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SCIENTIFIC OPINION

Scientific Opinion on porcine epidemic diarrhoea and emerging porcine deltacoronavirus[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2, 3]

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ABSTRACT

In the last decade, many porcine epidemic diarrhoea (PED) outbreaks have been reported by several countries in Asia whereas only a few Member States of the European Union (EU) have reported PED clinical cases and/or PED virus (PEDV)-seropositive animals. This alphacoronavirus was first reported in the USA in May 2013, followed by rapid spread throughout the country and outbreaks reported by several countries in the Americas. The recent PEDV-EU isolates have high level of sequence identity to PEDV-Am isolates. Based on nucleotide sequencing, multiple variants of PEDV are circulating in Europe, the Americas and Asia but any difference in virulence and antigenicity is currently unknown. Serological cross-reactivity has been reported between PEDV isolated in Europe and in the Americas; however no data regarding cross-protection are available. The impact of different PEDV strains is difficult to compare between one country and another, since impact is dependent not only on pathogenicity but also on factors such as biosecurity, farm management, sanitary status or herd immune status. However, the clinical signs of PEDV infections in naive pigs are similar in different countries with mortalities up to 100% in naive newborn piglets. The impact of recently reported PED outbreaks in Asia and the USA seems to be more severe than what has been described in Europe. Infected animals, faeces, feed and objects contaminated with faeces are matrices that have been reported to transmit PEDV between farms. Infectious PEDV has been detected in spray-dried porcine plasma (SDPP) in one study but the origin of the infectious PEDV in SDPP is not clear. Detection of porcine deltacoronavirus (PDCoV) has been reported in a few countries but only limited testing has been done. Based on the currently available information, it seems that PDCoV would have a lower impact than PEDV.

KEY WORDS

alphacoronavirus, deltacoronavirus, pig, diarrhoea, cross-protection, impact, matrix

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[4] A number of editorial changes were made on p. 19 and p. 24 which do not materially change the meaning or outcomes of the opinion. On p.19, 'of other tissues' has been replaced by 'to other tissues'. On p.24, the words 'and gut health' have been added as follows: 'improve the performance and gut health of piglets'. Also on p. 24, 'was effective in activating' has been replaced by 'effectively inactivated'. The original opinion is available on request as is a copy showing all the changes that were made.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Animal Health and Welfare (AHAW Panel) was asked to deliver a scientific opinion on porcine epidemic diarrhoea virus (PEDV) and porcine deltacoronavirus (PDCoV).

The approach used for this scientific opinion consisted of extensive literature searches (finalised by the end of September 2014), followed by extraction of the relevant information and a description of the current knowledge in accordance with the terms of reference. Data gaps and a lack of scientific evidence are identified and specified. Regarding the risk assessment of potential entry routes of PEDV and PDCoV into the European Union (EU), it was agreed with the European Commission that this scientific opinion would describe the currently available scientific evidence and identify data gaps, but a full risk assessment would not be performed. The following paragraphs summarize the knowledge first concerning PEDV and then concerning PDCoV, in relation to the terms of reference of the mandate.

PED

In the last decade, many porcine epidemic diarrhoea (PED) outbreaks have been reported by several countries in Asia. Only a few Member States of the EU have been reported PED clinical cases and/or PEDV-seropositive animals, the overall impact being very limited. This alphacoronavirus was first reported in the USA in May 2013, followed by a rapid spread throughout the country and outbreaks in several countries in the Americas. Vaccination has been used for many years in several Asian countries and might have influenced the epidemiological situation. New vaccines have been granted conditional licences in 2014 in the USA and no vaccines have been used in Europe.

Using a collection of 33 full-length PEDV genome sequences, it has been shown that all PEDV sequences (including the prototypic European isolate CV777) are closely related (the identity between the US and non-US strains varies between 96.3 and 99.5%) and that it was possible to group these into different clusters and sub-groups. Sequences from viruses circulating in Asia were present within each of the clusters and subgroups, indicating that a range of different viruses are circulating in Asia. All the US PEDV sequences were clustered into one group, but a sub-division of an original strain and a new variant strain can be made. Analysis of the European PEDV sequences is very limited, since there are only a few sequences available from viruses circulating in the 1970-80's, and the only others are from viruses circulating recently in Germany and Italy. There is high sequence identity between these very recently circulating German and Italian viruses and the US PEDV strains, but more studies are required in order to compare the virulence of these different viruses. Additional sequence data are required to understand PEDV evolution in Europe and the possible link with PEDV strains circulating in other parts of the world.

Although differences in the virulence of PEDVs have been suggested in the scientific literature, there are not enough data available at the moment to compare their phenotypic characteristics. Comparing the virulence/pathogenicity of different PEDV isolates would require comparative animal experiments. No experimental animal studies have been reported describing the cross-protection between different PEDV strains. Serological cross-reaction between the virus isolated in Europe (PEDV-EU) and that isolated in the Americas (PEDV-Am) has been described including neutralizing antibodies raised against the early PEDV-EU towards PEDV-Am.

The impact of recently reported PED outbreaks in Asia (after 2010) and the USA seems to be more severe than that described in Europe. However, it is difficult to compare the impact between one country and another, since impact is dependent not only on the pathogenicity of the virus but also on parameters such as the production system, biosecurity, the time of detection of an outbreak, farm management, herd size, the immune status of the population and herd sanitary status (e.g. presence of other infectious agents). The severity of disease associated with PEDV within a herd is variable and is highly dependent on the age of the infected pigs and on the level of immunity in the population. Based

on the scientific evidence available at the moment, the clinical disease of a PEDV infection in naive animals seems to be the same in different countries, with mortalities up to 100% in PEDV-naive newborn piglets. An apparent low impact of recent PED outbreaks caused by viruses with high sequence identity to US PEDV, has been reported in Italy and Germany. Factors which might influence the impact of a possible introduction of a US PEDV and spread of the virus into Member States include the level of cross-protection between different PEDVs and the seroprevalence (population immunity), both of which are currently unknown but are expected to vary between Member States. More knowledge on these factors is required before an accurate impact assessment can be performed.

Infected live animals and faeces have been reported to transmit PEDV. Infectious virus can survive in slurry, but at present there are no data available on the role of this matrix in PEDV transmission. High levels of infectious PEDV are shed in faeces and can contribute to contamination of various objects (e.g. vehicles, humans) and feed. Transmission of PEDV via feed has been shown, but more data are required to assess the source of PEDV contamination in feed. PEDV RNA has been detected at low levels in the serum fraction of whole blood but there are no data reporting infectious virus in this matrix to date. It is reported that spray-drying of porcine plasma (SDPP) can inactivate PEDV. However, the influence of variations in spray-drying processes has not been validated sufficiently for PEDV. Infectious PEDV has been detected in SDPP in one study but the origin of the infectious PEDV in SDPP is not clear (faecal cross contamination or inadequate spray-drying). Faecal cross-contamination of blood during collection at slaughterhouses cannot be excluded. Infectious virus has been detected in air collected under experimental conditions and so PEDV might be transmitted via the air for short distances. Low levels of PEDV RNA have been detected in semen but there are no data available on the presence of infectious virus in this matrix. Currently, there are no available data on the presence of PEDV in embryos, pork meat or other porcine derived feed components such as red blood cells, hydrolysed proteins, fat, gelatine and collagen. It can be assumed that porcine swill, particularly untreated pig intestines, can contain infectious PEDV, but there are no data available at the moment on the role of this matrix in PEDV transmission.

PDCoV

Detection of PDCoV has been reported in Hong Kong, the USA, Canada and China but only limited testing has been done. Serological tests specific to PDCoV and aimed at determining the immune status of the pig population have recently been developed and are currently in the process of validation.

Based on the currently available field observations from the USA, the current view is that PDCoV infections would have a lower impact than PEDV. However, the interpretation of field data is difficult since co-infections with PEDV or other intestinal pathogens are common. It is expected that further analysis of very recently performed experiments will provide a better understanding of the pathogenesis and clinical symptoms associated with PDCoV infection.

The available reports from the USA and Canada do not suggest a significant impact on animal health within these countries and no zoonotic potential of the virus has been reported. Therefore, the current knowledge of PDCoV leaves open questions on whether it can be classified as an emerging disease.

PDCoV RNA has been detected in porcine intestinal samples, faeces and feed, but no information on the presence of PDCoV in slurry, semen, embryos, porcine whole blood, SDPP, other porcine-derived feed components or air is currently available. It could be anticipated that the presence and survival of PDCoV in different matrices is comparable to that of other intestinal porcine coronaviruses such as PEDV and transmissible gastroenteritis virus.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Porcine epidemic diarrhea (PED) is a diarrheal disease of swine that was historically associated with a coronavirus of the *Alphacoronavirus* genus.

The PED virus (PEDV) appeared in Europe in the early 1970s, when the disease was detected for the first time in the United Kingdom in a pig holding affected by acute diarrhea in fattening pigs and sows. Afterwards, the disease has been detected in several countries in Europe causing outbreaks of watery diarrhea in swine of all age groups with high mortality in neonatal pigs. At the time of its emergence in Europe PED was confused with transmissible gastroenteritis (TGE), a diarrheal disease of pigs caused by a coronavirus of the same group as the one of PED. However, the two coronaviruses did not show a direct antigenic relationship and thus the two viruses were considered two different etiological agents. Then, in the 1980's and 1990's the evolution of the two viruses was rather different. In fact, as regards as TGEV, a mutant of the virus with tropism for the respiratory tract appeared in the pig population and become widespread in the world inducing a cross protecting immunity to TGEV that led to a gradual disappearance of TGEV, and the associated enteric disease. Instead, PED remained enzootic in the European pig population and the severity of the clinical disease was related to the immune-status of the affected herd. In cases where the disease agent was introduced into a non-immune, fully susceptible population, clinical symptoms were severe, and mortality in neonatal piglets could reach up to 80%.

In the 1980's and 1990's outbreaks of PED became less frequent in Europe, while the virus persisted in the pig population and occasionally limited outbreaks were reported in some countries. Serological surveys conducted in some Member States showed that the prevalence of the virus had become low but it appeared to persist in some localized pockets of infection.

The last documented evidence of the presence of the disease in Europe has been reported in Italy, where in the period 2005 – 2006 sixty-three outbreaks of PED occurred in the Po Valley. The disease affected pigs of all age groups, closely resembling the acute form observed in the seventies, when the disease emerged for the first time in Europe. Before that only sporadic outbreaks were observed in that area, affecting only grower and finisher pigs.

Although PED was first identified in Europe, in the nineties the disease has become increasingly problematic in many Asian countries, including Korea, China, Japan, the Philippines, and Thailand. Afterwards, in May 2013 PED was identified for the first time in the United States and since then it has spread rapidly in the US, apparently causing severe economic losses, at present 27 states have been affected. Recent studies have shown that all PEDV strains detected in the United States are clustered within the same sub-genogroup and they are closely related to a strain previously detected in China. In 2014 PED has also been reported in Canada, Peru', Japan and Mexico.

The PEDV currently circulating in the United States seems to be highly pathogenic, at least for certain categories of animals, and it appears that it causes significant production losses in the pig sector. An effective treatment does not exist, other than the control of secondary infections. Prevention is mainly based on the application of strict management and bio-security practices. Authorized vaccines exist only in Japan, South Korea and China but not in Europe and in the United States.

PED is not a notifiable disease in the EU and it is not amongst the OIE listed diseases. Moreover, contrary to the situation in the United States, currently there is no evidence that the disease is causing significant health or production problems in the European pig farming system. As a matter of fact last documented evidence of the presence of the disease was reported in Italy in 2006.

In addition, in February 2014 a new porcine *Deltacoronavirus* (PDCoV), similar to a coronavirus detected in Hong Kong in 2012, has been detected for the first time in the United States, where the virus has been identified in a breeding pig holding in Iowa with a history of acute severe diarrhea.

Furthermore, in March 2014, the PDCoV has been identified also in samples from six Ontario pig farms, in Canada.

The clinical signs associated with the occurrence of PDCoV are similar or identical to those caused by PEDV in the Americas. In some cases it appears that both the PEDV and the PDCoV were detected in the same farm where an epidemic of diarrhoea in pigs was ongoing. However, the role of this emerging virus in the ongoing epidemic of diarrhea in pigs in North America is still unclear.

Currently there is no evidence of the presence of the new emerging *Deltacoronavirus* in the European pig farming system.

Therefore, in order to better determine the extent of the problem and be prepared to face the possible re-emergence of the disease, the Commission needs specific advice to assess the risks posed by the PED strains currently circulating in Third Countries to evaluate their possible impact on the health status of pig holdings in Europe and on their production. The possible pathways of virus introduction into the EU should be evaluated as well. Furthermore, the appearance of the new porcine *Deltacoronavirus* needs attention as this emerging disease could make the picture even more complicated.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on:

1. The current epidemiological situation in North America and Asia and elsewhere in the world as regard PED and the new porcine *Deltacoronavirus*.

2. Characterization of the new porcine *Deltacoronavirus* as an emerging disease, especially as regards the severity of the disease induced.

3. Possible differences between the European classical PED *Alphacoronavirus* strains and the ones currently circulating in the rest of the world, in particular in the Americas, and possible existence of cross protecting immunity.

4. Impact of the different PED *Alphacoronavirus* strains and of the new porcine *Deltacoronavirus* in pigs in different immunological scenarios.

5. Risk assessment of potential entry routes of PED and the new porcine *Deltacoronavirus* in the EU ranking them on the basis of the level of risk with a view to enhance risk mitigation, prevention and preparedness.

ASSESSMENT

1. Introduction

This scientific opinion describes specific aspects of the porcine epidemic diarrhoea virus (PEDV) and porcine deltacoronavirus (PDCoV), which belong to the genera alphacoronavirus and deltacoronavirus, respectively.

PED was observed in Europe in 1971 and reported for the first time one year later (Oldham, 1972), and PEDV was described as the causative agent of PED by Pensaert and Debouck (1978). During the 1970s and 1980s, the virus spread throughout Europe, causing outbreaks of watery diarrhoea in swine of all ages. However, during the 1980s and 1990s, the number of PED outbreaks decreased markedly in the region. Some European countries (i.e. Scandinavian countries) have never reported outbreaks or the detection of PEDV. Only few severe outbreaks have been reported since the 1980s in Europe and so the impact on the swine production has been limited. In contrast, PEDV has been circulating in Asia for several decades. It was demonstrated for the first time during the 1980s in China (Xuan et al., 1984) and Japan (Takahashi et al., 1983). It was also confirmed in South Korea in 1992-1993 (Kweon et al., 1993) and in Thailand in 1995 (Srinuntapunt et al., 1995). According to the literature, PEDV was wide spread throughout the Asian continent and became an endemic infection during the 1990s. Feeding natural PEDV-infected material (e.g. piglet faeces and minced piglet intestine) to gestating sows has been used in Europe[5] and Asia (and later also in the Americas) to prime the immune response and promote lactogenic immunity in exposed dams to protect suckling piglets (Ayudhya et al., 2012).

This scientific opinion will further describe the epidemiological situation of PEDV infection based on information reported in the last 10 years (2004-2014), including the spread of PEDV to the Americas[6]. The virus may be present in more countries than those mentioned in this scientific opinion, as underreporting might exist.

In contrast, PDCoV was only recently described (Woo et al., 2012). Detection of PDCoV has currently only been reported in Hong Kong, the USA and Canada (see section 2.3).

The approach used for this scientific opinion consisted of extensive literature searches (finalised by the end September 2014), followed by extraction of the relevant information and a description of the current knowledge, in accordance with the terms of reference (TOR). Data gaps and a lack of scientific evidence are identified and specified. PEDV isolated in Europe, Asia or the Americas will be referred to as PEDV-EU, PEDV-As and PEDV-Am, respectively, independent of the year of isolation. Regarding the risk assessment of potential entry routes of PEDV and PDCoV into the EU, it was agreed with the European Commission that this scientific opinion would describe the currently available scientific evidence and identify data gaps, but that a full risk assessment would not be performed.

2. Epidemiological situation regarding PEDV and PDCoV

2.1. Collection of information

In order to describe the current epidemiological situation for PEDV and PDCoV (TOR1), information has been collected on cases and seroprevalence reported during the last 10 years in Europe, Asia and the Americas via an extensive literature review (see Appendix A), as well as through institutional contacts and a search of grey literature on the internet. Detailed information is provided in Appendix B.

[5] Feeding manure or the intestines of diseased pigs to animals in the EU, is not allowed under Animal By-Product Legislation (Regulation (EC) No 767/2009 Annex III: List of materials whose placing on the market or use for animal nutritional purposes is restricted or prohibited as referred in Article 6.

[6] There is one report of a coronavirus-like agent in Quebec (Turgeon et al., 1980), but there is no proof that this was PEDV.

2.2. Occurrence of PEDV

2.2.1. Europe

In the Czech Republic, Rodak et al. (2004) reported that 27 out of 219 faecal samples from diarrhoeic piglets (< 21 days old) were positive for PEDV. One year later, using a competitive blocking enzyme-linked immunosorbent assay (ELISA), PEDV antigens were detected in 15 faecal samples (out of 80 tested) from 6 farms (out of 38 farms with clinical diarrhoea) (Rodak et al., 2005). However, to our knowledge, there is no other information regarding PEDV occurrence (outbreaks) within the country after this particular report.

The only well-documented epidemic of PEDV in Europe during the last 10 years was reported in the Po Valley, northern Italy (Martelli et al., 2008). It occurred between May 2005 and June 2006 in an area densely populated with pigs. The outbreak started with four cases occurring in fattening farms in May (n=2), June (n=1) and July (n=1). No clinical cases were detected during August and September. In October, two new cases appeared: the first in a fattening unit and the second in the nursery of a three-site production unit. The disease spread during the winter of 2005-2006, affecting more than 60 farms including fattening units as well as farrow-to-finish or farrow-to-weaner farms. Some PEDV-positive farms (35 out of 476) were detected between mid-2006 and the end of 2007, but the disease progressively disappeared (Sozzi et al., 2010). From 2008 to 2014, only sporadic outbreaks were observed in grower and finisher herds: 71 cases in 58 different farms, out of 1 563 cases of enteritis (4.54%) (see Table 2 and Table 3 in Appendix B). Over the period 2007-2014, mild clinical signs involved pigs of all ages and mortality was observed in piglets only in the PEDV positive farms in Italy (Sozzi et al., 2014).

In Hungary, 12 piglets from one farm were PEDV-positive in 2009 (see Table 2 in Appendix B). No report was found describing these cases.

In Estonia, during 2010, an outbreak of PED was suspected and reported but was not confirmed. Some additional cases were suspected in 2011 and 2012 (see Table 2 in Appendix B). No report was found describing these cases.

In mid-2014, two independent PEDV outbreaks were reported in fattening farms in Germany: one in North-Rhine Westphalia and one in Baden-Württemberg (Henninger and Schwarz, 2014; personal communication, Sandra Blome, Friedrich Loeffler Institute, Riems, Germany, October 2014).

To our knowledge no PEDV outbreaks have been reported in the literature from any other European country during the last 10 years apart from the examples cited above (see Table 3 in Appendix B). However, most of the countries have not implemented active monitoring for this particular disease. In addition, antibody seroprevalence data are scarce and the sensitivity and specificity of the diagnostic tests remain unknown. Data from limited testing were provided to EFSA by Member State representatives (see Table 4 in Appendix B). All tested serum samples were PEDV antibody negative in Denmark (n=±2500 per year, 2000-2006) and in Belgium (n=460, 2014), whereas an estimate of PEDV-seroprevalence in slaughter pigs in Great Britain was 9.0% (95% confidence interval 6.3-11.7) based on samples taken in slaughter houses in the framework of a Salmonella study (n=558, 2013) (see Table 11 in Appendix E). A serosurvey in three Italian provinces revealed PEDV-specific antibodies in 11 out of 21 farms with 7 to 52% of the tested animals being positive (Alborali et al., 2014). However, the available data provide a low level of scientific evidence on the prevalence of PEDV in Europe, considering that the sampling method is not optimal, the testing is limited and the sensitivity and specificity of the diagnostic tests used is not well known. As only limited active monitoring is performed, underreporting of the disease cannot be ruled out. Underreporting is more likely for those cases with a low clinical impact, since more severe cases with significant impacts on production and pig health would presumably be more thoroughly investigated. At present, among the

EU Member States, PEDV is notifiable only in France, albeit on a temporary basis (Arreté Ministeriel of 12 May 2014[7]).

2.2.2. Asia

PEDV outbreaks have been reported from several countries of Asia during the last 10 years such as Thailand (Puranaveja et al., 2009; Olanratmanee et al., 2010; Ayudhya et al., 2012; Temeeyasen et al., 2014), Taiwan (Puranaveja et al., 2009; Lin et al., 2014), the Philippines (Morales et al., 2007), South Korea (Lee et al., 2010) and southern provinces of Vietnam (Duy et al., 2011). In October 2010, a large-scale outbreak of PEDV was reported in several provinces in southern China. PEDV also spread to other regions of the country, particularly in the northwest (Wang et al., 2013a). It is now circulating in at least 29 Chinese provinces (Feng, 2014). In October 2013, Japan confirmed a PEDV outbreak to the World Organisation for Animal Health (OIE) (OIE, 2014e) after an absence of seven years in the country. At present, 38 out of 47 prefectures are affected (Kawashima, 2014). Among 5 570 farms, 817 have been affected and at the peak of the epidemic, more than 100 newly affected farms per week were reported (Kawashima, 2014). According to the information provided by Japan's National Institute of Animal Health, PEDV isolates from this outbreak are genetically related to the PEDV isolates recovered from China and the USA in 2013. In addition, in late 2013, PEDV outbreaks were reported in South Korea and Taiwan (Choi et al., 2014; Lee and Lee, 2014; Lin et al., 2014). In August 2014, Taiwan reported 34 PEDV-positive farms in central and southern regions of the country (OIE, 2014b). More details on the different outbreaks are provided in Table 2 in Appendix B.

Attenuated or killed vaccines against PEDV, some of them combined with transmissible gastroenteritis virus (TGEV) (bivalent vaccines), have been used in China since 1995 (Chen et al., 2010). An attenuated virus vaccine using cell-culture-adapted PEDV has been administered on a voluntary basis to sows in Japan since 1997 (Song and Park, 2012). Oral vaccination with cell-culture attenuated vaccines has been used in South Korea since 2004 and in the Philippines since 2011 (Song and Park, 2012). However, several publications question the efficiency and/or safety of PEDV vaccines used in Asia (Ayudhya et al., 2012; Luo et al., 2012; Pan et al., 2012; Sun et al., 2012; Tian et al., 2013). It has been reported that PEDVs that have caused disease in China are very closely related to attenuated vaccine strains, which might be vaccine strains reverted to virulence (see section 3.2).

PED is a reportable disease in some countries of the region (i.e. Japan and South Korea). However, no active monitoring is conducted in any affected country in the region. Only one published report from Asia since 2004 included data on seroprevalence: 73.6% of the sows from 48 commercial farms sampled were seropositive in South Korea (Oh et al., 2005).

2.2.3. The Americas

PEDV was first identified within the USA in Iowa in May 2013, although testing of historical samples identified the earliest detection of the virus to have occurred in Ohio in April 2013. There was no previous description of PEDV in the region before that time and therefore, this pig population could be considered PEDV naive and fully susceptible to an infection with PEDV. Stevenson et al. (2013) described the disease in the first affected farms. It is relevant to point out that these farms were not related: they had no geographical connection (farms were separated by at least a 100 miles) and there was no evidence of shared personnel or links between feed mills/feed suppliers or trucks/trucking companies. PEDV rapidly spread throughout the country and was confirmed on farms from 32 states by the end September 2014 (see Table 2 in Appendix B for more information). It is not clear at the moment why PEDV spread so fast in the USA compared with other countries where the virus has been introduced. According to data provided, epidemics peaked in February and March 2014 and the number of positive submissions has been decreasing since then (see Figure 3 in Appendix B). PEDV is now a reportable disease in the USA (since June, 2014)[8]. A PEDV subunit vaccine based on replicon

[7] http://agriculture.gouv.fr/IMG/pdf/140512_categ_emergent_DEP_cle0f6c2e.pdf (accessed 1 August 2014)
[8] http://www.usda.gov/wps/portal/usda/usdamediafb?contentid=2014/06/0113.xml&printable=true (accessed 1 August 2014)

particle technology[9], using a single PEDV protein (the spike protein), as well as a PEDV vaccine with killed virus[10], have recently been granted a conditional licence in the USA[11].

PEDV was detected in Mexico for the first time in July 2013 (Fajardo et al., 2014). The virus spread through the country and, in May 2014, outbreaks were reported from 17 out of 32 federal entities (OIE, 2014c). No updates were available as of September 2014.

In October 2013[12], PEDV was identified for the first time in Peru (three outbreaks). In 2014, until September, six outbreaks were identified in the Lima region and one was identified in the Ica region (Quevedo-Valle, 2014). Preliminary studies suggested that Peruvian isolates are strongly related to North American strains, although no data were provided (More-Bayona et al., 2014; Quevedo-Valle, 2014).

In November 2013, PEDV was also identified as the cause of outbreaks of diarrhoea in farms in the Espaillat province, Dominican Republic. The isolates were closely related to US strains. This information was reported in June 2014 (OIE, 2014d). By September 2014, PED outbreaks were reported in seven of the 31 provinces of the country (Gómez, 2014).

In April 2014, Canada reported to OIE outbreaks of PEDV that started in January and affected 58 herds in four provinces. Again, sequencing of the PEDV genomes demonstrated that they were similar to those circulating in the USA (OIE, 2014a; Pasick et al., 2014). PED is not a federally reportable disease in the country although it is reportable in some of the provinces (e.g. Alberta[13], Manitoba[14] and Quebec[14]). The latest case in this country was reported in July 2014, and passive monitoring through the Canadian Swine Health Board[15] indicates that no new PED cases are being detected in the country.

An acute outbreak of diarrhoea and death in lactating piglets was observed in Colombia in March 2014. A total of 45 farms (including backyard farms) from five administrative departments were involved. PEDV isolates from Colombia were further characterized and found to be similar to PEDVs described in the USA (OIE, 2014e). This event was reported to OIE in June 2014. By September 2014, 54 samples from six departments were confirmed via laboratory testing (Rativa, 2014).

Finally, a PEDV outbreak occurred in a commercial pig farm in Ecuador in July 2014 and was reported to OIE in September 2014 (OIE, 2014f).

2.3. Occurrence of PDCoV

Viruses belonging to the genus deltacoronavirus were first reported in birds in 2009 (Woo et al., 2009), whereas PDCoV was identified for the first time in Hong Kong, as published by Woo and colleagues (2012).

However, the first association of PDCoV with a diarrhoeal disease was reported in February 2014 in a sow herd in Iowa. Viral enteritis was suspected based on clinical signs but neither PEDV nor TGEV was detected. Surprisingly, all faecal samples tested positive when a pan-coronaviridae reverse transcriptase polymerase chain reaction (RT-PCR) was performed. Further analysis using sequencing allowed the confirmation of PDCoV (Li et al., 2014). Since then, according to a US Department of Agriculture report of 19 June 2014, PDCoV has been detected on 277 farms distributed across 15 states (see Table 5 in Appendix B for more information). Sequence analysis of PDCoVs circulating in

[9] http://www.harrisvaccines.com/documents/filelibrary/images/2014/AASV_2014_PED_E5CB3D11FD6AE.pdf (accessed 27 August 2014)
[10] http://www.porknetwork.com/pork-news/Zoetis-granted-conditional-license-for-PEDv-vaccine-273753991.html
[11] http://content.govdelivery.com/accounts/USDAAPHIS/bulletins/be33f9 (accessed 22 July 2014)
[12] http://www.senasa.gob.pe/RepositorioAPS/0/1/JER/ANRIEVIEP_ESTATUTUSZOO/BOLET%C3%8DN%20OCTUBRE%202013.pdf (accessed 30 September 2014)
[13] http://www.producer.com/2014/01/alberta-lists-ped-as-reportable-disease-in-hogs/ (accessed 1 August 2014)
[14] http://www.agcanada.com/daily/manitoba-quebec-to-declare-ped-reportable (accessed 1 August 2014)
[15] http://www.swinehealth.ca/PED-Alert.php (accessed 1 August 2014)

several states revealed high sequence identity (Marthaler et al., 2013 and 2014a,b; Wang et al., 2014a). In many farms, PEDV and PDCoV were simultaneously detected (Wang et al., 2014a, b) and further research is needed to establish its role in swine disease. PDCoV infection was reported in April 2014 (OIE, 2014g). PDCoV is a reportable infection in the USA (June 2014)[16].

PDCoV has also been described in some Canadian farms with clinical signs of vomiting and diarrhea, that tested negative for TGEV and PEDV during 2014. This infection is not federally reportable in Canada at the moment, but it is reportable in several particular provinces (e.g. Alberta[17]).

Recently, PDCoV has also been detected in 20 out of 143 samples collected in five Chinese provinces: Heilongjiang, Liaoning, Tianjin, Shandong and Jiangsu (Feng, 2014).

Currently, there is no other description of PDCoV in any other country. However, given the recent identification of PDCoV, it is likely that diagnostic capabilities are limited in many countries and, hence, only very limited testing is carried out.

3. Differences between European, Asian and American PEDV isolates

3.1. Collection of information

In order to describe possible differences between PEDV-EU, PEDV-As and PEDV-Am isolates and the possible existence of cross-protecting immunity (TOR3), information has been collected on sequences and phenotypic characteristics of PEDV isolates via an extensive literature review (see Appendix A), as well as through a search of grey literature on the internet. Detailed information is provided in Appendix C.

3.2. Description

Like other coronaviruses, PEDV has a positive-sense, single-stranded, RNA genome of about 28 000 nucleotides. The genome includes seven known open reading frames (ORFs) encoding both non-structural proteins (including the replicase polyproteins (from ORF1a and ORF1b)) and structural proteins (including the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins). The S, E and M proteins are all present on the outside of virus particles and hence can be expected to be the target of host antibody responses. The S protein (encoded by ORF2) is a large glycoprotein (1383 amino acids, ca.180-220 kDa) and is believed to play a major role in mediating virus attachment to cells (for more information, see review by Song and Park, 2012). Multiple epitopes within the S protein have been identified, which are recognized by neutralizing antibodies (see Table 6 of Appendix C). The S protein has been the focus for the development of vaccines against PEDV, and mutations within the S gene are associated with growth adaptation *in vitro* (Sato et al., 2011). Differences in the S protein sequence may explain the ability (or not) of particular vaccines to confer protection against different strains of PEDV. However, the M protein (20-30 kDa) also induces antibodies that neutralize the virus in the presence of complement (Song and Park, 2012).

Prior to the development of next-generation sequencing, the large size of the coronavirus genome lead to many studies focusing on the sequence of specific genes, such as the S gene in particular, to differentiate strains. The full genome sequences of over 30 different PEDVs have now been determined, but the full-length S gene (ca. 4 100 nt) and even the S1 portion[18] (ca. 2 200 nt) are still considered useful to study the genetic relatedness between strains (Lee et al., 2010; Wang et al., 2013c; Chen et al, 2014a, b). The sequence of PEDV ORF 3 has also been used for phylogenetic comparisons; this ORF encodes a protein that is not essential for growth of the virus in cell culture (Li

[16] http://www.aphis.usda.gov/newsroom/2014/06/pdf/secd_federal_order.pdf (accessed 1 August 2014)
[17] http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/cpv12455/$FILE/2014-05-20-sdcv-announcement-final.pdf (accessed 29 Aug 2014)
[18] The S protein can be divided into S1 (1-789 amino acids) and S2 (790-1 383 amino acids) domains based on its homology with S proteins from other coronaviruses, although there is no cleavage site in PEDV (Duarte and Laude, 1994).

et al., 2013a) and its role in the virus life cycle is not entirely clear, but the product has been reported to be an ion channel protein (Wang et al., 2012).

Using a collection of 33 full-length PEDV genome sequences, including viruses from China, Korea, the USA and Europe, it was possible to group these into two clusters, termed group I and group II, with further sub-divisions possible (Figure 1). All the US PEDV sequences (99.7-99.9% nucleotide identity with each other) clustered in group IIa (Chen et al., 2014a, b) with some Chinese viruses from 2011 and 2012. Recent PEDV-As viruses are present within each of the clusters and sub-groups, indicating that a range of different viruses are circulating in Asia (Figure 1; see Table 7 in Appendix C for more detailed information). The sequences from PEDVs circulating in China after 2010 cluster together in groups separated from clusters of sequences of PEDVs circulating before 2010 (see Figure 1; Huang et al., 2013; Wang et al., 2013c). It should be noted that all the PEDV sequences (including the prototypic European isolate CV777) are closely related (the identity between the US and non-US strains varies between 96.3 and 99.5%). The complete genome sequences include some regions that are highly conserved, and thus nucleotide differences are more apparent within the S protein sequence when evaluated alone. However, the grouping of sequences based on the entire S gene (or the S1 or S2 regions) gave the same clustering of strains as observed for the whole genome (Figure 1). The clustering into groups I and II also applied to the M, N, E and ORF 3 sequences, when considered separately, but sub-divisions within these groups were less clear for trees based on the M and N gene sequences (Chen et al., 2014a, b).

There are two recent reports of a new variant PEDV strain in the USA. This variant strain has 99%[19] nucleotide identity at the whole-genome level and 97%[19] nucleotide identity in the full-length S gene, compared with the original PEDV isolates clustered in the original strain reported in the USA (Figure 1). The variant strains, also referred to as the S INDEL strain by some laboratories in the USA, have some small deletions and insertions within the S1 coding region, resulting in only 89-93.8 % nucleotide identity within part of the S1 coding region compared with the original PEDV isolates reported in the USA (Chen et al., 2014a; Wang et al., 2014a). The new variant strain was reported to appear less virulent than the original US strains based on field reports, with piglets from infected sows showing minimal clinical signs and no mortality (Wang et al., 2014a). However, it remains to be confirmed whether the variant induces less severe clinical disease in PEDV naive animals. This new variant is more closely related to a Chinese strain than to the other PEDV strains currently circulating in the US, and it has been shown, retrospectively, to have been present in the USA pig population since May 2013. This indicates that at least two strains of PEDV were introduced into the US at a similar time and are now co-circulating in the USA pig population (Huang et al., 2013; Chen et al., 2014a).

[19] The percentage refers to PEDV strain OH851 and might be slightly different for other variant PEDV strains

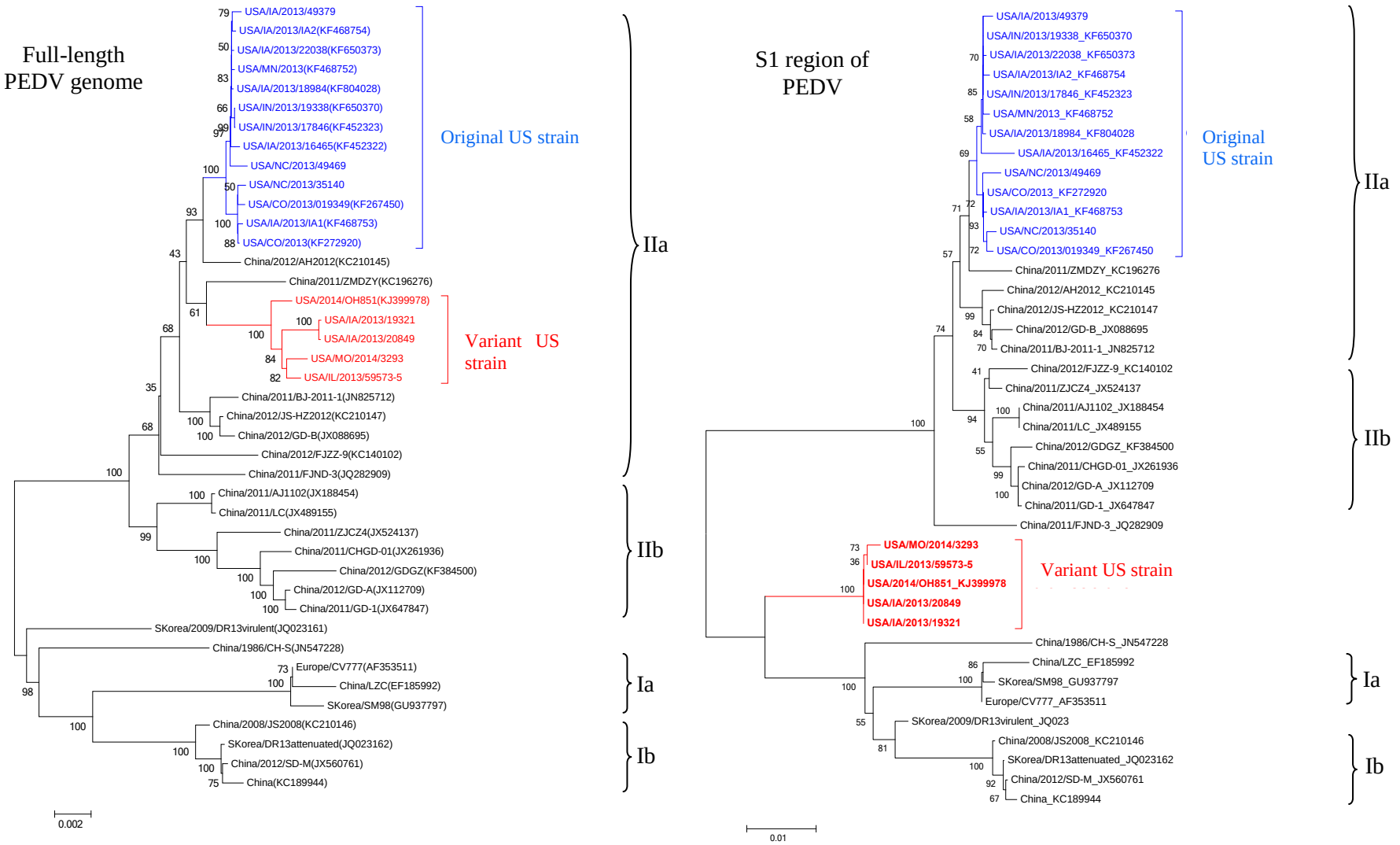
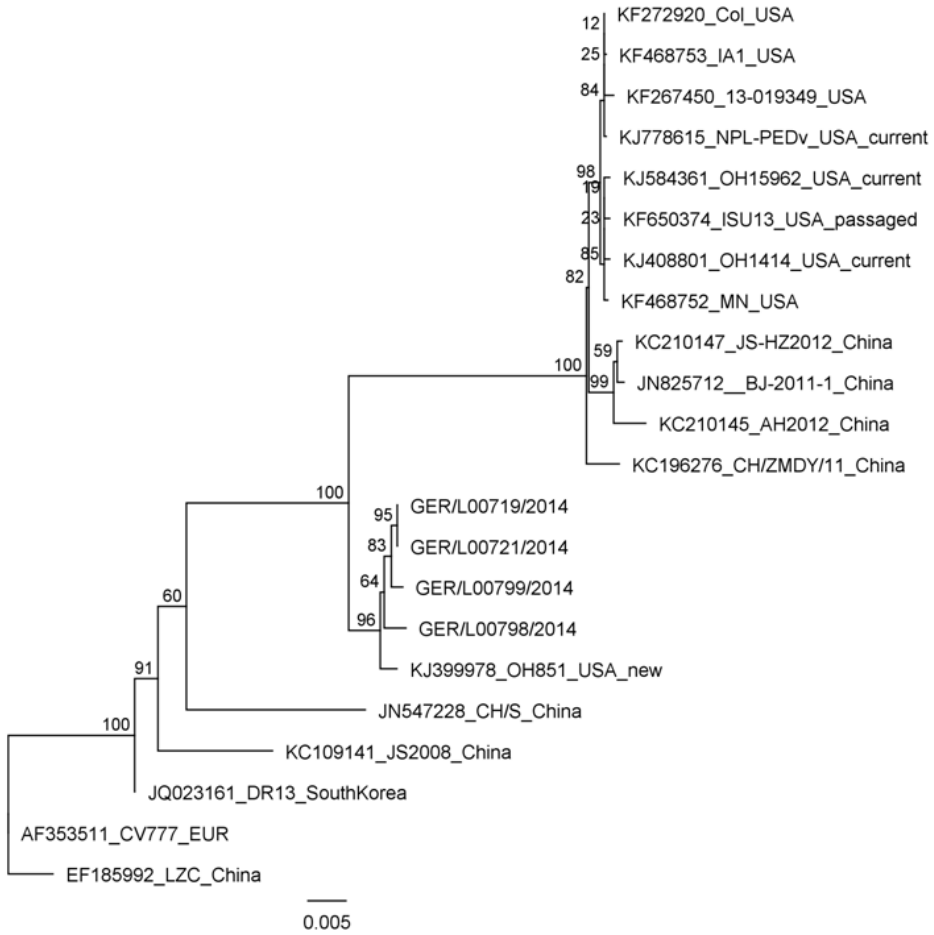


Figure 1: Phylogenetic analysis of the full-length PEDV genome and S1 region. (Adapted from Chen et al., 2014a, b)

Until data were reported recently by Germany and Italy, there was no information available on the sequences of the PEDVs that have circulated in recent years within Europe. The only PEDV-EU sequence information[20] that has been reported relates to a virus sampled in the 1970s and 1980s. Blome and colleagues (personal communication, Sandra Blome, Friedrich Loeffler Institute, Riems, Germany, October 2014) revealed the sequence of a virus that circulated in Germany (Baden-Württemberg), inducing watery diarrhea and killing approximately 20 infected pigs in a fattening herd of 1400 animals. Sequence analysis showed the closest identity was to the new variant PEDV-Am strain (99.5% identity of the full genome compared with PEDV-Am OH851). A phylogenetic tree based on the S gene is presented in Figure 2. Another German PEDV recently circulating in North-Rhine Westphalia showed high sequence identity to PEDV circulating in the USA (99% of a 651-nucleotide fragment of the S gene) (Henniger and Schwarz, 2014). A large fattening farm was affected. Despite the very high morbidity in feeders and finishers (almost 100%), none of the infected animals died. The sequences of several Italian PEDVs circulating in the period 2007-2014, which induced diarrhea and mortality in piglets, show that these recently circulating viruses cluster into three groups that are different from the cluster of the old PEDV-EU isolates (Alborali et al., 2014; Sozzi et al., 2014). One of the Italian clusters, including the only two sequences from 2014, has higher sequence identity to the PEDV-Am variant strain when analyzing the full S gene sequence. Taken together, it can be concluded that PEDVs with high sequence identity to the US PEDV strain are currently circulating in Europe, but more analyses and additional sequence data are required to understand PEDV evolution in Europe and a possible link with PEDV strains circulating in other parts of the world.



[20] CV777 isolated in Belgium (whole genome sequence available), Br1/87 isolated in France (sequence of some genes available)

Figure 2: Phylogenetic tree based on the spike protein. A distinct cluster can be found comprising the recent German isolates and the new variant US strain OH851 (kindly provided by Sandra Blome, Friedrich Loeffler Institute, Riems, Germany, October 2014).

Although PEDV isolates from the USA and recent isolates (2014) from Germany and Italy cluster together, there are not enough data to compare their phenotypic characteristics. Comparing the virulence/pathogenicity of different PEDV isolates would require comparative animal experiments. As already mentioned above, some studies describe PEDV isolates which might have altered pathogenicity (Li et al., 2012a; Yang et al., 2013b; Wang et al., 2014a), but side-by-side animal experiments including the different strains are required to assess potential differences in pathogenic effect.

The high level of sequence identity between different strains of PEDV would suggest that there should be antigenic cross-reactivity between them, albeit changes in individual epitopes could occur (Hao et al., 2014). However, it has been noted that the recent cases of PEDV infection in China from 2011 to2012 have occurred in animals that had been vaccinated with a CV777-based vaccine (Li et al., 2012a; Sun et al., 2012; Tian et al., 2013a). For instance, analysis of the S gene from PEDVs circulating in the Gansu province, China, found that the level of nucleotide and amino acid identity was about 94% compared with the CV-777-derived vaccine strain (Tian et al., 2013a). Some of the sequence changes were reported to modify the S protein by changing glycosylation sites, influencing its hydrophobicity or substituting amino acids in B-cell epitopes (Sun et al., 2012; Tian et al., 2013a,b; Hao et al., 2014), which might influence the neutralizing effect of the antibodies.

Li and colleagues (2012a) indicated that, in the early stages of the recent outbreaks of PEDV in China, the morbidity and mortality rates were lower within vaccinated herds than within non-vaccinated herds; however the CV777-based inactivated vaccine did not completely prevent disease. This was interpreted as indicating that the inactivated CV777-derived vaccine was providing only partial protection. Other vaccines against PEDV have also been employed in China, including a dual combination live-attenuated vaccine against TGEV and PEDV. Overall, there appears to be little information on the use and efficacy of the PEDV vaccines in China. In addition, it appears that some farms have experienced disease resulting from a PEDV isolate that was very closely related to the attenuated DR13 vaccine strain (see group I in Figure 1; Wang et al., 2013c) that is routinely used in South Korea, and so this might be to the result of reversion of the DR13 vaccine strain to virulence.

No experimental animal studies have been reported describing the cross protection between different PEDV strains. Serologic cross-reaction between PEDV-EU and PEDV-Am has been described including neutralizing antibodies raised against PEDV-EU towards PEDV-Am (Melinda Jenkins-Moore, Diagnostic Virology Laboratory, Ames, Iowa, personal communication, August 2014).

4. Impact of PEDV and PDCoV infection

4.1. Collection of information

In order to describe the possible impact of different strains/isolates of PEDV and PDCoV in the EU (TOR4), information has been collected on clinical course and pathological lesions caused by PEDV and PDCoV via an extensive literature review (see Appendix A), as well as through a search of grey literature on the internet. Experts also identified relevant publications from the period before 2004. Detailed information is provided in Appendix D.

4.2. Description of PEDV infection

4.2.1. Clinical course

The impact of PED reported in Asia (after 2010) and the US seems to be more severe than that described in Europe (Sun et al., 2012; Mole, 2013; Williamson et al., 2013; Kawashima, 2014). However, it is difficult to compare the impact between one country and another, since impact is

dependent not only on the pathogenicity of the virus but also on parameters such as the production system, biosecurity, the time of detection of an outbreak, farm management, herd size, the immune status of the population and herd sanitary status (e.g. presence of other infectious agents) (Chae et al., 2000; Jung et al., 2006a,b; Song et al., 2007; Ayudhya et al., 2012; Stevenson et al., 2013; Wang et al., 2013a; Williamson et al., 2013; Dufresne and Robbins, 2014; McOrist, 2014). For instance, the impacts of PED in the USA and Canada are significantly different despite the temporal overlap of outbreaks in the two countries. This section, however, provides a general description on the clinical course of a PEDV infection in naive animals since there is a lack of evidence for any difference in clinical disease in naive animals between countries.

Newborn piglets can be protected against infection by maternal antibodies (Bandrick et al., 2014). The aim of control measures such as vaccination and feeding sows with naturally infected materials is to achieve protective levels of maternal PEDV-specific antibodies in colostrum (Kweon et al., 1999; Song et al., 2007; Song and Park, 2012; Dufresne and Robbins, 2014; Gerber et al., 2014a). The severity of disease associated with PEDV within a herd is variable and is highly dependent on the age of the infected pigs and on the level of immunity in the population (see section 4.2.1.4). The next three sub-sections describe the clinical course of PEDV in suckling piglets, weaned piglets, and fatteners and adults considering a PEDV-naive herd. The last subsection on clinical course describes the evolution from an acute outbreak to endemic infection in a herd.

4.2.1.1. Suckling piglets

The term ‘suckling piglets’ refers to newborn animals until the time of weaning. At the peak of the epidemic, a PEDV-naive population of sucking piglets usually experiences very high morbidity (up to 100%), characterized by watery, non-mucous-haemorrhagic, fetid diarrhoea containing flocculent undigested milk as well as vomiting (not in all affected animals) (e.g. Duy et al., 2011; Sun et al., 2012; Stevenson et al., 2013; Wang et al., 2013a; Yang et al., 2013b; Dufresne and Robbins, 2014; Lin et al., 2014). Diarrhoea induces severe dehydration and emaciation causing mortality. Particularly in the first 2-3 days after birth, mortality associated with PED can reach up to 80-100% (Martelli et al., 2008; Stevenson et al., 2013). One-day-old caesarian-derived/colostrums-deprived piglets inoculated with a PEDV-Am strain demonstrated lethargy and severe, watery diarrhea within 12-24 hours post inoculation (hpi) with intermittent vomiting in some piglets from 24 to 48 hpi. Loss of body condition and marked dehydration were apparent by 48 – 72 hpi when piglets were euthanized (Phil Gauger, Iowa State University, Ames, Iowa, USA, personal communication, September 2014). Very similar observations were made in colostrum-deprived one-day-old piglets inoculated with a Korean PEDV isolate (Kim and Chae, 2000). More details are provided for each study in Table 8 of Appendix D.

4.2.1.2. Weaned pigs

The term ‘weaned pigs’ refers to animals from the time of weaning until they are 60-80 days of age. Morbidity rates of up to 90% in pigs 28-75 days-old have been reported (Martelli et al., 2008). Watery, non-mucous-haemorrhagic diarrhoea and vomiting in some pigs are reported, accompanied by anorexia and lethargy. The majority of the affected pigs recover around one week after infection (Hesse et al., 2014), so that mortality in weaned pigs is only 1-3% (Pospischil et al., 2002; Martelli et al., 2008; Stevenson et al., 2013). Three-week-old weaned pigs challenged with a PEDV-Am strain demonstrated clinical diarrhea by 48 hpi, with vomiting observed at two and three days post-inoculation (dpi) in some pigs. Morbidity, including lethargy, anorexia and watery diarrhea, peaked at 6 dpi but subsided by 10 dpi. In addition, challenged pigs demonstrated a significant reduction in average daily gain during the first week post-inoculation compared with non-challenged control pigs (Madson et al., 2014). More details are provided for each study in Table 8 of Appendix D.

4.2.1.3. Fatteners and adults

The age group ‘fatteners and adults’ refers to pigs from 60-80 days of age to slaughter age and includes both sows and boars. Morbidity can be variable but, in a susceptible population, fatteners and adults can experience a morbidity rate of up to 90%, showing the typical clinical signs of PED (watery

diarrhoea and, to a lesser extent, vomiting) for a few days after infection (Martelli et al., 2008; Stevenson et al., 2013). The effect of PEDV on the epithelial cells of the villi is the same in young piglets and in adults but the severity of the disease depends on the capacity of the cells at the base of the villus to differentiate into mature cells and migrate to the top of the villus, restoring the villus anatomy and function. As reported for transmissible gastroenteritis, regeneration of the villi takes more time in young animals (6-7 days or more) than in adults (3-4 days) (Kelly et al., 1972). Systemic clinical signs such as fever, anorexia and lethargy may be present in adult pigs. Adults showing mild watery diarrhoea can be off-feed for some days, but recover promptly (Martelli et al., 2008; Li et al., 2012a; Sun et al., 2012; Lin et al., 2014). In fatteners and adults, mortality remains between 0 and 4% (Martelli et al., 2008; Lin et al., 2014; personal communication, Sandra Blome, Friedrich Loeffler Institute, Riems, Germany, October 2014). More details are provided for each study in Table 8 of Appendix D.

A reduction in the reproductive performance of PEDV-infected animals has been reported in the scientific literature (Olanratmanee et al., 2010), but this may be induced by other pathogens that may be transmitted to sows via feeding of naturally PEDV-infected material as a measure to control PED.

4.2.1.4. Evolution from epidemic outbreak to endemic infection in a herd

When PEDV-infected pigs are introduced into a PEDV-naive herd, clinical signs typically appear within four to six days (Martelli et al., 2008; Geiger and Connor, 2013). In most outbreaks, clinical signs appear first in piglets, followed by disease in farrowing, breeding and gestation rooms. In some cases, diarrhoea can be observed first in gestating sows and subsequently in the farrowing units (Martelli et al., 2008; Dufresne and Robbins, 2014). The whole herd can rapidly become infected. On a herd basis, the average initial pre-weaning mortality is expected to range from 30 to 80% decreasing following the development of protective immunity among farrowing sows (Pospischil et al., 2002;Martelli et al., 2008; Gao et al., 2013; Stevenson et al., 2013). The induction of a protective immune response, mainly related to the stimulation of secretory immunoglobulin A, coincides with reduced PEDV shedding about one week following infection of an animal, but data are lacking to determine the exact duration of a protective PEDV-specific immune response (Saif et al., 1994; Van Cott et al., 1994; Carvajal et al., 1995; De Arriba et al., 2002). The immunity is certainly not long lasting, but a rapid anamnestic response after exposure generally prevents the reappearance of the disease in previously immunized or exposed animals (Saif et al., 2012).

Depending on the size of the herd and the type of operation, the time-to-baseline production[21] was approximately six weeks (ranging from 4 to 8 weeks) (Martelli et al., 2008; Goede and Morrison, 2014). PEDV infections will gradually disappear when a sufficient level of herd immunity is achieved. Endemic infection can occur in herds with a continuous flow production system and clinical signs can appear in recently weaned pigs when protective lactogenic immunity declines. Infected pigs suffering from moderate disease (diarrhoea) with low or no mortality typically clear the infection and develop a natural active immunity within two weeks (Martelli et al., 2008). On the other hand, introduction of new susceptible animals may lead to reoccurrence of PED in a herd (Pijpers et al., 1993; Martelli et al., 2008). PED may also be involved in a multi-etiological diarrhoea syndrome in feeder pigs, appearing two to three weeks after entering the fattening units, particularly when pigs originate from different sources and when new pigs are continuously added to the fattening units (Van Reeth and Pensaert, 1994).

4.2.2. Pathological lesions

Necropsied piglets demonstrated congestion of the small intestine with segmental enteritis, which probably contributed to malabsorption. Severe atrophic enteritis characterized by blunting of the intestinal villi and sloughing of the intestinal epithelium is also prominent in affected piglets. Histopathological lesions characteristically include small intestinal villous blunting (Debouck et al.,

[21] Defined using statistical process control methods to represent the time to recover of the number of pigs weaned per week that herds had prior to PEDV-detection.

1981; Li et al., 2012a; Huang et al., 2013; Stevenson et al., 2013; Jung et al., 2014; Lin et al., 2014). Although some strains also replicate in cecum and colon epithelial cells, cellular necrosis and villous atrophy were not evident. Ultrastructural colon lesions have rarely been observed (Saif et al., 2012). Whether PEDV infection of the large intestine contributes to the severity of PED is unclear (Jung et al., 2014). These pathological findings were similar to those described in conventional pigs naturally infected with PEDV-Am or PEDV-As isolates and in caesarean-derived, colostrum-deprived pigs experimentally infected with PEDV-EU isolate CV-777 (Debouck et al., 1981; Sueyoshi et al., 1995; Kim and Chae, 2000; Stevenson et al., 2013). More details are provided for each study in Table 8 of Appendix D.

4.2.3. Possible impact of the introduction of PEDV-Am into the EU

Within an immune population, protecting sucking piglets from PEDV, the most critical group in terms of clinical impact, depends on the level of lactogenic immunity provided by an immune dam. The effect of introducing a PEDV-Am isolate into the European pig population will depend on the level of PEDV-EU-specific immunity and the cross-protection between PEDV-EU and PEDV-Am isolates.

The susceptibility of pigs to PEDV will probably differ between Member States and parts thereof as it depends on the local PED history. As described in section 2.2.1, only limited data are available from preliminary testing on the seroprevalence levels against PEDV-EU in Member States. In Member States or regions where no PEDV infections have ever occurred or where no seropositive animals are present, the pig population is expected to be highly susceptible to PEDV of EU, Asian or American origin. Although some PEDV-EU seropositive animals might be present in some Member States, it is not known which level of immunity is required in individual animals or at herd or regional levels to achieve protective immunity and hence to prevent infection. Therefore, any estimation of the susceptibility of the European pig population to PEDV-EU infection would have a high uncertainty at the moment.

The lack of data regarding cross-protection between PEDV-EU and PEDV-Am isolates described in section 3.2, makes it impossible to predict the potential impact of introducing a PEDV-Am strain into the current European pig population.

The apparent low impact of recent PED outbreaks in Italy and Germany caused by viruses having high sequence identity to US PEDV may be associated with the level of immunity in the pig population and/or farm type and management. Other factors which may influence the impact of possible spread of the virus to Member States are the level of cross-protection between different PEDVs and seroprevalence (population immunity), which are currently unknown but expected to vary between Member States.

4.3. Description of PDCoV infection

PDCoV has been detected in 39 out of 42 faecal samples from five farms reporting outbreaks of diarrheal disease in sows and piglets (Wang et al., 2014b). The reported death rate in piglets (30-40%) was lower than that typically observed with PEDV infection. However, the interpretation of field data is difficult, since co-infections with PEDV or other intestinal pathogens are common (ranging from 20 to 80% of the samples analysed; Marthaler et al., 2014a; Wang et al., 2014b). Preliminary results show that two-day-old piglets inoculated with PDCoV under experimental conditions showed diarrheic feces on post-inoculation day 2 with 100% morbidity, whereas mortality was variable among the litters. Sows developed diarrhea on post-inoculation day 3 and were clinically normal after post-inoculation day 8. (Dick Hesse, Kansas State University, personal communication, 25 September 2014). Further analysis of this experiment will provide a better understanding on the viral pathogenesis and clinical symptoms associated with PDCoV infection.

Serological tests specific to PDCoV and aimed at determining the immune status of the pig population have recently been developed and are currently in the process of validation (Dick Hesse, Kansas State University, personal communication, 25 September 2014). This would allow seroprevalence studies in

the near future, which would help our understanding of the spread and impact of PDCoV. Based on the currently available field observations from the USA (e.g. Wang et al., 2014b), the current view is that PDCoV infections would have a lower impact than PEDV.

5. Characterization of PDCoV as an emerging disease?

In order to determine if PDCoV infections as an emerging disease (TOR2), it was assessed whether PDCoV fulfils the criteria defined by OIE.

An emerging disease is defined by OIE as a new occurrence of a disease, infection or infestation in an animal, causing a significant impact on animal or public health resulting from (1) a change of a known pathogenic agent or its spread to a new geographic area or species; or (2) a previously unrecognized pathogenic agent or disease diagnosed for the first time (OIE, 2014h). As described in section 2.3, PDCoV was first described in 2012 in Hong Kong (Woo et al., 2012) and additional cases have been reported only by the USA, Canada and China.

As described in section 4.3, PDCoV can induce clinical signs and mortality in pigs, but the available reports from the USA and Canada do not suggest a significant impact on animal health within these countries. In addition, no zoonotic potential of the virus has been reported. Therefore, the current knowledge of PDCoV leaves open questions on whether it can be classified as an emerging disease.

6. Presence and survival of PEDV and PDCoV in matrices

6.1. Collection of information

In order to describe the presence and survival of PEDV and PDCoV in different matrices and their role in the transmission of the virus (TOR5), an extensive literature review has been performed to collect information on the detection of genetic material and/or infectious viruses in different matrices, survival of the virus in the matrices and the possible role of the matrices in the transmission of PEDV or PDCoV (see Appendix A). A search of grey literature on the internet was also performed and experts identified relevant publications from the period before 2004. Detailed information is provided in Appendix E. Sections 6.2 and 6.3 provide an overview of the available scientific evidence and identify the data gaps. Ranking of the different matrices according to their probability of PEDV transmission was not performed in this scientific opinion. It is interesting to note that a full risk assessment has recently been done by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2014) based on (1) the probability that a given matrix will be a source of virus, (2) the probability of contact occurring between pigs and the matrix and (3) information on the existence of imports of that matrix into France from an infected country. The outcomes of this scientific opinion and the ANSES risk assessment cannot be directly compared, as different approaches are used.

6.2. Description of PEDV

6.2.1. PEDV hosts and tissue tropism

Pigs[22] are considered the main host of PEDV. After infection through the oral route, the virus replicates in epithelial cells of the small intestinal villi, particularly in the jejunum and ileum, but also possibly in the colon (Jung et al., 2014). Faecal shedding of PEDV is detected 24 hpi and diarrhoea is observed between 22 and 36 hpi. Given the high levels of PEDV replication reported in the intestinal tract (up to 12.3 log10 genome equivalents (GE)/mL) (Jung et al., 2014), leakage of viral components (e.g. incomplete virus particles containing RNA) or even infectious viruses to other tissues, such as blood, might be a possibility[23]. PEDV RNA was detected in the serum of infected piglets after the appearance of clinical signs, in the serum of piglets with acute diarrhoea in field conditions and in the

[22] To date, there have been no reports of PEDV in wild pigs but there is no reason why the virus would not affect wild pigs

[23] PEDV RNA has been detected in serum. The term viremia has not been used, as the infectious virus has not been demonstrated in blood.

serum of piglets that were in direct contact with experimentally infected piglets (Hesse et al., 2014; Jung et al., 2014). However, the amount of PEDV RNA detected in serum has been reported to be 10.000 – 10 million times lower compared to the level in faeces (Jung et al, 2014). At present, there are no data showing PEDV replication in tissues outside the intestinal tract.

There is no evidence at the moment that any other animal species act as a host for PEDV. Some tissue samples of geese, buzzards and a stray cat have been reported positive for PEDV RNA, but it is not clear if the virus replicates in these animals (see Table 9 in Appendix E). On the other hand, neither PEDV RNA nor PEDV-specific antibodies were detected in samples of experimentally PEDV-infected mice and sparrows or in samples of naturally exposed sparrows (see Table 9 in Appendix E). Research is ongoing to assess the role of birds and rodents in the spread of PEDV.

6.2.2. Data on PEDV presence in matrices

This section focuses on matrices that may contain PEDV as a result of their porcine origin, and does not include materials which may have become contaminated from such porcine matrices. Matrices taken into consideration are live pigs, porcine faecal material (faeces and slurry), porcine semen and embryos, porcine whole blood, spray-dried porcine blood and plasma[24], other porcine products permitted in pig feed (e.g. red blood cells, hydrolyzed proteins, fat, gelatin and collagen)[25] and untreated pig products/swill[26], and the air.

Many other matrices could become contaminated with PEDV (e.g. via faeces) and might also be relevant for the transmission of PEDV. The risk from these matrices is dependent on (1) their probability of being contaminated with PEDV and (2) the likelihood of transfer of the infectious virus to susceptible pigs. However, as indicated in the introduction, this scientific opinion does not include a full risk assessment on potential entry routes of PEDV.

For each of the considered matrices, the text below will describe whether or not detection of RNA and/or infectious viruses[27] has been reported, the survival of the virus in the matrix and whether or not the matrix has been reported to contribute to transmission of the virus. A summary is presented in Table 1 and detailed information is available in Table 10-13 of Appendix E.

6.2.2.1. Live pigs

As described in section 6.2.1., PEDV RNA and viral replication has been detected in intestinal tissues of infected pigs. Faecal shedding of PEDV is reported from 24 to 48 hpi and lasts, in general, for about one week (Carvajal et al., 1995; Song et al., 2006; Hesse et al., 2014), although shedding for a period of one to two months has been reported (Hesse et al., 2014; Sun et al., 2014). Faecal shedding of PEDV is considered the main pathway that contributes to the spread of PEDV from live pigs. The viral titers are highest in faeces (see section 6.2.2.2 below) and the oral-faecal route is considered the main route of PEDV transmission. Indeed, a recent experiment showed seroconversion of animals in direct contact with infected animals, whereas animals exposed only to aerosols of infected animals did not seroconvert (Hesse et al., 2014). At the moment, specific measures are in place to prevent the import of PEDV-infected pigs into the EU[28].

[24] Based on the Commission Implementing Regulation (EU) No 483/2014

[25] Based on Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Available online: http://ec.europa.eu/food/fs/bse/bse36_en.pdf (accessed 23 July 2014).

[26] Low biosecurity pig holdings commonly use swill as supplementary feed, often including untreated pork or pig products. This is, however, illegal within the EU according to the Animal By-product Regulation no. (EC) 1069/2009.

[27] Positive result in virus isolation, bioassay or transmission experiment.

[28] Commission implementing regulation (EU) No 750/2014. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0750&from=EN (accessed 30 September 2014).

Table 1: PEDV in different porcine matrices

Matrix	PEDV RNA detected	Infective PEDV detected	Survival of the virus in the matrix	Role reported in transmission
Live pigs	Yes	Yes	Faecal shedding of PEDV initiates 24-48 hours post-infection and lasts, in general, about one week, although shedding for a period of one to two months has been reported	Yes
Faeces	Yes	Yes	PEDV remains infectious when faeces are heated to 62.7°C (145°F) for 10 minutes or when faeces are incubated between 40 and 60°C with a relative humidity ranging from 30 to 70% for up to seven days; low infective dose but exact viral titer is not known	Yes
Slurry	No data available	No data available[(b)]	PEDV remains infectious when spiked in slurry and stored for 14 days at room temperature and at least for 28 days when stored at 4°C and -20°C	No data available[(b)]
Semen	Yes	No data available[(b)]	No data available[(b)]	No data available[(b)]
Embryos	No data available[(b)]	No data available[(b)]	No data available[(b)]	No data available[(b)]
Whole blood	Yes	No data available[(b)]	No data available[(b)]	No data available[(b)]
Spray-dried porcine blood and plasma	Yes	Yes	It is reported that spray-drying of porcine plasma can inactivate PEDV. Infectious PEDV has been detected in SDPP in one study but the origin of the infectious PEDV in SDPP is not clear (cross-contamination or inefficient inactivation)	No experimental proof that pigs have been infected via feed containing PEDV-contaminated SDPP, but very low concentrations of infectious PEDV in feed containing PEDV PCR-positive SDPP cannot be excluded at the moment
Other porcine-derived feed components[(a)]	No data available[(b)]	No data available[(b)]	No data available[(b)]	No data available[(b)]
Air	Yes	Yes (within a room)/no (long distance)	No data available[(b)]	Yes (within a room)/no (long distance)

[(a)] Including red blood cells, hydrolyzed proteins, fat, gelatin, collagen and untreated pig products (swill)
[(b)] No studies found

6.2.2.2. Porcine faeces

PEDV is shed in large amounts in faeces (e.g. levels of up to $10^{6.85}$ GE/mL and up to $10^{12.3}$ GE/mL have been reported by Sun et al., 2014, and Jung et al., 2014, respectively) and PEDV has been isolated from this matrix (e.g. Marthaler et al., 2013). Faeces can be considered the main PEDV source for transmission between pigs through an oral-fecal process. In general, PEDV stability is adversely affected by increasing temperatures (Hofmann and Wyler, 1989; Pospischil et al., 2002; Song and Park, 2012). Two preliminary reports[29,30] from recently performed experiments suggest that PEDV remains infectious when faeces are heated to 62.7°C (145°F) for 10 minutes or when faeces are incubated at between 40 and 60°C with relative humidity ranging from 30 to 70% for up to seven days (see Table 11 and Table 12 in Appendix E). One of these reports[29] also describes an experiment to estimate the minimal infectious dose of a PEDV-Am isolate, using 10-fold serial dilutions from an initial clarified homogenate of intestinal mucosa sampled from a PED-affected piglet. The reported results suggest that piglets could be infected with dilutions of the inoculum containing PEDV levels below the detection limit of the RT-PCR (which is not specified) (seeTable 13 Table 12 in Appendix E). These preliminary results suggest that very low PEDV titers in faeces are infectious, but the minimum infectious viral titer is not known.

Many other objects can become contaminated with PEDV-containing faeces and hence contribute to the transmission of PEDV. Mechanical transportation of the virus by humans (e.g. on clothes or boots) or vehicles is an issue because of the apparent low infectious dose required to infect a piglet and the relative resistance of the virus especially in cold and wet conditions. Contamination of trailers with faeces has been shown to be an important vehicle of virus spread between farms in the USA leading to drastic changes in biosecurity and disinfection procedures of vehicles (Lowe et al., 2014). Preliminary experimental results[29] suggest that PEDV survives one week when spiked in water and stored at 25°C, but data underpinning this statement were not found.

6.2.2.3. Porcine slurry

No data were found on the identification of PEDV RNA or infectious virus in slurry or on transmission of PEDV via slurry. Preliminary experimental results[29] suggest that PEDV remains infectious when spiked slurry is stored for 14 days at room temperature (~25°C) or stored for 28 days (or more[31]) at 4 °C or -20 °C, with relative humidity ranging from 30 to 70%.

6.2.2.4. Porcine semen

PEDV RNA has been detected at low levels in semen from healthy boars located on three different farms in China, with copy numbers between $10^{1.46}$ and $10^{3.65}$/mL (Sun et al., 2014). Detection of PEDV RNA in semen is also mentioned by other authors, although no data are provided and these authors state that contamination of the samples cannot be excluded (Dufresne and Robins, 2014). There are no data available on the detection of infectious virus in semen or on the possible role of semen in the transmission of PEDV.

6.2.2.5. Porcine embryos

As PEDV RNA has been detected in serum, PEDV could be present in embryos, but there are no studies found that looked for the presence of PEDV in porcine embryos. There are also no data available on the detection of infectious virus in porcine embryos or on the transmission of PEDV via porcine embryos.

[29] http://www.pork.org/filelibrary/Goyal%2013-215%201-21-14.pdf (accessed 24 July 2014)
[30] http://www.pork.org/filelibrary/Holtkamp%2013-227%2012-20-13.pdf (accessed 24 July 2014)
[31] 28 days was last time point of sampling in the experiment.

6.2.2.6. Porcine whole blood

PEDV RNA has been detected at low levels in the serum fraction of whole blood but there are no data reported on the detection of infectious virus in this matrix (see section 6.2.1; Table 10 in Appendix E). No reports could be found describing a role of whole blood in the transmission of PEDV.

Whole blood is collected at slaughterhouses for further processing and used as an animal by-product. Blood from slaughterhouse animals can be collected using (1) an open draining system, in which blood from the animal is drained into buckets or trays, or (2) a closed draining system, in which blood from the slaughterhouse animal is not exposed to air and is drained directly from the body of the animal, for example using a hollow knife connected to vacuum piping (Bah et al., 2013). PEDV cross-contamination of blood cannot be excluded, especially when the open draining system is used (Davila et al., 2006).

6.2.2.7. Spray-dried porcine blood and plasma

SDPP incorporated in piglet feed is used to improve the performance and gut health of piglets. The PEDV-infectivity of artificially contaminated bovine plasma before and after the spray-drying process was evaluated on VERO cell monolayers. The study showed that, although still PCR positive, the spray-dried plasma did not contain infectious PEDV, meaning that the process inactivated the virus (Pujols and Segalés, 2014). However, isolation of PEDV in cell culture is difficult (Shibata et al., 2000; Chen et al., 2014b; Pasick et al., 2014) and may be a less sensitive model for evaluating PEDV infectivity than a swine bioassay. A very recent study from Gerber and colleagues (2014b), indicates that an experimental spray-drying process effectively inactivated infectious PEDV in plasma, since no PEDV RNA was present in faeces of inoculated piglets (at 3 dpi) and none of the pigs seroconverted (by 14 dpi).

The Ontario Ministry of Agriculture and Food reported in February 2014 that a particular lot of SDPP used in feed pellets contained PEDV genetic material[32] (no data shown). The Canadian Food Inspection Agency confirmed by RT-PCR that both the plasma and the feed pellets contained PEDV genetic material[33] (Pasick et al., 2014).

After the detection of PEDV RNA-positive SDPP, experiments were performed to assess whether or not this feed component contained infectious virus. Bioassay studies performed by Pasick and colleagues (2014) demonstrated that an implicated SDPP lot did contain PEDV capable of infecting and causing clinical disease in pigs. It is not clear if the presence of infectious PEDV in this lot of SDPP was due to inefficient inactivation of PEDV during spray-drying or whether cross-contamination after spray-drying took place. On the other hand, preliminary results of two other experiments could not demonstrate the presence of infectious virus in SDPP when piglets were inoculated with PEDV RNA-positive plasma, although the data from these studies are not yet published in a peer-reviewed journal (experiments from the US Food and Drug Administration and the University of Minnesota; see Table 10 in Appendix E). Cross contamination of SDPP with PEDV can occur at any point during the manufacturing, packaging and storage processes and/or during transportation of the product, owing to a breach in good manufacturing practices and/or biosecurity. Overall, it can be concluded that one study reported the presence of infectious PEDV in PEDV RNA-positive SDPP.

In addition it has been examined whether or not feed containing PEDV-contaminated SDPP could cause infection of piglets. Pigs that received feed supplemented with the Canadian infectious SDPP lot did not become infected with PEDV (Pasick et al., 2014). In a study by Opriessnig and collaborators (2014), commercial SDPP that was naturally RT-PCR positive for PEDV RNA (3.3 $\log_{10}$ PEDV RNA copies/g of the final diet) was fed to two-week-old piglets negative for PEDV. The piglets did not shed

[32] http://www.omafra.gov.on.ca/english/food/inspection/ahw/PED-advisory.html (accessed 30 July 2014)
[33] http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/2014-02-18/eng/1392762739620/1392762820068 (accessed 30 July 2014)

PEDV RNA and did not seroconvert. The authors concluded that the PEDV RNA present in the SDPP lot used in the diet was not infectious. Preliminary results of two other experiments also suggest that feeding pigs with a diet containing SDPP that was PEDV RNA-positive could not infect the animals, although the data of these studies are not yet published in a peer-reviewed journal (Campbell et al., 2014 and study from ISU; see Table 10 in Appendix E). Overall, there is no experimental evidence at the moment that piglets can become infected with PEDV when they receive feed containing PEDV-contaminated SDPP. However, testing large numbers of animals is required to detect very low concentrations of infectious PEDV in feed containing PCR-positive PEDV SDPP.

Experimental results suggest that PEDV can survive in SDPP for up to three weeks, two weeks and less than one week when SDPP contaminated with PEDV is stored at 4°C, 12°C and 22°C, respectively (Pujols and Segalés, 2014; see Table 10 in Appendix E). The virus survives longer in wet feed than in dry feed (28 and 7 days, respectively; see Table 11 in Appendix E). Further processing of SDPP to make pelleted feed might contribute to the inactivation of PEDV since a typical feed pelleting system uses temperatures between 70 and 100 °C (Nitikanchana, 2014).

The SDPP intended for the feeding of porcine animals that is produced and/or imported into the EU must be submitted to a heat treatment at a temperature of at least 80°C throughout the substance; should fulfill the physico-chemical and microbial requirements described in the European legislation and should be stored in dry warehouse conditions under room temperature for at least six weeks[34]. The design and implementation of adequate quality control systems is crucial to guarantee that every lot of SDPP meets these criteria. Spray-drying is affected by many parameters such as in-out air temperature, product flow, solids content, droplet size distribution and dryer configuration. Some of these parameters can vary amongst production cycles and/or methods. It is unknown whether or not such variations would affect PEDV inactivation. The current legal microbial requirements define maximum levels for *Salmonella* and *Enterobacteriaceae*, but do not require analysis for any virus.

It remains unclear if SDPP and pig feed in general are important in the epidemiology of PEDV. Epidemiological studies carried out in Ontario (Canada) in the first farms infected by PEDV showed that 18 of the 20 first-affected farms received their feed from the same company, as did the isolated case in Prince Edward Island, Canada[35]. On the other hand, PEDV PCR-positive plasma was shipped from the USA to Brazil and Western Canada in 2013, but no PEDV cases have been reported in these regions (Crenshaw et al., 2014). All experiments reported so far suggest that PEDV PCR positive SDPP in feed is incapable of transferring PEDV, since all animals remained negative (see Table 10 in Appendix E). It might be that infectious PEDV is present at very low concentrations in feed containing PEDV PCR-positive SDPP, suggesting that the probability of detecting at least one infected piglet during a bioassay would be very low (Pasick et al., 2014). There is one experiment reported (Dee et al., 2014) in which pigs received feed mixed with PEDV PCR-positive material collected from the interior wall of feed bins (using Swiffer pads and paint rollers) from four clinically affected farms. The exact source of PEDV contamination in the feed is undetermined. The pigs demonstrated diarrhea and their faeces were PEDV PCR-positive from four days after treatment until study termination at seven days after treatment. Overall, PEDV can be transmitted via feed but more data are required to assess the importance of PEDV spread via feed.

6.2.2.8. Other porcine-derived feed components

Detection of PEDV RNA or infectious virus in other processed swine products that could be used in animal feed (hydrolyzed proteins, gelatin, collagen, animal fat) has not been reported to date. Although not allowed in the EU[25], pigs may be fed with untreated pork meat and other pig products, often referred to as swill. As mentioned earlier (see section 6.2.1), a high level of PEDV replication has been detected in the intestinal tissue, and PEDV RNA has been detected at low levels in serum.

[34] Annex X, Chapter I of Commission Regulation (EU) No 142/2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council; Commission implementing Regulation (EU) No 483/2014

[35] http://www.farmscape.com/f2ShowScript.aspx?i=24577&q=Contaminated+Feed+Most+Likely+Source+of+Ontario+PED+Outbreak (accessed 30 July 2014)

There are no data showing PEDV replication in tissues outside the intestinal tract. Based on this, porcine swill (particularly untreated pig intestines) may contain infectious PEDV, but no data are available that describe the detection of PEDV RNA or the infectious virus in porcine swill.

There are also no data available on the detection of PEDV RNA or the infectious virus in pork muscle tissue, or on the role of this matrix in the transmission of PEDV. The consumption of pork muscle tissue by humans is not a problem, since PEDV has no zoonotic capacity[36].

6.2.2.9. Air

PEDV RNA has been detected in air samples collected in and around naturally infected herds (up to 10 miles downwind) and in isolation rooms where piglets were experimentally infected (Alonso et al., 2014) (see details of different studies in Table 10 in Appendix E). Air samples collected in field conditions were found to be negative in a bioassay[37], indicating that the RNA detected did not belong to infectious virus particles. In contrast, air samples collected under experimental conditions were demonstrated as infectious. On the other hand, preliminary results of another experimental study indicate that no viral antigen or seroconversion could be detected in animals of an aerosol control group, despite the presence of PEDV RNA in nasal and oral fluids sampled from these animals (Hesse et al., 2014). Overall, the current available data suggest that PEDV may be transmitted via air for short distances (within an isolation room), but there is no proof that PEDV can be transmitted naturally between pigs via air. More data are required to confirm the reported findings and to obtain knowledge on the survival of PEDV in air.

6.3. Description of PDCoV

PDCoV RNA has been detected in porcine intestinal samples, faeces and feed (Li et al., 2014; Marthaler et al., 2014a; Wang et al., 2014b;) but there is currently no information on the presence of PDCoV in slurry, semen, embryos, porcine whole blood, SDPP, other porcine-derived feed components or air. At the moment, there are no reports on the isolation of infectious PDCoV or experimental infection of pigs with PDCoV-contaminated material. It could be anticipated that the presence and survival of PDCoV in different matrices is comparable to that of other intestinal porcine coronaviruses such as PEDV and TGEV.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

PEDV and PDCoV are a rapidly evolving area of knowledge. This scientific opinion reflects current understanding at the time of publication.

TOR1: The current epidemiological situation in North America and Asia and elsewhere in the world regarding PED and the new porcine deltacoronavirus.

PED in Europe:

- Only limited active monitoring is conducted.
- Only a few Member States reported PED clinical cases and/or PEDV-seropositive animals within the last 10 years.
- In 2014, some outbreaks have been reported in Germany and Italy.
- No vaccination has been used.

PED in Asia:

- Only limited active monitoring is conducted.
- Many outbreaks have been reported in several countries within the last 10 years.

[36] There are no human cases reported
[37] Two mL pool of three positive air samples diluted at 1:10 to obtain 20 mL of inoculation material per pig.

- Vaccination has been used in several countries, which might influence the epidemiological situation.

PED in the Americas:

- Only limited active monitoring is conducted.
- The first outbreak was reported in May 2013 in the USA, followed by a rapid spread throughout the country and outbreaks reported by several countries in North, Central and South America.
- In 2014, new vaccines were granted conditional licences in the USA, which may influence the epidemiological situation.

PDCoV:

- Diagnostic capabilities are limited in many countries and, hence, only very limited testing is carried out.
- PDCoV has only been reported from Hong Kong, US, Canada and China

TOR3: Possible differences between the European classical PED alphacoronavirus strains and the ones currently circulating in the rest of the world, in particular in the Americas, and the possible existence of cross-protecting immunity.

- Few sequence data from PEDV-EU isolates are available, limited to historic (1970s and 1980s) and very recent (2014) cases. A high level of sequence identity was found between recent German and Italian viruses (2014) and PEDV-Am viruses.
- An original and a variant PEDV-Am strain, both having high nucleotide sequence identity to PEDV-As isolates from 2011-2012, are now co-circulating in the Americas. Retrospective studies indicate that at least two PEDV strains were introduced into the USA at a similar time.
- Differences in the nucleotide sequence of PEDVs have been identified, but their effects (if any) on virulence of the virus is currently unknown. No comparative experimental studies have been conducted or reported.
- Serological cross-reactivity between PEDV-EU and PEDV-Am is reported; however, no data regarding cross-protection are available.
- The evolution of PEDVs in Europe and the link to PEDV strains circulating in other parts of the world is not well understood at present

TOR4: Impact of the different PED alphacoronavirus strains and of the new porcine deltacoronavirus in pigs in different immunological scenarios.

PEDV:

- The impact of recently reported PED outbreaks in Asia (after 2010) and the US seems to be more severe than what has been recently described in Europe.
- The clinical signs of PEDV infections in naive pigs are similar in different countries indicating that different PEDV isolates induce similar clinical signs.
- The different impacts of PED outbreaks in different countries cannot be directly compared owing to variation for instance, in age group of the affected pigs, production systems, biosecurity, farm management, herd size, the immune status of the population and herd sanitary status.
- Mortality of up to 100% has been reported in suckling piglets for PEDV-EU, PEDV-Am and PEDV-As.
- An apparent low impact of recent PED outbreaks caused by viruses that have high sequence identity to US PEDV, has been reported in Italy and Germany. Factors which might influence the impact of a possible introduction of a US PEDV and spread of the virus to Member States include the level of cross-protection between different PEDVs and sero-prevalence (population immunity), which are currently unknown but expected to vary between Member

States. The recent impact of PED in Europe needs to be interpreted with care because only a small number of outbreaks have been described.

PDCoV:

- Diagnostic tools to detect PDCoV-specific antibodies have recently been developed and are currently in the process of validation
- Based on the currently available field observations from the USA, the current view is that PDCoV infections would have a lower impact than PEDV.

TOR2: Characterization of the new porcine deltacoronavirus as an emerging disease, especially as regards the severity of the disease induced.

- At present, there is no clear evidence that PDCoV infections is causing a significant impact on animal or public health.

TOR5: Risk assessment of potential entry routes of PED and the new porcine deltacoronavirus in the EU ranking them on the basis of the level of risk with a view to enhance risk mitigation, prevention and preparedness.

PEDV:

- Infected live animals and faeces have been reported to transmit PEDV. The infectious virus can survive in slurry, but at present there are no data available on the role of this matrix in PEDV transmission.
- High levels of infectious PEDV are shed in faeces and contribute to contamination of various objects (e.g. vehicles, humans) and feed.
- The transmission of PEDV via feed has been shown but more data are required to assess the importance of PEDV spread via feed.
- PEDV RNA has been detected at low levels in the serum fraction of whole blood, but, to date, no data exist on the infectious virus in this matrix.
- Faecal cross-contamination of blood during collection at slaughterhouses cannot be excluded.
- It is reported that spray-drying of porcine plasma can inactivate PEDV. However, the influence of variations in spray-drying processes has not been sufficiently validated for PEDV.
- Infectious PEDV has been detected in SDPP in one study, but the origin of the infectious PEDV in SDPP is not clear (cross-contamination or inadequate spray-drying).
- The infectious virus has been detected in air collected under experimental conditions and so PEDV may be transmitted via the air for short distances. Low levels of PEDV RNA have been detected in semen, but there are no data available on the presence of infectious virus in this matrix.
- There are currently no data available on the presence of PEDV in embryos, pork meat or other porcine-derived feed components such as red blood cells, hydrolysed proteins, fat, gelatine and collagen.
- Porcine swill, particularly including untreated pig intestines, can contain infectious PEDV but there are no data available at the moment on the role of this matrix in PEDV transmission.

PDCoV:

- There is a lack of data on the presence and survival of PDCoV in different matrices. It could be anticipated that the presence and survival of PDCoV in different matrices is comparable to that of other intestinal porcine coronaviruses such as PEDV and TGEV.

RECOMMENDATIONS

TOR1: The current epidemiological situation in North America and Asia and elsewhere in the world as regard PED and the new porcine deltacoronavirus.

- Promote harmonized diagnostic tools for PEDV as well as for PDCoV.

TOR3: Possible differences between the European classical PED alphacoronavirus strains and the ones currently circulating in the rest of the world, in particular in the Americas, and the possible existence of cross-protecting immunity.

- The genetic sequence of further recent PEDV-EU isolates should be determined to understand PEDV evolution in Europe and the possible link with PEDV-Am and/or PEDV-As strains.
- Comparative animal studies including PEDV-EU, PEDV-Am and PEDV-As strains should be performed to obtain knowledge on their differences in virulence.
- More knowledge is required regarding the cross-protection between PEDV-EU, PEDV-Am and PEDV-As strains, which could be acquired by performing cross-infection experiments.

TOR4: Impact of the different PED alphacoronavirus strains and of the new porcine deltacoronavirus in pigs in different immunological scenarios.

- The assessment of the possible impact of PEDV infection in the EU would require monitoring of the PEDV-seroprevalence level in Europe.

TOR2: Characterisation of the new porcine deltacoronavirus as an emerging disease, especially as regards the severity of the disease induced.

- Experimental studies are needed to obtain more knowledge on the pathogenesis and clinical signs of PDCoV infection.

TOR5: Risk assessment of potential entry routes of PED and the new porcine deltacoronavirus in the EU ranking them on the basis of the level of risk with a view to enhance risk mitigation, prevention and preparedness.

- More knowledge is required to assess the importance of feed components, blood and semen in the spread of PEDV.
- Cross-contamination of any object or feed with intestinal contents and faeces from PEDV-infected pigs should be prevented.
- The influence of variations in spray-drying processes should be validated more extensively for PEDV.

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APPENDICES

Appendix A. Extensive literature review

An extensive literature search was performed to collect information on PEDV and PDCoV published in the last 10 years (2004-June 2014). The search string (Pig or porcine) AND ((“porcine epidemic diarrh*” or PED or PEDV) OR (“deltacoronavirus” or PDCoV or SDCV or SCDV or PCDV) OR SECoV) was entered in Web of Science and resulted in the identification of 450 records. The number of papers reduced to 399 after removing the duplicates.

A publication was included in the review if (1) it reported on PEDV and/or PDCoV and (2) the main topic of the paper was on (a) prevalence, incidence, occurrence of the virus(es), (b) seroprevalence against the virus(es), (c) immune response against the virus(es), (d) sequence and/or phenotypic characteristics of the virus(es), (e) impact of the virus(es) (e.g. morbidity, mortality, production losses) and/or (f) virus presence in matrices (e.g. faeces, semen, plasma). A publication was excluded when it did not meet the inclusion criteria or when it was not written in English.

Screening of the titles reduced the number of included papers to 199, which were then screened based on the abstract and full text. An update of the search was done end Sep 2014 and resulted in the identification of 23 additional publications. Data were extracted from 138 publications and are mainly presented in the Appendices below.

Appendix B. Information on PEDV and PDCoV outbreaks in the period 2004-2014

1. PEDV

The members of the EFSA network on Animal Health and Welfare were contacted to provide information on the occurrence of PEDV in their country in the period 2004-2014. Information was provided by twenty-one countries (
Table 3). PEDV outbreaks were identified in Germany, Italy, Estonia and Hungary, whereas the other countries indicated that no data were available, no cases were reported or the country was negative for PEDV. However, none of the European countries performs active monitoring, meaning that the absence of identified cases provides only limited assurance that the virus is absent.

Table 2: Information on PEDV occurrence in the period 2004-2014 obtained by an extensive search of the scientific literature and relevant websites

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
Europe							
Czech Republic						15 samples from 6 herds/80 samples from 38 herds	Rodak et al., 2005
Italy	Po Valley	2005	2006	63		22/55	Martelli et al., 2008
		2006-2007				Faeces: 44/215 in PCR and 42/215 in ELISA, Intestinal content: 6/291 in PCR and 13/291 in ELISA	Sozzi et al., 2010
Germany	North-Rhine Westphalia	Spring 2014		1			Henninger and Schwarz, 2014
Asia							
Japan	Okinawa	01/10/2013		4			OIE, 2014e
	Ibaraki	18/11/2013		5			
	Kagoshima	11/12/2013		156			
	Miyazaki	13/12/2013		68			
	Kumamoto	28/01/2014		20			
	Aichi	16/02/2014		20			
	Aomori	24/02/2014		10			
	Kochi	04/03/2014		3			
	Okayama	13/03/2014		2			

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
	Tottori	13/03/2014					
	Saga	14/03/2014		9			
	Oita	16/03/2014		5			
	Fukuoka	20/03/2014		4			
	Chiba	27/03/2014		29			
	Nagasaki	28/03/2014		6			
	Saitama	28/03/2014					
	Mie	29/03/2014		14			
	Kagawa	02/04/2014					
	Ehime	04/04/2014		2			
	Tochigi	07/04/2014		9			
	Gunma	07/04/2014		6			
	Niigata	10/04/2014		13			
	Shizuoka	10/04/2014		5			
	Ishikawa	11/04/2014					
	Toyama	11/04/2014		2			
	Fukushima	11/04/2014		2			
	Yamagata	12/04/2014		2			
	Gifu-	14/04/2014		2			
	Hokkaido-	14/04/2014		3			
	Fukui	15/04/2014					
	Iwate	16/04/2014		8			
	Akita	19/04/2014					
	Miyagi	21/04/2014		3			
Thailand	Nakornpathom	December 2007	March 2008	8		33/33	Puranaveja et al., 2009
		2008	2012	50			Temeeyasen et al., 2014
China	Qinghai						Lan et al., 2005
	Heilongjiang	2005			39,128 (2,317)		Junwei et al., 2006
	Shanghai	2006					
	five provinces						Chen et al., 2008

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
	Gansu, Heilongjiang, Henan, Hunan, Inner Mongolia, Jiangsu, Jilin and Shanghai						Chen et al., 2010
	12 provinces	January 2011	October 2011	45		278/455	Li et al., 2012a
	Shandong	November 2010	April 2012			175/217	Wang et al., 2013b
	Shanghai	September 2011	January 2012			25/95	Ge et al., 2013
	29 provinces					361/504	Chen et al., 2013
	15 provinces	Jan 2006	Aug 2011			127 samples	Chen et al., 2013b
	Beijing, Hebei and Zhejiang	2011 Jan	2012 Mar			10/10	Gao et al., 2013
	5 provinces	February 2010	March 2012	55			Li et al., 2013b
Philippines		2006			(60,000)		Morales et al., 2007
		2007			(2,179)		
Vietnam	Three provinces in southern provinces	2009	2010				Duy et al.. 2011
South Korea						754/1024	Oh et al., 2005
						35/107	Jung et al., 2006.
						319/737	Park et al., 2007a
	Gyeongbuk	2008	2009				Lee et al., 2010
		Dec 2013	Jan 2014			10/10	Lee and Lee, 2014
Taiwan	central and southern Taiwan	n/a	n/a	n/a	16000	n/a	http://focustaiwan.tw/news/aeco/201402170041.aspx
	central and southern Taiwan	Dec 2013	Jan 2014	25			Lin et al., 2014
The Americas							
USA	Ohio	15/04/2013[a]	Ongoing[b]	366[b]	276[a]		[a]OIE 2014c; [b]USDA, 2014;

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
	Indiana	22/04/2013[a]	Ongoing[b]	430[b]	324 [a]		[c]http://www.wisconsinagconnection.com/story-national.php?Id=1388&yr=2014; [d]Mole, 2013; [e]Stevenson et al., 2013
	Iowa	29/04/2013[a]	Ongoing[b]	2314[b]	1912 [a]		
	Colorado	06/05/2013[a]	17/08/2014[b]	103[b]	77 [a]		
	Minnesota	06/05/2013[a]	Ongoing[b]	1410[b]	976 [a]		
	Pennsylvania	06/05/2013[a]	Ongoing[b]	96[b]	82 [a]		
	Missouri	20/05/2013[a]	Ongoing[b]	319[b]	132 [a]		
	Oklahoma	20/05/2013[a]	Ongoing[b]	445[b]	411 [a]		
	South Dakota	27/05/2013[a]	Ongoing[b]	146[b]	61 [a]		
	Michigan	27/05/2013[a]	Ongoing[b]	228[b]	130 [a]		
	Illinois	27/05/2013[a]	Ongoing[b]	957[b]	542 [a]		
	Kansas	03/06/2013[a]	Ongoing[b]	278[b]	243 [a]		
	New York	18/06/2013[a]	03/08/2014[b]	7[b]	5 [a]		
	North Carolina	24/06/2013[a]	Ongoing[b]	830[b]	585 [a]		
	Tennessee	22/072014[a]	24/08/2014[b]	18[b]	11 [a]		
	Texas	26/07/2013[a]	Ongoing[b]	107[b]	59 [a]		
	Wisconsin	07/08/2013[a]	07/09/2014[b]	25[b]	13 [a]		
	Kentucky	08/10/2013[a]	03/08/2014[b]	23[b]	15 [a]		
	Maryland	29/10/2013[a]	29/10/2014[b]	1[b]	1 [a]		
	Nebraska	02/12/2013[a]	Ongoing[b]	202[b]	89 [a]		
	California	27/12/2013[a]	Ongoing[b]	25[b]	10 [a]		
	Wyoming	30/12/2013[a]	27/04/2014[b]	32[b]	8 [a]		
	South Carolina	06/01/2014[a]	01/05/2014[b]	2[b]	2 [a]		
	Arizona	28/01/2014[a]	Ongoing[b]	12[b]	5 [a]		
	Idaho	07/02/2014[a]	14/09/2014[b]	6[b]	3 [a]		
	Montana	08/02/2014[a]	29/06/2014[b]	3[b]	2 [a]		
	North Dakota	26/02/2014[a]	14/09/2014[b]	2[b]	2 [a]		
	Vermont	25/03/2014[a]	25/03/2014[b]	1[b]	1 [a]		
	Mississippi	04/04/2014[a]	04/04/2014[b]	1[b]	1 [a]		
	Virginia	13/04/2014[b]	13/04/2014[b]	1[b]			
	Arkansas	23/06/2014[c]	Ongoing [c]	1 [c]			
	Utah	31/08/2014	Ongoing [b]	6[b]			
Canada	Ontario	22/01/2014[a]	Ongoing[b]	62[b]			[a]OIE, 2014e; [b] http://www.ontariopork.on.ca/ped/Home.aspx;
	Manitoba	14/02/2014[a]	09/05/2014[c]	2[a,c]			

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
	Prince Edward Island	14/02/2014[a]	14/02/2014[c]	1[c]			[c]http://manitobapork.com/2014/05/09/second-on-farm-porcine-epidemic-diarrhea-virus-pedv-case-confirmed-in-manitoba/
	Quebec	23/02/2014[a]	07/03/2014[a]	1[c]			
Colombia	HUILA	07/03/2014	09/06/2014	12	750 (426)		OIE, 2014g
	Cundinamarca	08/03/2014	09/06/2014	30	2476 (564)		
	Tolima	04/04/2014	09/06/2014	1	3 (3)		
	Boyacá	24/04/2014	09/06/2014	1	27 (21)		
	Santander	12/05/2014	09/06/2014	1	72 (40)		
Dominican Republic	Espaillat	12/11/2013	03/12/2013	1	3200 (2450)		OIE, 2014h
	Santiago	08/02/2014	31/03/2014	1	25065 (20691)		
	Santiago Rodrigu	28/03/2014	11/04/2014	1	1300 (71)		
	Peravia	02/04/2014	20/05/2014	1	135 (38)		
	La Vega	16/04/2014	14/05/2014	1	4790 (684)		
	Salcedo	13/05/2014	20/05/2014	1	950 (627)		
	Distrito Nacional	27/05/2014	12/06/2014	1	3602 (1509)		
Mexico	Aguascalientes, Baja, California, Colima, Federal District, Guanajuato, Guerrero, Jalisco, State of Mexico, Michoacán, Morelos, Nuevo León, Puebla, Querétaro, Sinaloa, Sonora, Tlaxcala and Veracruz	30/07/2013		83			OIE, 2014f

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Number of positive /tested samples	Reference
Peru	Lima	10/10/2013					http://www.perulactea.com/2013/10/10/charla-nueva-enfermedad-diarreica-afecta-la-produccion-porcina-ingreso-libre/ http://www.oie.int/doc/ged/D4547.PDF

Table 3: Info on PEDV occurrence in European countries in the period 2004-2014 provided to EFSA by country representatives of the AHAW Network

Country	Info provided to EFSA
DE, GR, IS, LT	No data
BE, DK, ES, FI, FR, LV, NO, SE, SI, UK	Not reported
AT, UK	Not reported (diarrhoea submissions negative in PCR)
FI	Not reported (samples of pigs with clinical symptoms are tested using the Antigen Rapid PED Ag Test kit and were negative in 2010 (n=3), 2011 (n=6), 2012 (n=23), 2013 (n=38) and 2014 (n=1 until May)
NL	Negative since 2003 (autopsy)
HU	Outbreak in one farm in 2009 (12 piglets)
EE	Outbreak in 2010 (not documented), few cases in 2011 and 2012, no data 2013
IT	Outbreak 2005-2006 (see Table 2). From 2008 to 2014 only sporadic outbreaks were observed in growers and finishers herds: 71 PED cases in 58 different farms, out of 1563 cases of enteritis (4.54%). 2011 (n=18), 2012 (n=19) and 2013(n=8)

Table 4: Information on current PEDV-specific seroprevalence in European countries obtained from country representatives of the AHAW Network

Country	PEDV-specific seroprevalence
AT, DE, EE, ES, FI, FR, GR, HU, IS, LT, LV, NL, NO, SE, SI	No data
BE	Negative (IPMA assay, 92 farms throughout the country, 5 serum samples per farm, 2014)
DK	Negative (in-house ELISA; approximately 2500 swine sera per year between 2000 and 2006 with no positive results reported during this period)
IT	Antibodies were found in 11 out of 21 farms in 7% to 52% tested animals
UK	94/206 sera were positive for PEDV-antibodies between 2007 and 2012; estimated GB national level seroprevalence is 9% based on samples collected in framework of a Salmonella study in 2013 (558 pigs sampled in 12 abattoirs and originated from 395 farms)

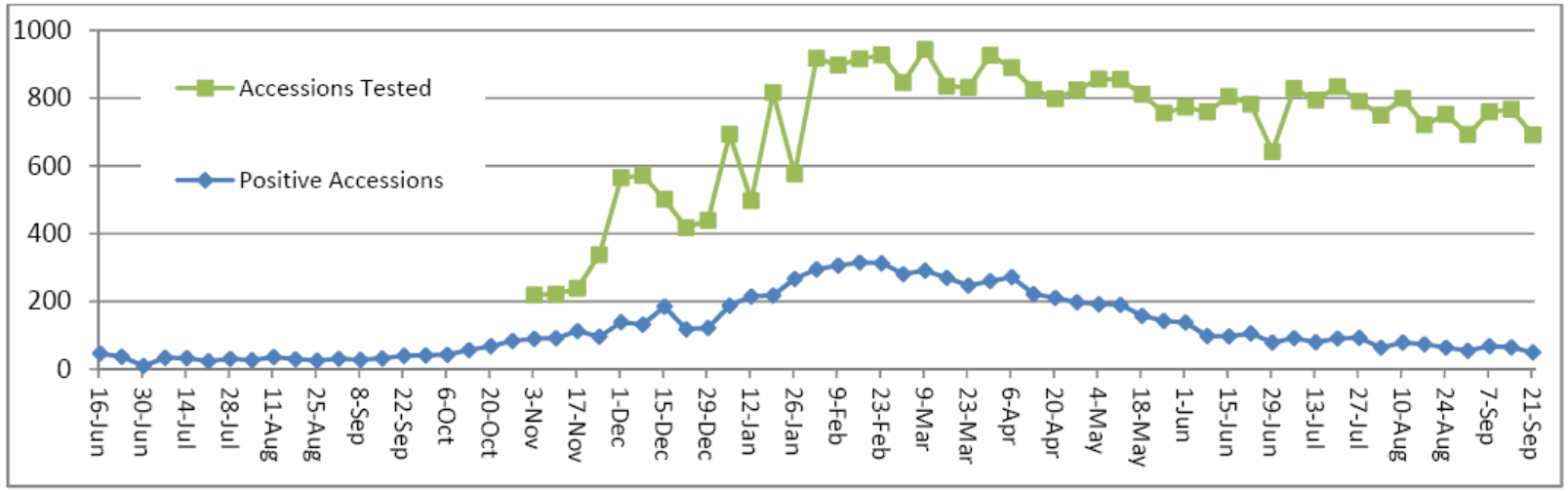

Figure 3: Number of laboratory biological accessions positive for PEDV positive in each week in the United States (info obtained from USDA Swine enteric coronavirus disease testing summary report (version 01 Oct 2014); https://www.aasv.org/pedv/SECoV_weekly_report_141001.pdf)

2. PDCoV

Table 5: Information on PDCoV occurrence in the period 2004-2014 obtained by an extensive search of the scientific literature and relevant websites

Country	Region	Date first outbreak (year, month)	Date last outbreak (year, month)	Number of reported outbreaks	Number of reported cases (deaths)	Reference
US	IA	30/03/2013[a]	ongoing[a]	43[a]		[a]USDA, 2014
	IL	30/03/2013[a]	10/08/2014[a]	54[a]		
	IN	30/03/2013[a]	ongoing[a]	44[a]		
	KS	27/04/2013[a]	01/06/2014[a]	2[a]		
	MI	30/03/2013[a]	10/08/2014[a]	15[a]		
	MN	30/03/2013[a]	27/07/2014[a]	88[a]		
	MO	20/04/2014[a]	17/08/2014[a]	9[a]		
	MT	30/03/2014[a]	29/06/2014[a]	2[a]		
	NC	13/04/2013[a]	ongoing[a]	11[a]		
	NE	06/04/2013[a]	17/08/2014[a]	14[a]		
	OH	30/03/2013[a]	24/08/2014[a]	56[a]		
	OK	01/06/2013[a]	01/06/2014[a]	1[a]		
	PA	06/04/2013[a]	06/07/2014[a]	13[a]		
	SD	06/04/2013[a]	13/07/2014[a]	17[a]		
	TX	06/04/2013[a]	27/07/2014[a]	4[a]		
	Unknown			10		
Canada	Ontario	01/02/2014	14/03/2014	6		http://www.thepigsite.com/swinenews/36095/swine-deltacoronavirus-detected-on-canadian-pig-farms
China	5 provinces					Feng et al., 2014

Appendix C. Information on differences between European, Asian and American PEDV strains

Table 6: Studies describing B-cell epitopes in the PEDV S protein

Description	Reference
Identification COE neutralizing epitope on S protein: Data on the reduction of the plaque-forming ability of PEDV by the COE-specific polyclonal antisera revealed that the COE region of the PEDV spike protein might contain the major neutralizing epitope for the virus. The antisera was produced using a recombinant protein using the sequence of the European PEDV strain Brl/87, neutralized European PEDV strain CV777.	Chang et al., 2002
A truncated form of S1 gene (aa 636-789) was fused to GST and expressed in *Escherichia coli*. The purified recombinant protein was able to react with PEDV antiserum and to elicit formation of neutralization antibodies in mice. The immune serum against the recombinant protein showed binding ability to the native S protein of PEDV.	Sun et al., 2007
Identification SS2 and SS6 neutralizing epitopes on S protein: Mini-GST fusion proteins were scanned by ELISA and Western blotting with the six McAbs, and the result showed that S1D5 (residues 744–759) and S1D6 (residues 756–771) are two linear epitopes of the PEDV S protein. The antisera of the epitopes S1D5 and S1D6 could react with the native S protein of PEDV. Furthermore, Pepscan of the two linear epitopes demonstrated that SS2 ($_{748}$YSNIGVCK$_{755}$) and SS6 ($_{764}$LQDGQVKI$_{771}$) are two core epitopes on S1D5 and S1D6, respectively, located on the S protein of PEDV.	Sun et al., 2008
Identification 2C10 neutralizing epitope on S protein: A synthetic peptide whose linear sequence is identical to the 24 aa carboxy-terminal portion of the spike protein (S-CT24) elicited a strong antibody response in BALB/c mice. These antibodies exhibited neutralizing activities against the KPEDV-9 strain in focus reduction neutralization tests suggesting that the GPRLQPY motif induces neutralizing antibodies against PEDV.	Cruz et al., 2006 and 2008

Table 7: Info on sequence differences between PEDV strains reported since 2004 and obtained by an extensive search of the scientific literature

Country (region)	Sequence info	Reference
Germany (North-Rhine Westphalia)	The German virus circulating in North-Rhine Westfalia has 99% sequence identify compared to US PEDV strains, based on the sequence of a 651 bp fragment of the S gene.	Henniger and Schwarz, 2014
US	This study reported a PEDV-Am isolate with a 197 aa deletion in the S gene. This large S deletion was not detected in the original sample but was introduced in the first cell line passage. At the moment, it is not clear if large S deletion strains circulate naturally in the US swine population.	Oka et al., 2014

Country (region)	Sequence info	Reference
US	Among 178 US PEDVs collected from 23 states, S1 sequences of 156 cases from 22 states had 99.0-100% nucleotide (nt) identity to each other, including the PEDVs initially sequenced after the outbreak in April 2013 (hereafter designated as original US strain). In contrast, S1 sequences of the remaining 22 cases from 10 states had only 92.4-93.8% nt identity to the original US strains, while they shared 99.6-100% nt identity to each other (hereafter designated as variant US strain). It seems probable that at least two genotypes of PEDV have been introduced into the US concurrently and are co-circulating in US swine now.	Chen et al., 2014a
US	Sequences were also compared to those of 4 additional U.S. PEDV strains and 23 non-U.S. strains. All US PEDV strains were genetically closely related to each other (>99.7% nucleotide identity) and were most genetically similar to Chinese strains reported in 2011 to 2012. Phylogenetic analyses using different genes of PEDV suggested that the full-length spike gene or the S1 portion is appropriate for sequencing to study the genetic relatedness of these viruses. Based on sequence comparison and phylogenetic analysis, it appears that the U.S. PEDV strains are genetically closely related to some PEDV strains that were circulating in China in 2011 to 2012. The full-length S gene or the S1 portion is appropriate for sequencing to study the genetic relatedness and molecular epidemiology of PEDV.	Chen et al., 2014b
US (Ohio)	Strain OH851 showed 99% and 97% nt identity to PEDVs currently circulating in the United States (Colorado, Iowa, Indiana, Minnesota) for the whole genome and the full-length spike (S) gene, respectively. By distinct contrast, strain OH851 showed only 89% or even lower nucleotide identity to PEDVs currently circulating in the United States in the first 1,170 nt of the S1 region. It is highly possible that the sequence deletions, insertion, and mutations found in variant strain OH851 might have contributed to the reduced severity of the clinical disease in the piglets.	Wang et al., 2014a
US	The genome sequences of a PEDV strain isolated from an infected piglet was compared against its in vitro adapted version. The original PEDV strain was grown in Vero cells and passed 10 times serially in a MARC145 cell line. The sequence analysis of the native PEDV strain and in vitro passaged virus shows that the cell culture adaptation specifically modifies PEDV spike protein whereas the open reading frame 1a/b (ORF1a/b)-encoded polyprotein, the nucleoprotein, NS3B (ORF3), and membrane and envelope proteins remain unchanged	Lawrence et al., 2014
China	The S gene sequences of 10 Guangdong isolates, four PEDV vaccine strains and the USA-Colorado-2013 strain were analysed. The same amino acid sequence was found in SS2 (AA 748-755) and 2C10 epitopes (AA 1368-1374) were identical in all strains, whereas 2 AA changes were identified in epitope SS6 (AA 764-771) and 16 AA changes were identified in epitope COE (AA 499-638). Forty-one strains were analysed for N-glycosylation sites. They all contained 9-10 high-specificity N-glycosylation sites, three of which are conserved (213NVTS, 778NISI, 1246NKTL). The 127NKTL site seems to be absent in strains isolated after 2010. In most after-2010 strains, a palmitoylation motif is newly present at site 230 and the 122 site lost, the latter being conserved in vaccine and Chinese pre-2011 strains.	Hao et al., 2014
China (5 provinces)	The fifteen Chinese field PEDV strains had 96.1-100 % nucleotide and 94.8-100 % deduced amino acid sequence identity to each other. Sequence comparison with the other seven selected strains of PEDV revealed that the Chinese field PEDV strains had nucleotide sequence identities of 94.2-99.7 % and deduced amino acid sequence identities of 94.1-99.5 %. In addition, the fifteen strains showed a high degree of nucleotide sequence identity to the early domestic strains (98.4-99.7 %) except the LZC strain, but less identity to the vaccine strain (CV777) used in China (94.7-97.7 %). Phylogenetic analysis showed that the Chinese PEDV strains are composed of a separate cluster including three early domestic strains (JS-2004-02, LJB/03 and DX) but differ genetically from the vaccine strain (CV777) and the early Korean strains (Chinju99 and SM98).	Li et al., 2014

Country (region)	Sequence info	Reference
Taiwan	Eighteen PEDV isolates collected from the Dec 2013 – Jan 2014 outbreak in Taiwan, shared 99.5-100% nucleotide sequence identity of the partial S gene (COE domain). Two major clusters, based on the phylogenetic relation of the partial nucleotide sequences of the COE domain in the S gene, were detected (Fig. 1). The first cluster was comprised of prototype isolates (TW1/A/2013 and TW2/B/2013) and Chinese strains LJB/03 and DX/2007. The second cluster consisted of all 18 Taiwanese isolates from this outbreak and 7 US isolates.	Lin et al., 2014
South Korea	The complete genome of PEDV strain K14JB01 showed high nucleotide sequence homology (99.7 to 99.8%) with U.S. strains (e.g. USA/Colorado/2013) identified in 2013, and with Chinese strains (AH2012, BJ-2011-1, GD-B, and JS-HZ2012) identified in 2011 and 2012 (99.1 to 99.4%). The ORF1a and ORF1b genes of K14JB01 show 99.8% homology (at the nucleotide level) with those of USA/Iowa/16465/2013. In addition, the S genes are 99.6% similar, the ORF3 genes are 100% identical, theMgenes are 99.9% similar, and theNgenes are 99.9% similar.	Cho et al., 2014
South Korea	Three PEDV strains were isolates from dead piglets from two pig farms in Korea during the outbreak late 2013. Comparative genome analysis of the reemerging Korean PEDV isolates and other strains revealed that the complete genome sequences of the recent Korean strains were almost identical (99.9%) to those of the US PEDV strains isolated in 2013. According to the phylogenetic analysis, the reemerging Korean PEDV isolates were closely clustered with the US strains isolated in 2013 and Chinese strains isolated in 2012.	Choi et al., 2014
South Korea	The full-length spike glycoprotein sequences were determined of ten PEDV strains isolated during the outbreak late 2013. The authors determined that the full-length spike genes of the PEDV strains were 9 nt longer than that of the prototype PEDV strain, CV777; this difference was caused by the presence of genetic signatures for recent PEDV field isolates as described elsewhere (Lee et al., 2010 VR). Nucleotide sequence analysis showed high homology (98.8%–99.9%) among the 10 tested isolates. In contrast, the isolates all shared only 94.3%–94.7% nt sequence identity with a previously sequenced field isolate from South Korea, KNU-0801. However, the sequences of the 10 isolates were compared with those of other published PEDV strains and found to consistently share 99.2%–99.9% nt identity with recently emergent US strains. The complete genomic sequence of KNU-1305 was determined to be 28,038 nt in length, excluding the 3′ ploy(A) tail. The complete PEDV genome of KNU-1305 shared 96.3%–99.9% nt identity with other complete PEDV genomes available in GenBank; the highest nucleotide identity (99.9%) was with US strains CO/13, IA1, IN17846, and MN. The full-length spike gene–based phylogenetic analysis revealed that the PEDV strains were clearly defined into 2 separate clusters, designated genogroup 1 (G1) and genogroup 2 (G2); each of the groups can be further divided into subgroups 1a, 1b, 2a, and 2b (Figure 1, panel A). All 10 PEDV strains from South Korea were classified into subgroup 2b and most closely clustered together with the recent US strains in an adjacent clade with the same subgroup.	Lee and Lee, 2014
Thailand	Based on the analysis of the partial S genes, the Thai PEDV isolates were clustered into two groups related to Korean and Chinese field isolates. The results for the complete S genes, however, demonstrated that both groups were grouped in the same cluster.	Temeeyasen et al., 2014
Vietnam	Full-length genome sequences are reported for three PEDV isolates from pigs displaying severe diarrhea from farms located in northern and southern provinces of Vietnam. A comparison to PEDV sequences available in GenBank demonstrated that the three new PEDV isolates share high similarity (98.6% to 98.7% and 97.7% to 98.0% at nucleotide and amino acid levels, respectively) with more recent isolates from China responsible for 2010–2012 outbreaks. They all have unique characteristics including deletion and insertion in spike genes, which make them genetically distinct from CV777 and other earlier Chinese isolates.	Vui et al., 2014

Country (region)	Sequence info	Reference
China	PEDV CHYJ130330 was isolated from southern China and shown to be highly virulent when inoculated into neonatal pigs. This isolate has a high nucleotide identity of 99.1% with the U.S. strain IA1.	Jia et al., 2014
China	S protein identity among the 3 new isolates was 99.4%–99.6% and shared 93.6%–93.7% identity with classical CV777 strain	Wang et al., 2013b
China	Sequence analysis showed that ten post-2010 isolates shared high homology with each other and were always clustered together with the virulent DR13 strains (South Korea) and/or one earlier Chinese strain, CH-S, in phylogenetic analysis. All post-2010 isolates possessed common sequence changes in each gene. These results suggest that current Chinese PEDV isolates originated from either South Korea and/or Chinese ancestors that underwent some genetic variation, thereby forming a new PEDV genotype in China.	Wang et al., 2013c
China (Shanghai)	Phylogenetic analysis based on a complete ORF3 gene fragment of Shanghai PEDV field isolates, together with other PEDV reference strains, confirmed that all PEDVs fell into three groups. One group comprised the CV777, Br1/87, and LZC strains and the SH4 isolate. The second group consisted of vaccine strains (the attenuated strains DR13 and CV777 vs), the CH/GSJIII/07 strain, and the SH5 isolate. The third group was made up of eight Shanghai field isolates (SH1, SH2, SH3 and PF1-5), Chinju99, and the parent strain DR13. From the M gene phylogenetic tree, we found that all isolated strains in Shanghai and most of the strains in China since 2006 were in the same clade with those isolated in Thailand and South Korea but differed genetically from the European strain (Br1/87), LZC from China isolated in 2006, and strain CV777, which is used as a vaccine in China. PEDV exhibits rapid variation and genetic evolution, and the currently prevailing PEDV strains in Shanghai represent a new genotype. The ORF3 of SH5 clusters with CV777 vs, and SH4 clusters with the wild-type CV777. Compared with attenuated DR13 and CV777 vaccine strains, ORF3 of SH5 contains no nucleotide deletions.	Ge et al., 2013
China (Beijing, Hebei and Zhejiang)	15 complete M genes were analyzed and revealed 99.1-100% amino acid similarity with each other. Phylogenetic analysis including M gene sequences available in the GenBank indicated that the M genes obtained in this study were relatively conserved and exhibited minor variations although the M gene of PEDV was in general genetically diverse. The analysed S genes had an amino acid deletion between positions 155 and 156 compared with CV777, previous Chinese strains (LZC, CH/S, JS-2004-2, LJB-03 and DX), Korean strain (Chinju99) and Japanese strains (MK, 83P-5). This deletion was identical in Korean KNU-serial strains (KNU-0802) reported in recent years and similar to Korean strain Spk1.	Gao et al., 2013
Korea (4 provinces)	Phylogenetic analysis based on the complete E gene fragments of the Korean PEDV field isolates, PEDV vaccine strains, and PEDV reference strains confirmed that all PEDVs, including the Korean field isolates, fell into three groups. One group comprised the virulent DR13 strain, eight Korean field isolates, and five Chinese strains. The second group consisted of the live vaccine strains, attenuated DR13, KPED-9 and P-5V. The third group included the CV777, Chinju99, virulent SM98-1 strain of the Korean inactivated PED vaccine, and four Chinese strains.	Park et al., 2013
China (5 provinces)	The N genes of 15 PEDV strains were amplified by RT-PCR. The nucleotide sequences were 96.1-100% identical to each other and the deduced amino acid sequences were 94.8-100% identical. Phylogenetic analysis showed that the Chinese PEDV strains are composed of a separate cluster including three early domestic strains (JS 2004-02, LJB/03 and DX) but differ genetically from the vaccine strain CV777 and the early Korean strains (Chinju99 and SM98).	Li et al., 2013b
China (15 provinces)	The deduced amino acid sequences of 32 field strains showed 95-100% sequence identity to each other for the N gene and 96.2-100% sequence identity to CV777. The number of predicted phosphorylation sites of N proteins varied from 5 to 12.	Chen et al., 2013

Country (region)	Sequence info	Reference
China (southern China)	The isolated porcine epidemic diarrhea virus (PEDV) CH/GDGZ/2012 strain was obtained from the faeces of diseased pigs in 2012 in southern China. The complete genome sequence of CH/GDGZ/2012 exhibits 96.6%, 97.4%, 97.9%, and 97.0% nucleotide homologies with the genomes of PEDV strains CV777, DR13, CH/FJND-3/2011, and CH/S, respectively. Among the genes, the S gene of CH/GDGZ/2012 has 93.6% to 97.1% nucleotide sequence identity with those of the strains reported previously.	Tian et al., 2013b
Chine (Gansu Province)	The S genes' nucleotide sequences of 5 strains isolated in China (Gansu province) have 98.0-98.1% identity with the Chinese strain CH8 (isolated in 2011), 92.6-95.7% identity with previously isolated Chinese strains (DX, LZC, LJB-03, JS-2004-2 and CHS), 93.6-93.7% identity with the European strains CV777 and Brl-87. All 5 strains have 8 mutations in the COE epitope, 1 mutation in the SS6 epitope and no mutations in epitopes SS2 and 2C10 compared to the vaccine strain CV777.	Tian et al., 2013a
China (Honan, Shanxi, Anhui and Hebei provinces in central China)	Based on the phylogenetic analyses of M and ORF3 genes, PEDVs from central China and reference strains could be separated into three groups: G1, G2, and G3. The 15 PEDV strains (collected from different areas in central China during the 2010-2011 outbreak) belonged to G3 group and showed a close relationship with Korean strains (2007), Thai strains (2007–2008), and partial other Chinese strains (2010–2011), but differed genetically from strains isolated in China from 2003 to 2006, European strains (Br1/87) and the vaccine strain (CV777) being used in China.	Yang et al., 2013b
China	Phylogenetic analysis based on the N protein sequences of Asian PEDV strains indicated that there are two major groups of Chinese PEDV strains, a Japanese PEDV group and a Korean PEDV group.	Yang et al., 2013a
US	The complete PEDV genome of CO/13 has a nucleotide identity of 96.5 to 99.5% with other complete PEDV genomes available in GenBank, with the highest nucleotide identity (99.5%) with Chinese strain AH2012.	Marthaler et al., 2013
US	Phylogenetically, the PEDV isolate USA/Iowa/18984/2013 is 99.8 to 99.9% similar to other U.S. PEDVs reported earlier, 97.2 to 99.6% similar to recent Chinese PEDVs, with AH2012 being the closest, and 96.9% similar to the prototype PEDV strain CV777.	Hoang et al., 2013
China	Phylogenetic analyses based on the whole genome of strain CHGD-01 revealed that it shared nucleotide sequence identities of 98.2–98.4% with two other Chinese isolates reported in the same year. Amino acid sequence analysis based on individual virus genes indicated a close relationship between the spike protein gene of CHGD-01 and the field strain KNU0802 in Korea. Its ORF3 and nucleoprotein genes, however, were divergent from all other sequenced PEDV isolate clusters and therefore formed a new group, suggesting a new variant PEDV isolate in China.	Pan et al., 2012
China	Seven PEDV strains isolated in Hebei province of China in 2010 showed 99.4–99.9 % nucleotide sequence identity of the M gene and 98.2–99.1 % deduced amino acid identity. When compared with other Chinese isolates and foreign isolates, the seven isolates showed high nucleotide identity with the Thailand isolate M-NIAH1005 (99.6–99.9 %) and Korea isolate PFF188 (99.7–100 %), but low identity with other Chinese isolates (96.6–99.1 %) and with the vaccine strain CV777 used in China (97.8–98.2 %).	Fan et al., 2012
China	A phylogenetic tree based on the entire genome sequence of representative PEDVs showed that Chinese PEDVs could be divided into three subgroups. AJ1102, together with three field strains isolated in 2011 (WUH1-2011, BJ-2011-1, CH/FJND-3/ 2011), forms a separate branch, supporting the concept that AJ1102 is the epidemic PEDV in China. Compared to CH/S (a Chinese virulent PEDV strain isolated in 1986 in China) and the classical strain CV777 (3, 6), the AJ1102 S gene has a 6-nt insertion. Interestingly, similar insertions could be observed in the S gene of three field strains (GenBank accession numbers JN980698, JQ239429, and JQ638915) which were recently isolated in China.	Bi et al., 2012

Country (region)	Sequence info	Reference
Thailand and Vietnam	Phylogenetic analysis of the partial S gene of recent Thai and Vietnamese PEDV isolates indicated that they originated from the same Chinese PEDV ancestor and these isolates were gradually undergoing genetic variation and forming a new PEDV subcluster in each country.	Ayudhya et al., 2012
China	The partial S gene deduced amino acid sequences of 9 Chinese PEDV isolates were compared and showed a high degree of homology (98.0%–100.0%); they had 98.0%–98.7% identity with Thailand strains, and 93.3%–94.7% with vaccine strain CV777 (9 amino acids are changed in Chinese isolates compared to CV777).	Sun et al., 2012
China (Guangdong)	The complete genome sequence of GD-1 shared 98.3%, 98.1%, 97.6%, and 96.8% nucleotide sequence identity with those of GD-B, CH/FJND-3/2011, DR13, and CV777, respectively. Phylogenetic analysis of the complete genome revealed that Chinese PEDVs could be divided into three subgroups, among which GD-1 and other recent isolates, such as GD-A and AJ1102, belong to the same subgroup, which was distant from the CV777 vaccine strain and other foreign PEDV strains.	Wei et al., 2012
China	The complete genome sequence of CH/FJND-3/2011 has 97.34% nucleotide sequence identity with that of CH/S. Among the six genes of CH/ FJND-3/2011, the S ene has the lowest sequence identity (93.75%) with that of CH/S and is 9 nt longer than those of CV777 and CH/S. The alignment in the S1 region (nt 1 to 2217) f the S gene reveals two domains exhibiting increased divergence compared to the remaining part of the sequence. The first domain is composed of the 1,100 N-terminal nucleotides. In this domain, the CH/FJND-3/2011 S gene has three insertion regions (nt 162, nt 170 to 180, and nt 413 to 415) and one deletion region (nt 70 to 475). Furthermore, the largest number of nucleotide differences is clustered in the N-terminal region of the S1 gene. The second domain is located at positions 1428 to 1914. These regions are also found in the S genes of Korean PEDV isolates.	Chen et al., 2012a
China (Guangdong)	The virulent PEDV isolate LC was isolated from suckling piglets with severe diarrhoea on an immunized-swine breeding farm. The complete genome sequence of LC shares 96.9 to 98.9% nucleotide sequence identities with those of other PEDV isolates deposited in GenBank. Phylogenetic analysis based on the complete genome shows that LC together with BJ_2011-1, CH/FNJD-3/2011 and GD-B forms a new cluster and is distant from vaccine strain CV777.	Chen et al., 2012b
China	The full-length PEDV S gene sequence was determined from 9 diarrhea samples from pigs at 9 farms with high mortality rates. The full-length S gene sequences of the 9 isolates from this study showed overall high conservation with the reference strains, up to 94.9%–99.6% homology. By phylogenetic analysis, 4 of the field isolates (CH2, CH5, CH6, CH7) clustered with the previously described strain JS-2004–2 from China. Three field isolates (CH1, CH8, CHGD-01) formed a unique cluster with the sequence-confirmed variant strain CH-FJND-3, which had been isolated from China in 2011 (Chen et al., 2012 JV).	Li et al., 2012a
China (Guangdong)	The complete genome of the PEDV isolate GD-A has 96.5% to 98.4% nucleotide sequence identities with those of the reference strains reported in GenBank. Phylogenetic analysis of the complete genome shows that GD-A and other recent isolates (CH/FJND-3/2011 and BJ-2011-1) belong to the same group distant from the CV777 vaccine strain and other foreign strains. Furthermore, CH/FJND-3/2011 and BJ_2011_1 can be clustered into one subgroup and differ genetically from GD-A.	Fan et al., 2012b

Country (region)	Sequence info	Reference
Vietnam (three provinces in southern Vietnam)	Genetic characterisation of 8 southern Vietnamese isolates revealed 15 amino acid changes in part of the S gene (region AA 500-700 analysed) compared with CV777. Analysis of the full M gene sequence from 6 isolates identified 2 amino acid substitutions compared with CV777. The phylogenetic relationship of both partial S and M protein genes indicated that the current Vietnamese PEDVs were in the same cluster with the Chinese isolates (JS-2004-2 and DX), the Thai isolates (07NP01, 08NP02 and 08CB01) and the recent Korean isolates (KNU-0802 and CPF299). The results suggested that the current Vietnamese PEDV isolates might have originated from the same Chinese ancestor undergoing genetic variation and possibly forming a new PEDV genotype in Vietnam.	Duy et al., 2011
	Serial passage of the PEDV 83P-5 strain in Vero cells resulted in growth adaptation of the virus in cultured cells. Sequence analyses revealed a strong selection for the S gene and virtually all mutations occurring at the 34th and 61st passages had been carried over to the 100th-passaged virus. In contrast, the viral M and N genes showed a strong conservation during the serial passage.	Sato et al., 2011
Korea (6 provinces)	The complete ORF3 gene sequence and phylogenetic analysis showed that all Korean PEDV field isolates (except DBI865) have a close relationship to Chinese field strains and differ genetically from European PEDV strains. The Korean PEDV field isolates (except DBI865) are also genetically different from the vaccine strains (attenuated DR13, KPED-9 and P-5V), which have been used for prevention of PEDV infection in Korea. Most of the Korean PEDV field isolates analyzed in this study differ from members of the group that includes the vaccine strains, and only a few isolates belonged to that group.	Park et al., 2011
	H1381 in the cytoplasmic tail of the S protein is a component of the KxHxx motif which is a retrieval signal of the S protein for the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Loss of this motif could allow for the efficient transfer of S proteins from ERGIC onto the cell surface and subsequent increased fusion activity.	Shirato et al., 2011
China	The complete genome sequence of strain CH/S was reported.	Chen et al., 2011
China: Gansu, Heilongjiang, Henan, Hunan, Inner Mongolia, Jiangsu, Jilin and Shanghai	Chinese PEDV field strains (excluding CH/GSJIII/07) differ genetically from European field strains (CV777 and Br1/87) and have a close phylogenetic relationship to Korean field strains (DR13 and Chinju99). The Chinese PEDV field strains (excluding CH/GSJIII/07) are genetically different from the CV777 vaccine strain, which is used to prevent PEDV infection in China at present. There is a new genotype of PEDV prevailing in China that differs from the genotype of the vaccine strains.	Chen et al., 2010
Korea (Gyeongbuk)	The sequence analysis data indicate the diversity of the PEDV isolates currently prevalent in Korea that represents a heterogeneous group. Phylogenetic analyses showed two separate clusters, in which all Korean field isolates were grouped together in the second cluster (group 2). The results indicate that prevailing isolates in Korea are phylogenetically more closely related to each other rather than other reference strains. data implicates a potential usefulness of the partial S protein gene including the N-terminal region in unveiling genetic relatedness of PEDV isolates.	Lee et al., 2010

Country (region)	Sequence info	Reference
China	The 3' UTR existing on the extreme 3' end of the genome is 334 nt in length and possesses an octameric sequence, GGA AGAGC, beginning at base 73 upstream from the poly(A) tail. The genome of CH/S contains six genes, the replicase (Rep), the spike (S), ORF3, envelope (E), membrane (M), and nucleoprotein (N) genes, arranged in the order 5 -Rep-SORF3- E-M-N-3 . Two long ORFs (ORF1a and ORF1b) are of 12,351 nt (nt 293 to 12643) and 8,037 nt (nt 12598 to 20634) in length and overlap by 46 nt. At the overlapping region, there is a specific seven-nucleotide "slippery" sequence (UUUAAAC) and a pseudoknot structure ribosomal frameshifting signal), which are required for the translation of ORF1b. Sequences at the 5' end of each gene represent signals for the transcription of subgenomic mRNAs of coronavirus. These sequences, known as transcription-regulating sequences (TRSs), include a stretch of a highly conserved sequence designated the core sequence (CS), located at sites immediately upstream of most of the genes. The CSs in the genome of CH/S are the hexameric motifs 5 -XUA(A/G)AC- 3 . The sequence CUAAAC found at the starting sites of gene 1 and the N gene, the GUAAAC sequence found at the starting site of the S gene, the CUAGAC sequence found at the starting sites of gene 3 and the E gene, and the AUAAAC sequence found at the starting site of the M gene are identical to those found in the same sites in the CV777 strain.	Chen et al. 2010
Thailand (8 provinces)	The M gene sequence analysis of 31 PEDV isolates obtained in Thailand indicated that the nucleotide sequence of the entire M gene was highly conserved. All recent PEDV isolates in Thailand had 99.3%–100% nucleotide homology. The lowest sequence identity 96.5%, was with the Chinese strain, EF185992/LZC, and the highest sequence identity (99.2%–99.7%) was with the Chinese strain, JS-2004-2, and concurrent isolates from the National Institute of Animal Health, Thailand, NIAH 07-08. findings demonstrated that the recent PEDV isolates in Thailand were genetically diverse in their S genes either within their group or with the reference strains. These point mutations may lead to genetic diversity among these isolates. Recent Thai PEDV isolates clustered in the same group were highly homologous with the Chinese strains, JS-2004-2 and LJB/03. The phylogenetic relationship of the Thai PEDV strain indicated that the recent Thai PEDV isolates differed genetically from previous Thai isolates. Our data suggested that all recent Thai PEDV isolates are genetically similar to the Chinese isolates identified in 2004.	Puranaveja et al., 2009
China (five provinces)	Sequence homology of M protein genes indicated that six Chinese PEDV isolates were highly homologous to CV777, Br1/87, Chinju99, JMe2, and JS-2004-2 rather than to QH, LZC, LJB/03, KPEDV-9, and KPEDV-9F. Although CH/SHH/06, CH/HNCH/06, CH/IMB/06 were isolated in different provinces, they had identical nucleotide sequences. It showed that the three isolates maybe originated from the same ancestor. By phylogenetic analysis, the six Chinese PEDV isolates with S-2004-2 formed a separate group, which excluded European strains, Korean strains, Japanese strain and three Chinese strains (QH, LZC, LJB/03). The phylogenetic relationship indicated that Chinese PEDV isolates were different from foreign PEDV strains and a new genotype PEDV was prevailing in China.	Chen et al., 2008
Korea	The nucleotide and deduced amino acid sequence identities among field isolates were 96–99% and 94–98%, respectively, suggesting that these field isolates are more closely related to each other than the cell-adapted CV777 or the vaccine virus.	Lee et al., 2008
Korea	The coding region of the S gene of attenuated PEDV DR13 had 20 nucleotide changes that appeared to be significant determinants of function in that they produced changes in its predicted amino acid sequence. Notably, attenuated PEDV DR13 has previously been found to exhibit reduced pathogenicity in pigs. The regions containing these 20 nucleotide changes may therefore be crucial for PEDV pathogenicity. Phylogenetic analysis suggested that attenuated PEDV DR13 is closely related to CV777, Br1/87, JS-2004-2 and parent DR13, rather than to Spk1 and Chinju99 and is especially close to the Chinese PEDV strain JS-2004-2.	Park et al., 2007

Country (region)	Sequence info	Reference
China	The polymerase gene, a non-structural gene from strain TS of transmissible gastroenteritis virus (TGEV), was amplified by RT-PCR primers designed based on the Purdue nucleotide sequence in GenBank. The expected 20054 bp product was obtained. The nucleotide sequence of ORF1 of TS shared nucleotide and amino acid identities of 98.8% and 99.0%, respectively with that of strain Pur46-MAD. The identity at the amino acid level for the ORF1 between TS and FIPV, PEDV, HCV299E,SARS was 87% 57% 57% 45%, respectively. RdRp was regarded to have an important role in replication and the results indicated that it was a conserved protein. The data also showed that there was a ribosomal slippage site UUUAAAC and three stem-loop structures in the ORF1a and ORF1b overlapping regions.	Li et al., 2006
China (Heilongjiang)	Sequence comparison with other PEDV strains selected from GenBank revealed that the N gene of the PEDV strain LJB/03, isolated in China (Heilongjinag), has a high sequence homology to those of other PEDV isolates, 97.4% with JS2004, 95.6% with chinju99, 96.6% with Br1/87, and 96.8% with CV777. The encoded protein shared 96.4% amino acid identities compared with CV777, 96.1% with Brl/87, 98% with JS2004, 96.90% with chinju99, respectively. N protein sequences demonstrated that PEDV strains LJB/03 and JS2004 which comes from China and chinju99 comes from Korea are more closely related to each other than they are to those two isolates European CV777 and Brl/87.The deduced amino acid sequence of the N gene is 99.8% identical between the European strains CV777 and Brl/87.	Ge et al., 2006
China (Qinghai)	The membrane (M) gene of porcine epidemic diarrhea virus (PEDV) QH strain previously isolated in Qinghai, China, was cloned and sequenced then the sequences of nucleotide and deduced amino acid from PEDV-QH M gene was compared with PEDV-CV777, JMe2, Br1/ 87, JS-2004-2 and KPEDV-9 strains. The nucleotide sequence encoding M protein's entire ORF of PEDV-QH was 681 bp in length and encoded a protein of 226 amino acids with predicted molecular weight of approximate 25 ku, it consisted of 154 adenines (22.61%), 160 guanines (23.49%), 217 thymines (31.86%) and 150 cytosines (22.03%). The PEDV-QH M gene nucleotide sequence shared 98.5%, 98.4%, 98.4%, 98.2% and 97.9% of homologous rates with that of CV777, JMe2, Br1/ 87, JS-2004-2 and KPEDV-9, respectively. The PEDV-QH M protein revealed 99.1%, 99.1%, 98.7%, 98.2% and 97.8% amino acid identity with that of CV777, JMe2, Br1/ 87, JS-2004-2 and KPEDV-9, respectively. Analysis predicted that M protein transmembraned 3 times; the amino acid sequence contained one potential site for prokaryotic membrane lipoprotein lipid attachment, three sites for glycosylat ion and four for serine (S-) or threonine (T-) linked phosphorylation by protein kinase C or casein kinase. Sequences of nucleotides and amino acids correspondingly genetic derivation analysis showed that the mutation of M gene was belong to synonymous mutation mainly.	Lan et al., 2005

Appendix D. Info on impact of PEDV and PDCoV

Table 8: Reported info on clinical signs and pathological lesions for different age groups of PEDV since 2004 obtained by an extensive search of the scientific literature and relevant websites

Country	Age of the animals	Description clinical signs and/or pathological lesions	Morbidity	Mortality	Production losses	Reference
Taiwan	Piglets under 2 weeks of age	Severe vomiting and watery yellowish diarrhea	80-100%	90-100%	Different degrees of weight loss	Lin et al., 2014
	Sows, gilt, finishing, growing and nursery pigs	Only developed appetite loss, anorexia and soft faeces for 3-7 days		0%		
China	4-day-old Duroc crossbred piglets	The dead piglets showed hemorrhage and shedding in the gastric mucosa, swelling and congestion in the mesenteric lymph nodes, and hemorrhage in the intestinal wall	100%	100%		Wang et al., 2013b
United States	Neonatal piglets	The piglets had signs of emaciation and dehydration. The gross pathological lesions were confined to the small intestine and were characterized by thin translucent intestinal walls that contained moderate amounts of yellow watery faeces without macroscopic traces of blood. No other gross abnormalities were noticed. Histological evaluation revealed regions of small intestines with villus blunting and fusion and minimal lymphoplasmacytic infiltration of the villi of the lamina propria. The gross and histological lesions from the PEDV outbreaks in the United States are similar to those observed in China (Li et al., 2012 EID).				Huang et al., 2013
United States	piglets within 24 hr of birth	Watery fetid diarrhea that contained flocculent undigested milk in 90% of piglets and many were observed vomiting. Piglets dehydrated rapidly, were covered with feces.		mortality was 95% within 2–3 days		Stevenson et al., 2013
	sows with parity ≤ 1	Animals showed diarrhea and were anorectic. Some vomited.	90%			
	gilts		90%			
	parity 2 and older sows		15%			

Country	Age of the animals	Description clinical signs and/or pathological lesions	Morbidity	Mortality	Production losses	Reference
		Acutely affected suckling pigs had yellow faeces coating the skin and hair, the stomachs contained little milk curd, the small and large intestines were distended by watery contents that contained floccules of white-to-yellow undigested milk, and small intestinal walls were thin. Microscopic lesions were limited to the small intestines. The most acute lesions were of degenerate epithelial cells on the lateral surfaces and tips of villi. Villi were variably shortened with condensation of the lamina propria near the tips of villi. Lesions in other pigs, presumably slightly later in the viral infection, were observed throughout the entire length of the small intestine.				
China	Suckling piglets	watery diarrhea, severe dehydration		83.5-100%		Gao et al., 2013
Vietnam	Suckling piglets	severe watery diarrhoea, dehydration	Up to 100%	65-91%		Duy et al., 2011
Italy	farrowing unit	watery diarrhoea without evidence of blood or mucus		up to 34.5% (average due to diarrhoea 0.73% before outbreak and 11.9% during outbreak)		Martelli et al., 2008
	nursery unit (28 to 60 days)	watery diarrhoea without evidence of blood or mucus	70%	2%		
	weaned piglets (40 to 75 days)	watery diarrhoea without evidence of blood or mucus	90%	0%	feed consumption decreased, according to the farmers, by up to 50%	
	finishing unit	watery diarrhoea without evidence of blood or mucus	20-80%	0-4%		
	Pregnant sows		80%	0%		
	Lactating sows		90%			
Thailand	Suckling piglets	acute watery diarrhea		49.2% (3519/7153)	reduction in reproductive performance	Olanratmanee et al., 2010

Country	Age of the animals	Description clinical signs and/or pathological lesions	Morbidity	Mortality	Production losses	Reference
Korea	Sows				sows exhibited signs of mastitis resulting in an inadequate transfer of lactogenic immunity against PEDV to newborn piglets.	Park and Lee, 2009
Korea		watery diarrhea and dehydration at the time of sample collection				Park et al., 2013
China	Piglets	watery diarrhea and dehydration				Chen et al., 2013b
China	Suckling piglets	Diarrhea, mild hemorrhage, undigested curdled milk in the stomach, thin-walled intestines with severe mucosal atrophy and foamy fluid	100%	80-100%		Sun et al., 2012
	sows and boars	few animals showed clinical diarrhoea				
China	piglets	severe watery diarrhea, dehydration with milk curd vomitus, mild hemorrhage, undigested curdled milk in the stomach and thin-walled intestines with severe mucosal atrophy and foamy fluid		60-85%		Wang et al., 2013a
China	Suckling piglets	severe diarrhea and dehydration		Up to 100%		Yang et al., 2013a
China	Pigs of all ages	Watery diarrhea, dehydration with mild curd vomitus and thin-walled intestines with severe villus atrophy and congestion. Loss of appetite with different degrees of severity, which were determined to be age dependent. The disease progressed within a few days.				Li et al., 2012a
	Suckling piglets		100%			
	Pigs >2 weeks of age	Mild diarrhea and anorexia, which resolved within a few days				
United States	10- to 35-day-old gnotobiotic pigs	Acute, severe watery diarrhea and vomiting developed in all inoculated pigs 24-48 hours after inoculation. Thin and transparent intestinal walls (duodenum to colon) and accumulation of large amounts of yellowish fluid in the intestinal lumen. The stomach was filled with curdled milk, possibly due to reduced intestinal peristalsis. Histological lesions included acute diffuse, severe atrophic jejunitis and mild vacuolation of superficial epithelial cells and subepithelial edema in cecum and colon.				Jung et al., 2014

Country	Age of the animals	Description clinical signs and/or pathological lesions	Morbidity	Mortality	Production losses	Reference
United States		sows were known to be infected but piglets showed minimal to no clinical signs and no piglets had died				Wang et al., 2014
United States		diarrhea started on day 2-3 following inoculation and continued for 7-8 days				http://www.pork.org/filelibrary/Yoon%2013-226%2011-27-13.pdf
Czech Republic		diarrhoea appeared 24 48 hpi and the animal died 48 hpi				Rodak et al., 2005
United States	Suckling piglets			100% ranging from 3.5 to 5 weeks of production		Dufresne and Robbins, 2014
	Weaned piglets			Slight increase in mortality		
	All age groups				Reduction of growth rate	

Appendix E. Info of PEDV and PDCoV in matrices

Table 9: Reported info on occurrence of PEDV in species other than pigs since 2004 obtained by an extensive search of the scientific literature and relevant websites

Host	Matrix	Detection method	Number positive/tested samples	Reference
Samples of geese and buzzards	Mouth	RT-PCR	1/2	http://www.cvm.umn.edu/sdec/prod/groups/cvm/@pub/@cvm/@sdec/documents/content/cvm_content_475778.pdf
	Lung	RT-PCR	0/3	
	Stomach	RT-PCR	2/3	
	Intestine	RT-PCR	1/4	
	Vent/cloaca	RT-PCR	2/3	
	Faeces	RT-PCR	0/2	
	Feet	RT-PCR	1/6	
	Area landed	RT-PCR	0/1	
Starling	Feet, cloaca and crop	RT-PCR	0/14	http://www.pork.org/filelibrary/Thomas%2014-171%20main%206-16-14.pdf
	ileum	Immuno-histochemistry	0/9	
Mice (inoculated with PEDV)	Blood	RT-PCR	0/10	Kamau et al., 2010
	Intestine	RT-PCR	0/10	
	Lung	RT-PCR	0/10	
	Spleen	RT-PCR	0/10	
	Kidney	RT-PCR	0/10	
	Faeces	RT-PCR	0/10	
	Serum	ELISA	0/36	
Sparrows (inoculated with PEDV)	Small intestine	RT-PCR	0/16	Lee et al., 2014
Mice (inoculated with PEDV)	Small intestine	RT-PCR	0/16	
Rodents	Brain, tonsils, lungs, heart, spleen, liver, kidneys, mesenteric lymph nodes, small and large intestines	RT-PCR	0/102	Truong et al., 2013
Stray cats	Brain, lungs, heart, spleen, liver, kidneys, mesenteric lymph nodes, small and large intestines	RT-PCR	0/24	
	Tonsils	RT-PCR	1/24	

Table 10: Reported info on PEDV detection in matrices since 2004 obtained by an extensive search of the scientific literature and relevant websites

Matrix	Detection method	Description	Reference
Faeces	RT-PCR	Ten samples tested positive for PEDV alone, while all of the other samples also tested positive for other enteric pathogens	Ge et al., 2013
Intestine	RT-PCR	Positive	Chen et al., 2013a
Milk	RT-PCR	Positive	
Faeces		Virus shedding (up to $10^{12.3}$ $\log_{10}$ genomic equivalents per ml) from 24 h up to 72h after inoculation with PEDV strain PC21A	Jung et al., 2014
Intestine	Immuno-histochemistry	Immunofluorescence-stained cells were observed mainly in the epithelium of atrophied villi of small (duodenum to ileum) and large intestines. Lung tissues of the infected pigs did not show immunofluorescence staining.	
Serum	RT-PCR	All infected pigs tested at acute or later stages of infection had viral RNA titers of 4.8–7.6 log10 GE/mL in serum samples. These titers were similar to those for field samples tested by real-time RT-PCR; 11 (55%) of 20 acute phase serum samples collected from 13- to 20-week-old pigs with diarrhea from Ohio had viral RNA titers of 4.0–6.3 GE/mL. The early, severe diarrhea and vomiting and the PEDV fecal shedding at high titers may be accompanied by viremia.	
Transport vehicle	RT-PCR	Before unloading, 38 (6.6%) of the 575 trailers were contaminated with PEDV. The proportion of contaminated trailers ranged from 2% to 14.6% among the 6 harvest facilities; the facility level median was 5.0%. Of the remaining 537, 28 (5.2%) that were not contaminated at arrival were contaminated in the unloading process. Of the 38 trailers that were contaminated on arrival, environmental samples from 13 (34.2%) were negative for PEDV after unloading. Environmental samples from these 13 trailers tended to have higher cycle threshold values than those from the 25 trailers that were positive before and after unloading: 32.3 versus 30.6, respectively.	Lowe et al., 2014
Faeces	RT-PCR	faecal shedding of PEDV mainly from 2 till 21 days after oro-nasal inoculation with a pool of gut-derived intestinal contents that has been used as 'feedback' inocula for controlled exposure of a sow herd in a commercial swine production unit	http://www.pork.org/filelibrary/Hesse%2013-228%2012-20-13.pdf; Hesse et al., 2014
Nasal swab	RT	nasal shedding of PEDV from 2 till 14 days after oronasal inoculation	
Serum	RT	PEDV detection in serum in 3/5 contact animals and 9/22 inoculated animals (2-8 days following oronasal inoculation)	
Serum	immuno-fluorescence assay	there is no evidence of seroconversion in the aerosol control group at day 35 or 43 in spite of clear demonstration of PEDV nucleic acid in nasal and oral fluid samples	
Faeces	RT-PCR	virus shedding between day 1 until day 25 after oral inoculation	http://www.pork.org/filelibrary/Yoon%2013-226%2011-27-13.pdf
Oral fluid	RT-PCR	PEDV RNA was detected in oral fluids between day 1 and day 28 after oral inoculation	
Plasma	Bioassay	Plasma collected from PEDV infected pigs at peak disease did not contain infectious PEDV	Gerber et al., 2014a

Matrix	Detection method	Description	Reference
Spray-dried bovine plasma (SDBP)	Vero cell culture	Bovine plasma was inoculated with PEDV at an average final titer of $10^{4.2}$ $TCID_{50}$/ml. Using a laboratory scale drier, inoculated plasma was spray dried at 200°C inlet temperature and either 70 or 80°C throughout substance. Liquid samples contained infective virus, but none of the spray dried samples were infectious. Commercial SDBP powder was inoculated with PEDV to an average final titer of $10^{2.8}$ $TCID_{50}$/g. The virus was non-infectious for all samples (n=5 per time and temperature condition) stored at 22°C at 7, 14 and 21 days. PEDV was infective in 1 out of 5 samples stored at 12°C at 7 days, but none of the samples stored for 14 and 21 days were infectious in Vero cell culture. For samples stored at 4°C, 4 out of 5 samples were infectious at 7 days, 1 out of 5 samples was infectious at 14 days but none was infectious at 21 days. These results suggest that survival on SDBP was dependent upon storage temperature and time, the virus was not found infectious on cell culture within 7 days when stored at room temperature and within 21 days when stored at refrigerated temperature.	Pujols and Segalés, submitted; https://www.aasv.org/pedv/research/NASDBPP_research.pdf
SDPP	Bioassay	Bioassay studies performed by CFIA demonstrated that the implicated plasma did contain PEDV capable of infecting and causing disease in pigs; data not shown	Pasick et al., 2014; http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/2014-02-18/eng/1392762739620/1392762820068
SDPP	PCR and bioassay (experiment 1); clinical signs, RT-PCR and immunohistochemistry (experiment 2)	17-19-day-old pigs were inoculated with PEDV PCR+ plasma samples and remained negative in PCR and serology testing (3 plasma samples; 5 pigs/sample) (experiment performed by FDA). In a second experiment (performed by University of Minnesota), four PEDV PCR+ plasma samples were tested in piglets. All animals (3 per sample) remained negative for clinical signs (up to 72 h post inoculation), in RT-PCR (jejunum) and immunohistochemistry.	https://www.aasv.org/pedv/research/NASDBPP_research.pdf
SDPP in feed	Bioassay	A bioassay using 3-week-old piglets could not demonstrate that the feed pellets containing the PEDV PCR+ blood plasma were capable of causing disease	Pasick et al., 2014; http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/2014-03-03/eng/1393891410882/1393891411866

Matrix	Detection method	Description	Reference
SDPP in feed	RT-PCR and bioassay	3-week old piglets received a non-pelleted diet with 5% SDPP containing 5.1±0.1 $\log_{10}$ PEDV RNA copies per gram. The diet contained 3.3±0.3 $\log_{10}$ PEDV RNA copies per gram. The interval from SDPP production to initiation of feeding the pigs in this trial was between 28 and 32 days, which are considered typical in the US by the authors since it accounts for the time from production, transport to the feed mill, distribution to the farm, and administration to the pigs. Feed samples were obtained on a weekly basis and contained 3.2±0.5 $\log_{10}$ PEDV RNA copies per gram without apparent reduction over time. The animals (n=3) remained PEDV RNA negative and did not seroconvert until at least 28 days (end of experiment), no PEDV was detected using immunohistochemistry, no villus atrophy was detected and none of the animals had colitis.	Opriessing et al., 2014
SDPP in feed	PEDV-specific antibodies and immunohistochemistry (experiment 1); PEDV-specific antibodies (experiment 2)	Experiment 1 showed that feeding pigs a diet containing 5% commercial spray-dried porcine plasma that was PEDV PCR+ (26.2 Ct) did not demonstrate evidence of PEDV infectivity in these pigs through 21 days post-weaning (n=24) (negative for PEDV-specific serum antibodies and immunohistochemistry). Experiment 2 performed by Iowa State University indicated that feeding pigs a diet with PEDV PCR+ spray-dried porcine plasma (30.0 Ct) did not result in PEDV infectivity over the 28-day study period (negative in PEDV-specific antibodies).	Campbell et al., 2014; https://www.aasv.org/pedv/research/NASDBPP_research.pdf
SDPP in feed	Clinical signs	Millions of pigs in Brazil and Western Canada fed diets containing PEDV PCR+ SDPP imported from the US since summer 2013 have not developed PEDV (no cases reported)	Crenshaw et al., 2014
Feed	Bioassay	Piglets receiving PEDV PCR-positive feed bin samples from three clinically affected breeding herds, showed clinical signs of PEDV infection and viral shedding was detected. The exact source of PEDV contamination in the feed lots is undetermined.	Dee et al., 2014
Faeces	RT-PCR and real-time PCR	PEDV was shed in faecal excretions at high concentration (up to $1x10^{6.85}$ copies mL^{-1}) and the shedding time lasted for 56 days under field conditions.	Sun et al., 2014
Air	RT-PCR and real-time PCR	Air samples were collected at three infected farms. All samples from farm A were PEDV negative, the overall PEDV positive rate of farms B and C was 6.7% and 12.2% respectively.	
Semen	RT-PCR and real-time PCR	Twenty boars without symptoms of PED were selected from PEDV-infected farms and 16 from them tested positive ($10^{1.46}$ to $10^{3.55}$ copies/mL). The study did not confirm whether PEDV could be transmitted by semen.	

Matrix	Detection method	Description	Reference
Air	RT-PCR	Experimental (intragastral) infection of 7 to 8 week-old pigs with an inoculums derived from mucosal scrapings of a PEDV case. Air samples were detected in the center of the isolation rooms and pigs did not have direct contact with the sampler. All air samples (taken between 8 and 63 hours post inoculation) were positive and the estimated number of RNA copies per m^3 of air ranged between $1x10^6$ to $1x10^9$. Air samples were also collected around eight swine herds experiencing acute PEDV outbreaks in Oklahoma (US), at different locations downwind (distance to the farm ranging from 9.6 m to 24.14 km). Eleven out of 62 (18%) air samples tested RT-PCR positive. Genetic material of PEDV ($7.98x10^3$ PEDV RNA copies/m^3) was detected up to 10 miles downwind of farm C. At least one air sample from each farm (except farms E and H) tested positive. None of the air samples collected under field conditions was positive on bioassay.	Alonso et al., 2014
Air	RT-PCR and bio-assay	Three-week-old piglets were inoculated with a pool of gut derived intestinal contents that has been used as feedback inoculum in a commercial swine production unit. Group-C pigs were not inoculated, but were housed in a separate pen in the common animal rooms as Groups A (inoculated) and Group B (not inoculated but direct contact with Group A). There is no evidence of seroconversion in the aerosol control group C in spite of the clear demonstration of PEDV nucleic acid in nasal and oral fluid samples.	Hesse et al., 2014
Air	RT-PCR and bio-assay	Ten 8-week old pigs were experimentally infected with PEDV. Air samples were collected for 3 days from the isolation room this group of pigs was housed in. All samples put into bioassay were PEDV PCR positive. Clinical signs (diarrhoea), necropsy findings and/or significant replication (15-16 Ct in small intestine) indicated live virus in the air samples.	http://www.cvm.umn.edu/sdec/prod/groups/cvm/@pub/@cvm/@sdec/documents/content/cvm_content_474046.pdf

Table 11: Stability of PEDV in different matrices under different conditions (http://www.pork.org/filelibrary/Goyal%2013-215%201-21-14.pdf)

Matrix	Temperature	RH	cT value in intestinal samples from inoculated piglets					
			0 days	1days	3 days	7 days	14 days	28 days
Fresh feces	40°C	30%	16.48	NR	32.00	-	-	NR
		50%	NR	16.35	-	-	-	NR
		70%	13.79	NR	13.30	15.16	-	NR
	50°C	30%	15.65	15.75	-	-	NR	NR
		50%	16.63	NR	19.08	-	-	NR
		70%	16.12	NR	14.99	35.33	-	NR
	60°C	30%	NR	13.26	35.10	37.24	NR	NR
		50%	17.94	16.31	-	-	NR	NR
		70%	NR	33.93	35.61	-	NR	NR
Slurry	Room temperature (~25°C)	30%	17.01	NR	NR	15.81	36.86	-
		50%	NR	NR	NR	17.60	17.56	-
		70%	NR	NR	NR	15.75	35.86	-
	4°C	30%	16.26	NR	NR	16.41	17.19	16.08
		50%	NR	NR	NR	17.03	16.04	17.90
		70%	NR	NR	NR	16.19	17.08	36.69
	-20°C	NR	16.77	NR	15.79	16.27	15.51	14.81
Wet feed	Room temperature (~25°C)	NR	15.52	NR	NR	15.21	27.63	29.67
Dry feed	Room temperature (~25°C)	NR	NR	NR	NR	16.52	-	-

NR, not reported

Table 12: Stability of PEDV in faeces under different conditions (Thomas et al., 2014; http://www.pork.org/filelibrary/Holtkamp%2013-227%2012-20-13.pdf)

Group	Treatment simulates	Number of PEDV positives/tested in pig bioassay
No treatment, pigs received a gavage of PEDV-negative faeces	No exposure to PEDV	0/4
No treatment, pigs received a gavage of PEDV-positive faeces	Exposure to a PEDV-contaminated hog trailer with no decontamination attempted	4/4
PEDV-positive faeces were placed on an aluminium tray and heated to 160 °F (71.1 °C) in an incubator and held at this temperature for 10 minutes.	Exposure to a PEDV-contaminated hog trailer that was heated via thermo-assisted drying and decontamination (TADD) to a temperature of 160F (71.1°C) and held at this temperature for 10 min. This is consistent with TADD protocols in some systems.	0/4
PEDV-positive faeces were placed on an aluminium tray and heated to 145 °F (62.7 °C) in an incubator and held at this temperature for 10 minutes	Exposure to a PEDV-contaminated hog trailer that was heated via TADD to a temperature of 145F (62.7°C) and held at this temperature for 10 minutes. This is consistent with TADD protocols in some systems.	1/4

Group	Treatment simulates	Number of PEDV positives/tested in pig bioassay
PEDV-positive faeces were placed on an aluminium tray and heated to 130 °F (54.4 °C) in an incubator and held at this temperature for 10 minutes	Exposure to a PEDV-contaminated hog trailer that was heated via TADD to a temperature of 130F (54.4°C) and held at this temperature for 10 min. This was done to demonstrate a TADD protocol that is not reaching a temperature that is probably not sufficient to inactivate PEDV.	1/4
PEDV-positive faeces were placed on an aluminium tray and heated to 100F (37.7 °C) in an incubator and held at this temperature for 12 hours	Exposure to a PEDV-contaminated hog trailer that was not decontaminated via acceptable TADD procedures. This would stimulate leaving a trailer in a heated garage of bay overnight to encourage drying.	2/4
PEDV-positive faeces were placed on an aluminium tray and left at room temperature for 24 hours	Exposure to a PEDV-contaminated hog trailer that was not heated, but left to sit for 24 hours between loads of hogs.	1/4
PEDV-positive faeces were placed on an aluminium tray and left at room temperature for 24 hours	Exposure to a PEDV-contaminated hog trailer that was not heated, but was left to sit unused for 1 week between loads.	0/4

Table 13: Determination of the minimal infective dose of PEDV (http://www.pork.org/filelibrary/Goyal%2013-215%201-21-14.pdf)

PEDV dilution	Experiment 1		Experiment 2	
	Extent of diarrhea	cT value in mucosal samples from inoculated piglets	Extent of diarrhea	cT value in mucosal samples from inoculated piglets
10^{-2}	++	17.24		
10^{-3}	++	16.92		
10^{-4}	++	15.32		
10^{-5}	+	17.10		
10^{-6}	+	16.02	+	15.52
10^{-7}	-	15.70	++	15.52
10^{-8}			+	16.03
10^{-9}			-	30.29
10^{-10}			-	-
10^{-11}			-	-
10^{-12}			-	-

GLOSSARY AND ABBREVIATIONS

GLOSSARY

Case	One infected animal
Isolate	Virus that has been isolated using cell culture
Outbreak	The occurrence of one or more cases in an epidemiological unit
Strain	A group of viruses and/or isolates having a high sequence identity and hence clustering together in phylogenetic trees

ABBREVIATIONS

aa	Amino acids
dpi	Days post infection
EFSA	European Food Safety Agency
EU	European Union
GE	Genomic equivalent
hpi	Hours post inoculation
nt	nucleotides
PED	Porcine epidemic diarrhoea
PEDV	Porcine epidemic diarrhoea virus
PEDV-Am	PEDV isolated in Americas
PEDV-Asia	PEDV isolated in Asia
PEDV-EU	PEDV isolated in Europe
PDCoV	Porcine deltacoronavirus
SDPP	Spray-dried porcine plasma
TGEV	Transmissible gastroenteritis virus
TOR	Terms of reference