Veterinary Parasitology: Regional Studies and Reports 43 (2023) 100902

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Regional report

Heartwater: A situation report of the Southern district of Botswana from 2017 to 2019

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ARTICLE INFO

Keywords:
Heartwater
Amblyomma hebraeum
Ehrlichia ruminantium
Botswana
Tick-borne disease
Management

ABSTRACT

Heartwater is a tick-borne haemoparasitic disease that can limit agro-business expansion in Botswana. It poses a threat to national food security due to low animal production as well as livestock morbidity and mortality. This report gives a snapshot view of heartwater in the Southern district of Botswana. Ixodid ticks parasitizing livestock in four Southern sub-districts of Botswana were collected and identified using morphological and molecular methods. A wide distribution of *Amblyomma hebraeum* in all four Southern sub-districts was revealed. The annual number of heartwater cases across the Southern district of Botswana was determined from veterinary clinical case reports and confirmed through Giemsa-stained brain smears. A concerning gradual annual increase in heartwater cases was shown in the Moshupa sub-district - a hardveld terrain with rock outcrops where the vector thrives. Goats were affected most (55%) by heartwater followed by sheep (37%) and then cattle (8%). Farmers were interviewed on the management of the heartwater burden within their respective sub-districts and they reported that their animals were affected by heartwater despite 17 out of the 27 farmers interviewed attempting to control vectors through acaricide use. The presented heartwater situation warrants further investigation of the prevalence of heartwater and the effectiveness of existing disease control interventions in the disease-endemic Southern district of Botswana.

1. Background

Heartwater is a notifiable disease in Botswana and it is caused by an intracellular bacterium, *Ehrlichia ruminantium*, which belongs to the Rickettsiaceae family (Thomas, 2016). *Ehrlichia ruminantium* can parasitize *Amblyomma hebraeum,* and the vector can drive transmission of the disease in the presence of its wide range of domestic and wild ruminant hosts. Heartwater is a major threat to national food security in agro-established countries in the tropics and sub-tropics (Mahabile et al., 2002). In Southern Africa, it has been reported that approximately 90% of stock losses can occur due to untreated heartwater cases alone (Bath and Leask, 2020). Consequently, it is vital to manage this economic livestock disease. Over the last 50 years, heartwater has been managed with preventative measures such as tick eradication programs, vaccination with a live blood vaccine followed by antibiotic administration.

Botswana has a long history of both arable and pastoral farming. In Botswana, subsistence pastoral farmers can purchase small-stock (goats and sheep) to improve their livelihoods through funds from poverty eradication programmes (Sharma, 2014). In order to support this initiative, there is significant movement of small stock around the country. However, the movement of any livestock, regardless of the reason, poses a threat of introduction or spread of heartwater through infected animals and the disease-transmitting three-host ticks.

Ixodid ticks such as *Amblyomma* spp., *Rhipicephalus* spp., and *Hyalomma* spp. have previously been reported in Botswana (Paine, 1982). *Amblyomma variegatum* (Raboloko et al., 2020) and *Amblyomma hebraeum (*Walker et al., *1978)* have both been reported to transmit heartwater in Botswana. *Amblyomma hebraeum* in particular as a vector of heartwater has specifically been reported in the south-east of Botswana (Paine, 1982), although the presence of the disease in the Southern district has not been formally reported.

Tick distribution is governed by aspects such as the presence of

Abbreviations: DVS, Department of Veterinary Services; BNVL, Botswana National Veterinary Laboratory; GPS, Global positioning system; COI, Cytochrome c oxidase I; DNA, Deoxyribonucleic acid; PCR, Polymerase chain reaction.

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https://doi.org/10.1016/j.vprsr.2023.100902
Received 16 August 2021; Received in revised form 1 May 2023; Accepted 16 June 2023
Available online 17 June 2023
2405-9390/

suitable hosts, temperature, humidity and vegetation cover. Heavy tick infestation in Botswana is generally seen in the months of November to March, which constitute the rainy season with relatively warm weather. Mitigation of heartwater transmission can be done through use of acaricides to control tick populations (Allsopp, 2009). In Botswana, there is no formal tick control policy (Fanikiso and Ndzinge, 1992); instead, farmers are responsible for effectively balancing disease control methods to support growth of the agro-economy. Botswana farmers succeed in this endeavor with the support of veterinary extension officers.

The work described herein aimed to report the current (2017 to 2019) situation of heartwater in the endemic Southern district of Botswana. We report; the distribution of ticks that potentially carry *Ehrlichia ruminantium* in the established heartwater-endemic district, the annual clinical cases of heartwater, the different types of livestock affected by heartwater, the tick control strategies used by farmers across the Southern district of Botswana.

2. Materials and methods

2.1. Study context

Ticks and clinical heartwater reports were collected with permission from the Department of Veterinary Services (DVS) in the Southern district of Botswana. The district is divided into four sub-districts i.e., Kanye, Jwaneng, Moshupa, and Good Hope. Each sub-district serves as a livestock advisory center for surrounding villages and towns. Each center has an established veterinary office with three staff members - a veterinary officer and two veterinary assistants attached to the office. These offices located in various sub-districts report to the district DVS headquarters based in Kanye.

2.2. Case reports

The Department of Veterinary Services provided 326 clinical case reports from 2017 to 2019 that were used to compile the data. The 301/326 heartwater reports that were evaluated all included DVS veterinary officials' descriptions of the symptoms and confirmed diagnosis through gross pathology and presence of *Ehrlichia ruminantium* on post-mortem, Giemsa-stained brain smears performed by the Botswana National Veterinary Laboratory (BNVL).

2.3. Animals for tick collection

A total of 391 ruminants (60 cows, 234 goats, and 97 sheep) were purposively selected for tick collection from 27 purposively selected subsistence farms with no particular regard to a specific age, sex, or breed. The selected farms had animal numbersranging from 29 to 140. All the animals at each farm were sampled for ticks. The locations of the farms were mapped using global positioning system (GPS) coordinates and are shown in Fig. 1. The sampling sites are divided into sub-districts as follows: Moshupa crush includes Mokwenatlhogo and Makudu crushes, Kanye crush includes Mathethe, Molapowabojang, and Rakololo crushes, Good Hope crush includes Digawana and Gatwana crushes, and Jwaneng crush includes five (5) farms around the township of Jwaneng. The map was created using free and open-source QGIS 3.0.

2.4. Tick collection and morphological identification

Ticks were hand-picked from under the tails, around the anus, on belly or groin regions, and between hooves of purposively selected livestock. Ticks were collected during the summer months (November–March) of 2017, 2018, and 2019. Ticks were carefully transferred into labeled 2 mL tubes and kept in a labeled Ziploc bag at room temperature until refrigeration at the end of the sampling day. Ziploc bags were labeled with the sub-district, site coordinates, and date of collection. The ticks collected were morphologically identified using a Leica M205 C microscope (Leica Microsystems Ltd., Switzerland), which had a Leica MC190 HD camera connected to it. Key features used for identification were adopted from guidelines provided by Walker (Walker, 2003). Molecular analysis was done to confirm morphological identification.

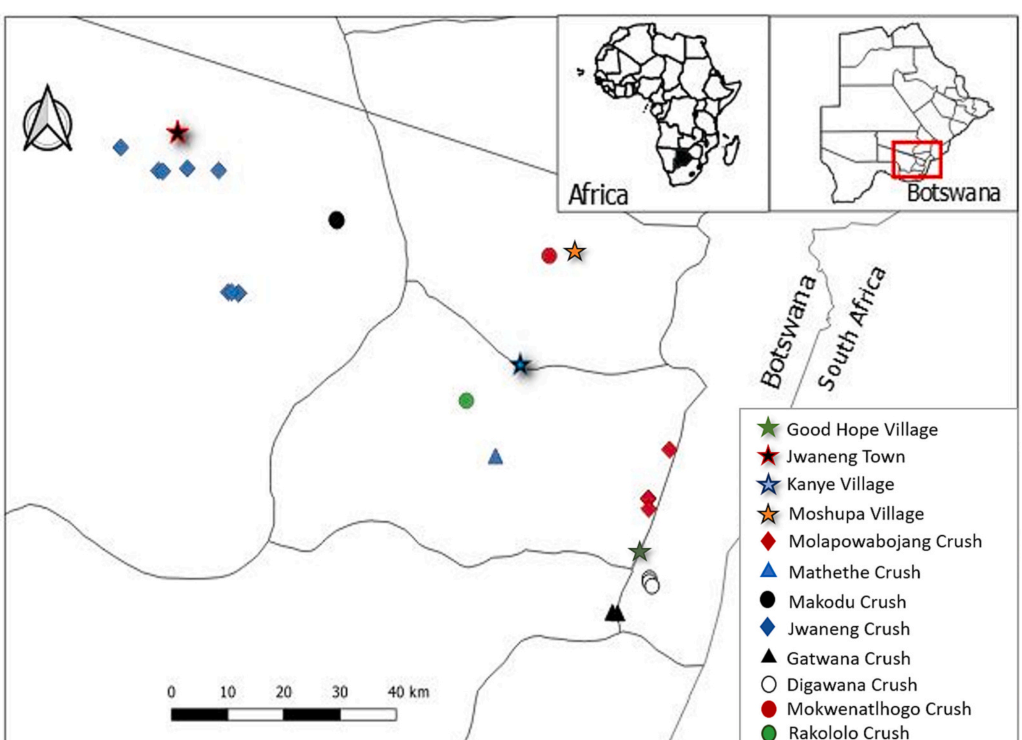

Fig. 1. Study area map indicating sample sites within four (4) sub-district of the heartwater-endemic Southern district, Botswana.

2.5. Molecular identification of ticks

▪ Tick genomic deoxyribonucleic acid (DNA) extraction

Ticks were incubated in 1 mL of Quick-DNA Tissue/Insect Miniprep Kit lysis buffer at 56 °C overnight. Tick tissue was dash frozen with liquid nitrogen and ground using a pestle and mortar. Tick tissue was then transferred to bashing tubes from the Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA). DNA extraction was then undertaken according to the manufacturer's instructions. Genomic DNA was eluted using 25 μL nuclease-free water.

▪ Polymerase Chain Reaction (PCR)

A fragment of the mitochondrial marker cytochrome *c* oxidase subunit I (COI) was amplified using PCR using universal primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATT GG-3′) and HCO 2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3) described by Folmer (Folmer et al., 1994) to yield a 710 bp amplicon. 25 μL PCR reaction samples were prepared in PCR vial tubes. Each reaction comprised of 12.5 μL of Q5® High-Fidelity 2× Master Mix PCR Master Mix (Thermo Fisher Scientific, USA), 6.5 μL of nuclease-free water (VWR International LLC, USA), 1 μL each of 10 μM forward and reverse primers and 2 μL of extracted genomic DNA. PCR conditions were as follows; initial denaturation at 98 °C for 30 s, followed by 35 cycles of; denaturation at 98 °C for 10 s, annealing at 48 °C for 30 s, and extension at 72 °C for 30 s; this was followed by a final extension step at 72 °C for 7 min. The reaction was then stopped at 4 °C. The presence of amplicons in PCR products was verified through agarose gel electrophoresis. PCR products were electrophoresed on 1% agarose gel at 120 V for 90 min. The agarose gel was stained with ethidium bromide to visualize the amplicons. PCR products were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

▪ Sequencing

Bidirectional, Sanger sequencing of the partial COI gene was performed at Inqaba Biotechnical Industries (Pretoria, South Africa) and the contiguous sequences were analyzed using the basic local alignment search tool (BLAST) to confirm matches with submissions made to the NCBI Genbank.

2.6. Questionnaire survey

Questionnaires were administered to 27 farmers or farm staff in November 2018. The questionnaire (Additional file 1) had 17 questions with open and closed questions that generally covered aspects relating to heartwater presence, impact, control, and management. The interview was translated into Setswana by the assisting veterinary officer, and the responses were recorded in English. Descriptive statistics were computed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

3. Results

3.1. Diversity and geographical distribution of tick species

Three hundred and ten feeding ticks belonging to three tick genera namely, *Amblyomma* (262), *Hyalomma* (10), and *Rhipicephalus* (38) were found to parasitize livestock (90 ticks on cattle, 25 ticks on sheep and 195 ticks on goats) in the Southern district of Botswana. Out of the total ticks found, most ticks were identified as *Amblyomma hebraeum,* and this tick species was found in all four sub-districts of the Southern district of Botswana. The other ticks identified were *Rhipicephalus evertsi mimeticus* (34), *Rhipicephalus evertsi evertsi* (4), and *Hyalomma truncatum* (10).

Molecular characterization of *Amblyomma hebraeum* by PCR and sequencing revealed that the partial COI sequences had 99.56% sequence identity to GenBank: KY457512.1, and these results confirmed the morphological identification. Likewise, the molecular identity of the other ticks (*Rhipicephalus evertsi mimeticus, Rhipicephalus evertsi evertsi,* and *Hyalomma truncatum*) concurred with the outcomes of the morphological identification. The partial COI sequences matched GenBank submissions MF425990.1 (99.41%), MK551208.1 (99.26%) and KT999717 (99.41%) respectively.

3.2. Heartwater cases

The analysis of the 301 heartwater cases collected from the Veterinary Service Centers of four sub-districts showed the highest annual number of confirmed disease in Kanye in 2017 (53%). In 2018 and 2019, the highest annual number of confirmed heartwater cases were reported in Moshupa (Fig. 2). Over the three-year study period, Moshupa, had both the highest number of heartwater vectors (Table 1), and the most heartwater cases. The general trend of heartwater cases over the study period showed a gradual increase in Moshupa, while there was a gradual decrease observed in Good Hope (Fig. 2), where the least number of ticks was recorded (see Table 1 and Table 2). Over the three-year period and across all 4 sub-districts, small stock - caprine and ovine species - were most affected by heartwater (Table 2) with 55.6% and 36.2% of total cases reported for each species respectively. Cattle were the least affected at 8.2% of total cases.

3.3. Livestock breeds affected by heartwater

Heartwater cases were reported in indigenous (Tswana breed), exotic (Boer goat, Kalahari red goat, Dorper sheep, Damara sheep, Brahman cattle, Seitz cattle & Simmental cattle) and crossbred livestock belonging to three species, that is, caprine, ovine and bovine (Fig. 3).

3.4. Farmer's Questionnaire: vector control and disease management by subsistence farmers

Having determined the types of ticks present, their distribution, and the annual level of disease detection in the Southern district of Botswana, we sought to determine the impact of heartwater on subsistence farmers and to determine the disease management practices of the farmers. Almost 60% of the interviewed farmers (16/27) reported that heartwater is a disease that afflicts their livestock as they reported that they have lost livestock to the disease. However, 8 out of 27 were able to identify high stepping and respiratory distress as symptoms of heartwater. All 27 farmers indicated that disease vector control is one of the strategies that they use to control heartwater. The popular method used by respondents was acaricide dipping (17 out of 27 farmers) compared to other control methods viz. grease application (8 out of 27 farmers) and building of a brand-new kraal (2 out of 27 farmers).

The choice of disease management strategies used by subsistence farmers to handle heartwater disease varied across 16 out of 27 farmers who reported that they have had suspected heartwater cases. Six out of 16 farmers reported self-treating suspected heartwater cases with broad-spectrum tetracycline antibiotics, 3 out of 16 reported using homemade remedies (such as traditional, plant-based treatments), 6 out of 16 preferred consulting with veterinary officers, and 1 out of the 16 farmers reported self-treating cases using a variety of approaches.

In terms of disease prevention, responses from the questionnaires revealed that 25 out of 27 farmers do not prevent disease by using the available heartwater live vaccine. Two farmers however said that they use the live vaccine and that they found it helpful in managing heartwater. When asked a follow-up question, 24 out of the 25 farmers who do not use the heartwater vaccine explained that they did not use it because the majority (20 out of 24) were yet to be educated on the live vaccine as a prevention method while the minority (4 out of 24) said that they could not afford the live vaccine.

4. Discussion

Information on heartwater and *Amblyomma hebraeum* distribution is scarce in Botswana with last published information of more than a

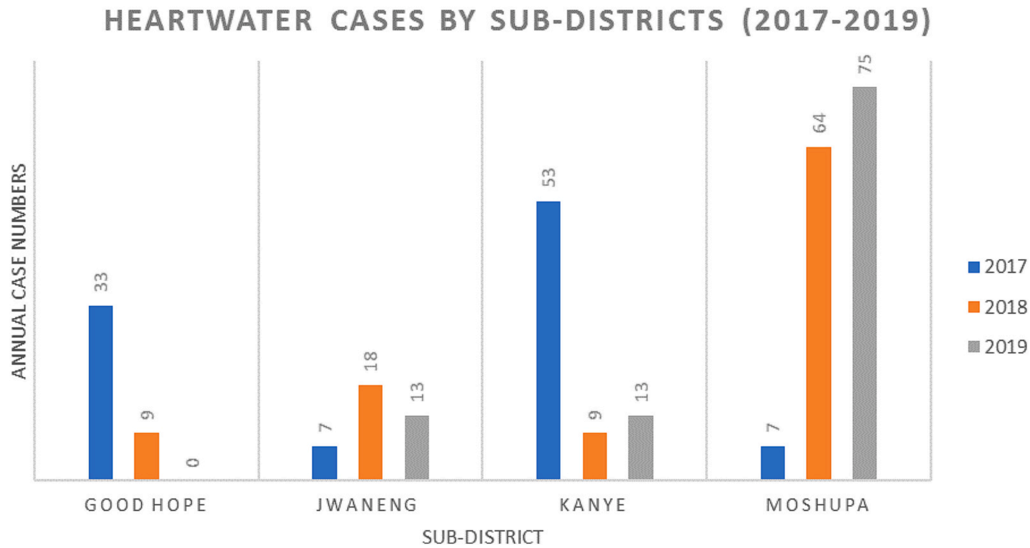

Fig. 2. Annual heartwater cases in the years 2017, 2018, and 2019 across four Southern sub-districts in Botswana.

Table 1
Distribution of ticks parasitizing livestock (cattle, sheep and goats) in the Southern district of Botswana during summer months of November to March 2017–2019.

Sub-district	Name of tick identified	Number of ticks collected	Proportion (%)
Good Hope	*Amblyomma hebraeum*	49	15.81
Jwaneng	*Amblyomma hebraeum*	58	18.71
Jwaneng	*R*[1]*. evertsi mimetucus*	34	10.97
Jwaneng	*R*[1]*. evertsi evertsi*	4	1.29
Jwaneng	*Hyalomma truncatum*	10	3.22
Kanye	*Amblyomma hebraeum*	51	16.45
Moshupa	*Amblyomma hebraeum*	104	33.55
Total ticks		310	100

[1] *Rhipicephalus.*

Table 2
Types of livestock affected by heartwater across four Southern sub-districts in Botswana between 2017 and 2019.

Location	Number of Cases	Caprine (%)	Ovine (%)	Bovine (%)
GOOD HOPE	42	35 (83%)	4 (10%)	3 (7%)
JWANENG	38	20 (53%)	16 (42%)	2 (5%)
KANYE	75	38 (51%)	27 (36%)	10 (13%)
MOSHUPA	146	73 (50%)	63 (43%)	10 (7%)
Total	301	166	110	25

decade ago (Batisani et al., 2012; Musuka et al., 2001; Bahia et al., 2021; Chepkwony et al., 2020; Ruff et al., 1993; Simpson et al., 1987; Mahan et al., 2001; Eygelaar et al., 2015). This study adds to and updates the body of knowledge on this important disease and its vector in Botswana. The findings of this work illustrate the presence of both *Amblyomma hebraeum* and heartwater throughout Botswana's Southern district and shows that the heartwater endemicity of the Southern district is associated with the geographic extension of vector species.

The clinical heartwater cases observed in this study showed correlation to the ecological preferences of the heartwater vector species under consideration. Ecological conditions influence the distribution of heartwater vectors (Batisani et al., 2012; Musuka et al., 2001). Hardveld districts (Good Hope, Kanye, and Moshupa) had higher proportions of heartwater vectors and clinical heartwater cases than the transitional sandveld-hardveld district (Jwaneng), indicating that *Amblyomma hebraeum* ticks prefer the denser vegetation of hardvelds to the dry, sandy, and less dense vegetation of sandvelds (Bahia et al., 2021; Chepkwony et al., 2020).

Heartwater cases were reported in three livestock species namely cattle, sheep and goats. The current report presents data that shows that while cattle seemed to be less affected by heartwater when compared to small ruminants, all types of breeds of goats, sheep and cattle were affected by heartwater. Literature has shown that there is variation in predisposition to clinical manifestation of heartwater (Ruff et al., 1993). The finding that goats and sheep were more affected by heartwater as compared to cattle corroborates with the findings of van den Heever et al. who made the same observationin the conxtext of the South African livestock industry (van den Heever et al., 2022).

Farmers were interviewed to gain perspectives of their local heartwater contexts. Few of the farmers who reported that they have previously suspected their livestock of having heartwater could describe the signs and symptoms of the disease. This finding is similar to Sungirai et al. (Sungirai et al., 2016) who found that famers could name tick-borne diseases but could not describe the signs and symptoms. Therefore, this implies that farming communities need regular training to learn about the symptoms of heartwater. About 40% of farmers indicated that despite experiencing tick infestations, they had not suspected their livestock of having heartwater. This result brings to the fore, a need for investigation of aspects of vector competence and host susceptibility to infection which are both requirements for disease transmission.

Controlling ticks by acaricide dipping was the approach most frequently utilized by questionnaire respondents. Similar studies in Africa (Sungirai et al., 2016; Masika et al., 1997; Mugambi et al., 2012) have also reported dipping as the most common tick control strategy. This strategy however has inherent fragility and thus must be used strategically for its maximum efficiency (Moyo and Masika, 2009). Despite the implementation of acaricide use by most respondents, the tick populations persist. Reports from 1982 (Paine, 1982) had already started to detect resistance to arsenic and camphechlor as *Amblyomma hebraeum* control agents in Botswana pointing to some potential weakness in heartwater management. The specific cause of tick persistence and the resultant heartwater cases across the study districts in the reported context therefore requires further investigation. Furthermore, we propose that further work be undertaken to better understanding of the disease including the appropriate symptoms.

In this study we found that few farmers use antibiotics to treat heartwater cases and vaccines to prevent the disease. This outcome is in line with observations made by Masika and Afolayan in the South Africa, who confirmed that farmers used plants to treat livestock diseases

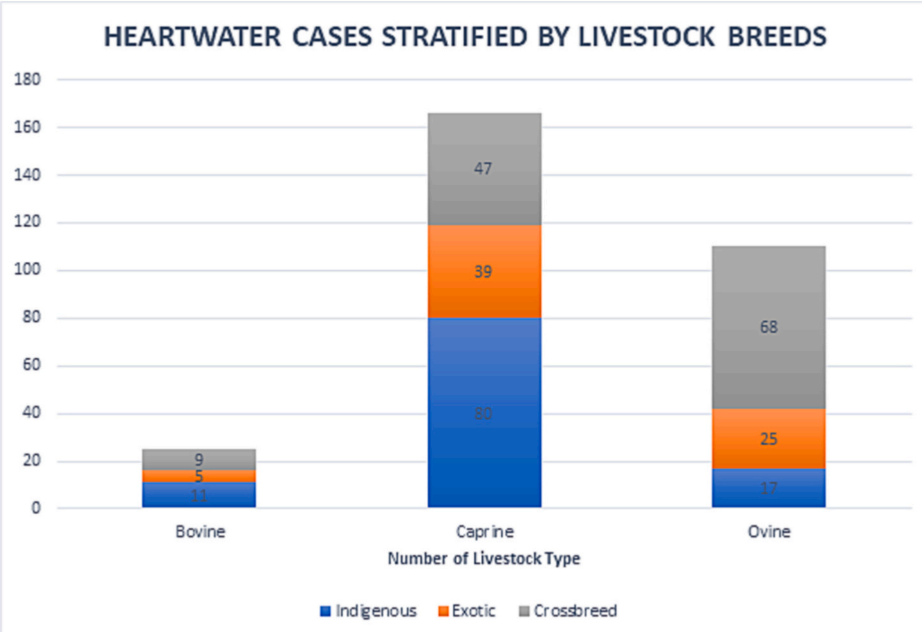

Fig. 3. Number of heartwater cases stratified according to breeds (Indigenous, Exotic and Crossbreed) per species within the southern district, Botswana. Bovine breeds belonged to; indigenous (Tswana breed), exotic (Brahman cattle, Seitz cattle & Simmental cattle) and crossed breeds. Caprine breeds belonged to; indigenous (Tswana breed), exotic (Boer goat and Kalahari red goat) and crossed breeds. Ovine breeds belonged to; indigenous (Tswana breed), exotic (Dorper sheep, Damara sheep) and crossed breeds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Masika and Afolayan, 2003). Masika and Afolayan reported that farmers resorted to herbal treatment since they lacked knowledge on conventional remedies and/or found conventional remedies expensive or inconvenient to administer. The findings further support the implementation of training programs for farmers to support treatment as a disease control approach.

The actual heartwater situation reported in this study is likely grossly underestimated as the current diagnostic method relies on post-mortem diagnostic procedures. Therefore, all affected and at-risk animals in a single kraal or farm are not included in the presented report. The reported data is limited by; i) low sampling numbers which restrict the extent of extrapolation of findings to the rest of the district and ii) sampling only during the summer months and missing out on the opportunity to establish the phenology of the tick species and the epidemiology of the disease. Further work hinged on serology could be undertaken to further understand the epidemiology of heartwater in the district.

The presence of *Amblyomma hebraeum* is not only limited to the Southern district of Botswana. Movement of livestock from the district poses conceivable risk of spread of the vector where conducive environments exist. Holistic heartwater management is therefore required in order to ease the impact of the disease. What is being done well in terms of disease management, such as is the case for Good Hope with reducing heartwater cases, needs to be well investigated and documented. Education of farmers on heartwater disease prevention and management is also pivotal. Furthermore, the determinants of the persistence of the vector need to be investigated. These efforts will assist in reducing the impact of heartwater on subsistence farmers and improving livestock productivity.

Ethics approval and consent to participate

The collection of tick specimens from livestock was approved by the Director of Veterinary Services [DVS 8/2 II (21)] – Ministry of Agricultural Development Food Security, Republic of Botswana. All specimens were collected under the supervision of staff from the Department of Veterinary Services. The supervising staff from the DVS also obtained verbal consent for study participation and specimen collection from farmers before farm visits.

Consent for publication

Verbal consent for publication of questionnaire data was obtained from participants. The research contained no more than minimal risk of harm to participants.

Funding

This work was financially supported by the Botswana International University of Science and Technology initiation grant R00081 proposed by KL. The funder had no role in the design of this study nor its execution, analyses, interpretation of the data, and the decision to publish results.

Authors' contributions

IR – Investigation, formal analysis, data collection, and data analysis. Wrote the manuscript.

JH – Conceived work to be performed, assisted in data analysis and writing of the manuscript.

KL – Conceived work to be performed, assisted in data analysis and writing of the manuscript.

MS- Assisted in data analysis and writing of the manuscript.

All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data generated or analyzed during this study are included in this published article. Case data that support the findings of this study are available from the Department of Veterinary Services (Ministry of Agricultural Development and Food Security, Republic of Botswana).

Acknowledgements

We are very grateful for the permission, support, and facilitation of the Director of Veterinary Services (Dr Letlhogile Modisa) and sub-district Veterinary Officers; Drs Kitosi, Sedupelela, Mthethwa, Motseolapile, and Ndlovu. We acknowledge the Chief Technical Officer, Ms. Keitumetse Mangate, for the permission to store samples and sharing of collection equipment during the fieldwork. We thank Associate Professor Nyamukondiwa, Department of Biological Sciences and Biotechnology (Invertebrate Biodiversity & Physiological Ecology) at BIUST for the provision of morphological identification expertise for the identification of ticks. We appreciate Mr. Mokola Letia who assisted in the collection of ticks.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2023.100902.

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