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Technical note

Evaluation of sow seroconversion with the use of inoculum at different doses and vehicles against porcine epidemic diarrhea

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Abstract:

Porcine epidemic diarrhea (PED) is a highly contagious enteric disease of pigs, which has caused great economic losses to the swine industry worldwide. The known measure for PED control prior to the development and launch of vaccines in 2017 in Mexico, was "feedback" or "liquefaction". It was a widely used measure during the PED outbreak in 2013; however,

there is no homogeneity in its use among the various authors who recommend it. Currently, several studies have experimented with other types of prophylaxis, such as oral immunization with PED virus obtained from cell culture isolation, which allows quantification of the infectious virus and ensures that only the virus, and no other agent, is being used as inoculum. The objective of the present study was to compare the time of seroconversion in sows inoculated with the quantified virus with four different vehicles (milk, wheat, direct, and water) and different doses of vehicle (1 ml, 2 ml, and 3 ml) at different pregnancy stages and with a different number of farrowings. The study was conducted at CEIEPP, a full-cycle farm with 170 females. The present study showed that the vehicles with the best results were the inoculum with water and the direct inoculum combined with the 1 ml dose, as the combination of these vehicles and an inoculum dose resulted in seroconversion in more than 90 % of the sows from the second week post inoculation.

Key words: Inoculum, Porcine Epidemic Diarrhea, Seroconversion.

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Porcine epidemic diarrhea is a highly contagious enteric disease in pigs caused by the Porcine Epidemic Diarrhea (PED) virus[1], a single-stranded positive-sense enveloped RNA virus that belongs to the genus *Alphacoronavirus*, family *Coronaviridae*[2]. It infects mainly the epithelial cells of the intestine of pigs, causing atrophy, necrosis, and detachment of the intestinal villi, which affects nutrient absorption[3], causing problems such as watery diarrhea, acute vomiting, anorexia, extensive dehydration, imbalanced blood electrolytes, and weight loss in pigs of all ages[4]. It is especially severe in seronegative piglets, among which the morbidity and mortality rate is up to 100 %[5]. The virus was identified in the 1970s in the United Kingdom and Belgium. In 1976 a similar epidemic occurred in several European countries, and was named EVD2; since then, the disease has been reported in many other countries[6,7]. In October 2010, a highly pathogenic variant of the PED virus strain was identified in China, and later in May 2013, this same variant caused disease in the U.S.A, from where it spread to Canada and other countries in Central and South America, including Mexico[6,8]. It was estimated that, in the U.S.A, the PED outbreak affected more than 8,400 farms[9], killing more than 7 million pigs equivalent to 10 % of their swine population[10], with losses of $1.1 billion dollars for producers[11].

The known measure for control of the disease has been "feedback" or "liquefaction; however, there is no homogeneity in the use of this technique[12,13]. It consists in the ingestion of small intestine, gastric contents, or diarrhea of pigs showing clinical signs of PED in the first 6 to

12 h after the onset of the disease. It can also be prepared from the intestinal scraping of slaughtered piglets that had diarrhea in their last 4 h and can be mixed with evaporated milk, trying to achieve a liquid (not pasty) consistency[14]. Another type of prophylaxis is oral immunization with PED virus obtained from cell culture isolation, which allows quantification of the infectious virus and calculation of a protective dose, and ensures that only the virus —and not any other agent— is being used as inoculum[15,16]. Most of the vaccines marketed in Mexico are live attenuated or inactivated vaccines that use strains similar to CV777 and are administered orally[15], and they are recommended for use in pregnant females in the 2nd and 3rd week prior to farrowing[16]. Stress has been observed with the use of the vaccine in pregnant sows[17], and its effectiveness is still under evaluation.

The objective of the present study was to compare the time of seroconversion in sows inoculated with quantified viruses with four different vehicles (milk, wheat, direct, and water) and different doses of vehicle (1 ml, 2 ml, and 3 ml), at different pregnancy stages and with parity of the sows.

The study was conducted in a semi-technified full-cycle farm located in the northeast of the State of Mexico, with an average of 170 Landrace x Yorkshire females in the inventory.

The method for animal handling was submitted to and approved by the Institutional Subcommittee for the Care and Use of Experimental Animals (SICUAE) of the Faculty of Veterinary Medicine and Zootechnics FMVZ CU-UNAM, with approval number MC-2020/4-4.

The virus was obtained from the Virology Laboratory of the Faculty of Veterinary Medicine and Zootechnics, Universidad Nacional Autónoma de México (UNAM), identified in the Gen Bank with the accession number KM044335.1, which has a titer of 1x108 DICC50%/ml[18,19].

Sows were immunized with 12 different intervention protocols against PED on January 26, 2018. The variants of this protocol were to administer the quantified virus in four different vehicles, which were: milk, wheat, water, and without a vehicle, i.e., direct (viral suspension in a culture medium), with three different doses of each vehicle, 1 ml, 2 ml and 3 ml (Table 1). The inoculation protocol was performed on all sows on the farm in the form of a sheet.

Table 1: Summary of experimental design

Group	Vehicle	Dose	Virus concentration ($DICC_{50\%}$/ml)	No. of animals
1	Milk	1 ml	$1x10^8$	8
2	Milk	2 ml	$2x10^8$	6
3	Milk	3 ml	$3x10^8$	10
4	Wheat	1 ml	$1x10^8$	8
5	Wheat	2 ml	$2x10^8$	8
6	Wheat	3 ml	$3x10^8$	10
7	Water	1 ml	$1x10^8$	8
8	Water	2 ml	$2x10^8$	8
9	Water	3 ml	$3x10^8$	10
10	Direct	1 ml	$1x10^8$	7
11	Direct	2 ml	$2x10^8$	8
12	Direct	3 ml	$3x10^8$	9

After the administration of the different intervention protocols, blood samples were taken from the sows at wk 2, 4, 8, and 13, in tubes for blood sample collection without additive. These samples were transferred in ice boxes at 2 to 8 °C to the Virology Laboratory of the Faculty of Veterinary Medicine and Animal Husbandry of the UNAM. Samples were processed using the ELISA technique of the ID Screen® PEDV Indirect Kit (ID-VET), according to the supplier's specifications, ID Screen® PEDV Indirect - IDVet[18]. The ELISA test was used to monitor the different immunization protocols of the females and to identify the females that exhibited seroconversion.

The seroconversion data were analyzed with descriptive statistics and with the Kaplan-Meier survival curve and the Mantel-Cox log-rank test, respectively. A value of $P<0.05$ was considered statistically significant. Table 2 shows intervention protocols.

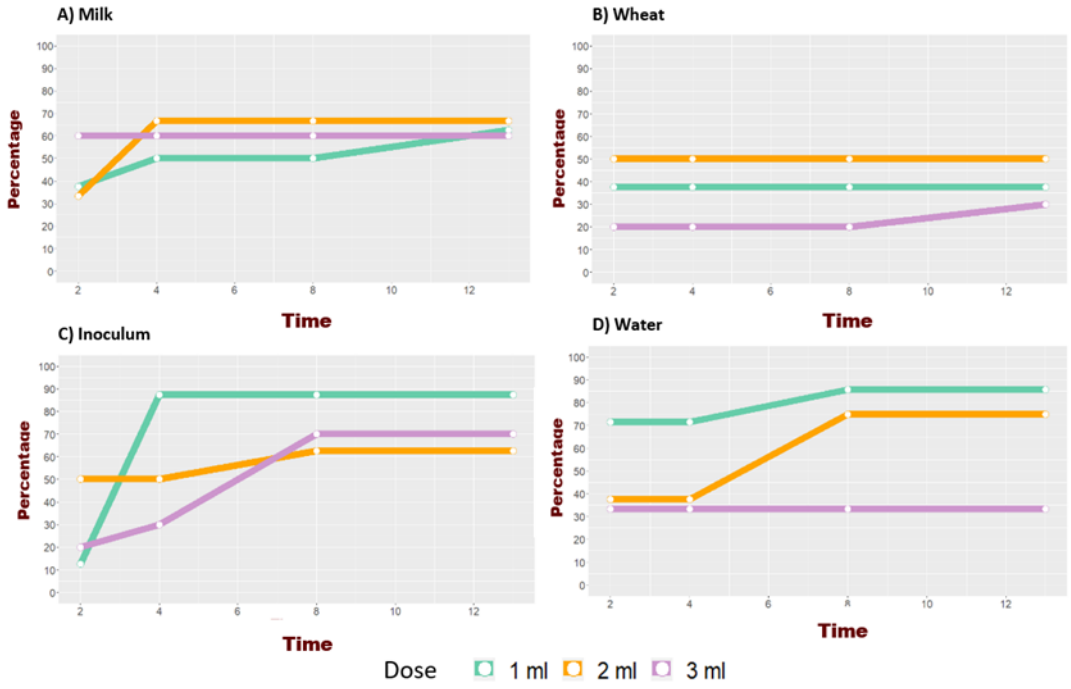
Table 2: Comparison groups of different vehicles and doses using the Kaplan-Maier survival curve test and the Mantel-Cox log-rank test

Groups	Comparison
1	1 ml milk, 2 ml milk, and 3 ml milk
2	1 ml wheat, 2 ml wheat, and 3 ml wheat
3	1 ml direct, 2 ml direct, and 3 ml direct
4	1 ml water, 2 ml water, and 3 ml water
5	1 ml milk, 1 ml wheat, 1 ml direct, and 1 ml water
6	2 ml milk, 2 ml wheat, 2 ml direct, and 2 ml water
7	3 ml milk, 3 ml wheat, 3 ml direct, and 3 ml water

Each ml of vehicle contains 1x108 DICC50%/ml of the PED virus.

It was observed that two vehicles showed the best seroconversion response with the 1 ml dose (direct vehicle and vehicle with water), while the vehicle with the lowest seroconversion was wheat (Figure 1).

Figure 1: Percentage of seroconversion of the four different inoculum vehicles (water, direct, milk, and wheat) at three different doses, at four different times after inoculum administration

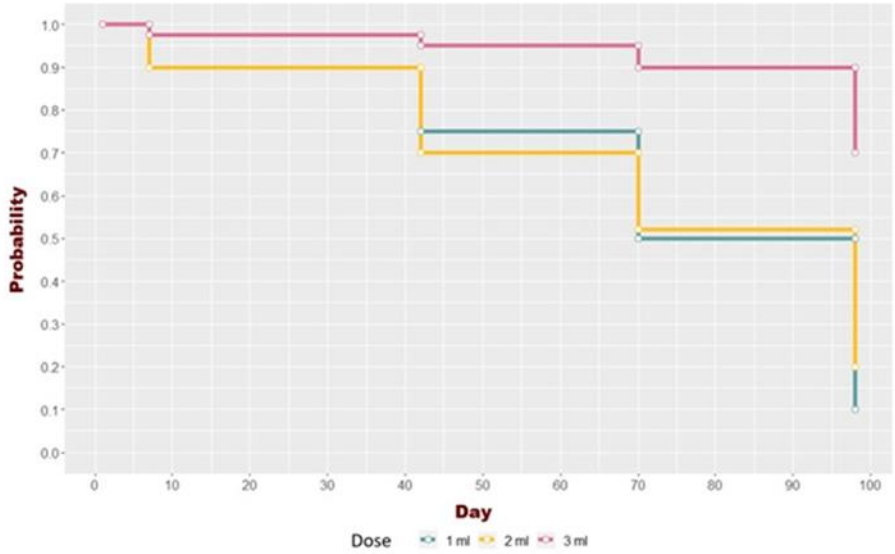


Differences in survival time were found only in groups 2 and 7: Mantel-Cox, χ^2 = 12.56, 2 gl; *P*= 0.0019 and χ^2 = 15.75, 3 gl; *P*= 0.0013 (Figure 2), respectively, The water and milk vehicles showed a seroconversion above 45 % and 60 % at wk 2 and 6, respectively.

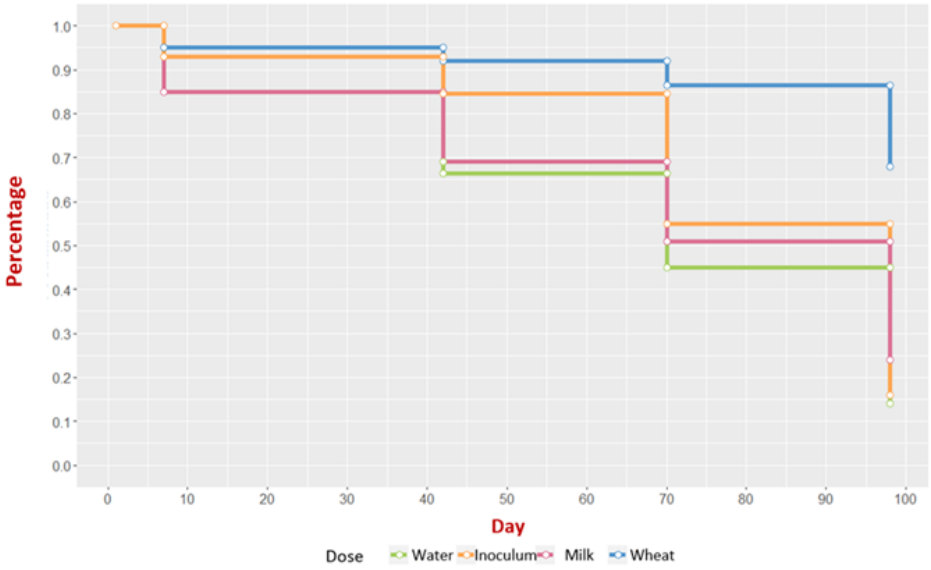
The use of different vehicles and doses has been implemented and described by various authors[12,13,20], although the effectiveness of one over the other has not been proven. In this work, the water and direct inoculum with 1 ml were the best. However, there was no difference between the different vehicles and doses, with the exception of the wheat inoculum and the three-milliliter doses with different vehicles (Figure 2).

Figure 2: Kaplan-Meier survival curve, A) curve of domestic pigs inoculated with the wheat vehicle, days after inoculation. B) domestic pigs inoculated with 4 different vehicles at 3 ml doses, days after inoculation

A



B



In serological assays, on average, antibodies are first detected in serum 6 to 14 d after contact with the virus[21]. The present work revealed that a small percentage of sows already exhibited PED antibodies from the second week on, while most of the sows exposed to the PED virus exhibited antibodies during the 10th and 14th wk. It was also found that the seroconversion

effect differed according to the vehicle or dose at which the sows were exposed to the PED virus.

Antibody levels in sows naturally infected with the PED virus remain high for up to six months, although the recovered fecal levels disappear within one to two months' post-infection[22].

Immunization of pregnant sows is reportedly important for controlling PED and reducing piglet deaths[23,24]. 100 % of piglets from sows immunized at 57 to 59 d of gestation have been reported to survive[24], while those exposed at 19 to 22 d of gestation and those exposed after 96 d of gestation showed piglet survival rates of 87 and 56 %, respectively[25].

The efficacy of the vaccine and of the intramuscular booster depends on the induction of IgA antibody memory B-cells in sows previously exposed to the field virus or orally immunized[26]. This work utilized oral immunization protocols with different vehicles and different doses.

Certain studies highlight the better performance of oral inoculation (including feedback) versus intramuscular inoculation. However, both protocols can be inefficient as a consequence of: 1) the lack of standardized feedback protocols; 2) the poor ability of current intramuscular vaccines to induce lactogenic immunity; 3) the antigenic difference between the vaccine and the epidemic strains, and 4) the potential and continuous re-infection with PEDs due to the use of feedback[27].

The present study showed that the vehicles with the best results were the inoculum with water and the direct inoculum combined with the 1 ml dose, as the combination of these vehicles and doses with the inoculum resulted in seroconversion in more than 90 % of the sows from the second-week post-inoculation.

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