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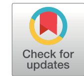
Disinfection of transboundary animal disease viruses on surfaces used in pork packing plants



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ABSTRACT

In the event of an intentional or accidental incursion of a transboundary animal disease (TAD) virus into the US, a major concern to the meat industry would be the potential contamination of packing plants by processing infected animals. TAD agents such as foot and mouth disease virus (FMDV), African swine fever virus (ASFV) and classical swine fever virus (CSFV) are found in swine products such as blood and feces and are present in the tissues of infected animals. To test the disinfection of TAD viruses in a pork-packing environment, a previously developed disinfection assay was used to test two biocides currently used by industry sanitarians, against TAD viruses dried on industry relevant surfaces in saline or swine products. With the exception of one virus, both commercial disinfectants tested were effective against the TAD viruses dried on steel, plastic, and sealed concrete surfaces in the absence of the swine products. Disinfectant activity was greatly inhibited in the presence of dried blood and meat juices. The acidic disinfectants were able to inactivate the viruses in swine feces whereas fecal material generally inhibited sodium hypochlorite-based disinfectants. These results highlight the importance of manufacturer-recommended pre-cleaning steps to remove gross soil before surface disinfection. Taken together, these data support the use of acid- and surfactant-containing commercial products for packing plant disinfection during a TAD virus outbreak event.

1. Introduction

All meatpacking plants focus on the safe processing of livestock to ensure optimal products are delivered to customers. A large part of the safety aspect is determined by direct bacterial testing of meat products to determine their shelf life (Sun and Holley, 2012). Proper disinfection of meat processing equipment and plant premises is crucial to keeping packaged meat fresh for the longest period possible. Thus, from the point of view of plant managers and sanitarians, spoilage bacteria are the main targets of disinfection procedures. During the event of a virus outbreak, however, the packing plant needs to ensure that meat products are not shipped contaminated with viral pathogens. In a worst-case scenario, the introduction of a transboundary animal disease (TAD) virus-infected animal into the plant could enhance virus spread if the disinfection procedures used for bacterial elimination were ineffective against the outbreak virus.

Introduction of certain TAD viruses, especially foot-and-mouth disease virus (FMDV), into a non-endemic country can have serious consequences for the meat industry; trade with other countries is generally halted until proof of eradication has been demonstrated (Terrestrial Animal Code, 2016). Packing plants that processed infected livestock must ensure that they have successfully disinfected the premises to prevent potential transmission to new animals via feed, fomites or human vectors. The resistance of small, non-enveloped viruses to inactivation by chemical disinfectants is generally regarded to be higher than that of intact bacteria (McDonnell and Russell, 1999), calling into question the efficacy of standard bacteria-centric industry disinfection procedures against many viruses. While there is limited published data on the surface disinfection of TAD viruses in general, there is little published knowledge regarding the use of food industry-compatible chemical disinfectants against TAD virus-contaminated fomites in a packing plant setting.

Simulating the disinfection of TAD viruses in a packing plant requires specific attention to the surfaces onto which swine products come into contact. The main surfaces that could act as a vector for virus fomites are stainless steel blades and processing surfaces, plastic cutting boards and conveyor belts and concrete floors. In the case of an infected animal processed by the plant, the virus is likely present in swine products such as blood and meat juices during processing, as well as in feces during animal holding. It is known that organic material inhibits

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the activity of many chemical disinfectants (Weber et al., 1999), making the inactivation of viruses less effective. Further, experiments modelling hospital disinfection demonstrated that viruses desiccated in blood products had increased viability and increased resistance to disinfectants (Terpstra et al., 2007). By using disinfectants that are used by the food industry on surfaces similar to those found in the packing plants, with virus inoculated into swine products, the disinfection of a packing plant can be more closely simulated.

Here we demonstrated the disinfection of three TAD viruses, FMDV, African swine fever virus (ASFV) and classical swine fever virus (CSFV) in swine blood, meat juices and feces after drying on steel, plastic and concrete surfaces. These viruses have been found at concentrations in swine excretions that can successfully infect naïve animals (de Carvalho Ferreira et al., 2012; Alexandersen et al., 2003; Ribbens et al., 2004). Disinfectants used by the food industry proved highly effective against virus dried in the absence of organic material, however the efficacy of the disinfectants tested against the TAD viruses was strongly inhibited when viruses were dried in most swine products.

2. Materials and methods

2.1. Cells and viruses

FMDV serotype A24 stocks were generated in BHK-21 cells (ATCC# CCL-10). FMDV infection was identified by the presence of cytopathic effects 2 or 3 days post infection in highly FMDV-sensitive LFBK-αvβ6 cells (LaRocco et al, 2013). CSFV strain Brescia and the swine kidney cell line SK6 were obtained from Dr. Manuel Borca (PIADC). CSFV replication was detected by immunohistochemistry as described by Risatti and coworkers (Risatti et al., 2005). ASFV strain BA71/v was obtained from the PIADC virus repository and grown in Vero cells (ATCC# CCL-81). ASFV was identified by plaque formation 5 to 7 days after infection. The propagation of the cell lines has been described previously (Krug et al., 2011). Media used for virus serial dilutions and infections was Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen) supplemented with 1% heat-inactivated Fetal Bovine Serum (Hyclone) and Antibiotic/Antimycotic (Invitrogen). All virus work was conducted under biosafety level 3-Ag containment in accordance with the APHIS select agent regulations in title 9 part 121 of the code of United States federal regulations.

2.2. Virus stock production

All virus stocks were prepared as described previously (Krug et al., 2011). Briefly, cells were infected at an MOI of 0.01 PFU/cell in 850 cm^2 roller bottles and incubated at 37 °C until either 100% cytopathic effect was observed (FMDV and ASFV) or 5 days post infection (CSFV). To harvest the FMDV stocks, roller bottles were frozen and thawed to release virus from infected cells, then the supernatants were clarified by centrifugation, aliquoted and stored at −70 °C prior to use. For CSFV and ASFV stocks, the infected cells were scraped from the roller bottles and centrifuged at low speed to remove the medium. The cell pellets were resuspended in 5 ml of fresh media and subjected to 2 cycles of freezing at −70 °C and thawing at 37 °C, then sonicated 3 times for 30 s each on ice. The cell debris was clarified by centrifugation and the supernatants were aliquoted and stored at −70 °C prior to use.

2.3. Disinfectants and neutralizers

Citric acid (Acros Organics) was neutralized with sodium bicarbonate (Invitrogen) and sodium hypochlorite (Baker) was neutralized with Fluid Thioglycolate Broth (FTB, Fluka) as previously described (Krug et al., 2011). All concentrations of citric acid and sodium hypochlorite were used at 500 µl for disinfection and pre-determined concentrations of neutralizers were also used at 500 µl. Samples of two packing industry disinfectants were obtained from a disinfection company based on the recommendations of packing plant sanitarians. A specific neutralizer for each commercial disinfectant was made to counteract each component of the disinfectant. Commercial disinfectant A was used according to the manufacturer's recommendations at 800 ppm for disinfection in a 400 µl volume. Disinfectant A was neutralized with 1.5 ml of a solution containing 0.6X Dey/Engley broth (Difco) to counteract quaternary ammonia compounds, 7.8 µM sodium hydroxide (Ricca Chemical) to equilibrate pH and 70% calf serum (Invitrogen) to quench surfactant. Commercial disinfectant B was used at 600 ppm as recommended by the manufacturer for disinfection in a 500 µl volume and neutralized with an equal volume of 1.2% FTB to counteract sodium hypochlorite and equilibrate pH. Commercial disinfectant C, a commonly used agricultural disinfectant, was used at 2% and neutralized with a mixture of 70% calf serum to quench surfactant and 2.25% sodium bicarbonate to equilibrate pH. The mixtures of the commercial disinfectants and their specific neutralizers were confirmed experimentally not to be virucidal and the pH of these mixtures was verified to be in the range of 7.0 to 7.5 prior to use in each experiment. The general composition of the commercial disinfectants used herein is described in Table 1. All disinfectants were diluted in 400 ppm calcium carbonate to simulate hard water conditions.

Table 1
Commercial Disinfectants Used in this Study.

Disinfectant	Composition	Use Dilution	pH
A	Quaternary Ammonia, Surfactant	800 ppm	1.8
B	Stabilized Sodium Hypochlorite	600 ppm	10.8
C	Potassium Peroxymonosulfate, Surfactant	2% w/v	2.2

2.4. Disinfection assay

This protocol is a modification of ASTM E1053: Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces (ASTM International, 1997) and as previously described to enhance the recovery of enveloped viruses (Krug et al., 2012). ASFV, FMDV and CSFV stocks were diluted in 1X phosphate buffered saline (PBS). The final concentration of calf serum in the virus inoculum was 1%. 100 µl of this mixture was pipetted on the surface coupons, either stainless steel base molds (Fisher Scientific #15182505C) or non-tissue culture treated polystyrene 6-well plates (Falcon #351,146) and dried for 30 to 60 min at ambient temperature (20 °C −24 °C) in a biosafety cabinet with the lights off. Once dried, virus was exposed to disinfectant for the indicated contact time at room temperature. At the end of the contact time, a predetermined volume of the appropriate neutralizer was added and the submerged dried virus was scraped from the bottom of the coupon into the mixture, which was then added to 1 ml of cell culture media. In each experiment, one control coupon with dried virus was exposed to 500 µl of a mixture of the disinfectant and neutralizer (recovery control) and one coupon without dried virus was exposed to 500 µl of cell culture media (surface cytotoxicity control). All control coupons were incubated at ambient temperature for the maximum indicated contact time simultaneously with the coupons exposed to disinfectant. After the contact time was complete, the recovery control received another 500 µl of the neutralizer: disinfectant mixture prior to scraping, and then the entire mixture was added to 1 ml of cell culture media after scraping. For liquid disinfection assays, the same procedure was performed except the virus was not dried prior to the addition of the disinfectant.

These neutralized samples were serially diluted in infection media and titrated on susceptible cells in 96-well plates. The titer of the recovered virus was calculated using the Spearmann-Karber endpoint titration method (Hierholzer and Killington, 1996). Because of virus dilution and the number of replicate wells infected per dilution, the lower limit of detection in this assay is 0.8 log_{10} $TCID_{50}$, except for the

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