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Brief communication

Weight of the evidence linking feed to an outbreak of porcine epidemic diarrhea in Canadian swine herds

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Summary

The weight of the evidence gathered during an outbreak of porcine epidemic diarrhea (PED) in Canada in January 2014 supports an association with feed containing spray-dried porcine plasma contaminated with the virus. Many questions remain regarding the importance of feed and (or) feed ingredients in the transmission of PED virus.

Keywords: swine, porcine epidemic diarrhea virus, infectious disease outbreaks, animal feed

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Resumen - Peso de la evidencia vinculando el alimento a un brote de diarrea epidémica porcina en hatos porcinos Canadienses

El peso de la evidencia reunido durante un brote de diarrea epidémica porcina (PED por sus siglas en inglés) en Canadá en Enero del 2014 apoya una asociación al alimento que contiene plasma porcino secado por aerosol contaminado con el virus. Aún quedan muchas preguntas con respecto a la importancia del alimento y (o) ingredientes del alimento en la transmisión del virus PED.

Résumé - Fardeau de la preuve liant l'aliment à une épidémie de diarrhée épidémique porcine dans des troupeaux porcins canadiens

Le fardeau de la preuve accumulé durant une épidémie de diarrhée épidémique porcine (DEP) au Canada en janvier 2014 supporte une association avec de l'aliment contenant du plasma porcin séché au jet contaminé par le virus. Plusieurs questions demeurent quant à l'importance de l'aliment et (ou) des ingrédients alimentaires dans la transmission du virus de la DEP.

Porcine epidemic diarrhea (PED) is a highly contagious disease of swine caused by the porcine epidemic diarrhea virus (PEDV), an Alphacoronavirus of the Coronaviridae family.[1] Swine enteric coronavirus diseases (SECDs) have been known for decades, but PEDV was reported for the first time in Canada in January 2014, nine months after it was first discovered in the United States in May 2013. Even though genetic and phylogenetic analyses of three US PEDV strains suggest that they likely originated from China,[2] the exact pathway for introduction has yet to be identified. A root cause investigation conducted by the United States Department of Agriculture Animal and Plant Health Inspection Services (USDA-APHIS) suggested that the use of flexible intermediate bulk containers, contaminated in the country of origin and reused in the United States for the transport of bulk feed or feed ingredients, could have been the source of introduction of SECD viruses into the United States, as well as contributing to their widespread introduction onto individual farms all over the country.[3]

In Canada, the initial investigation of the outbreak by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) led to the hypothesis that swine feed containing imported spray-dried porcine plasma (SDPP) was a possible route of introduction of PEDV in swine herds,[4] and polymerase chain reaction (PCR) testing revealed that the feed and SDPP both contained PEDV genetic material.[5] As part of its mandate to safeguard the food supply and the plant and animal resource base in Canada, including the assurance that livestock feed sold in Canada is safe and effective, the Canadian Food Inspection Agency (CFIA) conducted a feed investigation. The aim of the study presented here was to assess the weight of the evidence gathered during the feed investigation and to determine whether swine feed or feed ingredients were linked to cases of PED in Canadian swine herds.

Material and methods

A positive case herd was defined as a Canadian swine herd with laboratory confirmation of PEDV in pigs reported between January 22 and March 7, 2014. Secondary cases, which were attributed to a direct or indirect contact with another case farm, were excluded from the investigation.

Trace-back and trace-forward activities were conducted to determine the origin of the feed and its ingredients, to determine where the feed was distributed, and to ensure that

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other possibly contaminated products were identified. Case-herd owners were questioned on the feed or feed ingredients that were used on their farm during the 2 weeks prior to the onset of clinical disease. A distribution list was obtained from the distributor of the feed containing the imported PEDV-positive SDPP, and all farms that had received this feed were contacted. Additional lines of inquiry related to swine by-products, such as dried porcine solubles, spray-dried porcine red blood cells, and SDPP manufactured at other plants, as well as other feeds manufactured in the same time period as the feed containing the contaminated SDPP, were also investigated by the CFIA.

Confirmatory testing of the feed and SDPP was conducted at the National Centre for Foreign Animal Disease as described in Pasick et al.[6] Briefly, PEDV N gene real-time reverse transcription-polymerase chain reaction (RT-PCR), PEDV N and S gene conventional RT-PCR, and gene sequencing were conducted following nucleic acid extraction. In addition, naive piglets were inoculated with the samples in a swine bioassay experiment to determine whether the detection of genetic material corresponded with the presence of live virus.

The weight of the evidence linking the feed to cases of PED was assessed in a framework developed in Canada for the investigation of foodborne illness outbreaks.[7] The weight of the evidence gathered during the investigation was evaluated for the following criteria: consistency of the laboratory results with the epidemiological evidence, consistency of temporal and (or) spatial clustering of cases with the availability and distribution of the feed, temporal association between feed consumption and disease, strength of the statistical association between the feed and the disease, whether a single specific feed appeared to be the vehicle of infection, and whether the strength of the association increased with increasing consumption of the feed (dose response). A literature review was conducted to evaluate the plausibility that the feed pellets containing contaminated SDPP were the vehicle of infection. Finally, alternate explanations were considered. The proportion of positive cases exposed to the feed was compared, using exact probability testing, to the proportion expected to be exposed, on the basis of market-share estimates. Attack rates were computed as the number of cases divided by the size of the population exposed.

Results

This study covers the initial period of the 2014 Canadian outbreak of PED, which started in a swine herd in southwestern Ontario. During the period of the investigation, a total of 27 cases of PED were confirmed in Ontario, but spread to the rest of the country was limited. Only three cases were reported outside of the province: one case each in Manitoba, Prince Edward Island (PEI), and Quebec.

Laboratory evidence. It was discovered early on in the investigation of the outbreak by the OMAFRA that a single feed company delivered creep or nursery feeds to many of the case herds investigated. Samples from these feeds and from one lot of imported SDPP used as a feed ingredient were positive for PEDV on RT-PCR testing.[4] Confirmatory molecular diagnostic testing and swine bioassay studies demonstrated that the SDPP, but not the feed, did contain PEDV capable of infecting inoculated piglets, as well as transmitting the infection to contact piglets.[6]

Space and time consistency. Clinical signs at the index farm started on January 21, 2014, one week after the feed containing PEDV-positive SDPP was delivered to that facility. Pigs had consumed feed containing the PEDV-positive SDPP on 60% of the case herds (Ontario, n = 17; PEI, n = 1) (Figure 1).

Approximately 288 tonnes of the feed containing the PEDV-positive SDPP was distributed from January 3 to February 9, 2014, when it was voluntarily withdrawn from the market by the manufacturer. The SDPP was manufactured in the United States in November 2013, imported to Canada in December 2013, and used in the manufacture of three lines of pelleted swine nursery (piglet) feed by a third-party manufacturer in Canada. The feed contained no other ingredient of porcine origin. The feed was delivered to 84 farms, located primarily in Ontario (n = 75), but also in Alberta (n = 3), Manitoba (n = 5), and PEI (n = 1). For 20 of the farms it was not possible to confirm whether the feed had been consumed.

Strength of the association. The attack rate for the cohort of 84 exposed farms in which pigs presumably consumed the feed was 21.4% (18 of 84). Considering only farms where the consumption of feed was confirmed, the attack rate was 28.1% (18 of 64). The attack rate for unexposed farms was estimated at 0.17% (12 cases for approximately 7000 hog farms in Canada).

In Ontario, cases of PED were significantly more likely (exact binomial probability test; $P < .001$) to have been exposed to the feed (17 of 27; 63.0%) than expected from the 10% to 15% market share reported by the distributor.

Specificity. The attack rates were similar for the three different lines of feed that were manufactured using the PEDV-positive SDPP. Other products not containing SDPP were produced in the same feed mill during January and February 2014; these feeds were not linked to cases of PED.

Dose response. Each line of the feed was available in different SDPP concentrations. The attack rates were higher for the farms that received feed containing higher concentrations of SDPP (Figure 2). The risk of disease was significantly higher (relative risk = 9.0; 95% confidence interval 1.3-64.0) on farms that received feed containing high SDPP concentrations (3% to 6%) than on farms that received only feed containing low SDPP concentrations (1.0% to 1.5%). The PEI case farm was the only one of the nine exposed farms outside of Ontario that became infected; it was also the only farm outside of Ontario which received feed with an SDPP inclusion rate of 3% or more.

Alternate explanations. Investigation of the initial cases by the provincial authorities found no association with other exposures, such as feed transporters, service providers, a rendering company, or livestock haulers. Environmental contamination with PEDV was discovered at a major assembly yard in Ontario, but it was not possible to determine whether this contamination preceded the initial cases of PEDV infection in Ontario.[4]

Discussion

There was a good temporal and geographical correlation between cases and distribution of the feed; timing of the cases was also consistent with the incubation period of the disease. A single lot of SDPP was identified as the vehicle of infection, and the proportion of cases that were exposed to feed containing this SDPP was significantly higher than expected, based on market share. The attack rate calculated for the exposed farms was significantly higher than the attack rate estimated for unexposed farms. The strength

Figure1: Number of Canadian swine herds with confirmed cases of porcine epidemic diarrhea (PED) between 22 January and 7 March, 2014, by epidemiological week of onset of clinical signs (n = 30). Pigs in herds indicated in red consumed feed containing a specific lot of PEDV-positive (PEDV+) SDPP, whereas pigs in herds indicated in blue consumed feed that did not contain SDPP, or that contained PEDV-negative (PEDV-) SDPP. The feed containing the PEDV+ SDPP was voluntarily withdrawn from the market by the manufacturer on February 9, 2014. For nine of 15 farms that did not receive feed containing the specific SDPP lot, clinical signs were absent (n = 3) or the date of onset of clinical signs was missing (n = 6) and was replaced by the date of laboratory confirmation. PEDV = porcine epidemic diarrhea virus; SDPP = spray-dried porcine plasma; VMW = voluntary market withdrawal of the feed containing the specific lot of PEDV+ SDPP.

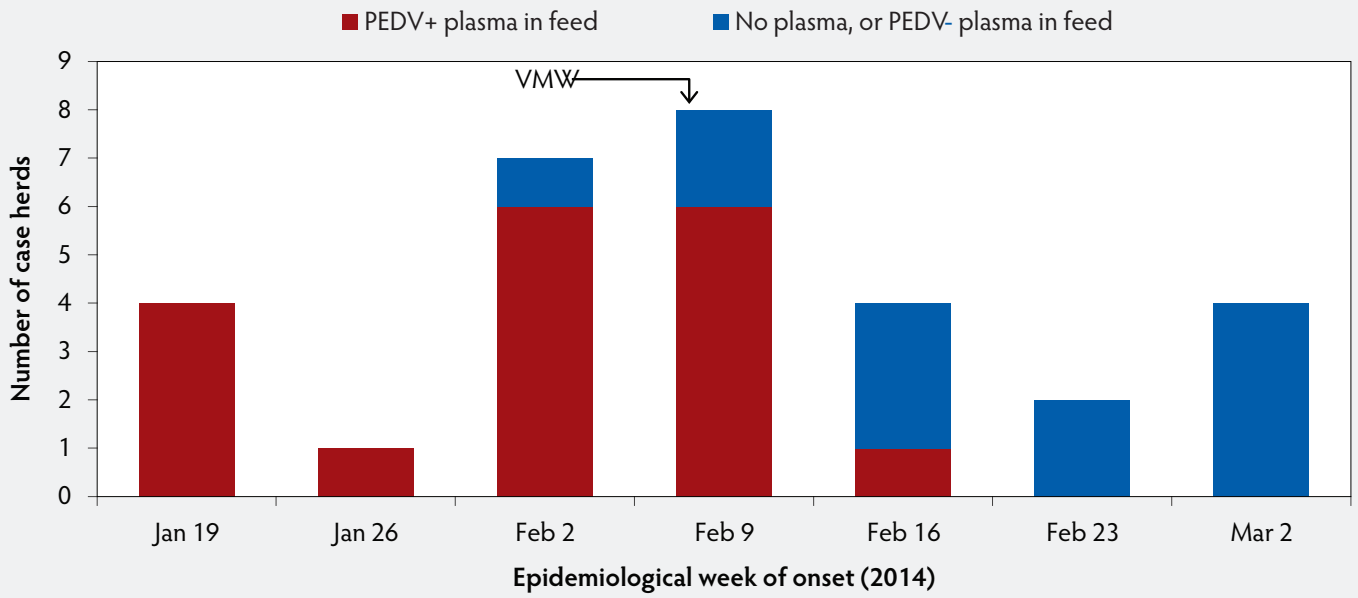
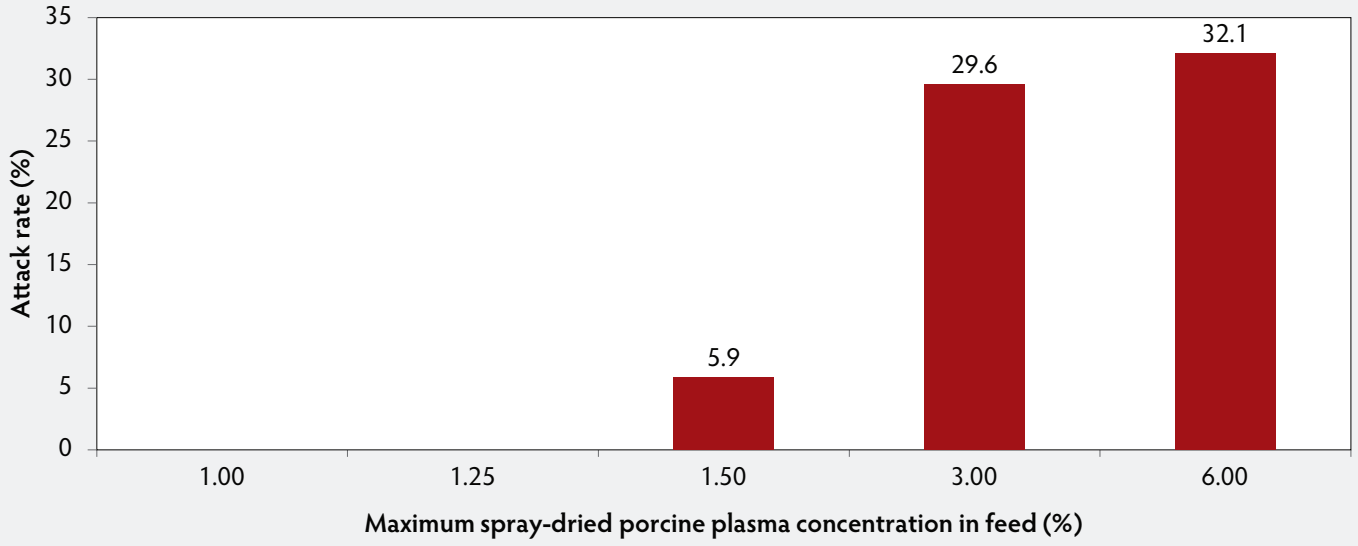


Figure 2: Attack rates for PED increased with increasing concentration of SDPP in feed for the cohort of 84 exposed Canadian swine herds. The risk of disease was significantly higher (RR = 9.0; 95% CI, 1.3-64.0) on farms that received feed containing high SDPP concentrations (3% to 6%; n = 55) compared to farms that received only feed containing low SDPP concentrations (1% to 1.5%; n = 29). 95% CI = 95% confidence interval; PED = porcine epidemic diarrhea ; RR = relative risk; SDPP = spray-dried porcine plasma.



of the association increased with increasing concentration of SDPP in feed, but this could have been confounded by the fact that the concentration of SDPP in nursery feed is typically higher for younger piglets, which are also more susceptible to PEDV infection than older pigs. The laboratory results confirmed the presence of live PEDV in the SDPP, but not in the feed.[6] This is compatible with infectious PEDV being present in the feed at very low concentrations, thereby causing infection on a few farms when fed to thousands of pigs for many consecutive days, but not in limited bioassay studies (low-dose, single-hit concept of infection; multiple repeated exposures).

On the other hand, there is evidence that the spray-drying process is effective at inactivating PEDV[8-10] as well as other viruses, such as the porcine reproductive and respiratory syndrome (PRRS) virus, pseudorabies virus,[11] and porcine circovirus (PCV2), which is one of the most resistant porcine viruses.[12,13] Good manufacturing practices, which include collection of blood only from animals fit for slaughter for human consumption, a closed system, cleaning and disinfection of holding tanks and equipment, and monitoring of the parameters of the spray-drying process, are in place to ensure that commercial SDPP is a safe product.[14] Nevertheless, a breach in good manufacturing practices and (or) biosecurity could potentially lead to cross-contamination during processing, and (or) post processing during packaging, storage, and (or) transportation.[15] A recent study[16] described outbreaks of PED that appeared to be linked to contaminated feed (not containing any animal by-products) on three different farms, and it provided proof of concept that feed can serve as a vehicle for PEDV infection of naive piglets. It is unknown whether contaminated flexible intermediate bulk containers could have played a role in this outbreak, but one would have then expected PED cases to be associated with a greater diversity of feed or feed ingredients, as appears to have been the case in the early cases the United States.[3]

While many questions remain regarding the plausibility or the importance of PEDV transmission through spray-dried porcine plasma or swine feed in the epidemiology of the disease, the weight of the evidence gathered during this outbreak supports that this first Canadian outbreak of PED was associated with swine feed containing a contaminated lot of SDPP.

The potential for PEDV contamination of SDPP or swine feed to occur at any point throughout the production and distribution chain needs to be investigated further in order to evaluate the importance of PEDV transmission via feed in the epidemiology of the disease.

Implications

- A systematic framework developed for the investigation of foodborne illness outbreaks can be used to assess the weight of evidence gathered during a feed investigation.
- It is possible for swine feed containing spray-dried porcine plasma (SDPP) contaminated with PEDV to be linked to clinical cases of porcine epidemic diarrhea, especially when the SDPP concentration in feed is ≥ 3%.
- Research is needed to elucidate the conditions under which swine feed or feed ingredients can become contaminated with PEDV and other swine pathogens, and potentially introduce new agents of disease into naive swine herds.

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Conflict of interest

None reported.

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