

National Pork Board Project - Final Update

The effect of disinfection practices on RT-PCR detection of PEDv

Courtesy of Dr. Andrew Bowman, The Ohio State University

Porcine epidemic diarrhea virus (PEDv) is causing significant damage to the United States pork industry. Rapid dissemination of this highly contagious virus across the nation has occurred, likely via contaminated transportation equipment. Cleaning and disinfection of trucks and trailers is widely practiced in the swine industry today and post-disinfection assessment is an important part of any protocol. Currently the detection of PEDv is limited to reverse transcription (RT)-PCR, a test which does not offer insight into the infectivity of detected PEDv but rather only identifies the presence of PEDv nucleic acid. Several classes of disinfectants render PEDv biologically inactive but do not disrupt the viral RNA. This situation results in samples testing positive when in fact the virus has been inactivated. In the present study we sought to test currently available disinfectants for their ability to both inactivate PEDv and disrupt the viral RNA so that PEDv cannot be detected by RT-PCR.

Objective 1: In vitro evaluation of disinfectants.

In the first objective, five commonly used classes of disinfectants were evaluated against PEDv in plastic petri dishes at varying concentrations, both in the presence and absence of swine feces to simulate field conditions. The testing was conducted under three different temperatures (37°C, 4°C, or -20°C) to mimic seasonal variations in ambient temperature. The disinfectants included in this study were a phenolic disinfectant, (One-stroke Environ); a quaternary ammonia compound (Roccal-D Plus); a chlorine compound, sodium hypochlorite (household bleach); an oxidizing agent, (Virkon S); and a quaternary ammonium/glutaraldehyde combination product (Synergize). Oxidizing agents and sodium hypochlorite are known to disrupt the RNA of other viruses thus three different dilutions of Virkon S (0.5%, 1% and 2%) and four different dilutions of sodium hypochlorite (0.17%, 0.52%, 1.03%, and 2.06%) were tested. All of the tested disinfectants were able to render PEDv non-infectious in cell culture. All the disinfectants except for 0.17% sodium hypochlorite produced significant reductions in the estimated number of PEDv copies on RT-PCR in all tested settings; however, none of the disinfectants were able to produce RT-PCR results that were completely negative across all replicates. Strong solutions of sodium hypochlorite (1.03% and 2.06%) and 0.5% oxidizing agent did produce several negative or nearly negative RT-PCR test results.

Objective 2: Evaluation of a selected disinfectant on PEDv bioassay

In the second objective, results from Objective 1 were used to select 2.06 % sodium hypochlorite and 0.5% oxidizing agent as disinfectants that showed potential to produce RT-PCR negative test results when used in the field. Aluminum coupons were pitted with 5% acetic acid to simulate a used livestock trailer. PEDv was applied to the aluminum coupons, allowed to dry, and then treated with either 2.06 % sodium hypochlorite or 0.5% oxidizing agent. The surface of the aluminum was swabbed and the swabs were tested with RT-PCR and used to inoculate both cells and naïve pigs. Neither of these two disinfectants was able to produce completely negative PCR results on pitted aluminum. While still PCR positive, the samples collected from the aluminum coupons were not infectious in cell culture or to naïve pigs.

All of the disinfectants tested in the present study were able to render PEDv non-infectious but few were able to disrupt the viral RNA to the point that PEDv could not be detected by RT-PCR. Because most PEDv strains do not grow in cell culture, the pork industry must rely upon RT-PCR for testing. Pork producers can expect to receive PCR positive test results even after proper disinfection with most commercially available disinfectants. Results of the present study indicate that oxidizing agents and sodium hypochlorite are most likely to produce negative RT-PCR results.

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Editor's comments: This study explores a phenomenon experienced by many producers and researchers which has caused uncertainty in disinfection practices after heavy contamination with PEDv. Even in BSL-2 isolation facilities after standard disinfection protocols using bleach which Dr. Bowman's study investigates, surface samples tested by RT-PCR for PEDv have been positive (even <30 Ct) without demonstrating infectivity. There was, however, a single instance during PEDv bioassay studies where the PEDv material remaining after disinfection protocols was able to infect a room of 11-day-old piglets. In light of this, it is important to remember that Dr. Bowman's study represents an ideal disinfection circumstance with a dried sample on a metal surface in the absence of organic material. In worse conditions or without proper power washing or scrubbing, any of these disinfectants including bleach may leave infectious virus on the surface.

High Ct value from RT-PCR testing of environmental samples, feed or other materials can be an indicator of infectious potential, but as Dr. Bowman mentions infectivity can only be objectively evaluated using cell culture or live animal bioassay. Both of these methods also have their limitations some of which include length of time to get results, feasibility/resources of diagnostic laboratories to handle large numbers of samples, and sensitivity to different strains or variants of PEDv. Dr. Torremorell at the University of Minnesota is conducting a study to evaluate viability-PCR (v-PCR) as a feasible alternative for detection of infectious virus. Preliminary updates suggest that v-PCR can differentiate between infectious and non-infectious virus in fecal samples.

Both Dr. Bowman's and Dr. Torremorell's research updates and other NPB research updates can be found at <http://www.pork.org/pork-checkoff-research/pedv/pedv-research/>