Veterinary Microbiology 197 (2016) 151–160

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Inter-laboratory study to characterize the detection of serum antibodies against porcine epidemic diarrhoea virus

Bertel Strandbygaard[a], Antonio Lavazza[b], Davide Lelli[b], Yannick Blanchard[c,d], Béatrice Grasland[c,d], Sophie Le Poder[e,f,g], Nicolas Rose[c,d], Falko Steinbach[h], Wim H.M. van der Poel[i], Frederik Widén[j], Graham J. Belsham[a], Anette Bøtner[a,*]

[a] National Veterinary Institute, Technical University of Denmark, Lindholm, Kalvehave 4771, Denmark
[b] IZSLER – Virology Unit, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" Via Bianchi 7/9, 25124 Brescia, Italy
[c] ANSES – Laboratory of Ploufragan-Plouzané – BP 53, 22440 Ploufragan, France
[d] Université Bretagne Loire, Rennes, France
[e] Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, UMR 1161 Virology, Maisons-Alfort, France
[f] INRA, UMR 1161 Virology, Maisons-Alfort, France
[g] ANSES – Laboratory of Animal Health of Maisons-Alfort – UMR 1161 Virology, 23 avenue du Général de Gaulle, France
[h] APHA, Dept of Virology, Weybridge, Addlestone, Surrey. KT15 3NB, UK
[i] Wageningen University and Research Centre, Edelhertweg 15, 8219PH, Lelystad, The Netherlands
[j] The National Veterinary Institute (SVA), 75189 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 17 November 2016
Accepted 20 November 2016

Keywords:
ELISA
Porcine coronavirus
PEDV
Ring trial
Serology

ABSTRACT

Porcine epidemic diarrhea virus (PEDV) has caused extensive economic losses to pig producers in many countries. It was recently introduced, for the first time, into North America and outbreaks have occurred again in multiple countries within Europe as well. To assess the properties of various diagnostic assays for the detection of PEDV infection, multiple panels of porcine sera have been shared and tested for the presence of antibodies against PEDV in an inter-laboratory ring trial. Different laboratories have used a variety of "in house" ELISAs and also one commercial assay. The sensitivity and specificity of each assay has been estimated using a Bayesian analysis applied to the ring trial results obtained with the different assays in the absence of a gold standard. Although different characteristics were found, it can be concluded that each of the assays used can detect infection of pigs at a herd level by either the early European strains of PEDV or the recently circulating strains (INDEL and non-INDEL). However, not all the assays seem suitable for demonstrating freedom from disease in a country. The results from individual animals, especially when the infection has occurred within an experimental situation, show more variation.

1. Introduction

Porcine epidemic diarrhoea virus (PEDV) is a member of the *Alphacoronavirus* genus within the family *Coronaviridae*. Infection of swine by this virus causes disease characterized by diarrhoea and vomiting which can lead to severe dehydration and results in high (90–100%) mortality in newborn piglets (Stevenson et al., 2013). Older animals (≥14 days) normally recover from the infection and seroconvert against the virus. The disease was initially identified within the United Kingdom (UK) in 1971 and the detection of the aetiological agent, PEDV, was first achieved in Belgium (Pensaert and de Bouck, 1978); afterwards the virus spread within Europe and also to Asia (reviewed in Jung and Saif, 2015; Lee, 2015). In 2013, outbreaks of the disease occurred for the first time in the USA (Huang et al., 2013; Chen et al., 2014) and rapidly spread within both North and South America. About 7 million piglets died in a single year as a result of the outbreaks in the USA alone (Jung and Saif, 2015; Lee, 2015).

The genome of PEDV, like other coronaviruses, is a single-stranded positive sense RNA of about 28 kb. The virus produces a number of sub-genomic mRNAs which encode the various

* Corresponding author.
E-mail addresses: bstr@vet.dtu.dk (B. Strandbygaard), antonio.lavazza@izsler.it (A. Lavazza), davide.lelli@izsler.it (D. Lelli), Yannick.BLANCHARD@anses.fr (Y. Blanchard), Beatrice.GRASLAND@anses.fr (B. Grasland), slepoder@vet-alfort.fr (S.L. Poder), Nicolas.ROSE@anses.fr (N. Rose), Falko.Steinbach@ahvla.gsi.gov.uk (F. Steinbach), wim.vanderpoel@wur.nl (W.H.M. van der Poel), frederik.widen@sva.se (F. Widén), grbe@vet.dtu.dk (G.J. Belsham), aneb@vet.dtu.dk (A. Bøtner).

http://dx.doi.org/10.1016/j.vetmic.2016.11.020
0378-1135/

structural and non-structural proteins within infected cells. The virus particles include the spike (S) protein, the envelope (E) protein, the membrane (M) protein and the nucleocapsid (N) protein (Brian and Baric, 2005). The spike protein is exposed on the virus surface and gives the virus particles their characteristic morphology. A number of different variants of PEDV are known (see Huang et al., 2013; Wang et al., 2014; Lee, 2015). The early European viruses are represented by the CV777 strain (these are sometimes classified, on the basis of the S gene sequence, as being within genogroup 1a) while two different variants of PEDV (classified, on the same basis, within genogroups 1b and 2b) have been identified within the USA. The US PEDVs are also referred to as "INDEL" (e.g. OH 851, from the genogroup 1b) and "non-INDEL" (e.g. MN, 1A1 and 1A2 strains from 2013, in genogroup 2b); these differ by the presence of certain deletions and insertions within the S gene sequence (Huang et al., 2013; Wang et al., 2014; Lee, 2015).

Within infected pigs, antibodies are generated against the PEDV proteins and these can be detected by a variety of methods including ELISA, immunoblotting and immunostaining of infected cells.

The recent reappearance of PEDV (closely related to the INDEL OH 851 strain) infections within Europe, including in Germany, France, Italy, Portugal, The Netherlands and Slovenia (Hanke et al., 2015; Grasland et al., 2015; Boniotti et al., 2016; Mesquita et al., 2015; Toplak et al., 2016; EFSA, 2014), has led to the need for an assessment of existing diagnostic assays for the detection of PEDV infections. The PEDV can be identified by RT-PCR (e.g. Kim et al., 2007; Chen et al., 2014) in faecal or intestinal samples from acutely infected animals and a low level of viremia has also been observed in serum from acutely infected pigs (Jung et al., 2014, 2015; Lohse et al., 2016). However, the virus is only present in infected animals for a limited period (typically less than 1 month, see Lee, 2015) while the serological response can be expected to be much longer lasting (Crawford et al., 2015).

Extensive serological screening of swine (2500 samples/yr) within Denmark during the period 2000 to 2006 did not detect any sign of PEDV infection. More recently, following the disease outbreaks in the USA, additional Danish sera (2400 samples in 2014 and 3960 samples in 2015) were also tested and, again, all gave negative results. It is important to ensure that national diagnostic laboratories are able to detect PEDV infection efficiently when outbreaks of disease occur. For PEDV-free countries, like Denmark, Sweden and UK, or when planning to export animals from PEDV negative herds it is also important to be able to declare freedom from disease. Therefore, assays with both high sensitivity and high specificity are needed. Different laboratories use a variety of "in-house" assays; in addition, commercial tests for the detection of antibodies to PEDV are available. However, the properties of these different tests have not been analysed, in parallel, previously. An assessment of a range of tests, performed in different reference laboratories from Denmark (DK), Italy (IT), France (FR), The Netherlands (NL), Sweden (SE) and the UK using shared panels of porcine sera, collected from animals in the field and from experimental infection studies, has now been undertaken and the results of these analyses are presented.

2. Material and methods

2.1. Description of porcine serum panels

Panel 1 included 54 sera collected in different countries including known positive sera (diluted or neat) from pigs experimentally infected with the Br1/87 or CV777 early European strains of PEDV, negative control sera plus field sera from farms with PED clinical disease (in the US and Canada,) and also field sera from farms without clinical disease (in DK, FR and SE).

Panel 2 included 8 sera collected in DK from experimentally infected pigs at either 14 or 28 days post inoculation (dpi) with either the early European (Br1/87 strain) or a recent "non-INDEL" US strain of PEDV (described in detail by Lohse et al., 2016).

Panel 3 included 20 sera from finisher pigs from a single herd in Italy that had experienced clinical signs of PED a few weeks prior to sampling; they were collected in 2015. The presence of an INDEL strain of PEDV (closely related to OH 851) on this farm was confirmed by RT-qPCR and sequencing (data not shown).

Panel 4 included two sets of 40 sera collected from farms in Italy, during 2015. One set of 40 sera was collected from 6 different farms that had each experienced clinical signs of PED and from which PEDV (very closely related to the INDEL OH 851 strain) had been identified. The second set of 40 samples was collected from 5 other Italian farms, localized in PEDV-free areas, that had no history of enteric signs, and which had tested negative by a PEDV specific RT-PCR and using a PEDV-Ab ELISA (see below) (note: due to limitations in availability of sera, this panel was only tested in two different laboratories using three separate assays).

2.2. "In house" blocking ELISA (DK); ELISA 1

The presence of anti-PEDV antibodies in sera was determined (as in Lohse et al., 2016) using an "in-house" blocking ELISA (analogous to that used for PRRSV (Sørensen et al., 1997)) using antigen prepared from PEDV (Br1/87, closely related to CV777)-infected Vero cells. Briefly, Vero cells were infected with the Br1/87 strain of PEDV and after 24–48 h, when CPE was apparent, the cells (and medium) were frozen. After thawing, cell debris was removed by centrifugation at 5500 × *g* for 10 min at 5 °C. The virus antigen was harvested from the supernatant by further centrifugation (30000 × *g* for 4 h) and resuspended in PBS (1/100th of initial volume). The antigen was coated (typically at 1:1000 dilution but titrated for each batch) onto 96-well ELISA plates, washed and then stored frozen until use. Sera (diluted 1:10) were added to the wells and incubated overnight at 20 °C, prior to further incubation for 1 h with a biotin-conjugated pig anti-PEDV polyclonal antibody (diluted 1:100 in 10% normal pig serum) prepared essentially as described previously (Sørensen et al., 1997). Following washing, the bound biotinylated-antibody was detected using avidin-conjugated horseradish peroxidase (eBioscience, diluted as recommended by manufacturer) plus 3,3′,5,5′- tetramethylbenzidine substrate and the OD was measured at 450/630 nm. The cut-off value for a positive reaction is set at 40% blocking, values below 35% are considered negative while intermediate values are considered inconclusive.

2.3. "In-house" blocking ELISA (NL and UK); ELISA 2

Sera were tested using the ELISA essentially as described by van Nieuwstadt and Zetstra (1991). For this assay, ELISA plates coated with cell culture grown virus antigen (CV777) were incubated with serum and then unblocked virus is detected using two different monoclonal antibodies. Samples were tested using two-fold dilutions and the presented results were obtained using 1:2 or 1:4. Blocking values >50% are positive, values <40% are negative and values of 40–50% are considered inconclusive.

2.4. "In-house" blocking ELISA (IT); ELISA 3

Sera were tested using an in-house blocking ELISA based on a double antibody sandwich that has been described previously (Sozzi et al., 2010). In brief, the ELISA microplates were coated with the 1F12 capture monoclonal antibody (MAb). Serum samples diluted 1:2 or 1:4 were mixed with equal volumes of whole PEDV (CV777), inactivated with ß-propiolactone, and pre-incubated in