Prevalence and factors associated with the contamination of porcine epidemic diarrhea virus during pig transport to slaughterhouses in Colombia

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Abstract

Abstract: Background: Porcine epidemic diarrhea (PED) is an infectious disease that mainly affects neonatal piglets with a morbidity rate of 80%–100% and a mortality rate of 50%–90%. Prior to March 2014, PED was an exotic disease in Colombia with no historical epidemiological data and this study was conducted at the beginning of the spread of the virus in the country. The aim of the present study was to determine the prevalence and factors associated with porcine epidemic diarrhea virus (PEDV) contamination in pig transportation to slaughterhouses in Colombia through a cross-sectional study involving a sample of 518 pig trucks visiting the 32 main slaughterhouses authorized by the regulatory agency of the govern, the INVIMA. Samples were obtained using proportional allocation stratified sampling of pig trucks entering and leaving slaughterhouses for the diagnosis of PEDV through RT-PCR, as well as conducting a survey of the drivers of the sampled trucks. The sampling was performed from June to November 2014, during the first outbreak. Results: The prevalence of PEDV at the time of entry of the plant was 71.8% (CI 95%: 70.8–72.8) versus 70.5% (CI 95%: 69.5–71.5) on exit (p=0.375). Associated factors found to increase the possibility of contamination included: the type of slaughterhouse either national (OR 15.9, CI 95%: 4.9–51.85) or national–exportation (OR 9.0, CI 95%: 2.20–36.91), the zone of highest slaughter (OR 9.05, CI 95%: 2.9–27.63), the non-exclusive use of vehicles for the transport of pigs (OR 3.75, CI 95%: 1.55–9.08) and visiting animal feed plants (OR 13.5, CI 95%: 4.1–44.12). Factors identified to reduce the possibility of contamination included: the cleaning of vehicles, the use of disinfectants on the vehicle and the exclusive of the vehicle for pig transportation. Conclusions: The results showed that the high degree of truck mobilization, with poor biosafety compliance and dissemination characteristics of the virus, facilitated the spread of PEDV throughout the national territory. These factors contributed to establishing the disease as an endemic problem in Colombia. Keywords: coronavirus, epidemiology, PEDV, prevalence, swine.

Background

Porcine epidemic diarrhea (PED) is a common type of viral enteritis in pigs, caused by the PED virus (PEDV), an RNA-type virus that belongs to the order Nidoviridales, family Coronaviridae, subfamily Coronavirinae and genus Alphacoronavirus [1,2]. Diarrhea is the main clinical sign associated with this disease, but other signs are vomiting, anorexia, dehydration and weight loss.

PEDV can infect pigs of any age, from newborns to sows or breeders, but the severity of PED in pigs differs according to age [3]. The incubation period of the virus is approximately 2 days, with a range from 1 to 8 days depending on the field or experimental conditions [4,5]. This virus has the ability to survive in fresh feces for up to 7 days at temperatures between 40ºC to 60ºC with a relative humidity of 30% to 70%. It has been evidenced that PEDV can survive for 14 days in liquid manure at room temperature, even at low infective dose (10^{12} to 10^{18} dilutions) [6]. It was reported that the longest duration of fecal elimination of PEDV was 24 days, with intermittent elimination up to 42 days after inoculation [4].
PEDV is commonly transmitted from animal to animal via the fecal–oral route through contact with infected pigs. It can also be introduced into susceptible farms via pigs, manure, fomites (equipment, boots, clothes, implements, materials that can carry manure) and personnel (hands). Contaminated transport [7–9] and animal feed concentrates [7] can also be important sources of virus transmission.

PED was an exotic disease in the American continent until 2013, when it appeared in the United States, causing the death of 7 million pigs, most of which were suckling pigs [10,11]. Subsequently, it presented in Mexico [12], Peru [13], Canada [14], the Dominican Republic [15] and Ecuador [16]. In Colombia, the first report of PED was in March of 2014 [17].

Following this first report of the disease, the incidence of PEDV and the detailed procedures involved in the transport of pigs to slaughterhouses remained unknown. Changes to pig transportation practices, focusing particularly on slaughterhouses and the management of the pigs in trucks, can help control and even prevent the spread of the disease. The implementation of such measures would be expected to lower the dissemination of PEDV. The cases of PED in Colombia between 2014 and 2015 decreased productivity in the pork sector, increased spending and slowed growth, therefore making the control and prevention of this disease an important focus for producers.

Results

Prevalence of PEDV contamination in trucks

Among the 518 trucks sampled on admission, we found a PEDV contamination prevalence of 71.8% (CI 95%: 70.8% -72.8%) and found a 20.12% of suspicious trucks (CI 95%: 19.12% -21.12%); regarding among the 518 trucks sampled at the exit, we found a PEDV contamination prevalence of 70.5% (CI 95%: 69.5% -71.5%) and we found a 19.5% (CI 95%: 18.5% -20.5%) of suspicious trucks. (See table 1)

Table 1 Prevalence of PEDV contamination in tucks

| At the time of PCR | On entry of the slaughterhouse | On exit of the slaughterhouse |
|-------------------|-------------------------------|-------------------------------|
| RT-PCR result     | n (%)                         | n (%)                         |
| (%)               | 42 (8.1)                      | 52 (10)                       |
| (+)               | 372 (71.8)                    | 365 (70.5)                    |
| (+/%)             | 104 (20.1)                    | 101 (19.5)                    |
| Total             | 518                           | 518                           |

In total, 54.4% of the surveys (282 surveys) were collected at 11 slaughterhouses in Antioquia, 19.3% were collected at 4 slaughterhouses in Cundinamarca, 7.7% were collected at 3 slaughterhouses in the Valle del Cauca and 7.1% were collected at 4 slaughterhouses in the Colombian coffee region (a zone composed of the departments of Caldas, Quindío and Risaralda), which was the area containing the
greatest number of slaughterhouses. Of the total surveys conducted, 59.3% (307) were carried out in national plants, 30.1% (156) in national export-type plants and 10.6% (55) in local plants. Furthermore, 88.6% (459) of the trucks were sampled at plants located in the zone containing the greatest number of slaughterhouses (Figure 1).

With respect to the practices used in slaughterhouses, it was evident that 57% (299) of the trucks were washed at the entrance, for 67.8% (351 trucks) the disinfection bow/back pump system was used, while 25.1% (130 trucks) were not subjected to any disinfection process. In addition, in 44.8% (232) of the trucks, beds were collected and in 59.5% (308) of the trucks, the beds were not disinfected.

*Mobilization practices*: Regarding the number of farms and slaughterhouses visited, it was evident that 68.1% trucks (353 trucks) visited another location. Among the trucks, 96.7% (501 trucks) do not visit the fair, 48.8% (253 trucks) visited a second plant animal food plants and 45.9% (238 trucks) visited only one plant. Among the trucks, 37.3% (193 trucks) were exclusively engaged in the transport of pigs, 62.2% (322 trucks) transported pigs and other products and 0.5% (3 trucks) of the drivers did not answer the question. Of the trucks that transported other products, 9.7% (50 trucks) carried other species of animals.

When evaluating the number of departments visited by the trucks, it was found that 83.4% (432 trucks) were mobilized within the same department. Regarding the frequency of transportation of animals or other products, it was found that 54.8% (284 trucks) journeyed at least once a week.

*Truck cleaning practices:*

Truck cleaning was a practice performed in 93.8% (486) of cases, for 49.2% (255 trucks) this was performed daily. More specifically, 95.8% (496 trucks) underwent cleaning of the body, 92.7% underwent cleaning of the tires (480 trucks) and 80.5% (417 trucks) underwent cleaning of the cabin.

In terms of cleaning practices, it was found that for 96.9% (502 trucks) water was used, for 47.7% (247 trucks) soap was used and for 77.4% (401 trucks) disinfectant was used for cleaning. Regarding the type of disinfectant used, for 12% (62 trucks) oxidant agents were used. The practice of removal of organic matter was reported for 93.8% (486 trucks).

*Transport practices*: In relation to the use of equipment during transportation, 53.9% (279 drivers) reported not using any equipment, 66.4% (344 drivers) stated that they did not wear overalls during transport, 53.9% (279 drivers) do not use boots, 88% (456 drivers) do not use gloves and 91.5% (474 drivers) stated that they did not use any other equipment during transportation. In addition, 81.7% (423 drivers) indicated that they descend from the vehicle on arrival at farms or slaughterhouses.

*Practices on the farm in preparation for transportation:*

The process of washing and disinfecting trucks on the farm was carried out by 89% (461 trucks). The systems used for washing and disinfecting were predominantly a back pump 80.9% (419 trucks) and a disinfection arc 10.2% (53 trucks).
Regarding the practices implemented on entry to the farm, it was found that 79.3% (411 drivers) were not asked to shower and 68.9% (357 drivers) were not asked to use any protective elements (gaiters).

Of the drivers, 49.4% (256 drivers) stated that they were not provided endowment, 60.2% (312 drivers) reported that they were not given overalls, 52.3% (271 drivers) stated that they were not supplied with boots, 82% (425 drivers) did not receive gloves and 82.4% (427 drivers) indicated that they had not received any other protective equipment.

About the use of the truck for the transportation of farm products, 58.9% (305 trucks) indicated exclusive use of the vehicle for the farm, while 40.7% (211 trucks) were not used exclusively for the farm. In addition, 59.5% (308 drivers) reported that the farms had a parking lot and a jetty outside the farm.

Factors associated with the presence of PEDV and the transport practices to slaughterhouses:

Washing practice vs. RT-PCR result: The results showed that in 98% (391) of the trucks there were no changes in the result of RT-PCR at admission and at exit of the slaughterhouse, and only 1% (4) of the trucks that were positive before washing gave a negative result after this process (McNemar value \( p: 0.375 \)). This means that there is no evidence to claim that washing at the slaughterhouse is associated with the PCR result. The factors associated with the contamination of PEDV were: the type of national slaughterhouse or national-exportation slaughterhouse, the washing system, the non-exclusive use of vehicles for the transport of pigs, visits to animal feed plants, drivers alighting the vehicle and not using personal protective equipment, as shown in Table 2.

| Type of Slaughterhouse | OR (CI 95%) | p value |
|------------------------|-------------|---------|
| National               | 42.6 (16.8 -107.7) | 0.000   |
| National - Export      | 26.2 (9.4 -72.8)  | 0.000   |
| Local                  | 1            |         |

| Washing System          | OR (CI 95%) | p value |
|-------------------------|-------------|---------|
| None                    | 1           |         |
| Arc decontamination system / back pump | 5.17 (2.53 - 10.56) | 0.000   |
| Other                   | 0.53 (0.18 - 1.59) | 0.262   |

| Zone of slaughter        | OR (CI 95%) | p value |
|--------------------------|-------------|---------|
| Zone of greater slaughter (> slaughter zone) | 1 |         |
| Zone of lesser slaughter (< slaughter zone)    | 0.04 (0.018-0.086) | 0.000   |

| Visit animal feed plants | OR (CI 95%) | p value |
|--------------------------|-------------|---------|
| Yes                      | 14.3 (5.00-40.9)   | *0.000  |
| No                       | 1            |         |
Use of the vehicle

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Transport of pigs exclusively   | 1       |        |         |
| Transport pigs and other products| 2.41 (1.26-4.60) | 0.006  |         |

Vehicle cleaning

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.089 (0.03-0.23) | 0.000  |         |
| No                              | 1       |        |         |

Cabin cleaning

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.16 (0.08-0.31) | 0.000  |         |
| No                              | 1       |        |         |

Use of disinfectant in cleaning

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.32 (0.16-0.62) | 0.001  |         |
| No                              | 1       |        |         |

Alighting the vehicle

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 3.50 (1.05-11.6) | *0.039 |         |
| No                              | 1       |        |         |

Use of pressurized water in the farm

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.38 (0.15 - 0.95) | 0.033  |         |
| No                              | 1       |        |         |

Use of a back pump in the farm

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.17 (0.08-0.35) | 0.000  |         |
| No                              | 1       |        |         |

Use of protection equipment

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 1       |        |         |
| No                              | 2.63 (1.07-6.46) | 0.028  |         |

Vehicle for the exclusive use of the farm

| Practice                        | Yes     | No     | p-value |
|---------------------------------|---------|--------|---------|
| Yes                             | 0.36 (0.19-0.70) | 0.002  |         |
| No                              | 1       |        |         |

*Fisher’s exact test

Determination of the association between transport-related practices:

Two models were applied to associate the presence of PEDV with transport biosecurity measures. The variables that fitted and provided the best explanation for each model were used. The first model contained independent variables relating to slaughterhouses such as: the type of plant, the area where the slaughterhouse was located and the use of the vehicle, where the reference categories for each were: local plant, area of least sacrifice and transportation of pigs only, respectively. The second model
contained independent variables relating to biosecurity practices in pig mobilization, such as the type of plant, visits to the fair and visits to animal feed plants, where the reference categories for each were: local plant, do not visit fairs and do not visit animal feed plants, respectively.

For the goodness of fit of the models, p-values determined by the Hosmer–Lemeshow test of 0.240 and 0.950 were obtained, indicating good adjustment of the logistic model in both cases. This indicated that the models were calibrated and that the observed results were similar to the expected results (Table 3 and 4).

Regarding the positive detection of PEDV on entry of a slaughterhouse, it was observed that the national plant type (OR 15.9, CI 95%: 4.9–51.85) and national-export plant type (OR 9.0, CI 95%: 2.20–36.91), the highest slaughter area (OR 9.05, CI 95%: 2.96–27.63) and the use of vehicles for transporting pigs and other products (OR 3.75, CI 95%: 1.55–9.08) were factors that increased the possibility of a truck testing positive for PEDV. These data are shown in Table 3.

**Table 3** Model 1: RT-PCR of PEDV vs. plant variables

| Variable                        | Adjusted OR | CI 95%          | for the adjusted OR | p value |
|---------------------------------|-------------|-----------------|---------------------|---------|
| Plant type                      |             |                 |                     |         |
| National                        | 15.95       | 4.90–51.85      |                     | 0.000   |
| National - export               | 9.02        | 2.20–36.91      |                     | 0.002   |
| Slaughter zone (>slaughter zone)| 9.05        | 2.96–27.63      |                     | 0.000   |
| Vehicle use (pork and other products) | 3.75       | 1.55–9.08      |                     | 0.003   |

In addition, positive detection of PEDV at the entrance of slaughterhouses was found to be highly dependent on the following factors related to the movement of pigs: the type of national plant (OR 35.5, CI 95%: 12.7–111.1), national - export plants (OR 33.1, CI 95%: 10.0–109.63) and visits to animal feed plants (OR 13.56, CI 95%: 4.17–44.12). These data are shown in Table 4.

**Table 4** Model 2: RT-PCR of PEDV vs. movement variables
| Variable                              | Adjusted OR | CI 95%       | for adjusted OR | p value |
|--------------------------------------|-------------|--------------|-----------------|---------|
| Plant type                           |             |              |                 |         |
| National                             | 35.58       | 12.71–111.15 |                 | 0.000   |
| National - export                    | 33.16       | 10.03–109.63 |                 | 0       |
| Fair visits (Yes)                    | 2.05        | 0.27–15.78   |                 | 0.49    |
| Animal feed plants visits (Yes)      | 13.56       | 4.17–44.12   |                 | 0.000   |

**Discussion**

The prevalence of PEDV contamination in trucks at the entrance of slaughterhouses was 71.8%, along with 20.1% of trucks being classified as suspicious to PEDV contamination, among the 32 plants sampled across the country. These values are high when compared with the results of a study carried out in the United States on transport trucks [18], where 7.07% (38/537) of trucks tested positive at the entrance of plants and 3.4% (25/757) of the trucks tested for PEDV contamination on discharge of the plant.

Similarly, the prevalence of contamination with PEDV among trucks exiting the slaughterhouses was 70.5% in our study. This percentage was similar to that found at the entrance of the plants, and no significant differences were evident between these two prevalences. Our data indicates that currently in Columbia, approximately two thirds of the trucks leaving slaughterhouses are contaminated with PEDV, possibly due to deficiencies in the biosafety process during transport and non-compliance with prevention measures for trucks in slaughterhouses. This may be largely because washing and disinfecting in slaughterhouses will remain voluntary until decree 1500/2007 comes into force.

The lack of a significant difference between the detection rates of PEDV in trucks on entering and leaving slaughterhouses may indicate that current biosecurity measures are failing both in plants and in farms; however, further studies are needed to affirm this. Efforts are being made in the implementation of biosafety measures by plants to mitigate the spread of contamination, although these data indicate that at present they do not go far enough. The processes of cleaning, washing, disinfection and drying are not applied with the required rigorousness to ensure the desired effects. Therefore, transport has become one of the main risk factors for dissemination.

Regarding the type of slaughterhouse, we found that the national or national-export-type slaughterhouses were risk factors for PEDV contamination in trucks versus local plants. A possible explanation for this is the high frequency of trucks entering slaughterhouses where a large number of animals are sacrificed, which increases the risk of contamination of the vehicles. The areas of greater slaughter also showed greater levels of PEDV contamination, which may be related to the large concentration of national and
national-export-type plants in the area and the resulting demand for a higher flow of vehicles, which in turn facilitates the spread of the virus.

This association between the type of plant and PEDV contamination is consistent with the findings of Lowe et al. 2014 [9] who reported that trucks that are mobilized in slaughterhouses where there are more contaminated trucks present a higher incidence of contamination.

Visits to animal feed plants by pig transporting trucks increases the possibility of contamination with PEDV (OR 14.3, CI 95%: 5.00–40.94). This is in accordance with the findings of Morrison & Goede (2013) who reported that farms with higher numbers of feed trucks had higher PEDV positivity [19]. In addition, collection points may be a source of truck contamination, allowing for the propagation of diseases over wide distances as reported by Lowe et al. and O’Dea et al. 2016 [9,20].

These findings were also consistent with those reported by Sasaki et al. (2016) [21], who showed that an increase in the number of feed trucks visiting a farm increased the occurrence of PED to 16% (OR 1.16). In general, the farms in Colombia do not produce their own pig feed but instead store feed concentrate in a warehouse. Feed is provided weekly, increasing the flow of trucks coming from the animal feed plants in which the biosecurity measures may be insufficient or not be applied in farms. This is consistent with the finding that vehicles that transport pigs and other products are more likely to be contaminated with PEDV compared with those that only carry pigs (OR 2.41, CI 95%: 1.27–4.67).

In contrast to the report by Lowe et al. (2014) [9] that considered fairs as a source of contamination with PEDV, fairs were not found in the present study to be a factor that increased the possibility of contamination. This is due to the fact that, unlike American fairs, the fairs in Colombia involve small numbers of pigs from the surrounding areas, bred in a non-technical manner, limiting the movement of animals over short distances. In the present study, it was found that of the trucks dedicated to the transport of pigs few visited fairs; however, the risk of contamination of animals due to poor sanitary conditions and inadequate biosafety cannot be ruled out.

The results of this study suggest that prevention measures such as washing trucks with water, soap and disinfectant using a standardized protocol should become common practice in slaughterhouses. In addition, limiting the need for drivers to alight vehicles and reinforcing practices such as the use of sprinklers and the washing of staff’s boots every time they come into contact with another truck, are further measures that should be implemented.

The main factors that are able to reduce PEDV infection were identified with our bivariate model. These factors included: the location of the plant in the area of least slaughter, general cleaning of the vehicle, cleaning of the cabin, the use of disinfectant in cleaning, the use of high pressure water during washing on the farm, the use of a back pump on the farm and the exclusive use of the vehicle by that purpose only. Our findings were consistent with those of Lowe et al. (2014) who indicated that control measures such as segregation, hygiene and disinfection were important in limiting the spread of PEDV. The processes of cleaning, washing and disinfection should be governed by rigorous monitoring and
compliance, ensuring that the processes are managed in the correct order, for example: withdrawal of organic material, the use of pressurized water, soap, drying and disinfection. Bowman et al. (2015) [22] found that cleaning and surface preparation are the basis of any disinfection protocol since organic material is known to inactivate many types of disinfectants, whereas the presence of feces did not affect the total number of positive RT-PCR results.

It should also be noted that there are other important factors in the effectiveness of disinfection such as the concentration, the contact time and the temperature. Sasaki et al. (2016) [21] found that the probability of a positive PEDV result increased 2.5 times in farms that did not allow contact time of more than 20 min when applying disinfectant (OR: 2.63).

The characteristics of PEDV that facilitate its dissemination include the course of infection, the volume of secretions or excretions (feces and vomiting), the age of animals, the type and severity of clinical signs and the type of accommodation and airflow. It is worth noting that the amount of PEDV released per gram of feces in acutely-infected pigs, as well as the volume of liquid found in the diarrheal material, can generate increased amounts of PEDV RNA [23], demonstrating the importance of implementing cleaning, washing and disinfection protocols in a strategic and meticulous manner.

To ensure that the cleaning and disinfection of vehicles is effective in controlling the propagation of PEDV, it is important that the bodywork in which the pigs are housed in the truck is lined in a smooth, washable material, either metal or some similar material. This variable was not analyzed in the present study but should be considered in future investigations. In Colombia, the loading area of most trucks comprise wooden walls, irregular surfaces and metal stakes, and this construction does not guarantee adequate cleaning and disinfection. The presence of irregularities in the surfaces allows viruses to be harbored, which decreases the effectiveness cleaning practices [22].

The exclusive use of vehicles on farms is a factor that decreases the possibility of contamination with PEDV and should therefore be considered a key aspect of biosecurity practices. Some producers do not own a vehicle for economic reasons. Instead, they use rental vehicles, which are not therefore used exclusively on a single farm, thereby increasing the risk of PEDV contamination.

**Conclusions**

The high degree of truck mobilization, coupled with poor biosafety compliance and the dissemination characteristics of the virus, have facilitated the spread of PEDV throughout the national territory, resulting in PED becoming an endemic disease in Colombia. The present work provides an overview of the practices currently undertaken in the transport of pigs in Colombia. More in depth studies are now needed to evaluate the implementation of internal and external biosecurity practices on pig farms. Standardization of the biosafety protocols for slaughterhouses, pigs transport and farms, is needed to meet the country's sanitary regulations.
Methods

The present study is a cross-sectional observational study. The main objective of this study was to determine the prevalence of PEDV contamination in trucks transporting pigs to slaughterhouses. We aimed to find association between pig transportation practices from farms to slaughterhouses and the presence of PEDV in the trucks through sampling applied to trucks.

Sampling:

Sampling stratified by proportional allocation was carried out on 3,143 pig transporting trucks entering the 32 main slaughterhouses approved by the National Drug and Food Surveillance Institute (INVIMA) in 2014. These slaughterhouses account for 73% of the animals slaughtered legally in the country [24]. To calculate the sample size, the following parameters were taken into account, 1% prevalence, 95% confidence level and 30% expected loss percentage, resulting in a sample size of 518. The collection of samples was carried out in the second semester of 2014 during a one-week period in each of the slaughterhouses. The samples were distributed throughout the week according to the order of arrival of the trucks. Sampling was carried out in the trucks at the point of entry and exit of the slaughterhouses, and the environmental samples were analyzed by RT-PCR.

Techniques for collecting and obtaining information:

The present study included two primary information sources:

- Environmental samples analyzed by RT-PCR to determine the presence of PEDV. The samples were preserved and sent on the same day as collection to the Laboratory of Microbiology of the Faculty of Veterinary Medicine of the National University of Colombia, Bogotá.
- A survey was completed by the drivers of the trucks sampled in the slaughterhouses. The survey was used to characterize the practices employed for the transport of animals and to identify factors associated with PEDV contamination during the transport of animals.

Processing techniques:

RNA extraction was performed with the RNeasy extraction kit (QIAGEN®). Then, cDNA synthesis was accomplished using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s recommendations. The N and S genes were amplified by RT-PCR and a standard curve was constructed. The primers and probes for hydrolysis (TAQMAN labeled with FAM), as well as a synthetic positive control, were designed following the protocol developed by the Laboratory of Veterinary Diagnosis of the University of Minnesota. The positive control included regions of the N and S genes. A thermocycler (Roche®) was used for the PCR. All positive samples for gene N were re-checked for amplification of S (spike) and M genes. For the interpretation of the results, the crossing point (cp) value for the N gene was categorized as follows: positive, cp <35; suspicious, cp 35–40; negative, cp = 0. Statistical analysis:
We performed a descriptive analysis of the variables through frequency distributions, measures of central tendency and dispersion. The prevalence of PEDV contamination in the trucks at the entrance and exit of the slaughterhouses was estimated together with a characterization of the practices related to pig transport to slaughterhouses.

To determine the association between the dependent variable RT-PCR PEDV on admission of the slaughterhouse and independent variables, the chi-squared test for association or Fisher’s exact test was used and the association magnitude was estimate with OR 95% CI. The differences between the prevalence of PEDV on admission with the prevalence of PEDV to exit of the slaughterhouse were compared through a McNemar test.

A multivariable logistic regression model was used to determine the variables that help explain the probability of contamination with PEDV and for this model the variables that were taken into account were those that were statistically significant in the previous analyses and showed epidemiological importance in the transmission of the virus. Consequently, the possible effects of interaction and confusion were studied. Finally, the model that best explained the event probability was selected.

For the control of biases and errors, aspects such as sample type and sample size, clear inclusion and exclusion criteria, question writing, expert review and standardized training of interviewers were taken into account.

The software used for the analyses was EPI INFO 7 and SPSS 22, with a license provided by Rosario University.

**Abbreviations**

INVIMA: National Drug and Food Surveillance Institute.

OR: Odds Ratio

PED: Porcine Epidemic Diarrhoea

PEDV: Porcine Epidemic Diarrhoea virus

RT-PCR: Reverse Transcriptase Polymerase Chain Reaction

**Declarations**

**Availability of data and materials:**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.
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Contributions:

PP and PC: Designed and implemented the research, analyzed the data and wrote the manuscript.

GH: Statistical consultant, analyzed the research data and participated in the preparation of the publication.

CT: Methodological adviser, analyzed the research data and participated in the preparation of the publication.

CZ: Thematic adviser, conceptualized the study, designed and implemented the research and participated in the preparation of the publication.

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Ethics declarations
**Ethics approval:**

This study was approved by the bioethics committee of the faculty of veterinary medicine of the National University of Colombia, did not have intervention measures in the pigs since the sampling was carried out in the pig’s trucks at the point of entry and exit of the slaughterhouses. The objective of the study was explained verbally to the truckers from whom verbal consent was obtained to answer the survey and participated voluntarily.

**Consent for publication:**

Not applicable

**Competing interests**

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**Figures**

**Figure 1**

Distribution of slaughterhouses according to type and zone of slaughter classification. Design by the authors.