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

Vázquez-Flores, S[1]., Barrera-Almanza, S. [1], Sánchez, F. [2], Esteves-Lopes, R. [2], Nenov, V. [2]

[1]Tecnológico de Monterrey, México *svazquef@tec.mx*
[2]Huvepharma, Belgium.

Biosecurity is one of the areas of opportunity to control cryptosporidiosis. The environment where a calf is born in the maternity area is the main contamination source as peripartum cows can shed 2.4 x 10^9 per 100 g of feces. A calf starts shedding oocysts since the first meconium, and in longitudinal studies the prevalence is from 96.6 to 100% in the dairies if sampled continuously. The pathogen stays viable under different conditions of cold, humidity, and even direct sunlight. Removing manure and cleaning is not enough, classical disinfectants like chlorine, quaternary ammonium compounds, potassium monopersulfate are not useful to destroy oocyst.
Materials and methods. The 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). The treatments were distributed in blocks in triplicate. The samples were cleaned, concentrated, quantitated, and submitted to *in vitro* culture by Atwill et al., 1997. Oocysts were classified due to viability and non-viability status.
Analysis of oocysts post-disinfectant treatments.
H_2O_2 35% vol 1:100, showed the higher number of complete oocysts, damaged oocysts in partial sunlight.
Analysis of oocysts post-viability treatment submitted to direct sunlight, indirect sunlight and under the shade.
Direct sunlight: In the case of chlorocresol 1:50 the treatment generated damage to the oocysts, and detritus, the quantitation indicated larger numbers of destroyed oocysts, complete oocysts, and complete non-viable oocysts than the rest of the treatments.
Indirect sunlight: In the case of chlorocresol 1:100 the number of non-viable oocyst and detritus was larger than the other treatments.
Shade: The H_2O_2 35% vol 1:100 treatment had the highest number of complete oocysts, and the highest number of non-viable oocysts in treatments under the shade. The highest destruction of oocysts was with chlorocresol 1:100.
The H_2OD_1 control treatment showed the larger number of destroyed oocysts during all the environmental conditions when submitted to viability test. When analyzed with a correlation test, the damage indicated that the viability test was successful due to the amount of viable oocysts.
As conclusions, the study aimed to assess the effectiveness of different disinfectants on cryptosporidiosis oocysts under various lighting conditions. Hydrogen peroxide (H_2O_2) and chlorocresol were tested at different concentrations and compared to a control. The 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:

1. ARROWOOD, M. J., STERLING, C. R. Isolation of Cryptosporidium oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. *The Journal of parasitology*, 1987;314-319.
2. ATWILL, E. R., JONES, T., VÁZQUEZ-FLORES, S. . Assessing the environmental risk from rangeland cattle shedding *Cryptosporidium parvum* oocysts in bovine feces: optimization of in vitro excystation rates. *American Veterinary Medicine Foundation*. 2nd Ed. Beltsville, MD.: CRC Press. 1997;43-64.

3. QUILEZ, J., SANCHEZ-ACEDO, C., AVENDANO, C., DEL CACHO, E., LOPEZ-BERNAD, F. Efficacy of two peroxygen-based disinfectants for inactivation of Cryptosporidium parvum oocysts. *Applied and environmental microbiology*, 2005;*71*(5), 2479-2483.