



Inactivation of porcine epidemic diarrhea virus using heated water

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is a very contagious swine pathogen that spreads easily via the fecal-oral route, notably from contaminated fomites. The present study investigated heated water as a method for rapid thermal inactivation of PEDV. Cell-culture adapted PEDV was treated with water at varying temperatures and viral titers were measured at multiple time points post-treatment. Viable PEDV was not recovered after a ten second or longer treatment with water heated to ≥ 76 °C; however, PEDV nucleic acid was detected in all samples regardless of treatment. Hot water decontamination could be considered in settings where chemical disinfection is impractical.

1. Introduction

Porcine epidemic diarrhea virus (PEDV) is a very contagious pathogen that has become a major economic burden for U.S. swine producers since its initial North American detection in May 2013. This coronavirus is estimated to have killed over 7 million pigs in 2013 alone with significant additional swine morbidity and mortality in subsequent years (Chen et al., 2014; Cima, 2013, 2014). Infection with PEDV induces severe diarrhea, dehydration, and vomiting in all age groups of swine but most notably results in extremely high mortality (80–100%) in neonatal piglets (Cima, 2013; Stevenson et al., 2013). Fecal-oral transmission involving contaminated fomites, such as vehicles, farm equipment, clothing, and feedstuffs, has been implicated in wide-spread distribution of the virus within and between farms (Dee, Neill, Clement, Christopher-Hennings & Nelson, 2014; Dee et al., 2015; Lowe et al., 2014).

The now nearly global distribution of PEDV requires that swine producers and veterinarians use strict biosecurity practices to control the virus and prevent accidental spread. The vast majority of PEDV control studies have focused on various applications of chemical disinfectants, heat, alterations to pH, and drying. Chemical disinfectants have received the most attention and many classes are able to inactivate PEDV (Bowman et al., 2015b; Schneider, Zhang, Ramirez, Wang & Holtkamp, 2015). However, some chemical disinfectants are corrosive to metal surfaces, hazardous to human and animal health, and inactivated with organic material and water with high mineral content (Babb, Bradley & Ayliffe, 1980; Racioppi et al., 1994). Additionally, most chemical disinfectants require prolonged contact

times to achieve maximum effectiveness (Rutala, Weber, David & the Healthcare Infection Control Practices Advisory Committee (HIPAC), 2008), which can be problematic in various sectors of the high-throughput swine production chain.

One commonly used alternative to chemical disinfection is thermal inactivation of virus on inanimate objects. PEDV has been shown to maintain infectivity at temperatures as high as 50 °C, but infectivity is lost when heated above 60 °C for 30 min (Hofmann & Wyler, 1989). Within the U.S. swine industry, it has been advised for animal hauling trailers to be heated to 71 °C for 10 min to ensure inactivation of PEDV (Thomas et al., 2015b). However, use of heat in this setting requires specialized equipment and a prolonged period, which can be a challenge in such a high turn-over industry. While not targeting PEDV, animal harvest facilities routinely use the application of 83 °C water for approximately five seconds to thermally inactivate many pathogens on meat cutting equipment (Taormina & Dorsa, 2007). The present study sought to investigate hot water as a method for rapid thermal inactivation of PEDV.

2. Materials and methods

Forty milliliters of sterile distilled water were continuously mixed while heated to one of seven treatment temperatures: 4.4, 65.6, 71.1, 76.7, 82.2, 87.8, and 98.9 °C (with 4.4 °C serving as a positive control). Water at each temperature was inoculated with 1.5 ml (10^6 TCID₅₀/ml) of cell-culture adapted PEDV (strain PC22A, (Oka et al., 2014)) and following inoculation, 1 ml aliquots of inoculated water removed at specified time points: 5, 10, 30, 60, 90, 120, 300, and 600 s.

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Immediately after collection, aliquots were mixed 1:1 with maintenance medium (MM) being held at 4 °C in an ice block (Bowman et al., 2015b). In the same manner, each vial of heated water was sampled immediately prior to inoculation with PEDV to serve as a negative control. All above mentioned procedures were completed in triplicate.

All samples were inoculated in quadruplicate with 24 well cell culture plates containing monolayers of Vero cells (CCL-81; American Type Culture Collection, Manassas, VA) with 600 µl of the sample. The remainder of each sample was frozen at –80°C for additional testing. Inoculated wells were observed daily and all samples demonstrating cytopathic effects (CPE) three days post-inoculation in any of the wells were classified as positive. Previously described methods were used to measure tissue culture infective dose (TCID₅₀) for all positive samples (Bowman et al., 2015b). Mean TCID₅₀ values were calculated for each triplicate, which were subsequently log-transformed, normality visually assessed, and compared using a two-way analysis of variance (ANOVA). A p-value < 0.05 was considered significant. In addition, each sample was tested with rRT-PCR targeting the N gene using previously described methods (Jung et al., 2014, Bowman et al., 2015b) in order to determine if the hot water degraded viral nucleic acid.

3. Results

Infectious PEDV was recovered from all samples in the 4.4 °C control group. Samples from treatments of 65.6 °C at 5, 10, and 30 s; and 76.7 °C and 87.8 °C at 5 s contained viable virus post-treatment. Heated water was effective at rendering PEDV nonviable at all other temperature and time points (Table 1). All samples, regardless of treatment, were positive for PEDV nucleic acid via rRT-PCR post-treatment (Ct range: 20.26–25.99).

Treatment with 87.8 °C water for 5 s resulted in a > 4 log reduction in viable virus (from 10^{4.6} to 10^{0.3} TCID₅₀/ml). Viable PEDV was not recovered after a ten second or longer treatment with water heated to ≥76.7 °C (Table). The ANOVA showed that the effects of time, temperature, and the interaction between time and temperature were all significant predictors of the viral titer of infectious PEDV (p < 0.0001).

4. Discussion

Inactivation of PEDV in heated water was accomplished using a wide range of temperature and time combinations demonstrating the potential use as an alternative disinfection method. Most importantly the ability of heated water to rapidly inactivate PEDV in as little as 10 s would make it an asset for many areas of swine production where economic demands require great attention to time-sensitive approaches.

The importance of fomites in PEDV transmission was highlighted when contaminated flexible intermediate bulk containers were implicated as the likely method for intercontinental movement of the virus to United States (U.S. Department of Agriculture (USDA), 2015). Once the virus was introduced to the United States, PEDV spread rapidly across the country, likely through gaps in biosecurity protocols (Bowman, Krogwold, Price, Davis & Moeller, 2015a; Lowe et al., 2014). The relative ease with which this virus can spread via fomites is due in part to the extremely low dose required to infect naïve pigs (Thomas et al., 2015a); even miniscule amounts of viable virus appear to be adequate to initiate large swine outbreaks. Furthermore, with the ability to survive in the environment for up to 180 days in soybean meal (Dee et al., 2015) and 9 months in manure storage structures (Tun, Cai, & Khafipour, 2016), PEDV can persist long enough in the swine production chain to circumvent typical management practices designed to protect the health of pigs. Heated water may be useful in situations where penetration of objects with chemical disinfectants is difficult and where corrosion of the target materials (e.g. metal

surfaces, rubber gaskets, etc.) should be minimized.

The swine industry is reliant upon nucleic acid detection to identify the presence of PEDV in pigs and on fomites; however, this diagnostic tool does not determine if there are intact viral particles capable of causing infection. This limitation is important to consider when developing disinfection strategies to prevent the transmission of PEDV to naïve swine. One challenge with the use of many classes of disinfectants is the inability to disrupt RNA beyond the threshold of detection, which makes it impossible to discern disinfection effectiveness from a PCR positive test result (Bowman et al., 2015b; Thomas et al., 2015b). Heated water is not immune from this challenge as PEDV nucleic acid was detected via rRT-PCR in all samples in the present study, including those rendered non-infectious by their treatment.

One limitation in the present study is the cyclic operating heat element which made it difficult to maintain a perfectly constant temperature over the entire 10-min treatment period. However, the desired treatment temperature was always verified at the time of inoculation and given the observed rapid results, we concluded the temperature fluctuations were of minimal impact. Additionally, the first sampling time point (5 s post inoculation) may not have provided sufficient time for thorough mixing prior to sample collection. It is also important to note that cell culture assay may underestimate the actual viral infectivity when compared to animal bioassays. Further studies might include observing the infectivity in neonatal pigs in comparison to weaned pigs which would validate whether hot water would be a feasible disinfectant for a swine farm (Thomas et al., 2015a).

Overcoming the above mentioned limitations would be essential in conducting a follow-up experiment using field conditions (e.g. fecal material, pavement, bedding, etc.) to mimic materials found in swine production and harvest facilities. It is well known that the presence of organic material can inactivate many classes of chemical disinfectants (Lewis & Arens, 1995). While thermal inactivation is less impacted by organic material, it could potentially serve as viral protectant during the application of hot water.

Since viable PEDV was not recovered after a ten second or longer treatment with water heated to ≥76 °C, hot water decontamination could be considered in settings where chemical disinfection is impractical and/or rapid inactivation of PEDV is needed. While heated water may not be practical in some situations due to animal and human health risks from scalding hot water, heated water is another tool in the veterinarian's tool box to combat PEDV.

Conflict of interest

The authors declare that they have no competing interests.

Table 1

Porcine epidemic diarrhea virus survivability over time when treated with water heated to specified temperatures.

Temperature (°C)	Time (s)							
	5	10	30	60	90	120	300	600
98.9	–	–	–	–	–	–	–	–
93.3	–	–	–	–	–	–	–	–
87.8	10 ^{0.3}	–	–	–	–	–	–	–
82.2	–	–	–	–	–	–	–	–
76.7	10 ²	–	–	–	–	–	–	–
71.1	–	0 ^a	–	–	–	–	–	–
65.6	10 ^{3.4}	10 ^{3.1}	10 ^{0.8}	–	–	–	–	–
4.4 (pos. control)	10 ^{4.6}	10 ^{4.5}	10 ^{4.5}	10 ^{4.5}	10 ^{4.4}	10 ^{4.7}	10 ⁵	10 ^{4.6}

Mean TCID₅₀/ml values are reported and there was a significant difference between 4.4 °C controls and all treatment groups (all p ≤ 0.036).

^a One of three samples was positive via initial inoculation but viral titer was below the limit of quantification for subsequent TCID₅₀ assay.

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