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Retrospective analysis of laboratory diagnostic data to assess the prevalence and seasonal variation of *Ehrlichia ruminantium* in commercial farms of Lephalale Municipality, Limpopo, South Africa

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ABSTRACT

Heartwater is an economically important disease of livestock and some wild ruminants in Southern Africa. The study used retrospective laboratory data from Lephalale Veterinary Laboratory, Limpopo to establish the prevalence and seasonal occurrence of heartwater in commercial farms of Lephalale Municipality between 2010 and 2022. A total of 472 brain samples from livestock (cattle, goats, sheep) and game carcasses brought to the laboratory for postmortem examination were subjected to heartwater testing using Giemsa staining technique. The overall prevalence of heartwater in Lephalale Municipality was 34.1% (95% CI = 29.9–38.6, $p < 0.001$). During the years under investigation, there was a significant ($p < 0.001$) variation, with the highest and lowest prevalence in 2022 (72.2%) and 2019 (6.3%), respectively. The prevalence of heartwater was high in springbok (57.1%), sheep (49.3%), cattle (36.6%) and goats (32.2%). Heartwater was detected throughout the year with autumn having the highest (40.6%) frequency followed by summer (40.3%), spring (30.5%) and winter (25.5%). This study is the first to establish the prevalence and seasonality of heartwater in Lephalale Municipality which will serve as a baseline for prevention and control strategies as well as future epidemiological studies. Official surveillance programmes, more research on the distribution and genotypes of *E. ruminantium* in the area need to be undertaken for better understanding of the disease in the area.

1. Introduction

Tickborne diseases (TBDs) and their vectors have a significant global economic impact and severely constrain livestock production globally, especially in Africa (Bishop et al., 2023). The economic and social impact of livestock ticks on resource-poor livestock keepers in Africa is substantial with TBDs being among the most significant and serious challenges to improving livestock production and efficiency (Mukhebi et al., 1999; Dinkisa, 2018; Bishop et al., 2023). Livestock plays a crucial role in poverty alleviation, food security, and livelihoods, and any decline in livestock production affects not only the farmer but also the entire country (Allsopp, 2015; Jongejan et al., 2020; Ngoshe et al., 2022). Improved global control of tickborne diseases would contribute substantially to improved meat and milk production, as failure to keep up with local and international market demands could have far-reaching consequences on the economy and livelihoods of the country's inhabitants.

Heartwater also known as cowdriosis, is a socioeconomically important disease of livestock (sheep, goats, cattle) and some wild ruminants (Allsopp, 2015). The disease is caused by *Ehrlichia ruminantium*, a tiny, intracellular, pleomorphic, gram-negative cocci bacteria of the order *Rickettsiales* and family *Anaplasmataceae*. It is transmitted by members of the tick genus *Amblyomma*, principally *A. hebraeum* in Southern Africa and *A. variegatum* throughout much of the rest of Sub-Saharan Africa and on several islands in the Caribbean (Peter et al., 1998). Ticks become infected with *E. ruminantium* while feeding on

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infected animals and remain infectious for their entire lifespan (Bath et al., 2005). However, transovarial transmission can also occur (Bezuidenhout and Jacobsz, 1986).

Heartwater is regarded as an important disease in South Africa (van den Heever et al., 2022). *Amblyomma hebraeum* has been reported to occur in Limpopo, North-West, Gauteng, Free State, Mpumalanga, the coastal regions of KwaZulu-Natal and the west of the Eastern Cape provinces (Pfitzer et al., 2004; Makwarela et al., 2023). This disease is characterised by high mortalities (up to 90%) in all susceptible domestic ruminants (WOAH, 2018) with the estimated costs at R1 266 million per annum in South African livestock industry (van den Heever et al., 2022). The direct costs contribute 66.47%, while indirect costs contribute 33.57% to the total cost of heartwater (van den Heever et al., 2022). Heartwater is prevalent in extended areas of the north and eastern parts of South Africa, with estimates that 35%, 54%, and 12% of total cattle, goat, and sheep populations, respectively, are at risk of infection (Leask and Bath, 2020).

Different forms (per acute to subclinical) of the disease have been described which may depend on factors including age and breed of animal, severity of the tick challenge and the virulence of the genotype of the *E. ruminantium* strain involved (Allsopp, 2015). The characteristic clinical signs of heartwater in domestic animals includes: nervous signs, respiratory distress, hanging head, stiff gait, exaggerated blinking and chewing movements, hyperaesthesia, lacrimation, convulsions and recumbency (Van de Pypekamp and Prozesky, 1987; Allsopp, 2015). In South Africa, Angora goats are particularly susceptible to heartwater which places a severe economic constraint on mohair producers of the Eastern Cape province (Latif et al., 2020).

The diagnosis of heartwater is primarily based on clinical, necropsy findings and detection of *E. ruminantium* colonies in the brain smear. The detection by Polymerase Chain Reaction (PCR) based assays in necropsy tissues or blood samples collected from the febrile stage of the diseased animals has been reported (Steyn et al., 2008).

Traditional methods for the control of heartwater involves the use of acaricides and vaccination. However, the rising costs of these approaches have posed challenges for both farmers and government institutions (Mukhebi et al., 1999). Furthermore, the emergence of tick resistance to acaricides is a growing global concern. In addition, tick free, healthy treated stock may serve as a disease reservoir which may remain infective for up to 12 months (Allsopp, 2015). A commercial heartwater vaccine that involves infecting animals with *E. ruminantium* live strain and subsequently treating them with antibiotics is available in South Africa. However, it is important to note that this vaccine does not provide protection against all *E. ruminantium* field strains. Moreover, revaccination is considered risky due to the potential for anaphylactic reactions.

Heartwater has been extensively researched in various regions of South Africa. It is prevalent in the northeastern parts of the country, ranging from the northeast area of North-West province, across Limpopo and the north-eastern regions of Mpumalanga, along the coastal zones of KwaZulu-Natal an Eastern Cape provinces (Allsopp et al., 2004; Leask and Bath, 2020). However, in the recent years, there has been numerous heartwater suspected cases and deaths of livestock reported in the Lephalale municipality (Limpopo Department of Agriculture and Rural Development, Veterinary Services, unpublished data). Despite the high number of livestock cases and deaths, there has been a notable absence of efforts to investigate their cause and the epidemiology of this disease in the area. Additionally, there is currently no official surveillance programs in place to address this problem.

The study will use retrospective laboratory data from Lephalale Veterinary Laboratory between 2010 and 2022 to establish the prevalence and seasonal occurrence of heartwater in commercial farms of Lephalale municipality. The study will also serve as a baseline for prevention, and control strategies as well as future epidemiological studies.

2. Materials and methods

2.1. Study area and design

The study used a facility-based cross-sectional design to investigate the prevalence of heartwater in commercial farms within the Lephalale Municipality, located in Limpopo province, South Africa. The assessment relied on the examination of laboratory test records archived at the Lephalale Provincial Veterinary Laboratory. This methodology was selected for its cost-effectiveness and efficiency in data collection. The Lephalale Veterinary Laboratory, a government-run facility, currently plays a pivotal role in supporting numerous farmers through the provision of laboratory services, technical advisory assistance, and diverse farming inputs. The selection of commercial farms for the study was deliberate as they constituted most sample submissions to the laboratory during the study period spanning from 2010 to 2022.

2.2. Data source

This facility-based study conducted a retrospective and longitudinal analysis of laboratory diagnostic data obtained from carcass samples, routinely received at the Lephalale Provincial Veterinary Laboratory over a period of 12 years. The inclusion criterion encompassed all records with sufficient and valid results for heartwater in commercial farms within the Lephalale Municipality. Consequently, the sample structure for this analysis consisted of 472 purposively selected files containing raw datasets from tests conducted between January 2010 and December 2022. Animals with the clinical history indicative of heartwater and postmortem findings such as hydropericardium, hydrothorax, and lung oedema were tested for the presence of *E. ruminantium* on brain smear.

Lephalale Municipality is divided into 15 administrative wards which consist of residential areas (villages, town, and townships), commercial and communal farms. Lephalale Municipality has approximately 518 farms (Limpopo Department of Agriculture and Rural Development, Veterinary Services, unpublished data). As a result, the dataset used in this study represented a diverse range of animal species, including domestic and wildlife. Notably, variables such as breed, age, and sex were excluded from the analysis due to their absence in a significant number of records. For this study, the summer period ranged from December to February, while the autumn season covered the period from March to May, June to August was categorized as the winter season, and the period from September to November as the spring season.

2.3. Laboratory diagnosis

The examination of *E. ruminantium* in brain (cerebrum, cerebellum, or hippocampus) tissues adhered to the methods and recommendations by the World Organization for Animal Health (WOAH) terrestrial manual, (2018). Brain smears, prepared during post-mortems conducted at the laboratory, followed a laboratory standardized operation procedure. In summary, a vascularized portion of the brain (cerebrum) was incised, and a brain crush smear was made by placing a small piece of the incised brain tissue between two slides. The resulting smear was air-dried, fixed with methanol, and stained with Giemsa stain. Smears were then observed under the light microscope for *E. ruminantium* colonies. Positive colonies of *E. ruminantium* (clusters) appeared reddish-purple to blue in the capillary endothelial cells of the brain.

2.4. Statistical analysis

The data was entered and analysed using the Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM SPSS, 2021) to generate the descriptive statistics such as frequencies and prevalences of heartwater. Pearson's chi-square ($\chi 2$) test was used to test for association between prevalences and potential risk factors. The magnitude of effects of potential risk factors on the prevalence were analysed using binary logistic regression method. Variables considered for the multivariable model were the year, animal species and seasonality. A *p*-value $\leq$0.05 was considered statistically significant and the 95% confidence intervals from 1000 bootstrap samples were determined.

2.5. Ethics statement

A permission to use the laboratory diagnostic results data was granted by the Limpopo Department of Agriculture and Rural Development Director of Veterinary Services. These were results of animal samples or specimens submitted to the laboratories for diagnostic purposes.

3. Results

A total of 472 brain samples were collected during postmortem examinations and tested for heartwater using Giemsa staining method as described in the methodology. Samples were collected from cattle (123), sheep (146), goats (115), buffaloes (5), wildebeest (1), zebra (2), and a variety of antelopes including blesbok (1), bushbuck (2), eland (7), gemsbok (4), impala (8), kudu (4), nyala (15), roan (4), sable (28), and springbok (7). The overall prevalence of heartwater in commercial farms of Lephalale Municipality over a 12-year period was 34,1% (95% Cl: 29.9–38.6). Table 1 shows the distribution of heartwater according to years in which a statistically significant difference was observed in different years ($p < 0.001$). The highest prevalence was recorded in 2022 (72.2%) while the lowest was recorded in 2019 (6.3%). A statistically significant difference was observed on the prevalence of heartwater among species of animals ($p < 0.001$) (Table 2). The highest prevalence was recorded in springbok (57.1%), sheep (49.3%), cattle (36.6%) and goats (32.2%), while the lowest was recorded in sable (3.6%). Furthermore, it was observed that no heartwater was detected in wildebeest, buffaloes, zebra, blesbok, buffalo, bushbuck, eland, kudu, nyala and roan. The overall prevalence of heartwater was significantly higher ($p < 0.001$) in domestic animals (32.6%) compared to wild animals (1.5%). The prevalence of heartwater varied significantly ($p < 0.001$) according to seasonality, with the highest prevalence recorded in autumn (40.6%) while the lowest (25.5%) was registered in winter (Table 3). The multivariable regression analysis (Table 4) shows the potential risk factors (year, species, and seasonality) on heartwater prevalence. From this table it can be observed that the risk of heartwater occurrence were relatively higher during the year 2016, (OR = 1.03, 95% CI = 0.47–2.28, $p = 0.937$); 2021 (OR = 1.31, 95% CI = 0.49–3.45, $p = 0.588$) and 2022 (OR = 3.40, 95% CI = 1.12–10.34, $p = 0.031$). However, the OR was only statistically significant for the year 2022. For animal species, the risk of heartwater was associated with springbok (OR = 2.31, 95% CI = 0.49–10.79, $p = 0.287$) and sheep (OR = 1.67, 95% CI = 1.03–2.75, $p = 0.036$). For seasonality, the risk of heartwater occurrence was high in autumn (OR = 1.97, 95% CI = 1.12–3.49, $p = < 0.019$) and spring (OR = 1.99, 95% CI = 1.11–3.58, $p = < 0.020$) than in winter (OR = 1.28, 95% CI = 0.73–2.26, $p = < 0.386$).

4. Discussion

The current study was conducted to assess the prevalence and seasonal occurrence of heartwater in commercial farms of Lephalale Municipality based on the laboratory test (brain smear) results. The overall prevalence of heartwater between 2010 and 2022, was found to be 34.1% in both domestic and wild animals. The low prevalence in this study may be attributed to the fact that animals used in this study were brought to the laboratory for routine post-mortem examination and were diagnosed for diseases other than heartwater. Moreover, it may be due to the low diagnostic sensitivity of the Giemsa-smears. Therefore, other sensitive diagnostic methods such as PCR may be used in conjunction with Giemsa-smear for confirmation in the future. It is also important to note that very few farmers confirm suspected cases of heartwater by means of laboratory testing which could underestimate the true reflection of the prevalence of this disease in the field (Leask and Bath, 2020). The prevalence was higher in domestic animals than wild animals, which is to be expected since it is documented that domestic animals (goats, sheep, and cattle) are more susceptible to heartwater than wildlife (Allsopp, 2010). The susceptibility of wild animals to *E. ruminantium* infections is quite diverse. Deaths due to infection with *E. ruminantium* have been reported in blue wildebeest, springbok, bushbuck, black wildebeest, blesbok, steenbok, sitatunga and eland (Peter et al., 2002). Some wild animals such as African buffalo, eland, kudu, blue wildebeest, giraffe, sable and tsessebe are reported to be subclinical carriers or reservoirs of the heartwater parasites (Pfitzer et al., 2004). The findings of this study corroborate the previous reports since most positive cases in wild animal were detected in springbok, and few in impala, gemsbok and blesbok.

The epidemiology of heartwater depends upon many interacting circumstances which includes among others the vertebrate hosts, the tick vectors, and suitable environmental conditions. The distribution of *Amblyoma hebraeum* occurs across the Limpopo province which could suggest that the actual *E. ruminantium* infections in both domestic and wild animals in the field may be higher than reported in this study, since only clinical disease or fatalities are normally reported by the farmers. (Allsopp et al., 2004; Uys and Horak, 2005; Schroder et al., 2006; Jongejan et al., 2020; Makwarela et al., 2023).

However, the discovery of 25 *E. ruminantium* genotypes (Allsopp et al., 2004; Steyn and Pretorius, 2020), which differ in infectivity and virulence (Pfitzer et al., 2004; Allsopp et al., 2007), suggests that the presence of *A. hebraeum* alone may not inherently result in infection,

Table 1
Distribution of heartwater prevalence according to years.

Year	Samples tested	No. of positive samples	Prevalence (%)	95% Cl	$\chi 2$	*df*	*P*-value
2010	90	39	43.3%	33–54	37.63	12	<0.001
2011	58	19	32.8%	21–45			
2012	48	9	18.8%	8–29			
2013	50	10	20.0%	10–33			
2014	44	10	22.7%	11–35			
2015	44	18	40.9%	26–56			
2016	34	15	44.1%	27–61			
2017	17	5	29.4%	8–55			
2018	18	7	38.9%	17–62			
2019	16	1	6.3%	0.0–21			
2020	15	5	33.3%	11–59			
2021	20	10	50.0%	27–73			
2022	18	13	72.2%	50–92			
Total	**472**	**161**	**34.1%**	**29.9–38.6**			

CI: Confidence intervals based on 1000 bootstrap samples.

Table 2
Distribution of heartwater prevalence by species.

Species	Samples tested	No. of positive samples	Prevalence (%)	95% CI	χ2	df	P-value
Bovine	123	45	36.6%	28–45	51.85	7	<0.001
Caprine	115	37	32.2%	24–41			
Gemsbok	4	1	25.0%	0.0–75			
Impala	8	1	12.5%	0.0–43			
Ovine	146	72	49.3%	41–58			
Sable	28	1	3.6%	0.0–12			
Springbok	7	4	57.1%	20–100			
Other game[a]	41	0	0.0%	ND			
Total	**472**	**161**	**34.1%**	**29.9–38.6**			

CI: Confidence intervals based on 1000 bootstrap samples. ND: Not done.
[a] Other game = blesbok, buffalo, bushbuck, wildebeest, eland, kudu, Nyala, roan, zebra.

Table 3
Distribution of heartwater prevalence by season.

Seasons	Samples tested	No. of positive samples	Prevalence (%)	95% CI	χ2	df	P-value
Summer	119	48	40.3%	31–50	8.357	3	0.001
Autumn	106	43	40.6%	32–50			
Winter	106	27	25.5%	17–34			
Spring	141	43	30.5%	22–39			
Total	**472**	**161**	**34.1%**	**29.9–38.6**			

CI: Confidence intervals based on 1000 bootstrap samples.

Table 4
Logistic regression analysis of potential risk factors on the prevalence of heartwater.

Variables	Category	OR	95% CI for OR	p-value
Year	2010	Ref	–	–
	2011	0.64	0.32–1.27	0.200
	2012	0.30	0.13–0.69	0.005
	2013	0.33	0.15–0.73	0.007
	2014	0.38	0.17–0.87	0.022
	2015	0.90	0.44–1.88	0.790
	2016	1.03	0.47–2.28	0.937
	2017	0.54	0.18–1.67	0.289
	2018	0.83	0.29–2.34	0.728
	2019	0.08	0.01–0.69	0.021
	2020	0.65	0.21–2.06	0.471
	2021	1.31	0.49–3.45	0.588
	2022	3.40	1.12–10.34	0.031
Species	Bovine	Ref	–	–
	Ovine	1.67	1.03–2.75	0.036
	Caprine	0.82	0.48–1.41	0.474
	Springbok	2.31	0.49–10.79	0.287
	Impala	0.25	0.03–2.08	0.198
	Gemsbok	0.58	0.06–5.72	0.639
	Sable	0.06	0.01–0.49	0.008
	Other game	ND	–	–
Seasonality	Winter	Ref	–	–
	Summer	1.97	1.12–3.49	<0.019
	Autumn	1.99	1.11–3.58	<0.020
	Spring	1.28	0.73–2.26	<0.386

OR: Odds Ratio; CI: Confidence intervals. ND: Not done.

unless specific, virulent genotypes are involved. This highlights a knowledge gap, emphasizing the need for further research on disease prevalence, the distribution of the vectors and the *E. ruminantium* genotypes currently circulating in the Lephalale area, to gain a more comprehensive understanding of this disease.

During the years under investigation, heartwater detection in commercial farms of the Lephalale Municipality varied, with the highest and lowest prevalence recorded in 2022 and 2019, respectively. The decline or low prevalence of this disease during the years under scrutiny, particularly from 2017 to 2020, can likely be attributed to the COVID-19 pandemic, which significantly hampered the functionality of laboratories. Additionally, the pandemic drastically altered the lives of communities, making it challenging to maintain proper farm management and animal husbandry practices. This shift in focus and resources towards managing the pandemic itself further contributed to the decreased attention on controlling the disease. According to previous reports (Bath et al., 2005; Allsopp, 2010), heartwater infestation depends on many factors including the presence and activity of a vector as well as susceptible hosts. Since *A. hebraeum* is a three-host tick, it is expected to take longer (up to 12 and 36 months) to mature under favourable and unfavourable conditions, respectively. Furthermore, if the disease is not transovarially acquired, ticks must then acquire the disease from infected hosts which can only be passed onto a new host in the next active stage (Nyangiwe et al., 2018). In a study conducted by Norval in the early 1970s, it was established that it is highly unlikely for a matured female *A. hebraeum* tick to attach to the host unless a sexually matured male has attached. The author further observed the inability of an unfed adult male to attract a female adult for copulation, an important part of the tick's life cycle. This shows that there is more to heartwater transmission than just the host-vector presence in the area. Furthermore, according to Allsopp (2010), ticks in the heartwater endemic areas exhibit surprisingly low infection rates with *E. ruminantium*. The author further proposed that during the larval or nymphal stages, numerous ticks may be feeding on non-susceptible or non-infected hosts. Therefore, the low detection during these years may be attributed to the absence of infective and sexually matured ticks as well as susceptible hosts. However, the possibility of non-reporting, heartwater control measures, host resistance and meteorological factors cannot be ruled out.

Heartwater is a disease of domestic and some wild ruminants. In the current study, detection of heartwater by species was high in springbok. The total prevalence of wild ruminants was low compared to that of domestic ruminants, which was as expected. No disease was detected in blesbok, wildebeest, buffalo, eland, zebra, bushbuck, kudu, nyala and roan. These results are in correlation with the previous studies (Neitz, 1935; Oberem and Bezuidenhout, 1987; Andrew and Norval, 1989; Peter et al., 1998; Wesonga et al., 2001; Mapham and Vorster, 2017) in South Africa which reported similar wild ruminants as asymptomatic careers and important sources for the spread of heartwater. Sheep and goats are more susceptible to heartwater than cattle depending on the variation in breed (Bath et al., 2005). In our study, heartwater detection was high in sheep (49.3%) followed by cattle (36.6%) and goats (32.2%). The detection of heartwater in cattle was 4% more, compared to goats. According to previous studies (Horak et al., 1987; Schroder and Reilly, 2013), large host species have greater chances of harbouring large numbers of adult ticks, including *A. hebraeum* which might be the reason for the higher prevalence in cattle than goats. On the other hand, Andrew and Norval (1989) proposed that new introductions of susceptible cattle in the area as contribute to mortality, which might have been the case in this study. However, age and breed may have played a role in this species.

According to Jongejan et al. (2020), all stages of *A. hebraeum* development can often be found on hosts throughout the year. This pattern aligns with the findings of the current study, where heartwater was detected across all seasons. The tick population in an area is heavily influenced by temperature and humidity (Cumming, 2002; Allsopp, 2010). In the current study, the prevalence of heartwater by season was high in autumn (40.6%), followed by summer (40.3%) and spring (30.5%), while the lowest prevalence was recorded in winter (25.5%). Lephalale Municipality is a semi-arid area with average daily temperatures varying between 17 °C and 32 °C in summer and between 4 °C and 20 °C in winter. Furthermore, most of the precipitation falls between October and May, while little rain occurs between April and September (Mangani et al., 2020). These climate conditions support the development and maintenance of *A. hebraeum* in the area. The result of the current study is in correlation with a study conducted by Bryson et al. (2000) and Schroder and Reilly (2013) where they also established high and low *A. hebraeum* abundance and activity in the warmer and cooler temperatures, respectively. The current study has identified sheep, sable, autumn, and spring as the potential risk factors. However other factors such as age, sex, breed of animals could have added more valuable information to the study.

5. Conclusion

Heartwater is an economically important disease in Southern Africa. This study has established the prevalence and seasonality of heartwater in the commercial farms of the Lephalale Municipality. This information will form a baseline for future epidemiological studies. Furthermore, the current study has also highlighted the knowledge gaps in terms of the epidemiology of the disease, especially the host factors, the distribution of the vector and the *E. ruminantium* genotypes circulating in the area. Moreover, the study has also identified potential risk factors which may be used in disease control strategies. Therefore, authors recommend that more research should be conducted in the area to have a better understanding of this disease. The authors further recommend that official surveillance programmes be put in place to guide the prevention and control measures in the area.

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Animal welfare statement

No ethical permissions were necessary for this study as the samples were collected from specimens or carcasses sent in for routine veterinary diagnostics.

CRediT authorship contribution statement

Emmanuel Seakamela: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Itumeleng Matle:** Writing – review & editing, Investigation. **Nandipha Ndudane:** Writing – review & editing, Conceptualization. **Relebohile Lepheana:** Writing – review & editing, Conceptualization. **Sikhumbuzo Mbizeni:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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