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ScienceDirect
Veterinary Parasitology 143 (2007) 214–221
veterinary parasitology
www.elsevier.com/locate/vetpar

Epidemiological studies on *Theileriosis* and the dynamics of *Theileria parva* infections in Rwanda

T. Bazarusanga [a], J. Vercruysse [b], T. Marcotty [c], D. Geysen [c,*]

[a] *National Veterinary Laboratory of Rwanda, BP 804 Kigali, Rwanda*
[b] *University of Ghent, Faculty of Veterinary Medicine, Salisburylaan 133, B-9820 Merelbeke, Belgium*
[c] *Institute for Tropical Medicine, Department of Animal Health, Nationalestraat 155, B-2000 Antwerp, Belgium*

Received 17 November 2005; received in revised form 9 August 2006; accepted 5 September 2006

Abstract

An epidemiological analysis based on three country wide surveys was carried out to determine the prevalence of infections with *Theileria* spp. in Rwanda. In the 1998 dry season, a total of 264 blood samples were submitted to *Theileria* spp. characterisation using the 18S species-specific PCR-RFLP assay. The same samples together with 634 samples (317 samples/season) collected during the 2002 dry season and the 2003 wet season were further analysed using the p104 *Theileria parva* specific PCR. The results from the 18S characterisation showed the presence of four *Theileria* spp., namely *T. parva*, *T. mutans*, *T. taurotragi* and *T. velifera* in the field. Half of the animals had multiple *Theileria* spp. infections. *T. parva* was the most prevalent and a high correlation (94%) was found between the prevalence results using the 18S and the p104 PCR assays. The prevalence of *T. parva* infections was stable over time and over season but decreased significantly from the high land to the low land areas. This unexpected trend cannot be explained alone by ecology or the dynamics of the tick population in the different zones, many other components such as breed type, tick control practices and grazing system are likely to play a role. Another important finding was the fact that young animals are infected early in life in all regions except in the high land zone indicating the existence of a particular epidemiological situation in this part of the country.

Keywords: *Theileria parva*; Epidemiology; Prevalence; Agro-ecological zones; Rwanda; PCR

1. Introduction

East Coast fever (ECF) is caused by *Theileria parva*, a tick-borne protozoan parasite of cattle which leads to severe economic constraints of the cattle industry in eastern, central and southern Africa (Mukhebi et al., 1992). It is mainly transmitted by the three-host ixodid tick, *Rhipicephalus appendiculatus*. Ecological and climatic variations induce changes in tick population dynamics which result in different epidemiological situations of theileriosis in the endemic regions (Fandamu et al., 2005). The epidemiology of the disease is further complicated by the presence of other *Theileria* spp. which are less pathogenic to cattle. However, while *Theileria taurotragi* transmitted by *R. appendiculatus* or *Theileria velifera*, transmitted by *A. variegatum* are benign infections, benign and virulent strains of *Theileria mutans* have been described (Young et al., 1978) which is transmitted by *A. variegatum*.

Collection of epidemiological data on theileriosis is a first prerequisite on the road to develop a control strategy. Earlier investigations in many countries have been restricted to estimates of *T. parva* prevalence in limited geographic areas and none so far have addressed the question of the distribution of theilerial infection on a country wide basis. A gradient-like *T. parva* prevalence

* Corresponding author. Tel.: +32 3 247 6261; fax: +32 3 247 6264.
E-mail address: dgeysen@itg.be (D. Geysen).

0304-4017/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2006.09.009

has been reported from a single district of Kenya (Gitau et al., 2000) and from the Eastern Province of Zambia (Billiouw, 2005). These reports are based on serology results using the indirect fluorescent antibody test (IFAT) being so far the most commonly used detection method (Norval et al., 1992b). However, the performance of the IFA test in terms of sensitivity and specificity under field conditions is poorly documented. It is known that the test lacks specificity because of cross-reactions with *T. taurotragi*, which is of importance as the distribution of *T. taurotragi* overlaps with *T. parva* throughout much of eastern, central and southern Africa (Goddeeris et al., 1982; Norval et al., 1992b). Furthermore, IFAT lacks sensitivity in endemic regions when tick transmission is seasonal (Billiouw et al., 2005). The use of DNA based methods detect not only the presence of the parasite but can also be used to obtain prevalence data.

Animals can recover after a *T. parva* infection and become carriers for prolonged periods (Bishop et al., 1992; Kariuki et al., 1995). These animals are immune to homologous infections. Specific identification of low *T. parva* parasite levels in naturally infected animals in the field is essential for epidemiological studies. The recently developed polymerase chain reaction (PCR) techniques have a higher specificity and sensitivity than conventional diagnostic methods in determining theilerial infections in carrier animals (Bishop et al., 1992; d'Oliviera et al., 1995). The PCR methods have proven to be able to characterise and distinguish *T. parva* from multiple *Theileria* spp. infections in field samples (Geysen et al., 1999, Ogden et al., 2003).

In Rwanda, little is known about the prevalence of theilerial infections although ECF is considered as the most important disease of cattle (Paling and Geysen, 1981). High *T. parva* prevalence rates have also been recorded in neighbouring Uganda (Rubaire-Akiiki et al., 2004) and in Kenya (Gitau et al., 2000). The present study reports on results from three epidemiological surveys conducted both during dry and wet seasons and covering all four agro-ecological zones of Rwanda.

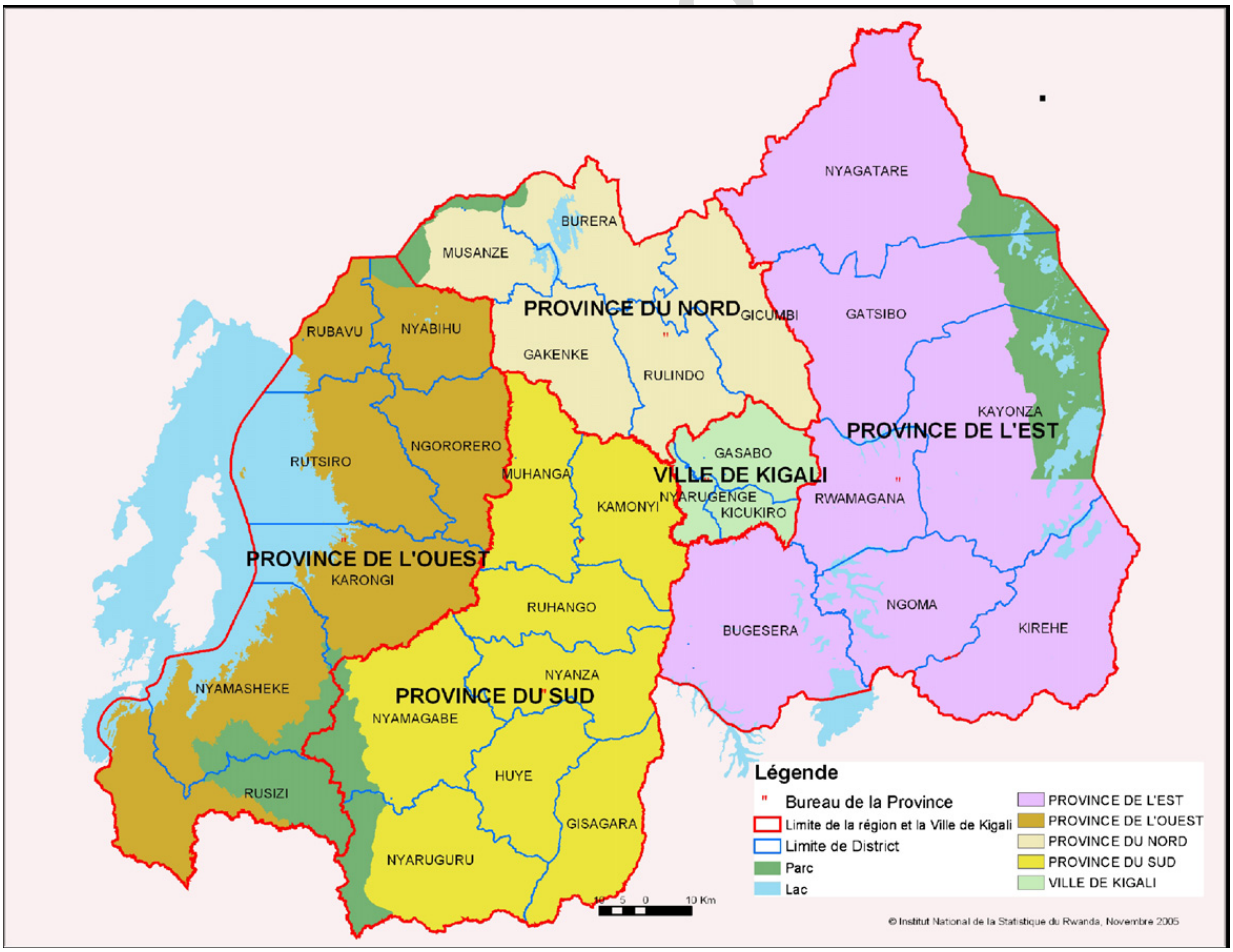

Fig. 1. Administrative map of Rwanda with the five provinces. Province boundaries match with the AE zones. Highland: Province du Nord; medium continental: Province du Sud; medium tempered: Province de l'Ouest; low land: Province de L'Est and Ville de Kigali.

2. Materials and methods

2.1. Study area

With approximately 26,000 km^2 and 7.8 millions inhabitants, Rwanda is situated between latitudes 1°S and 3°S and longitudes 29°E and 31°E. The country is ecologically quite diverse due to large variations in altitude (between 1000 and 3000 m above sea level). Four major agro-ecological zones have been defined, namely the high, the medium tempered (by Lake Kivu), the medium continental and the low land (Fig. 1; Table 1). Cattle are raised in all four agro-ecological zones under different management systems. Figs. 2 and 3 show the mean temperature and rainfall data for Rwanda. Table 2 summarises the farming systems in use over the country. Tick numbers range between 17 and 82 ticks per animal depending on season and AE zone (Table 3). For *R. appendiculatus*, all three tick stages can be found throughout the year.

2.2. Experimental set-up

Three surveys were organised: the first during the 1998 dry season, the second in the dry season of 2002 and the third in the wet season of 2003. All samples were tested for the presence of *T. parva* specific DNA. Full *Theileria* species determination was carried out on the samples from the 1998 survey only. The likelihood that animals were re-sampled over the different surveys was low as the sampling were spread over 34 districts, done randomly and the sample size for each site was low.

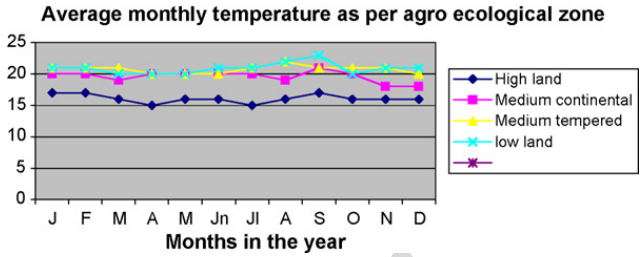

Fig. 2. Rwanda climatic data: average monthly temperatures as per agro-ecological zone of Rwanda.

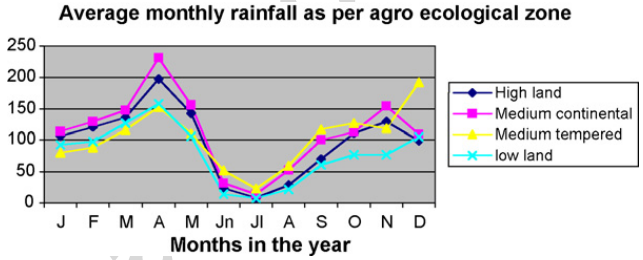

Fig. 3. Rwanda climatic data: average monthly rainfall as per agro-ecological zone of Rwanda.

2.3. Animals and samplings

A total of 898 cattle were sampled during three country wide transversal surveys. For each survey,

Table 1
Description of the four agro-ecological zones of Rwanda

Agro-ecological zone	Altitude (m)	Temperature (°C)	Annual rainfall (mm)	Period of rainy season
High land	>1950	14–17	>1400	September–July
Medium tempered	1550–1900	19–21	1200–1300	October–June
Medium continental	1650–1950	18–20	1100–1250	October–June
Low land	1000–1550	21–24	800–950	November–May

Table 2
Farming system data according to the AEZ in Rwanda

AEZ	Management system (%)			Breed type (%)			Age category (%)	
	Extensive	Semi-intensive	Intensive	Local	Cross	Pure	Heifer	In calf adult
H.L.	73.1	17.8	9.1	62.5	27.5	10	26	74
M. T.	55.8	39.8	4.4	89.2	10.5	0.3	18	82
M. C.	47.9	40.7	11.4	68.3	21.2	10.5	24	76
L. L.	44.1	47.6	8.3	67.8	23.2	9	17	83
Total	55.2	36.5	8.3	71.9	20.6	7.5	21.2	78.8

AEZ: agro-ecological zone; H.L.: high land; M.T.: medium tempered; M.C.: medium continental; L.L.: low land.

Table 3
Ecological and seasonal dynamics of tick distribution in the country

Agro-ecological zones	Number of animals	Average number of ticks per animal		
		Dry season	Wet season	Total
High land	36	25.3	9.5	17.4
Medium continental	60	70.5	46.9	58.7
Medium tempered	54	53.0	104.9	78.9
Low land	54	77.7	85.2	81.5
Total	204	56.6	61.6	59.1

similar numbers of animals were randomly selected from each agro-ecological zone. The majority of animals were Zebu local breed with a limited number of cross-breed and pure Taurine types.

In 1998, a total of 264 blood samples (half from calves under 7 months of age and half from adults) were collected. In the 2002 and 2003 surveys, a total of 317 animals of 1 year or older were sampled in each season. Blood samples were obtained by venopuncture into 10 ml venoject tubes containing EDTA. Tubes were kept on ice in a cool box and sent the same day to the National Veterinary Laboratory (NVL), Kigali. Upon arrival, blood drops were transferred to Whatman No. 4 (Whatman, United Kingdom) filter papers the same day, air-dried overnight and stored in plastic bags containing silicagel at −20 °C until use.

2.4. DNA extraction and PCR-RFLP assay

Parasite DNA was extracted from filter paper blots using a modified de Almeida et al. (1997) protocol as described by Geysen (2000). The presence of *T. parva* DNA was determined using the p104 semi-nested PCR-RFLP assay as described by Geysen (2000). Part of the samples were further analysed for the presence of other *Theileria* spp. using the 18S based PCR-RFLP assay (pan species PCR). In short, the reactions were carried out in a total volume of 25 μl. Each reaction contained 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 20 pmol of each primer, 0.5 U *Taq polymerase* enzyme (Goldstar, Eurogentec) and 5 μl of a DNA solution as template. In the semi-nested run, 0.5 μl of amplified product from the first run was added to the semi-nested mix at 82 °C (Hot start principle), containing the same ingredients and concentration, except that 0.3 U *Taq* was used. The amplification programme was as follows for both assays: Step 1: 94 °C for 4 min; Step 2: 94 °C for 30 s; Step 3: annealing for 45 s at 60 °C; Step 4: extension temperature of 72 °C for 1 min; Steps 2–4 were repeated for 39 times in case of the first run and 24 times in case of a semi-nested run. Step 5 was a final extension phase at 72 °C for 8 min and standard detection with ethidium bromide staining was used after electrophoresis of the amplified samples with a 100 bp molecular weight marker (MBI Fermentas, Lithuania). Species differentiation was done on profiles obtained on silver stained PAGE gels after digestion of 18S amplicons with *Msp*1 restriction enzyme. Confirmation of the p104 amplicons was done by analysis of the profiles obtained on silver stained PAGE gels after digestion with *Alu*I.

2.5. Statistical analysis

Data were analysed using a logistic regression in Stata 8.0 (StataCorp, 2003) software. A first model was based on the 1998 data to evaluate the effects of age classes, agro-ecological zones and the interaction between the two on the prevalence of the parasite as detected using the PCR based p104 gene assay. The effects of the respective surveys, the agro-ecological zones and the interaction between the two were assessed in a second model, using the whole dataset. Coefficients with a *P*-value less than 0.05 were considered significant.

3. Results

In the 1998 survey, 97 of the total 264 samples examined (36.7%) were found positive for *Theileria* spp., showing a fragment of approximately 1010 bp in the 18S pan species PCR (Fig. 4). These samples were considered positive for *Theileria* spp. Based on the different RFLP patterns, four *Theileria* spp. were identified: *T. parva* (62.9%), *T. mutans* (50.5%), *T. taurotragi* (29.9%) and *T. velifera* (15.5%). Table 4 shows that approximately half of the *Theileria* spp. positive samples were mixed infections (double (40.2%) and triple (9.3%)).

The prevalence of *T. parva*, determined by the p104 *T. parva* specific assay, was 66/264 (25.3%), 89/317 (28%) and 86/317 (27.1%) for the 1998, 2002 and 2003 surveys, respectively. There were no significant

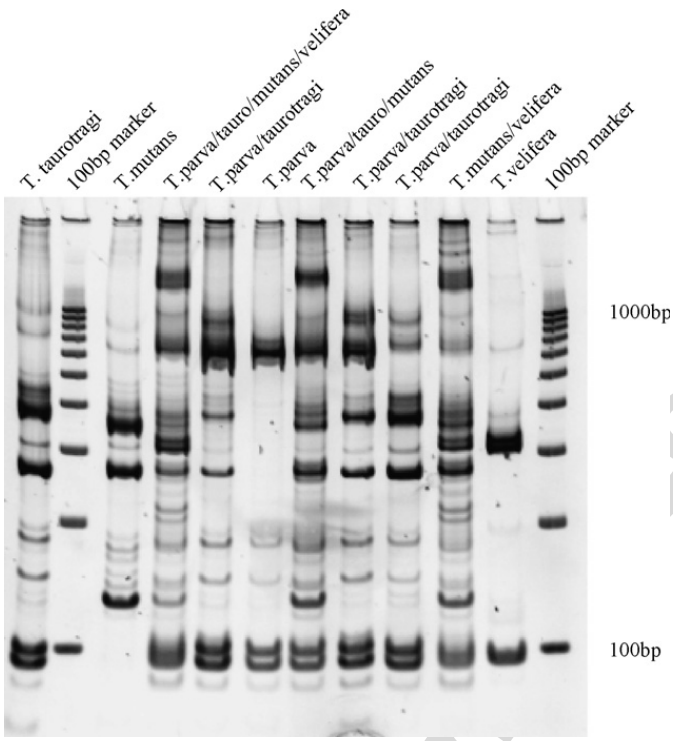

Fig. 4. *Theileria* species-specific profiles resulting from RFLP-PCR analysis of 18S loci using *Msp*1 enzyme digestion. Primers 18SF/18SR were used in the first run and 18SF/18SNR in the second run. 18S RFLP PCR results from DNA of various *Theileria* species were compared against a 100 basepair marker (lanes 2 and 15). Multiple theilerial infections were detected when the summed size of the resulting RFLP fragments exceeded the size of the unrestricted PCR product.

differences between the results of the three surveys showing the same prevalences during dry and wet seasons (2002 and 2003 surveys). A concordance of 94% was found for the 18S and p104 assays for all *T. parva* positives.

Fig. 5 shows the prevalence of *T. parva* during the three surveys for the four agro-ecological zones. The regional distribution of *T. parva* infections showed a geographically declining trend with highest prevalence in the high land zone and the lowest in the low land zone. The prevalence of *T. parva* was significantly higher in the high land zone compared to both continental and tempered medium and low land zones ($P < 0.05$). The *T. parva* prevalence did not differ between the medium continental and the medium tempered zones ($P > 0.05$) but both zones showed significantly higher prevalences compared to the low land zone ($P > 0.05$). Age related results were only available from the 1998 study. Here, higher *T. parva* infections were observed in calves compared to adult cattle in all agro-ecological zones, except the high land zones (Fig. 6).

4. Discussion

The results obtained by the 18S PCR assay revealed the coexistence of four *Theileria* spp. (*T. parva*, *T. mutans*, *T. taurotragi* and *T. velifera*) reflecting the

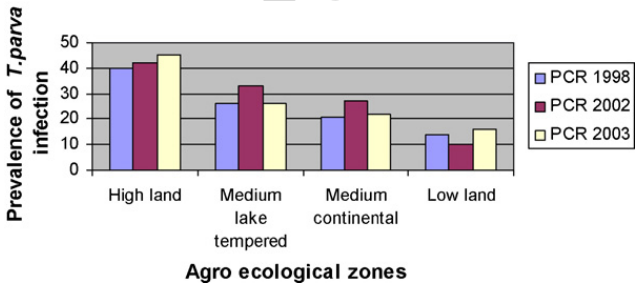

Fig. 5. The prevalence of *T. parva* according to the four agro-ecological zones in Rwanda.

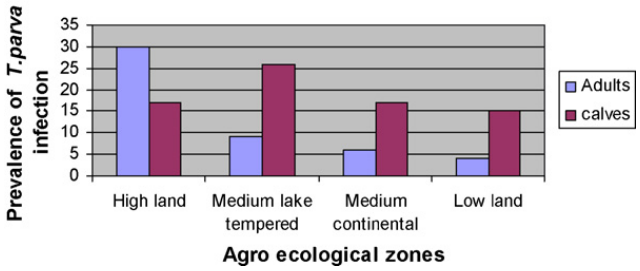

Fig. 6. The prevalence of *T. parva* in Rwanda (1998 survey) according to age.

Table 4
Single, double and triple infections with *Theileria* spp. as identified by the 18S species specific assay

Theileria spp.	Number	Percentage (%)
Single infections	49	50.5
T. parva	21	21.6
T. mutans	23	23.7
T. velifera	3	3
T. taurotragi	2	2.0
Double infections	39	40.2
T. parva/T. mutans	9	9.3
T. parva/T. taurotragi	20	20.6
T. parva/T. velifera	2	2
T. mutans/T. velifera	8	8.2
Triple infections	9	9.3
T. parva/T. mutans/T. taurotragi	7	7.2
T. parva/T. mutans/T. velifera	2	2
Total	97	100

presence of different tick species as previously described in Rwanda (FAO, 1982). The presence of multiple *Theileria* spp. is a common feature in bovine samples from eastern Africa (Norval et al., 1992a). In the present study, a high number of samples had mixed infections with *Theileria* spp. Despite this complexity, the pan species PCR method gave clear discrimination between the different *Theileria* parasites in field samples. *T. parva* and *T. mutans* were the most dominant infections, reflecting the relative importance in Rwanda of *R. appendiculatus* and *Ambyomma variegatum* tick populations, as described earlier by Paling and Geysen (1981). Similar results were obtained in a sero-survey conducted in neighbouring Tanzania (Swai, 2002), confirming that *T. parva* and *T. mutans* are the most important theilerial species in the region.

T. parva was the most prevalent species and there was a good correlation between prevalences obtained by the pan *Theileria* 18S and by the *T. parva* specific p104 assays. The high prevalence of *T. parva* infections is not surprising since the main vector, *R. appendiculatus* accounted for more than 80% in the total tick collections from Rwanda (FAO, 1982). This is to be expected as climatic conditions are very favourable for tick survival (Elb and Anastos, 1966; Newson, 1978) allowing intensive host-tick interactions and subsequent challenge of cattle with *T. parva*.

When considering the dynamics of *T. parva* infection in the country, it can be seen that the parasite is prevalent throughout the year in all regions of the country. Despite the limitation of PCR as a detection method for carrier animals due to the fluctuating nature of parasitaemia in these animals (Geysen, 2000), the prevalence of *T. parva* infections was relatively high throughout the country.

Despite the induction of a carrier status, infections were more prevalent in calves than in adult cattle in almost all regions. This could be explained by higher parasitaemias in primary infections as against fluctuating parasitaemias in carriers. Similar higher PCR prevalences in calves have been reported in Mbarara district of neighbouring Uganda (Oura et al., 2005) or in ECF endemic regions of Kenya where almost half of local Zebu calves were found infected by the age of 6–7 months (Moll et al., 1986). These high *T. parva* prevalences are an indication of the high transmission intensities in the region, giving infections early in life.

One exception was found in the high land areas where the majority of calves were free of *T. parva* infection. It is thought that in this region, tick populations reflect a seasonal occurrence. Paling and Geysen (1981) reported on the presence of *R. appendiculatus* ticks in the whole of Rwanda except in regions of over 2000 m altitude where climatic conditions are marginal for tick survival. However, recent data on tick populations are not available. But visual inspection of the animals was systematically done during the dry and wet season field surveys and ticks were found to be virtually absent during the rainy season in the high land zones. These observations suggest that *T. parva* transmission will be interrupted during the rainy season in these areas. Most of the calves in the high land would probably not have been in contact with infected ticks. In areas with less intensive *T. parva* transmission like Zambia, all calves were found free of *T. parva* infection up to the age of 6 months and only 50% were infected before the age of 15 months (Billiouw et al., 2002). Additional work on the dynamics of tick populations would help to clarify this particular situation of the high land areas.

Although *T. parva* was present throughout the country, significant prevalence differences were found among geographic regions. These are in line with the findings of Deem et al. (1993) and reflect different levels of exposure to *T. parva* infection. The results of this study are in agreement with these seroprevalence studies where positive correlations were found between prevalence rates and the intensity of *T. parva* infection (Gitau et al., 2000; Maloo et al., 2001a). The declining trend in the prevalence of *T. parva* infections from higher zones having a relatively less suitable ecology to low land areas is an unexpected result. The hypothesis of variable levels of vector competence amongst ticks from different ecological areas (Ochanda et al., 1998) is

probably a likely explanation apart from the altitude influencing tick population dynamics. However, the tick population is not the only factor that determines the *T. parva* epidemiology in a region. The breed type, the tick control practices and the grazing system in the different agro-ecological zones will bring different levels of interaction between hosts and vectors. These have been reported to play a significant role in the epidemiology of *T. parva* infection in the east African region (Maloo et al., 2001b; Rubaire-Akiiki et al., 2004) and this needs further investigation.

The present prospective study provides valuable epidemiological information on the endemic status of ECF in Rwanda which is central to any future elaboration of strategic control measures. Investigations into the dynamics of tick populations and managerial practices are needed to characterise and understand the epidemiology of ECF in the different agro-ecological zones of Rwanda.

Acknowledgements

This study was made possible through the financial support of the VLIR and the assistance from technical staff of the National Veterinary Laboratory of Rwanda (Francisca R. Mudaheranwa, Jeanne M. Uwamahoro, Ernest Munyagishali and J. Claude Ndolimana) and the Department of Animal Health of the Institute for Tropical Medicine, Antwerp, Belgium (Rita Beudeker and Ko De Witte) to whom we are grateful. We are thankful to Dr. Joram Sebatware for logistic support and useful advices.

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