

The Director General

Maisons-Alfort, 7 July 2014

OPINION
of the French Agency for Food, Environmental
and Occupational Health & Safety

regarding the risk of emergence of porcine epidemic diarrhoea (PED)
due to a new variant of the PED virus in France

ANSES undertakes independent and pluralistic scientific expertise.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 7 July 2014 shall prevail.

On 8 April 2014, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) received a formal request from the Directorate General for Food (DGAL) in order to assess the risk of a new variant of the porcine epidemic diarrhoea (PED) virus being introduced in France.

On 12 May 2014, in light of new available information on the situation in the United States and Canada, ANSES was also requested to address additional points regarding the risk of the PED virus being introduced.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Since April 2013, the United States has been facing an unprecedented epidemic of porcine epidemic diarrhoea that has resulted in the death of several million piglets (see Bulletin Épidémiologique 58, 21-22). Over 7447 farms have been affected in 30 US states since the beginning of the epidemic as of 15 June 2014 and the disease has spread to Canada (68 farms affected as of 15 June 2014: 62 in Ontario, four in Manitoba, one in Quebec and one recently on Prince Edward Island).

The early epidemiological information regarding the epidemic in the United States shows a first phase of likely farm contamination at a common source, with the virus then rapidly spreading, suggesting mechanical transport of the virus or through animal movements. The situation in the United States and Canada is of great concern due to the effective spread of the virus from herd to herd despite the widespread establishment of biosafety measures.

The new identified strain of the PED virus is very similar to a Chinese strain. It is different from the other PED strains that have been observed in Europe where PED was first clinically identified in 1972. After that date, the virus spread widely in the European pig population but the

disease has not been detected in Europe since the late 1990s with the exception of an epidemic in Italy from May 2005 to June 2006 (Martelli *et al.*, 2008).

Porcine epidemic diarrhoea was recently added to the list of emerging category-one health hazards for animal species, in Annex I.b of the Ministerial Order of 29 July 2013 on the definition of category-one and -two health hazards for animal species. Further to this decision, all cases appearing on French soil must be reported.

Given the risk of the virus spreading, considering that the United States, Canada, Mexico, Japan, Colombia and the Dominican Republic are experiencing an epidemic of PED, the DGAL has prepared a draft Order listing certain materials that are prohibited from being imported into France depending on their country of origin (see Annex 1).

In light of the available information, the Agency's opinion has been requested regarding the following points:

1: Identification of risk factors, assessment of the risk of introduction and measures intended to reduce this risk

- How can we qualify the risk of the PED virus being introduced in France by the various identified sources: live pigs, semen, feed, 'sensitive' raw materials, other sources?
- Are these risks the same for all the countries experiencing the epidemic?
- What would be the minimum production conditions for raw materials derived from pigs (blood products, hydrolysed proteins, fats, gelatines) to guarantee control of the risk for use of these materials in animal feed? The Order relating to the precautionary measures adopted in France against PED lists certain raw materials that are prohibited from being imported into France depending on their country of origin. Are there any products to be removed/added?

2: Risk of spreading and measures intended to reduce this risk

- If the risk of introduction is not negligible (if it is not less than or equal to near-zero according to the scale below), what control measures identified thus far could be implemented in order to limit the risk of spreading?
- Foreseeable impact for the sector if the virus is introduced in France (France has temporarily added it to the list of category-1 diseases to make reporting mandatory)
- If the risk of introduction is not negligible (if it is not less than or equal to near-zero according to the scale below), what would be the advantages/disadvantages of simple confinement (APDI¹ with or without zoning) versus preventive culling?
- If a first outbreak were to occur, could it be contained through simple confinement of the first reported cases?
- Would a preventive total cull with each report limit the consequences of PED introduction into France?
- Can the vaccines that may be available be expected to provide protection?

It should be noted that this Opinion is intended to answer these questions, while limiting the scope of the expert appraisal to the variant of the PED virus as defined above.

¹ APDI: Prefectural Declaration of Infection

2. ORGANISATION OF THE EXPERT APPRAISAL

2.1. Establishment of an Emergency Collective Expert Assessment Group (GECU)

This expert appraisal was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities".

Given the health urgency related to the possible emergence of a new pig disease in France, ANSES established an Emergency Collective Expert Assessment Group (GECU) on PED to respond to the questions in the formal request. The GECU report was presented at the SANT Expert Committee meeting of 2 July. The GECU met on 15 May, 3, 20 and 30 June and 3 July 2014. The work of the GECU was discussed with the Expert Committee on Animal health, which met on 2 July 2014.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public on ANSES's website (www.anses.fr).

2.2. Expert appraisal method

The GECU used the qualitative risk assessment method developed by AFSSA in 2008 (AFSSA, 2008). More specifically, the method used to assess the risk of the virus being introduced in France consisted in:

- Defining the context of the expert appraisal and establishing a conceptual model to describe factors likely to play a part in the occurrence of the hazard (see Annex 5);
- Providing available knowledge of the PED virus variant;
- Identifying the various sources of the virus and assessing their likelihood of excretion;
- Assessing the likelihood of exposure to these virus sources;
- Estimating the likelihood of the virus being introduced for each source;
- Assessing the impact of the virus spreading in France from an initial outbreak;
- Assessing various possible control measures to prevent this PED variant from emerging and spreading in France.

To undertake its assessment, the GECU relied on the following:

- The data taken from the TRACES database, provided by the DGAL, regarding imports of live pigs and pig products;
- The international information provided throughout the assessment by the DGAL;
- The information collected from various stakeholders:
 - o Mrs Anne LÉBOUCHER, national reference for animal by-products;
 - o Mrs Sandrine DELAFOSSE, national reference for animal feed;
 - o Mrs Marie-Alix Roussillon, IFIP;
 - o Mr Michel Dochez, Coop de France, Animal nutrition section;
 - o Mr Alain Coupel, member of the Scientific Board for Animal Nutrition (CSNA).
- The regulatory texts on the treatment of animal by-products;
- The scientific publications listed at the end of the report.

To ensure a common understanding of the terms used in this report, the scale of correspondence between the qualitative assessment and an ordinal scale, developed by AFSSA in 2008, is given in Table 1 below:

Table 1: descriptions of likelihoods for qualitative risk assessment (AFSSA, 2008)

Ordinal scale	Description
0	Nil (N)
1	Near-Nil (NN)
2	Minimal (M)
3	Extremely low (EL)
4	Very low (VL)
5	Low (L)
6	Not Very high (NVH)
7	Quite high (QH)
8	High (H)
9	Very high (VH)

The descriptions do not appear in the rest of the document. Only the ordinal scale has been used for the comparison of likelihoods.

Uncertainty as to data quality (weight-of-evidence) has been taken into account and classified into the levels presented in Table 2 below:

Table 2: Definition of 'uncertainty factors' for scoring

Uncertainty factor	Quality of data used for the expert appraisal (weight-of-evidence)
1 - Low	The assigned score is based on convergent results of scientific studies or on a data collection system with recognised reliability.
2 - Medium	The assigned score is based on a limited number of scientific studies or on a data collection system with limited reliability AND with convergence between authors and/or experts.
3 - High	The assigned score is based on: - a limited number of scientific studies or on a data collection system with limited reliability AND without consensus between authors and/or experts; - or an individual expert opinion without scientific studies or data collection systems.
4 - No data	Total absence of data (a score of '999') and expert opinion

This data quality is indicated in the assessment of likelihoods of excretion and exposure to the hazard. The uncertainty factor is abbreviated UF in the text.

3. ANALYSIS AND CONCLUSIONS OF THE GECU

The PED GECU adopted the collective expert appraisal work and its conclusions and recommendations, which are covered in this opinion, at its meeting of 3 July 2014 and informed ANSES's General Directorate.

3.1 Risk identification: new variant of the porcine epidemic diarrhoea virus

3.1.1 Causal agent: structure (UF: 1)

The new variant of the PED virus (PEDv) is a coronavirus, classified in the genus *Alphacoronavirus* (Song and Park, 2012). This virus infects pigs and is not zoonotic in nature. The viral genome has the same structure as all coronaviruses. The order of the genes is canonical: replicase- S-E-M-N. The replicase contains two open reading frames (ORF) 1a and 1b. The S gene encodes the spike protein on the surface of the virion, the E gene an envelope protein, the M gene the matrix protein and the N gene the nucleocapsid protein that surrounds and protects the viral RNA (Masters, 2006). PEDv has an additional ORF called ORF3 located between the S and E genes. By phylogenetic analysis, based on the S, M and ORF3 gene sequences, it is possible to identify various clusters by region of origin. More virulent forms were described in China in 2011 and 2012. According to the phylogenetic analysis of the full genome, the strains isolated in the United States in May 2013 are similar to a strain isolated in 2012 in the Chinese province of Anhui. The American strains of PEDv have insertions of amino acids 56 to 59 and 139, and deletions of amino acids 160 to 161 in the S protein in contrast to the European reference strain CV777. These specificities were also identified in the PEDv strains recently isolated in South Korea and China (Huang *et al.*, 2013).

Recently, in March 2014, a different coronavirus belonging to the genus *Deltacoronavirus* was isolated from a herd with the same symptoms and spread fairly widely in the pig population in the United States (https://www.aasv.org/pedv/SECoV_weekly_report_140425.pdf). This delta coronavirus is not covered in this opinion.

3.1.2 Pathogenesis (UF: 1)

After oral infection of piglets with the European reference strain CV777 or an American strain isolated in June 2013, PEDv multiplies in the epithelial cells of the small intestine villi, particularly the jejunum and ileum, and in the colon from 12 hrs. post-inoculation. Faecal excretion is detected 24 hrs. post-inoculation. Severe diarrhoea begins 22 to 36 hrs. post-inoculation. After inoculation with an American strain isolated in June 2013, viraemia is observed after the appearance of clinical signs in piglets (Debouck *et al.*, 1981; Jung *et al.*, 2014) as previously demonstrated with other older strains.

3.1.3 Epidemiology

An epidemic in a susceptible herd occurs four to five days after the introduction or sale of pigs. Therefore, the virus is probably introduced in farms through infected pigs or mechanical transport (boots, lorries, etc.). PEDv may persist more easily in an enzootic form after an initial epizootic phase. An enzootic cycle can thus be established in farms where turnover is high with mixed litters from different batches with different immune statuses.

- Minimum infectious dose (UF: 1)

The minimum infectious dose is very low. The disease was reproduced after inoculation with a 10^{-8} dilution of a clarified homogenate of intestinal mucosa from a piglet infected with the PEDv isolated in the United States in 2013 (Goyal, 2014). Inoculation of the 10^{-9} dilution, not

detectable by real-time RT-PCR, still caused piglets to become infected, underlining the very low minimum infectious dose.

- Oral-faecal transmission (UF: 1)

PEDv is essentially transmitted between pigs by the oral-faecal route.

- Airborne transmission (UF: 2)

The viral genome has been found up to 16 km from an infected farm (but without triggering the disease). This suggests that the virus may spread through the air but does not rule out other means of transport.

(http://www.cvm.umn.edu/sdec/prod/groups/cvm/@pub/@cvm/@sdec/documents/content/cvm_content_474046.pdf)

- Venereal transmission of the virus (UF: 2)

It is not known for PEDv. It is nonetheless possible since a viraemia phase is observed at least during primary infection. This transmission assumption is also supported by detection of the viral genome in the semen of infected boars in a boar stud affected by PED (Dufresne and Robbins, 2014). However, the possibility of cross-contamination by faeces during semen collection or preparation cannot be ruled out.

3.1.4 Clinical signs and lesions (UF: 1)

The main clinical sign of PED is liquid diarrhoea sometimes preceded by vomiting. In adult animals however, infection can be subclinical or only cause signs of anorexia and vomiting. During an epidemic, mortality rates can range from 50% on average to 95-100% in suckling piglets, which is the case with the current epidemic in North America. Older animals recover one week after clinical signs appear. Macroscopic lesions are concentrated in the small intestine whose wall becomes thin and transparent and whose content is watery and yellowish (Stevenson *et al.*, 2013) (Coussement *et al.*, 1982; Ducatelle *et al.*, 1982; Jung *et al.*, 2014; Pospischil *et al.*, 1982).

3.1.5 Resistance of the virus (UF: 1)

- pH

PEDv is stable from pH 5 to 9 at 4°C and from pH 6.5 to 7.5 at 37°C (Pospischil *et al.*, 2002). It therefore appears that the virus can survive in fairly wide pH ranges at low temperatures.

- Biocides

Disinfectants effective against PEDv are those containing phenol, peroxygen and chlorine and those containing quaternary ammonium and glutaraldehyde.

- Effect of temperature and humidity

Infectivity is maintained (Goyal, 2014):

- In experimentally infected faeces, for at least seven days at 40, 50 and 60°C with humidity levels of 30, 50 and 70%;
- In drinking water, for up to seven days at ambient temperature (25°C);
- In wet feed (slurry), stored at ambient temperature for up to 28 days, PEDv RNA is not degraded;
- In dry feed, for up to seven days;

- In manure:
 - o for at least 28 days at 4 and -20°C and for up to 14 days at 25°C;
 - o contaminated manure placed on a metallic medium:
 - Inactivation at 71°C for ten minutes and at 20°C for seven days;
 - No inactivation at 54°C or 62°C for ten minutes, 37°C for 12 hours or 20°C for 24 hours (University of Iowa).

3.2 Epidemiological description of the porcine epidemic diarrhoea situation in various countries

3.2.1 Background in Europe (UF: 1)

The first cases of PED were described in the early 1970s in the United Kingdom (Oldham, 1972) where a disease similar to transmissible gastroenteritis (TGE) was observed in several farms. The disease spread to several countries in Europe and the causal coronavirus was characterised (Chasey and Cartwright, 1978; Pensaert and De Bouck, 1978). In the 1980s, serological studies undertaken in several European countries (Belgium, Germany, France, Spain, Netherlands, United Kingdom, Switzerland, Bulgaria) showed that the virus had spread widely in the pig population. From the 1990s, the prevalence of the virus tended to decline in these countries (Pensaert and Van Reeth, 1998) but there were some sporadic cases until the end of the 1990s. The cases described in Europe in the 1990s contrasted with those in certain Asian countries where there were severe epidemics with very high mortality rates (Japan, South Korea). Thereafter, the disease was no longer described in Europe with the exception of the diarrhoea epidemic that affected pigs of all ages in Italy from May 2005 to June 2006 (Martelli *et al.*, 2008). Recently, a seroprevalence of 10% was observed in the United Kingdom (<http://www.defra.gov.uk/ahvla-en/science/bact-food-safety/2013-pig-abattoir-study/>; D. Armstrong, BPEX, personal communication).

3.2.2 Situation in the United States (UF: 1)

In late April 2013, the first cases of PED were detected in the United States whereas this disease had never before been described on the American continent. As of 21 June 2014, over 7440 farms had been affected in thirty states (Figure 1).

The incidence of positive-confirmed farms in the United States can be estimated through the number of laboratory accessions confirmed as positive for the PED virus (Figure 2). These numbers potentially overestimate the real incidence particularly if samples from the same farm were successively submitted over time or to separate laboratories. Nonetheless, insofar as these samples were taken to establish a diagnosis, it is unlikely that sampling was repeated once one had been established.

In the first part of the epidemic (from early April to 10 June 2013), the R_0^2 for the inter-farm spread of the epidemic was estimated at 48.3 95%CI [28.9; 81.6] (Figure 3). This suggests the grouped contamination of several farms through common exposure at the beginning of the epidemic. For the more recent part of the epidemic curve (from September 2013 to late January 2014), the R_0 was 2.4 95%CI [2.2; 2.5]. This estimate suggests the effective inter-farm spread of the disease, which is consistent with spreading through the mechanical transport of the virus by vectors (vehicles, personnel, etc.), animal movements or possibly aerosols from neighbouring farms. PEDv has been a notifiable disease in the United States since 21 April 2014, i.e. almost one year after it appeared in North America.

² The R_0 estimates the number of secondary farms infected by an infected farm. Estimation by the exponential growth rate method for the epidemic (Package 'R0', R), assuming a Gamma distribution of the epidemic's generation time with an average of 40 days (Poulin, M.C. and Klopfenstein, C., 2013. Évaluation et gestion du risque d'introduction et de dispersion de la diarrhée épidémique porcine (DEP) au Québec, Centre de Développement du Porc du Québec Inc., CDPQ, Québec, Canada.)

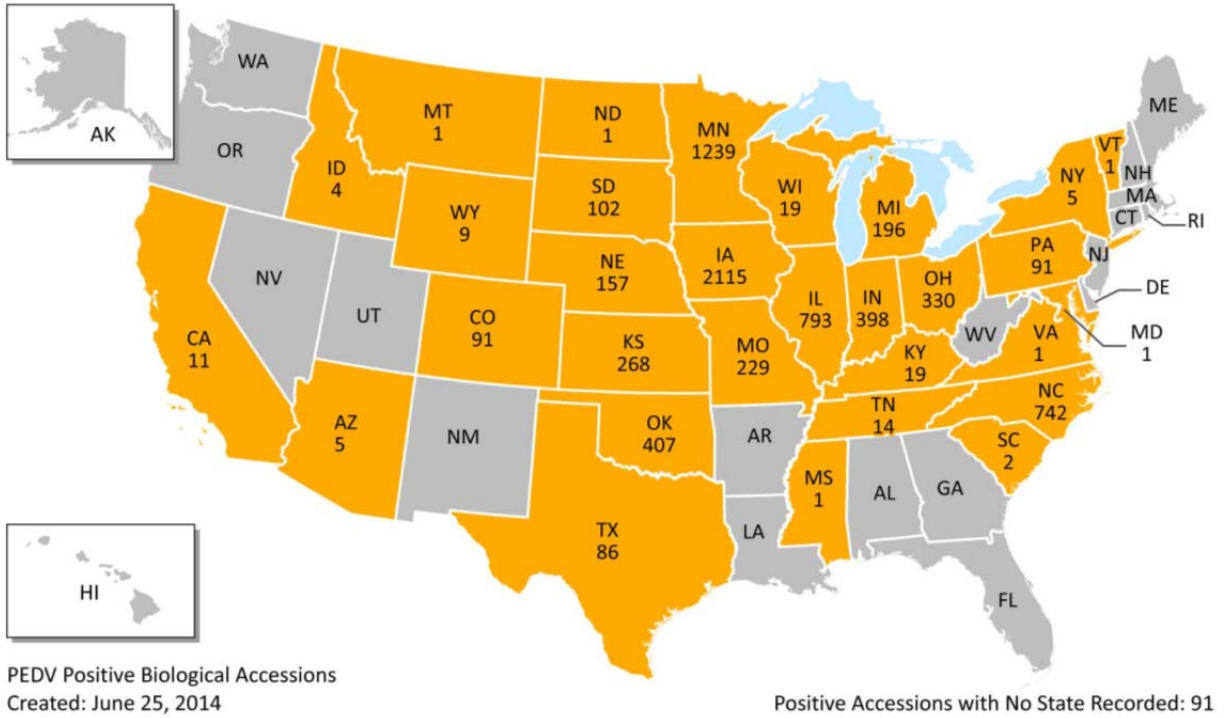


Figure 1: Geographic distribution of farms positive for the porcine epidemic diarrhoea virus (laboratory confirmations) in the United States on 25 June 2014.
<http://www.aasv.org/aasv%20website/Resources/Diseases/PorcineEpidemicDiarrhea.php>.

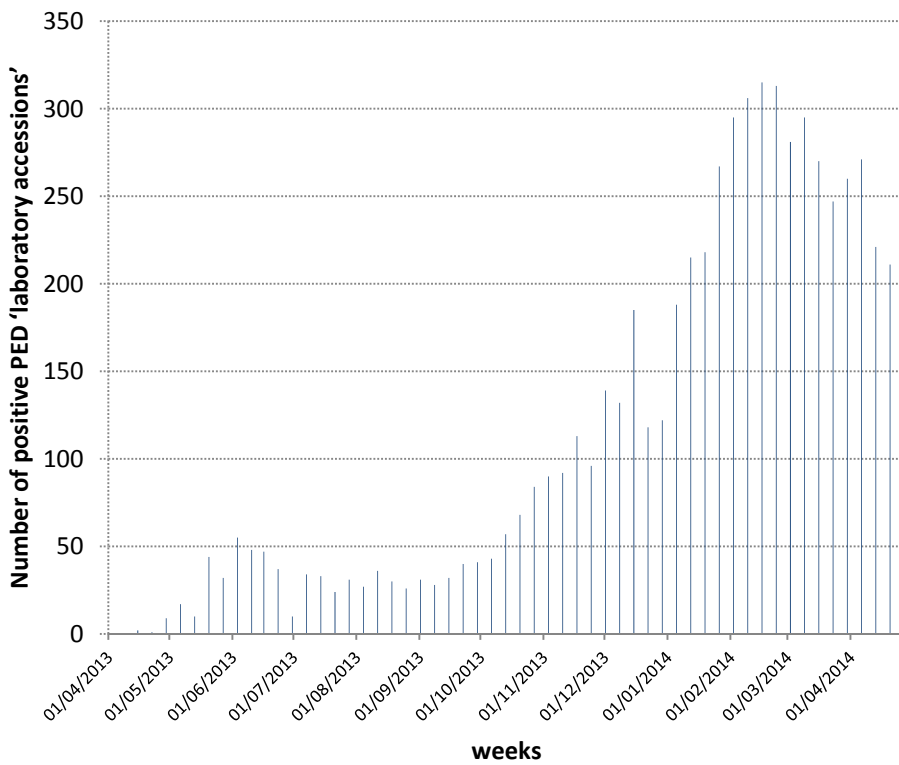


Figure 2: Number of positive PEDV laboratory accessions in the United States since the start of the epidemic. An accession is specific to a farm and can contain several samples from the same farm.
<http://www.aasv.org/aasv%20website/Resources/Diseases/PorcineEpidemicDiarrhea.php>

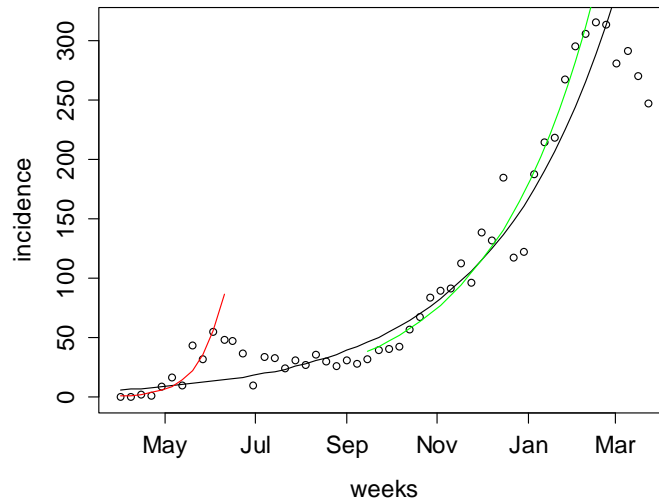


Figure 3: Modelled increase in incidence in the United States for various periods for R0 estimation (exponential growth rate method for the epidemic, Package 'R0', R).

3.2.3 Situation in Canada (UF: 1)

On 22 January 2014, Ontario reported the first case of PED in Canada, followed by Manitoba and the Montérégie region. As of 15 June 2014, a total of 68 PED cases had been diagnosed after laboratory confirmation (Figure 4). Note that a very isolated farm reported the disease on Prince Edward Island on 13 February. In Montérégie (Quebec), rapid culling followed by a fallow period on the affected farm undoubtedly kept the virus from spreading to other farms in this densely populated area. In April, Manitoba confirmed that pigs had contracted PED in two assembly centres. In Ontario, where the disease is most present, PED has risen relatively moderately (one to seven new cases per week, Figure 5).

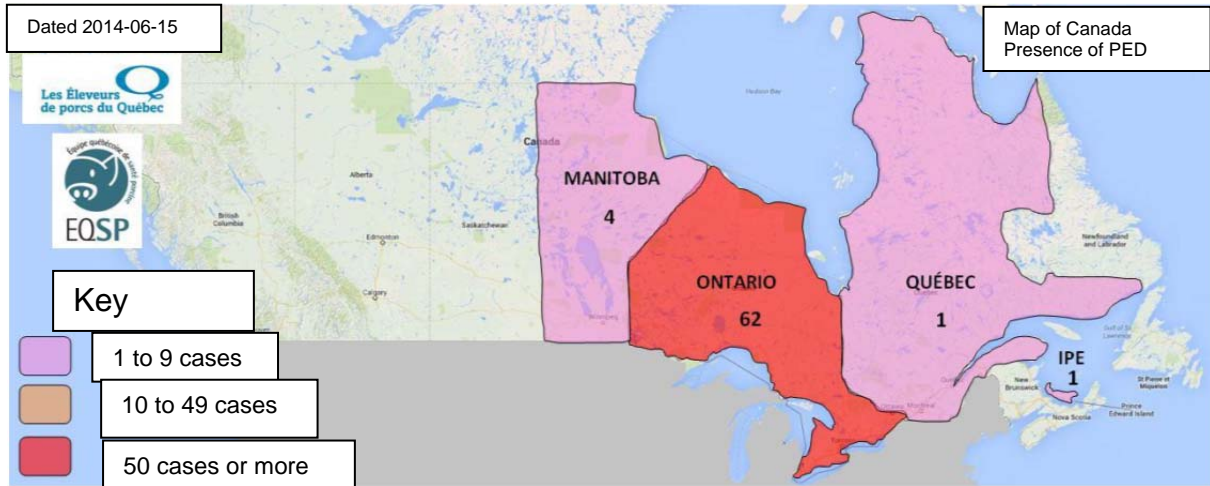


Figure 4: geographic distribution of PED-positive farms (laboratory confirmations) in Canada on 15 June 2014. (<http://www.leseleveursdeporcsduquebec.com/les-eleveurs-fr/actualites.php>).

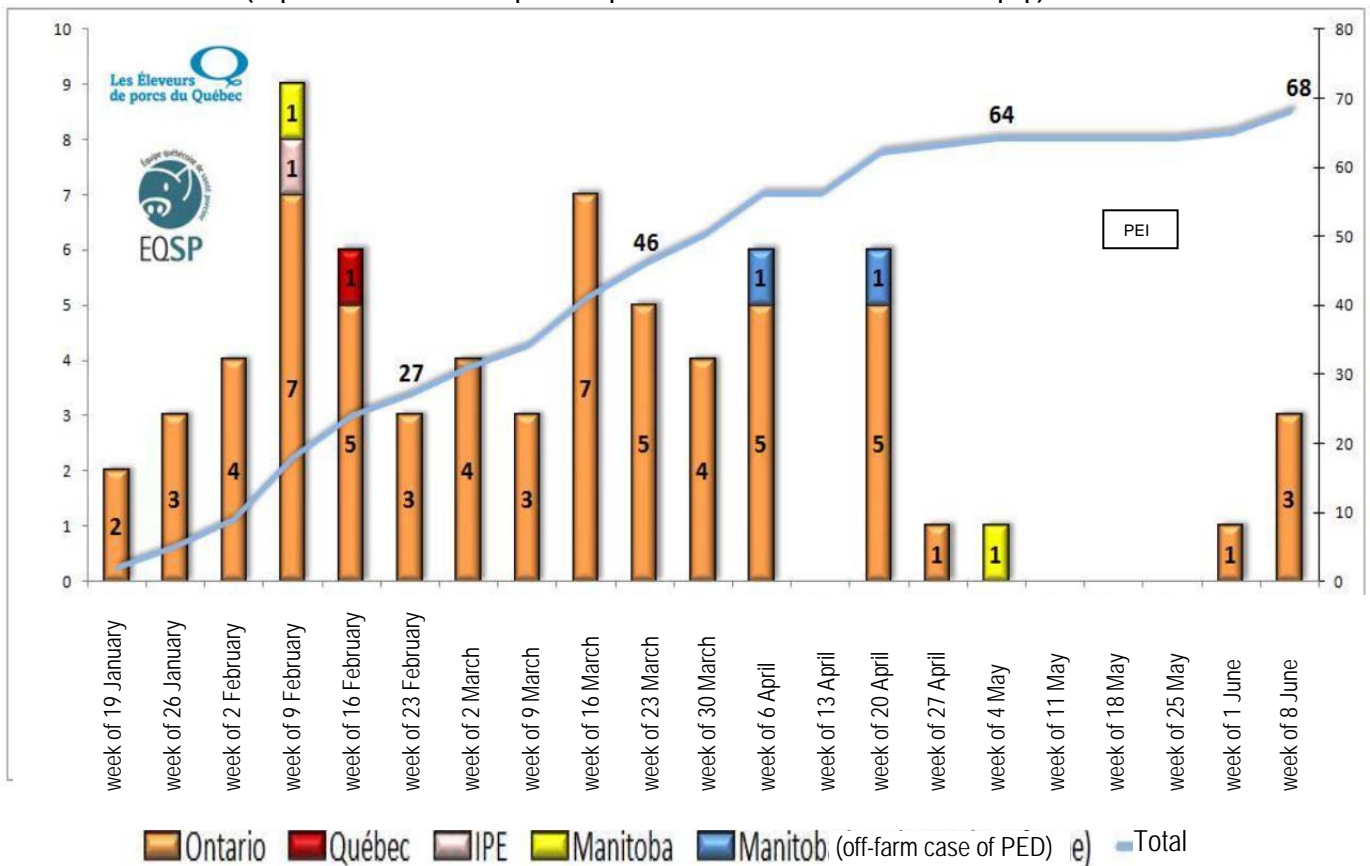


Figure 5: weekly incidence of PED-positive farms (laboratory confirmations) in Canada (weeks of 19 January 2014 to 8 June 2014).

(<http://www.leseleveursdeporcsduquebec.com/les-eleveurs-fr/actualites.php>)

3.2.4 Situation in Central and South America (UF: 2)

Mexico

The epidemic began on 30 July 2013 and spread to the centre and west of the country; it has affected 83 farms in 17 different states (OIE report of Dr Joaquin Braulio Delgadillo Alvarez [Director General for Animal Health, National Service for Health, Food Safety and Quality

(*SENASICA, Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food*], Mexico City, Mexico).

Of 2309 samples collected in 19 states between August 2013 and May 2014, 30% were confirmed positive for PEDv.

Dominican Republic

An OIE report dated 13 June 2014 describes seven separate outbreaks in the Dominican Republic; the first cases appeared in November 2013. To date, the epidemic has resulted in the death of over 26,000 piglets. Samples taken and analysed in the United States confirmed PEDv, and sequencing showed they were similar to the strains circulating in the United States.

(*OIE report of Dr Nimia Lissette Gomez Rodriguez, Director, Animal Health Directorate, Livestock General Directorate (DIGEGA), Ministry of Agriculture, Santo Domingo, Dominican Republic*)

Colombia

The information is less specific in other Latin American countries although 45 outbreaks have been confirmed in Colombia to date (commercial and backyard farms). The situation is apparently much less severe than in the United States (less mortality and return to a normal state within two weeks). PEDv was indeed confirmed as the etiological agent and the sequencing data also appear to indicate strong similarity to American strains.

(*OIE report of Dr Luis Humberto Martinez Lacouture [General Manager, Colombian Agricultural Institute (ICA), Ministry of Agriculture and Rural Development], Bogota, Colombia*)

3.2.5 Situation in China (UF: 3)

The first cases of PED in China were described in 1973. Twenty years later, an inactivated, adjuvanted vaccine was developed and has been widely used in the country's pig population. It appears that until 2010, the prevalence of the disease was relatively low, with only sporadic cases with low severity. At the end of 2010, the prevalence of PED cases sharply increased in provinces with high pig densities. The described cases were more severe than those previously encountered and vaccinated farms were also affected, although mortality may have been lower in this case (Li *et al.*, 2012). The analyses undertaken showed PEDv together with the strains typically isolated previously in the sporadic cases (Bi *et al.*, 2012; Pan *et al.*, 2012). These variant viruses were very similar to the emerging strains isolated in the United States (Huang *et al.*, 2013). However, no quantitative data are available to describe the development of the epidemic related to the new variant viruses in China.

3.2.6 Situation in Japan (UF: 1)

The disease reappeared in Japan in October 2013 after seven years of absence. Since then, 664 farms have been affected in 38 prefectures (Figure 6).

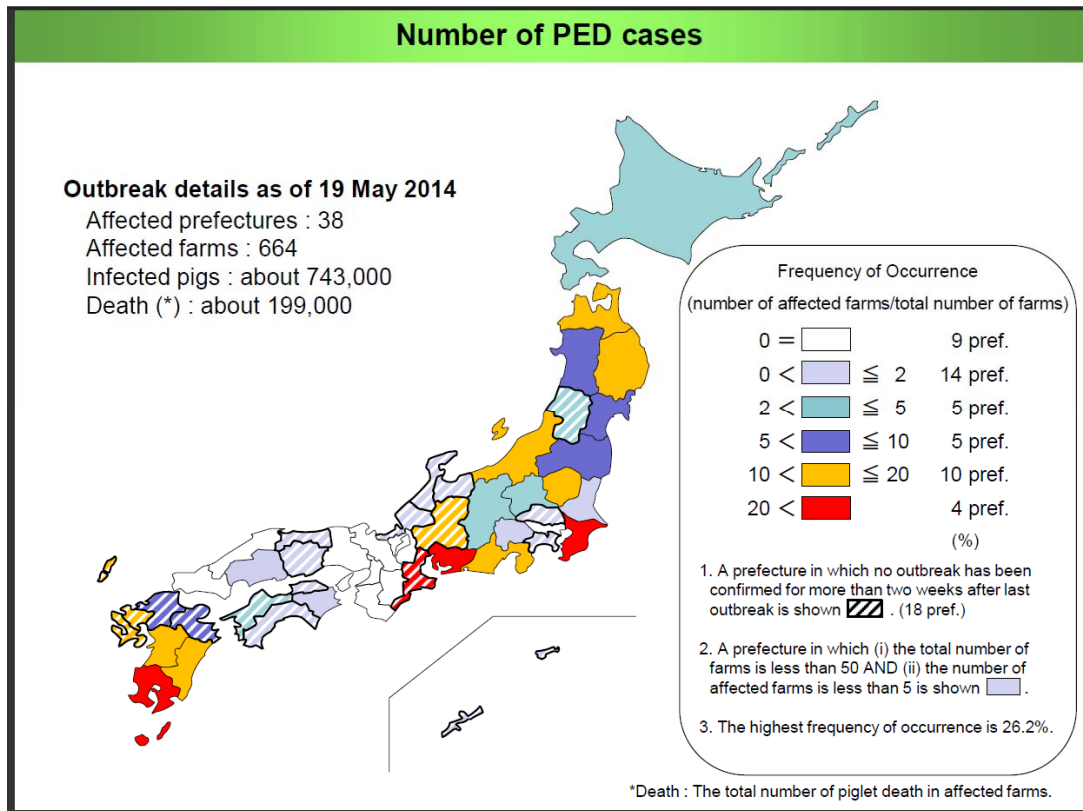


Figure 6: Geographic distribution of PED outbreaks in Japan (laboratory confirmations) on 19 May 2014.
(Source: Japanese embassy in France)

The change in weekly incidence in Japan since the beginning of the epidemic shows similarities to the situation in the United States: after a first phase, from December 2013 to January 2014, in which incidence rose moderately and then declined in the region of Kyushu/Okinawa, it increased sharply and very quickly at the beginning of March 2014, reaching an epidemic peak of nearly 100 new cases per week in mid-April. While incidence remained fairly stable in the region of Kyushu/Okinawa, which had been initially affected, the increase in overall incidence seemed to be due to the virus rapidly spreading to other regions (Tokai, Kanto and Tohoku in particular). Incidence appears to have decreased since mid-April 2014. However, it rose much faster than in the United States, resulting in a very high R0 for the spread of the epidemic (40.1 95%CI [26.2; 61.8]) using the same calculation assumptions as for the United States.

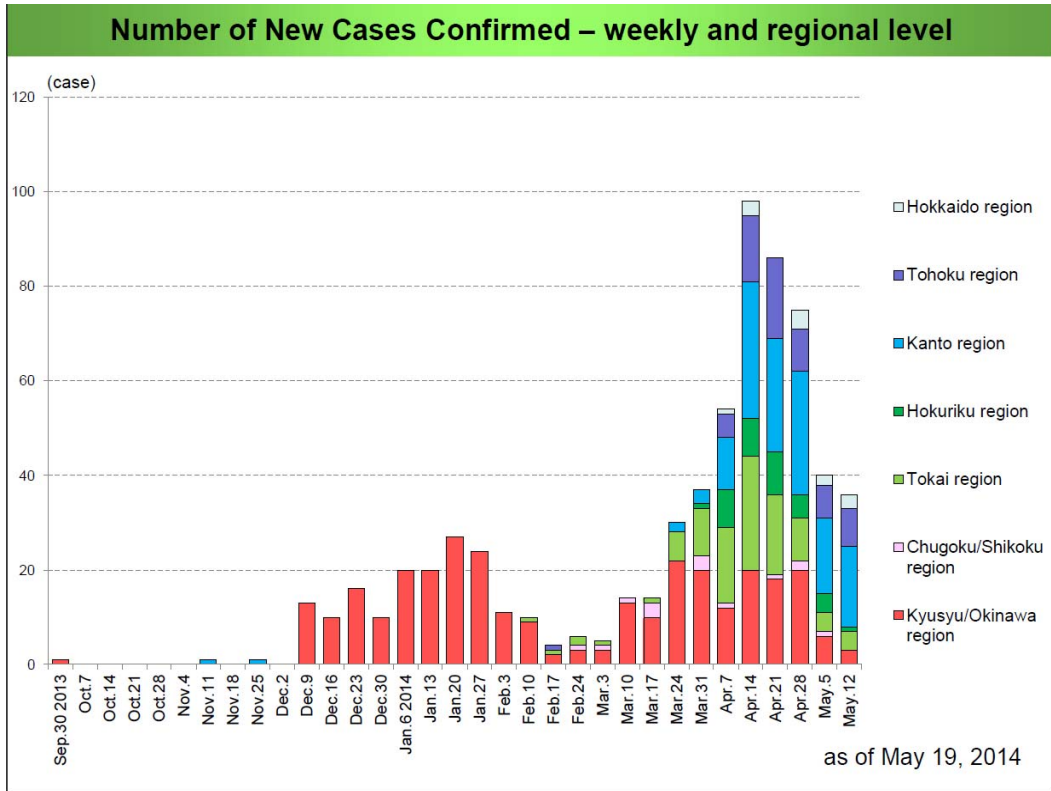


Figure 7: Weekly incidence of PED outbreaks in Japan from September 2013 to May 2014. (Source: Japanese embassy in France)

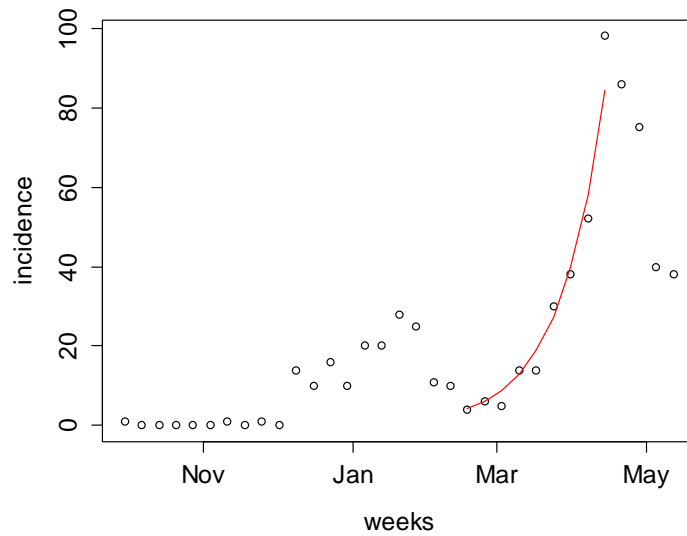


Figure 8: Modelled increase in weekly PED incidence in Japan for R0 estimation (exponential growth rate method for the epidemic, Package 'R0', R).

3.2.7 Situation in other Asian countries (UF: 4)

- South Korea

According to the latest quarterly report (January-March 2014) of the Korea Rural Economic Institute (KREI), 19% of the pig farms surveyed in South Korea have experienced a PED outbreak, reducing piglet production on these farms by nearly 25%. The KREI estimates that 5.8% of piglets were lost nationwide and forecasts a 6.4% decrease in slaughters for the June-August period due to PED.

- Southern Vietnam

In early 2009, emerging PED outbreaks were confirmed by pathology investigations and RT-PCR in most of the southern provinces of Vietnam. The investigations undertaken revealed that acute diarrhoea syndrome occurred in all age groups. The affected animals showed acute watery diarrhoea with a return to normal in adult animals only. Piglets suffered from severe watery diarrhoea and died within a few days. Morbidity in piglets reached 100% and mortality among provinces ranged from 65% to 91%. The Vietnamese isolates from these outbreaks were quite different from the European reference isolates (C777) but formed a homogeneous group with Chinese (JS-2004-2 and DX), Thai (07NP01, 08NP02 and 08CB01) and Korean (KNU-0802 and CPF299) strains identified in 2011 (Do *et al.*, 2011).

3.3 Response to question 1 on the risk of the new variant of the porcine epidemic diarrhoea virus being introduced and measures intended to reduce this risk

3.3.1 Risk of introduction

The various potential sources of PEDv have been considered using the conceptual model for the introduction and spread of PED (see Annex 5). Available knowledge has been taken into account to determine, by country, whether each identified source may contain an infectious dose of the virus (estimation of the likelihood of excretion) and also assess the likelihood of French pigs being exposed to these various sources (estimation of the likelihood of exposure).

- The likelihood of excretion depends on two factors: the possibility of the various identified sources containing the virus and the level of infection in the country of origin. While the situation in some contaminated countries (United States, Canada) is fairly well-known, epidemiological information is lacking for others: in Asia, the disease is endemic but its prevalence is unknown; in South America, the epidemic is real but poorly documented. It is thus particularly difficult, in the current state of knowledge, to take into account an objective difference between the various infected countries. Therefore, the GECU has decided to:
 - Only take into account the likelihood of PEDv being introduced from infected countries;
 - Not distinguish between these countries in terms of prevalence of the disease. Thus, the likelihood of excretion will depend only on the various sources of PEDv.
- Exposure of French pigs to PED depends on two factors:
 - The likelihood that one of these sources may be in contact with pigs.
 - The level of imports in France of the various products identified as potential sources.

Regarding imports, thanks to the TRACES tool, it is possible to obtain data on incoming flows in France through French border points or through the border points of Member States whenever France has been defined as the final destination at the time of shipping. However, it is not possible to access the flows entered by other Member States if they are not initially intended for France (free circulation of goods within the EU).

Thanks to Decision 2007/275/EC, it is possible to see which products are covered by the customs codes used to differentiate the various queries performed in TRACES. For most customs codes, there is not always a distinction between the animal species the product is derived from (e.g. blood products). For others, the animal species can be easily identified (semen and embryos, foodstuffs, live animals).

Thus, the import data remain partial in terms of both product identities and imported quantities. All of the collected data appear in Annex 6.

It should be noted that the risk assessment was undertaken at a given time, considering all available knowledge at the time of the assessment, but is likely to rapidly change, particularly regarding the list of infected countries, changes in the health regulations and knowledge of the virus.

3.3.1.1 *Live pigs (UF: 1)*

Potential sources include sick animals, subclinical carriers and animals in incubation. Regarding the risk of introduction, it is nonetheless necessary to also take into consideration the type of animal most likely to be exported by the infected country. These would more likely be animals

with subclinical infection and those in incubation, rather than sick animals which would be removed from the market.

The number of imported live animals is small (TRACES data: 23 animals in 2013 from Canada, eight from the United States and two from Canada from January to May 2014) and imports mainly involve adult animals less susceptible to PEDv infection. No live pigs entered French territory in 2013 or 2014 from other countries with PED such as Mexico, Japan, China, Dominican Republic, South Korea or Southern Vietnam (TRACES data).

Given the differences in the expression of the disease by age group, it is necessary to distinguish between the types of live animals that are likely to be imported:

- Breeders: these may be subclinical carriers (Dufresne and Robbins, 2014). However, the scope of viral shedding in such cases is not known. Since the infectious dose is extremely low, if these animals shed the virus, even at low levels, the likelihood they will then infect other animals is not zero. Given the low value of the infectious dose, this likelihood of excretion should be considered non-zero. Furthermore, with PEDv, even adults can show clinical symptoms. However, there are no data on the frequency of these situations. The experts can only indicate that there is a non-zero likelihood.
 - Piglets: these are usually animals with clinical signs, and a higher likelihood of being a source of the virus. Viral shedding in infected piglets starts 48 hrs. before the onset of symptoms. In animals that survive, shedding after symptoms disappear is not precisely known. It is considered relatively short and compatible, in terms of safety, with the 30-day quarantine period.
 - Slaughter pigs: the experts estimate the likelihood of excretion of the virus to be similar to that for breeders.
- Given this information, the experts estimate the likelihood of excretion of the virus by live pigs from a contaminated country at the following levels:
 - piglets: likelihood of 7 on an ordinal scale of 0 to 9. Indeed, even though the likelihood of shedding the virus for an infected piglet is close to 9, the fact that sick piglets are unlikely to be exported lowers this likelihood to 7;
 - breeders and slaughter pigs: likelihood of 6 on an ordinal scale of 0 to 9, to take into account less shedding of the virus than by piglets.
 - The likelihood of contact between contaminated live pigs and other pigs was assessed similarly by the experts irrespective of the age group:
 - Slaughter pigs and breeders: 9 on an ordinal scale of 0 to 9;
 - Piglets: 9 on an ordinal scale of 0 to 9.
 - Level of imports: the contact assessed above can only occur with French pigs if there are import flows of live pigs into France (directly or through another EU Member State). These flows are low but not zero.
The GECU considered that to assess the likelihood of a first case occurring in France, the scope of flows was of little significance. The experts therefore considered that the answer to this question was binary: no imports = 'no' / imports = 'yes'. In the first case, the lack of imports cancels out the likelihood of contact assessed above; in the other case, the likelihood of contact is confirmed.
Here, the answer is 'yes'.

3.3.1.2 Semen (UF: 2)

The presence of PEDv in pig semen cannot be ruled out since infection with this virus results in viraemia. Therefore, the virus circulates in the blood and can thus be found in the sex organs of

animals. However, it is not known whether there is viraemia in subclinical carriers. Moreover, contamination of samples during semen preparation cannot be ruled out.

In the United States during an outbreak of acute PED in a boar stud in 2013, it was reported that the viral genome had been detected in semen (Dufresnes, 2014). The likelihood of semen being a source of the virus is therefore not zero. If semen is contaminated, given the digestive tropism of this virus, the experts underline that transmission would more likely occur through contact with the animal than by transfer through the matrix.

Likewise, the risk of the virus being transmitted to breeders through contaminated blister packs during handling is not zero (the virus can survive for seven days on inert surfaces).

- The experts estimate the likelihood of excretion of the virus by semen at 5 on an ordinal scale of 0 to 9;
- Since the destination of pig semen is necessarily pigs themselves, the likelihood of contact between semen and pigs is estimated at 9 on an ordinal scale of 0 to 9;
- Level of imports: there are documented import flows for semen and embryos, but there is no way to differentiate between the two types of products. During the period from January 2013 to May 2014, identified pig semen and embryos were imported from Canada only (1191 in total). Unspecified animal semen and/or embryos were also sent to France from the United States (49) and Japan (3) (TRACES data). As indicated above, these data led the GECU to adopt the answer 'yes' for the imports factor.

3.3.1.3 Embryos (UF: 4)

Like for semen, viraemia can cause an embryo to become infected in a sick breeder. While this likelihood is theoretically not zero, there is no confirmed knowledge today. Indeed, although there is available information on possible semen contamination, the level of uncertainty as to the introduction of PEDv by embryos remains high.

- Lacking specific data on embryos, the experts decided to adopt the same estimate for the likelihood of excretion by semen, i.e. 5 on an ordinal scale of 0 to 9.
- Since the destination of pig embryos is necessarily pigs themselves, the likelihood of contact between embryos and pigs is estimated at 9 on an ordinal scale of 0 to 9;
- Level of imports: since there are documented import flows for semen and embryos, but there is no way to differentiate between the two types of products, the experts considered that there are imports of embryos from infected countries (answer 'yes' for the imports factor).

3.3.1.4 Humans as mechanical vectors (UF: 2)

Given the extremely low value of the infectious dose, the ability of the virus to persist in the environment and the large amounts of virus excreted by sick animals (Goyal, 2014; Jung *et al.*, 2014), humans are effective mechanical carriers (the virus is not zoonotic in nature in the current state of knowledge), either by intervening as such on farms or by interacting with products. This applies only to professional categories directly or indirectly related to the pig sector: professionals in the sector itself and service professionals working for the sector.

Their role should be assessed in contaminated zones and virus-free zones.

- In contaminated zones

The human carriers are mainly professionals who move from one farm to another: firstly veterinary practitioners and technicians, who are potentially more hazardous because they intervene on infected farms, ; and to a lesser extent, occasional workers such as maintenance personnel, who are potentially hazardous vectors since they are not farm professionals and are often less familiar with biosafety procedures.

Semen deliverers who move from one farm to another and can passively transport the virus.

Humans can be contaminated through direct contact with contaminated pigs as well as indirectly from lorries: wheels, steering wheels, seats, floor mats. Animal reception sites: farms supplied with deliveries of breeders, slaughterhouses and more hazardous than any other site, rendering plants. .

- In virus-free zones

At the beginning of the epidemic in the United States, 50% of farms were contaminated through feed and in 50% of cases by the usual routes of transmission, including humans as mechanical carriers.

Visitors are potential vectors: in endemic periods, farmers, technicians and veterinary practitioners travelling for research or even tourist purposes to previously infected farms or, a fortiori, to farms during the clinical phase, or simply to an infected area, can cause the virus to be introduced, including from long distances.

Fairs and markets are also sites of viral contamination from which humans can become contaminated and carry the virus. The biosafety conditions applied by exhibitors are generally highly inadequate (Thunes and Carpenter, 2007).

- Considering these points, the experts estimate the likelihood of the virus being excreted by humans as mechanical carriers at 4 on an ordinal scale of 0 to 9;
- Given the population targeted in this analysis (professionals from contaminated countries), the likelihood of contact between these people and pigs is estimated at 7 on an ordinal scale of 0 to 9 in the absence of restriction measures;
- Level of imports: there are currently no restrictions on movements of people between countries with regard to the PED situation. The answer is therefore 'yes' for the 'imports' level.

3.3.1.5 Farm equipment and transport vehicles (UF: 1)

For the same reasons that were given in the previous point, farm equipment and especially transport vehicles for animals or animal by-products are sources of the virus in affected countries. This equipment can also include imports of second-hand tower silos dismantled and transported by container from the United States. Such second-hand silos are currently marketed by French companies to be installed on farms producing feed on the farm (Nicolas Rose, personal communication).

In affected countries, and especially in Canada, there is extensive awareness-raising in the pig sector regarding this point³.

- In light of these points, the experts estimate the likelihood of excretion of the virus by farm equipment and transport vehicles, in a country with the epidemic, at 6 on an ordinal scale of 0 to 9;
- The likelihood of contact between this equipment and pigs is estimated at 6 on a scale of 0 to 9;
- Level of imports: there is no available quantitative information on the introduction of this equipment in France. However, since imports of second-hand farm equipment in France from the United States have been described, the GECU considers this level of imports to be non-zero: 'yes'.

3.3.1.6 Swill (UF: 2)

Swill refers to solid organic waste from meal preparation (catering, food processing industry) or leftovers. The recovery of this by-product is subject to a regulation (Ministerial Order of 28

³<http://www.lesleveursdeporcsduquebec.com/les-eleveurs-fr/dep/reenseignements-generaux.php>, consulted on 11 June 2014.

February 2008⁴) that establishes the positive list of authorised recipients which include collection centres and certain authorised end users. No farm establishments (aside from those for fur animals) appear on this positive list.

It should also be noted that through the prohibition of animal proteins in animal feed (Regulation (EC) No 999/2001⁵), the use of swill in feed intended for pigs is prohibited in France.

Depending on the end user, swill may or may not undergo certain treatments. For example, Article 17 of the Ministerial Order of 28 February 2008 stipulates that cooking and table waste intended for the feed of domestic carnivores must be subject to heat treatment respecting at least one of the following time/temperature combinations: 30 minutes at 60°C; 10 minutes at 70°C; 3 minutes at 80°C; 1 minute at 100°C. These treatments do not appear to be mandatory for other authorised end users.

According to a 2011 FAO report (FAO, 2011), swill can be contaminated. The international organisation underlines that the utmost rigour is required when swill is used in pig feed; indeed, it can include dry cured meats that have not been heat-treated.

Swill containing pig products contaminated by PEDv can therefore be a source of the virus when it is sufficiently resistant in these dry cured matrices. No data are currently available on this point. Due to the resistance of PEDv in the environment, the likelihood of its survival in such matrices should be considered non-zero.

A DGAL guidance note in force on the management of waste from third countries (flight/catering waste and waste from border inspection seizures or passenger/freight inspections) specifies that waste from international flights (leftover food that has been partly consumed and food not distributed during transport) is considered animal by-product category 1 waste. This waste must be accompanied by a support document (common format defined in Annex VIII, Chapter III of Regulation (EU) No 142/2011). All parties (operators consigning, transporting or receiving products) must keep a copy of each commercial document for a minimum period of two years. However, the non-regulatory use of this waste cannot be completely ruled out.

- Given this information and to take into account the possibility of the virus surviving curing techniques, the experts estimate the likelihood of excretion of the virus by swill, in a country with the epidemic, to be 2 to 4 on an ordinal scale of 0 to 9.
- In light of the regulations, the likelihood of contact between swill and pigs is estimated at 1 on a scale of 0 to 9;
- Level of imports: the TRACES data for swill imports provide a blank spreadsheet which suggests that the level of imports for these products is zero: 'no'.

3.3.1.7 Manure

As stated in Section 3.1, PEDv is widely excreted in faeces. Manure from a contaminated farm will therefore be infected and can transmit infection by ingestion (UF: 1).

The level of contamination cannot be accurately estimated, since it depends on the number of pigs excreting PEDv at the same time. Experimentally, contaminated manure maintained for 12 hours at 37°C infects two in four piglets by the oral route; maintained for 24 hours at 20°C, it infects one in four piglets (UF: 1) (Iowa State Univ, cited by Rose and Grasland). However, considering that the infectious dose is very low, it can be estimated that any litter or manure from a pig farm infected with PEDv will be infectious (UF: 3).

Resistance of the virus in manure:

- Liquid manure: resistance for more than 28 days at 4°C and more than 14 days at 25°C (Goyal, 2014) (UF: 1);

⁴ Ministerial Order of 28 February 2008 relating to the conditions of issuance for health accreditation and authorisation for establishments covered by Regulation (EC) No 1774/2002 of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption.

⁵ Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

- Dehydrated manure: due to the lack of data on the effect of dehydrating the virus, the resistance data for liquid manure have been taken into account (UF: 3);
- Storage data for manure: manure can be stored for "several months" (Quideau *et al.*, 2013) (UF: 1); this storage phase may have a 'cleansing' effect on the virus. However, the accumulation of manure from different dates in a pit should be considered. If two pits are available on a farm, the manure may be dated. If not, the manure from the last day is combined with that from previous days.
- In light of these points, the experts estimate the likelihood of excretion of the virus by fresh pig manure, in a country with the epidemic, at 8 on an ordinal scale of 0 to 9. Due to the lack of data on dehydrated manure, the same level of likelihood is used.
- The likelihood of contact between pigs and the virus in manure depends on the possibility of the virus being transmitted through spreading, which gives rise to aerosolisation and potential transmission to remote pig farms. Transmission of the virus by aerosolisation has been described in the United States. But the level of excretion of the virus from spreading is unknown, as is the distance that can be covered by the virus (UF: 4). Moreover, it is important to distinguish between wet manure and dehydrated manure (used in fertilisers). In light of these points, the likelihood of contact between manure and French pigs is estimated at 4 on a scale of 0 to 9.
- Level of imports: the TRACES data on the introduction of manure in France provide a blank spreadsheet which suggests that the level of imports for this product is zero. Moreover, the importation of unprocessed manure is prohibited according to Regulation (EU) No 142/2011, Chapter VIII, Article 25. The response for this factor is therefore 'no'.

3.3.1.8 Organic fertilisers (UF: 4)

Organic fertilisers of animal origin must comply with the NF U 42-001 Standard.

Several products are cited in these texts on organic fertilisers:

- Dried blood (product obtained by grinding dehydrated blood);
- Pig bristles, which can be used in fertilisers;
- NP fertilisers derived from dehydrated manure (obtained through extraction of the solid phase of manure followed by composting with or without addition of plant matter and/or drying and containing at least 40% dry matter)

The standard does not contain any processing/decontamination processes.

There are other processing procedures such as the methanation of manure (Quideau *et al.*, 2013). This involves anaerobic digestion in a digester heated to 38°C. The estimated storage time is five months. The pH of the digestate reaches 8.2 to 8.3.

- Due to the lack of data on the fertiliser processing/decontamination process, the same level of likelihood as for fresh manure is used for excretion of the virus by this source. The experts therefore estimate the likelihood of excretion of the virus by organic fertilisers, in a country with the epidemic, to be 8 on an ordinal scale of 0 to 9.
- The likelihood of contact between pigs and the virus from fertiliser (containing dehydrated manure) is lower than with fresh manure (no or little aerosolisation). This likelihood is estimated at 1-2 on a scale of 0 to 9.
- Level of imports: no information is available regarding the level of imports of organic fertilisers to be able to provide a 'yes' or 'no' answer. The possibility of importing fertiliser containing pig manure therefore cannot be ruled out ('yes').

3.3.1.9 Pig products

Pig products can have very different outlets, in human food, animal feed and industry. Table 3 summarises these various outlets (UF: 1).

Table 3: Description of meats and pig products intended for human consumption and animal feed (France Agrimer, 2013a; France Agrimer, 2013b; SIFCO, 2011)

Product type	Use	Outlets
Pork		Human food
Fat and Lard	Product casing	Meat industry (e.g. roast beef); human food
	Rendering	Soaps, candles, human food (insignificant), lipochemistry, oleochemistry, petfood, energy, biofuels
Offal		Human food
Pancreas	Insulin Pancreatin Fermentation enzymes	Pharmaceuticals
Intestinal mucosa		Pharmaceuticals Biogas
Rest of digestive tract, Reproductive system, Lung, Trachea		Petfood
Content of digestive tract, waste		Biogas production and composting
Cartilage		Pharmaceuticals, cosmetics, biochemical industry Petfood
Bones	Gelatine	Human food Pharmaceuticals, cosmetics Photo industry, textile production, glue, paper
	Bone meal	Petfood, fertiliser
Skin	Leather	Tanning
	Glue	Industry
	Gelatine	Human food Pharmaceuticals, cosmetics Photo industry, textile production, glue, paper
Gelatine by-product	Dicalcium phosphate	Animal feed, fertiliser
Bristles (1kg/pig)	AA extraction	Pharmaceuticals
	Brushes	Industry
	Pig bristle meal (no keratin)	Animal feed, petfood Fertiliser
Blood (4 kg/pig)	Whole blood (primary recovery)	Human food
	Whole blood meal	Petfood, animal feed for other species (fish)
	Plasma	Delicatessen meats, cured meats Petfood, animal feed for other species
	Dried red blood cells (cruor)	Red meat pigmentation (human food) Petfood, animal feed for other species Fertiliser
Slaughterhouse scraps	Processed animal protein (PAP)	Petfood, animal feed for other species (fish)

A) PIG BY-PRODUCTS FOR ANIMAL FEED

A *European regulatory framework*

Since the BSE crisis, there has been strict legislation on the use of animal proteins and animal products such as plasma in animal feed. Likewise, the European regulations regulate the treatment conditions for these various products intended for animal feed.

- **Raw materials of animal origin authorised in pig feed (UF: 1)**

Regulation (EC) No 999/2001⁶ limits and regulates the use of animal proteins in animal feed. The general principle of prohibiting animal proteins in the feed of ruminants is extended to other species, with some exceptions appearing in Annex IV, Chapter II of this regulation. Thus, the following are authorised in pig feed in Europe:

- blood products derived from non-ruminants (e.g. plasma, red blood cells);
- hydrolysed proteins;
- fats derived from non-ruminants;
- non-ruminant gelatines/collagen.

All of these four categories of by-products have therefore been taken into account in the request.

- **Treatment of raw materials of animal origin intended for pig feed**

Regulation (EC) No 142/2011⁷ regulates the production of products such as processed animal proteins, hydrolysed proteins, blood products, fats, gelatines and collagen authorised for animal feed (Annex 3 of this opinion).

Blood products must have been subject to:

- One of the processing methods 1 to 5 or processing method 7 described in Annex 4 of this opinion or;
- Another method guaranteeing the compliance of the blood product with the microbiological standards applicable to by-products, regarding two indicators: *Salmonella* and Enterobacteriaceae (Annex 3 of this report).

Regarding the established processing methods, methods 1 to 5 specify the technological parameters used in treatments. But this is not the case for method 7, which groups together various treatments that can be validated by the competent authority, when a performance obligation is met for three microbiological indicators: *Salmonella*, Enterobacteriaceae and *Clostridium perfringens*. The established microbiological standards are considered as corresponding to sterilisation processes (Anne Leboucher, national reference for animal by-products at the Ministry of Agriculture).

Regarding 'another method', it should be noted that the applicable microbiological standards are less stringent than for method 7, since they only cover *Salmonella* and Enterobacteriaceae, with no objective for *Clostridium perfringens*.

This prompts the question of whether the various possible treatments for blood products can guarantee the inactivation of PEDv.

While it is possible to interview French plasma manufacturers to determine the type of treatment applied in their establishments, it is more difficult to obtain such information from a Member State or third country.

⁶ Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

⁷ Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive.

When an importer indicates that these blood products comply with the European regulations, this means that they can be treated using one of these various methods. Only the performance obligation for *Salmonella* and Enterobacteriaceae is visible on import. It should be noted that if there are no bacteria in the product originally, the microbiological standards can be reached without treatment.

A new European regulation dating from 8 May 2014⁸ amends these provisions by requiring, for the blood and plasma of porcine animals, "*heat treatment at a temperature of at least 80°C throughout the substance and the dry blood and blood plasma is of not more than 8% moisture with a water activity (Aw) of less than 0.60*" and states that products must be "*stored in dry warehouse conditions under room temperature for at least 6 weeks*". The experts note that this regulation does not indicate any times of exposure to the temperature of 80°C. In light of the scientific knowledge currently available on the resistance of the virus (inactivated after heating at 71°C for ten minutes), the proposed treatment should have been validated as to its ability to inactivate PEDv. This validation (e.g. piglet testing) does not appear in the recitals of the Regulation.

Further to this analysis of the various treatments of raw materials of animal origin, the GECU concludes that if pig products have undergone one of the processing methods 1 to 5, the product can be considered cleaned of the PEDv virus.

However, the experts cannot form an opinion of method 7 or 'another method' and consider that the likelihood of excretion of the virus by these treated pig products cannot be zero.

Lacking more information on the treatment conditions for pig blood and plasma presented in the European regulation of 8 May 2014, and in light of the scientific knowledge currently available, the experts consider that the likelihood of the virus not being inactivated is not zero.

The very low infectious dose that characterises the virus demonstrates the significant risk of cross contamination: products or their packaging can indeed be contaminated when handled by personnel. In a plant processing blood products for example, the treatments applied to the products may be safe, but there is non-zero risk that the personnel receiving and handling the raw materials may be in contact with the finished product, with the ability to re-contaminate it if drastic measures to separate the production and packaging phases are not applied.

This risk of cross contamination should be taken into consideration with a great deal of vigilance in the various processing stages.

Lastly, the geographic origin of raw materials intended for the production of pig products and their traceability are also significant factors that can influence the safety of finished products. For example, products obtained by combining products of various origins pose a higher health risk than single-origin products from a virus-free country.

Case of plasma (UF: 2)

Benefits and use of blood plasma in pig feed

Blood plasma does not appear to be widely used by feed manufacturers located in France. Several reasons can be put forth to explain this situation. First of all, the fact that production

⁸ Regulation (EU) No 483/2014 of 8 May 2014 on protection measures in relation to porcine diarrhoea caused by a deltacoronavirus as regards the animal health requirements for the introduction into the Union of spray dried blood and blood plasma of porcine origin intended for the production of feed for farmed porcine animals.

sectors have specifications that exclude raw materials of animal origin significantly reduces the introduction of this type of raw material into animal-feed plants to prevent potential cross contamination.

However, some manufacturers of feed for young piglets use six to ten percent pig plasma in their feed formulas. This raw material is not essential for the formulation of feed but is positioned as providing very high-quality proteins and claims to have an immuno-modulating effect for piglets.

The tonnage represented by this feed is therefore limited. All of this feed is characterised by high energy and protein levels in order to meet the particularly high nutritional requirements of piglets at this stage in which feed intake is limited.

However, as indicated above, the benefits of blood plasma can be considered other than from this strict nutritional standpoint. Indeed, the literature mentions a positive effect of blood plasma on the palatability of feed, resulting in an increased growth rate since feed conversion is slightly higher. Nevertheless, this beneficial effect is limited to the first week post-weaning for plasma levels of up to 6% (van Dijk *et al.*, 2001). The difference in performance between feed containing plasma and control feed depends on the latter's characteristics; having feed with adequate nutrients and no unpalatability factors reduces the appeal of plasma. To explain the growth-promoting effect of plasma, Oswald and Gallois (Oswald and Gallois, 2009) advance the assumption of an improvement in animal health. In the event of infectious challenge, plasma prevents delayed growth and the related clinical signs, especially if the plasma comes from animals that have been vaccinated against the pathogenic *E. coli* strain used. But the positive effect persists when this is not the case, which suggests the role played by certain plasma constituents; regarding this point, Oswald and Gallois mention the glycan fraction of plasma glycoproteins which could compete with the adherence of pathogenic bacteria to the intestinal wall.

Health risk related to plasma

In February 2014, the Canadian Food Inspection Agency (CFIA) announced that PEDv RNA had been detected in plasma samples from the United States that were used to produce feed for young piglets and sows. RT-PCR results showed Ct values ranging from 30 to 33 or the equivalent of 10^2 - 10^3 infectious viral particles (Nitikanjana, 2014). The batches of feed in question were withdrawn from the market. The Canadian agency then advanced the assumption that feed could contribute to carrying the virus. A bioassay was then undertaken at the University of Minnesota in which piglets were inoculated with plasma positive for PEDv. The animals were infected. However, when the granules used in feed (approximately 5 to 10% plasma in the feed (Gallois *et al.*, 2009)) were distributed to the piglets, no animals were infected with PEDv.

After the PEDv-positive batches of piglet and sow feed were recalled, 190 bags of plasma were sampled and divided into groups of ten to be tested by RT-PCR. The 19 batches were positive for PEDv RNA. The investigations then undertaken in Ontario, Canada on the first farms affected by PED showed that 18 of these first 20 farms had been supplied by the same feed plant, as was the farm infected with PED located on Prince Edward Island, which is extremely isolated

(<http://www.farmscape.com/f2ShowScript.aspx?i=24577&q=Contaminated+Feed+Most+Likely+Source+of+Ontario+PED+Outbreak>).

Lastly, the possibility of PEDv being transmitted by feed was demonstrated by an experimental trial carried out by Scott Dee in 2014

(http://www.cvm.umn.edu/sdec/prod/groups/cvm/@pub/@cvm/@sdec/documents/content/cvm_content_474046.pdf). On farms with sows showing clinical signs of PED within 24 to 48 hours of feed delivery, samples were taken in the feed bins using swabs and rollers. Viral RNA was found in these samples, showing that feed could be contaminated by the virus. The PEDv-positive feed in the bins was mixed with virus-free feed and used in open access to feed piglets

in a contained facility. The piglets developed clinical signs of diarrhoea and vomiting four days after ingesting the feed.

This work establishes that plasma could be a risk factor for the introduction of the PED virus on virus-free farms.

As indicated above, it is also important to note the risk of cross contamination that would cause plasma, once treated, to become re-contaminated due to a lack of separation between the various production and packaging stages.

Lastly, the plasma used in animal feed is obtained from a mixture of raw materials derived from pigs of different origins, decreasing the traceability of the raw materials contained in the plasma. This increases the likelihood of excretion of the virus compared to a product with only one known origin.

- In light of these points, the experts estimate the likelihood of excretion of the virus by treated pig plasma, in a country with the epidemic, at 7 on an ordinal scale of 0 to 9.
- Plasma is used in France in the production of feed for piglets, which have the highest susceptibility to PED. Furthermore, blood products have high potential as raw materials of being used in the context of digestive disorders in piglets in order to reduce the use of antibiotics. They therefore represent an opportunity. The GECU estimates the likelihood of contact between blood products and pigs at 7 on a scale of 0 to 9 to take into account both the low level of current use and the potential for use.
- Level of imports: the TRACES data regarding the introduction of blood products in France show that the level of imports is non-zero but there is no way to differentiate between the species from which these blood products are derived. According to some companies, no blood products are imported from contaminated countries (UF: 3). Given the uncertainty related to these data, it is not possible to guarantee the lack of imports. The response for this factor is therefore 'yes'.

Case of red blood cells (UF: 3)

The use of red blood cells in pig feed appears fairly low, but its benefits as a source of highly digestible proteins makes it possible, particularly for piglets, which are the most susceptible to PED.

However, the high imbalance in certain amino acids (leucine) makes its use unlikely due to the cost of mandatory supplementation with synthetic amino acids (Alain Coupel, personal communication).

The treatment of these raw materials must adhere to the same regulation as for blood plasma. During the risk assessment, the experts considered that blood products encompassed both plasma and red blood cells.

Case of hydrolysed proteins

Hydrolysed proteins are defined as polypeptides, peptides and amino acids as well as their mixtures, obtained by hydrolysis of animal by-products. The treatment of pig products to obtain hydrolysed proteins involves an alkaline hydrolysis/temperature combination (Annex 3) used to consider that a product is safe in terms of PEDv risk (UF: 1).

However, no information is available as to the use of hydrolysed proteins in pig feed. This possibility was not ruled out in the stakeholder hearings, insofar as hydrolysed proteins can be a beneficial source of proteins and even amino acids, particularly for piglets. While the level of use is not known, these products are confirmed to be on the market (UF: 3).

- In light of the information regarding the treatment of hydrolysed proteins, the experts estimate the likelihood of excretion of the virus by hydrolysed proteins, in a country with the epidemic, at 1 on an ordinal scale of 0 to 9.
- Likelihood of contact with pigs: considering that use is similar to that of blood products in nutritional terms (piglet feed), the expert group also estimates that the likelihood of contact with pigs is 7 on an ordinal scale of 0 to 9.
- The level of imports of these products: the expert group was unable to obtain data. The possibility of importing hydrolysed proteins into France therefore cannot be ruled out ('yes').

There is a memo by the animal nutrition unions dating from 2 May 2014 who, "*pending the ANSES Opinion, recommend suspending supplies to feed plants of porcine hydrolysed proteins and blood products intended for pig feed (all origins)*". Since this recommendation has no regulatory value, the GECU decided not to take it into account in the risk assessment.

Case of fats derived from non-ruminants

While the processing standards for these animal by-products are similar to those for blood products (see Annex 3), with uncertainties for some of them, it should nonetheless be noted that PEDv, even in the viraemic stage, has essentially digestive tropism. Therefore, it is very unlikely that it is found in fats. Only cross contamination from other products is possible.

Moreover, the use of animal fats in animal feed is quite uncommon, since there are specifications mentioned above in the case of plasma.

- In light of these points, the experts estimate the likelihood of excretion of the virus by animal fats, in a country with the epidemic, at 3 on an ordinal scale of 0 to 9.
- Animal fats are used very seldom in pig feed, for the same reasons as for other products of animal origin. These raw materials, which essentially supply energy, tend to be used more in finishing. Thus, the likelihood of contact between animal fats and pigs is estimated at 2 on a scale of 0 to 9.
- The data on imports of animal fats indicate that there are flows with an imports factor: 'yes'.

Case of non-ruminant gelatine (UF: 3)

The treatment of by-products to obtain gelatine involves hydrolysis at pH 3 for three hours and heat treatment (60°C). This process is *a priori* inactivating for PEDv, but other treatments are possible according to the regulations (see Annex 3), also involving an acid or alkaline treatment followed by several heating operations (unspecified temperature). The lack of details on the parameters of these other treatments makes it difficult to conclude with certainty as to their inactivating nature.

The risk of cross contamination should not be neglected: gelatine can become re-contaminated after treatment if drastic measures to separate the production and packaging phases are not applied.

Qualitatively, gelatine is one of the ingredients that can be used in the production of certain additives for animal feed (coating). However, the quantities of these products used in pig feed are unknown.

- Given this information regarding the treatment of products to obtain gelatine and the risk of cross contamination which cannot be neglected, the experts consider the likelihood of excretion of the virus by gelatine derived from contaminated pigs, in a country with the epidemic, at 2-3 on an ordinal scale of 0 to 9.
- Likelihood of contact with pigs: the expert group was unable to obtain information regarding the level of gelatine found in pig feed. However, its use as a carrier of additives intended for animal feed makes it highly likely that this gelatine comes into contact with pigs through feed. Due to the lack of specific data on this point, the highest level of likelihood was adopted by the GECU: 9 on an ordinal scale of 0 to 9.
- The level of imports of these products is not documented either. As stated above however, it is recognised that additives for animal feed can contain gelatine as a carrier. Gelatine is therefore imported this way. The response for this factor is therefore 'yes'.

Case of non-ruminant collagen (UF: 3)

The collagen extraction process involves washing, pH adaptation using an acid or alkali, then one or more rinsing stages, filtration and extrusion. The extrusion stage can be omitted in the production of low molecular weight collagen from raw materials not derived from ruminants. Heating therefore may not take place. The information is inadequate to conclude with certainty as to the inactivating nature of these other treatments.

The use of collagen is not documented, but its nutritional benefits in piglet feed are considered low.

- In light of this information regarding the treatment for obtaining collagen, the experts estimate the likelihood of excretion of the virus by collagen derived from contaminated pigs, in a country with the epidemic, at 3 on an ordinal scale of 0 to 9.
- Likelihood of contact with pigs: the expert group did not have information regarding the level of collagen found in pig feed, but given its low nutritional benefits, the likelihood of contact with pigs was estimated at 1.
- Level of imports of these products: the expert group was unable to obtain data. The possibility of importing collagen into France therefore cannot be ruled out ('yes').

B MEATS AND PORK PRODUCTS FOR HUMAN FOOD

PEDv can be found in pig blood and can therefore potentially be found in all pig tissues and products including muscle, when they come from pigs in the viraemic phase.

The vast majority of meats and other pork products (delicatessen meats and cured meats) are derived from animals at the end of fattening. Viraemia cannot be ruled out in these animals, but the likelihood is lower than for piglets.

Amplification of the virus is zero in humans: indeed, the virus is not zoonotic in the current state of knowledge. Humans are therefore not vectors of contamination through consumption of these products.

Only direct contact between a pig product and pigs could be considered (in addition to the human mechanical transport role mentioned above). During the swine fever epidemic in the United Kingdom in 2000, the explanation given by the Ministry of Agriculture for the index case was the consumption of a discarded sandwich by outdoor sows (<http://www.pighealth.com/diseases/csfsources.htm>).

- In light of these points, the experts estimate the likelihood of excretion of the virus by meats and other pork products, in a country with the epidemic, at 4 on an ordinal scale of 0 to 9.
- The likelihood of contact between pork, delicatessen meat or cured meat and pigs is estimated at 1 on a scale of 0 to 9.
- Data on imports of meats and other pig products show that it is possible to import fresh pork and pork products from the United States under certain conditions (EU requirements for growth promoters / USDA Quality System Assessment Program) (the response for this factor is therefore: 'yes').

3.3.1.10 *Summary table of likelihoods*

The following tables (Tables 4 and 5) summarise the estimated likelihoods of excretion by the various sources of the virus as well as the exposure of French pigs to these sources.

Table 4: Estimated likelihood of the excretion of PEDv by the various sources from countries with recognised infection

Source	Likelihood of excretion by the source	
Live pigs	Piglets 7	Slaughter pigs and breeders 6
Semen	5	
Embryos	5	
Humans	4	
Equipment, vehicles	6	
Swill	2-4	
Manure	8	
Organic fertilisers	8	
Blood products	7	
Hydrolysed proteins	1	
Animal fats	3	
Gelatine	2-3	
Collagen	3	
Pork	4	

Table 5: Estimated likelihood of exposure to PEDv in French pigs through the various sources

Source	Likelihood of contact			Imports	Likelihood of exposure		
	Piglets 9	Slaughter pigs 9	Breeders 9		Piglets 9	Slaughter pigs 9	Breeders 9
Live pigs				yes			
Semen	9			yes	9		
Embryos	9			yes	9		
Humans	7			yes	7		
Equipment, vehicles	6			yes	6		
Swill	1			no	0		
Manure	4			no	0		
Organic fertilisers	1-2			yes**	1-2		
Blood products (plasma, red blood cells)	7			yes	7		
Hydrolysed proteins	7			yes**	7		
Animal fats	2			yes	2		
Gelatine	9*			yes	9		
collagen	1			yes**	1		
Pork	1			yes	1		

*Due to a lack of data, the highest likelihood was adopted: 9

**Due to a lack of data, the possibility of imports cannot be ruled out: yes

3.3.1.11 Conclusions on the likelihood of the porcine epidemic virus being introduced

Considering these estimates, the likelihood of PEDv being introduced is obtained by combining the likelihood of excretion and that of exposure, using the combination tables in the AFSSA method, 2008.

Table 6 shows the assessment of this likelihood for the various identified sources.

Table 7 ranks the likelihoods of introduction from lowest to highest, making it possible to identify the most at-risk sources. However, it should be noted that these likelihoods do not have the same levels of uncertainty for all sources of the virus.

Table 6: Assessment of the likelihood of the PED virus being introduced by source

Source	Likelihood of excretion		Likelihood of exposure		Likelihood of introduction	
	Piglets 7	Slaughter pigs and breeders 6	Piglets 9	Slaughter pigs and breeders 9	Piglets 7	Slaughter pigs and breeders 6
Live pigs						
Semen	5		9		5	
Embryos	5		9		5	
Humans	4		7		3	
Equipment, vehicles	6		6		5	
Swill	2-4		0		0	
Manure	8		0		0	
Organic fertilisers	8		1-2		1-2	
Blood products (plasma, red blood cells)	7		7		6	
Hydrolysed proteins	1		7		1	
Animal fats	3		2		1	
Gelatine	2-3		9		2-3	
Collagen	3		1		1	
Pork	4		1		1	

Table 7: Ranking of the various sources by likelihood of introducing the PED virus

Source	likelihood of introduction
Swill	0
Manure	0
Hydrolysed proteins	1
Animal fats	1
Pork	1
Collagen	1
Organic fertilisers	1-2
Gelatine	2-3
Humans	3
Equipment, vehicles	5
Semen	5
Embryos	5
Blood products (plasma, red blood cells)	6
Live pigs	6-7

Given the scientific knowledge currently available and in particular:

- the extremely low minimum infectious dose for this PEDv variant;
- its resistance in the environment;
- the quantity of the virus excreted by sick animals,

the GECU considers that there is a confirmed risk of PED being introduced in France from an infected country.

The collective expert assessment undertaken resulted in a characterisation of the various sources of the virus, ranked by the likelihood of introduction. This ranges from 0 to 7 on an ordinal scale of 0 to 9.

However, given the characteristics of the virus presented above, the experts point out that the risk of cross contamination by the virus from pig products is omnipresent. The utmost vigilance is therefore necessary when it comes to the origin of all inputs on pig farms.

3.3.2 Measures intended to reduce the risk of introduction

- What would be the minimum production conditions for raw materials derived from pigs (blood products, hydrolysed proteins, fats, gelatines) to guarantee control of the risk for use of these materials in animal feed?

In the current state of knowledge, the virus appears inactivated when it is heat treated at a minimum temperature of 71°C for ten minutes (see 3.1.5). Other time-temperature combinations are not currently documented in terms of their ability to inactivate the virus. Therefore, all other treatments need to be assessed.

Storing products in a dry environment for at least seven days at a temperature of 20°C also appears to inactivate the virus. However, inactivation of the virus during storage heavily depends on the temperature and humidity level. It is therefore difficult to conclude as to the effect of product storage on the inactivation of the virus, lacking precise hygrometry conditions and ambient temperature control.

The GECU notes that the minimum infectious dose is particularly low for PEDv. The risk of cross contamination is therefore high. Raw materials can thus be re-contaminated during packaging if all precautions are not taken to separate the various phases of product production. It is therefore necessary to comply with hygiene measures before and after heat treatment ('forward flow' measures, separation of clean and dirty areas, different personnel in the areas).

Moreover, inoculation of the 10⁻⁹ dilution of a clarified homogenate of intestinal mucosa, sampled from a piglet infected with PEDv, non-detectable by real-time RT-PCT, causes piglets to be infected (see 3.1.3). Thus, in the current state of knowledge, the assessment of product safety in relation to PED cannot be completely guaranteed by RT-PCR analysis.

Considering the most at-risk sources identified in the previous section, the GECU recommends the following for plasma and other porcine blood products:

- **Heat treating the products at a temperature of over 71°C for at least ten minutes; all other time-temperature combinations need to be validated based on relevant experimental tests or new bibliographic data;**
 - **Storing the heat treated products in a dry environment for at least seven days at a temperature of 20°C; this storage is not an alternative to heat treatment. Temperature and hygrometry parameters must be monitored;**
 - **Complying with hygiene measures in order to keep the treated products from becoming re-contaminated by contaminated products through cross contamination.**
- The Order relating to the precautionary measures adopted in France against PED lists certain raw materials that are prohibited from being imported into France by country of origin. Are there any products to be removed/added?

For reasons of time, the GECU did not make any decisions about this list of raw materials that are prohibited from being imported into France. The ranking of the various sources based on the risk of PEDv introduction provides some answers to this question regarding precautionary measures adopted in France (see 3.3.1).

3.4 Response to question 2 on the risk of the porcine epidemic diarrhoea virus spreading and measures intended to limit this risk

The various questions regarding the risk of PED spreading and the corresponding control measures have been grouped together and reorganised under the following three points:

1. Foreseeable impact for the sector if the virus is introduced in France (France has temporarily added it to the list of category-one diseases to make reporting mandatory) → 3.4.1
2. If the risk of introduction is not negligible, what would be the advantages/disadvantages of simple confinement versus preventive culling? If a first outbreak were to occur, could it be contained through simple confinement following the first reported cases? Would a preventive total cull with each report limit the consequences of PED being introduced into France? What control measures identified thus far could be implemented in order to limit the risk of spreading? → 3.4.2
3. Can the vaccines that may be available be expected to provide protection? → 3.4.3

3.4.1 Foreseeable impact for the sector if the porcine epidemic diarrhoea virus is introduced in France

The impact on the sector was analysed from two angles:

- The epidemiological angle through a PEDv between-herd spread model;
- The economic angle, for which a provisional estimate was made.

3.4.1.1 Model for the spread of the porcine epidemic diarrhoea virus after introduction in France

➤ Interface used

The modelling tool used is the North American Animal Disease Spread Model version 4.0.13 (Harvey *et al.*, 2007) which is used to represent the spread of an animal epidemic in one or more animal populations that interact through time and space. This open-access tool has been used on several occasions, including to model the spread of Aujeszky's disease and assess strategies for eradicating it (Ketusing *et al.*, 2014) and to represent a 'One Health' model for the transmission of the influenza virus between animal and human populations (Dorjee *et al.*, 2014). The tool was developed and is kept updated by the University of Guelph in Canada and University of Colorado in the United States.

➤ General principles of the model and main characteristics

The model is an agent-based, discrete-time (time-step = one day), stochastic model. It is based on a spatial representation of epidemiological units (geolocation) and used to represent various types of farms and possibly various animal sectors if the disease affects various species. Herd size can be taken into account especially if the transmission process requires that a likelihood of transmission be represented based on within-herd prevalence (not used for PED).

The infectious model relies on a compartmental model used to represent epidemiological units by status:

- susceptible: susceptible to infection (**S**);
- latent: infected without transmission of infection (**E**);
- subclinically infectious: possible transmission of infection during an asymptomatic phase (**I1**);
- clinically infectious: transmission of infection during a clinical phase (**I2**);

- naturally immune: acquisition of natural herd immunity, can no longer transmit infection and can no longer be infected (**R**);
- mortality due to the disease: herd eliminated due to mortality affecting all the animals (**M**);
- herd destroyed: in the framework of a control measure to cull the whole herd;
- vaccinated: acquisition of immunity related to vaccination through the establishment of a control measure.

Only states **S**, **E**, **I1**, **I2**, **R** and **M** are used for the PED model. The likelihood of mortality depends on the type of farm. The conceptual compartmental model used is represented in Figure 9.

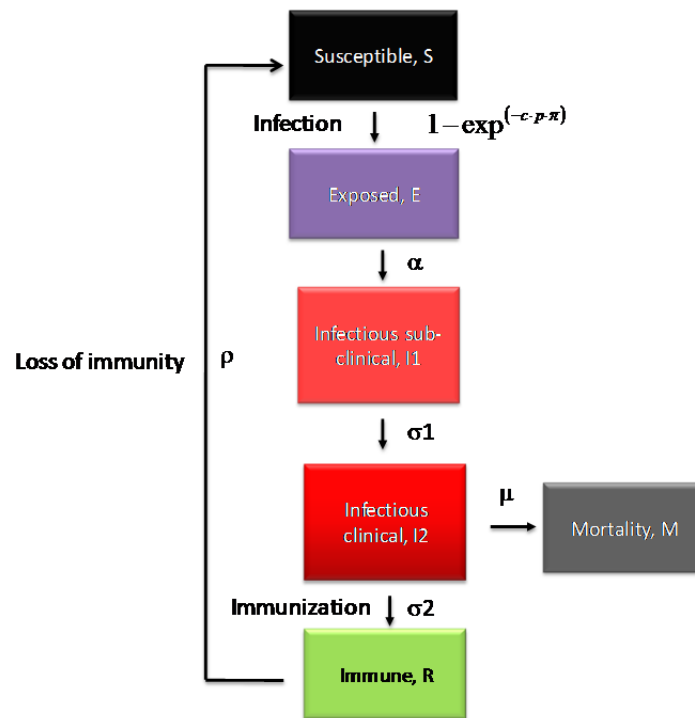


Figure 9: conceptual compartmental model used for modelling the spread of PED virus.

Parameters: c : daily contact rate, p : probability of virus transmission in case of contact, π : proportion of infectious units $1/\alpha$: length of the latent period, $1/\sigma_1$: length of the subclinically infectious period, $1/\sigma_2$: length of the clinically infectious period, μ : probability of mortality, $1/\rho$: immune period.

Various transmission processes can be represented and taken into account in the infection dynamic:

- **Transmission by direct contacts.** This transmission process represents a transfer of the infectious agent from one herd to another through animal movements. It is governed by a probabilistic process depending on the frequency of contacts and a probability of the agent being transferred in case of contact. The network of contacts between herds is not explicitly represented. The likelihood of a farm being infected for each time-step is governed by a process based on the Poisson distribution. In short, the likelihood of escaping infection for a farm is assumed to follow a Poisson distribution with mean $c.p.\pi$ where c is the daily contact rate, p the probability of the virus being transmitted in case of contact and π the proportion of infectious units. The likelihood of infection for each time-step is therefore $1 - \exp^{-c.p.\pi}$.
- **Transmission by indirect contacts.** This transmission process represents a transfer of the infectious agent from one herd to another through so-called indirect contacts, i.e. mechanical carriers of the infectious agent excluding animal movements. It is also

governed by a probabilistic process depending on the frequency of indirect contacts and a probability of the agent being transferred in case of contact.

- **Transmission by proximity.** This transmission process represents the spread of the infectious agent by proximity (contaminated aerosols) to very nearby herds by defining an at-risk radius around the infected farm.
- **Airborne transmission.** It is possible to represent the probability of the infectious agent being spread from an infected herd based on the prevailing winds, within a determined maximum distance.

To model PED spread, only the direct and indirect transmission processes were used.

➤ Data used for model parameterisation

➤ **Characteristics of the disease at herd level**

The data on the latent period, subclinically infectious period, clinically infectious period and immune period at herd level come from a study of the literature (Martelli *et al.*, 2008; Pensaert and De Bouck, 1978) and reports published in the United States (<http://www.aasv.org/aasv%20website/Resources/Diseases/PorcineEpidemicDiarrhea.php>) and Canada (Poulin and Klopfenstein, 2013).

➤ **database on farm characteristics and animal movements**

The data used for the geolocation of farms, their type, unit sizes and animal movements come from a BDPORC extraction for 2010 and for Brittany. The database describes all movements for each IDM (identification mark) and the type of movement in question: 8kg piglets, 25kg piglets, slaughter pigs, breeder pigs. This database is used to create the population initialisation file for representing farms in space and their characteristics (production type, unit size, geographic coordinates, status at the beginning of the simulation) (Figure 10).

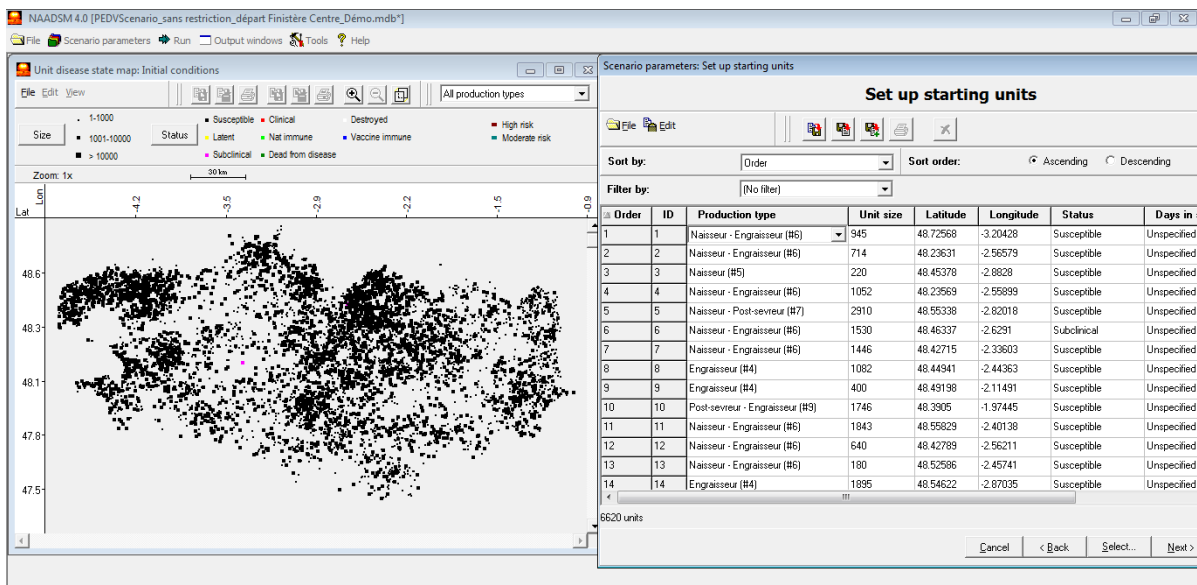


Figure 10: Screen-shot illustrating the geographic representation of geolocated farms and the initial database (production type, unit sizes, geographic coordinates and status at the beginning of the simulation).

➤ **Direct contacts**

For each farm type i , the average daily frequency of 'loading' movements (pigs leaving the farm) is calculated from average annual movements of this type for each animal category j (8kg piglets,

25kg piglets, breeder pigs). Let F_{ij} be this frequency. To take into account the proportion of farms with this type of movement in the population j , this frequency is weighted by this proportion φ_j . The weighted average daily rate of direct contacts to which a farm receiving category j animals is exposed is therefore:

$$C_i = \sum_j F_{ij} \cdot \varphi_j$$

The probability of transmission in case of direct contacts (p) is set at 0.95 irrespective of the category of source farm.

➤ **Indirect contacts**

In order to configure indirect contacts (spread by mechanical carriers), the data from a prior study undertaken in the framework of a European project on densely and sparsely populated animal production areas were used (Rose and Madec, 2002). These data quantitatively describe the features of farms by type (farrow-to-finish, farrow-to-grower, finishing) from a sample of 140 farms. Contacts with animal transport vehicles, feed vehicles, technician and veterinarian vehicles, dealer vehicles, manure vehicles, rendering vehicles and visitors (friends, neighbours) are quantified (annual frequency/farm). An annual median frequency is calculated for each type of vehicle and by farm type as well as a total number of contacts by farm (frequency of all visits combined/year/farm). A daily contact rate is calculated for each type of farm as described in the study (farrow-to-finish, finishing and farrow-to-grower). It is assumed that this rate results from exposure to indirect contacts from 'source' farms according to their respective proportion in the population. Let F'_{ij} be this daily frequency of indirect contacts for the category i farm; and η_j the proportion of category j 'source' farms in the population. Then the daily rate of indirect contacts for the category i farm related to category j source farms is:

$$CI_i = \sum_j F'_{ij} \cdot \eta_j$$

The probability of transmission in case of indirect contacts (p) is set at 0.15 irrespective of the category of source farm, except when the source farms are finishing farms, when it is 0.10; it is assumed to be lower since these farms do not have any young animals under the age of nine weeks.

A detailed description of the various parameters can be found in Annex 7.

➤ Discussion of the model and conclusions

Based on a farm situation in Brittany with a high density, the model results in the establishment of a **scenario in which the disease spreads rapidly, in the absence of control and management measures**, as shown in Figure 11.

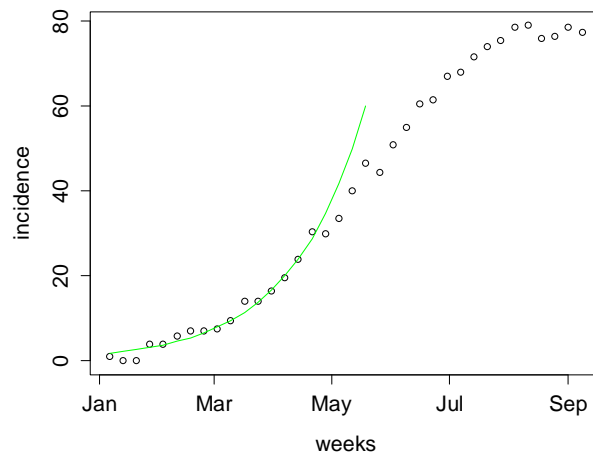


Figure 11: Brittany: simulated mean incidence of PED predicted by the model

Four initial locations were assessed that correspond to densely populated pig areas (Côtes d’Armor and Finistère Nord) as opposed to more sparsely populated areas (Finistère Centre, Ile et Vilaine). The impact of the location of the index case was highly limited in the simulations (Table 8). Over 3000 farms were infected on average during the simulation period; only 5 to 16% of the simulations undertaken resulted in an epidemic that ended before the 550th day of the simulation. The maximum weekly incidence ranged on average from 73 to 85 new cases per week.

The scenario would be different in regions of France with other farming characteristics.

Moreover, only the direct and indirect transmission processes were used to establish the PEDv spread model. Transmission by proximity (aerosols) and by air (wind) was not included in the assumptions. The spread model may therefore end up being less severe than reality, if these other routes of transmission were confirmed.

The model can also be used to simulate the implementation of the control measures presented in the following section.

The simulations were undertaken using only one index case. Therefore, simulated animal epidemics would *a priori* be more severe with multiple index cases (several contaminations at the same source).

Table 8: Impact of the index case's location on the output parameters of simulations in the absence of control measures (100 simulations, 550 days).

	No restriction, index case in Côtes d'Armor				No restriction, index case in Finistère Nord				No restriction, index case in Ile et Vilainene				No restriction, index case in Finistère Centre			
	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95
Total number of infected farms	3105.73	5.9	3681,0	4058.55	3393.47	13.9	3698,0	4054.95	3526.58	2749.95	3708,0	4037.5	3189.15	3.9	3724,0	4067.4
Total number of detected farms	3072.86	6.9	3641,0	4025.1	3358.33	14.85	3654.5	4016.25	3489.34	2701.5	3670.5	3995.25	3155.9	3,0	3691.5	4019.05
Percentage of iterations where the epidemic ends before the 550th day	16,0				9,0				5,0				15,0			
Duration of these epidemics that end (days)	82.13	39.5	75,0	157.5	100.11	43.8	85,0	187.2	100.8	59,0	105,0	127,0	72.13	21.9	84,0	110.5
Maximum weekly incidence	73.09	0,0	79,0	143.05	80.19	0,0	82.5	140.35	85.06	13.3	86.5	143.2	74.6	0,0	81.5	140.2

p5, p50, p95: 5th percentile, median and 95th percentile

3.4.1.2 Economic impact on the sector

The financial consequences of PED would be considerable, both for affected farms and for the entire sector, but would depend on the area where the index case occurs. The following simulations estimate the impact of the disease on the various levels of the sector and its potential cost for a medium-sized farm, which would vary depending on the severity. They are based on scenarios that may need to be re-assessed to take into account new knowledge and more in-depth economic calculations.

➤ **Direct consequences**

Piglet mortality can reach 100% on the 7th day after the start of symptoms and last for several weeks and even several months. For a farm of 200 productive sows, managed as seven batches of 28 sows, assuming that two consecutive batches are severely affected and the others are less affected since the sows were sick during gestation, the losses for 12 live-born piglets are estimated at €32,928⁹.

These losses are combined with the consequences of breeding disruption, since the sows lose their piglets before the usual weaning date, and returns to oestrus are scattered and can be deferred. The cost of one day lost over the weaning-to-service interval (WSI) is evaluated at €3.80. If two batches of sows are completely disrupted, with three additional days of WSI on average, the cost is then estimated at €638¹⁰.

Diarrhoea affects all pigs, irrespective of their age, with an impact on growth. Despite compensatory growth, 15 additional days are needed on average to reach slaughter weight, i.e. for two batches of 10x28 pigs at €2.30, an estimated €1,288¹¹.

Mortality, while not considerable during post-weaning or fattening, undoubtedly rises, by 2% in post-weaning and 1% in fattening, representing, according to the IFIP data, a loss of:

- €23 per sow for 1% losses during fattening;
- €32 per sow for 2% losses during post-weaning.

i.e. $23 \times 200 + 32 \times 200 = €11,000$.

Average losses for a 200-sow farm can therefore be evaluated at €45,854, under a favourable assumption as to duration without taking into account the costs of potential slaughter compensation for the State.

According to the estimation of the Centre de Développement du Porc au Québec (CDPQ), a 365-day PED epidemic in Quebec would represent a cost of \$14 million to \$50 million depending on the speed at which the disease spreads (Poulin and Klopfenstein, 2013).

➤ **Indirect consequences**

Regarding feed manufacturers and other suppliers, reduced unit sizes and movement restrictions would result in a significant shortfall that is difficult to accurately estimate but would clearly depend on the size of the affected herds, how many there are and the duration of the epidemic.

⁹ $12 \times 28 \times 2 \times 49$: Cost evaluated by the French Pork and Pig Institute (IFIP): *Gourmelen 2003*, updated in May 2014

¹⁰ $3.80 \times 28 \times 2 \times 3$

¹¹ $2 \times 10 \times 28 \times 2.30$, 10 being the number of pigs produced per sow and per batch, IFIP data

Regarding transport companies, the additional cost generated by the drastic biosecurity measures that would need to be taken would be very high and difficult to pass on to farmers (Poulin and Klopfenstein, 2013):

- increase in the number of lorries that would need to be decontaminated, if it were decided they all needed to be washed and disinfected between two deliveries;
- increase in needs for personnel, single-use clothing, washing products, water, electricity and fuel for drying.

Regarding slaughterhouses, the shortfall would relate to the decrease in the number of slaughtered pigs and the increase in cleaning-disinfection costs.

Regarding the pig market, after the available tonnage were reduced, prices would likely increase, in proportions depending on the scope of infection. However, it is difficult to predict how consumers would respond, even though the disease is not a zoonosis.

Regarding international trade: the disease would be harmful to exports. In France, exports account for 15% of production in terms of turnover, exports of live pigs 2.7%, cuts and carcasses 25%, and delicatessen meat products 9%¹².

3.4.2. Measures for controlling spreading: culling, confinement, biosecurity

The questions in the request on the spread of the disease have been grouped together in order to respond to the following points:

- Would simple confinement or culling make it possible to contain a first outbreak following the first reported cases?
- Would a preventive total cull with each report limit the consequences of PED being introduced into France?
- What are the advantages and disadvantages of culling/confinement?
- What biosecurity measures could help limit the spread of PED virus?

'Simple confinement' is defined as being all measures that can be adopted through a Prefectural Declaration of Infection, excluding culling¹³.

It includes two sets of measures: restriction of movements and external biosecurity measures.

Biosecurity refers to all measures taken to protect farms against the introduction of new infectious agents. It is divided into:

- external biosecurity: this is intended to prevent or limit the introduction of the virus in the farm;
- internal biosecurity: this is intended to reduce spreading of the virus within the farm.

When controlling the spread of a disease, external biosecurity measures are essential (internal biosecurity is an ongoing prerequisite for the management of a farm).

3.4.2.1 Controlling the first reported outbreak by culling or confinement

- In the United States, there were no total culls with the first outbreaks. Only the sick animals were culled and this did not limit the spread of the disease.

The spread of infection to neighbouring farms was examined in a retrospective study in the United States based on outbreak reporting data (University of Minnesota, 2013; <http://www.cvm.umn.edu/sdec/SwineDiseases/pedv/index.htm>). Based on the spatial analysis

¹² *Le Porc par les Chiffres 2013-2014. 2012 data. IFIP publication*

¹³ *Article L223-8 of the French Rural and Maritime Fishing Code*

of data from 2039 holding sites, the authors assessed the likelihood of being infected based on the distance from the closest known positive site:

- at less than 1.6 km, the risk is multiplied by 8.4;
- at less than 3 km, the risk is multiplied by 6.3;
- at less than 5 km, there is no increased risk.

However, these values depend on farm density. For example, in low-density areas, the risk of contamination decreases in relation to the distance from farms; but in high-density areas, the risk of spreading remains the same regardless of the distance from the closest known positive site. This suggests that simple confinement would have a very limited effect on the spread of the virus in areas where farm density is high.

Therefore, in light of the current knowledge on the spread of PEDv, simple confinement of the animals during the first outbreaks of infection does not appear sufficient to keep the virus from spreading. This would be even truer if the infected farms were located in an area with a high farm density.

- In Canada, in the province of Quebec (Montérégie), the authorities decided on a total cull with the first outbreak of contamination (fattening farm) on 22 February 2014 (in a region with high farm density). Accompanied by maximum biosecurity measures, this prevented spreading around the contaminated farm even though the pigs were transported to and slaughtered in a slaughterhouse. This site is still empty and under quarantine.

These points suggest that subject to drastic biosecurity measures (see 3.4.2.3), culling at the first detected outbreak would make it possible to limit the spread of the virus. It would also be necessary to comply with an adequate fallowperiod to decontaminate the building.

In light of these points, the GECU concludes:

- **that simple confinement of the first outbreak, in an area with a high farm density, would not be sufficient to keep the disease from spreading;**
- **that a total cull with the first outbreak, combined with the application of drastic biosecurity measures, would be more appropriate in an attempt to limit spreading;**
- **that biosecurity measures should also be strengthened as soon as the first case is detected and culled, to limit spreading through vehicles, equipment and personnel that may have come into contact with this farm;**
- **that the response time is important: a late response due to a long interval between detection, reporting and the establishment of control measures would multiply opportunities for the virus to spread, making the cull of this first outbreak ineffective against the spread of the disease.**

3.4.2.2 Controlling spreading from the first outbreak

The spread model presented in Point 3.4.1.1 can be used to simulate the impact of several types of epidemic control measures:

- **Restriction of movements** between farms after an infected farm is detected and reported. An area of restriction is set up around the reported infected farm with abolition of animal movements between the farms inside the area and those outside the area. Movements within the surveillance area are authorised. The speed at which the area is set up can be configured by representing a likelihood of the infected farm being detected and reported more or less early. The detection time includes the time required to identify the disease, report it to the veterinarian, undertake a confirmatory analysis and obtain a result. The

reporting time is the time required, after confirmation of the case, for the competent health authorities to make a management decision, and therefore runs until the control measure has actually been taken.

- **Strengthening external biosecurity** by decreasing the likelihood of infection being transferred through indirect contact.
- **Stamping out** of the reported infected units. In this case, the herds are culled once infection has been reported with a time-lag taken into account, like for the establishment of a restricted-movement area, in addition to a period for undertaking culling operations (total elimination of the infectious source).

Spread scenarios were simulated in combination with several control measures, by varying the parameters set out in Table 9. For the detection and reporting times represented in the restricted-movement and stamping out scenarios, relationship functions are used; they represent the change in the likelihood of detecting and reporting infection as a function of time (Appendix 8 and 9).

Table 9: Description of the assessed control options and the corresponding configuration in the spread model

		Origin of the index case	Detection time	Reporting time	Time to undertake culling operations	Radius of the restricted area	Decrease in the likelihood of transmission through indirect contacts
Control measures	Restriction of movements	Centre Finistère	. Relationship function A1 . Relationship function A3	Relationship function B	-	. 2 km . 5 km	-
	Biosafety strengthening	Centre Finistère	-	-	-	-	-30%
	Cull	Centre Finistère	. Relationship function A1 . Relationship function A2 . Relationship function A3	Relationship function B	7 days	-	-

• **Impact of the establishment of measures restricting animal movements (Table 10)**

In these scenarios, restricted-movement areas are established once infection has been reported (for the time lag shown). Various sizes of areas are assessed (2 or 5 km radius). Only animal movements between farms are abolished; movements of slaughter pigs to the slaughterhouse are not restricted in these simulations. These restrictions of movement have an impact on the length of the epidemic, since 100% of the simulations undertaken result in an epidemic that ends within 550 days. The average length of these epidemics is approximately one hundred days with the highest responsiveness (detection within ten days). The scope of the epidemic is also considerably reduced (a total of approximately thirty farms infected on average). Delayed detection (40 days) considerably decreases the effect of restrictions of movement (830 farms infected on average for an average epidemic duration of 337 days). Increasing the size of the restricted-movement area (5 versus 2 km) does not significantly change the effect of restricting movements.

• **Impact of the establishment of external biosecurity measures and measures restricting animal movements (Table 11)**

Here the impact of external biosecurity measures is represented by the 30% decrease in the likelihood of infection being transmitted through indirect contacts (Table 9).

- Strengthening external biosecurity alone: increasing external biosecurity between farms has an impact on the epidemic with a significant decrease in the total number of expected cases (533). However, only 51% of the iterations performed end before the 550th day of the simulation and the average length of these epidemics that end is 171 days. It appears that in this case, the disease is established in enzootic form.
- Combination of strengthened biosecurity measures with restriction of movement: this considerably improves the expected effectiveness (391 farms infected on average versus 831 for a detection time of 40 days with and without strengthened biosecurity respectively). With this combination of restriction of movements/strengthened biosecurity, nearly 100% of the simulated epidemics end before the 550th day for an average length of 178 to 292 days depending on the level of responsiveness.

Table 10: Impact of the establishment of restricted-movement areas
(100 simulations, 550 days)

	Restriction of movements, area with a 2km radius, detection within 10 days				Restriction of movements, area with a 2km radius, detection within 40 days				Restriction of movements, area with a 5km radius, detection within 10 days			
	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95
Total number of infected farms	29.31	0,0	10,0	112.4	831.33	7.9	912,0	1037.35	31.79	0,0	14,0	119.4
Total number of detected farms	30.01	1,0	11,0	113.4	722.94	6,0	788,0	904.35	32.37	1,0	15,0	118.45
Day of the first report	10.6	4,0	10,0	18,0	19.47	9.95	19.5	31.05	11.14	4,0	11,0	20.05
Number of farms infected before the 1st detection	4.91	1,0	4,0	11,0	14.42	3,0	12,0	31.4	5.45	1,0	5,0	14,0
Percentage of iterations where the epidemic ends before the 550th day	100,0				96,0				100,0			
Duration of these epidemics that end (days)	100.16	32.9	71,0	228.1	337.73	71,0	350,0	453.25	108.66	35.95	95,0	239.1
Maximum weekly incidence	2.85	0,0	0,0	14.05	39.59	0,0	42,0	79.05	2.89	0,0	0,0	13.1

p5, p50, p95: 5th percentile, median and 95th percentile
detection time: time between infection and confirmation of the diagnosis
reporting time: time between reporting of infection and actual implementation of the control measure

Table 11: Impact of strengthened biosecurity combined or not with the establishment of restricted-movement areas (100 simulations, 550 days)

	Strengthened biosecurity alone				Strengthened biosecurity, restriction of movements in an area with a 2km radius, detection within 40 days				Strengthened biosecurity, restriction of movements in an area with a 2km radius, detection within 20 days			
	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95
Total number of infected farms	533.11	4,0	437,0	1415.4	391.23	2.9	438.5	638.35	112.78	1,0	75.5	324.1
Total number of detected farms	526.44	5,0	433.5	1403.55	338.41	3.9	375.5	560.35	107.64	2,0	69,0	314.2
Day of the first report	11.99	4,0	11,0	21.05	22.59	9,0	22,0	40.3	16.92	8.9	16,0	27,0
Number of farms infected before the 1st detection	5.45	1,0	5,0	12,0	15.39	2,0	14,0	34,0	8.31	2,0	6,0	20.2
Percentage of iterations where the epidemic ends before the 550th day	51,0				98,0				100,0			
Duration of these epidemics that end (days)	171.06	56,0	115,0	504,0	292.28	55,0	316,0	440.15	178.68	44.85	171,0	360.35
Maximum weekly incidence	9.54	0,0	0.5	39.1	16.71	0,0	14,0	44.15	5.1	0,0	0,0	21.05

*p5, p50, p95: 5th percentile, median and 95th percentile
detection time: time between infection and confirmation of the diagnosis
reporting time: time between reporting of infection and actual implementation of the control measure*

- **Impact of stamping out detected infected herds (Table 12)**

The establishment of measures to cull the detected infected herds has the greatest impact on the size of the simulated epidemics.

- **Stamping out alone:** Responsiveness has a major influence: late detection within 40 days considerably decreases the effectiveness of the measure to cull the infected units (1023 farms infected on average in this case versus 16.5 with detection within ten days). With late detection, 13% of the simulated epidemics do not end before the 550th day of the simulation. The number of culled herds is also much higher (912 *versus* 17 for detection within ten days).
- **Combination of stamping out and biosecurity measures:** combining these rapid cull measures with strengthened biosecurity further increases effectiveness since in this case the length of the epidemic is very short (less than 30 days) and the total number of infected farms is ten on average.

Table 12: Impact of herd stamping out based on detection time
(100 simulations, 550 days)

	Detection within 10 days				Detection within 20 days				Detection within 40 days				Detection within 10 days + Strengthened biosecurity			
	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95	Mean	p5	p50	p95
Total number of infected farms	16.51	2,0	11,5	43,05	107.82	2,0	57,0	301,95	1023.08	11.95	1143,0	1514.35	10.49	1,0	7,0	28,05
Total number of detected farms	17.16	3,0	12,0	44,0	102.76	2.95	55,5	287,65	887.74	12.85	993,5	1313,15	11.23	2,0	8,0	28,05
Day of the first report	12.27	5,0	11,0	23,0	15.92	7,0	15,0	26,0	22.47	8.95	23,0	35,0	11.23	4,0	11,0	20,05
Number of farms infected before the 1st detection	5.92	1,0	5,0	14,05	9.48	2,0	7,5	22,0	18.01	3,0	15,0	45,05	5.15	1,0	4,0	12,05
Percentage of iterations where the epidemic ends before the 550th day	100,0				100,0				87,0				100,0			
Duration of these epidemics that end (days)	36.83	16.9	34,0	65,05	108.62	24.9	90,0	232,3	328.94	45.9	340,0	511,0	29.7	15.95	28,0	54,0
Maximum weekly incidence	4.29	0,0	0,0	19,05	7.51	0,0	6,0	26,05	34.62	0,0	32,5	79,15	3.03	0,0	0,0	13,0
Number of farms subject to stamping out	17.26	3,0	12,0	44,05	104.22	2.95	56,5	291,6	912.85	12.85	1018,5	1340,9	11.34	2,0	8,0	28,05

*p5, p50, p95: 5th percentile, median and 95th percentile
detection time: time between infection and confirmation of the diagnosis
reporting time: time between reporting of infection and actual implementation of the control measure*

From the information provided by the model, it appears that:

- **Restrictions of movements** reduce the length of the simulated epidemic as well as its scope;
- **Combining external biosecurity measures with restrictions of movements** further reduces the length and scope of the simulated epidemic;
- **Stamping out** is the measure that has the greatest impact on the size of simulated epidemics, as long as the detection of outbreaks is early and drastic external biosecurity measures are also undertaken, particularly thorough decontamination of means of transport to slaughterhouses.

The experts emphasize that:

- The likelihood of detecting the index case is high due to the very high morbidity and mortality seen in the infected herds. However, it is still possible that the virus could first spread without this index case being reported, especially if infection occurred in adults, which would increase the risk of dealing with several simultaneous outbreaks;
- A rapid response time is essential: a late response due to a long interval between detection, reporting and establishment of the control measure would have disastrous consequences and would likely result in a large number of cases that would be very difficult to manage through culling measures (see Table 12);

- Additional control measures can be considered depending on the epidemiological situation, such as:
 - if culling was performed at the first outbreak, the preventive cull of geographically close or epidemiologically related herds could be considered;
 - establishing an area where movements are banned;
- The practical conditions for implementing measures, the resistance of the virus in the external environment and the very low infectious dose are likely to refine the conclusions of the model. The results obtained from the model, with the chosen configuration, show variability given its stochastic nature. The interpretations given above rely on average values for the output parameters. Their variability should also be taken into account in the conclusions.
 Thus, the control measures considered (restriction of movements + external biosecurity measures / cull + external biosecurity measures) would help limit the spread of the disease but may not be sufficient to stop the virus from spreading. This would result in an epidemic.

3.4.2.3 Advantages and disadvantages of the two options for controlling the spread of the disease

Based on the points discussed above, there are advantages and disadvantages of the two control options: simple confinement (restriction of movements + external biosecurity measures) / stamping out + external biosecurity measures, as shown in Table 13.

Table 13: Advantages and disadvantages of confinement versus total stamping out

	Advantages	Disadvantages
Simple confinement (restriction of movements + external biosecurity measures)	<ul style="list-style-type: none"> - In low-density areas, decreased risk of contamination (based on the distance from the contaminated farm). - Movement banned in an area of 2 or 5km: limitation of spreading by transport, equipment 	<ul style="list-style-type: none"> - In high-density areas, no decrease in the risk of spreading regardless of the distance from the contaminated farm. - Maintenance of infection by re-infection of the animals present, risk of local spreading maintained. - Difficulties managing animals with rapid growth.
Stamping out + external biosecurity measures	<ul style="list-style-type: none"> - Elimination of the infected animals. - The concurrent application of drastic biosafety measures would make it possible to eliminate the source of infection. 	<ul style="list-style-type: none"> - Difficult to completely eliminate sources of infection on farms due to a very low infectious dose → sufficiently long fallow period essential or else there will be re-contamination with the reintroduction of animals. - Spreading by vehicles depends on the application of drastic biosecurity measures: taking essential precautions for the transport of animals to the slaughterhouse for culling operations (sealed lorries, controlled movements, etc.). - Measures encouraging early reporting are required. - The success of this culling measure heavily depends on responsiveness in the detection of outbreaks and implementation of measures.

3.4.2.4 Biosecurity measures to limit the spread of the porcine epidemic diarrhoea virus

This section describes the external biosecurity measures mentioned in the previous paragraphs.

Biosecurity measures have been described in details by technical pig institutes in France (IFIP – www.ifip.asso.fr@ifip.asso.fr¹⁴), the United States and Canada. The GECU has not listed in detail all of the measures that should be applied on farms to limit the spread of the virus, but insists on the very low infectious dose of the virus, which is why drastic biosecurity measures need to be applied.

The GECU underlines that these measures require means much greater than those usually granted in the sector.

The main principles of biosecurity revolve around three points:

- Isolation and segmentation: protecting pigs from exposure to viruses (quarantines, inspections of animal movements, loading platform, carcass storage room and rendering vehicle access, provisional ban on carcass composting when necessary);
- Control of movements: continuously limiting access to the farm and buildings (operators, visitors: management of human inputs) and incoming flows (animals and equipment from the exterior). Within the farm, the 'forward-flow' principle, from the least contaminated to the most contaminated sectors (flows of equipment, personnel and animals);
- Cleaning and disinfection: limiting contamination of the farm environment and buildings (disinfection of vehicles, washing and disinfection of equipment, air filtration, pest and rodent control in buildings (passive transport), foot baths, water points, etc.).

In the context of PED, external biosecurity measures, to prevent viruses from entering the farm, are the most significant. However, internal biosecurity measures remain essential to limit the number of infected animals and reduce the negative economic consequences of the disease within a farm.

3.4.3 Means of controlling spreading: vaccination

Immune response and protection from the PED virus

Antibodies against the PED virus are detected between two and three weeks after infection and can persist for more than two years in infected animals. The main immunoglobulin isotypes (IgA, IgM and IgG) are produced in pigs after experimental infection with the reference strain CV777 or with the PEDv strain isolated in 2013 in the USA (de Arriba *et al.*, 2002; Gimenez-Lirola *et al.*, 2014). However, the presence of antibodies in serum is not correlated with protection. Rather, protection from re-infection is reflected by intestinal mucosal immunity which is short-lived (Pensaert, 1989). Up to the age of 13 days, piglets are protected by IgG antibodies directed against the PED virus that are found in the colostrum and milk of immunised sows. There is also a passive transfer of IgA through colostrum and milk which offers protection to suckling piglets. The length of immunity depends on the mother's level of antibodies. IgG antibodies account for 60% of the immunoglobulins in colostrum, but IgA antibodies are more effective in neutralising the virus by oral route and inducing a local immune response in suckling piglets (Song and Park, 2012). After a

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http://ifip.asso.fr/sites/default/files/pdf-documentations/dep_biosecurite.pdf; http://ifip.asso.fr/sites/default/files/pdf-documentations/dep_transport.pdf; http://ifip.asso.fr/sites/default/files/pdf-documentations/dep_nettoyage-desinfection.pdf

primary infection, periodic re-infections have been described on certain farms (Dufresne and Robbins, 2014) and can occur from five months after the first PED episode even when the animals have antibodies (Pensaert, 1989). Deliberate contamination is used in countries where the disease has become enzootic but this method is uncontrolled and should therefore be ruled out.

Live attenuated vaccines

Two main strains are used as vaccine strains: the reference strain CV777 isolated in Belgium in 1978 and the Korean strain DR13 isolated in 1999. The two strains are adapted in an *in vitro* cell culture and attenuated with successive passages. The two attenuated strains CV777 and DR13 have 49 and 51 nucleotide deletions respectively in the ORF3 gene located between the S gene and the E gene. A recent study suggests that ORF3 encodes a protein functioning as an ion channel that regulates viral production and is related to pathogenesis (Wang *et al.*, 2012).

Live vaccines containing either of these two attenuated strains are used in Asia with intramuscular injection. In Japan, since 1997, a commercial attenuated vaccine containing a cell-culture adapted PEDv strain has been administered by intramuscular injection to sows with two injections four to eight weeks apart. The second injection is given two weeks before farrowing.

Oral vaccination of sows with the attenuated DR13 strain (100 *in vitro* passages) has been tested and has proven more effective than vaccines administered by intramuscular route. This vaccine has been used in South Korea since 2004 and in the Philippines since 2011.

Although these attenuated vaccines provide cross protection against PED virus strains and reduce clinical signs, they do not reduce the duration of viral shedding and do not enable sows to develop high lactogenic immunity (Song and Park, 2012). Furthermore, the use of these live attenuated vaccines can enable vaccine strains, by reversion, to evolve into more pathogenic forms on farms (Chen *et al.*, 2010).

Inactivated vaccines

An inactivated vaccine with the CV777 strain is also marketed in China.

A new biotechnological vaccine developed in the United States has been used by veterinary prescription since August 2013. This vaccine was recently granted a conditional licence for marketing in the United States by the United States Department of Agriculture (USDA). To produce this vaccine, the S gene of a variant isolated in China was cloned in a replicative vector containing the non-structural genes of an alphavirus, the Venezuelan equine encephalitis virus. The replicative vector is transfected by electroporation in Vero cells to produce defective viral particles with the PEDv S protein on their surface. These recombinant particles are then purified and injected in the muscles of animals.

Vaccination failures

Many cases of vaccination failures on farms using vaccines based on the attenuated or inactivated CV777 strain have been reported since 2010 in China (Li *et al.*, 2014; Li *et al.*, 2012; Wang *et al.*, 2013). The PEDv strains isolated in central China between 2012 and 2013 apparently evolved from strains previously found in this region. Most of the amino acid changes observed in these strains occurred in the two regions 7-146 and 271-278 with neutralising epitopes in the S protein of the PED virus. Amino acid mutations in these epitopes may be associated with changes in the antigenicity of PEDv strains and consequently result in vaccination failure (Li *et al.*, 2014).

Thus, the protection granted by vaccines reduces clinical signs in the best of cases but has no effect on viral shedding.

Vaccine protection is short-lived:

- **in sows: re-vaccination with each pregnancy is necessary;**

- **in piglets, it is related to the presence of colostrum antibodies whose levels decrease rapidly over time.**

Live attenuated vaccines appear the most effective. However, the vaccine strain risks reverting to virulence. They must be closely monitored.

Latest-generation vaccines will be developed from the PEDv variant strains circulating in the United States.

None of the vaccines that are currently available are sufficiently effective to be used for controlling PED.

If new vaccines are developed, they must be adapted to the virulent variant strain.

Even if their effectiveness is limited to reducing clinical signs, they would have a beneficial effect by reducing costs related to the disease.

Conclusions of the collective expert appraisal

There has been a new variant of the 'Porcine epidemic diarrhoea' virus (PEDv) in the United States in the past year and it has spread to other countries on the American continent including Canada and several South American countries. This strain is highly virulent and given its genetic proximity to certain Chinese viruses, it can be assumed that this PEDv variant may have been introduced in the United States from China.

The United States is experiencing a major PED epidemic, in spite of the biosecurity and hygiene measures taken. Given the epidemiological characteristics of PEDv, there are concerns about the risk of it being introduced in Europe, which has been free of PED for many years. It should be pointed out that this disease is not a zoonosis and therefore cannot be transmitted to humans.

In this context, the DGAL issued a formal request to ANSES to assess the risk of the virus being introduced in France and the risk of its spreading if it were introduced, and to provide answers as to the relevance of various control measures. Given the health urgency, ANSES created an Emergency Collective Expert Assessment Group (GECU) to answer the DGAL's various questions. Its conclusions are as follows:

- **Risk of introduction**

After assessing the various sources of the virus and considering the extremely low minimum infectious dose for PEDv, its resistance in the environment and the quantity of the virus shed by sick animals, the GECU considers that there is a confirmed risk of PED being introduced in France from an infected country. The likelihood of introduction depends on the product:

- 6 to 7 for live pigs on an ordinal scale of 0 to 9 (0 corresponding to zero risk and 9 corresponding to very high risk);
- 6 for blood products on the ordinal scale of 0 to 9;
- 5 on the ordinal scale of 0 to 9 for
 - semen
 - embryos
 - farm equipment and vehicles;
- 3 for people as passive carriers, on the ordinal scale of 0 to 9;
- 2 to 3 for gelatine, on the ordinal scale of 0 to 9;
- 1 to 2 for organic fertilisers, on the ordinal scale of 0 to 9;
- 1 for collagen, animal fats, hydrolysed proteins, pork and delicatessen and cured meat products, on the ordinal scale of 0 to 9;
- 0 for manure and swill (since these products are not imported).

However, it should be noted that the estimation of likelihood of the virus being introduced by embryos and organic fertilisers is associated with high uncertainty due to the lack of available data.

Data are also lacking for gelatine, which is a common constituent of additives intended for animal feed. While product treatment appears capable of guaranteeing inactivation of the virus, the risk of products becoming re-contaminated after treatment cannot be ruled out and depends on the organisation of the factories that produce these products.

Moreover, the zero likelihood for manure and swill is due to the fact that these products are not imported from countries currently infected. If this situation were to change, the risk assessment would be amended.

Given the characteristics of the virus presented above, the experts point out that the risk of cross contamination by the virus from pig products is omnipresent. The utmost vigilance is therefore necessary when it comes to the origin of all inputs on pig farms.

- **Minimum production conditions:** regarding blood products derived from pigs, for which the likelihood of the virus being introduced is at a value of 6 on an ordinal scale of 0 to 9, the experts recommend:
 - Heat treating the products at a temperature of over 71°C for at least ten minutes; all other time-temperature combinations need to be validated based on experimental tests or new bibliographic data;
 - Storing the heat treated products in a dry environment for at least seven days at a temperature of 20°C; this storage is not an alternative to heat treatment. Temperature and hygrometry parameters must be monitored;
 - Complying with hygiene measures in order to keep treated products from becoming re-contaminated by contaminated products through cross contamination ('forward-flow' measures, separation of clean and dirty areas, different personnel in the areas).

They underline that in the current state of knowledge, the assessment of product safety in relation to PED cannot be completely guaranteed by RT-PCR analysis.

- **Foreseeable impact for the sector** if the virus were introduced in France: the epidemiological analysis undertaken through a between-herd PEDv spread model results in the establishment of a scenario in which the disease spreads rapidly, in the absence of control measures, in areas with high pig densities.
- **Measures to control the spread of the virus** if it were introduced in France: the GECU assessed the possibilities of simple confinement, culling with the first outbreak and cull with each reported case. The collective expert appraisal shows:
 - For the first outbreak:
 - That simple confinement of the first outbreak, in an area with high farm density, would not be sufficient to keep the disease from spreading;
 - That a total cull with the first outbreak, combined with the application of drastic biosecurity measures, would be more appropriate in an attempt to limit spreading;
 - That biosecurity measures should also be strengthened as soon as the first case is detected and culled, to limit spreading through vehicles, equipment and personnel that may have come into contact with this farm;
 - That a rapid response time is essential: a late response due to a long interval between detection, reporting and establishment of the control measure would multiply opportunities for the virus to spread, making the stamping out of this first outbreak ineffective against the spread of the disease.
 - For each report, in light of the data provided by the model:
 - Restrictions of movements reduce the length of the simulated epidemic as well as its scope;
 - Combining external biosecurity measures with restrictions of movements further reduces the length and scope of the simulated epidemic;
 - Stamping out is the measure that has the greatest impact on the size of the simulated epidemics, as long as the detection of outbreaks is early and drastic external biosecurity measures are also undertaken.

In addition to the model, the experts underline that:

- Like for the detection of the first outbreak, a rapid response time is essential: a late response due to a long interval between detection, reporting and establishment of the control measure would have disastrous consequences and would likely result in a large number of cases that would be very difficult to manage through culling measures;
 - Additional control measures can be considered depending on the epidemiological situation, such as:
 - in case of culling with the first outbreak, considering the preventive cull of geographically close or epidemiologically related herds;
 - establishing an area where movements are banned;
 - The practical conditions for implementing measures, the resistance of the virus in the external environment and the very low infectious dose are likely to refine the conclusions of the model. The results obtained from the model, with the chosen configuration, show variability given its stochastic nature. The interpretations given above rely on average values for the output parameters. Their variability should also be taken into account in the conclusions. Thus, the various control measures under consideration would limit the spread of the disease but may not be sufficient to stop the virus from spreading. This would result in an epidemic.
- **Vaccination:** none of the vaccines that are currently available are sufficiently effective to be used for controlling PED.
If new vaccines are developed, they must be adapted to the virulent variant strain. Even if their effectiveness is limited to reducing clinical signs, they would have a beneficial effect by reducing costs related to the disease.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the analysis and conclusions of the GECU.

Marc Mortureux

KEYWORDS

Porcine epidemic diarrhoea, pigs, risk of introduction

APPENDIX 5: Conceptual model

