In vitro efficacy of novel antiprotozoal compounds against *Trichomonas foetus*

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There are no legal treatments for cattle infected with *Trichomonas foetus*. This obligate parasite of the reproductive tract in the bovine species represents a serious economic threat to the cattle industry in the United States. The hypothesis of this study was that oxfendazole combined with a pluronic lecithin organogel (PLO) to form a topical formulation may be an effective treatment for bulls infected with *T. foetus*. The specific aim of this experiment was to conduct in vitro testing of the antiprotozoal effects of the oxfendazole formulation and its components on *T. foetus* organisms.

Oxfendazole powder was reconstituted to a solution (OXF) with 70% ethanol (EtOH) and mixed with Velvachol (VC), an emollient, prior to addition of PLO. Viable *T. foetus* trophozoites (5 x 10⁴ mL⁻¹) were added to tissue culture wells containing 4 mL of Diamond’s medium (DM). At time 0, each of the following treatments were added to tissue culture wells in duplicate: 1) positive control 4 mL DM; 2) negative control 4 mL EtOH; 3) 150 mg OXF (1 mL) + 2 mL VC + 1 mL PLO + 4 mL DM; 4) 150 mg OXF (1 mL) + 3 mL VC + 4 mL DM; 5) 150 mg OXF (1 mL) + 3 mL EtOH + 4 mL DM; 6) 150 mg OXF (1 mL) + 2 mL EtOH + 1 mL PLO + 4 mL DM; 7) 1 mL EtOH + 2 mL VC + 1 mL PLO + 4 mL DM; 8) 1 mL EtOH + 3 mL VC + 4 mL DM; 9) 3 mL EtOH + 1 mL PLO + 4 mL DM; 10) 4 mL EtOH + 4 mL DM; 11) 4 mL VC + 4 mL DM; 12) 150 mg oxfendazole dissolved in 4 mL 99% DMSO + 4 mL DM; 13) 4 mL 99% DMSO + 4 mL DM (a known control). Cultures were incubated at 37℃ and aliquots taken every 8 hours over 24 hours to assess the number of viable organisms. A 20 μL sample was collected from each well. Surviving organisms were counted utilizing disposable Neubauer hemocytometers and the presence of pseudocysts noted. At 24 hours after treatment, contents were removed from the tissue culture wells, placed in centrifuge tube, and centrifuged at 4000g for 10 minutes. The supernatant was removed and placed in a labeled vial for evaluation of drug concentration. The pellet was re-suspended in DM and placed in a tissue culture well containing drug free DM and re-incubated at 37℃ for 24 hours. This process was repeated every 24 hours for a total of 5 passages (120 hours) to evaluate for re-emergence of *T. foetus*. Each sample was examined microscopically during this period for the presence of *T. foetus* organisms by counting the live organisms as described above. Non-motile trophozoites were characterized by the presence of the pear shaped bodies with externalized flagella but lack of motion. Pseudocysts were characterized by rounding of the cell with internalized flagella. Multiple formulations rapidly induced the state of non-motile trophozoites and pseudocysts. The antiprotozoal and topical components inhibited the in vitro growth of *T. foetus* to varying extents. However, formulations 2-13 lead to the complete kill of *T. foetus* by 24 hours. Re-emergence to the trophozoite state was not observed in samples from cultures 2-13. In conclusion, this study demonstrated that oxfendazole combined with PLO gel can inhibit the growth of the bovine strain CDTF3 of *Trichomonas foetus* in in vitro cultures with efficacy similar to 70% ethanol which is commonly used to destroy the organism in the laboratory.

Keywords: *Trichomonas foetus*, bull, topical treatment, oxfendazole