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In vitro assessment of the effectiveness of the disinfectant Stalosan-F® against *Lawsonia intracellularis*

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Introduction

Lawsonia intracellularis (LI) is transmitted among pigs via a fecal-oral route. Horizontal transmission occurs as a continuous cycle through fecal material and fomites. To break the cycle of infection, proper disinfection of housing, floors, and equipment is important to eliminate or reduce the number of LI in the environment. Few studies have been conducted to determine the efficacy of disinfectants against LI (1). The objective of this study was to determine the efficacy of the powder disinfectant Stalosan F® against LI in vitro using both a tissue culture system and a direct count method.

Materials and Methods

LI strains VPB4 and PHE-MN-01 were used and both strains were grown and harvested as described elsewhere (2). Stalosan F was prepared and tested both as a powder disinfectant and as an aqueous suspension. For use as a powder, 300 µl of bacterial solution containing 10^8 LI/ml were added in duplicate to 10x10 cm sterile petri dishes. Then 1g, 0.5g, or 0.25g of Stalosan F powder was applied and distributed to cover the entire surface of each dish. These yielded final concentrations of Stalosan F equivalent to 100 g/m^2, 50 g/m^2 (dose recommended on label), and 25 g/m^2, respectively. To test an aqueous suspension, Stalosan F was prepared to final concentrations of 1%, 4%, 8%, 16%, and 32% in Dulbecco's Modified Eagles Medium (DMEM). Eight ml of each concentration was aliquoted in duplicate. Then 300 µl of 10^8 LI/ml was added to each aliquot. In both applications, LI was exposed to Stalosan F for 0.5h, 1h, 2h, and 4h. The controls of each time point were live LI in DMEM without exposure to Stalosan F and LI killed with isopropyl alcohol (2). After incubation, the dishes were washed and the bacteria in both applications were separated from the powder by passing the suspension through 5 µm filters and washing twice. After the final wash, half of the pellet was resuspended with sterile distilled water for enumeration with the direct count method by staining the bacteria with live/dead BacLight as described elsewhere (2). The other half was resuspended with LI culture media and transferred to infect one-day-old McCoy cells in 96-well plates (3). After 5 days of incubation, the infected plates were stained with the IPMA procedure for counting the number of heavily infected cells (HIC), an indicator of LI viability.

Results and Discussion

The LI strains PHE-MN-01 and VPB4 were similar in their susceptibility to powder and aqueous suspension of Stalosan F. Using the tissue culture system to determine LI viability, there was no LI detectable in the cell culture after exposure to Stalosan F powder at levels of 0.25, 0.5, and 1 g/cm^2 for 30 minutes. Furthermore, the number of HIC was markedly reduced in the aqueous concentration of 4% (<20% compared to controls) and no LI was detected in cell culture at a concentration of at least 8% for 30 minutes.

Using live/dead staining to determine LI viability, detection of live bacteria was less than 1% after exposure to the powder at concentrations of 0.25, 0.5, and 1 g/cm^2 for 30 minutes. In aqueous form, the number of viable bacteria was significantly decreased in concentrations of at least 16%, with more than 99% of LI killed when compared to controls.

Our results indicate that Stalosan F in both powder and aqueous suspension is able to inactivate over 99% of both LI strains after 30 minutes of exposure. Both tissue culture system and live/dead staining techniques can be used to determine the effect of disinfectants on LI viability.

Reference

1. Collins A, *et al.* 2000. Swine Health and Prod. 8(5): 211-215.
2. Wattanaphansak S, *et al.* 2005. CJVR. 69: 265-271.
3. Wattanaphansak S, *et al.* 2007. Proceeding of 38th AASV. 255-256.