Impact of field vaccination with a *Theileria annulata* schizont cell culture vaccine on the epidemiology of tropical theileriosis

S. Singh\(^a\), N. Khatri\(^a\), A. Manuja\(^b\), R.D. Sharma\(^a\), D.V. Malhotra\(^b\), A.K. Nichani\(^b,*,1\)

\(^a\) Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Sciences, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, India

\(^b\) AICRP on Blood Protozoa, College of Veterinary Sciences, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, India

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Abstract

Tropical theileriosis, caused by *Theileria annulata*, is an important tick-borne disease of cattle. A cell culture attenuated vaccine has been developed in our laboratory by long-term in vitro propagation of the schizont stage of the parasite. A longitudinal study was conducted at selected farms housing indigenous, cross-bred and exotic animals to investigate the effect of vaccination on the epidemiology of the disease. A total of 120 animals in 4 age groups were vaccinated with the vaccine before the onset of disease season. An equal number of age-matched animals were kept as controls at the same sites. Animals were monitored for 14 months at monthly intervals. The 97.5% vaccinated animals showed a rise in antibody titres 1 month post-vaccination, as determined by single dilution ELISA. The 78.3% of non-vaccinated animals became sero-positive over the period of observation. Mean antibody titres were significantly higher in vaccinated than non-vaccinated animals. Cross-bred animals showed higher antibody titres followed by exotic and indigenous animals in both the vaccinated and non-vaccinated groups. However, the antibody titres in animals of different ages were similar. The 36.7% vaccinated and 64.2% non-vaccinated animals became carriers (<0.5% piroplasms in erythrocytes) during the observation period. Clinical cases of theileriosis were recorded only in the non-vaccinated group suggesting that vaccinated animals...
were sufficiently immune to withstand field tick challenge for at least 14 months. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Theileria annulata*; Field vaccination; Epidemiology

1. Introduction

Tropical theileriosis, caused by the protozoa *Theileria annulata* and transmitted by the ticks of genus *Hyalomma*, is an important disease of cattle in tropical and sub-tropical countries extending from North Africa and southern Europe in the West to India and China in the East (Robinson, 1982). The disease causes heavy economic losses in the form of high morbidity and mortality rates as well as reduced production in recovered animals (Brown, 1990). The disease assumed particular importance with the intensification of cross-breeding programmes aimed at enhancing production (Sharma and Gautam, 1977). Exotic cattle, their cross-breds and young indigenous calves are highly susceptible, whereas adult indigenous cattle are relatively resistant (Beniwal et al., 1997).

The control measures currently available include live attenuated *T. annulata* cell culture vaccines (Pipano, 1995). The intracellular macroschizont stage of the parasite can be propagated in vitro and become attenuated by continuous passaging (Brown, 1983; Sharma et al., 1998). Such cell culture vaccines are used in many countries for control of this disease (Hashemi-Fesharki and Shad-Del, 1973; Pipano, 1977; Stepanova et al., 1982). A cell culture vaccine was developed in our laboratory (Sharma et al., 1987; Shukla and Sharma, 1991; Khatri et al., 2001) and found to be safe, potent and protective for animals of all breeds and age groups (Beniwal et al., 1997; Gupta et al., 1998). The vaccine has shown to provide immunity for at least 6 months under laboratory conditions in the absence of tick challenge (Beniwal et al., 2000). The effect of vaccination has not been studied in the field where infected ticks are present. Therefore, a longitudinal study of cattle of different breeds and ages was undertaken under field conditions to determine the effect of vaccination with the cell culture vaccine on the epidemiology of tropical theileriosis.

2. Materials and methods

2.1. Farms, animals and vaccination

A longitudinal study was conducted at three dairy cattle farms I, II and III in Hisar, Haryana, India. Farm I housed indigenous animals of Haryana and Sahiwal breeds, farm III housed exotic Holstein Friesian and Jersey animals and farm II housed cross-bred animals from farms I and III. Twenty animals each in four age groups (0–2 months, 2–4 months, 4 months to 2 years and >2 years) were randomly selected from each of the three cattle farms. These animals were serologically negative for antibodies against *T. annulata* as tested by ELISA (Manuja et al., 2000) and showed no piroplasms in their blood smears. Animals were divided in two equal groups (120 each). One group was vaccinated with a *T. annulata* (Hisar) cell culture vaccine (Gupta et al., 1998; Khatri et al., 2001) just before the onset
of disease season (Beniwal et al., 1997) and the second group was kept as non-vaccinated controls. Animals were observed at monthly intervals for 14 months spread over nearly two disease seasons.

2.2. Serological observations

Serum samples were collected from all the animals before vaccination and at monthly intervals thereafter. The samples were stored at \(-20^\circ C\) until they were tested for antibodies against *T. annulata*. A single dilution ELISA, as described previously, using a soluble *T. annulata* piroplasm antigen (Khatri et al., 2001; Manuja et al., 2001), was used. Briefly, a regression equation \(Y = a + bX\); where \(Y\) is the predicted log titre by single dilution ELISA, \(X\) the positive:negative ratio at 1:200 serum dilution, \(a\) the constant, \(b\) the regression coefficient), already derived in the laboratory by Khatri et al. (2001) was used. The regression equation was \(Y = 1.167 + 1.132X\). The test serum samples were diluted to 1:200, optical density (OD) was read at 492 nm in duplicate wells using an ELISA reader (Organon Teknika). The mean OD for each sample was converted to a positive:negative ratio by dividing it by the mean OD of known negative sera at the same dilution. The antibody titres were then predicted using the above regression equation. An animal showing more than a two-fold increase in antibody titre over 1 month was recorded as sero-positive.

2.3. Clinical, parasitological and haematological observations

Clinical, parasitological and haematological observations were recorded on all the animals every month as described previously (Khatri et al., 2001). Animals showing a rectal temperature above 103°F (39.5°C) were considered to be suffering from fever. Thin blood smears were prepared from all animals at monthly intervals, fixed with methanol, stained with Giemsa and examined for the presence of *T. annulata* piroplasms. At least 50 fields per slide at 1000× magnification were examined for the presence of piroplasms. The percentage of infected erythrocytes was calculated. Blood was collected from animals in EDTA and haemoglobin concentration (Hb), packed cell volume (PCV) and total leukocyte counts (TLC) were estimated by standard procedures (Schalm et al., 1975).

2.4. Statistical analysis

The results obtained from the vaccinated and non-vaccinated animals were analysed by a randomised block design (RBD) and factorial RBD, depending upon their suitability (Panse and Sukhatme, 1978).

3. Results

3.1. Serological responses in vaccinated and non-vaccinated animals

Mean antibody titres of vaccinated animals at month 0 were similar to the corresponding titres of non-vaccinated animals. Vaccinated animals showed a sharp increase in antibody titres at 1 month post-vaccination followed by similar titres at month 2 (Fig. 1). Antibody
titres declined slightly at months 3–6, and were significantly lower than mean titres at month 1 \( (P < 0.01) \). Antibody titres at months 7–11 declined further and were significantly lower than titres at months 3–6 \( (P < 0.01) \). Mean titres increased again from months 12 to 14 with the start of new disease season, but were statistically similar to titres of months 10 and 11. The non-vaccinated animals also exhibited a significant increase \( (P < 0.01) \) in antibody titres during months 1–5 in the disease season. This was followed by a decline in antibody titres between months 6 and 12, which further rose slightly during months 13 and 14 with the beginning of new disease season (Fig. 1). Mean antibody titres at months 6–12 were similar to mean titres at month 0. The titres at months 13 and 14 were similar to titres at months 1–5. Antibody titres of vaccinated animals were significantly higher \( (P < 0.01) \) than those of non-vaccinated animals from months 1 to 10 and 12 to 14 (Fig. 1).

Most of the vaccinated animals (117 out of 120) became sero-positive 1 month post-vaccination. One animal sero-converted at 3 months post-vaccination. The remaining two animals showed positive antibody titres at the beginning of second disease season (Fig. 2A). In the non-vaccinated group, 41 out of 120 animals were found to be sero-positive when observed after 1 month. In the following 3 months, 19, 11 and 10 new animals became sero-positive, respectively (Fig. 2A). Nine animals showed antibodies for *T. annulata* in the second disease season. Out of 120 non-vaccinated animals, 94 animals (78.3\%) became sero-positive during the 14 months period.

### 3.2. Comparison of antibody titres in animals of different breeds and ages

Amongst the vaccinated animals (Table 1), highest antibody titres were observed in cross-bred animals followed by exotic and then indigenous animals. Mean titres of indigenous
animals were significantly lower than both mean titres of cross-bred and exotic animals ($P < 0.05$). The pattern of antibody titres in non-vaccinated animals was similar to that of vaccinated animals (Table 1). However, the antibody titres in all the three breeds were not statistically different.

In the vaccinated group, maximum antibody titres were observed in animals of 0–2 months of age and lowest in animals of 4 months to 2 years (Table 2). In the non-vaccinated
Table 1
Effect of breed on antibody titres against *T. annulata*<sup>a</sup>

<table>
<thead>
<tr>
<th>Breed</th>
<th>Reciprocal log&lt;sub&gt;10&lt;/sub&gt; antibody titre (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated animals</td>
</tr>
<tr>
<td>Indigenous</td>
<td>3.38 ± 0.647 a</td>
</tr>
<tr>
<td>Cross-bred</td>
<td>3.70 ± 0.858 b</td>
</tr>
<tr>
<td>Exotic</td>
<td>3.59 ± 0.867 b</td>
</tr>
</tbody>
</table>

<sup>a</sup> The values represent overall mean of 600 observations from 40 animals each. Values bearing different letters differ significantly (*P* < 0.05).

Table 2
Effect of age on antibody titres against *T. annulata*<sup>a</sup>

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Reciprocal log&lt;sub&gt;10&lt;/sub&gt; antibody titre (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated animals</td>
</tr>
<tr>
<td>0–2 months</td>
<td>3.62 ± 0.807 a</td>
</tr>
<tr>
<td>2–4 months</td>
<td>3.59 ± 0.669 a</td>
</tr>
<tr>
<td>4 months to 2 years</td>
<td>3.42 ± 0.880 a</td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>3.59 ± 0.848 a</td>
</tr>
</tbody>
</table>

<sup>a</sup>The values represent overall mean of 450 observations from 40 animals each. Values bearing different letters differ significantly (*P* < 0.05).

group, maximum titres were observed in animals above 2 years of age and lowest in animals of 4 months to 2 years. However, the differences between antibody titres of different age groups were not statistically significant.

3.3. Parasitological observations

Out of 120 vaccinated animals, 44 (36.7%) showed occasional piroplasms (<0.5% infected erythrocytes) in their blood smears at different months during the observation period (Fig. 2B). Ten animals exhibited occasional piroplasms in their blood smears when observed at 1 month post-vaccination. In the non-vaccinated group, 77 animals (64.2%) showed the presence of piroplasms in their blood smears at different months of the observation period (Fig. 2B). This was comprised of 18 indigenous, 29 cross-bred and 30 exotic animals.

Four animals suffered from acute clinical theileriosis in the non-vaccinated group, but no clinical disease was recorded in the vaccinated animals. Three cases occurred in calves below 2 months of age and one in calf of 2–4 months. Clinical cases were recorded in the first disease season during the months of April (one case), May (one case) and June (two cases). One exotic, one indigenous and two cross-bred calves suffered from the disease. These animals exhibited 6–16% piroplasms in their blood smears. All the four animals were treated with buparvaquone (Butalex, Mallinkrodt vet) at 2.5 mg/kg body weight (BW) by i/m injection.
3.4. Clinical and haematological observations

Mean rectal temperatures of vaccinated animals were similar to those of non-vaccinated animals over the period of observation. Mean Hb, PCV and TLC values in vaccinated animals also did not differ significantly from those of the non-vaccinated animals. However, vaccinated animals exhibited a slightly higher mean PCV than non-vaccinated animals (data not shown).

4. Discussion

Lack of fever and other clinical reactions in the vaccinated animals indicated that the vaccine was safe, non-pathogenic and suitable for field use. High antibody titres and very mild parasitological reactions following vaccination showed that responses to this cell culture vaccine were similar to those reported previously (Beniwal et al., 1997; Gupta et al., 1998; Khatri et al., 2001). The post-vaccination reactions of animals were comparable to those reported earlier with other attenuated cell lines (Pipano, 1977, 1995; Ouhelli et al., 1989). The presence of occasional piroplasms in some animals at 1 month post-vaccination is likely to be because of the live vaccine. The cell line used for vaccination is attenuated, yet it can produce piroplasms in a few animals.

Increase in antibody titres, occurrence of four clinical cases of theileriosis and appearance of piroplasms in blood smears of non-vaccinated animals during the disease season confirmed the presence of infected ticks at these farms. A tick infectivity rate of 32.8% has been reported around Hisar (Sangwan et al., 1986), which indicates that all the animals might not be exposed to infected ticks during one disease season. This is also evident from the present study where 78.3% non-vaccinated animals became sero-positive in 14 months over two disease seasons. During the winter months when ticks are not active, a gradual decrease in antibody titres was observed in both the vaccinated and non-vaccinated animals. However, antibody titres of vaccinated animals were slightly higher than the non-vaccinated animals even at the beginning of next disease season. Both vaccinated as well as non-vaccinated animals showed an increase in antibody titres and number of new piroplasm carriers indicating infected tick activity with the onset of the new disease season. No clinical cases and lower number of piroplasm carriers in the vaccinated animals showed that the vaccine was able to prevent infection. Both vaccinated as well as non-vaccinated animals were exposed to infected ticks during the disease season leading to maintenance of high antibody titres in vaccinated animals because of the tick booster and increase in titres of non-vaccinated animals in response to parasite challenge. These observations suggested that animals vaccinated before the onset of the first disease season were immune to the field tick challenge when they entered into the second disease season. This is unlikely to have occurred in the absence of infected ticks present in the field, and is supported by the observations that immunity starts waning after 6 months under laboratory conditions in the absence of parasite challenge (Beniwal et al., 2000).

Highest antibody titres were observed in cross-bred animals followed by exotic and indigenous animals in the both vaccinated and non-vaccinated groups during the present study. Numbers of piroplasm carriers were also higher in non-vaccinated cross-bred and exotic
animals than indigenous ones. The reasons for these differences are difficult to explain since animals were housed in farms with close proximity to one another and with similar management systems. Therefore, differences in tick infection rates at these farms seem unlikely to be the reason. Local indigenous animals are more resistant to *T. annulata* infection than exotic and cross-bred animals and show lower clinical reactions than cross-bred and exotic animals under experimental infection (Gill et al., 1980; Preston et al., 1992). The present experiments show that indigenous animals also show lower immunological responses.

Interestingly, similar antibody titres in animals of all age groups indicate that young calves are fully receptive to the attenuated cell culture vaccine and can be vaccinated at a young age. This is important particularly in the Indian scenario where young calves have been reported to be most susceptible to tropical theileriosis (Beniwal et al., 1998). Slightly higher antibody titres in non-vaccinated adult animals could be because they are sent out for grazing, where as young calves are mostly kept in sheds at these farms.

Observations on other clinical and haematological parameters viz. rectal temperature, Hb and TLC values exhibited no significant differences between vaccinated and non-vaccinated animals. However, overall mean PCV values were slightly but non-significantly higher in vaccinated animals than the non-vaccinated animals. This could be because of higher infection rates in non-vaccinated animals. Vaccination reduced the number piroplasm carriers and consequently the decrease in PCV. This is important because less piroplasm carriers will give less opportunity to ticks to pick up infection thereby having a long-term effect.

Immunity to *T. annulata* has mainly been reported to be cell mediated (Campbell and Spooner, 1999; Nichani et al., 1999). However, humoral immune responses against all the three major stages of parasite, i.e. sporozoite, schizont and piroplasm stages are generated and antibodies may have a neutralising role against extracellular sporozoite and merozoite stages (Preston et al., 1999). Antibody levels have been used as an indirect method to determine the level of immune activity to vaccination in the past (Pipano et al., 1973; Ozkoc and Pipano, 1981; Shukla and Sharma, 1991). Animals showing high antibody titres in the present experiments did not show clinical theileriosis. The four clinical cases occurred in animals showing low antibody titres. Appearance of clinical cases of tropical theileriosis in the months of April and May during the present study coincides with our earlier epidemiological observations in this area (Beniwal et al., 1997).

Observations on non-vaccinated animals confirmed that *T. annulata* infected ticks were present at these farms. Absence of clinical reactions, lower number of piroplasm carriers, lack of clinical cases and significant increase in antibody titres in vaccinated animals indicated that these animals were sufficiently immune to withstand field tick challenge. The study, thus showed that this *T. annulata* (Hisar) cell culture vaccine was safe, potent and protective in the field and was able to provide immunity against field challenge for at least 14 months spread over two disease seasons.

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