Cryptosporidium spp. oocyst viability submitted to disinfectants under different light conditions.

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Abstract

The study aimed to assess the effectiveness of different disinfectants on cryptosporidiosis oocysts under various lighting conditions. Tested disinfectants included hydrogen peroxide (H_2O_2) and chlorocresol at different concentrations, compared to a control. The findings indicated that chlorocresol, especially at 1:50 and 1:100 concentrations, was effective in damaging oocysts across diverse light conditions. H_2O_2 at a concentration of 1:100 also caused significant damage to oocysts, particularly in shaded environments. The control treatment showed the highest number of viable oocysts during cell culturing tests across all environmental conditions.

Introduction

To effectively control cryptosporidiosis in calves, it is crucial to focus on biosecurity measures, particularly in the maternity area where calves are born. Peripartum cows can significantly contaminate the environment by shedding a high volume of oocysts in their feces. Calves begin shedding oocysts from their first meconium, leading to a high prevalence of *Cryptosporidium* spp*.,* in dairies, reaching nearly 100% prevalence if monitored continuously. The pathogen remains viable under various conditions including cold, humidity, and direct sunlight. Traditional methods of manure removal and cleaning are insufficient, and conventional disinfectants like chlorine, quaternary ammonium compounds, and potassium monopersulfate are ineffective against oocysts.

Methods

A double blinded study was performed *in vitro*, with a sterilized substrate of sand, spiked with 3.3 x 10^5 oocysts obtained from calves with cryptosporidiosis, set under three different conditions: sunlight, partial sunlight, and shade.

Five treatments were used: **1.** H_2O_2 vol 1:50; **2.** H_2O_2 vol 1:100; **3**. Chlorocresol 1:50; **4.** Chlorocresol 1:100; and **5**. Control (H_2OD_1).

Treatments were distributed in blocks in triplicate. Samples were cleaned, concentrated, quantitated, and submitted to *in vitro* culture by the Arrowood system. Oocysts were classified due to their viability and non-viability status.

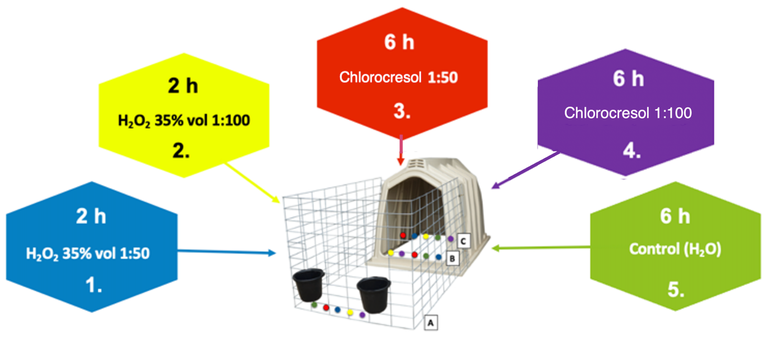
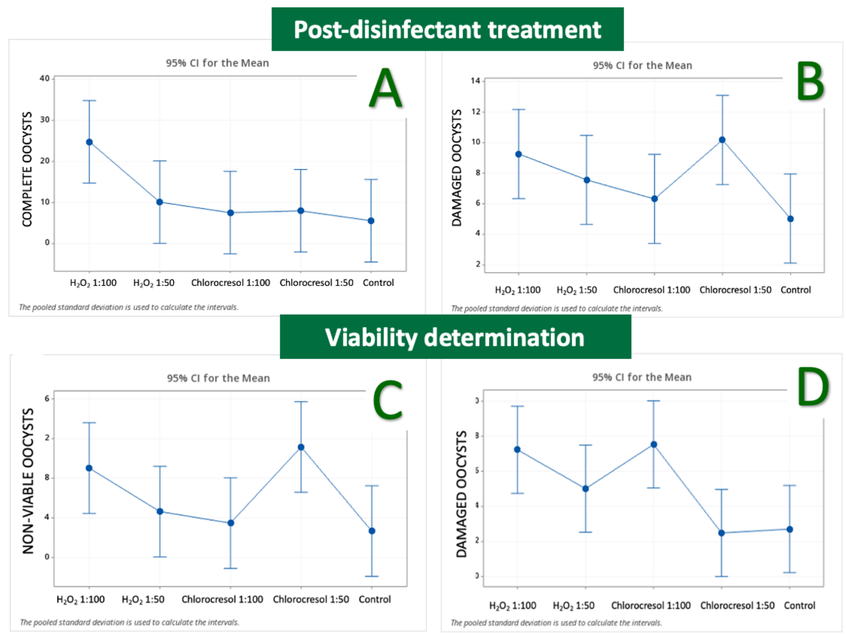


Image 1. H_2O2, Chlorocresol and Control treatments were assigned by dilution, blocking for sunlight, partial sunlight and shade exposure.

Results

Post-disinfectant treatment



Gr**aphs A-D.** Comparison of complete oocysts post-treatment with disinfectants, with 24.8 x 10^4 oocyst for the H_2O_2 1:100 $p<0.05$ (A).Chlorocresol and H_2O_2 show damage ($p< 0.001$ and <0.05 respectively (B). Non-viable oocyst post *in vitro* culture where 11.2 x 10^4 oocysts from the Chlorochresol 1:50 group, followed by H_2O_2 1:100 ($p<0.05$) (C). Damaged non-viable oocysts where Chlorocresol 1:100 showed the most damaged, followed by H_2O_2 1:100 ($p>0.001$ and $p>0.05$ respectively) (D).

Results

Direct Sunlight

1. Chlorocresol 1:50:

o Generated significant damage to oocysts and detritus.

o Quantitation indicated larger numbers of destroyed oocysts, complete oocysts, and complete non-viable oocysts compared to other treatments.

Partial Sunlight

1. H_2O_2 35% vol 1:100:

o Showed the highest number of complete oocysts.

2. Chlorocresol 1:100:

o Resulted in the highest number of non-viable oocysts and detritus compared to other treatments.

Under Shade

1. H_2O_2 35% vol 1:100:

o Exhibited the highest number of complete oocysts.

o Had the highest number of non-viable oocysts amongst all treatments under shade.

o Chlorocresol 1:100 led to the highest destruction of oocysts.

Control Treatment (H_2OD_1)

• Showed the largest number of destroyed oocysts across all environmental conditions when submitted to viability tests. A correlation test indicated that the viability test was successful due to the amount of viable oocysts in the control group.

Results

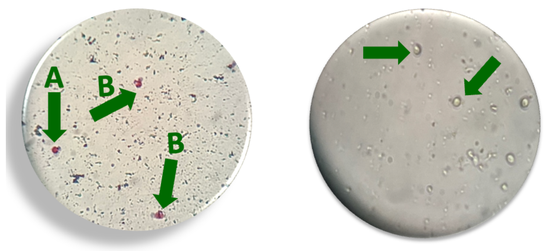


Image 2 left. Oocysts stained with acid-fast staining submitted to H2O2 at a 1:100 concentration. The image shows one complete oocyst (indicated with arrow A) and two destroyed oocysts (indicated with arrows B and C) under 40x magnification. The staining technique highlights the structural integrity of the oocysts, demonstrating the effectiveness of H2O2 in damaging the oocysts.

Imagen 3 right. Non-viable oocysts post- in vitro culturing from oocysts submitted to Chlorocresol at a 1:50 concentration and exposed to direct sunlight. The image, taken at 40x magnification, shows distinct non-viable oocysts (indicated with arrows). The chlorocresol treatment, in combination with direct sunlight, demonstrates significant damage to the oocysts, leading to their non-viability as confirmed by the culturing results

Conclusion

The study aimed to assess the effectiveness of different disinfectants on cryptosporidiosis oocysts under various light conditions. Hydrogen peroxide (H_2O_2) and Chlorocresol were tested at different concentrations in comparison to a control. Results indicated that Chlorocresol, especially at concentrations of 1:50 and 1:100, was effective in damaging oocysts across various light conditions. H_2O_2 at a concentration of 1:100 also showed significant damage to oocysts, particularly under shade conditions. The control treatment consistently showed the highest number of viable oocysts during the cell culturing tests across all environmental conditions.

References upon request