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Brief communication

Inactivation of porcine epidemic diarrhea virus in contaminated swine feed through inclusion of a dry lactic acid-based product

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Summary

Survivability and infectivity of porcine epidemic diarrhea virus within complete feed was tested in the presence or absence of a dry lactic acid-based feed acidifier product (Guardicate) at levels of 0.75%, 1.0%, or 1.5%. The virus was inactivated, and contaminated feed did not cause infection at all three inclusion rates.

Keywords: swine, diarrhea, virus, lactic acid, feed

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Resumen - Inactivación del virus de la diarrea epidémica porcina en alimento porcino contaminado mediante la inclusión de un producto seco a base de ácido láctico

Se evaluó la capacidad de supervivencia e infectividad del virus de la diarrea epidémica porcina en alimento terminado en presencia o ausencia de un producto acidificante seco a base de ácido láctico (Guardicate) a niveles de 0.75%, 1.0%, o 1.5%. El virus fue inactivado y el alimento contaminado no causó infección en los tres porcentajes de inclusión.

Résumé - Inactivation du virus de la diarrhée épidémique porcine dans de la moulée porcine contaminée par inclusion d'un produit sec à base d'acide lactique

La capacité de survie et le potentiel infectieux du virus de la diarrhée épidémique porcine dans de la moulée complète furent testés en présence ou absence d'un produit acidifiant sec à base d'acide lactique (Guardicate) à des concentrations de 0.75%, 1.0%, ou 1.5%. Le virus fut inactivé, et la moulée contaminée ne causa pas d'infection quel que soit le taux d'inclusion du produit.

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded, positive-sense RNA virus belonging to the order Nidovirales, the family Coronaviridae, and the genus *Alphacoronavirus*.[1] Following detection in the US swine population during May 2013, the virus spread rapidly across the country.[2] In 2014, contaminated feed was proposed as a possible risk factor for PEDV spread between farms and possibly countries. Initial reports indicated the ability of PEDV to survive in dry feed for 7 days and in wet feed for 28 days when stored at room temperature.[3] Proof of concept that contaminated complete feed could serve as a route of PEDV transmission to naïve pigs was published[4] and the minimum infectious dose in complete feed has been calculated[5] as 5.6×10^1 median tissue culture infectious doses/mL (Cycle threshold [Ct] = 37).

Given concerns regarding the transmission of PEDV via ingestion of contaminated feed, there has been considerable effort to identify commercially available products that can be incorporated into the feed allowing for a disease mitigation effect. The use of chemical feed mitigants such as formaldehyde-based products have been shown to effectively reduce the risk of PEDV survivability and infectivity in contaminated feed.[6,7] However, adoption of commercial formaldehyde-based products by US swine producers has been limited given worker safety concerns and the need for specialized feed mill equipment to administer liquid product. Medium chain fatty acid blends at 1% to 2% of the diet have been shown to enhance RNA degradation of PEDV in swine feed and ingredients and reduce infectivity[7]; however, commercial adoption is limited due to cost. Another potential candidate to mitigate viral risk in feed are the organic acids, possibly through the reduction in pH in the gastrointestinal tract.[8] Therefore, the objective of this study was to determine the impact of a commercial lactic acid (LA)-based

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product (Guardicate; Alltech) on the survival of PEDV in complete feed and whether inoculated, LA-treated feed could prevent PEDV infection in pigs.

Methods

All protocols involving animals were reviewed and approved by the South Dakota State University (SDSU) Institutional Animal Care and Use Committee, study approval No. 15-121A.

Diagnostic procedures

All diagnostic testing, including reverse transcription-polymerase chain reaction (RT-PCR), and virus isolation (VI) was conducted using protocols developed and validated by the SDSU Animal Disease Research and Diagnostic Laboratory.[4] Samples were submitted by code to the laboratory thereby blinding personnel to treatment identity.

Experiment 1: Survivability in feed

Experiment 1 was designed to evaluate the survivability of PEDV in corn and soy-based complete feed treated with a dry LA-based product. A 454-g sample of complete feed was acquired from a commercial swine herd and tested by RT-PCR to document a PEDV RNA-negative status prior to use. The experimental design included three treatments: Feed + 0.75% LA, Feed + 1.0% LA, and Feed + 1.5% LA. Thirty grams of feed was used for each treatment and 2 replicates were assigned per treatment. To promote proper mixing, each feed sample and its designated quantity of LA were combined, inverted 10 times, and vortexed for 2 minutes. Immediately after, the pH of each sample was measured using an Orion Star A100 Series pH meter (Thermo Fisher Scientific).

Following treatment, all complete feed samples were inoculated with 2 mL PEDV (total dose 4×10^5 fluorescent focus units (FFU); Ct = 14.46) and mixed as previously described. This quantity of PEDV was selected in an effort to provide a final mean Ct value in complete feed of approximately 25 (range: 19-30) following mixing, based on data from actual field cases of PEDV-contaminated feed and challenge levels used in published studies.[4,6] In addition, a positive control, a 30-g control sample of feed (PEDV RNA-positive by RT-PCR, no LA), a negative control (PEDV and LA-negative), and a stock virus control (total dose 4×10^5 FFU; Ct = 14.46) were included in the design. Samples were incubated for 24 hours at 20°C and then tested by PEDV RT-PCR and PEDV VI.

Experiment 2: Infectivity

Swine bioassay. The purpose of the swine bioassay was to determine whether viable PEDV was present in any feed ingredient sample that had tested positive for PEDV RNA on RT-PCR but negative for PEDV on VI. This study was conducted in a Biosafety Level 2+ room at the SDSU Animal Resource Wing (ARW).

Facilities and source of animals. Animals (n = 15; 7-day old piglets) were sourced from a PEDV-naïve herd and were tested on arrival to the ARW via blood sampling and collection of rectal swabs from each pig. Prior to animal arrival, all rooms (walls, ceilings, floors, and drains) were monitored for the presence of PEDV RNA by RT-PCR using sampling procedures previously described.[4,6] Five stainless steel gnotobiotic units measuring 0.6m wide x 1.2m long x 0.6m high were used to house the piglets. Units were divided into 3 semi-isolated housing units, allowing for 3 piglets per unit with individual feeding arrangements. Treatment or control groups were housed in one of five units: unit 1 = 0.75% LA treatment, unit 2 = 1.0% LA treatment, unit 3 = 1.5% LA treatment, unit 4 = positive control, and unit 5 = negative control. Flooring consisted of an open weave rubberized mat on a perforated stainless-steel grate raised 10 cm for waste collection. Each unit was covered with an inflatable 20 mil plastic canopy and fitted with 2 pair of dry-box gloves for feeding and procedures inside the canopy. Each canopy was secured and sealed to the unit with duct tape and ratchet straps. Ventilation was supplied by an electric fan maintaining sufficient positive pressure inside the canopy to keep it inflated above the unit. Incoming and outgoing air to each unit was HEPA-filtered. Each unit was initially sterilized using 47% aerosolized formalin, which was allowed to dissipate for 2 weeks prior to introduction of the animals. All incoming and outgoing materials needed during the study (eg, swabs, injectable medication, blood collection supplies) were passed through an air-tight stainless-steel port and sterilized using 5% peracetic acid before entering or exiting the port.

Preparation of bioassay inocula. For preparation of the inocula, new (30 g) samples of complete feed and varying amounts of LA (0.75%, 1.0%, or 1.5%) were mixed with 50 mL of sterile phosphate-buffered saline in a 250 mL centrifuge tube, inverted 10 times to mix, and vortexed for 2 minutes. Three inocula were prepared for each of the 3 concentrations of treated feed and 3 were prepared for each control group for a total of 15 inocula, one for each pig in the experiment. Each suspension was centrifuged at 5200*g* for 15 minutes, and the supernatant decanted and tested by RT-PCR prior to piglet inoculation. Each pig in the unit received 1 mL of the designated inoculum orally via syringe and was observed for 7 days. The 3 positive-control piglets were inoculated orally with a designated sample of feed spiked with 2 mL PEDV (total dose 4×10^5 FFU; Ct = 14.46) and the 3 negative-control piglets were inoculated orally with a designated sample of feed spiked with 2 mL sterile saline.

Piglet monitoring and testing. The ARW personnel inspected animals daily for clinical signs of PED. Showers were taken upon entry to the rooms and room-specific coveralls, footwear, hairnets, gloves, and P95 masks (3M) were worn. Rectal swabs (Dacron swabs, Fisher Scientific) were collected from each pig on days 0, 2, 3, 5, and 7 post inoculation (PI). Swabs were tested by RT-PCR for the presence of PEDV RNA. At the end of the 7 days, all pigs were humanely euthanized with intravenous sodium pentobarbital.

Results

Experiment 1: Survivability in feed

Porcine epidemic diarrhea virus RNA was detected by RT-PCR across all feed samples spiked with PEDV (Table 1). The negative-control sample was RT-PCR negative. Both the complete feed positive-control samples and the virus stock controls were VI positive at 24 hours PI. The complete feed negative-control samples and all LA-treated feed samples across all 3 inclusion rates were VI negative at 24 hours PI. Finally, while not analyzed statistically, addition of the LA product appeared to reduce pH of the feed on a numerical basis, as compared to control samples.

Table 1: Summary of survivability diagnostic data 24 hours post PEDV inoculation (experiment 1)

	Ct		FFU		pH	
Treatment	**Replicate 1**	**Replicate 2**	**Replicate 1**	**Replicate 2**	**Replicate 1**	**Replicate 2**
Positive control	19.6	19.6	7680	7680	5.8	5.8
Negative control	38.0	38.0	< 20	< 20	6.7	6.7
Virus stock	14.4	14.4	440,000	440,000	8.1	8.1
LA - 0.75%*	25.8	25.9	< 20	< 20	5.5	5.4
LA - 1.0%†	27.1	28.9	< 20	< 20	5.0	5.1
LA - 1.5%‡	30.6	31.1	< 20	< 20	5.0	4.9

* Samples in each replicate contained Guardicate at a 0.75% level.
† Samples in each replicate contained Guardicate at a 1.0% level.
‡ Samples in each replicate contained Guardicate at a 1.5% level.
PEDV = porcine epidemic diarrhea virus; Ct = cycle threshold; FFU = fluorescent focus units; LA = lactic acid.

Table 2: Summary of PEDV bioassay data (experiment 2)

	Inoculum	Ct values*						
Treatment	**pH**	**Inoculum**	**Day 0**	**Day 2**	**Day 3**	**Day 5**	**Day 7**	**Diarrhea†**
Positive control								
Sample 1	5.8	**21.68**	38.0	**34.4**	**28.5**	**23.6**		
Sample 2	5.6	**21.55**	38.0	**32.6**	**29.5**	**25.3**	Not tested	**positive**
Sample 3	5.8	**21.05**	38.0	**34.1**	**30.1**	**29.6**		
Negative control								
Sample 1	6.0	38.0	38.0	38.0	38.0	38.0	38.0	
Sample 2	6.0	38.0	38.0	38.0	38.0	38.0	38.0	negative
Sample 3	5.9	38.0	38.0	38.0	38.0	38.0	38.0	
LA - 0.75%‡								
Sample 1	5.5	**25.31**	38.0	38.0	38.0	38.0	38.0	
Sample 2	5.6	**24.83**	38.0	38.0	38.0	38.0	38.0	negative
Sample 3	5.4	**26.78**	38.0	38.0	38.0	38.0	38.0	
LA - 1.0%§								
Sample 1	5.2	**26.50**	38.0	38.0	38.0	38.0	38.0	
Sample 2	5.3	**27.23**	38.0	38.0	38.0	38.0	38.0	negative
Sample 3	5.1	**26.14**	38.0	38.0	38.0	38.0	38.0	
LA - 1.5%¶								
Sample 1	5.0	**28.60**	38.0	38.0	38.0	38.0	38.0	
Sample 2	4.8	**31.14**	38.0	38.0	38.0	38.0	38.0	negative
Sample 3	4.9	**33.75**	38.0	38.0	38.0	38.0	38.0	

* Ct values from rectal swabs collected from the 3 bioassay pigs in each group on designated days post inoculation. A Ct value of 38 is considered a RT-PCR-negative sample.
† Clinical observations in groups of pigs.
‡ Samples in each replicate contained Guardicate at a 0.75% level.
§ Samples in each replicate contained Guardicate at a 1.0% level.
¶ Samples in each replicate contained Guardicate at a 1.5% level.
PEDV = porcine epidemic diarrhea virus; Ct = cycle threshold; LA = lactic acid.

Experiment 2: Infectivity

All 3 piglets in the positive-control unit displayed evidence of diarrhea and shed PEDV RNA in feces (Table 2). In contrast, all piglets (n = 12) inoculated with LA-treated complete feed samples and the negative-control piglets remained healthy and all rectal swabs were negative by RT-PCR. As before, while not analyzed statistically, addition of the LA product appeared to reduce pH of the feed on a numerical basis, as compared to control samples.

Discussion

Feed contamination with PEDV is a significant concern, when considering the small quantity of virus to cause infection in complete feed. Therefore, given the potential risk of PEDV feed contamination within the feed supply chain, it highlights the importance of having proper biosecurity practices that minimize potential disease transmission events. In this brief communication, we describe the results of a study designed to provide proof of concept data regarding the efficacy of a lactic acid-based feed additive. While the design of the study was purposefully limited to small sample sizes and reduced replications, the results indicate that the product appeared to negatively impact the survivability and infectivity of PEDV. While the study was not designed to ascertain the mechanism of action, these observations may have been due to a reduction of sample pH. Furthermore, it provided potential inclusion rates for how the product may be used, should it gain acceptance in the field. As this is a very small study, more testing is required involving more replications, larger population sizes housed under controlled field conditions, and multiple pathogens, which are currently underway. However, if proven efficacious, this type of product may provide advantages regarding safety and ease of application resulting in a new option to provide a safe and efficacious means to reduce the risk of virus-contaminated feed for the swine industry.

Implications

- Contaminated feed may serve as a vehicle for PEDV transport and transmission.
- For risk mitigation, feed biosecurity changes are needed at the farm and mill.
- An LA-based feed additive may provide a solution pending a large-scale study.

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Conflict of interest

All authors and their employers developed the product offering discussed in this manuscript and have an ongoing financial interest in the sale of the product.

Disclaimer

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