

## **Short communication**

**Title:** Feed or feed transport as the potential route for a porcine epidemic diarrhea outbreak in a 10,000-sow breeding herd in Mexico

**Running Title:** Feed or feed transport role in a PED outbreak

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## **Summary**

Porcine epidemic diarrhea virus (PEDV) produces infection in pigs characterized by vomiting and diarrhea. PEDV is transmitted via oral-fecal and a very low oral dose is enough to infect susceptible pigs, causing devastating consequences in production. A 10,000-sow farrow-to-wean farm located in northwest Mexico was infected with PEDV. After the observation of the first clinical signs, an outbreak investigation was taken into place to determine the most probable source of infection. A systematic collection of samples including rectal swabs, gestation and lactation feed, surface swabs from the interior of feed bins and many points of the feed truck delivering the implicated feed was performed. Samples were tested for PEDV polymerase chain reaction (PCR). Positive PCR results showed the evidence of PEDV RNA in lactating feed, the interior walls of the feed bins and in the interior of the auger boom of the feed truck. This, connected with the location of first clinical signs, points that the most probable incursion of PEDV into this breeding herd was contaminated feed. This paper shows how feed or feed transport can be a potential source of PEDV infection in farms and highlights the importance of establishing biosecurity programs to mitigate the risk of PEDV infections.

**Key words:** Porcine Epidemic Diarrhea virus (PEDV), transmission, feed, pig, infection

## **Introduction**

Porcine epidemic diarrhea (PED) was introduced to the US in 2013 (Chen *et al.*, 2014). In the following months the disease was reported in other countries of North and South America including Mexico (OIE, 2014). PED virus (PEDV) causes explosive occurrence of diarrhea and vomiting affecting all ages, with 90–95% mortality in suckling pigs (Stevenson *et al.*, 2013). The economic impact of the disease has been estimated in retrospective studies. In breeding herds, a median of 2.7 piglets per inventoried sow were not weaned and the average time required to recover to baseline production was ten weeks (Goede and Morrison, 2015). In growing pigs, mortality and feed conversion were increased by 11% and 0.5, respectively, and average daily gain decreased by 0.07 kg/day in PEDV positive pigs (Alvarez, *et al.*, 2015).

PEDV is directly or indirectly transmitted via the oral-fecal route. Contaminated fomites, including livestock transportation trailers (Lowe *et al.*, 2014) and feed (Dee *et al.*, 2014), can work as routes for disease introduction to a region or farm. Since an extremely low oral dose of PEDV is capable of infecting susceptible pigs (Thomas *et al.*, 2015), and the virus can be infectious after several weeks at environmental conditions (Dee *et al.*, 2016) the disease can be introduced to a herd by multiple routes. However, the onset of clinical signs as soon as 24 hours after exposure (Jung *et al.*, 2014) generally allows for an accurate outbreak investigation. The goal of this short communication is to summarize the risk events and diagnostic findings before and after the detection of PEDV in a breeding herd in northwest Mexico.

## **Case Description**

### *Farm details*

The 10,000-sow farrow-to-wean farm is located in northwest Mexico (Sonora state). Although there are other fifteen pig production sites within 40 Kms of the farm, the closest site is located eight Kms away (Figure 1). The farm was populated in 2013 and had never been infected with PEDV, Porcine Delta Coronavirus (PDCoV) or Transmissible Gastroenteritis virus (TGEV). The producers of this region proactively share diagnostic results as part of a coordinated effort and PEDV, PDCoV or TGEV have not been reported.

Biosecurity procedures include mandatory change of clothes and shower in / out for employees and visitors, decontamination room for supplies, perimeter fence and a control point for inspection and re-disinfection of all vehicles including feed and livestock transport. Replacement gilts are born and raised within the farm. The company feed mill produces 18,000 tons per month and supplies 250 tons per week to this sow farm from 204 Kms. At the time of the PED outbreak, no chemical mitigants were in used in the feed but 100% of the feed was pelleted.

The farm has 35 feed bins and eight barns. Figure 2 shows the farm layout with two barns for gilt developing (GDU), three gestation barns, two farrowing barns and one barn for culled sows. Farrowing barns have ten rooms with 96 farrowing pens each and piglets are weaned at 24 days of age. Feed bins are located inside the perimeter fence and the feed truck delivers the feed from the outside road around the farm. Table 1 summarizes the frequency of events when people or fomites approach the site or enter the facilities every week.

#### *Timeline of clinical signs and diagnostics*

On December 28<sup>th</sup>, the head veterinarian of the system visited the farm and did not find clinical signs suggesting a disease outbreak in the herd. The next afternoon, the farm staff identified diarrhea and vomiting in farrowing rooms five (18-day-old piglets), twelve (6-day-old-piglets) fourteen (4-day-old piglets) and fifteen (3-day-old piglets). Rectal swabs were collected immediately for diagnostics. All six pools of five rectal swabs tested positive for PEDV PCR while negative for PDCoV and TGEV. Additional samples were collected for PEDV PCR testing as part of the outbreak investigation. Gestation and lactation feed samples were directly collected from the feed bins in plastic bags. Following a previously published method, paint roller pads and extension poles were used to individually sample the interior walls of feed bins (Dee *et al.*, 2014). Pads were immediately placed in plastic bags and distilled water was poured into the bag. An aliquot of the extracted liquid from each bag was tested by PCR. Multiple points of the feed truck delivering the implicated feed were also sampled. No clinical signs had been detected in the GDU or in the gestation barn at this time. PEDV or associated clinical signs were not detected in the nurseries receiving the weaned pigs from this breeding herd, including the group weaned on December 28<sup>th</sup>. On December 30<sup>th</sup>, the whole herd was orally exposed to intestinal

content obtained from acutely affected piglets. A non-production window of 16 days with no piglets in farrowing was created to allow for development of immunity and decontamination of the facilities. The herd reached the transitionally negative status with three consecutive PCR-negative tests (Cano *et al.*, 2016) in the following weeks.

### *Outbreak investigation*

Table 3 shows a chronological timeline of events starting on December 17<sup>th</sup>. Nothing out of the ordinary was reported during the investigated period in terms of vehicles or people entering or approaching the farm. No PED had been detected in the downstream flow or in the neighboring farms; however, the Sonoran Swine Health Committee had reported numerous PED outbreaks in the state in the previous two months. Hot water supply was affected between five and three days before the detection of clinical signs, potentially compromising the quality of staff/visitor showering process; however, the clothing and footwear change outside before approaching the showers and the final change of clothing and footwear in the shower area was confirmed to occur as protocols require.

On December 26<sup>th</sup>, three days before the outbreak, a feed truck identified as #34, delivered feed on bins 24, 25, 26 and 28 supplying lactating feed to the farrowing rooms (Figure 2). Feed truck 34 is dedicated to deliver feed to this farm. Samples from this vehicle, the feed bins and the feed were tested by PCR. First clinical signs were reported in farrowing rooms offering lactating feed from these feed bins filled on December 26<sup>th</sup>. Farm staff had no recollection on any abnormal situation regarding visitors, loading pigs, mortality removal or wildlife during the investigation period.

### *Economic impact*

The largest cost of the PED outbreak consisted on piglet mortality and pregnancy losses including those necessary to create the 16 days non-production window in farrowing. Detergent, disinfectant and equipment utilized during the cleaning and disinfection process of the farrowing rooms, as well as additional labor to complete the task have been included. Porcine epidemic diarrhea killed virus vaccine (Zoetis, Kalamazoo, Michigan, USA) was used two weeks pre-farrowing to boost maternal immunity during the first weeks following the outbreak. The

increased mortality and reduced gain of affected pigs in the downstream flow was not included in this summary (Table 4).

## **Discussion**

The lack of PEDV detection and clinical signs in the area minimizes the likelihood of exposure for area spread, at least from known sources. Similarly, the absence of infection in the downstream flow right up to the time of the outbreak minimizes the likelihood of exposure from weaned pig transport vehicles. The location of the appearance of the first clinical signs, four specific farrowing rooms at the same time, suggests a lower likelihood of exposure from the cull sow transport vehicle and from the mortality removal process. Although, these or other potential routes of virus introduction, such as people or supply entry, cannot be completely eliminated, the detection of PEDV RNA in the lactating feed itself, in the interior walls of the feed bins and in the interior of the auger boom of the feed truck, connected with the location of first clinical signs indicate that the most plausible hypothesis for PEDV incursion to this breeding herd was contaminated feed.

Multiple biosecurity improvements had been made in the feed mill regarding people and vehicle flow, covering of ingredient receiving pit, decontamination of feed trucks and consistent source validation of procured ingredients. However, significant regional exposure of PEDV was present in the weeks preceding this case. A chemical mitigant was included in all feed diets used in breeding herds right after the confirmation of this outbreak. No further PEDV infections have been reported from breeding herds receiving feed from this feed mill. The economic impact of swine enteric coronaviruses, the inherent complexity of feed mill operations and the demonstrated efficacy of some chemical mitigants to reduce the risk of transmission of several infectious agents (Dee *et al.*, 2021) indicate that chemical risk mitigation in breeding herds feed needs to be considered as part of the health and biosecurity program.

## **Conflict of Interest**

All authors disclose any potential sources of conflict of interest.

<b>Event</b>	<b>Frequency (average/week)</b>
Feed deliveries	6
Pig weaning (loading out)	3
Cull sows (loading out)	1
Propane (vehicle and operator stay outside of perimeter fence)	0.5
Supply deliveries (therapeutics, vaccines, office items)	3
Semen deliveries	4
Staff showering in	300

Table 1. Frequency of entry events.

<b>Sample identification</b>	<b>PEDV PCR result</b>	<b>TGEV PCR result</b>	<b>PDCoV PCR result</b>
Pool 1: rectal swabs	POS: Ct 18.48	NEG	NEG
Pool 2: rectal swabs	POS: Ct 19.03	NEG	NEG
Pool 3: rectal swabs	POS: Ct 17.56	NEG	NEG
Pool 4: rectal swabs	POS: Ct 17.44	NEG	NEG
Pool 5: rectal swabs	POS: Ct 18.97	NEG	NEG
Pool 6: rectal swabs	POS: Ct 19.01	NEG	NEG
Gestation feed, bin 20	NEG	NEG	NEG
Gestation feed, bin 21	NEG	NEG	NEG
Gestation feed, bin 22	POS: Ct 38.11	NEG	NEG
Lactation feed, bin 25	NEG	NEG	NEG
Lactation feed, bin 27	POS: Ct 35.87	NEG	NEG
Lactation feed, bin 28	POS: Ct 38.71	NEG	NEG
Paint roller pad GDU bin 9	NEG	NEG	NEG
Paint roller pad Gestation bin 12	NEG	NEG	NEG
Paint roller pad Gestation bin 14	NEG	NEG	NEG
Pain roller pad Gestation bin 16	NEG	NEG	NEG
Paint roller pad Gestation bin 17	NEG	NEG	NEG
Paint roller pad gestation bin 21	POS: Ct 39.25	NEG	NEG
Paint roller pad lactation bin 24	POS: Ct 34.79	NEG	NEG
Paint roller pad lactation bin 25	POS: Ct 37.51	NEG	NEG
Paint roller pad lactation bin 26	POS: Ct 34.91	NEG	NEG
Paint roller pad lactation bin 28	POS: Ct 36.15	NEG	NEG
Inoculum	POS: Ct 19.85	NEG	NEG

Truck 34 / seats	NEG	NEG	NEG
Truck 34 / back mudguard	NEG	NEG	NEG
Truck 34 / front mudguard	NEG	NEG	NEG
Truck 34 brake pedals	NEG	NEG	NEG
Truck 34 interior of auger boom	POS: Ct 37.56	NEG	NEG
Truck 34 exterior of auger boom	NEG	NEG	NEG
Truck 34 cab rubber mats	POS: Ct 36.55	NEG	NEG
Truck 34 wheels	POS: Ct 35.83	NEG	NEG

Table 2. PEDV, PDCoV and TGEV PCR results for samples collected during the outbreak.

<b>Date</b>	<b>Event</b>	<b>Detail</b>
17-Dec-2020	Veterinarian visit	Last pig contact Dec 16th
	No clinical signs	
	Wean pig load out	Truck #11 inspected and disinfected
18-Dec-2020	Cull sows load out	Truck #21 inspected and disinfected
19-Dec-2020	Feed reception	Truck #31 inspected and disinfected
	Wean pig load out	Truck #12 inspected and disinfected
21-Dec-2020	Feed reception	Truck #31 inspected and disinfected
	Wean pig load out	Truck #11 inspected and disinfected
22-Dec-2020	Feed reception	Truck #31 inspected and disinfected
	Supply reception	Truck #01 inspected and disinfected
23-Dec-2020	Cull sows load out	Truck #21 inspected and disinfected

	Wean pig load out	Truck #11 inspected and disinfected
24-Dec-2020	Supply reception	Truck #01 inspected and disinfected
	Hot water failure	Shower quality could had been compromised
25-Dec-2020	Hot water failure	Shower quality could had been compromised
26-Dec-2020	Feed reception	Truck #34 inspected and disinfected
	Supply reception	Truck #01 inspected and disinfected
	Hot water failure	Shower quality could had been compromised
27-Dec-2020	Hot water failure	Shower quality could had been compromised
28-Dec-2020	Cull sows load out	Truck #21 inspected and disinfected
	Wean pig load out	Truck #11 inspected and disinfected
	Feed reception	Truck #33 inspected and disinfected
	Veterinarian visit	Last pig contact Dec 20th
	Supervisor visit	Last pig contact Dec 22nd
	Propane reception	Truck inspected and disinfected
29-Dec-2020	Feed reception	Truck #33 inspected and disinfected
	Clinical signs in suckling piglets	Acute diarrhea in rooms 5, 12, 14 and 15
30-Dec-2020	Diagnostic confirmation of PEDV infection	

	Feedback and farrowing room all-in-all-out process	
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Table 3. Chronological timeline of events

<b>Item</b>	<b>Cost (USD)</b>
Pig mortality	416,121.83
Decontamination	16,909.77
Labor (extra time)	8,152.86
PEDV vaccination	14,510.30
Pregnancy losses	371,206.76
<b>TOTAL</b>	<b>826,901.52</b>

Table 4. Costs associated to the PED outbreak and elimination in the breeding herd.

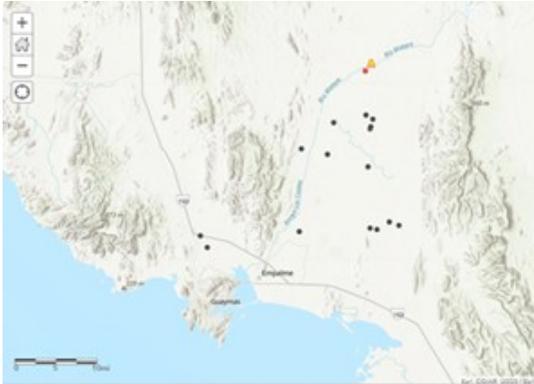


Figure 1. Map representing the location of the study farm (A – red) and the neighboring pig sites (black).

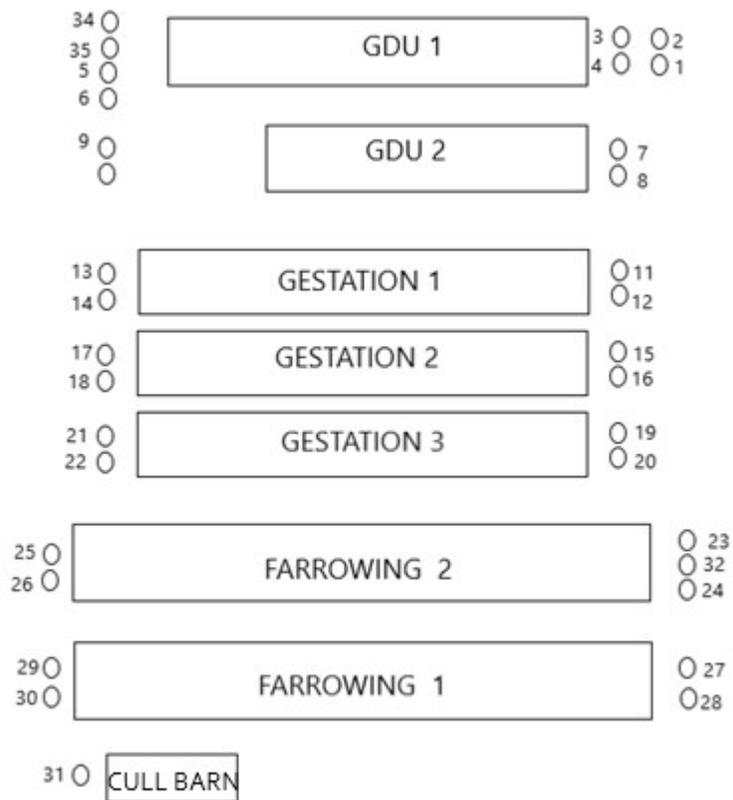


Figure 2. Farm diagram showing barn and feed bin location.

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