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# Identification of hard ticks (Acari: *Ixodidae*) and seroprevalence to *Theileria parva* in cattle raised in North Kivu Province, Democratic Republic of Congo

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**Abstract** This study aimed to identify tick species and to determine their relationship with the *Theileria parva* seroprevalence in cattle raised under an extensive farming system in North Kivu Province, Democratic Republic of Congo in two agro-ecological zones namely medium (1,000–1,850 m) and high (>1,850 m) altitude. Among the 3,215 ticks collected on 482 animals, from February to April 2009,

*Rhipicephalus appendiculatus* (64.26 %), the main vector of *T. parva*, was the most abundant species followed by *Rhipicephalus decoloratus* (35.49 %) and *Amblyomma variegatum* (0.25 %). The mean burden of *R. appendiculatus* tick per infested animal appeared significantly higher at medium ( $6.5 \pm 0.22$  ticks) than at high ( $0.07 \pm 0.3$  ticks) altitude ( $P < 0.05$ ). However, an indirect fluorescent antibody test carried out on 450 blood samples revealed a global *T. parva* seroprevalence of 43 % (95 % CI: 38–47) which was not significantly ( $P > 0.05$ ) different between medium (48.4 %; 95 % CI: 38–49) and high (41.9 %; 95 % CI: 35–49) altitude. These relatively low seroprevalences suggest that there is a state of endemicity to *T. parva* infection in the study area. The presence of the tick vector on animals was associated with an increased risk of being seropositive to *T. parva* infection (odds ratio=2.04; 95 % CI: 1.8–2.3;  $P < 0.001$ ). The results suggest the need for a longitudinal study to investigate the seasonal dynamics of tick species and *T. parva* infection. The rate of tick infection should also be evaluated in order to determine the intensity of *T. parva* transmission to cattle.

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## Introduction

In veterinary medicine, hard ticks are major vectors of many important cattle diseases (Morel 2000; Ashford et al. 2001). Studies provided information on the presence of ticks in cattle livestock of the Democratic Republic of Congo (DRC; Lessard et al. 1990; Makumyaviri and Habimana 1993), ticks belonging to the genus *Rhipicephalus* being the most abundant (Makumyaviri and Mwilambwe 1998).

Indeed, climatic (high mean temperature and rainfall) and ecological conditions (extensive breeding, presence of wild animals, luxuriant vegetation all year round) prevailing in DRC are optimal for *Rhipicephalus appendiculatus*, *Rhipicephalus duttoni* and *hipicephalus decoloratus* (Norval et al. 1992).

A preliminary survey on three tick-borne diseases in North Kivu carried out among veterinarians working in large animal practices revealed that the perceived prevalence of East Coast fever (ECF; 49 %) was higher than those for anaplasmosis (36 %) and babesiosis (15 %; Kalume et al. 2009). This survey indicated also that the tick burden was fluctuating between 10 and 50 ticks per animal. However, a relatively low tick burden (<10 ticks/animal) was recorded in animals with a suspicion of ECF. Buparvaquone (Butalex®) and Parvaquone (Clexon®, Parvaxone®) were used to treat ECF cases (Dolan 1986; Mbwambo et al. 2002, 2006). However, these drugs were expensive and not readily available in North Kivu. Thus, control of ticks and tick-borne diseases is difficult to achieve in this province.

The present study aimed to: (1) identify ticks present on cattle and their pastures in North Kivu, (2) study their distribution in two different agro-ecological zones (medium and high altitude) and (3) estimate *T. parva* seroprevalence. These information are crucial in order to set up appropriate strategies for tick control and consequently of tick-borne diseases, ECF in particular.

## Materials and methods

### Study area

The study was carried out during the short rainy season (February–April 2009). The different cattle herds, located in the Lubero and Beni territories, North Kivu province (longitude 29° 12' to 29° 17' E and latitude 0° 8' N to 0° 40' S; Fig. 1), were visited once. At the time of the study, an estimated cattle population in the two territories was of 104,336 in 3,478 herds. These territories were selected because they were relatively secure and easy to reach by road. They include two different agro-ecological zones (AEZ) separated by the equator line: a zone at medium altitude (1,000–1,850 m above sea level; latitude 0° 7' to 0° 8' N) and a zone at high altitude (>1,850 m above sea level; latitude, 0° 19' to 0° 40' S). Meteorological data recorded from the nearest meteorological station between January and April 2009 were as follows: mean temperatures at medium and high altitudes reached 19.4 and 17.4 °C, respectively, whereas mean monthly rainfalls at medium altitude (256.5 mm) were higher than at high altitude (144 mm; Table 1).

### Cattle herds and management

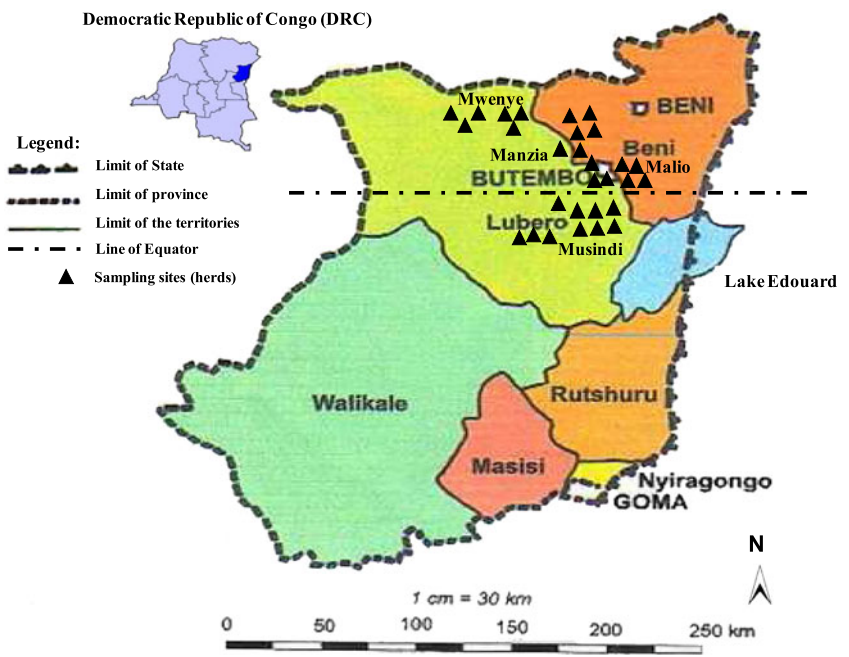
The sample size was determined according to the method described by Martin et al. (1987):  $n = [1.96^2 \times p(1-p)] / L^2$ , where 1.96 is the *z* value for the desired confidence level (95 %), *p* was an estimated expected prevalence of infection and *L* was the tolerable error. As the antibody prevalence to *T. parva* in the study area was not known a priori, a 50 % prevalence was assumed and a 5 % tolerable error used. A previous study on breeding practices and land management reported an average herd size between 15 and 20 heads (Mararo 2001). Consequently, 26 herds (totalizing at least 390 cattle heads) were considered as the minimal sample size. The herds were enrolled through a stratified randomization method and the final sample size resulted from the number of eligible animals available in each herd.

The data pertaining to animal numbers and herds localization were obtained from the offices of veterinarian surgeons responsible for Agriculture, Livestock and Fisheries (AGRIPEL) in Lubero and Beni territories. Three main criteria were retained for the selection of enrolled herds: (1) the accessibility of the herds (insecure areas being excluded) and the occurrence of ECF in cattle (Kalume et al. 2009), (2) a minimal herd size of 15 cattle of both sexes and of at least 12 months to eliminate the confounding effect of colostral antibodies (Gitau et al. 1999) and (3) the availability of a crush pen to restrain and sample the animals. Twenty-nine herds totalizing 482 cattle heads participated in the study: 19 (315 heads) and 10 (167 heads) herds were located at medium and high altitude, respectively. In a first step, all villages (defined as agropastoral groups) meeting the first selection criteria were classified as follows: Malio, Musindi, Mwenye and Manzia villages and Butembo town. Next, two to four herds were selected randomly from each village in order to have at least 10 herds in each AEZ (medium versus high altitude). In a final step, about 50 % of the animals belonging to each selected herd was examined and sampled. Tick collection and blood sampling were performed once in each herd. In two herds located at medium altitude, the animals could not be restrained properly and ticks only were collected from animals, during milking. Consequently, in this AEZ, blood was collected from 17 herds (283 cattle heads) instead of 19. All animals were of cross-breed (Ankole or Zebu crossed with European breeds). In many herds, a control scheme against ticks and tick-borne diseases was applied. Under such circumstances, acaricides are usually applied on a weekly basis by spraying or dipping. The availability and the cost of the different products are the main selection criteria.

### Tick collection and identification

In each participating herd, at least 15 cattle were examined and all attached ticks (3,215 ticks) were collected by manual

**Fig. 1** Map of North Kivu Province showing territories limits, villages visited and sampling sites ( $n=29$  herds)



extraction and grouped according to the anatomical site of collection (ears, head, neck and dewlap, upper and lower perineum, belly and legs, tail). Ticks were stored in 70 alcohol. The age, breed and sex were also recorded as well as any additional useful information such as intercurrent disease, recent acaricide treatment and treatment against ECF. The identification of ticks was carried out according to Walker et al. (2003).

Additionally, 4,353 ticks were collected from the pastures used by the selected herds by flagging according to a standardised technique described by Short and Norval (1981a). The flag had a surface of 0.86 m<sup>2</sup> and was drawn over a distance of 200 m. Ticks were collected, preserved and identified as described above.

#### Blood sampling and serological analysis

A total of 450 blood samples were collected from the 27 herds which could be restrained. At least 15 animals were sampled in each herd. Blood spots were made on 22 mm

diameter Whatmann no. 4 filter papers. After drying at room temperature, the papers were stored at -70 °C in air-tight bags containing silicagel. The indirect fluorescence antibody test (IFAT) was performed according to Burrige and Kimber (1972). The *T. parva* schizont antigen was produced as described by Goddeeris and Morrison (1988). A titre of >1/160 was considered as positive.

#### Statement of the body that oversees animals

The offices of veterinarian surgeons responsible for AGRIPPEL oversee animals in Lubero and Beni territories and authorised the sampling on animals in these agroecological zone.

#### Statistical analysis

The relationship between qualitative variables was analysed using Fisher's exact test and the comparison between the

**Table 1** Weather data recorded at the stations of the Technical Agro-Veterinary Institute (ITAV/Butembo, northern latitude) and of the Centre for Improved Seeds Production (CAPSA/Luoto, southern latitude)

Month	Medium altitude (1,000–1,850 m; northern latitude)			High altitude (>1,850 m; southern latitude)				
	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Relative humidity (%)		
	Min.	Max.		Min.	Max.			
Jan 2009	12.8	27.0	238.6	90.7	12.6	22.8	153	84.2
Feb 2009	12.2	27.3	308.6	89.2	12.5	21.8	208.2	82.7
Mar 2009	13.0	24.5	175.4	88.4	12.8	22.6	138.4	81.4
Apr 2009	13.5	24.4	303.2	85.2	12.5	21.9	76.2	81.1
Average	12.9	25.8	256.5	88.4	12.6	22.3	144.0	82.4

means was carried out using the test of Welch (Dagnelie 1998). *Theileria parva* seroprevalence was calculated as percentages of seropositive animals. A herd was considered positive if at least one animal was found positive in IFAT (Dohoo et al. 2003). Similarly, a herd was considered infected by *R. appendiculatus* if at least one tick was collected on the animals or in their pasture. The relationship between the presence of *R. appendiculatus* on cattle and the seroprevalence to *T. parva* was evaluated by calculating the odds ratio (OR) with the software Winepiscope 2®. The OR is a relative measure of risk that describes how much more likely it is that an animal which is exposed to the factor under study will develop the outcome as compared to an animal which is not exposed. The density of ticks per square metre in the pastures was calculated by multiplying the number of ticks collected and the surface of the flag (0.86 m<sup>2</sup>) and divided by a predefined distance (200 m).

## Results

### Allocation of animals

The allocation of the 482 cattle heads within the different agropastoral groups is presented in Table 2. A majority (92.9 %) of the animals were over 24 months of age. Most of them were females (97.1 %). Two third and one third of the herds were located at medium (1,000–1,850 m) and high (>1,850 m) altitude, respectively.

### Tick species and abundance

In total, 3,215 ticks were collected from the animals (Table 3). *R. appendiculatus* was by far the most abundant

**Table 2** Distribution of the major characteristics of the 482 selected cattle in the study area

Variables	Levels	Frequency	Percentage
Age group (Months)	12 to 24	34	7.1
	>24	448	92.9
Sex	Males	14	2.9
	Females	468	97.1
Agro-ecological zone	Medium altitude	315	65.4
	High altitude	167	34.6
Village (agropastoral group)	Malio	171	35.48
	Musindi	167	34.65
	Mwenye	86	17.84
	Butembo town	43	8.92
	Manzia	15	3.11
Tick control methods by acaricides	Spraying	270	56.0
	Dipping	212	44.0

species on cattle (64.26 %) followed by *R. decoloratus* (35.49 %) and *Amblyomma variegatum* (0.25 %). These tick species were not equally distributed in the two AEZ ( $P < 0.001$ ). A higher proportion of ticks were collected at medium altitude when compared to high altitude (78.1 versus 21.9 %). *R. appendiculatus* was the most abundant species at medium altitude (99.4 %) whereas *R. decoloratus* predominated at higher altitude (60.7 %). The mean tick burden reached  $7 \pm 0.17$  ticks per infested animal. Mean tick burden per infested animal appeared significantly higher at medium ( $8 \pm 0.22$  ticks) than at high ( $4.2 \pm 0.3$  ticks) altitude ( $P < 0.001$ ). Furthermore at medium altitude, cattle sprayed at weekly intervals with acaricide had a significantly higher tick burden ( $9.8 \pm 0.23$ ) than cattle immersed in dipping tanks ( $2.6 \pm 0.26$ ;  $P < 0.05$ ). At high altitude, most animals were sprayed and consequently it was not possible to make any comparison. The anatomical site of collection had a significant effect on tick species composition regardless of the stage (adult/immature;  $P < 0.0001$ ; Table 4). The adult *R. appendiculatus* ticks were particularly abundant on the ears (91.2 %) whereas nymphs were found on the ears (41.3 %) and the head (40.2 %). No larvae of this species were found. In contrast, the adults of *R. decoloratus* were mostly found on the belly and the legs (73.5 %). The nymphs of this species were located mainly on the neck and dewlap (68 %) and larvae were found on abdomen, legs (50 %) and neck dewlap (36 %) whereas a majority of the adult *A. variegatum* ticks were attached to the belly and legs (75 %).

In the pastures grazed by the studied herds, only the immature stages of *R. appendiculatus* (larvae and nymphs) and *R. decoloratus* (larvae) were collected by flagging (Table 5). The numbers of ticks collected from successfully flagged pastures were higher at medium altitude ( $P < 0.001$ ). At high altitude, only larvae of *R. decoloratus* were collected by flagging. Tick densities were higher alongside streams of water (13.8 ticks/m<sup>2</sup>) than near hedges used for fencing (4.9 ticks/m<sup>2</sup>).

### Seroprevalence to *T. parva*

Results are presented in Table 6. A total of 450 blood samples were examined for specific antibodies to *T. parva*. A global seroprevalence of 43 % (95 % CI: 38–47) was recorded and varied significantly across the different villages visited ( $P < 0.05$ ). However, these seroprevalences did not differ between the AEZ ( $P > 0.05$ ) with 43.5 % (38–49) and 41.9 % (35–49) at medium and high altitude, respectively. At medium altitude, the sprayed herds had a significantly higher *T. parva* seroprevalence (48.4 %; 95 % CI: 42–54) than the herds treated by dipping (35.4 %; 95 % CI: 29–42;  $P < 0.05$ ). Dipping is not performed at high altitude and it was not possible to make any comparison. When the variables were offered to the multivariable analysis, two of

**Table 3** Identification and distribution of 3,215 ticks collected from cattle in the study area in relation with the agro-ecological zones and tick control methods

Tick species	Variables	Levels	Number of examined animals	Number of ticks collected (%)	Ticks burden per infested animal ±95 % CI
	Infested	Herds (n=29)	482	3,215	7±0.17
<i>R. appendiculatus</i>	AEZ	MA	315	2,054 (99.4)	6.5 a±0.22
		HA	167	12 (0.6)	0.07 b±0.30
<i>R. decoloratus</i>	AEZ	MA	315	448 (39.3)	1.42 a±0.22
		HA	167	693 (60.7)	4.15 b±0.30
<i>A. variegatum</i>	AEZ	MA	315	8 (100)	0.02 a±0.22
		HA	167	0	0 b
	TCM	Spraying	270	2,655 (82.6)	9.8 a±0.23
		Dipping <sup>a</sup>	212	560 (17.4)	2.6 b±0.26

The values with different letters differ significantly at a threshold of 5 % for levels of each variable along the column

AEZ agro-ecological zones, TCM tick control methods by acaricide, MA medium altitude, HA high altitude

<sup>a</sup>At high altitude, dipping is not performed

them (the presence of the *R. appendiculatus* tick in a herd and tick control methods) were significantly ( $P<0.05$ ) associated with *T. parva* seroprevalence (Table 7). Cattle from herds in which the vector was found during the herd visit were more likely to be seropositive to *T. parva* than those from herds in which the vector was not found (OR=2.04; 95 % CI: 1.8–2.3;  $P<0.001$ ). Tick control by spraying at weekly intervals increased the risk of being seropositive to *T. parva* by 4.1 times (95 % CI: 3.0–5.6) when compared to dipping ( $P<0.01$ ).

**Discussion**

The present study provides preliminary results on ticks and *T. parva* seroprevalence in North Kivu an Eastern province of the DRC. Although cross-sectional studies are not powerful at showing cause–effect relationships (Dohoo et al. 2003), this study provides nevertheless useful information on the distribution of different tick species feeding on cattle in the study area. It confirms also, under field conditions, the relationship between the presence of *R. appendiculatus* on the animals and the seropositivity to *T. parva*. The 482 animals were enrolled in 29 herds distributed into two main

AEZ (medium versus high altitudes) and most of them were females (97.1 %). This is due to the fact that males are usually slaughtered before 12 months of age. Moreover, one third of the herds were enrolled at high altitude because most of farmers who were contacted for participating to the study did not accept visits due to insecurity in this zone.

The fact that *R. appendiculatus* was the most abundant tick species (64.26 %) is in agreement with previous works carried out in Eastern DRC in the Province of South Kivu (57.2 %; Makumyaviri and Habimana 1993), in Rwanda (96 %; Nshimiyimana and Mutandwa 2010) and in Uganda (50.47 %; Rubaire-Akiiki et al. 2004). However, a marked difference between the tick distributions was found in the AEZ. Indeed at medium altitude, *R. appendiculatus* is by far the most abundant tick (99.4 %) whereas at higher altitudes *R. decoloratus* predominates (60.7 %). *A. variegatum* thrives in savannahs of semi-arid or humid tropical areas (Yeoman and Walker 1967). A few individuals of this species were observed in a single herd of cattle at medium altitude. The species was not found at higher altitude which confirms previous studies carried out in Rwanda at altitudes comprised between 1,000 and 1,550 m (Bazarusanga et al. 2007a) and in Uganda between 1,100 and 1,350 m (Rubaire-Akiiki et al. 2004).

**Table 4** Tick abundance according to the different anatomical sites (n=3,215)

Tick species	Stage	Total tick number	Ears (%)	Face (%)	Neck dewlap (%)	Abdomen–legs (%)	Ano-génital (%)
<i>R. appendiculatus</i>	Adults	1,974	1,801 (91.2)	161 (8.2)	10 (0.5)	2 (0.1)	0
	Nymphs	92	38 (41.3)	37 (40.2)	17 (18.5)	0	0
<i>R. decoloratus</i>	Adults	1,102	0	3 (0.3)	150 (13.6)	810 (73.5)	139 (12.6)
	Nymphs	25	0	0	17 (68.0)	6 (24.0)	2 (8.0)
	Larvae	14	0	1 (7.0)	5 (36.0)	7 (50.0)	1 (7.0)
<i>A. variegatum</i>	Adults	8	0	0	0	6 (75.0)	2 (25.0)
	Total (%)	3,215	1,839 (57.2)	202 (6.3)	199 (6.2)	831 (25.8)	144 (4.5)

**Table 5** Number and density of ticks collected by flagging over a distance of 200 m in pastures grazed by cattle at medium (grass length, >12 cm) and high (grass length, <10 cm) altitudes in the study area (flag surface=0.86 m<sup>2</sup>)

Tick species	Stage	Variables	Levels	Total numbers of ticks	Density (ticks/m <sup>2</sup> ) ±95 % CI
<i>R. appendiculatus</i>	Larvae	AEZ	Infested Pastures	4,353	18.7±0.06
			MA	1,980	8.5 a±0.09
	Nymphs	AEZ	HA	0	0 b
			MA	1,753	7.5 a±0.09
			HA	0	0 b
			MA	0	0 b
<i>R. decoloratus</i>	Larvae	AEZ	MA	413	1.8 a±0.19
			HA	207	0.9 b±0.27
	PNH	MA	MA	3,006	12.9 a±0.07
			HA	207	0.9 b±0.27
			MA	1,140	4.9 a±0.11
			HA	0	0 b

The values with different letters differ significantly at a threshold of 5 % for levels of each variable along the column

AEZ agro-écological zones, PNS plots near a stream, PNH plots near a hedge, MA medium altitude, HA high altitude

It is well known that tick distribution and abundance are largely determined by different bio-climatic factors (Estrada-Peña 2003; Moorling et al. 2004) including the availability of appropriate hosts (Cummings 2002; Olwoch et al. 2003). During the present work, rainfalls were low at high altitude. This could explain the low numbers of tick collected in this sector of breeding. In areas close to the equator, like in the present work, the temperature and the photoperiod are optimal throughout the year (Short and Norval 1981a, b; Mwangi et al. 1991), rainfalls play a key role and this is particularly true as far as *R. appendiculatus* is concerned (Morel 2000). Under such conditions, *R. appendiculatus* does not undergo a diapause (Madder et al. 1999, 2002; Speybroeck et al. 2003) and it exhibits a preference for areas where altitude varies between 1,000 and 2,000 m (Burkot and Graves 2000). *R. decoloratus*, the second most abundant tick species in the present work is widely distributed with a preference for fairly high altitudes (Walker et al. 2003). Matthyse and Colbo (1987) reported the presence of this tick at altitudes between 600 and 2,300 m in Uganda.

Other factors such as cattle movements and tick control methods may influence the distribution and the abundance of ticks in the study area. It is generally known that cattle movement facilitates tick and associated pathogen dissemination (Bazarusanga et al. 2007a; Madder et al. 2007, 2011) and has a relatively high impact on their distribution (Pearson and Dawson 2003). Unprotected cattle movement is the rule in North Kivu an Eastern province of the DRC which is regularly under a state of war (Kabamba and Malumalu 2010). Breeding bulls and animals to be slaughtered are also moved over long distances (Byavu et al. 2000) and this could also enhance the spreading of ticks and associated pathogens. Furthermore, the abundance of ticks depends on the method used in their control (Morel 2000). In the present study, cattle treated against ticks at weekly intervals by spraying had higher parasite burdens than the animals treated by dipping at the same interval ( $P<0.001$ ). This can be explained by the fact that spraying is a time-consuming technique and its use is justified in small herds only (<50 heads; Morel 2000). Additionally, the efficacy of

**Table 6** Seroprevalence to *T. parva* in relation with the different variables recorded in participating cattle herds

Variables	Levels	Numbers of examined animals	Numbers of seropositive animals	Seroprevalence to <i>T. parva</i> (%) [95 % CI]	
Presence of antibodies against <i>T. parva</i> Villages or agropastoral groups	Herds (n=27)	450	193	42.9 [38–47]	
		Malio	168	78	46.4 a [39–54]
		Musindi	167	70	41.9 a [35–49]
		Mwenye	68	24	35.3 b [25–47]
		Town of Butembo	32	16	50.0 a [33–67]
		Manzia	15	5	33.3 b [15–59]
Agro-ecological zones	Medium altitude	283	123	43.5 a [38–49]	
		High altitude	167	70	41.9 a [35–49]
Tick control method by acaricides	Spraying	258	125	48.4 a [42–54]	
		Dipping <sup>a</sup>	192	68	35.4 b [29–42]

The values with different letters are significantly ( $P<0.05$ ) different for levels of each variable along the column

<sup>a</sup>At high altitude, dipping is not performed



**Table 7** Estimate of the *T. parva* seroprevalence risk at the herd level according to the presence of the tick *R. appendiculatus* and the tick control method

Effect	Variable	Level	Odds ratio	95 % confidence interval	<i>P</i>
Herds	Vector tick found on herd	Yes	2.04	1.8–2.3	0.001
		No	0	–	
Prevention of infection	TCM at medium altitude	Spraying	4.1	3.0–5.6	0.01
		Dipping <sup>a</sup>	0.009	–	

TCM Tick control methods by acaricide

<sup>a</sup> At high altitude, dipping is not performed

this technique relies essentially on the manipulator and some anatomical sites (internal parts of the ears, inguinal regions) are easily missed. A high proportion of *R. appendiculatus* ticks were found on the ears (57.2 %), a typical predilection site for this species (Ashford et al. 2001; Taylor et al. 2008). These factors explain probably the difference abundance of ticks recorded between cattle treated by spraying and dipping at medium altitude. At high altitude, the insecurity has led to the abandonment of dipping tanks. In this AEZ, it is very difficult to muster large groups of animals (>1,500 heads) which justify the use of dipping as described by Morel (2000). Thus, it was not possible to make any comparison regarding tick control methods.

At the pasture level, a difference was observed between the two AEZ, tick yield being 17 times lower at higher altitude. This difference could be due to the much lower rainfall at high altitude. It is noteworthy that only larvae and nymphs of *R. appendiculatus* and larvae of *R. decoloratus* were collected on pastures. The latter species is a one-host tick; consequently, the larval stage is the only one to be found in the environment (Morel 2000). In contrast, *R. appendiculatus* is a three-host tick, consequently both stages (larvae, nymphs and adults) of this tick can be found in the pastures (Swai et al. 2006). Tick densities were higher alongside streams of water than near hedges used for fencing. The hedges used for fencing or against erosion represent an ideal shelter for small mammals, the hosts for immature ticks (Walker et al. 2003). However, the presence of water nearby is highly favourable to the ticks.

The global *T. parva* seroprevalence was 43 % (95 % CI: 38–47) and there was no significant difference between the two AEZ. This relatively low seroprevalence suggests that a state of endemicity to *T. parva* infection exists in the province as it has been described by Norval et al. (1992). However, overall seroprevalence is not the only indicator that determines the *T. parva* epidemiology in a region. Other factors such as morbidity and mortality of animals, age of calves at first contact to *T. parva*, tick control methods and grazing system will bring different levels to assess the state of ECF epidemiology. These indicators have been reported

to play a significant role in the *T. parva* infection in the Eastern African region (Maloo et al. 2001; Rubaire-Akiiki et al. 2006). In the present work, the situation is probably due to the low *R. appendiculatus* tick burden ( $4.3 \pm 0.17$  ticks/animal) recorded in the enrolled herds. Low-tick abundance and a low rate of infection in the tick population for a given pathogen are related to low seroprevalences to *T. parva* (Gilioli et al. 2009; Odongo et al. 2009). However, data about the seasonal abundance and the rate of infected ticks by *T. parva* are not available in the study area.

In the present study, the presence of the vector in a given herd was a good indicator of seropositivity to *T. parva* in cattle. This supports the well-known relationship between the presence of *R. appendiculatus* and *T. parva* reported both in cross-sectional (Deem et al. 1993; Gachohi et al. 2011) and longitudinal studies (Rubaire-Akiiki et al. 2006; Swai et al. 2009). Under these conditions, the herds located at medium altitude (where the tick vector was more abundant) would present a higher *T. parva* seroprevalence when compared to herds grazing at higher altitude. However, this was not the case. This would suggest that tick abundant is not the only factor that determines the level of *T. parva* transmission. Tick infection proportions are also considered helpful (Perry 1996). The situation can be well explained by the variable levels of vector competence to *T. parva* transmission amongst tick from different AEZ as it was reported by Ochanda et al. (1998). Thus, the rate of tick infection should be evaluated in order to determine the intensity of *T. parva* transmission to cattle in the study area. Bazarusanga et al. (2007b) reported a similar situation in Rwanda in which a higher *T. parva* seroprevalence was observed in cattle from a region with low tick numbers compared to regions with higher tick abundant.

At medium altitude, spraying at weekly intervals increased the risk of being seropositive to *T. parva* by 4.1 times (95 % CI: 3.0–5.6) when compared to dipping ( $P < 0.01$ ). This confirms that the technique used for tick control can have a marked effect on tick abundance and consequently on *T. parva* seroprevalence. However, lack of association between tick control practices and *T. parva* seroprevalence was reported in several endemic areas of ECF (Rubaire-

Akiiki et al. 2006; Swai et al. 2009). In North Kivu, cattle breeders tend to apply tick control measures depending on occurrence, perceived incidence and severity of the clinical disease experiences and economic impacts of the disease at the farm level. Consequently, acaricides are used very regularly at weekly intervals and this represents a potential risk factor if control measures are discontinued for a reason or another (Mugabi et al. 2010; Phiri et al. 2010).

However, the presence of seropositive animals to *T. parva* indicates a failure of tick control by acaricides. Thus, the control of *T. parva* infection would base on a good knowledge of the population dynamics of the vector tick through longitudinal studies and effective treatment of clinical cases and immunisation against ECF. In several African countries, control of ECF relies on the so-called infection and treatment technique. In this approach, animals are actively infected with *T. parva* sporozoites and treated at the same time with long acting oxytetracycline (Marcotty et al. 2001; Kivaria et al. 2007). At the present time, this approach seems difficult to implement in North Kivu province and more generally in DRC.

These findings also suggest that: (1) an epidemiological study on the economical losses dues to tick infestations and *T. parva* infection must be carried out in order to justify the implementation of a coordinated action for an integrated control; (2) tick control by spraying should target preferential anatomical sites such as the ears, the abdomen and legs, the head, the neck and the dewlap; (3) the movements of unprotected cattle should be restricted and (4) dipping tanks should be restored at high altitude in the study area. These measures are unrealistic as long as the political stability of the region is not guaranteed. Acaricides are not always readily available and often fairly expensive (Kalume et al. 2009) and their extensive use can lead to tick resistance. In this context, the use of pesticides plants is an alternative option (Kasonia and Yamalo 1994). This approach requires additional in vitro and in vivo studies.

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