Bioassay of the Effectiveness of Xtreme Bio® in Inactivating Porcine Epidemic Diarrhea Virus

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Objective:
To determine the effectiveness of XTREME BIO® (XB) in inactivating the infectivity of Porcine Epidemic Diarrhea Virus (PEDv).

Study Design:
Twenty, approximately two-week old pigs were blocked by litter and randomly assigned to one of two treatment groups. Each treatment group was housed in a separate isolation room. After one day of acclimation to the test facility, each pig was challenged intragastrically with the contents of an individual tray (metal) that had been inoculated with a pure culture of PEDv and treated with XB. The treatment groups are given below in Table 1:

Table 1

Group	Treatment	Tray type	No. of Pigs
1	Positive control (PEDv material with no disinfectant)	Metal	10
2	0.5 oz per gallon XB treated w/ PEDv	Metal	10

The pigs were observed for seven days for clinical signs of PEDv. Fecal samples were collected on Days 0, 3 and 7. The fecal samples were tested for PEDv qPCR. On Day 7, all pigs were euthanized and necropsied.

- ## MATERIALS AND METHODS

Study Management

Animals: Twenty pigs (~14 days old at delivery) from a herd that had historically tested negative via fecal PCR and that had never been diagnosed with PEDv were delivered to a BSL-2 swine research facility. Each pig was double ear tagged with a unique tag. The pigs were blocked by litter and randomly assigned to treatment groups using Excel® random number generator.

Housing: All animals were housed at the VRI BSL-2 research facility. Each treatment group was housed in separate hepa-filtered isolation room. Each group was housed in two pens (5 pigs per pen). The pens were raised plastic tubs (~5ft. x 4ft.) with plastic slatted flooring and a self -

contained waste system. Each pen contained a six-hole plastic feeder and one nipple waterer. Supplemental heat was provided via heat lamps.

Feed and Water: Upon arrival, all pigs were fed a commercially available starter feed (Purina Ultracare® 100). Water was provided via a single nipple waterer source from the on-site well. Feed and water were made available *ad libitum*.

Biosecurity: Per VRI SOPs, all study personnel were required to shower in and out of each isolation room. Nitrile gloves and tyveks were worn when handling the animals. Boot baths were placed and used upon entry and exit from the rooms.

Disinfectant Phase

Trays: Ten trays were used to simulate a trailer surface (aluminum diamond plate metal trays). The trays were approximately 6" x 6" x 1".

Disinfectant: The XB was provided as a concentrate by the sponsor/manufacturer. Per the label instructions, the concentrate was prepared to a 0.5 oz/gallon solution. Solution was put in to a 32 oz. spray bottle and labeled with the concentration. In addition, a second spray bottle was filled with Phosphate Buffered Saline (PBS) and labeled.

Tray Inoculum: Fifty-five milliliters (55mls) of pure culture of PEDv (Lot no. 13-49469, 10^5 TCID/ml) was prepared by Dr. Jianqiang Zhang, Iowa State University Veterinary Diagnostic Laboratory virologist. On the day of tray inoculation, the culture was diluted (1:10) with 495 mls of Minimum Essential Media (MEM) to create a tray inoculum of 10^4 TCID/ml.

Tray Inoculation and Disinfection: On Day 0, individual trays (10 per group) were inoculated with pure culture PEDv. Ten trays were placed in a row. Ten milliliters (10mls) were withdrawn from the bulk culture preparations and delivered to each tray. Each tray was then gently, tilted back and forth several times to ensure coverage of the entire tray surface. Each individual tray was then sprayed with the respective dilution of XB or PBS five times. Each tray was again tilted back and forth to ensure coverage of the entire tray surface. The XB or PBS was then allowed ten minutes of contact time for each tray.

Bioassay Phase

Inoculation of Pigs: At the completion of ten minutes for each tray, the contents of the tray were collected by pipetting all of the liquid from the trays and placing each tray's contents in to a 50 ml plastic centrifuge tube. Each pig was manually restrained. A 10 Fr red rubber feeding tube was placed intragastrically and the contents of each tray were removed from the centrifuge tube and administered to each pig with a 20 ml syringe affixed to the feeding tube.

Daily Observations: Pigs were observed for clinical symptoms from Day -1 through Day 7 and scores were recorded. Clinical scores were based off the following criteria:

Behavior	**Body Condition**	**Fecal Score**
0 = Normal	0 =Normal	0 = Normal
1 = Mild Lethargy	1 = Thin	1 = Mild diarrhea, soft stool that lacks solid form
2 = Severely Depressed	2 = Gaunt	2 = Moderate diarrhea, liquid present in stool with some solid
		3 = Severe diarrhea, watery stools with no solids present

Fecal collection: Feces from each pig were collected on Days 0 (prior to inoculation), 3, and 7. The fecal samples were obtained using polyester tipped swabs. The swabs were then submitted to Iowa State University Veterinary Diagnostic Lab for PEDv qPCR testing.

Necropsy: All pigs were necropsied on study day 7. All pigs were euthanized, and the small intestine, cecum, and colon were examined. A section of ileum on each pig was collected, placed in formalin, and stored for possible future testing.

- # RESULTS

Fecal PEDv qPCR: Results for the fecal PCR are given in Table 2:

Table 2: Fecal PCR Results

		Day of Collection							
		Day 0		**Day 3**			**Day 7**		
ID	**Trt Gp**	**PEDv PCR -CT**	**Result**	**PEDv PCR - CT**	**PEDV Quant - PCR**	**Result**	**PEDv PCR - CT**	**PEDV Quant - PCR**	**Result**
605	1	>=36	Negative	23.7	12805123	Positive	14.9	4560538757	Positive
583	1	>=36	Negative	19.6	199922292	Positive	18.5	405301848	Positive
594	1	>=36	Negative	16.4	1628975221	Positive	18.5	399907257	Positive
576	1	>=36	Negative	16.9	1170110854	Positive	21.5	55971881	Positive
587	1	>=36	Negative	17.6	719584816	Positive	22.2	35236027	Positive
579	1	>=36	Negative	16.6	1407794053	Positive	22.3	31289235	Positive
599	1	>=36	Negative	19.0	297469556	Positive	26.3	2224620	Positive
596	1	>=36	Negative	14.1	7616261778	Positive	26.4	2120506	Positive
612	1	>=36	Negative	16.8	1246528482	Positive	26.6	1838459	Positive
609	1	>=36	Negative	16.2	1834452510	Positive	31.9	52571	Positive

581	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
582	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
584	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
586	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
592	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
598	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
603	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
604	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
607	2	>=36	Negative	>=36	0	Negative	>=36	0	Negative
614	2	>=36	Negative	30.1	174266	Positive	>=36	0	Negative

Daily Observations: No statistical analysis or summary was done for the daily observations. All pigs were observed as normal for Study Days -1 and 0. Abnormal fecal observations (fecal score ≥ 1) were observed beginning on Day 1 for both groups.

Necropsy: No statistical analysis or summary was done for the observations made at necropsy. In general, the abnormal observations recorded were "thin walled intestines" and/or "liquid contents for the intestines".

- ## CONCLUSIONS

For the purposes of this study, the primary variable to determine the efficacy of XB to inactivate PEDv would be the results from the fecal PCR testing. Based on the data provided in Table 2, XB is efficacious in inactivating the infectivity of a pure culture of PEDv when applied at the concentration of 0.5 oz/gallon and allowed 10 minutes of contact time with a non-porous metal surface. Further studies are warranted to determine XB effectiveness for concrete surfaces and surfaces contaminated with organic material. Variables such as increased concentrations and/or longer contact times may be considered.