Bovine anti-gp40 antibodies neutralize *Cryptosporidium* infections *in-vitro* and are reactive with different *Cryptosporidium* stadia

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INTRODUCTION

Neonatal calf diarrhea (NCD) is the leading cause of morbidity and mortality worldwide.

Cryptosporidium is one of the major pathogens associated with NCD and causes severe diarrhea in newborn calves[1-6]. Currently, no effective vaccine is available for the treatment or prevention of cryptosporidiosis.

A published known important antigen involved in the prevention of *C. parvum* infection is the gp40 or gp60 antigen[7-9], supporting the development of a recombinant gp40-based vaccine.

OBJECTIVE

The objective of this study was to investigate the antibody response of a recently developed *Cryptosporidium* gp40 vaccine in cattle in relation to *Cryptosporidium parvum* (*C. parvum*) parasitic infection stadia and *in-vitro* infection model.

MATERIALS AND METHODS

Healthy pregnant heifers (n=11) were vaccinated twice in the last trimester of pregnancy with the experimental *Cryptosporidium* gp40 vaccine and once with Bovilis® Rotavec® Corona.

A control group (n=12) was included that was only vaccinated once with Bovilis® Rotavec® Corona.

Serum samples were collected before and after vaccination and before calving. After calving, colostrum samples were collected.

All samples were measured in an antigen specific Elisa for anti-gp40 titers and the serum samples on ability to neutralize *C. parvum* using an *in-vitro* infection system.

The serum and colostrum Elisa antibody titers were statistically evaluated with P=0.05.

Sample pools of high positive anti-gp40 samples were used to stain different *C. parvum* infection stadia 24h after infection to show relevance of gp40 antibodies in prevention of *C. parvum* infections.

This study showed that high level anti-gp40, in-vitro neutralizing antibodies were raised when animals were vaccinated with *Cryptosporidium* gp40 vaccine. Staining of different *C. parvum* infection stadia revealed that gp40 was detected in at least two stadia, providing evidence that gp40 is an important protein expressed on the exterior of different *C. parvum* infection stadia

RESULTS

The *Cryptosporidium* gp40 vaccinated heifers showed significant (P<0.001) higher antibody levels compared to the non-vaccinated control group heifers (Table 1).

Colostrum anti gp40 titers from the *Cryptosporidium* gp40 vaccinated heifers were significantly higher (P<0.001) compared to the control group for milking 1 and 2 (Table 1).

Inhibition was observed with serum samples from *Cryptosporidium* gp40 vaccinated heifers in the *in-vitro* inhibition assay, while no inhibition was observed with the control animal samples (Fig. 1).

High positive anti-gp40 serum sample used to stain slides with infected and non infected HCT-8 cells after 24h showed positive staining of various *C. parvum* stages (trophozoites, meronts and merozoites) (Fig. 2), while no specific staining was observed with low positive anti-gp40 serum (not shown).

TABLE 1. Serology of vaccinated and non vaccinated heifers with the experimental *Cryptosporidium* gp40 vaccine

Group	Anti-gp40 titers (log2)									
	Before vaccination		4 weeks post prime		1 week post boost		Milking 1		Milking 2	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Vaccine	10.2	1.2	16.8	2.2	17.5	1.3	21.4	1.6	20.1	1.2
Control	9.9	0.9	nt				13.8	1.5	11.6	1.8

nt = not tested

FIGURE 1. Boxplot serum samples tested in the in-vitro inhibition assay

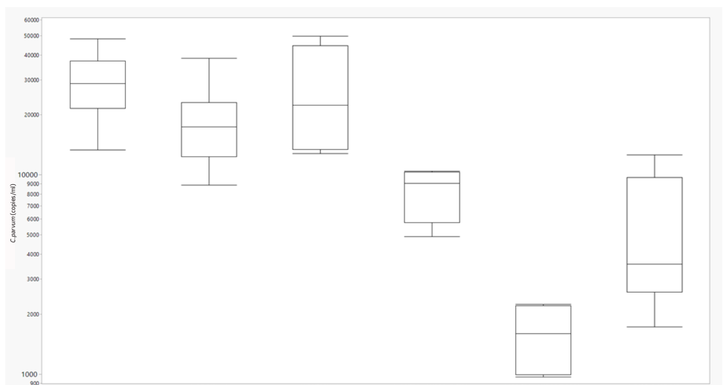
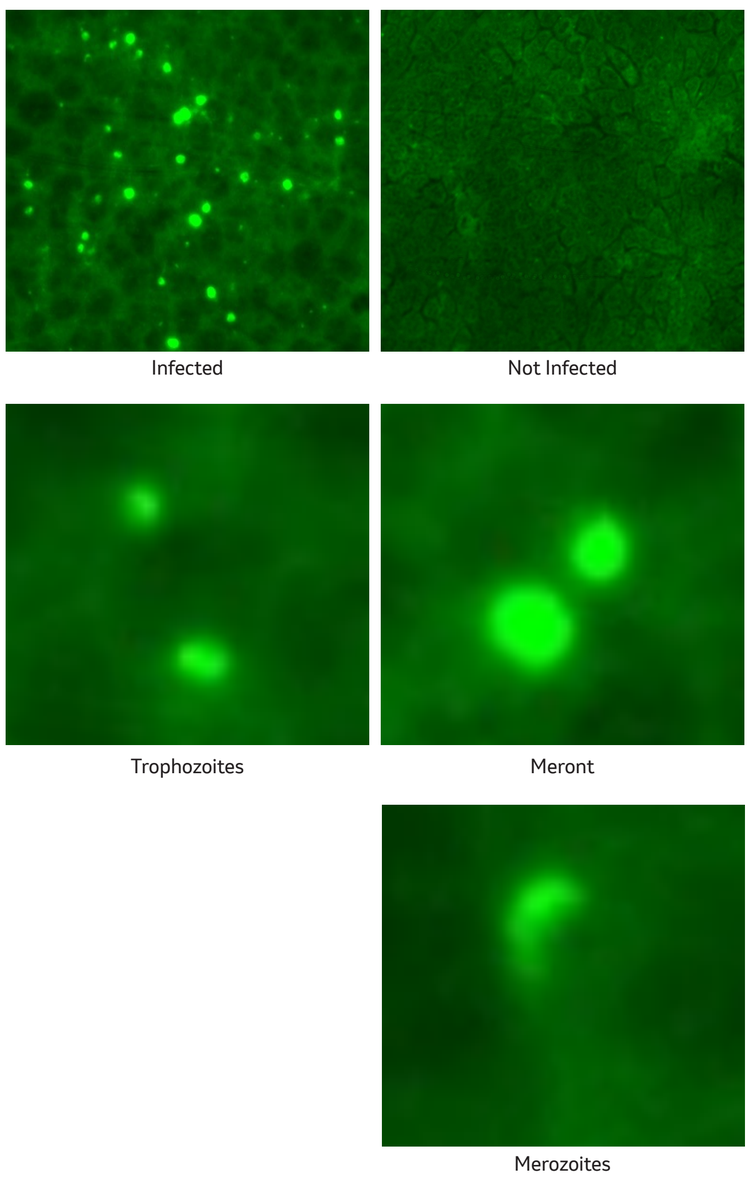


FIGURE 2. Anti-gp40 immune fluorescence staining of infected HCT-8 cells



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