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Tick load and prevalence of *Theileria parva* infection in cattle husbandry in Ituri, North Kivu and South Kivu provinces, East Democratic Republic of Congo

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Abstract

The present investigation was undertaken in January 2013 to assess the load of tick and prevalence of Theileria parva on 756 cattle raised in three provinces of eastern Democratic Republic of Congo (DRC): Ituri (250), North Kivu (255) and South Kivu (251).

Through whole body counts, the study came up with tick load data whereas the antibody percent positivity was determined by the polymorphic immunodominant molecule (PIM)-based T. parva enzyme-linked immunosorbent assay (ELISA).

A nested polymerase chain reaction (PCR) based on p 104 gene was used for the detection of the prevalence of T. parva parasites on the seropositive samples.

A total of 12,672 ticks was extracted manually on cattle. These ticks were distributed in five species. The Rhipicephalus appendiculatus was the most abundant tick species (68.04%), followed by Rhipicephalus decoloratus (18.16%), Amblyomma variegatum (7.87%), R. evertsievertsi (5.7%) and Hyalomma rufipes (0.24%).

South Kivu cattle carried the highiest mean load of R. appendiculatus tick per infested animal (18.46±0.33) ($p<0.05$) compared to North Kivu (7.93±0.32%) and Ituri cattle (7.87±0.33%). The lowest seroprevalence of T. parva infection was found in North Kivu cattle (7%; 95%CI=4.5-10.9) compared to South Kivu (35.5%; 95%CI=29.8-41.6) and Ituri cattle (40.4; 95%CI=34.5-46.6). Conversely, these seropositive cattle presented a higher T. parva prevalence in North Kivu cattle (100%) compared to South Kivu (87.6%, 95%CI=79-93) and Ituri cattle (85.1, 95%CI=76.8-90.8), which did not significantly differ ($p>0.05$). There was a significantly higher conditional probability of sampling a positive PCR result with a subsequent positive ELISA in the three strains cattle ($p<0.0001$). R. appendiculatus tick load significantly affected the seropositivity to T. parva ($p=0.0044$) and the prevalence of T. parva parasite ($p=0.0115$). However, no association was detected between R. appendiculatus and the occurrence of ECF

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clinical signs (p>0.05). The high parasite prevalence and its subsequent low antibody levels suggest many T. parva carriers which may not support clinical disease and confirm an ECF endemic instability state. Thus, in addition to ticks control regime and treatment of sick animals by appropriate drugs especially the buparvaquone, cattle must be protected against ECF clinical disease by infection and treatment method (ITM) using injection simultaneously of MugugaCocktail vaccine and Oxytetracycline (Alamycin LA 30%). However, the safety and efficiency of this vaccine must be evaluated on cattle under field conditions in the eastern DRC.

***Keywords**: Tick burden, Seropositivity, Theileria parva, endemic instability, Eastern DRC*

Résumé

Cette étude a été menée en janvier 2013 pour déterminer la charge de tiques et la prévalence de Theileria parva sur 756 bovins élevés dans trois provinces à l'Est de la République démocratique du Congo (RDC) : Ituri (250), Nord-Kivu (255) et Sud-Kivu (251).

La charge de tiques a été déterminée par le nombre de corps entiers et la positivité en pourcentage d'anticorps a été déterminée par un dosage immuno-enzymatique (ELISA) basé sur une molécule immunodominante polymorphe (PIM).

La prévalence des parasites T. parva a été détectée sur les échantillons séropositifs par une réaction en chaîne par polymérase (PCR) nichée basée sur le gène p 104.

Au total, 12 672 tiques ont été collectées par extraction manuelle et parmi cinq espèces identifiées, Rhipicephalus appendiculatus (68,04 %) le principal vecteur de T. parva, était le plus abondant suivi de Rhipicephalus decoloratus (18,16 %), Amblyomma variegatum (7,87 %), R .evertsi evertsi (5,7%) et Hyalomma m. rufipes (0,24%).

La charge moyenne par animal infesté de la tique R. appendiculatus est apparue significativement élevée (p<0,05) pour les bovins du Sud-Kivu (18,46±0,33) par rapport aux bovins du Nord-Kivu (7,93±0,32%) et des bovins de l'Ituri (7,87±0,33%).

La séroprévalence de l'infection à T. parva était plus faible chez les bovins du Nord-Kivu (7 % ; IC à 95 % = 4,5-10,9) par rapport aux bovins du Sud-Kivu (35,5 % ; IC à 95 % = 29,8-41,6) et les bovins de l'Ituri (40,4 ; 95 % IC=34,5-46,6).

A l'inverse, ces bovins séropositifs ont présenté une prévalence de parasites T. parva qui était plus élevée chez les bovins du Nord-Kivu (100%) par rapport au Sud-Kivu (87,6%, IC95%=79-93) et les bovins de l'Ituri (85,1, IC95% =76,8-90,8), mais la différence n'était pas significative (p>0,05).

La probabilité conditionnelle qu'un échantillon ait un résultat PCR positif alors qu'il avait un test ELISA positif était significativement élevée chez les trois souches bovines (p<0,0001). La charge de tiques R. appendiculatus a eu un effet significatif sur la séropositivité à T. parva (p = 0,0044) ainsi que sur la prévalence du parasite T. parva (p = 0,0115), mais n'a pas été associée à des différences significatives dans la survenue de signes cliniques de FEC (p>0,05). La détection d'une forte prévalence de parasites en combinaison avec de faibles niveaux d'anticorps indique de nombreux porteurs de T. parva qui peuvent ne pas supporter la maladie clinique et confirmer un état d'instabilité endémique ECF. Ainsi, en plus du régime de contrôle des tiques et du traitement des animaux malades par des médicaments

appropriés, en particulier la buparvaquone, les bovins doivent être protégés contre la maladie clinique ECF par une méthode d'infection et de traitement (ITM) utilisant l'injection simultanée de vaccin MugugaCocktail et d'Oxytetracycline (Alamycin LA 30%). Cependant, l'innocuité et l'efficacité de ce vaccin doivent être évaluées sur des bovins dans des conditions de terrain dans l'Est de la RDC.

Mots-clés : *Charge de tiques, Séropositivité, Theileria parva, instabilité endémique, Est de la RDC*

Introduction

The intracellular protozoan parasite *Theileria parva* causes the east coast fever (ECF) disease (Adl, S.M et al., 2005; Anon, 1988; Levine et al., 1980). The disease is transmitted by *Rhipicephalus appendiculatus*, a three-host tick dropping from diseased cattle in the previous life cycle stage, in a cyclo-propagative and transstadial pattern (Ashford et al., 2001; Norval et al., 1992). The distribution of *T. parva* as well as its main vector are similar and does not cover all areas infested by ticks due to the discontinuous distribution of ticks (Estrada-Peña, 2003; Moorling et al., 2004) which is affected by bio-climatic factors (Nshimiyimana & Mutandwa, 2010; Olwoch et al., 2008). Tick distribution is also related to host distribution (Kalume et al., 2012). In Eastern, Central and Southern Africa, the *T. parva* antibodies have been diagnosed in more than 15 countries (Lessard et al., 1990). Nevertheless, on the regional scale, the threat mainly affects 11 countries among others Kenya, Uganda, Tanzania, Burundi, Rwanda, Malawi, Mozambique, southern Sudan, Democratic Republic of Congo (DRC), Zambia and Zimbabwe (Lawrence & Perry, 1992; Mukhebi & Perry, 1992). The first occurrence of the East Coast fever in Comoros dates back in 2003 and 2004 (De Deken et al., 2007). This outbreak might have resulted from cattle imported from Tanzania and fed upon by native ticks. The latter subsequently infected the susceptible indigenous cattle population.

Across the eastern DRC, studies have provided information on cattle infection by *T. parva* (Kalume et al., 2012; Makumyaviri & Mwilambwe, 1998; Mukhebi & Perry, 1992) and data on the ECF epidemiology status were reported in North Kivu province where the seropositivity to *T. parva* was estimated at 43% (95% CI = 38-47) in a cross-sectional study (Kalume et al., 2013). An extensive farming system-based *T. parva* sero-prevalence of 70 % (95 % CI= 47-86) was reported in a longitudinal study when susceptible animals (crossbreed cattle) developed severe and lethal clinical

ECF and the disease was estimated to be in endemic instability state in the area (Kalume et al., 2012)

The reported information were serological results based on indirect fluorescent antibody test (IFAT). This method is mainly referred to for the detection of these infections (Norval et al., 1992). Under field conditions however, data related to sensitivity and specificity of the IFAT test are still lacking. The knowledge gap in the specificity of this test has to do with cross-reactions with *T. taurotragi*, since its distribution overlaps with *T. parva* in the east, central, and south African region (Norval et al., 1992;). Furthermore, IFAT sensitivity is affected by seasonal transmission of tick in endemic regions (Bisusa & Kalume, 2013).

Since *R. appendiculatus* tick and the transmission of the *T. parva* infection are continuously reported in eastern DRC throughout the year (Kalume, 2011; Kalume et al., 2012), the DNA based methods will be of help for the detection of not only the presence of the parasite but also data on its prevalence in eastern DRC. Following a *T. parva* infection, animals can recover but will remain carriers for years and hence become immune to the same infections. For epidemiological studies, it is crucial to specifically identify low levels of *T. parva* parasite in naturally infected herds. The supremacy of Polymerase Chain Reaction (PCR) technique over conventional diagnostic methods in determining theilerial infections in carrier animals lies in its higher specificity and sensitivity (Bishop et al., 1992). In field samples, PCR methods systematically characterize and discriminate *T. parva* from multitudes of *Theileria* spp. infections (Geysen et al., 1999; Ogden et al., 2003).

The present study was undertaken to assess the tick load and to investigate the prevalence of *T. parva* antibodies and parasites on cattle in Ituri, North Kivu and South Kivu provinces, Eastern RDC. The research findings of the present study are key for informed recommendations about the state of *T. parva* infection in the area.

Materials and methods

Study area

The study was conducted during the dry season (in January 2013) in eastern DRC. The cattle herds raised under an extensive farming system, visited once, were located in different territories amongst three provinces (**Fig. 1**) included: Irumu and Mambasa in Ituri (altitude 888 to 958 m above sea level; longitude 29° 31’ to 29° 48’ E and latitude 1° 23’ to 1° 74’ N),

Lubero and Beni in North Kivu (altitude 1,217 to 2,217 m above sea level; longitude 29°11′ to 29°24’ E and latitude 0°7′ N to 0°26′S), Kabare, Walungu and Uvira in South Kivu (altitude 890 to 1,680 m above sea level; longitude 28° 40’ to 29° 03’ E and latitude 2° 14’ to 2° 52’ S). These are areas where the most cattle population of each province was located (insecure areas being excluded) and constitute a suitable agroecological zone for the *R. appendiculatus* tick. There was little dispersion according to the latitude and longitude in Ituri province because territories were occupied by the war during the period of sampling. This was also the reason to exclude Walikale and Rutshuru territories where indigenous cattle populations are located in North Kivu province Mwenga territory where the forest hosts the wildest animals in South Kivu was not visited because of insecurity due to an on-going war.

The herd data were recovered from the Agriculture, Livestock and Fisheries (AGRIPEL) offices who are in charge of animal health in each territory. In the present study, herds were selected based on: (i) accessibility of the herds by road and the occurrence of ECF disease in cattle, (ii) a minimum herd size of 15 cattle of regardless of their sexes and of at least 6 months old in order to get rid of interference of the maternal antibodies and (iii) availability of animal handling facilities for the restriction of animals. However, in Ituri and South-Kivu provinces most of herds had no crush-pens. Thus, it required additional personnel to restrain animals properly for sampling.

Before any sampling, the selection procedure was explained to all cattle farmers. From each village, each village provided 2 to 4 herds. In total, 10 herds were selected in each province from which approximately 50% of the animals of the selected herd were sampled. This gave a total of 756 cattle distributed into three provinces. Three strains cattle were constituted: Ituri (250), North Kivu (255) and South Kivu (251). Most of these animals were females (74.34 %) mainly aged at least 24 months (70.37%) since males are slaughtered before 12 months of age.

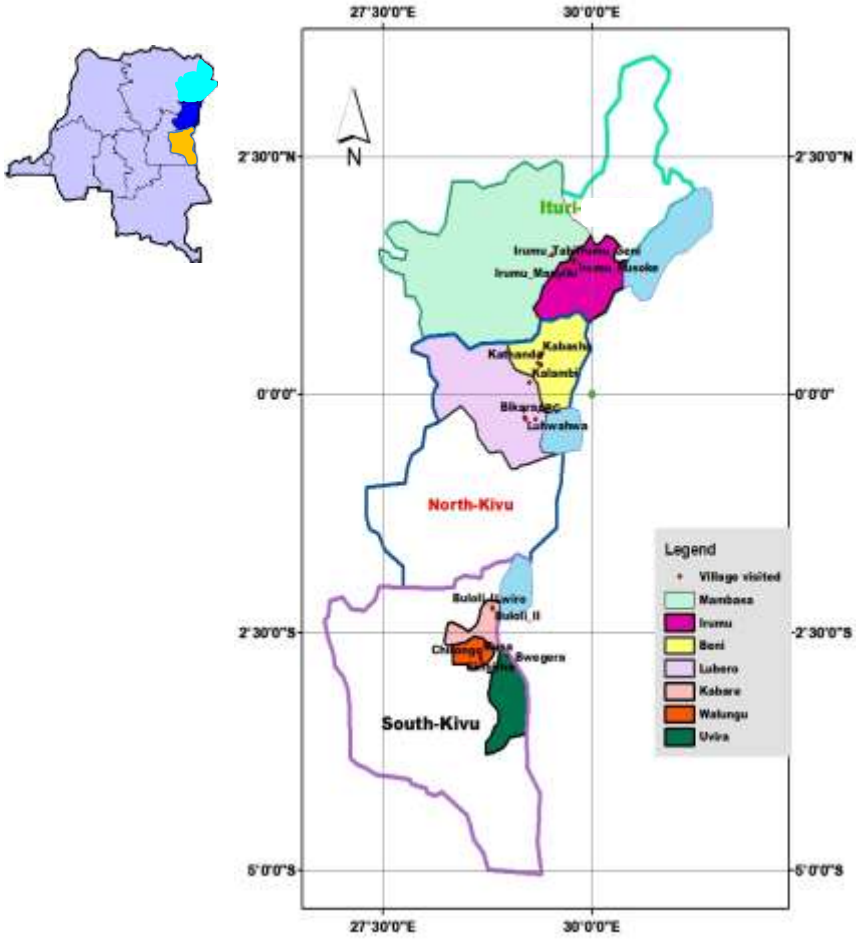

Fig. 2: Map of the three Province in Eastern DRC showing territories visited and sampling sites (n=756 cattle). Cattle herds and Allocation of animals

Sample collection and handling

In a herd, animals in poor condition of health were targeted to have a better chance of detecting *T. parva* infection. The presence of ticks on the body and any ECF clinical signs (rectal temperature above 39.5°C, swelling of parotid and prescapular lymph nodes, acute respiratory distress, lacrimation, nasal discharge and diarrhea) allowed for a good selection of the animals to be sampled. The animal age and sex were recorded along with information related to inter-current disease, recent acaricide prophylaxis and ECF treatment. However, most of the herds reported no consistent control of ticks and tick-borne diseases in. Through jugular

venipuncture, blood sample was collected from selected cattle (EDTA and for serum). For future DNA extraction, blood sample from each animal was spotted in duplicate onto FTA filter paper (Whatmann n°4, Bio-Science). For transportation to the laboratory, blood was kept on ice packs in vacutainers. Blood samples were aliquoted in 1.5ml eppendorf tubes coated with EDTA and kept at -20ºC until used for DNA extraction. Blood for serum was centrifuged and serum separated and kept at -20ºC at the Department of Parasitology and parasitic diseases, Faculty of Veterinary Medicine, Catholic University of Graben. An aliquot of serum, blood in EDTA and spotted FTA were shipped to Center for Tick and Tick-borne Diseases (CTTBD) in Lilongwe / Malawi for further analysis.

Tick identification and counts

On each selected animal, tick were counted after Londt et al. (1979). All visible ticks were collected by manual extraction from whole animal body and were stored immediately in 70° alcohol. Thanks to the keys of Walker et al. (2003), ticks were identified to species level at the parasitology laboratory of the Faculty of Veterinary Medicine, UCG/Butembo.

Indirect ELISA for *T. parva* antibodies

The 756 serum samples were submitted to the PIM-based enzyme-linked-immunosorbent assay (ELISA) (Katende et al., 1998) for the assessment of specific antibodies to *T. parva* (sensitivity > 99%, specificity 94 to 98%). The Optical density (OD) of blood samples was read at 405 nm on an ELISA reader (Mannheim GmbH, Germany). These readings were necessary for the computation of antibody percent positivity (PP) for each sample following the formua: Mean OD (sample or negative control) divided by mean OD of positive control multiplied by 100. The PP values greater than or equal to 20% were considered positive.

Genomic DNA extraction and detection of *Theileria parva*

Blood samples that were positive to ELISA test (208 samples) were submitted genomic DNA extraction. The screening of samples for the presence of *T. parva* was done by nested p104 polymerase chain reaction (nPCR) following Lams et al. (1990) and Odongo et al. (2010). The primary PCR was conducted consisting of a forward primer p104F2 (5´-CCA CCA TCT CCT AAA CCA CCG TT-3´) and reverse primer p104R (5´-TAA GAT GCC GAC TAT TAA TGA CAC CAC AA-3´). The initial denaturation was performed at 95°C for 5 min, followed by a second

denaturation at 95°C for 60 second. The annealing was done at 60°C for 60 s and the extension at 72°C for 60 seconds. The amplification was done in 35 cycles. This was then followed by secondary PCR where 5µl aliquot was used against forward primer p104nF (5´- ACC ACC GTT TGA GCC GAC TAT TAA TGA CAC CAC AA-3´) and reverse primer p104R (5´-TAA GAT GCC GAC TAT TAA TGA CAC CAC AA-3´). The conditions of a secondary PCR were the same as those of primary PCR except the number of cycles during the amplification was reduced to 30. The DNA fragments (products of secondary PCR) were then separated by horizontal electrophoresis in 0.5×TAE transferred onto a 2% high resolution agarose gel electrophoresis at 100V for 1 hour and visualized under ultraviolet (UV) trans-illuminator after staining with ethidium bromide.

Statistical analysis

Preliminary ELISA and PCR data processing was performed using Microsoft excel as well as the computation of descriptive statistics. Analytical analyses (Chi-square) were performed using StatView (Version 5.0.1) at the 95% significance level.

Results

Tick infestation among Ituri, North Kivu and South Kivu cattle

In total 12,672 ticks were collected from 756 animals and 5 species of ticks were identified. *Rhipicephalus appendiculatus* was the most dominant species (68%) prior to *R. decoloratus* (18.2%), *Amblyomma variegatum* (7.9%), *R. evertsievertsi* (5.7%) and *Hyalomma m. rufipes* (0.2%). Based on tick counts, the three provinces' cattle were significantly different ($p<0.0001$). Significantly higher population of ticks was recovered on South Kivu cattle (47.5%) when compared to North Kivu (30.5%) and Ituri cattle (22%). Significantly higher mean *R. appendiculatus* tick load per infested animal ($P<0.05$) was recovered in South Kivu cattle (18.46±0.33 ticks) compared to the one recovered on North Kivu (7.93±0.32 ticks) and Ituri cattle (7.87±0.33 ticks).

Prevalence of the overall *T. parva* antibody and parasite in the study herds

Table 2 highlights the antibody responses to *T. parva*. Regardless of the strain (province), all cattle showed a positive response for *T. parva* antibodies. The ELISA test with 20% cut-off point shows that 208 out of

the 756 selected cattle were positive to *T. parva* antibodies. In most cattle strain, the *T. parva* antibodies prevalence ranged from 7 to 40% (95%CI= 34.5-46.6). A significantly lower seroprevalence to *T. parva* ($p<0.05$) (7%; 95% CI=4.5-10.9) was found in North Kivu cattle compared to South Kivu (35.5%95% CI= 29.8-41.6) and Ituri cattle (40.495% CI= 34.5-46.6). After the removal of 524 negative and 24 inconclusive samples by ELISA test, only 208 samples were used for PCR detection of *T. parva* parasites. The PCR analysis depicted highly prevalent *T. parva* parasite (ranging from 85 to 100%) which was not statistically significant across the three cattle strains (**Table 3**). The antibody and parasite prevalence data proved the presence of *T. parva* infection in all the three provinces.

Relationship between tick load and the occurrence of ECF clinical signs, antibody response and *T. parva* parasite in the three provinces cattle

Based on the *R. appendiculatus* tick load, data of the occurrence of ECF clinical signs, antibody response and the *T. parva* parasites at the herd level are presented in **Table 4**. Out of 756 cattle examined clinical signs of ECF were observed on 50 cattle corresponding to 6.61% (95% CI=5-8.6) of ECF clinical cases. South Kivu cattle had relatively higher (20 cattle) clinical cases compared to Ituri (18 cattle) and North Kivu (12 cattle). However, the vector tick burden was not associated to significant differences in the occurrence of ECF clinical signs in the three strains cattle ($p>0.05$). A significantly higher conditional probability of having a positive PCR result on samples with a positive ELISA was found in the three cattle strains ($p<0.0001$). Besides, *R. appendiculatus* tick load significantly affected the seropositivity to *T. parva* ($p=0.0044$) as well as the prevalence of *T. parva* parasite ($p=0.0115$).

Table 1. Identification and distribution of 12,672 ticks collected from cattle in the study area

Strainscattle	Number of examined animals	*Rhipicephalus appendiculatus* % (n ±SE)	*Rhipicephalus decoloratus* % (n ±SE)	*Amblyomma variegatum* % (n ±SE)	*Rhipicephalus evertsi evertsi* % (n ±SE)	*Hyalomma m. rufipes* % (n ±SE)	Whole body tick count (%)
Ituri	250	22.8 (7.9±0.33^{a})	0.3 (0.03±0.33^{a})	66 (2.63±0.33^{a})	20.9 (0.6±0.33^{a})	0 (0.00±0.33^{a})	2,783 (22)
North Kivu	255	23.4 (7.9±0.32^{b})	79.7 (7.19±0.32^{b})	1.1 (0.04±0.32^{b})	0 (0.0±0.32^{b})	0 (0.00±0.32^{b})	3,865 (30.5)
South Kivu	251	53.8 (18.5±0.33^{a})	20 (1.84±0.33^{a})	32.9 (1.31±0.33^{a})	79.1 (2.3±0.33^{a})	100 (0.12±0.33^{a})	6,024 (47.5)
Overall	756	68.04 (11.4±0.19^{c})	18.16 (3.04±0.19^{c})	7.9 (1.32±0.19^{c})	5.7 (0.1±0.19^{c})	0.2 (0.04±0.19^{c})	12,672

n= Tick load per infested animal; ^{a,b,c} Means bearing different indices within column in each species are significantly different

Table 2. Prevalence of *T. parva* antibody responses using ELISA test in the study sites

Straincattle	Number of cattle sampled	ELISA positive (%)	Confidence interval at 95%	ELISA inconclusive	ELISA negative
Ituri	250	101 (40.4)	34.5-46.6	9	140
North Kivu	255	18 (7)	4.5-10.9	5	232
South Kivu	251	89 (35.5)	29.8-41.6	10	152
Overall	756	208 (27.5)	24.4-30.8	24	524

Table 3. Prevalence of *T. parva* PCR among seropositive samples in the study sites.

Straincattle	Number of seropositive samples	PCR positive (%)	Confidential interval at 95%	PCR negative
Ituri	101	86 (85.1)	76.8-90.8	15
North Kivu	18	18 (100)	-	0
South Kivu	89	78 (87.6)	79-93	11
Overall	208	182 (87.5)	82.3-91.3	26

Table 4. Influence of *R. appendiculatus* tick burden on ECF clinical signs, antibody percentage positivity by ELISA and prevalence of *T. parva* parasites by PCR in Ituri, North Kivu and South Kivu cattle

Strain cattle (*R. appendiculatus* tick burden)	ECF clinicalsigns	Chi-square	ELISA	Chi-square	PCR	Chi-square
Ituri (7.87±0.33[a])	18		101		86	
North Kivu (7.93±0.32[b])	12	1.9886	18	10.8734	18	8.9278
South Kivu (18.46±0.33[a])	20		89		78	

[a,b] Means bearing different indices within column in each species are significantly different

Discussion

The present study provides an overall insight on the tick load and the prevalence of *T. parva* infection on cattle in Ituri, North Kivu and South Kivu provinces, eastern DRC. Three findings of the present study were informative on tick infestation and its relationship with seroprevalence to *T. parva*, prevalence of parasite and ECF clinical signs on the three cattle strains.

First, the study reveaved that *Rhipicephalus appendiculatus* species, vector of *T. parva* and causal agent of ECF, was the most dominant (68.04%) tick species found on the cattle of both provinces. This situation is in accordance with previous studies reported in eastern DRC on North Kivu cattle (85.23%; (Kalume, 2011) and 64.26%; (Kalume et al., 2013) and South Kivu cattle (57.2 %; (Makumyaviri & Habimana, 1993) and

68.5%; (Bisusa & Kalume, 2013) The dominant tick species *R. appendiculatus*, outnumbered all others species in the total tick collection. A similar trend has also been reported in Rwanda (91.84%; (Bazarusanga et al., 2007) and 96 %; (Nshimiyimana & Mutandwa, 2010) as well as in Uganda (50.47 %; (Rubaire-Akiiki et al., 2004). Nevertheless, *R. appendiculatus* count significantly differed ($p<0.05$) between the three cattle strains. South Kivu cattle carried significantly higher tick burdens (18.46±0.33 ticks per infested animal) than North Kivu (7.93±0.32) and Ituri cattle (7.87±0.33). The highest *R. appendiculatus* load on South Kivu cattle may have resulted from a wide range of factors including management practices probably related to scarce dipping of cattle especially during the dry season in South Kivu (Amzati, 2011). However, the irregular tick control has also been reported in other endemic areas such as on North Kivu cattle (Kalume et al., 2009). The high tick loads on South Kivu cattle may be more clearly explained by animal factors in this ecological area especially on Ruzizi plain cattle in Kamanyola village, Uvira territory where animals seem be tolerant to ticks infestation and ECF clinical cases. Such perception agrees with Mathee et al. (1997) who reported that regardless of breed, the lack of appropriate control measures exposes animals to always carry more ticks all the year round with high mortality if ticks are not controlled. Furthermore, in the evaluation of risk factor, the current study showed that the *R. appendiculatus* tick burden was not associated to significant differences in the occurrence of ECF clinical signs among the three cattle strains ($p>0.05$). Since the results of a single herd visit cannot be used to reliably predict the phenology of *R. appendiculatus* tick, there is still limitations to the estimation of the epidemiological condition of ECF through a cross-sectional investigation. Therefore, the results of the present study might not reflect the right trend of transmission of *T. parva* and the occurrence of some ECF clinical signs such as fever (body temperature above 39.5°C) for more than 3 consecutive days.

The present study found a wide range of levels of antibody percent positivity for the three cattle strains which clustered in low seroprevalence to *T. parva* up to 40.4% (95%CI= 34.5-46.6) in comparison to 43% (95% CI = 38-47) previously reported in a cross-sectional study on North Kivu cattle (Kalume et al., 2013). However, most of North Kivu cattle showed a very lower antibodies PP categories than South Kivu and Ituri cattle. Besides Norval et al. (1992) indicated that antibodies levels are insightful ECF epidemiological indicator in an endemic area which can reflect

resistance to the pressure of infection in the study area. The low level of specific antibodies in endemic area can be associated with the response of very few resistant individuals. Under these conditions, the herds located in North Kivu province (where antibody percent positivity level was low) would present a higher proportion of ECF clinical signs cases when compared to South Kivu and Ituri cattle. However, this was not the case, and the situation seems to lead to the low tick load recovered on North Kivu cattle since the seropositivity to *T. parva* can accurately be predicted by the presence of *R. appendiculatus* on cattle (Deem et al., 1993; Gachohi et al., 2010; Swai et al., 2009). Nevertheless, low vector tick load was reported in Ituri in combination with high proportion of ECF clinical signs. This would suggest that the transmission of *T. parva* and the occurrence of ECF clinical cases does not depend on tick abundance only. The proportions of tick infection are also regarded as useful (Perry, 1996) since it determines the competence of different vectors to transmit *T. parva* amongst tick from North Kivu and Ituri cattle (Ochanda et al., 1998). Low infection rates in the population of ticks are associated with low seropositivity to *T. parva* (Gilioli et al., 2009; Odongo et al., 2010) and therefore low resistance of cattle. This agrees with the findings of the present study where lower ECF clinical signs were reported in North Kivu cattle compared to Ituri and South Kivu cattle. However, the study area is still lacking data on the rate of *T. parva* infection among tick populations.

Comparing the PCR results on the prevalence of *T. parva* parasites in the three cattle strains, the present study found that the parasite prevalence in North Kivu cattle was slightly higher (100% of seropositive cattle), although no significant difference was observed between Ituri and South Kivu cattle. In line with the results of the present studies, previous investiogations have reported increasing acquisition of blood parasites when less resistant cattle breeds with low level of antibodies are kept in *T. parva* endemic area (Laisser et al., 2016; Gachohi et al., 2012; Marufu .2008). The overall high *T. parva* parasite prevalence ranging from 85.1 to 100% across the three strains cattle implies that cattle are under constant exposure to the parasite in the area. This estimated prevalence agrees with one reported in majority of ECF endemic areas where nearly 100% of the animals can be carriers of *T. parva* (Bazarusanga et al., 2008). The *T. parva* prevalence in the three provinces confirms the significant role of the tick species *R. appendiculatus* as a suitable vector (Gitau et al., 1997; Rubaire-Akiiki et al., 2004). Serology and PCR results were strongly correlated and

R. appendiculatus infestation levels occur in all areas which agrees with previous findings in Rwanda (Paling & Geysen, 1981). Moreover, *T. parva* prevalence and *T. parva* seroprevalence are biologically associated (Norval et al., 1992). Previous studies found that this association can be affected by acaricide application, animals' age as well as the resistance of the hosts to the parasites and their tick vectors (Gachohi et al., 2010). However, influence of tick control regime, age of animals and host resistance level were not evaluated in the present study.

In the present study, there was a significantly higher conditional probability of a positive PCR result with positive ELISA in the three cattle strains. This result did not agree with reports by (Gachohi et al., 2010) who found that the association between prevalence of parasites and antibodies was site specific.

The overall results show that tick load and antibody level significant differed between the three cattle strains. Significantly higher tick load was recovered on South Kivu cattle compared to the North Kivu cattle which had a very lower seroprevalence to *T. parva*. In general, the low level of specific antibodies observed in the present study reflects the response of very few resistant individuals in a *T. parva* endemic area under natural tick challenge. In this context, most of cattle are expected to be carriers as a high prevalence of *T. parva* parasites was indicated for both seropositive cattle strains. These findings provide the first testimony to hitherto studies' assertions that ECF endemic instability exists in eastern DRC. This paves a way to designing programs for the protection of cattle against ECF disease by Infection and Treatment Method (ITM) using injection simultaneously of Muguga Cocktail vaccine and Oxytetracycline (Alamycin LA 30%) in tick control regime, and alongside treatment of clinical cases.

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