Can Nanoparticles be Used to Control PEDv?

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This project set out to evaluate the efficacy of novel metal nanoparticles (with previously demonstrated antimicrobial properties), either alone or in combination with alternative disinfecting strategies for inactivation of PED. Conducting the work under Biosafety Level 3 containment, experiments that simulated hog production environments showed that copper nanoparticles (Cu-NP) and lime treatments were effective in deactivating the PED virus. These treatments, after being fully validated in subsequent field trials, can form the basis for alternative strategies for controlling PEDv and can be used in conjunction with existing chemical disinfectants to expand biosecurity protection to gaps where current available techniques may not be effective or impractical to apply.

INTRODUCTION

Porcine epidemic diarrhea (PED) is a serious and highly contagious swine disease that is characterized by severe watery diarrhea followed by dehydration, leading to almost 100% mortality in nursing pigs. The primary strategies employed to prevent the spread of PEDv are sanitation regimes incorporating the heavy use of chemical disinfectants. While found to be adequate in critical situations and certain applications, some drawbacks of chemical disinfectants include high cost, adverse impact on certain metal structures, reduced efficacy in the presence of organic matter, and incompatibility for use in certain risk situations (e.g., large areas such as assembly yards, high-moisture or wet surfaces, non-wettable areas such as truck cabs, and heavily-soiled areas).

This project focused on finding alternative agents with proven antiviral properties that can be used against PEDv in situations where the primary chemical disinfectants being used at present cannot be applied, or have limited or unknown efficacy. The goal was to evaluate the efficacy of novel metal nanoparticles with previously demonstrated antimicrobial properties, either alone or in combination with alternative disinfecting agents for inactivation of PEDv.

MATERIALS AND METHODS

Phase I – Evaluation of various antimicrobial agents for inactivation of PED virus (in a clean environment without any organic debris)

In order to test the virucidal effect of nanoparticles (NPs) against porcine epidemic diarrhea virus (PEDv), an "infectivity assay" was conducted to determine whether the NPs treatment affected the ability of PEDv to infect a normally-susceptible host (Vero 76 cells)

Testing lethality of NPs against purified PEDv

Four 250 mL bottles, each containing 20 mL of sterile water, were inoculated with 1 mL of purified PEDv, and 1 mL of ZnO NP solution was added to each of three bottles at three different final concentrations, including: 0.27 mg/mL, 2.5 mg/mL and 5 mg/mL. The fourth bottle served as a no-NP control (just PEDv). The fourth bottle served as a no-NP control (just PEDv). Samples were taken from each treatment, including the control at 0 hr (immediately), 3 hours, 24 hours and 48 hours of NP exposure.

Phase II – Evaluation of various antimicrobial agents for inactivation of PED virus (with organic debris representative of the hog production environment)

In this part of the research, Cu-NP and Lime were evaluated for their respective effects against PEDv under simulated conditions that represent the hog production environment. Studies have shown that nanosized copper exert antiviral activity by generating hydroxyl radicals under aqueous conditions (Fujimori et al., 2011) and also by direct contact effects on dry surfaces (Warnes and Keevil, 2013). Lime has a long history of use as a high-pH disinfectant or antimicrobial agent. More recent studies have shown that lime (slaked) can also serve as an effective antiviral agent (Thammakarn et al., 2015). Accordingly, PEDv particles were mixed with sterile swine feces and then individually combined with either Cu-NP and lime under aqueous conditions.

A total of five replicates were used in this study for each treatment and for each of the three time periods (0, 3 and 6 hrs). A fresh PEDv sample was also incubated along with other treatment samples and quantified at all time periods. This control was implemented in order to monitor the natural decay of the PEDv. All tubes received a 1.3 mL aliquot of feces-PEDv suspension, 2.6 mL of each of the treatment (Lime and Cu-NP) and were then incubated for 0 (10 sec), 3 and 6 hr. After incubation, the tubes were centrifuged and the supernatant separated from the pellet. Both the pellet and the supernatant were subjected to RNA extraction and quantification. Also, the supernatant was further processed for Vero cell infection and culturing to examine the infectivity of the PEDv particles. The cell cultures were observed for 5 days for cytopathic effect and later harvested for RNA extraction. Lastly, samples from all Vero cell infection assays were transferred to fresh Vero cells and incubated to confirm the presence or absence of viable PEDv.

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RESULTS AND DISCUSSION

Infectivity assay

In this assay, the number of infectious PEDv particles in the "slurry" (host cell fragments) and the supernatant of previously-infected Vero 76 cells were determined. The results from both the slurry and supernatant infectivity assays showed similar trends. The highest number of infectious PEDv particles was detected in the control groups at 0 time. The number of PEDv able to infect after being treated with ZnO NPs progressively declined with increasing concentration of ZnO NPs, probably due to physical interference between the ZnO NPs, PEDv and Vero 76 cells. This trend could lead to the conclusion that ZnO NPs have a potency to inactivate PEDv, but not to physically destroy the viral particle.

These experiments suggest that ZnO-NPs interacted with the PEDv particles and generally tended to trap or capture them in the pelleted material. The completed tests clearly suggested the effect of ZnO-NPs in capturing the viruses, but also showed that the PEDv that remained in the supernatant were virulent and hence caused infection.

Experiments with Cu-NP and Lime under simulated field conditions

Table 1 summarizes the variation in levels of infection for the different treatments. For the first two days (48 hr), the cell cultures had no signs of infection. The PEDv in the control samples clearly showed heavy infection after day 5 but the lime and Cu-NP remained uninfected, indicating that the PEDv in these wells have been inactivated by the treatment. Also it can be seen that at T=0 hr (Table 1), the Cu-NP showed lower signs of infection as compared to the control and lime, indicating early onset of cidal effect imposed by the Cu-NP on the PEDv particles.

Table 1. Qualitative assessment of viral infection (e.g., syncytia formation) during cell culture infection.

Treatments	Samples at different incubation time		
	0 hrs	3 hrs	6 hrs
Control	+++	+++	+++
Lime	+++	0	0
Cu-NP	+	0	0

–Note: "+" sign indicates level of infection, "0" – no visible infection; "+" is 1-3 loci of infection; "++" is > 10 loci of infection; "+++" – Too many infection loci to count.

In addition, the cell culture plates when observed for the cytopathic effect, showed characteristic variations in infection between treatments and also at different incubation times (Figures 1 and 2).

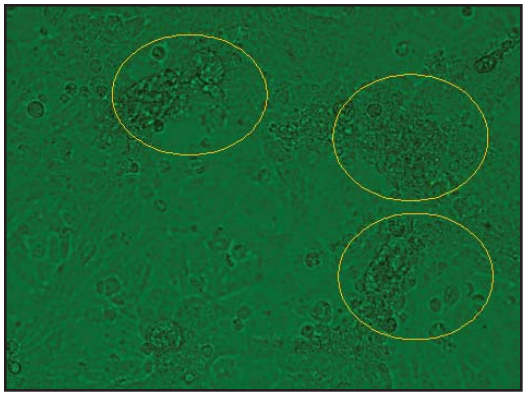

Figure 1. Image showing the sites of infection with PEDv in the Vero 76 cell culture in the 6-well plate. The circled regions show the cytopathic effect (CPE) caused by the PEDv in the control sample.

Figure 2 Image showing no infection (cytopathic effect) in the Vero 76 cell culture in the 6-well plate incubated with Cu-NP. A clear monolayer of cells is visible.

CONCLUSION

- A diagnostic methodology utilizing real time PCR techniques and modified primers was developed for reliable detection of PED virus. In addition, an infectivity assay using Vero cell cultures was also developed to accurately assess the degree of deactivation of PED virus.
- Direct evidence that both Cu nanoparticles and lime treatment exerted a significant anti-PEDv effect was measured. Although this was not shown definitively by the qPCR results, the Vero culture wells containing PEDv exposed to both Cu-NP and lime (for 3 and 6 hours) failed to show any sign of infection, thus confirming the PEDv-inactivation effect of Cu-NP and lime treatments.
- From the 0-time anti-PEDv effect observed for Cu-NPs, it seems that rapid, strong interactions occurred between PEDv and Cu-NPs, leading to an almost instant measurable decline in the number of viable virus.
- Given the results obtained from Cu-NP and lime treatment in the final set of experiments, these two agents appear to have potential to be used individually or in combination, as part of existing anti-PEDv strategies. In such an approach, these "bulk agents" can be applied on large surface areas which may or may not be contaminated with organic materials (e.g., service or assembly yards, loading docks, parking lots) that pose potential risks for PEDv contamination but to which current anti-viral disinfectants cannot or would be impractical to apply.

ACKNOWLEDGEMENTS

We would like to acknowledge the financial support for this project provided by the Agriculture Council of Saskatchewan Inc. through the Advancing Canadian Agriculture and Agri-Food Saskatchewan (ACAAFS) program. The authors would also like to acknowledge the strategic program funding provided by Sask Pork, Alberta Pork, Ontario Pork, the Manitoba Pork Council and the Saskatchewan Agriculture Development Fund.