Prevalence and Risk Factors of East Coast Fever in the Copperbelt and Central Provinces of Zambia

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ABSTRACT

East Coast fever (ECF) is an infectious tick-borne disease of cattle, caused by a protozoan parasite *Theileria parva.* It is a disease of major economic importance in Zambia, being the main cause of cattle morbidity and mortality. Despite its economic importance, the epidemiology of ECF in Zambia is poorly understood, thereby making ECF prevention and control difficult. Further, there is limited published literature on this disease in Zambia, with the little available research concentrating on Southern and Eastern provinces.

We conducted a cross-sectional study to determine the prevalence and associated risk factors of ECF in Copperbelt and Central provinces of Zambia. The provinces and districts were selected based on their vast potential for livestock production and the previously reported incidence of ECF. From each district, two veterinary camps were randomly selected and from each camp herds were randomly selected, from which individual animals were randomly sampled. Lymph node biopsies were collected from Mpongwe and Masaiti districts (Copperbelt province) and Kapiri Mposhi and Chibombo districts (Central province). Lymph smears were prepared and stained with Giemsa for microscopic examination.

Microscopic examination of lymph smears revealed that 6.4% (95%, CI=4.9-7.9) of the samples were positive for *T. parva* schizonts. The Copperbelt province prevalence was 6.1% (95%, CI=4.0-8.2) while in Central province it was 6.7% (95%, CI=4.5-8.9). Among the districts in these provinces, Kapiri Mposhi did not record any schizont positive cattle, while Chibombo had the highest prevalence at 13.6% (95%, CI=9.4-17.9). Risk factors that were identified to be associated with *Theileria* schizonts in cattle were locality (district), previous experience of ECF, tick burden, and age.

These results indicate that ECF is prevalent in Copperbelt and Central provinces of Zambia. Concerted efforts are needed to control ticks and prevent ECF through farmer sensitization, routine, regular, mandatory and supervised dipping or spraying of cattle and stringent livestock movement control to help bring down ECF prevalence.

Keywords: Epidemiology, lymph smear, microscopy, schizonts, *Theileria parva*.

1.0 INTRODUCTION

East Coast fever (ECF) is an infectious tick-borne disease (TBD) of cattle caused by a protozoan parasite *Theileria parva*, which is transmitted by the brown ear tick, *Rhipicephalus appendiculatus.* The disease is characterized by lymphadenopathy, pyrexia, laboured breathing, frothing and often death (Fandamu *et al*., 2005; Mtambo *et al*., 2008; Norval *et al*., 1992). In Zambia, ECF is one of the most economically important cattle diseases associated with significant losses to the livestock industry (Chizyuka *et al*., 1985). The disease has been reported in all the provinces in the country except for Luapula, Western and Northwestern provinces. The impact of ECF is due to the high morbidity and mortality as well as production losses and costs related to the control of ticks and treatment of the affected cattle (Anon., 2010; Gacholi *et al*., 2012; Makala *et al*., 2003;). This has resulted in the loss of sources of livelihoods for most farmers, especially the rural small scale resource-poor households (Gacholi *et al*., 2012). ECF has further been a restraint to the genetic improvement of indigenous breeds of cattle, as it prevents the introduction of more productive exotic breeds and has hindered the growth of the livestock sub-sector which is an essential component of Zambian agriculture (Gacholi *et al*., 2012; Simuunza *et al*., 2011).

Despite the importance of ECF to the cattle industry, there is inadequate systematic documented data on ECF prevalence in most parts of Zambia including Copperbelt and Central provinces (Simuunza *et al*., 2011) thereby making ECF prevention and control difficult. Although Simuunza *et al* (2011) described the epidemiology of tick-borne diseases (TBDs) in Zambia's Lusaka, Central and Eastern provinces, the sample size for the former provinces was very low, and, likely, this study did not give a clear view of the status of ECF in the three provinces. Fandamu *et al* (2005) also described the prevalence of ECF in Southern province using indirect fluorescent antibody test (IFAT) as the diagnostic tool. The study did not fully address the risk factors associated with the occurrence of ECF in the Province. For effective disease control planning, there is a need to have adequate information on the epidemiology of the diseases. Therefore, this study aimed to determine the prevalence and risk factors associated with ECF in the Copperbelt and Central provinces of Zambia.

2.0 MATERIALS AND METHODS

Study area

The study was conducted in Mpongwe and Masaiti districts of the Copperbelt province and Kapiri Mposhi and Chibombo districts of the Central province, Zambia (Figure 1).

The provinces and districts were selected based on their vast potential for livestock production and the previously reported incidence of ECF (Anon, 2010; Nambota *et al*., 1994).

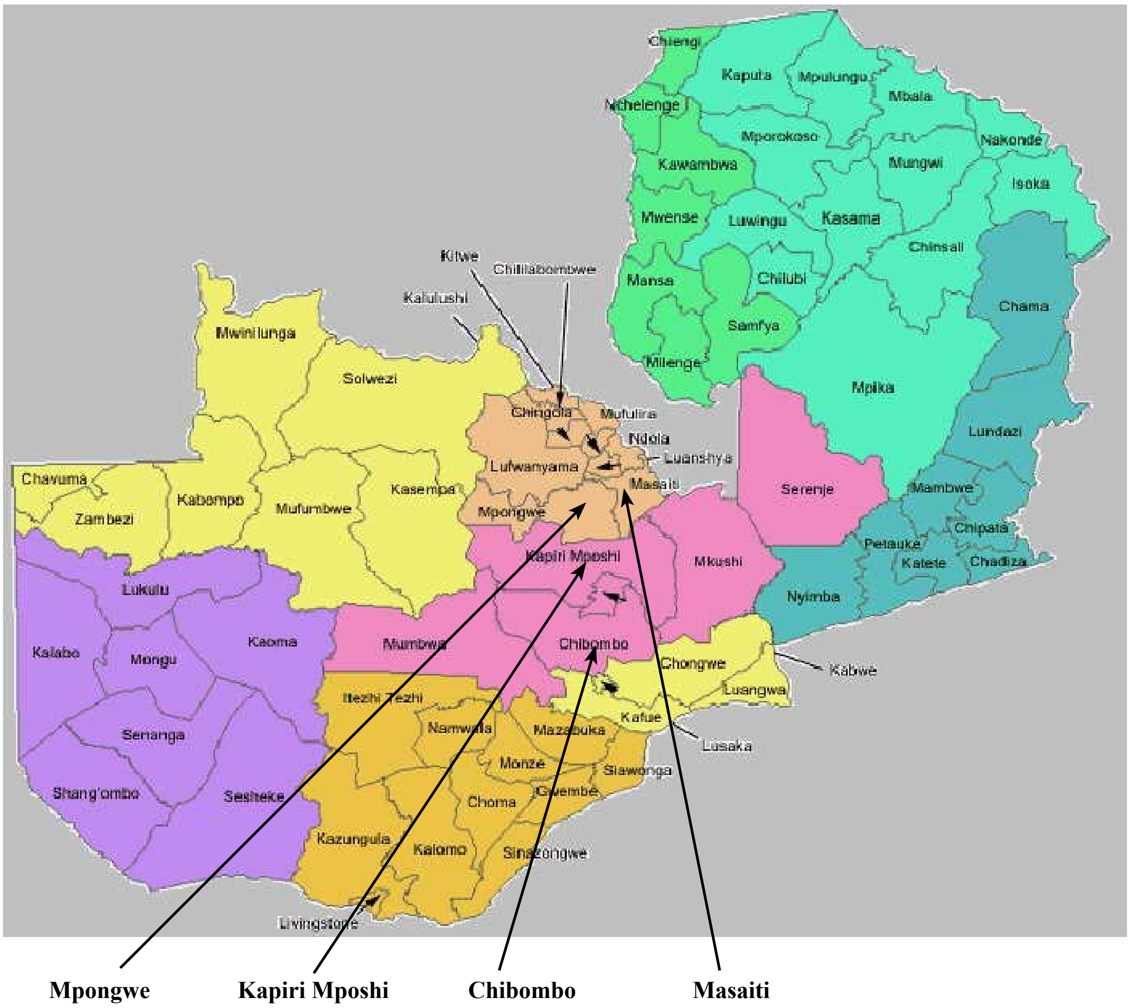

Figure 1: Map of Zambia showing Mpongwe, Kapiri Mposhi, Chibombo and Masaiti districts (Anon., 2015)

Study design and sample collection

The study was cross-sectional in design. The sample size was determined as described by Martin *et al.,* (1987), for a cross-sectional study. The assumptions were that ECF prevalence was 20% (95% CI, 17.5-22.5) and samples would be collected from four districts, giving a total of 984 animals for the whole study area (Ahmadu *et al*., 2004; Jongejan *et al*., 1988). For Mpongwe district, samples were collected in December 2011, and in January, February, August and December 2012. Samples from Masaiti district were collected in February, March and December 2012. Samples from Kapiri Mposhi district were collected in June 2012, while samples from Chibombo district were collected in July and August 2012. Herds of cattle were randomly selected from each veterinary camp. Each randomly selected herd lymph node biopsies was collected from the prescapula lymph node of randomly selected cattle. At the sample collection time, a semi-structured questionnaire was also administered to collect information on the farming system, management, ECF vaccination status, tick burden, breed, age and sex.

Laboratory Analysis

Lymph smears were prepared whilst in the field from the lymph node biopsies for microscopic examination. The smears were made by placing a drop of lymph node aspirate onto one end of a clean and labelled slide. The slides label was checked with the samples to make sure it was the same. The edge of another clean slide (pusher slide or spreader) was brought in contact with the drop, and the drop was allowed to bank evenly behind the spreader. The angle between the pusher slide and specimen slide was maintained at 45^0. The spreader was then pushed with the right hand to the left in a smooth and quick motion. The smear covered about half of the slide and consisted of a head, middle part and tail (anon., 2014a; anon., 2014b). The pusher slide was discarded in a biohazard discard bag and the smear allowed to air dry in a dust-free area.

The smears were fixed in methanol for 3-5 minutes, dried and placed in the freshly prepared Giemsa stain solution diluted with water at 1:9 (Giemsa: water) for 30 minutes, washed in distilled water, then air-dried and microscopically examined for the presence of *T. parva* schizonts under oil immersion at a magnification of X100 and at least ten sites of the slide were examined (Aiello *et al*., 1998; Salih *et al*., 2007).

Data analysis

Data from the questionnaire and laboratory analysis were entered into Microsoft Excel spreadsheets and later transferred to SPSS version 16 (IBM, USA) for analysis. Descriptive statistics were calculated for each of the variables included in the study. Binary logistic regression was used to quantify the effect of each risk factor on cattle being *T. parva* positive.

3.0 RESULTS

Descriptive Statistics

Of the 997 samples collected 12.4% (95%, CI=10.4-14.5) cattle came from the commercial farms and 87.6% (95%, CI=85.5-89.6) came from the traditional farming system. A few farms 9.2% (95%, CI=7.5-11.0) reportedly had no access to veterinary care, whereas most of the farms 90.8% (95%, CI=89.0-92.6) had access to veterinary services. None of the farms from which samples were collected practised ECF vaccination for their cattle.

Most of the farms 99.3% (95%, 95.9-103.1) reportedly practiced tick control. The majority of farms (95.9%, 95%, CI=92.7-99.1) practiced tick control throughout the year, but a few (3.4%, 95%, CI=0.5-6.3) practiced strategic tick control during the period from November to July. Of the 997 heads of cattle sampled, 5.7% (95%, CI=3.6-6.4) were owned by women, 91.1% (95%, CI=89.3-92.9) were owned by men and 3.2% (95%, CI=2.1-4.3) were owned by institutions. Based on age, 27.1% (95%, CI=24.3-29.8) were calves and 72.9% (95%, CI=70.2-75.7) were adults. The majority of the cattle sampled (54.0%, 95%, CI=50.9-57.1) were females and (46%, 95%, CI=42.9-49.1) were males. Of these cattle sampled, 12.1% (95%, CI=10.1-14.1) were commercial breeds, 5.6% (95%, CI=4.2-7.0) cross breeds and 82.3% (95%, CI=79.9-84.7) local breeds.

The prevalence of the disease according to a number of variables under study is shown in Table 1.

Table 1: **Schizont prevalence according to various factors**

Variable	Categories	n	Prevalence (%)	95% CI	p-value
Study area	Central and Copperbelt provinces	997	6.4	4.9-7.9	
Province	Central	505	6.7	4.5-8.9	0.390
	Copperbelt	492	6.1	4.0-8.2	
District	Chibombo	250	13.6	9.4-17.9	<0.001
	Kapiri Mposhi	255	0	-	
	Masaiti	245	2.4	0.5-4.3	
	Mpongwe	247	9.7	6.0-13.4	
Sex	Female	538	6.1	4.1-8.1	0.393
	Male	459	6.8	4.5-9.1	
Age	Adult	727	4.5	3.0-6.0	<0.001
	Calf	270	11.5	7.7-15.3	
Breed	Commercial	121	0.8	-3.0-4.6	<0.001
	Cross	56	17.9	7.5-27.9	
	Local	820	6.5	4.8-8.2	
Farming system	Commercial	124	1.6	-0.6-3.8	0.009
	Traditional	873	7.1	5.4-8.8	
Access to the vet. Service	No	92	9.8	3.7-15.9	0.126
	Yes	905	6.1	4.5-7.7	
Frequency of vet. Service	Undefined	100	9.0	3.4-15.6	0.383
	Every week	81	8.6	2.5-14.7	
	Monthly	520	6.3	4.2-8.4	
	Rarely	296	5.1	2.6-7.6	
Service provider	Community livestock worker	3	0	-	0.671
	Veterinary assistant	890	6.2	4.6-7.8	
	Unknown (or not serviced)	104	8.7	3.3-14.1	
Method of tick control	Dipping	57	19.3	9.1-29.6	0.001
	Spraying	934	5.7	4.2-7.2	
Tick burden	High	55	18.2	8-28.4	<0.001
	Low	147	4.8	1.3-8.3	
	Medium	681	6.8	4.9-8.7	
	Nil	114	0.9	-3.0-4.8	

Predictors of cattle being positive to *T. parva* schizonts

A stepwise binary logistic regression model was used to determine predictors of cattle being positive to *T. parva* on lymph smear examination. All variables with p ≤0.250 were included in the model. The Hosmer and Lemeshow test was a non-significant (p>0.05), and the Omnibus Test of Model coefficients was significant (p<0.05) indicating that the model adequately fitted the data.

Cattle from Chibombo district were 0.375 (95%, CI=0.797-3.470) times less likely to be positive for schizonts than Mpongwe cattle. Cattle from Masaiti district were 0.535 times less likely to be positive for schizonts than cattle from Mpongwe.

Cattle from farmers that reported to have previously experienced ECF were 0.300 (95%, CI=0.293-0.952) times less likely to be positive for schizonts than cattle from farmers that reported to have no previous experience of ECF. Cattle that had a low tick burden were 1.161 (95%, CI=1.307-123.785) times more likely to be positive for schizonts than cattle with no ticks. Calves were 0.320 (95%, CI=0.215-0.755) times less likely to be positive for schizonts than adult cattle. Therefore predictors of cattle being positive for schizonts include district (locality), previous experience of ECF, tick burden, and age.

Table 2: Maximum likelihood estimates of the binary logistic regression model of factors for the prediction of cattle being positive *Theileria parva* infection on lymph smear examination

Variable	Category	Odds Ration	p-value	95.0% C.I for Odds Ratio	
				Lower	Upper
District	Mpongwe		0.142		-
	Chibombo	0.375	0.176	0.797	3.470
	Masaiti	0.535	0.139	0.159	1.292
Previously experienced ECF?	No				
	Yes	0.300	0.034	0.293	0.952
Tick burden	Nil		0.014		
	Low	1.161	0.028	1.307	123.785
Age	Adults				
	Calves	0.320	0.05	0.215	0.755

4.0 DISCUSSION

Few studies have previously been done in Zambia to describe the epidemiology of ECF, yet it is a significant disease of livestock that continues to affect millions of people's income and livelihood (Fandamu *et al*., 2005). This study's objective was to establish the prevalence of ECF in cattle in Copperbelt and Central provinces and identify risk factors associated with its prevalence. The study used a microscopic examination of lymph smears to determine the prevalence of ECF and binary logistic regression to determine risk factors of being *T. parva* positive. Therefore, microscopic examination of lymph smears was used, which has limitations of low sensitivity and may miss schizonts in carriers that are not clinically sick (Fandamu *et al*., 2005).

The study established that ECF was prevalent in both the Copperbelt and Central provinces. The difference in prevalence between the two provinces was not significant. This could be because the two provinces are situated next to one another and receive almost the same amount of rainfall, although the Northern part of the Copperbelt province may receive slightly more. The activity and abundance of the adult tick vectors is dependent on the amount of rainfall (Norval *et al*., 1992).

Variability in prevalence across districts could be attributed to high variation in the spatial distribution of ECF for different areas partly due to differences in the distribution of the vector *R. appendiculatus* (Bazarusanga *et al*., 2008; Muhanguzi *et al*., 2014) and differences in tick densities and their infection rates, parasite virulence, cattle resistance, tick control strategies, tick control infrastructure and availability of veterinary service (Tembo *et al*., 2012). Geographical factors such as rainfall, altitude, temperature and vegetation cover for the different areas may also account for the differences in prevalence across districts as these affect the presence, growth and survival of the ticks (Fandamu *et al*., 2005; Pegram *et al*., 1986).

The prevalence of ECF obtained in this study was lower than that reported by Simuunza (2011) in Eastern, Central and Lusaka provinces, Fandamu (2005) in Southern province and Bazarusanga (2007) in Uganda, but was higher than the prevalence reported by Tembo (2012) in Mungwi District of Northern province. The prevalence estimated in this study was lower than that reported by Simuunza *et al* (2011a) and Fandamu (2005) because the diagnostic method used in this study is less sensitive than the ones used in the latter studies. In addition, microscopy was used to determine the presence of schizonts in lymph node smears. This parasite stage is more visible in animals with clinical disease and may be missed in carriers (Fandamu *et al*., 2005; Norval *et al*., 1992).

Calves had a higher prevalence than adult cattle which is in agreement with the findings of Salih (2007), who reported high infection of *T. parva* among calves compared to adults. The high prevalence among calves, when compared to adult cattle may indicate the susceptibility of indigenous calves partly due to low immunity especially between one month and six months to ECF (Oura *et al*., 2005). Salih (2007) also reported that calves were more are at risk when subjected to infection for the first time with high mortalities in calves of up to 6 months old.

Crossbreed cattle had the highest prevalence, followed by local breeds and commercial breeds had the lowest. This result indicates that the different cattle breeds have different susceptibilities to ECF (Minjauw *et al*., 1999) and agrees with the findings of Oura *et al* (2005) and Tembo *et al*., (2012) who reported a higher ECF prevalence among cross breeds compared to local breeds. The high prevalence among local breeds might be related to the traditional management system which local breeds of cattle are subjected to, where tick control is not consistent. Therefore despite being more resistant, local breeds had a high prevalence and ranked second to crossbreeds, and this result agrees with the findings of Minjauw *et al* (1999) and Simuunza *et al* (2011), who reported a higher prevalence among local breeds. Results obtained in this study may indicate effective tick control in the commercial breeds of cattle which are predominantly owned by commercial farmers. Further in the traditional sector often communal and open grazing is practised allowing for more interaction among cattle from different herds and in pastures where ticks can easily attach and actively transmit ECF from carrier animals to the susceptible in contact (Minjauw *et al*., 1999). Gitau (2000) also reports that the ECF challenge is high in areas where open grazing is practised and low for areas where cattle are zero grazed.

Cattle on which ticks were controlled by dipping had a higher prevalence than those that were sprayed. This may be an indication of inadequacies in the management and use of the dipping facilities and inadequate knowledge on drug dilution and administration as well as inadequate veterinary personnel and resources for effective monitoring and supervision of dipping activities. Cattle with a high tick burden had the highest prevalence, and those with no tick burden had the lowest. This result may indicate a high intensity of ECF transmission in cattle infested by high tick numbers and underscores the need for effective tick control. Risk factors associated with schizont prevalence were identified as a district (locality), previous experience of ECF, tick burden and age. The locality is most likely related to the presence and distribution of the tick vector and therefore the *T. parva* parasite which is more abundant in some districts than others. The acquired immunity may explain previous ECF experience that the animals develop and the farmers get more sensitized, concerned about ECF and control ticks on their cattle. The association between tick burden and ECF is most likely related to the transmission of the parasites leading to a higher prevalence following a high tick burden. Adult cattle have had a cumulative transmission of *T. parva* parasites compared to the calves, and this explains the high likelihood of finding schizonts amongst adults compared to calves.

5.0 CONCLUSION

East Coast fever is prevalent in Copperbelt and Central provinces of Zambia and is a constraint to cattle production. Risk factors that were identified for ECF transmission include district, previous experience of ECF, tick burden and age. There is, therefore, need for concerted efforts by all stakeholders to control ticks and prevent ECF through farmer sensitization, routine, regular, mandatory and supervised dipping and spraying of cattle and stringent livestock movement control. There is also need for further studies to ascertain the extent of ECF burden in the Copperbelt and Central provinces using more sensitive techniques such as polymerase chain reaction (PCR) and Loop-mediated isothermal amplification test (LAMP).

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7.0 COMPETING INTERESTS

The authors declare that there were no competing interests. Financial support was received from the Ministry of Agriculture and Livestock, where David Chabala was a paid employee. However, the Ministry did not play a role in the study design, study conduct, data analysis, and manuscript review.

8.0 AUTHORS CONTRIBUTIONS

David Chabala (Veterinary Department, Mpongwe District, Zambia) conceived the project and was the principal investigator who carried out sample and data collection, laboratory examination of samples, analysis of results and drafted the manuscript. Martin Simuunza (University of Zambia) and Boniface Namangala (University of Zambia) supervised the project and assisted in project design, laboratory examination, analyzing results and reviewed the manuscript.

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