

Research Article

Immunization with Live Attenuated Schizonts against Tropical Theileriosis (*Theileria Annulata* Infection) Increases Antibody Titers in Chronically Infected Cattle in Sudan

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Abstract

In Sudan, two isolates of *T. annulata*, namely Atbara and Hantoub were isolated and successfully attenuated in cell culture. An immunization trial to monitor the antibodies induced by attenuated *T. annulata* schizont candidate vaccine injection to crossbred cattle under field conditions was conducted in Atbara town, Northern Sudan during June and July, 2013. In this trial, 25 cattle of ages ranging from 1 month to 6 years were divided into five groups: one group as control and the other groups were inoculated with different doses of the candidate vaccine (0.05; 0.1; 0.5 and 1 million cells). Blood samples were collected before immunization and four weeks after. The antibody profile against *T. annulata* was assessed using ELISA and IFAT. All the animals were naturally seropositive using both techniques. Titration of antibodies using IFAT at four weeks after immunization showed significant increase in antibody titers against *T. annulata*. These results indicated that the candidate vaccine induced an increase of antibody titers in immunized animals even if they were seropositive because of natural infection.

Keywords: *Theileria annulata*; Attenuated vaccine; Immunization; IFAT; Sudan

Abbreviations

TBD: Tick-Borne Disease; *T. annulata*: *Theileria annulata*; GMEM: Glasgow Minimum Essential Medium; NaHCO₃: Sodium Bicarbonate; TaSP: *Theileria annulata* Surface Protein; IFAT: Indirect Fluorescent Antibody Test

Introduction

Tropical theileriosis is a major Tick-Borne Disease (TBD), which affects cattle throughout a large geographical area in the world including North Africa, Southern Europe and a large part of Asia. Since several years, the total cattle population at risk was estimated to be 250 millions [1]. The disease is caused by an apicomplexan parasite: *Theileria annulata*, which is transmitted by several tick species belonging to the genus *Hyalomma* [2]. Tropical theileriosis is one of the most important TBDs of cattle in Northern Sudan; it represents a major constraint to the intensification of cattle farming [3]. Schizont attenuated vaccine is to date the most effective measure for controlling this disease [4-6].

Immunization using attenuated *T. annulata* schizont vaccines usually but not always result in a response that involves both cellular and humoral immune responses. Cellular immune response include both specific (MHC-1 restricted) and non specific (MHC-1 non restricted) T cell response [7,8]. Humoral (antibodies) response has also been shown to be protective against the sporozoites and merozoites stages of the parasite [9]. The presence of humoral immune response detected by either IFA or ELISA is taken as indirect

evidence of stimulation of cellular immune response [10].

In the Sudan, two isolates of *T. annulata*, namely Atbara and Hantoub were isolated and successfully attenuated in cell culture [11,12], they were evaluated as vaccine candidates in a series of immunization and field challenge trials [13,14] (Sharieff et al. unpublished data).

Owing to the prevalence of tropical theileriosis in most of the Sudan, especially in exotic dairy cattle and their crosses with local type, the present trial was carried out to monitor the antibodies induced by attenuated *T. annulata* schizont candidate vaccine application to crossbred cattle under field conditions in Atbara town, a known endemic area in Northern Sudan during June and July, 2013.

Material and Methods

Cell culture vaccine

Atbara attenuated *T. annulata* schizont candidate vaccine was used at passage 98 of culture in Glasgow Minimum Essential Medium (GMEM) (Sigma, USA) supplemented with 1.875 g of Sodium Bicarbonate (NaHCO₃), 12.5 ml of sterilized Hepes buffer, 20% foetal calf serum, 100 mg of Streptomycin and 100000 IU penicillin per liter.

Immunization of animals using different doses

Twenty five crossbred cattle aged between 1 month and 6 years were randomly divided into five groups of four animals (group 1 and 3), five animals (group 2 and 4) and seven animals (group 5). The experimental animals in groups 1, 2, 3 and 4 were inoculated

Table 1: IFAT Ab-titers of crossbred cattle before and after immunization with different doses of attenuated *Theileria annulata* schizont candidate vaccine at passage 98 during June – July, 2013.

Animal group	Age group (body weight in kg)	Dose ($\cdot 10^6$ cells)	Pre-immunization	Post-immunization	P value
Group 1	1 < 3 months (30)	0.05			0.000*
1A			80	640	
1B			80	640	
1C			160	640	
1D			80	1280	
Geometric mean			95	761	
Group 2	≥ 4 months (40)	0.1			0.000*
2A			80	640	
2B			80	320	
2C			80	320	
2D			80	640	
2E			80	640	
Geometric mean			80	485	
Group 3	2-5 years ($40 \leq 120$)	0.5			0.001*
3A			80	640	
3B			160	1280	
3C			160	1280	
3D			160	160	
Geometric mean			134	640	
Group 4	≥ 6 years (≥ 200)	1			0.003*
4A			160	320	
4B			320	640	
4C			160	640	
4D			80	640	
4E			160	640	
Geometric mean			160	557	

Significant at $p < 0.05$

subcutaneously with 0.05; 0.1; 0.5 and 1 million cells of Atbara vaccine candidate and group 5 was kept as unimmunized control group.

Blood sample collection

The day of immunization, all animals were examined for the presence of *T. annulata* IgG antibody using *T. annulata* Surface Protein (*TaSP*) ELISA. All samples with a percentage positivity values higher than 31.6 were considered positive [15]. On the other hand, titration of sera using Indirect Fluorescent Antibody Test (IFAT) was performed on the day of immunization and four weeks as described before [16].

Statistical analysis

All IFAT titers were log-transformed then comparisons between the titers on the day of immunization and four weeks after were performed by Tukey test using SPSS 20.0 for Windows Software package. A probability of 0.05 was used as cut-off value.

Results

On the day of immunization, all the experimental crossbred

Table 2: Antibodies titers in the control group determined at day 0 and day 28 of the experiment.

Animal group	Day 0	Day 28	P value
Group 5 (Control)			0.001*
5A	80	320	
5B	80	320	
5C	80	320	
5D	160	320	
5E	160	160	
5F	80	640	
5G	160	640	
Geometric mean	108	353	

Significant at $p < 0.05$

cattle were positive to *T. annulata* IgG antibodies using *TaSP* ELISA and IFAT. Titration of antibodies with IFAT after four weeks of immunization revealed significant increase in antibody titers ($p < 0.05$) (Tables 1 & 2).

Table 3: IFAT log-titers from different age groups of crossbred cattle before and after immunization with attenuated *Theileria annulata* schizont candidate vaccine at passage 98.

Age group	Mean Ab titers before immunization ± SD	Mean Ab titers after immunization ± SD
1 < < 3 months	1.98 ± 0.2 ^b	2.88 ± 0.2
≥ 4 months	1.90 ± 0.0 ^b	2.69 ± 0.2
2-5 years	2.13 ± 0.2 ^a	2.81 ± 0.4
≥ 6 years	2.20 ± 0.2 ^a	2.75 ± 0.1

SD: Standard deviation.

a, b: letters in the same column followed by different superscripts are significantly different (p<0.05).

Table 4: Proportional increase in titre of crossbred cattle before and after immunization with different doses of attenuated *T annulata* schizont candidate vaccine at passage 98.

Group	Age	Dose	Proportional increase in titre (Post-titre / pre-titre)
1	1 < < 3 months	0.5×10 ⁵	8 (761/95)
2	≥4 months	1×10 ⁵	6 (485/80)
3	2 – 5 years	5×10 ⁵	4.8 (640/134)
4	≥ 6 years	10×10 ⁵	3.5 (557/160)
5(control)	1month < < 6years	-	3.3 (353/108)

Pre-immunization mean antibody titers were significantly higher (p<0.05) in older animals (2 years and above) than in the youngest one (1 - 4 months) However, no significant difference (p>0.05) was noted between different age groups after immunization (Table 3).

Analysis of antibody titers of tested cattle before and after immunization showed that the youngest animals (1 - 4 months) had the highest antibody response (Table 4).

Discussion

A well-established technique to produce attenuated live vaccine against tropical theileriosis has been used in Iran [17], Turkey [18], Tunisia [19,20], Uzbekistan [21] and Sudan [7]. In the present study, pre-immunization low titers were observed mostly in the youngest cattle group (1 - 4 months). This may probably due to maternal antibodies and/or low natural challenge history. Interestingly, this age group showed the highest antibody response four weeks after immunization indicating that pre-existing antibodies may not interfere with vaccine intake. Whilst, the lowest antibody response was observed in old cattle (aged ≥ 6 years). This may also indicate that the 1 - 4 months old animals are the most suitable age group to target for vaccination.

Doses used in the current trial were based on prior experience in the field where sero-negative animals were used [9] (El Hussein *et al.*, unpublished data). The present results showed that these doses could be injected safely to cattle in the field. Indeed, the antibody increase observed in the immunized animals, indicated that the use of the vaccine in seropositive cattle could afford protection in partially immune animals that may succumb to the disease because of the high field challenge in some areas of Sudan although no reports of vaccination trials in naturally infected animals with theileria or any other apicomplexan parasites are recorded however therapeutic immunization has been tried in cases of viruses e.g HIV, bacteria

e.g *Helicobacter pylori*, *H. mustelae* and parasites e.g *leishmania donovani*. The vaccines used were well tolerated and immunogenic and in some instances such as *H. mustelae* infection the infecting bacteria was cleared [22-25].

Moreover, no untoward side effects were observed among vaccinated animals. El Haj, [9] reported also that this cell culture vaccine using comparable doses was able to protect crossbred animals against disease occurrence.

Conclusion

It could be concluded that the present live attenuated schizont *T. annulata* candidate vaccine can be used safely in apparently healthy animals without regard to their natural infection status. Moreover, this kind of vaccine is known to be safe and reversion to virulence has never been demonstrated and that natural repeated challenges in the field induce long-lasting immunity without need to revaccinate these animals [6,26]. It is highly recommended that mass production and distribution of the vaccine be encouraged from the available strains of vaccine seeds in the Sudan. This vaccine could be injected once to young calves regardless of their serological status. The presence of high infected tick population will then boost the immunity. Because of the economic importance of tropical theileriosis in Sudan it is reasonable that the concerned animal health authorities in the country should consider adoption such policy that would lower costs of disease control in addition to the environmental benefits. Finally and to the best of our knowledge this study represents the first report on the reaction of *T. annulata* seropositive animals to immunization with attenuated schizont vaccine. However, increased titer in non vaccinated animals can indicate low level tick transmission of the parasite during the trial period. This might have also induced a booster effect in the vaccinated animals.

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