

## Epidemiological observations of Zimbabwean theileriosis: Disease incidence and pathogenicity in susceptible cattle during *Rhipicephalus appendiculatus* nymphal and adult seasonal activity

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### ABSTRACT

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Fifty-nine Hereford cattle susceptible to tick-borne diseases were used as tracer animals to assess the tick challenge and pathogenicity of *Theileria parva* under field conditions in Zimbabwe. They were moved periodically in groups of five to three commercial farms (one group consisted of four) during seasons of *Rhipicephalus appendiculatus* nymphal and adult activity. All tracer cattle were herded together with the farm cattle but were not dipped. The nymphal tick counts were high on two of the farms (up to 2000 per animal) but were very low on the third farm (less than ten per animal). On the three farms, 19 out of 24 (76%) tracers had patent *Theileria* schizonts. There was a range of clinical manifestations of theileriosis with acute and fatal infections occurring on one farm. The adult *R. appendiculatus* infestations during the wet season numbered 120–800 per animal on the three farms. The disease transmitted by the adults was very pathogenic on the three farms; 30 out of 35 (86%) had severe theileriosis infections. Cattle, which survived the nymphal diseases challenge, showed various degrees of immunity to subsequent *T. parva* challenge transmitted by adult ticks. Therefore, 13 out of 18 (72%) of these cattle had a second disease episode and the case fatality rate on the three farms was 46%. The factors which determined the epidemiological status of *Theileria* challenge on the farms, such as the farming systems and presence of wild animals, are discussed.

**Keywords:** Cattle, epidemiology, *Rhipicephalus appendiculatus*, *Theileria parva*, Zimbabwe

### INTRODUCTION

Zimbabwean theileriosis caused by *Theileria parva* (bovis) (Lawrence 1979; Uilenberg, Perie, Lawrence, Paling & Spanjer 1982) is a complex disease syndrome related to its tick vector *Rhipicephalus appendiculatus* population changes and other factors which

determine the epidemiology of the disease, namely, type of cattle, cattle management practices and tick control. In Zimbabwe and other countries within the region, the different instars of *R. appendiculatus* exhibit a strict pattern of seasonal occurrence with one generation occurring each year (Jooste 1966a; Matson & Norval 1977; Short & Norval 1981a). The adults are mostly active and abundant in the rainy season (mid December to May), larvae in the cool dry season (March to October) and nymphs in the dry months of June to October.

In the years 1914–1946, deaths from the then recently introduced East Coast fever (ECF) caused by *T. parva* occurred throughout the year in Zimbabwe (Matson 1967). However, after the supposed eradication of ECF in 1954, the disease occurrence pattern has changed (Matson 1967; Koch 1990; Lawrence 1991). A variant form of theileriosis, believed

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to produce milder infections, occurs mainly during the rainy season each year and was known initially as "Specific disease" and was later named "January disease" or "Rhodesian-Zimbabwean theileriosis" (Koch 1990). Since then, more than 95% of the theileriosis outbreaks reported in the country have been recorded during the period of adult tick activity. Nymphal ticks have not been related to any significant disease transmission (Koch 1990).

A number of explanations have been put forward to explain the observed change in disease occurrence and its pathogenicity. Norval, Lawrence, Young, Perry, Dolan & Scott (1991) and Lawrence (1991) postulated that the current populations of *R. appendiculatus* in Zimbabwe are different from the populations which were introduced with the cattle from East Africa and were responsible for the transmissions of ECF prior to 1946. The eradication of ECF from Zimbabwe may have been associated with the simultaneous eradication of the introduced East African strains of the non-diapausing tick populations. Diapausing tick strains, which are thought to have survived, continued to transmit the *T. parva* in a seasonal pattern. These theories have not been derived either from field studies or through regular close monitoring of the disease forms over the passage of time.

The objective of this study was to examine the disease occurrence more closely and to attempt to understand the epidemiology of Zimbabwean theileriosis. Field experiments were carried out during 1990–1992 in which susceptible cattle were exposed periodically under natural conditions during the season of adult or nymphal tick activity in order to assess the prevalence of theileriosis associated with adult activity and to investigate the potential role played by nymphs in the transmission of the disease.

## MATERIALS AND METHODS

### Selection of study farms

About 54% of cattle on commercial farms in Zimbabwe are found in Mashonaland West Province (Koch 1990). This province is situated within the high rainfall area where *R. appendiculatus* and Zimbabwean theileriosis are most common (Koch 1990).

Three commercial farms located between 100 and 180 km west of Harare were selected for intensive epidemiological studies from June 1990 to April 1992.

#### *Ayrshire farm—Banket*

Six hundred *Bos taurus* and high-grade crossbreds of different types were present on Ayrshire farm. Tick management was by regular weekly dipping in Baricade acaricide. The farm had no previous record of theileriosis for the 9 years preceding 1989. An outbreak of Zimbabwean theileriosis in which 20 cattle

died occurred during June to July 1989 coinciding with the period of *R. appendiculatus* nymphal activity. A further 18 cattle died and another 20 were treated for theileriosis during the following rainy season (February to March). The wild animals present on the farm included kudu (*Tragelaphus strepsiceros*), bushpigs (*Potamochoerus porus*) and guinea fowls (*Numida meleagris*).

#### *Chikeya farm—Banket*

The Chikeya farm in the Banket district borders Ayrshire farm and 210 crossbred cattle of different types were present. It had a previous history of theileriosis outbreaks for three consecutive years (1987–1990). The farm was poorly managed and tick control using pour-on formulations of synthetic pyrethroids was introduced in 1990. The same species of wild animals were also present on the farm but occurred in larger numbers than on Ayrshire farm.

#### *Botha farm—Karoï*

Botha Farm in the Karoï district had 660 crossbred cattle of different types. Theileriosis outbreaks had occurred in 1989–1990 but cattle management and tick control by dipping were subsequently improved. The farmer was more interested in wild animals and various species had been introduced. About 700 head of different species were recorded at the start of the experiment including kudu, impalas (*Aepyceros melampus*), sable antelopes (*Hippotragus niger*), reedbucks (*Redunca arundinum*), bushbucks (*Tragelaphus scriptus*), duikers (*Sylvicapra grimmia*), bushpigs, warthogs (*Phacochoerus aethiopicus*), hares (*Lepus capensis*) and guinea fowls. They grazed the same paddocks as the cattle.

### Cattle husbandry

Cattle on three the farms grazed on natural pastures and were rotated between paddocks. Maize was also cultivated. In 1991 cattle on Ayrshire farm were moved to maize crop residue fields (early maize) from April to June and again from September to November after the main crop harvest. There was a good harvest in 1992 and the cattle were kept on the maize fields from April to mid-August. On Botha and Chikeya farms, the maize fields were smaller and the cattle were kept in these fields during May and June 1991 and July and August 1991 on the two farms, respectively.

### Animals and observations

A total of 59 susceptible Hereford cattle (7 months old) were obtained from a farm near Harare, where theileriosis outbreaks had not occurred during the previous 16 years, and used as tracers. Blood smears

from these animals were free of piroplasms and serum samples were negative for antibodies to *T. parva* schizont antigen in the indirect fluorescent antibody test (IFA) (Burrige & Kimber 1972). The cattle were periodically moved in groups of five onto the three farms (Table 1) during the period of nymphal or of adult tick activity to assess the possible incidence of the tick-borne theileriosis challenge from 1990–1992.

In the rainy season of 1991, two groups were introduced—one in January and one in February—onto each of Ayrshire and Chikeya farms. Only two groups were moved to Botha farm—one (four animals) in the nymphal and the other in the adult tick seasons of 1991–1992. All tracer cattle were herded together with the farm cattle but were not dipped. They were given Imizol (imidocarb dipropionate) at a dose rate of 2.5 ml/100 kg body mass on the day before they were placed on the farms to control *Babesia* and *Anaplasma* infections and the same dose was repeated on day 14.

Cattle which survived the nymphal tick season, were kept on the farms and monitored during the following rainy season. The observations made included: daily rectal temperature, examination of blood smears on a weekly bases, daily palpation of superficial lymph nodes and examination of biopsy smears made from these nodes when they were enlarged.

The development of antibody titres to *T. parva* antigens was assessed using the IFA test after 30 days of their introduction on Ayrshire and Chikeya farms during the first exposure in 1991–1992. *Theileria parva* reactions in cattle was classified according to that of Hove, Musisi, Kanhai, Latif, Masaka, Munatswa, Pegram, Kamwendo, Quiroga, Mwangondwe & Dolan (1995). East Coast fever was confirmed as the cause of death in most fatal cases by necropsy and examination of liver and spleen impression smears (Norval, Perry & Young 1992). *Rhipicephalus appendiculatus* adults and nymphs were counted *in situ* on the animals twice a month.

Some of the tracer cattle were allowed to undergo severe to fatal infections during the first year of observation for two reasons. Firstly, there is a general

belief that *T. parva* in Zimbabwe produces mild infections in cattle when compared with the disease caused by *T. parva* in East Africa and it was considered imperative to monitor the disease syndrome in the tracers, with minimum intervention, to assess the pathogenicity of *T. parva* in Zimbabwe. Secondly, there were very few cases of theileriosis during the winter season, the period of nymphal tick activity. In the second year of the experiment, all animals developing severe theileriosis reactions were treated with buparvaquone (Butalex, Coopers-Moore) at a dose rate of 2.5 mg/kg.

### *Theileria* tick pick-up and cattle infectivity

On each of the farms laboratory-reared *R. appendiculatus* nymphs were allowed to feed on some of the tracer cattle when they were suffering from acute theileriosis and piroplasms were detected in their blood smears. Engorged nymphs were collected and the infection rates with *Theileria* parasite in the adults were assessed according to the method of Blewett & Branagan (1973). In addition, the infectivity of some of the ticks was assessed by allowing them to feed on susceptible cattle under laboratory conditions. Four tick batches were obtained and examined in this way—from animal 270 introduced on Chikeya farm on 30 June 1990, animals S7 and S22 introduced on Chikeya in January to February 1991 and animal S24 from Ayrshire in January 1991.

## RESULTS

### Ayrshire farm

*First exposure of susceptible cattle during nymphal and adult tick activity seasons (1990–1991) (Fig. 1)*

In the first group of five cattle that was placed on the farm in June 1990 the counts of *R. appendiculatus* nymphs were high (up to 2000 per animal) by July to August 1990 and then decreased towards the end of October. The infections with *Theileria* parasites in the tracer cattle were mild during the season. All of

TABLE 1 Summary of theileriosis reactions in susceptible cattle introduced periodically, in groups of five animals each, on three farms during the season of adult *R. appendiculatus* activity

Farm	Year	January Animal reactions	Days* to death/treatment	February Animal reactions	Days* to death/treatment
Ayrshire	1991	5 S/F	42.0 ± 20.5	2M, 1S/T, 2S/F	22.0 ± 2.6
	1992	4S/T, 1S/F	27.4 ± 1.3		
Chikeya	1991	5 S/F	34.4 ± 11.8	1M, 4S/T	25.0 ± 0.0
	1992	1M, 1S/T 3S/F	27.0 ± 4.7		
Botha	1992	1M, 2S/T, 2S/F	58.0 ± 16.6		

M = moderate reaction; S = severe reaction; T = treated; F = fatal

\* After introduction on the farms

them showed a schizont parasitosis, which was detected only once or twice in each animal. Four out of the five animals developed significant antibody titres (1/640) to *T. parva* schizont antigens by day 30 after being placed on the farm. Adult ticks were first noticed on them by mid-December and the highest counts were recorded in February 1991 (750 per animal). One animal died on 25 December 1990 but the cause of death was not determined. The remaining four animals all suffered from a second theileriosis infection. The reactions in two animals (A32 and A36) were severe and both died—one on 15 January and the other on 22 January 1991. The two remaining animals recovered spontaneously without treatment.

The adult tick counts on the second group of five tracer cattle which were introduced on 17 January 1991 were also high, ranging from 1 500 to 2 000 per animal in January and February. It was responsible for a severe *Theileria* parasite challenge as all of them developed fatal infections (Table 1).

In the third group of five animals that was placed on the farm in February 1991, two developed mild theileriosis reactions and the remaining three had severe reactions; two died and one recovered following treatment (Table 1).

*Second exposure during 1991–1992 (Fig. 1)*

The nymphal tick infestations in the first group of tracers that were introduced in June 1991 were low during June to end of July and became higher between August and September (350–1 600 per animal). All five animals became infected with *Theileria* parasites. Schizonts were detected for 2 weeks but only two of them showed moderate reactions. One animal (R18) developed two disease episodes but survived. The responses of the cattle to the subsequent *Theileria* challenge that coincided with adult tick activity were different. Two of the cattle (R11 and R13), which had reacted mildly, developed a second

theileriosis episode with prolonged illness and slow recovery. On the other hand, the two cattle (R15 and R18) which reacted moderately during the nymphal season, resisted further challenge and survived the rainy season. The fifth animal (R5) died in December 1992 but a necropsy was not done.

The adult tick counts on the second group of susceptible cattle exposed in January 1992 during the rainy season were high (up to 1 000). The subsequent theileriosis challenge was severe. They were all treated by day 25–28 after initial exposure (Table 1) but in spite of this treatment, one animal died acutely.

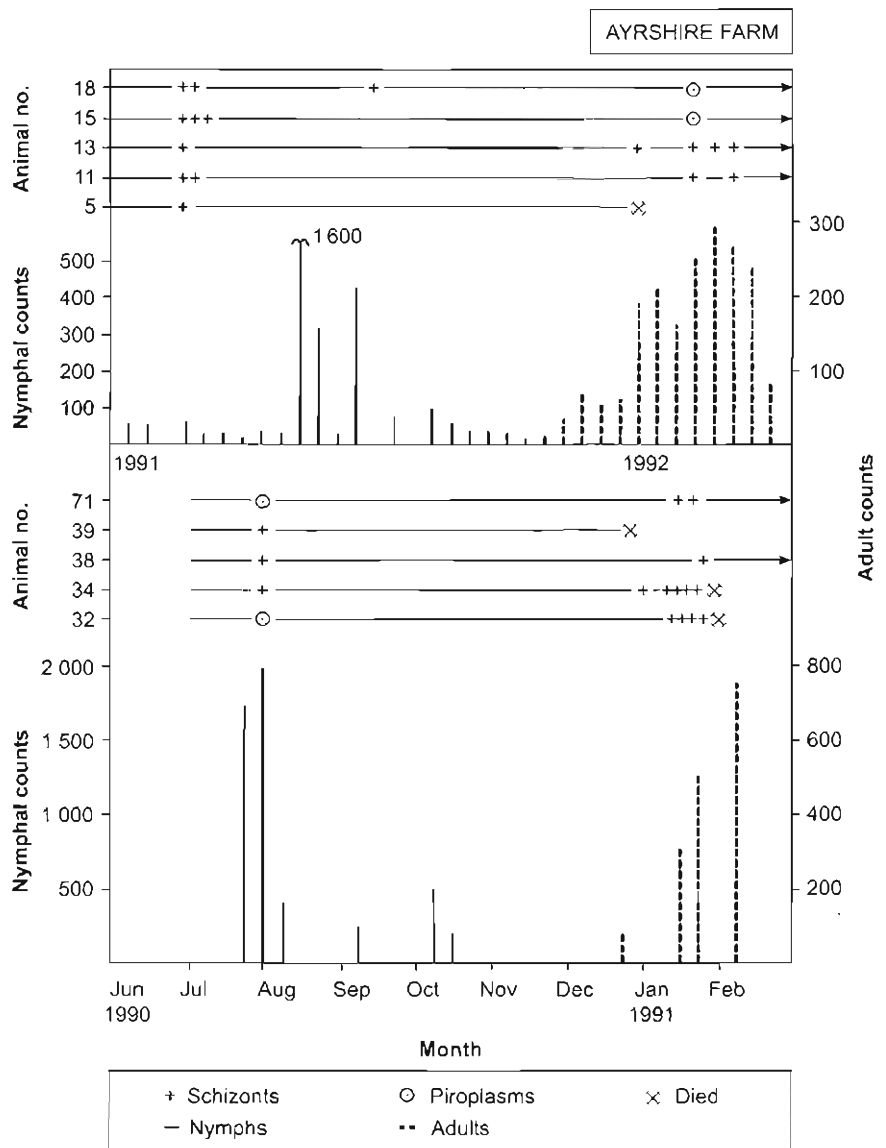


FIG. 1 Performance of susceptible Hereford cattle introduced on Ayrshire farm during the period of *Rhipicephalus appendiculatus* nymphal activity in July 1990 and June 1991 and monitored for theileriosis reactions

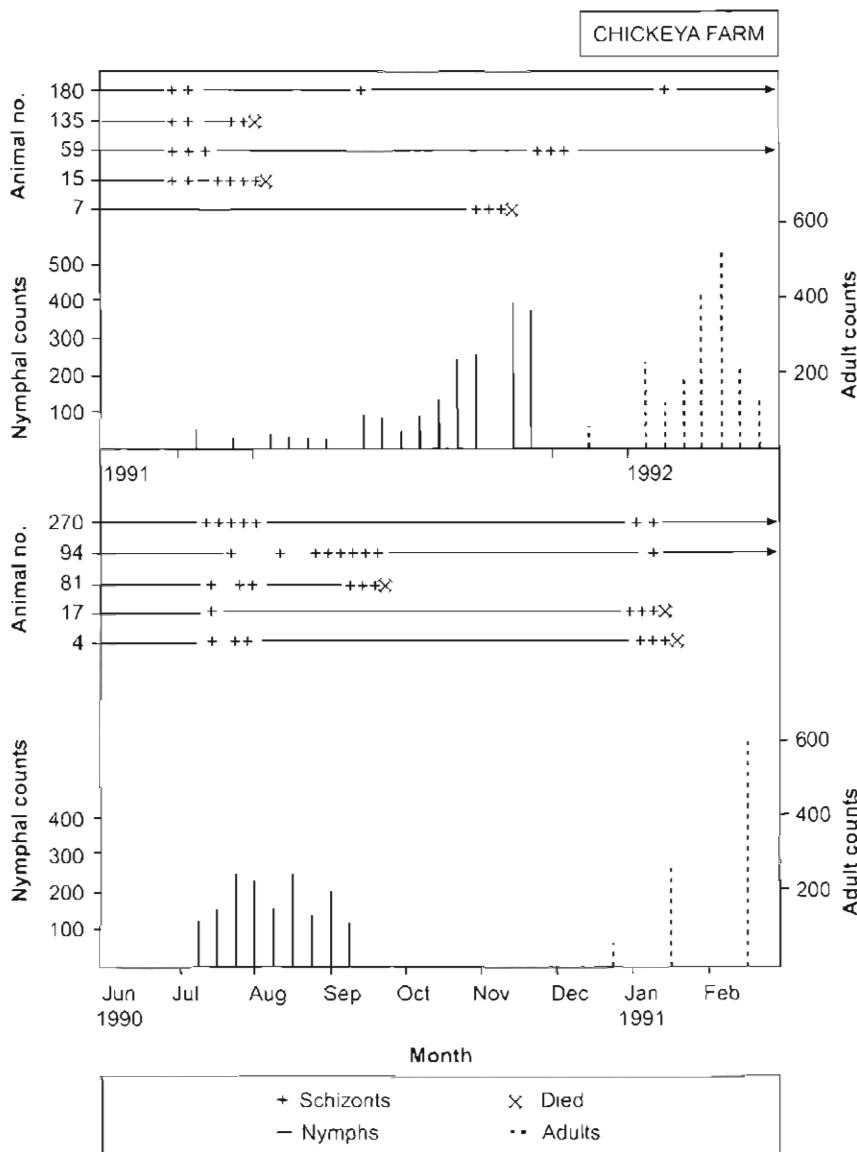
**Chikeya farm**

*First exposure during the nymphal and adult tick activity season 1990–1991 (Fig. 2)*

In the first group of five tracer animals that was released on the farm in June 1990 the nymphal tick counts during June to September were low and ranged between 100–300. However, the theileriosis challenge was high. All animals had severe reactions for up to two months but only one animal died of acute theileriosis (day 71). Two animals had prolonged convalescences. Four of the five animals developed significant antibody titres (1/640) to *T. parva* antigens by day 30 after exposure. Adult ticks were

seen on the animals by mid-December 1991. The cattle had different responses to the challenge. Two had a second severe and fatal infection, one died on 8 January 1991 and the other on 14 January 1991. The remaining animals, which had severe infections during the previous challenge showed mild to moderate reactions and recovered.

The second and third groups, each comprising five tracer animals were introduced in January and February 1991, respectively. They became infested with 600 adult ticks per animal during February. The *Theileria* challenge in the two groups was severe—five cattle died, four were treated for acute infection and one had mild reaction and recovered (Table 1).



*Second exposure 1991–1992 (Fig. 2)*

The nymphal infestation on the five tracers that were released on the farm in June 1991 was low during June to August. The tick counts increased from October to November.

Despite these low infestations during June and July, the theileriosis challenge was high. Four of the five cattle developed severe reactions within the first 2 weeks of exposure and two died of acute infections on days 38 and 42. There was a second disease episode as nymphal counts increased during October and November. One animal (B59) reacted moderately while animal (B7), which reacted during this period only, developed a severe and fatal theileriosis reaction on day 143. The two surviving cattle resisted further *Theileria* challenges during the following adult tick season.

The adult tick activity started in mid-December. Higher infestations (400–500 per animal) were counted on the second group of five tracers that was introduced in January 1992.

All animals in this group developed acute theileriosis—three died, one was treated and one recovered spontaneously (Table 1).

FIG. 2 Performance of susceptible Hereford cattle introduced on Chikeya farm during the period of *Rhipicephalus appendiculatus* nymphal activity in July 1990 and June 1991 and monitored for theileriosis reactions

FIG. 3 Performance of susceptible Hereford cattle introduced on Botha farm during the period of *Rhipicephalus appendiculatus* nymphal activity in June 1991 and monitored for theileriosis reactions

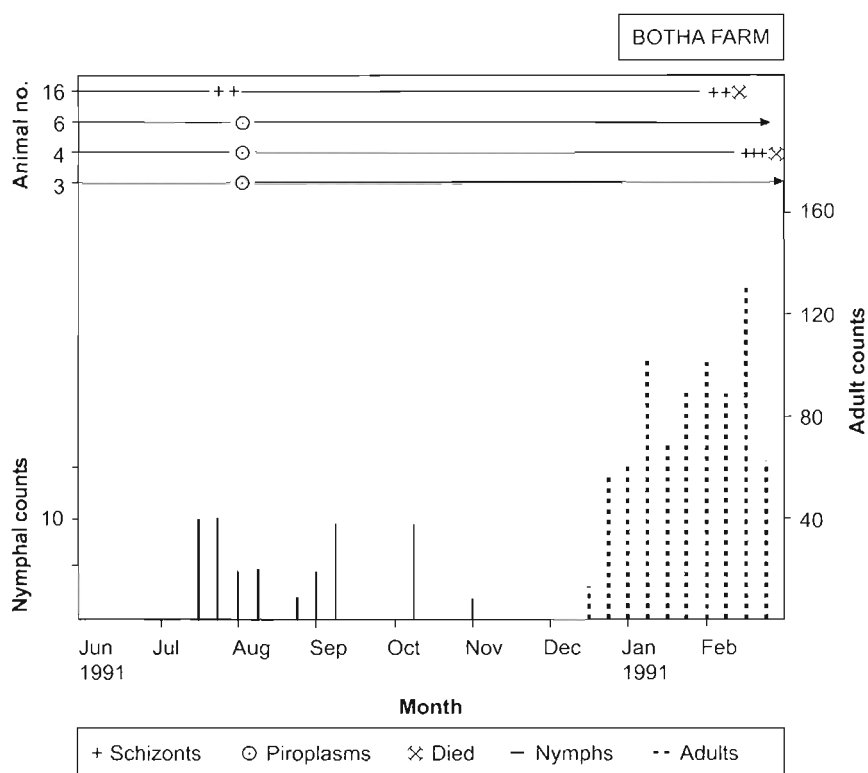


TABLE 2 Infection rates with *Theileria* in salivary glands of *R. appendiculatus* adults infected as nymphs on severely reacting tracer cattle

Animal no.	Farm	<i>Theileria</i> tick pick-up (date)	Tick season	Infection rate (%)	Average no. of infected acini per infected tick
270	Chikeya	27-7-90	Nymph	80.0	6.1
S7	Chikeya	18-2-91	Adult	86.6	38.0
S22	Chikeya	18-3-91	Adult	74.0	10.0
S24	Ayrshire	13-2-91	Adult	26.7	5.8

TABLE 3 Summary of theileriosis reactions in susceptible cattle challenged with adult ticks fed previously as nymphs on reacting animals (Table 1) or received stabilate inoculations prepared from these ticks (20 ticks/m<sup>2</sup>)

Animal source of ticks	Susceptible animal no.	No. ticks applied to feed	Stabilate dose	Animal theileriosis reactions
270	59	22	1.0 ml	Mild
	4431			Mild
S7	89-2	40	1.0 ml	Severe/fatal
	89-1			Moderate
S22	25 B	46		Severe/long illness
S24	89-5	152		Severe/fatal

### Botha farm

#### Cattle exposure during 1991 and 1992 (Fig. 3)

The nymphal tick infestation on the first group of four tracer cattle that was released on the farm in June 1991 was very low, about ten per animal from July to October. The subsequent theileriosis challenge

was also very low and only one animal had a few schizonts on day 21.

The adult tick numbers on the animals during January and February 1992 was about ten times the nymphal counts. During this period two of the animals developed fatal infections while the other two did not show any apparent reaction and survived.

The second group of tracers was introduced in January 1992 and developed severe theileriosis infections—two died, two were treated and one recovered spontaneously (Table 1).

### Transmission attempts and parasite isolation

The details of the *R. appendiculatus* adult ticks fed as nymphs on some reacting tracers and their infectivity to susceptible animals are shown in Tables 2 and 3. The infection rates with *Theileria* parasites in the salivary glands were high (27–80%) but the average number of infected acini per infected tick was low (6–10 in three of the four batches). Three of the tick batches, two from Chikeya and one from Ayrshire farms, were obtained from tracers which became sick during the period of adult tick activity, and produced severe reactions when fed on susceptible cattle in the laboratory. However, the ticks obtained from animal 270 that became sick during the nymphal activity on Chikeya farm produced only mild reactions in the recipient calf.

## DISCUSSION

The study has demonstrated the occurrence of clinical theileriosis in the susceptible tracers which were exposed to unlimited tick challenge on commercial farms during the season of *R. appendiculatus* nymphal activity. The nymphal tick counts on the animals were high on two of the farms and low on the third farm, where a large number of wild animals were present. On the three farms, 19 out of 25 tracer cattle (76%) had patent *Theileria* schizonts and a range of clinical manifestations were seen. Acute and fatal infections occurred on only one farm which confirms out-of-season theileriosis, also known as "winter theileriosis".

Results of laboratory transmission studies of *T. parva* with nymphs are conflicting. Koch (1990) was able to transmit *T. parva* with six out of 13 different tick batches using both high and low numbers of nymphs. Some of the recipient cattle developed severe and fatal infections. *Rhipicephalus zambeziensis* nymphs were also shown to transmit both *T. parva* and *T. taurotragi* (Walker, Norval & Corwin 1981). However, in one attempt with *R. appendiculatus* nymphs these workers failed to transmit *T. parva*. The failure of *R. appendiculatus* nymphs to transmit *Theileria* in these few laboratory studies led to the belief that nymphs are not involved in the transmission of clinical diseases, contradicting previous results. The results were also used to support the field observations on the seasonal occurrence of theileriosis outbreaks (Matson 1967; Koch 1990) while many assumptions and much speculation have been made on the supposed eradication of ECF from Zimbabwe (Lawrence 1991; Norval *et al.* 1991).

In Zimbabwe, *R. appendiculatus* adults and nymphs occur in succession during a 12-month period (Jooste 1966a, b; Matson & Norval 1977; Short & Norval 1981a, b). The present study has shown the transmission of *Theileria* by nymphs between June and December and by adult ticks between January and April. The disease transmitted by adult ticks was pathogenic on all the farms. However, there were variations in disease incidence and virulence during the nymphal season. These variations were related to factors which determine the epidemiological status of the disease on the farms such as the presence of wild animals and the type of cattle management practices as set out below.

Ecological studies on *R. appendiculatus* (Macleod & Colbo 1976) have shown that larvae fed on cattle only are not able to maintain nymphal or adult infestation levels. They suggested that alternative hosts play an important role in maintaining the tick population. Yeoman (1966) has observed that in the presence of wild animals such as hares, mongooses and duikers, cattle may be less important hosts for the larvae. Elands, sables, impalas, kudus, reedbucks and scrub hares were found to be good natural hosts for the larvae and nymphs of *R. appendiculatus* (Macleod & Colbo 1976; Horak 1982; Norval & Lightfoot 1982).

In one study, Horak (1982) showed that impalas harboured significantly more larvae and nymphs (87% of the total tick counts) than adult ticks, while cattle grazing in the same reserve were heavily infested with adult ticks. Moreover, the seasonal patterns of the ticks on scrub hares were identical to those on cattle (Horak & Fourie 1991). Most of these wild animals were present on the farms in varying numbers because of an increasing interest in keeping species of wild animals, other than buffaloes, together with cattle in order to diversify farming systems. The role played by these animals in maintaining tick populations on the three farms can be clearly identified in the present study. On Botha farm nymphal counts on tracer cattle did not exceed ten per animal during the period of nymphal activity, but subsequent adult counts were about 10–16 times higher. It seems probable that wild animals were supporting most of the larval population. This ecological association between cattle, ticks and wild animals has epidemiological implications on the transmission of theileriosis and its pathogenicity on commercial farms. Firstly, the *Theileria* parasites derived from these wild animals are of no or low pathogenicity in cattle hosts (Grootenhuys, Young, Dolan & Stagg 1979). Secondly, the challenge from *Theileria parva*-infected nymphal population may be reduced by feeding on wild animal "refractory hosts". Therefore, the incidence and pathogenicity of theilerial infections in cattle will be reduced.

The second parameter, which would influence the epidemiology of theileriosis on the farms, was the

cattle husbandry practice. Ecological studies on ticks have shown that the spatial distribution of the three developmental stages of *R. appendiculatus* is probably determined by the spatial distribution of their hosts (Minshull 1981). In the present study, cattle were grazed on paddocks during the rainy season (adult tick activity) and moved to maize-residue fields during the dry season (nymphal activity). These maize fields were probably free of ticks as preparation of the land would have started by November to December (rainy season) while crop harvesting was completed by August of the following year. Therefore, cattle were not available as hosts when nymphs were abundant on the pasture. This management practice had partly contributed to a restriction of cattle and tick contact for varying period of time (1–4 months) depending on the size of the maize fields, and may have reduced the incidence of "winter theileriosis" by breaking the transmission cycle.

Cattle, which survived the *T. parva* challenge transmitted by nymphs and had seroconverted, showed various degrees of immunity to the subsequent challenge transmitted by adult ticks. Thirteen out of 18 (72%) of these cattle had a second disease episode, and the case fatality on the three farms was 46% (6 out of 13). This suggests that endemic stability to *T. parva* could not be maintained by natural exposure of the calves during the season of nymphal activity. By using molecular techniques some of these infections on the farms have been characterized as being due to *T. taurotragi* (Bishop, Spooner, Kanhai, Kiarie, Latif, Hove, Masaka & Dolan 1994) which is wide-spread in Zimbabwe (Lawrence 1979; Lawrence & Mackenzie 1980; Koch, Ocama, Munatswa, Byrom, Norval, Spooner, Conrad & Irvin 1988). Cattle, which have recovered from *T. taurotragi* infections, are fully susceptible to *T. parva* challenge (Young, Grootenhuis, Kimber, Kanhai & Stagg 1977; Grootenhuis *et al.* 1979).

In conclusion, the Zimbabwean theileriosis transmitted by adult *R. appendiculatus* in natural situations can be highly pathogenic and is comparable to ECF in East Africa. Any breakdown in the present efficient tick control regimen on the commercial farms may result in disastrous outbreaks. *Rhipicephalus appendiculatus* nymphal transmission of *T. parva* can occur and the parasite may be highly pathogenic and cause economic problems. Under most farming systems applied in Zimbabwe the nymphal transmission of theileriosis is limited by factors which restrict cattle/tick contact such as the presence or absence of wild animals as alternative tick hosts. This epidemiological situation may have existed for several decades and probably explains the gradual change in the pattern of the seasonal occurrence of the disease, which now generally coincides with adult tick activity. The assumptions drawn from the present study may not agree with those of Norval *et al.* (1991) or with the hypothesis of Lawrence (1991) on the

change in pattern of theileriosis transmission related to the elimination of non-diapausing East African strain of *R. appendiculatus*.

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