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Exposure of cattle immunized with different stocks of *Theileria parva* to buffalo-associated *Theileria* challenge on two game parks in Zimbabwe

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ABSTRACT

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Eight cattle immunized with cattle-derived *Theileria parva* Boleni stabilate together with six susceptible controls were released in Dombawera Game Park on the Highveld of Zimbabwe. This coincided with *Rhipicephalus appendiculatus* nymphal activity. The cattle grazed together with African buffaloes (*Syncerus caffer*) and were not treated against tick infestation. The nymphal tick infestation was high, and seven of the eight immunized cattle and three of the controls had severe and fatal reactions. Subsequently, two stocks of *Theileria parva* to be tested for their immunizing abilities were prepared—one from adult ticks which were fed as nymphs on one of the sick control animals (Dom 268) and the other from adult ticks collected from pastures grazed by buffaloes (Bv-1). Two groups of cattle were immunized with either the Dom 268-derived strain (eight animals) or the Bv-1-derived strain (four animals). These together with three non-immunized controls, were released in Bally Vaughaun Game Park in the Highveld, where buffaloes are present, during the season of nymphal tick activity. A third group of five cattle, immunized with stabilate Bv-1, and three non-immunized controls were released at the same site during the season of adult tick activity. The nymphal and adult tick infestations of the cattle were large and more than 2 000 nymphs and 1 000 adult ticks were counted per animal. Cattle were treated with a pyrethroid pour-on preparation to control the tick infestation and screw-worm strike. The immunized cattle in the three groups survived the theileriosis challenge for a period of 18 months, but the non-immunized control cattle suffered a severe and fatal theileriosis 19–23 days after being placed on the pasture.

Keywords: Buffalo, cattle, immunization, *Rhipicephalus appendiculatus, Theileria parva*

INTRODUCTION

It is generally believed that Corridor disease (buffalo-derived *Theileria parva* infection in cattle) in Zimbabwe is confined to African buffalo (*Syncerus caffer*) environments, and the present disease control measures in this country accordingly limit possible cattle or buffalo contact. The number of Corridor disease outbreaks reported in the early 1980s was about 10% of the total theileriosis outbreaks recorded (Thomson 1985). Recently, there has been an increase in the introduction of game animals, including buffaloes, on the Highveld to establish private game reserves. Ten farms have been authorized to keep buffaloes on condition that they are confined in separate and secure paddocks (S.K. Hargreaves, personal communication 1992). Due to the drought during the years 1990–1991 and consequent shortages of pasture, some farmers introduced cattle into their buffalo paddocks. Three outbreaks of buffalo-derived theileriosis were reported (Anon 1993), which were considered to be the result of a breakdown in farm management and therefore not a national problem. Since a carrier state occurs in buffaloes and cattle in Corridor dis-

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ease (Maritim, Kariuki, Young & Mutugi 1989), the risk of transmission of pathogenic buffalo-derived theileriosis through cattle movement on the Highveld could disturb the present theileriosis control system.

Several reports have described the broad spectrum of immunity conferred by the cattle-derived *T. parva* (Boleni) stock from Zimbabwe against *Theileria* stocks from East and Central Africa. In three of these reports, it was stated that the Boleni stock protected against buffalo-derived *T. parva* (Serengeti-transformed laboratory strain) (Uilenberg, Perie, Lawrence, De Vos, Paling & Spanjer 1982; Irvin, Morzaria, Munatswa & Norval 1989; Hove, Musisi, Kanhai, Latif, Masaka, Munatswa & Dolan 1995). The objectives of the present study were to assess the immunity conferred by *T. parva* (Boleni) in cattle against buffalo-derived theileriosis, and to prepare buffalo-derived stabilates to be used for immunization, if required.

MATERIALS AND METHODS

Experimental animals

Susceptible Hereford cattle, 7–8 months old, were obtained from a farm near Harare, where theileriosis was not recorded during the preceding 16 years. The blood smears from these animals were free of piroplasms and serum samples were negative for antibodies to *T. parva* schizont antigens using the indirect fluorescent antibody test (IFA) (Burridge & Kimber 1972).

Exposure of cattle immunized with *T. parva* Boleni-stabilate to buffalo-derived *T. parva* challenge (experiment 1)

The privately owned Dombawera Game Park in Bindura district, had nine African buffaloes and various other species of wild animals, including impalas (*Aepyceros melampus*), kudus (*Tragelaphus strepsiceros*) and sable antelopes (*Hippotragus niger*). The buffaloes were brought into the park four years before the commencement of the present study. Eight cattle were immunized with cattle-derived *T. parva* (Boleni) stabilate (Lawrence & Mackenzie 1980; Uilenberg *et al.* 1982; Hove *et al.* 1995) and together with six susceptible controls were released in the game park in July 1990. The cattle grazed together with the buffaloes during the period of *Rhipicephalus appendiculatus* nymphal activity. All cattle were treated with imidocarb dipropionate (Imizol) (2.5 mℓ/100 kg) on the that day they were released and again 6 weeks later to protect them against babesiosis (*Babesia bigemina*). Clinical and parasitological examination of thin blood smears and lymph node biopsy smears of the cattle was performed weekly. *Rhipicephalus appendiculatus* nymphs and adults were counted on the animals *in situ* twice a month. Infecting clean laboratory reared *R. appendiculatus* nymphs with *Theileria* parasites was attempted from any control animal that developed severe clinical signs of theileriosis. The criteria used to record animal reactions to theileriosis have been described previously (Hove *et al.* 1995).

Exposure of cattle immunized with different stocks of buffalo-derived *T. parva* to buffalo-associated *T. parva* challenge (experiment 2)

Bally Vaughaun Game Park, Actrus district, also a privately owned farm, had 18 African buffaloes and, similar to Dombawera Game Park, also had various other species of game animals. Most of the buffaloes had been in the park for several years and only three of them were newly introduced at the time of start of the experiment. Two stabilate stocks of buffalo-derived *T. parva* were used to immunize experimental cattle. Stabilate Dom 268-1 was prepared from *R. appendiculatus* adults previously fed as nymphs on a susceptible control animal when it was suffering from a severe theileriosis reaction after grazing with the buffaloes (experiment 1). Stabilate Bv-1 was prepared from unfed ticks collected from the grass in buffalo-grazed paddocks on Bally Vaughaun Game Park. The method of preparation of the *Theileria* stabilates has been previously described (Anon 1993). The details of the immunization protocol are shown in Table 1. The two groups of immunized cattle (12) and the non-immunized controls (three) were released in the game park in July 1991 during the period of *R. appendiculatus* nymphal tick activity and herded together with the buffaloes. All the cattle also received the same chemoprophylactic treatment with imidocarb against babesiosis, as described in experiment 1. A third group of five animals was immunized with stabilate Bv-1 and together with three susceptible controls, was released in the park in January 1992, during *R. appendiculatus* adult activity. Clinical and parasitological monitoring of the cattle and tick counts were carried out as described in experiment 1.

Challenge of cattle immunized with *T. parva* (Boleni) stabilate with ticks collected from buffalo-grazed paddocks (experiment 3)

Unfed *R. appendiculatus* adults were collected from the grass within the buffalo-grazed pastures of Dombawera and Bally Vaughaun Game parks. Ticks from each location were allowed to feed on the ears of *T. parva* Boleni immunized animals together with non-immunized controls (Table 3). Approximately 100 ticks were applied, which is considerably fewer than the infestations recorded on exposed cattle in experiments 1 and 2. In addition, two Boleni-immunized cattle received a homologous stabilate challenge to ascertain their immunity. Clinical and parasitological monitoring was carried out daily after tick application.

TABLE 1 Immunization protocol for cattle before release in Bally Vaughaun Game Park during the seasonal activity of *Rhipicephalus appendiculatus* nymphs and adults

Group	No. cattle	First immunization	Second immunization	Animal reactions to challenge
1	4 4	Dom 268* Boleni**	Dom 268 Dom 268	4 no reaction 4 no reaction
2	4 3	Boleni Non-immunized	Bv-1* Non-immunized	4 no reaction 2 severe/fatal, 1 severe/recovered
3	5 3	Bv-1 Non-immunized	None Non-immunized	3 no reaction, 2 mild reaction 2 severe/fatal, 1severe/treated

* *Theileria parva* buffalo-derived
** *T. parva* cattle-derived
Second immunization given two months after initial immunization

TABLE 2 Reaction of *Theileria parva* Boleni-immunized and control cattle following buffalo-derived *T. parva* field challenge in Dombawera Game Park

Animal number	Days to macroschizonts	Died/recovered (D/R) (days)
Boleni immunized		
A45	15, 29	D (31)
A52	11, 43	D (53)
A59	15	D (22)
A62	11	D (29)
A63	15	D (25)
261	15	R
267	15, 36	D (39)
269	15, 36	D (53)
Susceptible controls		
A73	15	D (32)
260	15	R
263	15	R
265	15	D (42)
266	15	R
268	11	D (80)

RESULTS

Experiment 1

The clinical reactions of *T. parva* Boleni-immunized and the non-immunized control cattle following exposure in Dombawera Game Park are shown in Table 2. The *R. appendiculatus* nymphal infestations were high during July and August 1990 and more than 2 000 nymphs were counted on each animal. The resultant *Theileria* parasite challenge was severe. All immunized animals developed severe reactions—seven died and one recovered. Death occurred after 36.0 ± 11.8 days. All the affected animals showed the typical clinical signs and lesions of a *T. parva* infection (Norval, Perry & Young 1992). Four of them had a second episode of theileriosis, which was severe and two of the animals became blind before death. All control cattle showed severe reactions; three died from the acute disease and three recovered. The majority of cattle (9/14) had very high schizont counts while the parasitaemia was low (2 % and 4 % recorded in only two cases).

Experiment 2

The nymphal tick counts on cattle exposed in Bally Vaughaun Game Park were high with up to 2 000 nymphs per animal. Adult ticks were also recorded during July, although in smaller numbers. The highest counts being 40 per animal.

The resultant disease challenge was severe. Two of the three control cattle died of acute theileriosis on the 21st and 23rd day, respectively, after their release in the game park, while the third animal suffered severe disease but recovered. All 12 immunized cattle in groups 1 and 2 survived the challenge (Table 1) and also survived an artificial challenge with the buffalo-derived stabilate (Bv-1) given on the 76th day after their release in the park. The only surviving control animal which was challenged similarly, developed a severe reaction and died on day 22 following the challenge. The immunized cattle also survived the natural challenge during the subsequent period of adult tick activity.

The third group of five cattle immunized with stabilate Bv-1 and the three controls released in January 1992 had large *R. appendiculatus* adult infestations. More than 1 000 ticks were counted on each animal by day 12 after their introduction into the park. All immunized and control cattle were treated with flumethrin (Drastic Deadline, Bayer) to control ticks and protect them from screw-worm (*Chrysomyia bezziana*) strike. Two of the immunized animals had mild reactions but recovered spontaneously (Table 1). The control animals suffered from severe infections and two died on days 19 and 21, respectively. The third was treated with Butalex (buparvaquone; Coopers Pitman-Moore) on day 20 and subsequently recovered.

TABLE 3 Reactions of *Theileria parva* Boleni-immunized cattle and of non-immunized controls following challenge with either *Rhipicephalus appendiculatus* adults collected from buffalo-grazed pastures or with *T. parva* Boleni stabilate

Animal number	Challenge stock	No. female ticks engorged	Stabilate dose (mℓ)	Theileriosis reaction
291	Dombawera	63		Severe/fatal (17)*
314 control	Dombawera	22		Severe/fatal (19)
259	Bally Vaughan	30		Severe/recovered
276	Bally Vaughaun	16		Severe/fatal (28)
302 control	Bally Vaughaun	42		Severe/treated (13)
282	Boleni stabilate		3.0	No reaction
284	Boleni stabilate		3.0	No reaction

* Days to death or treatment (Butalex) after challenge

Experiment 3

The clinical reactions of Boleni-immunized animals challenged with ticks collected from the two game parks are shown in Table 3. All the cattle and the non-immunized controls developed severe infections. The two immunized cattle that were inoculated with a homologous challenge did not show any apparent reaction.

DISCUSSION

Following outbreaks of Corridor disease in privately owned game reserves on the Highveld, it was decided to investigate the possibility of immunizing cattle against buffalo-derived theileriosis. Previous experience had shown that Boleni-immunized cattle resisted a challenge with buffalo-derived *T. parva* (Serengeti transformed) (Uilenberg *et al.* 1982; Irvin *et al.* 1989; Hove *et al.* 1995) and stabilate Ngong 1 (Chumo, Taracha, Morzaria, Irvin, Voigt & Purnell 1985). The Boleni-immunized cattle in the present study were not protected against natural buffalo-derived *T. parva* challenge. A lack of protection was also recorded following a challenge using low numbers of ticks collected from paddocks grazed by buffaloes. It is most likely that the Boleni stock in previous experiments protected against the carrier-parasites derived from the original buffalo stocks. Thus, Serengeti transformed *T. parva* (Purnell, Young, Brown, Burridge & Payne 1974) had been passaged through cattle for several years while Ngong 1 (Chumo *et al.* 1985) was derived from cattle exposed to buffalo-grazed pastures harbouring infected *R. appendiculatus*. It is worth mentioning that Ngong 1 stock did not afford adequate protection against a field challenge comprising the source of the immunizing parasite isolate (Chumo *et al.* 1985). The high schizont parasitaemia recorded in the experimental cattle is not a common feature of typical buffalo-derived *T. parva* (Young & Purnell 1973). It might have been due to a mixed infection of *T. parva* and *T. taurotragi* which is of wide distribution in Zimbabwe including the two game parks (Bishop, Spooner, Kanhai, Kiarie, Latif, Hove, Masaka & Dolan 1994).

It has been shown in the present study that the stabilates prepared either from ticks collected from buffalo-grazed pastures or derived from reacting cattle provide cattle with adequate protection against natural challenge. Stabilates, which are prepared from field ticks contain a mixture of parasite components and may be superior to stabilates obtained from an individual animal (Dolan 1985). The immunized cattle survived a continuous challenge for over 18 months.

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