Bioassay of the Effectiveness of Xtreme Bio® in Inactivating Porcine Reproductive and Respiratory Syndrome Virus

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Objective:
To determine the effectiveness of XTREME BIO® (XB) in inactivating the infectivity of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv).

Study Design:
Twenty, approximately three-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 intranasally with the contents of an individual metal tray that had been inoculated with a pure culture of PRRSv and treated with either XB or Phosphate Buffered Saline 1X (PBS). The treatment groups are given in Table 1.

Table 1: Treatment Groups

Group	Treatment	Tray type	No. of Pigs
1	PRRSv treated w/ PBS (Positive Control)	Metal	10
2	PRRSv treated w/ 0.5 oz per gallon XB	Metal	10

The pigs were observed for fourteen days for clinical signs of PRRSv. Serum samples were collected on Days 0, 3, 7 and 14. The serum samples were tested for PRRSv PCR. On Day 14, all pigs were euthanized and necropsied. Bronchoalveolar lavage (BAL) samples were collected at the time of necropsy. The BAL samples were tested for PRRSv qPCR.

MATERIALS AND METHODS

Study Management

Animals: Twenty pigs (~21 days old at delivery) from a herd that was historically naïve for PRRSv were delivered to the 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 research facility. Each treatment group was housed in a separate isolation room. Each group was housed in a single pen (10 pigs per pen).

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: Twenty stainless steel metal trays were used. The trays were approximately 7.5" x 3.5" 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. The diluted solution was put in to a 32 oz. spray bottle and labeled with the concentration. In addition, a second spray bottle was filled with PBS and labeled.

PRRSv: A pure culture of PRRS virus (RFLP1-7-4, titer of 4.2 $TCID^{50}$/ml) was used to inoculate each metal tray.

Tray Inoculation and Disinfection: On Day 0, individual trays (10 per group) were inoculated with two milliliters (2mls) of pure culture PRRSv. Ten trays were placed in a row. Two milliliters (2mls) were withdrawn from the bulk culture 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 (virus w/XB or PBS) of the tray were pipetted from the trays and transferred to a 50 ml plastic centrifuge tube. Each pig was manually restrained. The contents of each tray were removed from the centrifuge tube and administered to each pig with a 6 ml syringe affixed to a nasal atomizer. Each tray was used to inoculate one pig. The total volume administered to each pig was approximately 5 mls and this total volume was delivered equally to each nostril.

Daily Observations: Pigs were observed for clinical symptoms from Day -1 through Day 14 and scores were recorded. Clinical scores were based off the criteria in Table 2.

Table 2: Clinical Signs

Behavior	**Respiratory**	**Cough**
0 = Normal	0 =Normal	0 = Normal
1 = Moderate depression, must stimulate to rise	1 = Moderate increased respiratory rate	1 = Mild, occasional cough
2 = Severe, recumbent	2 = Severe increased respiratory rate with abdominal effort may have nasal/ocular discharge	2 = Severe, persistent cough

Serum: Blood from each pig was collected on Days 0 (prior to inoculation), 3, 7, and 14. The blood samples were collected using 20-gauge x 1.5inch needles and serum separator tubes. The blood samples were centrifuged, and serum was aliquoted for testing. Serum samples were submitted to Iowa State University Veterinary Diagnostic Lab for PRRSv PCR testing.

Bronchoalveolar Lavage (BAL): Bronchoalveolar lavage (BAL) was collected on Day 14 at time of necropsy. BALs were collected via twenty-five milliliter (25ml) pipette using Phosphate Buffered Saline (PBS). BAL samples were placed in fifty milliliter (50ml) centrifuge tubes and submitted to Iowa State University Veterinary Diagnostic Lab for PRRSv qPCR testing.

Necropsy: All pigs were euthanized and necropsied on study day 14. Lungs were grossly examined, and percent of each lobe affected by lesions was noted. BALs were collected and tested as stated above.

RESULTS

Serum PRRSv PCR: No statistical analysis was done for serum PRRSv PCR results. All pigs tested negative for PRRSv on Day 0. All pigs in Treatment Group 1 (Positive Control) tested positive for PRRSv on Days 3, 7 and 14. All pigs in Treatment Group 2 (XB treated) tested negative for PRRSv on Days 3, 7 and 14. Individual results for the serum PCR are given in Table 3.

Table 3: Serum PCR Results

		Day of Collection							
		Day 0		Day 3		Day 7		Day 14	
ID	Trt Gp	PRRSv PCR CT	Result	PRRSv PCR CT	Result	PRRSv PCR CT	Result	PRRSv PCR CT	Result
97	1	>=37	Negative	27.3	Positive	20.9	Positive	15.5	Positive
112	1	>=37	Negative	28	Positive	18.5	Positive	19.1	Positive
113	1	>=38	Negative	25.8	Positive	20.1	Positive	16.1	Positive
121	1	>=37	Negative	24.1	Positive	17.1	Positive	16	Positive
123	1	>=37	Negative	21.5	Positive	16.8	Positive	20	Positive
124	1	>=37	Negative	23.8	Positive	15.4	Positive	18.8	Positive
125	1	>=37	Negative	27.5	Positive	20.3	Positive	16.9	Positive
132	1	>=37	Negative	23.3	Positive	14	Positive	13.8	Positive
133	1	>=37	Negative	26.1	Positive	21	Positive	14.1	Positive
134	1	>=37	Negative	24.1	Positive	18.8	Positive	15	Positive
106	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
107	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
108	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
109	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
110	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
111	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
114	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
115	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
122	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative
131	2	>=37	Negative	>=37	Negative	>=37	Negative	>=37	Negative

Daily Observations: No statistical analysis or summary was done for the daily observations. All pigs were observed as normal for Study Days -1 to 14. No abnormal behavior, respiration, or coughing was observed throughout the duration of the study. Although, it was noted that the pigs in Gp 1 had rough haircoats in comparison to pigs in Gp 2.

Necropsy: No statistical analysis was done for the observations made at necropsy. All pigs in Treatment Group 2 (XB treated) were free of lung lesions and had a total lung lesion score of 0.00%. All pigs in Treatment Group 1 (Positive Control) developed lung lesions to some degree, total lung lesion scores ranging from 15.00% to 71.00%. Individual lung lesion scores are given in Table 4.

Table 4: Lung Scores

ID	Trt Gp	L Apical	L Cardiac	L Caudal	R Apical	R Cardiac	R Caudal	Interm.	Total
97	1	30.00%	20.00%	10.00%	50.00%	50.00%	50.00%	10.00%	31.00%
112	1	10.00%	20.00%	50.00%	20.00%	30.00%	50.00%	20.00%	23.75%
113	1	10.00%	10.00%	10.00%	10.00%	10.00%	30.00%	10.00%	15.00%
121	1	30.00%	30.00%	50.00%	30.00%	50.00%	70.00%	20.00%	46.00%
123	1	70.00%	70.00%	50.00%	90.00%	90.00%	70.00%	90.00%	71.00%
124	1	10.00%	20.00%	10.00%	30.00%	50.00%	50.00%	50.00%	31.00%
125	1	50.00%	50.00%	40.00%	90.00%	70.00%	90.00%	50.00%	63.50%
132	1	50.00%	50.00%	20.00%	90.00%	90.00%	50.00%	50.00%	50.50%
133	1	30.00%	50.00%	20.00%	50.00%	100.00%	50.00%	50.00%	47.50%
134	1	50.00%	70.00%	40.00%	70.00%	90.00%	50.00%	90.00%	59.50%
106	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
107	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
108	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
109	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
110	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
111	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
114	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
115	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
122	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
131	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

BAL PRRSv qPCR: No statistical analysis was done for BAL PRRSv qPCR results. All pigs in Treatment Group 1 (Positive Control) tested positive for PRRSv on Day 14. All pigs in Treatment Group 2 (XB treated) tested negative for PRRSv on Day 14. Individual results for BAL PRRSv qPCR are given in Table 5.

Table 5: BAL qPCR Results

		Day of Collection	
		Day 14	
ID	Trt Gp	PRRSv PCR CT	Result
97	1	16.8	Positive
112	1	17.4	Positive
113	1	20	Positive
121	1	19.9	Positive
123	1	17.4	Positive
124	1	17.2	Positive

Table 5 continued: BAL qPCR Results

		Day of Collection	
		Day 14	
ID	Trt Gp	PRRSv PCR CT	Result
125	1	16.3	Positive
132	1	14.4	Positive
133	1	18.6	Positive
134	1	17.1	Positive
106	2	>=37	Negative
107	2	>=37	Negative
108	2	>=37	Negative
109	2	>=37	Negative
110	2	>=37	Negative
111	2	>=37	Negative
114	2	>=37	Negative
115	2	>=37	Negative
122	2	>=37	Negative
131	2	>=37	Negative

CONCLUSIONS

For the purposes of this study, the primary variable to determine the efficacy of XB to inactivate PRRSv would be the results from the serum PCR testing and bronchoalveolar lavage qPCR testing, and lung scores. Based on the data provided in Tables 3, 4 and 5, XB is efficacious in inactivating the infectivity of a pure culture of PRRSv 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.