signs of PED, we would recommend that samples are re-tested with one of the serological methods from the OIE PED technical factsheet [ELISA, either commercial or in-house, immunofluorescence test, immunohistochemistry test or serum neutralisation test (OIE, 2014)].

The detection of PEDV in Slovenia and other confirmed PED cases in Europe in 2015 represent a potential threat to the swine industry. The reason for the high variance in results obtained by the different methods used in this study is unclear. Commercially available methods for detecting PEDV antibodies efficiently for at least one year after exposure would be greatly appreciated in cases of PED emergency in Europe and for the purpose of PED prevalence monitoring.

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