Veterinary Microbiology 179 (2015) 213–218

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Effects of disinfection on the molecular detection of porcine epidemic diarrhea virus

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ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 28 May 2015
Accepted 30 May 2015

Keywords:
Porcine epidemic diarrhea virus
Sodium hypochlorite
Bleach
Reverse transcriptase polymerase chain reaction
Infection control
Biosecurity
Swine
Virus inactivation

ABSTRACT

Routine detection of porcine epidemic diarrhea virus (PEDV) is currently limited to RT-PCR but this test cannot distinguish between viable and inactivated virus. We evaluated the capability of disinfectants to both inactivate PEDV and sufficiently damage viral RNA beyond RT-PCR detection. Five classes of disinfectants (phenol, quaternary ammonium compound, sodium hypochlorite, oxidizing agent, and quaternary ammonium/glutaraldehyde combination) were evaluated *in vitro* at varying concentrations, both in the presence and absence of swine feces, and at three different temperatures. No infectious PEDV was recovered after treatment with evaluated disinfectants. Additionally, all tested disinfectants except for 0.17% sodium hypochlorite dramatically reduced qRT-PCR values. However, no disinfectants eliminated RT-PCR detection of PEDV across all replicates; although, 0.52%, 1.03% and 2.06% solutions of sodium hypochlorite and 0.5% oxidizing agent did intermittently produce RT-PCR negatives. To simulate field conditions in a second aim, PEDV was applied to pitted aluminum coupons, which were then treated with either 2.06% sodium hypochlorite or 0.5% oxidizing agent. Post-treatment surface swabs of the coupons tested RT-PCR positive but were not infectious to cultured cells or naïve pigs. Ultimately, viable PEDV was not detected following application of each of the tested disinfectants, however in most cases RT-PCR detection of viral RNA remained. RT-PCR detection of PEDV is likely even after disinfection with many commercially available disinfectants.

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1. Introduction

The recent emergence of porcine epidemic diarrhea virus (PEDV) in the United States swine herd has had severe detrimental impacts on the pork industry. Before 2013 PEDV was seen only in Asian and European swine herds but since the first reports of PEDV in Iowa in May, 2013 (Chen et al., 2013; Cima, 2013b), the highly contagious and deadly coronavirus has rapidly spread across North America. Common clinical signs include diarrhea and vomiting, which can lead to dehydration and electrolyte imbalance in infected animals. High mortality (70–100%) among neonates has led to significant economic losses (Cima, 2013a,b).

Transmission of PEDV occurs mainly through the oral-fecal route with acutely infected animals shedding large quantities of virus for several days post infection. The rapid emergence of highly similar strains across the United States and the frequent detection of PEDV in livestock trailers indicates that swine transportation plays a major role in the spread of PEDV in the country (Lowe et al., 2014). Contaminated transportation equipment has been linked to the spread of several other important swine diseases (porcine reproductive and respiratory syndrome virus, *Salmonella*, and *Escherichia coli*) making trailer disinfection common among United States pork producers (Dee et al., 2004, 2006; Rajkowski et al., 1998). Efficient disinfection for PEDV in animal contact spaces, including trailers and trucks, is currently one of the primary methods used to control the spread of the disease.

However, PEDV is difficult to culture outside of an animal model; thus, RT-PCR assays are currently the only tests available to pork producers and swine veterinarians to directly detect PEDV. Because RT-PCR only detects the viral nucleic acid, a positive RT-PCR result only indicates detection of PEDV viral RNA, but does not mean viable and infectious virus is present. Due to the limited testing options and the implications of environmental contamination, individuals are using RT-PCR to test trailers

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http://dx.doi.org/10.1016/j.vetmic.2015.05.027
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following disinfection to ensure that the equipment is free of PEDV before contact with naïve animals. However, RT-PCR tends to underestimate disinfection efficacy compared to infectivity assays; meaning, RT-PCR positive results are obtained when in fact the trailer has been effectively disinfected. This drawback of RT-PCR has been recognized for various pathogens (Pecson et al., 2011; Poschetto et al., 2007; Suarez et al., 2003), as most disinfectants damage the protective capsid, but often, this mode of action has limited or no effect on the viral nucleic acid (Pecson et al., 2009). Although the disinfection treatments result in loss of infectivity, RT-PCR can still detect the intact viral RNA that remains within a noninfectious viral particle. While rapid progress is being made on viral culture methods, there is an immediate need for practical solutions to address the discrepancy between RT-PCR and infectivity assays. In practical terms, pork producers must consider all RT-PCR positive trailers as contaminated; the consequences of not doing so could be disastrous to their operation and the entire swine industry. However, the cost associated with extra cleaning and disinfection and additional time until a trailer tests negative is very expensive for pork producers.

As an enveloped virus, a wide variety of disinfectants effectively inactivate PEDV (Pospischil et al., 2002) but we cannot detect this biological inactivation with RT-PCR. Presently there is a paucity of data examining disinfectant usage on PEDV RT-PCR results. Data from other pathogens indicate that some disinfectants (*e.g.*, accelerated peroxide-based compounds and/or sodium hypochlorite) would better disrupt the viral RNA and produce more meaningful RT-PCR results (Charrel et al., 2001; Ma et al., 1994; Ojeh et al., 1995; Suarez et al., 2003). Therefore, we examined the effect of disinfectants on RT-PCR results for PEDV and explored practical solutions to produce RT-PCR negative trailers after they have been contaminated with PEDV.

2. Methods

2.1. In vitro *evaluation of disinfectants*

Five commonly used disinfectants were evaluated for efficacy in inactivating PEDV and for their capability to disrupt PEDV RNA beyond the detection limits of RT-PCR. The disinfectants included were a phenolic disinfectant; a quaternary ammonia compound; sodium hypochlorite; an oxidizing agent; and a quaternary ammonium/glutaraldehyde combination product (Table 1). Since oxidizing agents and sodium hypochlorite are known to disrupt the RNA of other viruses, three different dilutions of the oxidizing agent and four different dilutions of sodium hypochlorite were tested. All disinfectants were tested at three different temperatures (37 °C, 4 °C, or −20 °C).

The samples were generated using 147.8 cm^2 plastic petri dishes marked on the exterior with 5 dots in a 7 cm square (1 dot per corner with the 5th dot in the center of the square); three petri dishes were used for each substrate to be tested. A cell-culture-adapted PEDV strain (PC22A) was used for all experiments in the present study (Oka et al., 2014). One mililiter (1×10^6 $TCID_{50}$/ml) of PEDV suspension was added to each petri dish and spread evenly to cover the surface of each dish using a sterile cell spreader. Dulbecco's modified eagle medium (DMEM) with 7 ug/ml trypsin, 1% penicillin-streptomycin, and 0.3% tryptose phosphate broth was used as the negative control. The inoculum was allowed to dry completely in each petri dish in biosafety cabinets. Once dry, the dishes were incubated for 15 min at the selected temperature. After the incubation, 1 ml of each disinfectant was added to its respectively labeled petri dishes and spread evenly with a spreader. All disinfectants were allowed to dry in open petri dishes in biosafety cabinets (60 min). Once the disinfectants were dry, the lids were replaced on the petri dishes and the dishes were incubated for 15 min at the selected temperature. Double distilled

Table 1
Disinfectants and concentrations tested against a tissue culture adapted porcine epidemic diarrhea virus strain in both cell culture media and 10% (v/v) swine feces slurry. Testing was performed in triplicate resulting in 72 samples for each temperature tested. This procedure was replicated for each temperature (37 °C, 4 °C and −20 °C) for a grand total of 216 samples.

Study group	PEDV status to contaminate petri dishes	Treatment with disinfectant (dilution)	Temperatures	Contact time (min)
Negative control	Neg; in culture medium or fecal slurry	Water	37 °C, 4 °C, or −20 °C	60 or 90
Positive control	Pos; in culture medium or fecal slurry	Water	37 °C, 4 °C, or −20 °C	60 or 90
Quaternary ammonium[a]	Pos; in culture medium or fecal slurry	1.5:128	37 °C, 4 °C, or −20 °C	60 or 90
Phenol[b]	Pos; in culture medium or fecal slurry	1:256	37 °C, 4 °C, or −20 °C	60 or 90
Quaternary ammonium/glutaraldehyde combination[c]	Pos; in culture medium or fecal slurry	1:256	37 °C, 4 °C, or −20 °C	60 or 90
Oxidizing agent[d] (0.5%)	Pos; in culture medium or fecal slurry	1:200	37 °C, 4 °C, or −20 °C	60 or 90
Oxidizing agent[d] (1%)	Pos; in culture medium or fecal slurry	1:100	37 °C, 4 °C, or −20 °C	60 or 90
Oxidizing agent[d] (2%)	Pos; in culture medium or fecal slurry	1:50	37 °C, 4 °C, or −20 °C	60 or 90
Sodium hypochlorite[e] (0.17%)	Pos; in culture medium or fecal slurry	1:50	37 °C, 4 °C, or −20 °C	60 or 90
Sodium hypochlorite[e] (0.52%)	Pos; in culture medium or fecal slurry	1:16	37 °C, 4 °C, or −20 °C	60 or 90
Sodium hypochlorite[e] (1.03%)	Pos; in culture medium or fecal slurry	1:8	37 °C, 4 °C, or −20 °C	60 or 90
Sodium hypochlorite[e] (2.06%)	Pos; in culture medium or fecal slurry	1:4	37 °C, 4 °C, or −20 °C	60 or 90

[a] Roccal-D Plus; Zoetis, Florham Park, New Jersey.
[b] 1-Stroke Environ; STERIS Corporation, Mentor, Ohio.
[c] Synergize; Preserve International, Reno, Nevada.
[d] Virkon S; DuPont, Wilmington, Delaware.
[e] Clorox Regular-Bleach (8.25% sodium hypochlorite); The Clorox Company, Oakland, California.

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