Effectiveness of Xtreme Bio in deactivating PRRSv and PEDv in the presence of organic matter (Proof of Concept Study)

Hans Rotto, DVM, MS; Darin Madson, DVM, PhD, DACVP;

Lori Layman, MS TRB, LLC Ames, Iowa, USA

Objective: To determine the effectiveness of XTREME BIO in deactivating the infectivity of PRRSv and PEDv in the presence of organic matter on porous concrete (hog barn slats) and aluminum panels (livestock hauling trucks).

Summary:

Two flooring substrates, representing porous concrete slats typical in confinement swine housing and aluminum paneling typical of livestock trailers, were covered with fecal matter inoculated with either PRRSv or PEDv. Four (4) oz. of fecal matter was spiked with either 12 mL of PRRSv ($TCID_{50}$ 10^4) or 10 mL of PEDv (2.84×10^8 genomic copies/mL) and spread over the flooring samples. An additional 4 oz. of fecal material was spread on the flooring to cover a 4 ft^2 surface area. The fecal material was allowed to remain in contact with the flooring for one
hour. The flooring was subjected to either a rough (partial) or NO wash treatment and then treated with XTREME BIO at a rate of 4 oz./gal. XTREME BIO was allowed to remain in contact with the flooring samples for one hour.
Remaining organic material was collected using a dry cleaning cloth dampened with 50 mL of distilled water wiped over the surface of the flooring; effluent was captured into a zipper topped plastic bag. Ten (10) mL of the effluent was inoculated into individually housed swine and animals were observed for clinical and diagnostic signs of disease for 20 days. One replicate of each combination of flooring (slat vs. aluminum) and wash treatment (rough vs. NO) was utilized for both PRRS and PED. Neither specific viral testing or antibody generation was detected in any animal for the duration of the study period. This proof of concept study showed favorable results that XTREME BIO is able to deactivate the infectivity of PRRSv and PEDv on flooring surfaces with mild to moderate fecal contamination.

Materials & Methods: Flooring Samples:

- Porous concrete: Representing open slat flooring inside confinement hog barns. These pieces were

exposed to natural UV radiation for more than 6 months prior to use. Slat pieces were laid side by side with a 4 ft^2 section of area marked with duct tape. (Figure 1)

Figure 1

- Aluminum panels: Aluminum hog sorting panels with grooves (Hog Slat Inc., Newton Grove, NC, Item #807705090) to represent the flooring pattern typical of the interior surface of livestock hauling trailers. The grooved area of one panel equal to 4 ft^2 was identified with duct tape. (Figure 2)

Figure 2.

Fecal Substrate/Virus Inoculum:

- Feces were collected from 3-5 week-old pigs negative for PRRSv and PEDv.
- PRRSv (1-8-4 RFLP, wild-type virus) was sourced from Iowa State University and was titered at 1 x $10^4 TCID_{50}$.
- PEDv was sourced from a private clinic feedback pool and tested to contain 2.86 x 10^9 genomic copies/mL.

Wash/Disinfectant Treatment:

- Rough Wash: The rough wash treatment utilized a standard hose end sprayer nozzle and cold water. The flooring was washed for no more than two minutes until the visible fecal material was removed from the flooring.
- NO Wash: The NO wash treatment was not subjected to any rinsing prior to disinfection.
- Disinfection: XTREME BIO was applied to the flooring samples at a rate of 4 oz./ gallon in an adequate volume to cover the 4 ft^2 surface area using a commercial hose end sprayer (Ortho® Dial N Spray, Scotts Miracle-Gro Company, Marysville, OH).)

Swine Bioassay:

- Pigs were healthy, four weeks of age, known naïve to PRRSv and PEDv and weaned approximately 7- 10 days.

Study Design:

A 2 x 2 block design was utilized for each challenge virus to represent slat vs. trailer and rough vs. no wash combinations. (Table 1)

Table 1

PRRSv Challenge

	Slat	Aluminum Panel
Rough Wash	n=1	n=1
No Wash	n=1	n=1

PEDv Challenge

	Slat	Aluminum Panel
Rough Wash	n=1	n=1
No Wash	n=1	n=1

Procedures:

PRRSv challenge: 12 ml of PRRSv ($TCID_{50}$ 10^4) was added to four (4) oz. (113 g) of fecal material. The spiked fecal mixture was spread by hand to evenly cover the pre-marked 4 ft^2 area of flooring. An additional 4 oz. (113 g) of non-spiked feces was spread over the top of the spiked material to cover the flooring. (Figure 3 and 4).

Figure 3 Figure 4

This procedure was repeated three additional times to represent each flooring/wash combination. The fecal material was allowed to remain in contact with the flooring samples for one hour prior to washing.

At the end of the elapsed hour, the flooring was subjected to the assigned wash treatment. The 'rough wash' treatment consisted of the use of a non-pressurized hose end sprayer nozzle and cold water for an elapsed time of approximately two minutes to flush away visible fecal material. (Figures 5 & 6).

Figure 5 Figure 6

The 'no wash' treatment was not subjected to any rinsing of fecal material from the flooring samples.

Immediately following the washing treatment, XTREME BIO was applied at a rate of 4 oz. /gal in a sufficient volume to fully saturate each flooring model. The disinfectant formula was allowed to contact the surface for one hour.

*Any excess/pooled disinfectant was drained off the aluminum panels after 10 minutes of contact time to avoid bias from excess disinfectant exposure and to replicate the action of trailer movement while driving. (Figure 7)

Figure 7

After one hour contact time, the flooring samples were wiped down with a dry cleaning cloth (Tuff & Tidy® dry mop cloth, Rochline Industries, Sheboygan, WI) moistened with 50 mL of distilled water. The cleaning cloth was used to wipe the entire surface of the flooring sample and wrung out back into a zipper top bag. The wiping and wringing techniques were repeated until at least 25 mL of effluent was collected. The bag was labeled with flooring type and wash treatment. The effluent was kept cool until bioassay inoculation.

PEDv challenge: 4 ml of PEDv (2.86 x 10^9 genomic copies/mL) was added to 36 mL of distilled water and swirled to mix. 10 mL of the resulting solution (2.86 x 10^8 genomic copies/ mL) was added to four (4) oz. (113 g) of fecal material.

The same procedures used for spreading, exposure and capture of effluent as listed in the PRRSv challenge model were repeated for the PEDv challenge replicates.

Bioassay inoculation: Pigs were randomly assigned to treatments by random number generator. PRRSv challenge pigs were inoculated with 5 cc of wash effluent IN (intranasally) and 5 cc of wash effluent orally (Figure 8). PEDv challenge pigs were inoculated with 10 cc of wash effluent orally. New syringes were used with each pig.

Figure 8.

Following inoculation; pigs were housed individually in crates and physically separated into two rooms by challenge virus.

Pigs were fed 1-2 pounds/day of a medicated, total mixed ration suitable for their weight and age. Pigs were given access to one gallon of water daily over two observation points.

Pigs were monitored twice daily for clinical signs consistent with PEDv (diarrhea, dehydration) and PRRSv (dyspnea, rough hair coat, coughing, lethargy, anorexia)

Diagnostic testing to confirm disease state was performed when representative clinical signs were present or at the end of the trial.

Results:

Diarrhea was present in 50% of the PEDv challenge pigs at days 3, 4 and 5. PEDv PCR testing on fecal swabs was performed on all four PEDv pigs on day 5 and showed negative results.

PRRSv challenge pigs were asymptomatic throughout the 20 days of the trial.

At study termination, serum was negative on all pigs for antibodies of known inoculation for each group (PRRSv and PEDv).

Conclusions:

This proof of concept study using XTREME BIO was able to deactivate the infectivity of PRRSv and PEDv viruses in the presence of minimal and moderate organic matter. Caution is advised when interpreting these data to larger surface areas and larger populations of animals.