Laboratory and field investigations into the *Theileria parva* carrier-state in cattle in Zimbabwe

A.A. LATIF[1], T. HOVE[2], G.K. KANHAI[3] and S. MASAKA[3]

ABSTRACT

LATIF, A.A., HOVE, T., KANHAI, G.K. & MASAKA, S. 2001. Laboratory and field investigations into the *Theileria parva* carrier-state in cattle in Zimbabwe. *Onderstepoort Journal of Veterinary Research*, 68:203–208

The *Theileria parva* carrier-state in cattle on commercial farms on Zimbabwe was investigated using parasitological and serological methods. The proportion of cattle showing *Theileria* piroplasms on two farms, which had recent histories of disease outbreaks, were 64 % (n = 106, total of heifers and weaned calves examined) and 71.5 % (n = 60) while the proportion of *T. parva* antibodies for the same animals were 59 % and 98.5 %, respectively. On four farms where no cases of the disease occurred for over 10 years, the average proportion of animals showing piroplasms and antibodies were 55.4 % (range 32–82, n = 223) and 73 % (range 47–91, n = 223), respectively. However, on another three farms which had no history of theileriosis outbreaks these proportions were very low, being 11.4 % (0–24, n = 157) for piroplasms and 12.2 % (5–23, n = 157) for antibodies. The mean infection rate in unfed *Rhipicephalus appendiculatus* adults collected from farms with a high prevalence of cattle which were carriers of *Theileria* piroplasms during the tick activity season was 29 % (range 12–60 %) with 9.3 (range 2–18.7) mean infected acini per infected tick. The infectivity of different tick batches to susceptible cattle produced a wide spectrum of theileriosis reactions. Laboratory controlled experiments were carried out to study the persistence of *T. parva* (Boleni) piroplasms in cattle immunized with this strain as well as its infectivity for ticks and its subsequent transmissibility to cattle. Examination of the salivary glands of 15 batches of ticks collected from six immunized cattle on three different occasions over 18 months showed that none were infected with *Theileria* parasites. However, the infectivity of other ticks in the same batches to susceptible animals was demonstrated 6, 10 and 18 months after cattle had been immunized with Boleni stabilate.

Keywords: Carrier-state, cattle, epidemiology, *Theileria parva*

INTRODUCTION

In Zimbabwe, in spite of over 80 years of intensive tick control by chemicals, the annual number of theileriosis outbreaks during the years 1954–1988 has increased (Koch 1990; Anon. 1993) and it has proved impossible to eradicate ticks (Norval 1982; 1983). More recently, however, there has been a move away from strict tick control to one of selective control and the establishment of endemic stability in cattle belonging to the communal sector (Thomson 1985; Madzima & Mutugi 1996). Immunization against *Theileria parva* and other tick-borne diseases could play a major role in an integrated control programme (Lawrence 1985). An initial immunization trial using the locally isolated *T. parva* Boleni stock was conducted successfully in 1990 by Koch, Kambeva, Ocama, Munatswa, Franssen, Uilenberg, Dolan & Norval. The infection and treatment method of immunization has also been shown to be effective in Malawi (Musisi, Quiroga, Ngulube & Kanhai 1989) and Zambia (Berkvens, Geysen & Lynen 1989). However, there has been considerable concern about the application of the method particularly identifying the

[1] Midrand University, Faculty of Science, P.O. Box 2986, Halfway House, 1685 South Africa

[2] University of Zimbabwe, P.O. Box 167 MP, Harare, Zimbabwe

[3] Veterinary Research Laboratory, P.O. Box 8101, Causeway, Harare, Zimbabwe

Accepted for publication 4 June 2001—Editor

role of immunized carrier-cattle in the overall epidemiology of the disease and the magnitude of the present *Theileria* carrier-state in the cattle population on different farms. Therefore, controlled laboratory experiments on the immunizing *T. parva* Boleni stock and field investigations on cattle theileriosis were carried out in this study to address these epidemiological concerns.

MATERIALS AND METHODS

Prevalence of *Theileria* piroplasms and antibodies in cattle on commercial farms

Seven farms that had no history of theileriosis outbreaks and two, which had a history of outbreaks (Anon. 1993), were selected to investigate the prevalence of *Theileria* piroplasms and antibodies in their cattle. Blood for serum and thin blood smears were taken from 30–56 animals from each of the weaner (7 months old) and the heifer (1–2 years old) groups on each farm. Sera were screened for *T. parva* schizont antibodies using the indirect immunofluorescent antibody test (IFA) (Burridge & Kimber 1972). Blood smears were stained with Giemsa's stain and examined microscopically for *Theileria* piroplasms.

Infection rates with *Theileria* parasites in ticks collected from five farms and their infectivity to cattle

Unfed *Rhipicephalus appendiculatus* adults were collected from the grass on five commercial farms on the Highveld which had reported incidence of theileriosis outbreaks. A total of 12 collections were made from five farms between January and April in 1991 and 1992, the season of adult tick activity (Short & Norval 1981). The infection rates in ticks from 11 collections from four farms were assessed using Feulgen's staining of whole salivary glands (Blewett & Branagan 1973). The number of ticks examined varied from 36–66 and in one occasion only 15 ticks were examined since most of them were found engorged and unsuitable for dissection. The infectivity of five tick collections from four farms to susceptible cattle was assessed using either live ticks (50–200 were applied per animal) or by injection of stabilate (1.0–2.0 mℓ) prepared from these ticks. One to two susceptible animals were infected with ticks or stabilate from each collection. The method of preparation of the *Theileria* stabilate has been previously described (Anon. 1993).

The infectivity of *Theileria* parasites derived from primary infections of theileriosis on two farms to susceptible cattle

Ten Hereford cattle (6–8 months old) were obtained from a farm on which no cases of theileriosis had occurred for over 16 years. Blood smears prepared and examined from these animals were free of piroplasms and their sera were negative for antibodies to *T. parva* schizont antigens in the IFA test. Five cattle were released on Ayrshire and five on Merton Park farms during the season of adult *R. appendiculatus* activity and were allowed to graze with the farm animals. Confirmed outbreaks of theileriosis had occurred on both farms during the preceding two years. If any of the experimental cattle on the two farms showed clinical signs of theileriosis which was confirmed by lymph node biopsy smears and blood smear examination they were isolated and uninfected *R. appendiculatus* nymphs from the laboratory colony maintained at the Veterinary Research Laboratory in Harare were applied to their ears. Tick pick-up of *Theileria* parasites was successful in two cases, bovines number A32, from Ayrshire and number MP-1 from Merton. The *Theileria* infection rates in adult ticks dropped as engorged nymphs from the two animals were assessed as described above. The infectivity of the adult ticks was also determined by applying ticks from each batch to the ears of one susceptible calf. The theilerial reactions in cattle was assessed according to those described by Hove, Musisi, Kanhai, Latif, Masaka, Munatswa, Pegram, Kamwendo, Quiroga, Mwangondwe & Dolan (1995).

Dynamics of transmission of *Theileria parva* Boleni parasites in carrier animals under laboratory conditions

Experiments were carried out using cattle immunized with *T. parva* Boleni to determine for how long the *Theileria* piroplasms in these animals could be picked-up by ticks and subsequently transmitted to susceptible animals. Six Hereford calves, 7–8 months old, were immunized with the *T. parva* Boleni stock (Lawrence & Mackenzie 1980; Anon. 1993) by the infection and treatment method as has been previously described (Anon. 1993). They were kept free of ticks for 18 months. Uninfected *R. appendiculatus* nymphs from the laboratory colony maintained at the Veterinary Research Laboratory in Harare were used to pick-up the *Theileria* piroplasms from the immunized animals after 6, 10 and 18 months of immunization. The infection rates with *Theileria* parasites in the subsequent adult ticks were determined as described above. The infectivity of each batch of ticks obtained from an individual immunized animal was tested in one susceptible cattle. The six immunized cattle received homologous challenges with the *T. parva* Boleni stabilate after 19 months of their initial immunization to ascertain their immunity. Similarly, the six animals receiving the ticks derived from the 18-month pick-up and recovered were also challenged after 2 months with the same Boleni stabilate to assess their immunity. The animal reaction to theileriosis was assessed as described above.

RESULTS

Prevalence of *Theileria* piroplasms and antibodies in cattle in commercial farms

The proportion of cattle that were parasitologically and serologically positive to *Theileria* piroplasms and *T. parva* antibodies is shown in Table 1. On the two farms (Ayrshire, Dunninue) on which cases of theileriosis had previously occurred the prevalence of *Theileria* piroplasms in blood smears of heifers was 60 % and 80 % and antibodies was 68 % and 100 %, respectively. The prevalence rate in the weaner group on the two farms was also high, being 68 % and 63 % for piroplasms and 50 % and 97 % for antibodies. On the farms which had no history of previous outbreaks of theileriosis, the proportion of animals showing piroplasms and antibodies in four out of the seven farms was 55.4 % (range 32–82, $n = 223$) and 73 % (range 47–91, $n = 223$), respectively. These proportions were very low in the remaining three farms where 11.4 % (range 0–24, $n = 157$) and 12.2 % (range 5–23, $n = 157$) of animals were found to be positive, respectively.

Infection rates in ticks collected from different farms and their infectivity to susceptible cattle

Unfed *R. appendiculatus* adults were found questing on grass tips on the farms from January to April. The mean infection rate with *Theileria* parasite masses in the ten tick batches collected from Ayrshire, Chikeya and Botha farms was 29 % ($n = 410$) and ranged between 11.7–60 %. The mean infected acini per infected tick was 9.3 (range 2–18.7) (Table 2). The infection rate was low in ticks collected from Mazuri

TABLE 1 Prevalence of *Theileria* piroplasms and antibodies to *T. parva* schizont antigen in cattle on different farms in Zimbabwe

Farm	Type	History of theileriosis	Piroplasm %		Ab* %	
			Weaner (*n*)	Heifer (*n*)	Weaner	Heifer
Ayrshire	Beef	Case	68 (56)	60 (50)	50	68
Dunninue	Dairy	Case	63 (30)	80 (30)	97	100
Windsor	Dairy	No-case	–	67 (30)	–	90
Rydale	Dairy	Mo-case	60 (25)	82 (33)	72	91
Highburry	Beef	No-case	32 (28)	57 (47)	64	72
Woodleigh	Beef	No-case	50 (30)	40 (30)	47	77
Magog	Beef	No-case	20 (30)	24 (30)	7	13
Gomo Estate	Beef	No-case	0 (30)	13 (30)	13	23
Mazoe Station	Mixed	No-case	0 (37)	–	5	–

(*n*) Number examined
Ab* Antibody titre 1/640 or higher was considered significant in the IFA test
Case Farms on which theileriosis had previously occurred
No-case Farms on which theileriosis had not previously occurred

TABLE 2 *Theileria* infection rates in *R. appendiculatus* adults collected from commercial farms in the Highveld with previous history of theileriosis outbreaks

Farm	Date tick collection	Number examined	Infection rate (%)	Mean infected acini/tick	Maximum parasite masses in single tick
Ayrshire	Feb. 1991	52	34.9	16.4	64
	Apr. 1991	56	51.8	18.7	100
	Jan. 1992	41	24.4	09.3	20
	Feb. 1992	42	21.4	06.5	25
Mazuri	Feb. 1991	66	32.0	09.4	60
	Apr. 1991	15	60.0	10.5	50
	Jan. 1992	39	25.6	05.3	11
	Feb. 1992	36	11.7	02.0	3
Botha	Feb. 1992	38	34.2	13.3	150
	Mar. 1992	40	13.0	03.0	5
Chikeya	Mar. 1992	50	03.2	03.7	7

Infection rate in ticks from the 5th farm was not determined

TABLE 3 Infectivity in susceptible cattle of *R. appendiculatus* adults collected from the pasture on four of the commercial farms

Source of ticks	Susceptible animal no.	Tick infection rate %	Stabilate/tick challenge	Animal reaction
Ayrshire Farm	310 5–89	34.9 (16.4)	1.0 mℓ 2.0 mℓ	Moderate Severe/prolonged
Chikeya Farm	B14	11.2 (2)	120 ticks	Severe/treated day 24
Mazuri Farm	B19	3.2 (3.7)	50 ticks	No
Felton Farm	084	Not done	200 ticks	Mild

Number of infected acini per infected tick shown between brackets

TABLE 4 Infectivity in susceptible cattle of *R. appendiculatus* adults derived from theileriosis primary infections in commercial farms

Source of ticks	Parasite stock	Calf no.	Tick infection rate	Stabilate/tick challenge	Animal reaction
Ayrshire Farm	A32	89-4	90 (19.2)	2.0 mℓ	S/F, D14
Merton Farm	MP-1	317	38 (2.2)	60 ticks	S/prolonged

Number of infected acini per infected tick shown between brackets
S Severe reaction
F Fatal
D Days to death

farm (3.2%). The infectivity in susceptible cattle of the four batches of ticks collected in the field is shown in Table 3. Ticks collected from Ayrshire and Chikeya farms produced severe reactions in susceptible animals, but the ticks collected from Mazuri and Felton farms induced only mild reactions.

Infectivity of ticks derived from primary infections of theileriosis on the two farms

The infectivity in susceptible cattle of *R. appendiculatus* adults derived from theileriosis primary infections in two commercial farms is shown in Table 4. Ticks dropped from animal number A32 in Ayrshire and MP-1 in Merton Park farms when they were showing severe theileriosis reactions had high infection rates, 90% and 38%, respectively and produced severe infections in susceptible animals (Table 4).

Dynamics of transmission of the *Theileria* Boleni parasites in carrier cattle under laboratory conditions

The results of the tick pick-up and transmission attempts from the six immunized cattle over 18 months period are shown in Table 5. None of the ticks in the 15 batches harvested from the six immunized cattle on three occasions showed any infections with *Theileria* parasites in their salivary glands. However, the infectivity of the these ticks to susceptible cattle was demonstrated in five out of six batches six months after immunization, one out of three batches ten months after immunization and four out of six batches 18 months after immunization. The theileriosis reactions in animals were moderate in five batches (320 ticks dropped per animal), mild in five batches (302 ticks dropped per animal) and five tick batches were not infective (326 ticks dropped per animal). The six cattle that were infected with the tick batch derived from the immunized animals 18 months after immunization were challenged with *T. parva* Boleni, the immunizing stabilate. Four out of the six animals, which had previously reacted to the carrier-parasites were found to be immune. The remaining two animals, which had not reacted, together with the two susceptible controls were proved to be fully susceptible. At the same time, all the six immunized cattle were found to be immune when challenged 19 months after immunization.

DISCUSSION

This parasitological and serological survey on theileriosis in Zimbabwe has yielded valuable epidemiological information on the disease. The data obtained on antibody titre and piroplasm prevalence rates were comparable. The piroplasms were associated to *T. parva* and *T. taurotragi* which are transmitted by *R. appendiculatus* while the vectors for other *Theileria* species of cattle are not present on the farms used in this investigation. In six of the nine farms surveyed, the prevalence rates were high suggesting that the majority of the cattle population were exposed to

TABLE 5 Dynamics of transmission using *R. appendiculatus* from cattle immunized with *Theileria parva* Boleni after 6,10 and 18 months of initial immunization, to susceptible animals

Immune animal	6 Months			10 Months			18 Months		
	Suscept. animal no.	No.ticks fed	Animal reaction	Suscept.. animal no	No. ticks fed	Animal reaction	Suscept. animal no	No. ticks fed	Animal reaction
079	4408	230	Mo (13)	ND	ND		4000	22	M (5)
197	057	15	Mo (12)	ND	ND		4420	144	Mo (6)
200	110	156	Mo (13)	325	130	NR	4414	170	NR
253	032	228	NR	323	74	NR	4430	77	M (5)
254	084	305	M (3)	ND	ND		4919	123	Mo (7)
294	065	270	Mo (10)	324	212	Mo (9)	4426	215	NR

Animal reactions:
NR no reaction
M Mild
Mo Moderate
ND Not done
Number between brackets indicates duration of schizont parasitosis

theilerial infections. Higher prevalence rates of theileriosis were obtained on farms which had recorded outbreaks as well as on farms on which no cases had been reported; this indicates widespread parasite transmission of the disease. Previous serological surveys (Norval, Fivaz, Lawrence & Brown 1985; Koch, Norval, Ocama & Munatswa 1987) showed that the prevalence of antibody positive cattle was widespread including areas where theileriosis outbreaks had not been officially reported. Whether these infections were due to pathogenic *T. parva* or to the non-pathogenic *T. taurotragi* could not be ascertained in the IFA test as the two species cross-react (Uilenberg, Perie, Lawrence, De Vos, Paling & Spanjer 1982). However, it has been possible to characterize these infections on some of the farms using molecular techniques (Bishop, Spooner, Kanhai, Kiarie, Latif, Hove, Masaka & Dolan 1994) confirming the presence of *T. taurotragi*.

Another important component in the epidemiology of theileriosis related to carrier animals is to determine the infection rates in field ticks (Young 1981; Young, Leitch & Newson 1981). These infection rates need to be determined before a quantitative study of the epidemiology can be undertaken (Walker, Young & Leitch 1981). *Rhipicephalus appendiculatus* ticks collected from farms with previous histories of theileriosis had very high average infection rates (29 %) and a high percentage of infected acini. However, in Zimbabwe *T. taurotragi* is widespread (Bishop *et al.* 1994) and one tick batch or a single tick may harbour and transmit more than one *Theileria* species. Therefore, in the present study, field ticks were applied to infect susceptible cattle to partly confirm the identity of the *Theileria* species. The infectivity of the field ticks in susceptible cattle produced a wide spectrum of clinical signs of theileriosis infections confirming the earlier results obtained by Matson (1967). In the present investigation only conventional methods have been used to determine some of the important factors related to the epidemiology of theileriosis. However, improved methods using molecular techniques are now available which can detect infection rates in ticks collected from cattle carrying very low levels of piroplasm parasitaemia (d'Oliveira, Van der Weide, Jacquiet & Jongejan 1997), as well as mixed infections and carrier animals (Gubbels, De Vos, Van der Weide, Viseras, Schouls, De Vries & Jongejan 1999).

In the experiments that were carried out to examine the persistence of *T. parva* Boleni in immunized cattle, its infectivity for ticks and the subsequent transmissibility to cattle, the immunized cattle maintained sporadic infection rates in ticks for 18 months and the infectivity of these parasites in susceptible cattle was demonstrated. The regulating factor, which was responsible for low infection rates in ticks, might have been the very low piroplasm parasitaemia in the immunized carrier cattle. In contrast, higher infection rates were demonstrated in ticks collected from the field and some batches produced severe theileriosis infections in susceptible cattle. In the field, however, superinfection of ticks can occur (Young 1987). Koch, Norval, Ocama & Munatswa (1992) compared the pathogenicity of *T. parva* Boleni carrier-parasites derived from carrier cattle either kept tick-free or maintained in the field. The study showed that ticks derived from carriers in the field produced severe reactions in susceptible cattle.

In conclusion, the present epidemiological studies on the carrier-state of theileriosis in cattle have produced valuable information, which is central in the future design of a theileriosis control policy in Zimbabwe. They have shown that the carrier-state is widespread and that long-term tick control has not reduced the rate and frequency of transmission. Moreover, field ticks can be highly infective for cattle

and constitute continuous risks for the cattle industry. The immunization method of infection and treatment using the *T. parva* Boleni stock produced a carrier-state in the immunized cattle for over 18 months. The carrier parasites transmitted to susceptible cattle produced mild theileriosis reactions under controlled laboratory experiments but afforded solid immunity in recipient animals when challenged with the original stock.

ACKNOWLEDGEMENTS

We are grateful to the commercial farms owners for their cooperation. We thank Prof. F. Jongejan who made valuable comments on the manuscript. The work was supported by the Food and Agriculture Organization (FAO), DANIDA and the Government of Zimbabwe.

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