Research Article

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Retrospective analysis of laboratory diagnostic data to assess the seasonal and proportional distribution of major tick-borne diseases in cattle from selected smallholder farms, Zambia

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Abstract: Tick-borne diseases (TBDs) are endemic in Zambia and several other Sub-Saharan countries. They affect livestock production by causing high morbidity and mortality, resulting in increased production losses in terms of live-weight gain, milk production, off-take rates, and draft power. Herein, we applied a facility-based design to assess the proportion and seasonal distribution of theileriosis, babesiosis, anaplasmosis, and heartwater in cattle from selected smallholder farms in Lusaka and Central provinces of Zambia using laboratory records for microscopic examination of haemoparasites. The overall proportions for theileriosis, babesiosis, and anaplasmosis were 33.7% (95% CI: 30.7–38.2%), 8.3% (95% CI: 6.4–10.9%), and 0.5% (95% CI: 0.1–1.4%), respectively. Heartwater was detected in all the six laboratory records reviewed. Lusaka province had the highest proportion of theileriosis at 30.2% (95% CI: 25.5–35.3%), followed by babesiosis, 8.5% (95% CI: 5.8–11.9%), and anaplasmosis, 0.3% (95% CI: 0.01–1.6%). Similarly, Central province had the highest proportion of theileriosis at 39.5% (95% CI: 33.8–45.4%), followed by babesiosis, 8.4% (95% CI: 5.5–12.2%), and anaplasmosis, 0.7% (95% CI: 0.1–2.5%). Among the districts, Rufunsa district had the highest proportion for theileriosis at 70.6% (95% CI: 44.0–89.7%), followed by Kafue, 54.5% (95% CI: 23.4–83.3%), and Shibuyunji, 51.4% (95% CI: 34.0–68.6%). Analysis of seasonal variation revealed no statistical difference between the dry and wet seasons for theileriosis, babesiosis, and anaplasmosis. Furthermore, there was no statistical difference between Central and Lusaka provinces for babesiosis and anaplasmosis except for theileriosis. These data may have implications on farmers' herd health management strategies with respect to TBDs, and farmers should be sensitized for effective implementation of risk-based disease control.

Keywords: tick-borne diseases, cattle, smallholder farmers

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1 Introduction

Tick-borne diseases (TBDs) are endemic in Zambia and several other Sub-Saharan countries. They affect cattle production by causing high morbidity and mortality, resulting in increased production losses in terms of live-weight gain, milk production, draft power, and off-take rates [1]. The most prevalent tick-borne pathogens in Zambia include *Babesia bovis*, *Babesia bigemina*, *Anaplasma* spp, *Ehrlichia ruminantium, Theileria parva*, *Theileria mutans,* and *Theileria taurotragi* [2]. These pathogens cause major TBDs such as babesiosis, anaplasmosis, heartwater, and theileriosis. Animals infected with *Babesia* parasites often manifest with haemolytic anaemia disease process but signs may vary according to agent and host factors. *Anaplasma* species cause anaemia, jaundice, and sudden death. Heartwater occurs in four different clinical forms (per acute, acute, subacute, and mild) depending on the host susceptibility, agent virulence, and infective dose. *Theileria* infections often show enlarged lymph nodes, fever, a gradually increasing respiratory rate, dyspnoea, and occasional diarrhoea [3]. Among the TBDs, theileriosis accounts for most of the total losses and causes significantly more deaths than the other TBDs combined [4,5].

Cattle is one of the most economically important livestock species. As of 2022, the total Zambian cattle population was estimated to be 4,698,972, and the sector has generally continued to record an upward growth [6,7]. However, the sector's development continues to be threatened by a high burden of TBDs, particularly among Smallholder livestock keepers [8]. Smallholder farming is among the critical sectors that provide families with financial flexibility in several underdeveloped countries. Appallingly, many smallholder farms are poorly resourced and are run by small to medium family farming systems under extensive production management systems, and hence are more vulnerable to the negative impacts of TBDs. Furthermore, the transition by many smallholder farmers, as seen in Zambia and other areas in Sub-Saharan Africa, from rearing indigenous to exotic breeds of cattle and their crosses may result in an upsurge of tick-borne diseases and worsen the situation since the improved livestock breeds tend to be more susceptible to ticks and TBDs [9].

Control of TBDs is mainly through dipping and spraying of animals with acaricides to eliminate the tick vectors. The successful control of tick vectors and tick-borne pathogens require timely and appropriate acaricide application. However, not all farmers dip or spray their animals as required due to various constraints and practices. For instance, some smallholder farmers in Zambia believe that most cases and mortalities from TBDs occur during the wet season and this belief often influences their decision to dip or spray animals [10]. The wet season is also known to have high activity and abundance of tick vectors [11], perhaps the reason why farmers selectively choose to intensify tick-control strategies during the wet season. The dangers of this selective approach to applying tick-control strategies based on seasonality are that animals can potentially remain exposed to TBDs in case of shifts from the known epidemiological conditions. Considering the detrimental impact and economic losses from TBDs, efforts to increase monitoring and surveillance and appropriate acaricide use, improved farmer support services, and sensitization of farmers on tick-control strategies and emerging acaricide resistance are necessary.

This study aimed to assess the proportional differences and seasonal distribution of major TBDs in cattle reared by smallholder farmers in consequence of the adverse impacts of climate change that Zambia has been experiencing, characterized by an increase in frequency and severity of seasonal droughts, occasional dry spells, increased temperatures in valleys, and flash floods. The data generated will provide useful information on the prevailing distributional trends and relative abundance of TBDs in the smallholder cattle system and potentially support the herd health management strategies and risk-based application of control measures.

2 Materials and methods

2.1 Study area and design

A facility-based design was applied in this study, and this allowed us to assess the seasonal and proportional distribution of TBDs of cattle from selected smallholder farms based on the records of laboratory test results archived in the Department of Laboratory and Diagnostics at Livestock Services Cooperative Society (LSCS) in Lusaka (capital city of Zambia). This was an affordable and less time-consuming way of collecting data. LSCS is a large nonprofit-making organization established in Zambia for purposes of increasing access to farming and veterinary services. The Cooperative currently supports many farmers through the provision of laboratory services, technical advisory services, and various farming inputs. Smallholder farmers were included in the study because they were the majority who made sample submissions to the laboratory during the study period.

Ethical approval: The conducted research is not related to either human or animal use.

2.2 Sampling

This was a facility-based retrospective review of laboratory diagnostic data at Livestock Services diagnostic laboratory for a period of 1 year. The inclusion criterion was based on all records with sufficient and valid results for any of the major TBDs in cattle samples from smallholder farms. Therefore, the sample structure used in this analysis comprised 756 purposively selected files of raw datasets of tests conducted between May 2021 and April 2022. In this study, the period from May to October 2021 was categorized as dry season while the period from November 2021 to April 2022 as wet season (https://www.zambiatourism.com/about-zambia/climate/). There were insufficient records from four districts to reliably determine the proportions within this sample set. The data for an additional one district suggested substantial pre-analysis errors, while additional two districts could not be unambiguously resolved due to post-analysis errors. For ehrlichiosis, only six records were available from two districts, probably because of the type of samples required for testing (brain crush smears). These data were excluded from the subsequent main analysis, and ultimately the final dataset analysed comprised 640 test records of cattle from 105 farms in nine districts of Lusaka and Central provinces of Zambia known to have numerous cattle farming activities and hence likely to be good representatives. The nine districts in the study area were Chibombo, Chisamba, Chongwe, Shibuyunji, and Mumbwa in Central province and Lusaka, Rufunsa, Chilanga, and Kafue in Lusaka province (Figure 1). Variables such as breed, age, and sex were not included in this analysis because the data were missing on most records. In terms of population distribution, there were more farms and samples from Chongwe and Chisamba recorded in this study compared to other districts within the study area, likely due to the high population and many cattle farms.

2.3 Laboratory analysis procedures for the data used

The methods and recommendations described in the World Organization for Animal Health terrestrial manual [3]. Diagnosis of theileriosis, babesiosis, and anaplasmosis is principally based on clinical signs, knowledge of disease and vector distribution, and identifying parasites in Giemsa-stained blood smears. Ehrlichiosis is often diagnosed in the brain (cerebrum, cerebellum, or hippocampus) samples at necropsy. As a general practice, however, farmers submitted the blood samples to the laboratory after collection and transportation in a cooler box (except for dried blood smears). For analysis, blood smears were prepared using the wedge or push technique and stained in 10% Giemsa stain for approximately 30 min after fixation in absolute methanol for 1 min. The slides were examined under oil immersion with the ×100 objective lens using a light microscope. The lymph node biopsy smears were stained in the same manner as blood smears to identify theileria parasites by examining multinucleate intra-lymphocytic and extracellular schizonts, a characteristic diagnostic feature of acute infections. Intracellular and free-flying schizonts were detected if present. For all three haemoparasites – theileria, babesia, and anaplasmosis – the piroplasm (intra-erythrocytic stage) was detected if present. Of

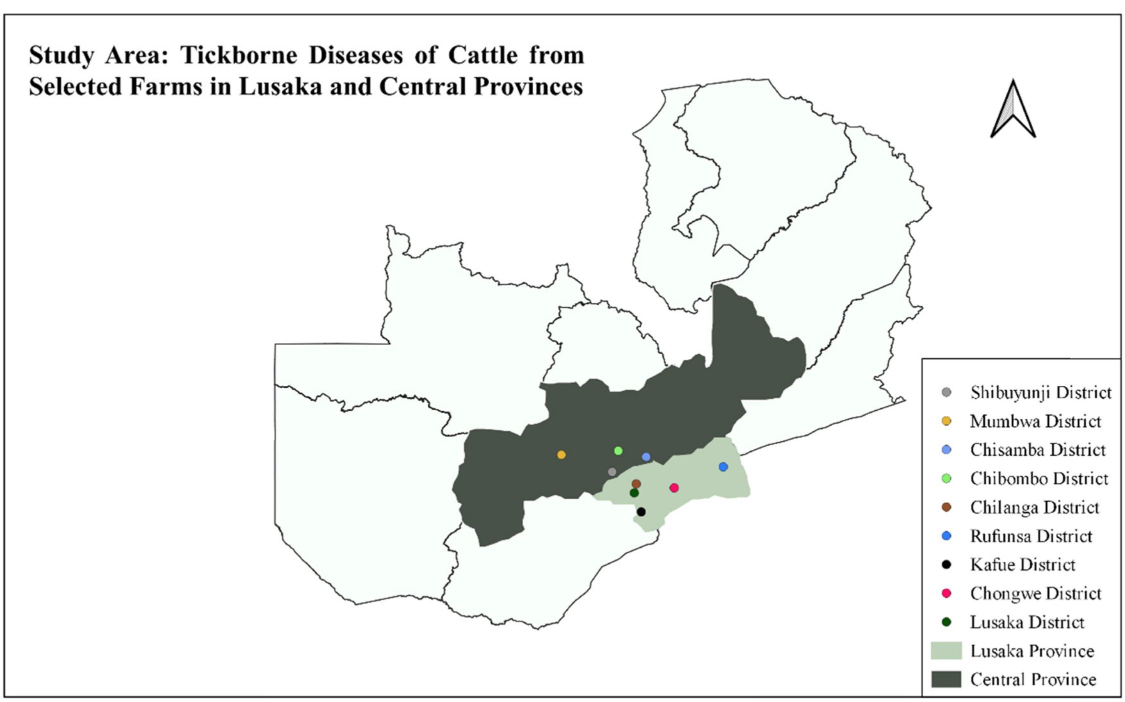

Figure 1: Study area showing districts and location of samples used in the analysis. (Map generated using QGIS Desktop 3.24.1.).

the total smears analysed, only 13 were lymph node smears. For ehrlichiosis, brain crush smears were either submitted by vets/farmers or prepared during necropsy conducted at LSCS. A vascularized portion of the brain (cerebrum) was incised and a brain crush smear was prepared by placing a small piece of the incised brain tissue between two slides. The brain crush smear was air dried, fixed with methanol, and stained with Giemsa stain. Observation of clumps of reddish-purple to blue, coccoid to pleomorphic organisms in the brain's cytoplasm of capillary endothelial cells under a light microscope was considered a positive case for ehrlichiosis.

2.4 Data analysis

Data collected were entered in Excel® (Microsoft Office Plus 2019), cleaned, and organized to generate tables and to perform descriptive analyses using proportions and frequencies. The data were then exported to SPSS (Version 20 IBM Statistics) to test the equality of two proportions. The Chi-Square test for comparing two proportions was used to test the hypothesis of whether differences in proportions were significant, and where this was not suitable, the Fisher's exact test was applied. Following export into SPSS, all cases and summaries of proportion were weighted and compared using the two-sided continuity correction factor to determine the statistical differences at a 95% confidence interval. Graph Pad Prism (Version 9.3.1, 471) was used for the analysis and generating figures for seasonality trends.

3 Results and discussion

The overall proportions for theileriosis, babesiosis, and anaplasmosis were 33.7% (95% CI: 30.7–38.2%), 8.3% (95% CI: 6.4–10.9%), and 0.5% (95% CI: 0.1–1.4%), respectively (Table 1). Lusaka province had the highest proportion of theileriosis at 30.2% (95% CI: 25.5–35.3%), followed by babesiosis, 8.5% (95% CI: 5.8–11.9%), and anaplasmosis, 0.3% (95% CI: 0.01–1.6%). Similarly, Central province had the highest proportion of theileriosis at 39.5% (95% CI: 33.8–45.4%), followed by babesiosis, 8.4% (95% CI: 5.5–12.2%), and anaplasmosis, 0.7% (95% CI: 0.1–2.5%). Among districts, Rufunsa district had the highest proportion for theileriosis at 70.6% (95% CI: 44.0–89.7%), followed by Kafue, 54.5% (95% CI: 23.4–83.3%), and Shibuyunji, 51.4% (95% CI: 34.0–68.6%). The significantly high detection of theileriosis compared to other major TBDs in all the provinces and districts studied was not surprising as theileria pathogens are known to be prevalent in the study area [2]. The areas are also known to have widespread distribution of competent vectors for TBDs due to weak animal movement control strategies. The high proportion of theileriosis in Rufunsa was equally not surprising because the district geographically borders Eastern province, which is known to have a high prevalence of theileriosis in Zambia and uncontrolled movement of cattle [12]. However, the low detection of anaplasmosis may need further explanation because the disease is known to have relatively high occurrence in cattle [13–16]. A probable explanation for the low detection of anaplasmosis is because the disease has a relatively long incubation period and often manifests late following immunosuppression caused by other parasites such as *theileria* (Nick Mchardy, Personal communication). Taken together, Chongwe district had the highest number of farms included in this study which may have somewhat increased the chances of detecting high babesia parasites in this area. Chongwe had 269 samples from 32 farms, while Chisamba had 155 samples from 20 farms. Fewer samples and farms were recorded in Chilanga, Kafue, and Rufunsa. Despite excluding heartwater from further analysis, it suffices to mention that all the six records from the two districts (Chisamba and Chibombo) in Central province had positive cases of heartwater.

Analysis of seasonal variation (Table 2) revealed no statistical differences between the dry and wet seasons for theileriosis, babesiosis, and anaplasmosis. Furthermore, there was no difference between Central and Lusaka provinces for babesiosis and anaplasmosis except for theileriosis ($P = 0.018$). The fact that proportions did not differ between the dry and wet seasons appears to be a major finding in this article because it contrasts the putative seasonal disposition of tick-borne pathogens in Zambia and a common view held by most farmers that cases of TBDs mainly occur during the wet season. This finding could possibly be explicated by the adverse impacts of climate change that Zambia has been experiencing, characterized by an increase in frequency and severity of seasonal droughts, occasional dry spells, increased temperatures in valleys, flash floods, and ecological and other biodiversity factors. This postulation is supported by studies that predicted the impact of climate change on TBD epidemiology in Zambia and few other sub-Saharan African countries [17]. Nevertheless, caution should be applied when interpreting these data because seasonal

Table 1: Proportion of TBDs in Lusaka and Central provinces

Provinces ($n = 2$)	Districts ($n = 9$)	Total no. of farms ($n = 105$)	Total no. of samples ($n = 640$)	Theileriosis		Babesiosis		Anaplasmosis	
				Total no. positive	% Positive	Total no. positive	% Positive	Total no. positive	% Positive
Lusaka	Lusaka	15	52	19	36.5	3	5.8	1	1.9
	Chongwe	32	257	68	26.5	26	10.1	0	0
	Kafue	2	11	6	54.5	0	0	0	0
	Rufunsa	2	17	12	70.6	0	0	0	0
	Chilanga	7	17	2	11.8	1	5.9	0	0
	Total	58	354	107	30.2	30	8.5	1	0.3
Central	Chisamba	20	144	52	36.1	10	6.9	2	1.4
	Chibombo	10	47	13	27.7	2	4.3	0	0
	Mumbwa	10	60	30	50	9	15	0	0
	Shibuyunji	7	35	18	51.4	3	8.6	0	0
	Total	47	286	113	39.5	24	8.4	2	0.7
Overall		105	640	220	33.7	54	8.3	3	0.5

variation could have other influencing factors such as area ecology. Seasonal variation could be real on the river flood plains, where the floods and burning of plains kill most of the vectors, thus reducing the tick burden. Unfortunately, in this study we could not establish whether there had been widespread flooding over the period of investigating or whether farmers burned off plants. The monthly trend analysis (Figure 2) showed increase in theileriosis cases in May and July and during the transition from dry to wet season (October to December). An increase in theileriosis cases during these transition periods could be attributable to stress-related factors during the transition. A single peak for babesiosis was seen during the dry and wet seasons. Conversely, anaplasmosis was flat due to fewer cases.

Smallholder livestock in Zambia plays several socioeconomic roles and contributes approximately 45% to household incomes [18]. The relatively high proportions of TBDs reported in this study show that these diseases are one of the major issues affecting smallholder cattle production in Zambia resulting in significant financial losses. Although data on financial losses attributable to TBDs are lacking in Zambia, evidence from other African countries shows that huge losses are incurred. In Uganda and Tanzania, for example, over USD 1.1 billion and USD 364 million are lost annually due to the TBD complex, respectively [5,19]. The transition by many smallholder farmers, from rearing indigenous to exotic breeds of cattle, may worsen the TBD situation since the improved livestock breeds tend to succumb easily [9]. Exotic cattle breeds have also been found to be significantly more likely to suffer from anaplasmosis [13]. Unfortunately, there is scarcity of data on the TBD incidence in exotic *versus* Zambian breeds and commercial *versus* smallholder.

Table 2: Statistical and proportional (%) analysis of TBDs according to province and season

Variables	*n*	Proportion					
		Theileriosis		Babesiosis		Anaplasmosis	
		Total no. negative	Total no. positive	Total no. negative	Total no. positive	Total no. negative	Total no. positive
Wet season	299	230	111	314	27	298	1
Dry season	341	190	109	272	27	339	2
Total	640	420	220	586	54	637	3
P value		0.340		0.717		0.601	
Lusaka province	354	247	107	324	30	353	1
Central province	286	173	113	262	24	284	2
Total	640	420	220	586	54	637	3
P value		0.018[a]		1.000		0.853	

[a]*P* values are significant; *n* – total number of records. Chi square/Fisher's exact, 95% confidence interval.

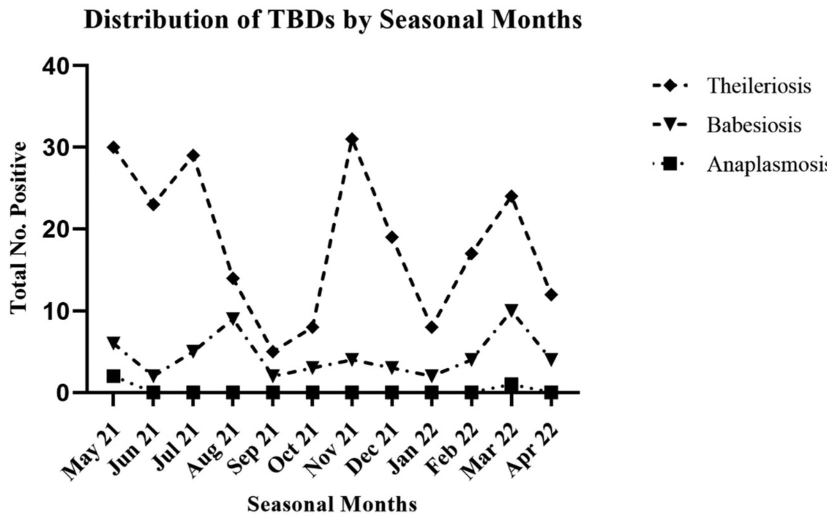

Figure 2: Monthly distribution of TBDs.

However, smallholder farmers in resource-poor settings may benefit more from rearing of indigenous cattle that are more resistant to TBDs, given that some genetic alleles associated with resistance to TBDs such as theileriosis have been found in the Zambian indigenous cattle [20]. Importantly, this study highlights the need for regular surveillance for TBDs and conducting farm-level studies to understand the smallholder farmer's disease-control priorities. As previously observed, farmers take a more holistic approach to animal health when prioritizing diseases, considering mortality, economic impact, risks, and coping mechanisms [21]. This observation is important because disease-control strategies are more likely to fail when disease priorities differ between animal health providers and farmers. Regarding TBDs, it is important to note that a vast majority of farmers have general awareness [22] and hence the need to engage them.

Although our study report provides useful information on the burden of TBDs, the data generated have some limitations. This analysis screened samples from only two provinces of Zambia and did not include variables such as breed, age, and sex because the data were missing in most of the records. We recommend that diagnostic laboratories should improve on how they capture data by including animal characteristics, disease history, and management practices when receiving sample submissions, as such information is important for passive surveillance and in-depth analysis of patterns of disease. For example, a longitudinal study on morbidity and mortality in young stock smallholder dairy cattle in Tanzania based on an analysis of survival times to death indicated that calves less than 6 months of age and those out on pasture had higher death rates than older calves and those kept under zero-grazing systems [15]. Furthermore, since the study was retrospective based on diagnostic data, it could have errors due to low quality control procedures and under-detection of the diseases in the laboratory due to the inherent limitations of using microscopy diagnostic tests. For future studies, we recommend field epidemiological studies and a wider sample distribution by including more provinces and the use of test methods with higher diagnostic indices such as polymerase chain reaction. This study, however, generates hypothesis on seasonal disposition and provides more evidence that the burden of theileriosis among other major TBDs in cattle reared by smallholder farmers in Zambia is still high. These data add to the existing wealth of information for supporting risk-based application of control measures. We further recommend that policymakers consider developing a national dipping strategy towards achieving endemic stability as a sustainable measure for mitigating clinical diseases.

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Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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