CELMANAX™

EFFECT OF CELMANAX™ LIQUID ON THE ATTACHMENT OF *CRYPTOSPORIDIUM* SPOROZOITES TO BOVINE EPITHELIAL CELLS *IN VITRO*

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Introduction: CELMANAX™ is a yeast culture product which has been improved to express more of the functional carbohydrates associated with the yeast culture fermentation process. One of the functional carbohydrates associated with this production process is Galactosamine. N-acetyl galactosamine has demonstrated an ability to interfere with *Cryptosporidium* spp. ability to attach to intestinal epithilium[1]. Inhibition of *Cryptosporidium* sporozoites to adhere to and penetrate host cells in the presence of sugars and complex carbohydrates has been reported[2]. In this study, the effect of CELMANAX on attachment of *Cryptosporidium parvum* sporozoites to bovine cells is investigated.

Objective: To determine the efficacy of CELMANAX to inhibit attachment of *Cryptosporidium parvum* sporozoites to bovine epithelial cells *in vitro*.

Materials and Methods: This investigation was done at Rural Technologies Inc., Brookings, South Dakota, an independent testing facility. The experiment was set up in six replicates. *C. parvum* sporozoites were co-incubated on Madin Darby Bovine Kidney (MDBK) epithelial cell monolayers for 2 hrs. at 37°C with 1 X PBS used as a negative control and 10 µM galactose N-acetyl galactosamine (Gal/GalNAc) used as positive controls. Effect of 0.1 mg/mL Bovine Submaxillary Mucin (BSM) and 40 mg/mL, 20 mg/mL or 2 mg/mL of CELMANAX LIQUID co-incubation on sporozoite attachment to epithelial cells was tested. An Immuno Fluorescence Assay (IFA) was done to microscopically visualize fluorescently stained sporozoites attached to cells *in vitro*. Fluorescing sporozoites were visualized at 400X and sporozoites from 30 random fields (5 fields from each of the 6 replicates) were counted for each treatment. Results are expressed as the number of attached sporozoites per field (sporoz./field).

Results: There was a significant decrease ($P < 0.001$) in the number of attached sporozoites when CELMANAX was present at the two highest concentrations (i.e., 40 and 20 mg/mL), compared to PBS (11.97 and 19.60 vs. 40.80 sporoz./field, respectively) (Table 1). While a trend was evident, there was no difference ($P > 0.05$) in the number of attached sporozoites between the two highest CELMANAX concentrations (Table 1 and Figure 1). The BSM (positive control) showed levels of attachment similar to the 20 mg/mL CELMANAX concentration. The difference in attachment was not significant from PBS control when CELMANAX 2 mg/mL or Gal/GalNAc were used.

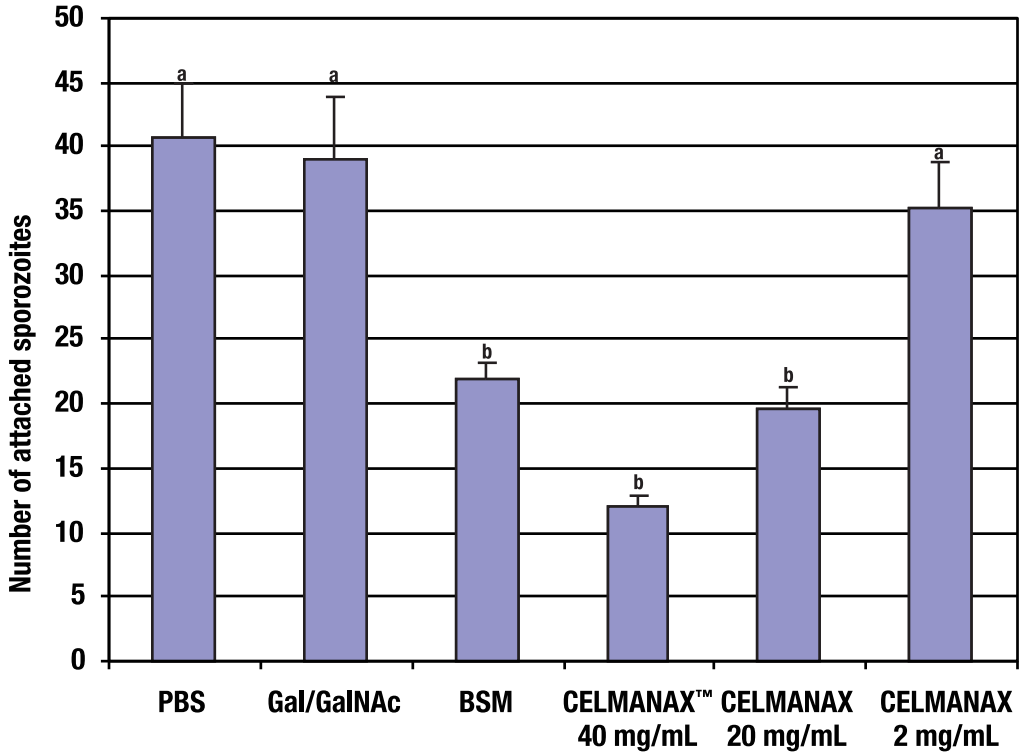
Conclusions: The results from this study suggest that CELMANAX LIQUID significantly inhibits the binding of *C. parvum* sporozoites to bovine epithelial cells *in vitro* in a dose-dependent manner.

Table 1: **Mean Number (30 Fields) of Sporozoites After Incubation with Products and Controls (Sporoz./Field)**

Item	Mean	Std. Error	Difference from PBS (P-value) [a]
PBS	40.80	4.17	NA
Gal/GalNAc	39.10	4.86	Not Significant
BSM	22.00	1.29	<0.001
CELMANAX 40 mg/mL	11.97	0.89	<0.001
CELMANAX 20 mg/mL	19.60	1.78	<0.001
CELMANAX 2 mg/mL	35.20	3.52	Not Significant

[a]Statistics performed on Graphpad, InStat software using Tukey-Kramer analysis for means comparison

Figure 1: **Mean Number of *C. parvum* Sporozoites Bound to MDBK Cell Monolayers Per Visual Field**
Error Bars Represent Standard Error of Means



References:

[1] Hashim *et al* (2006) *Infection and Immunity* 74:99–107

[2] Chen and LaRusso (2000) *Gastroenterology* 118:368-79