Presence and Persistence of *Amblyomma Hebraeum* Augments the Burden of Heartwater in the Southern District of Botswana

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Research

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Abstract

BACKGROUND

Heartwater is a tick-borne, haemoparasitic disease that has the potential to limit the growth of agro-businesses in Botswana. This threat to national food security is attributed to low production and mortality of livestock in subsistence farming of small stock than in commercial farms. This study gives a view of the epidemiological burden of heartwater in Botswana.

METHODS

Feeding ticks on livestock kept in four Southern sub-districts of Botswana were identified through morphological and molecular analysis. Farmers were interviewed on the management of the heartwater burden within their respective sub-districts. Veterinary clinical case reports were collected to assess the frequency of cases across the Southern District of Botswana.

RESULTS

Outcomes of the study revealed; a wide distribution of *Amblyomma hebraeum* in all four studied sub-districts, gradual annual increase in heartwater cases in Moshupa sub-district and indication of increased impact on indigenous caprine species despite the effort to control vectors by 63% of interviewed farmers.

CONCLUSION

Amblyomma hebraeum, the principal vector of *Ehrlichia ruminantium* in Southern Africa is widely distributed in the Southern District of Botswana and thus poses a challenge to livestock productivity in this district and, perhaps, elsewhere in the country where the tick infests livestock. There is therefore a need to re-examine the existing tick control interventions with an intention to reduce the heartwater disease burden particularly in sheep and goats.

Background

Amblyomma hebraeum is an invasive tick vector that can contribute to devastating repercussions for the agricultural industry when both the vector and its parasite are present. This tick species acts as a vector of the economically important livestock disease commonly known as heartwater. Heartwater is caused by an intracellular bacterium, *Ehrlichia ruminantium*, which belongs to the Rickettsiaceae family [1]. *Ehrlichia ruminantium* can parasitize *Amblyomma hebraeum* and the vector can drive transmission of the disease in the presence of a suitable ruminant host. Heartwater is a major threat to the national food security in agro-established countries worldwide [2]. In Southern Africa, it has been reported that approximately 90% of stock losses can occur due to untreated heartwater cases alone [3]. Consequently, it is important to manage this economic livestock disease. Heartwater has been managed over the last 50 years through preventative measures such as tick eradication programs, administration of antibiotics and vaccination

with a live blood vaccine. The vaccine contains live *Ehrlichia ruminantium* and is the only commercially available vaccine while the development of the new generations of the vaccine are explored [2], [4].

Botswana has a long history of both arable and pastoral farming. Poverty eradication programmes in Botswana fund the purchase of small stock (goats and sheep) by subsistence farmers to improve their livelihoods [4]. Movement of small stock around the country from heartwater endemic areas can lead to devastating losses of heartwater infection-prone breeds in non-endemic areas. This movement of small stock poses a threat in subsistence farming, as opposed to commercial farming, through the introduction of the heartwater disease in non-endemic areas in the absence of rigorous disease prevention measures as infected or tick-infested ruminants are transported in such areas [5]. This work reports the distribution of ticks that could potentially carry *Ehrlichia ruminantium* in an established heartwater endemic district (Southern District) in Botswana, details the frequency of heartwater clinical manifestations between 2017 and 2019, presents the most susceptible species and breeds and outlines tick control strategies that are used by farmers across the Southern district.

Methodology

Study area

Ticks and heartwater diagnostic 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 Goodhope. 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 headquarters of DVS that is based in Kanye. Ticks were collected and questionnaires administered in 13 subsistence farms holding cattle, sheep and goats within the Southern District of Botswana. The locations of the farms were mapped using GPS coordinates and are shown in Fig. 1.

Questionnaires

Questionnaires were administered to 27 farmers/staff concurrently with tick collections. The questionnaire of 18 questions, contained open and closed questions that generally covered aspects relating to heartwater burden, susceptibility, control and management. The interview was translated in Setswana by the assisting veterinary officer and the responses were recorded in English. Questionnaires were analyzed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

Sample period and sample collection

Sample collection was carried out during the summer seasons of 2017, 2018 and 2019. Three hundred and ninety-one small livestock ruminants (60 cows, 234 goats and 97 sheep) were randomly selected for tick collection from communal farms with no particular regard to a specific age, sex or breed.

Tick Collection and Identification

Ticks were hand-picked from under the tails, around the anus, on belly or groin regions and between hooves of randomly selected livestock. 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 MC190HD camera connected to it. Key features used for identification were adopted from guidelines provided by Walker et al (2003) [6].

Tick genomic 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 manufacturer's instructions. Genomic DNA was eluted using 25 µL of nuclease-free water.

Polymerase Chain Reaction

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 [7] to yield a 710 bp amplicon. Twenty five µL PCR reaction samples were prepared in PCR vial tubes. Each reaction comprised of 12.5 µL of the Q5® High-Fidelity 2X Master Mix PCR Master Mix (Thermo Fisher Scientific, USA), 6.5 µL of nuclease free water (VWR International LLC, USA), 1 µL each of forward and reverse primers, and 2 µL of extracted genomic DNA. PCR reaction conditions were as follows; initial denaturation at 98 °C for 30 seconds, followed by 35 cycles of; denaturation at 98 °C for 10 seconds, annealing at 48 °C for 30 seconds and extension at 72 °C for 30 seconds. This was followed by a final extension at 72 °C for 7 minutes. The reaction was then stopped at 4 °C. The presence of amplicons in PCR products was verified through agarose gel electrophoresis.

Agarose gel electrophoresis

PCR products were electrophoresed on 1% agarose gel at 120 V for 90 minutes. The agarose gel was stained with ethidium bromide to visualize the amplicons.

Amplicon clean-up

PCR products were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA), according to manufacturer's instructions.

Sequencing

Bidirectional, Sanger sequencing of the partial COI gene was performed at Inqaba Biotechnical Industries (Pretoria, South Africa).

Molecular identification of ticks

Contiguous sequences were analyzed using basic local alignment search tool (BLAST) to confirm matches with submissions made to the NCBI genbank.

Results

Diversity and geographical distribution of tick species

A total of 310 feeding ticks collected from 27 different livestock farms during the summer seasons of 2017, 2018 and 2019 were identified on basis of morphological features and three tick genera namely, *Amblyomma*, *Hyalomma* and *Rhipicephalus* were found to parasitize livestock (cattle, sheep and goats) in the Southern District of Botswana. Out of the total ticks found, 262 ticks (84.5%) were identified as *Amblyomma hebraeum* and this tick species was found in all four sub-districts of the Southern District of Botswana (Tables 1a & 1b). The other ticks identified were *Rhipicephalus evertsi mimeticus* (11%), *Rhipicephalus evertsi evertsi* (1.3%) and *Hyalomma truncatum* (3.2%). These ticks are vectors for theileriosis, anaplasmosis and babesiosis diseases respectively.

Table 1a
Frequency of ticks found parasitizing livestock (cattle, sheep and goats) in the Southern District of Botswana during summer period 2017–2019

Genus of tick	Number of ticks found	Relative frequency (%)
<i>Amblyomma</i>	262	84.5
<i>Hyalomma</i>	10	3.2
<i>Rhipicephalus</i>	38	12.3
Total ticks	310	100

Table 1b
Distribution by sub-district of ticks found parasitizing livestock (cattle, sheep and goats) in the Southern District of Botswana during summer period 2017–2019

Sub-district	Name of tick identified	Number of ticks found	Relative frequency (%)
Good Hope	<i>Amblyomma hebraeum</i>	49	15.81
Jwaneng	<i>Amblyomma hebraeum</i>	58	18.71
Jwaneng	<i>R¹. evertsi mimetucus</i>	34	10.97
Jwaneng	<i>R¹. evertsi evertsi</i>	4	1.29
Jwaneng	<i>Hyalomma truncatum</i>	10	3.22
Kanye	<i>Amblyomma hebraeum</i>	51	16.45
Moshupa	<i>Amblyomma hebraeum</i>	104	33.55
Total ticks		310	100
¹ <i>Rhipicephalus</i>			

Molecular characterization of *Amblyomma hebraeum* by PCR and sequencing revealed that the partial cytochrome c oxidase (COI) sequences had 99.56% sequence identity to GenBank: KY457512.1 and these molecular identification results confirmed the outcomes of the morphological analysis. Likewise, the molecular identity of the other ticks (*Rhipicephalus evertsi mimeticu*, *Rhipicephalus evertsi evertsi* and *Hyalomma truncatum*) concurred with the outcomes of the morphological identification shown in Fig. 2. The partial COI sequences matched GenBank submissions MF425990.1 (99.41%), MK551208.1 (99.26%) and KT999717 (99.41%) respectively. The main interest in this study was the tick vector for heartwater. *Amblyomma hebraeum*, as pictured in Fig. 3, which was identified in all four sub-districts of the Southern District of Botswana.

Morphological landmarks that are unique to *Amblyomma hebraeum* are: 1) the exceptional ornate patterns that embellishes the tick scutum, 2) sensilla distributed through-out the leg, 3) protruding internal and external spur, 4) yellowish bands on the legs, 5) prominent festoons and 6) the anal groove that is present posterior to the tick anus.

Frequency of heartwater cases

The analysis of the heartwater cases collected from the veterinary service centers of four sub-districts showed the highest frequency of confirmed disease in Kanye in 2017 and Moshupa in 2018 and 2019 (Fig. 4).

Moshupa, which had the highest number of heartwater vectors (Table 1b), had the highest number of heartwater cases over the 3 year study period (Table 2). The trend showed a gradual increase in the

number of heartwater cases observed in Moshupa while there was a gradual decrease observed in Goodhope cases (Fig. 4) where the least number of vectors was recorded (see Table 1b and Table 2). Further analysis revealed that small livestock were more affected in all sub-districts (Table 2) with caprine breeds being the most affected.

Table 2
Relative frequency (%) of clinical manifestation in sub-districts between 2017–2019

Location	Number of Cases	Caprine (%)	Ovine (%)	Bovine (%)
GOODHOPE	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%)

Livestock breeds affected by heartwater

The indigenous breeds of caprine and bovine species were found to be more affected than other breeds. On the other hand, crossbreeds of ovine species were cumulatively found to be more affected across sub-districts during the study period (Fig. 5).

Vector control and burden management by subsistence farmers

Having determined the types of vectors, their distribution and the burden of disease that they caused in the southern district of Botswana, we then sought to determine the disease management practices of farmers. The responses of 27 communal farmers show that vector control is practiced across the district. The popular method used by respondents is acaricide dipping (63%) compared to other control methods viz. grease application (30%) and building of a brand-new kraal (7%) (Fig. 6). This observation highlights the effort by some communal farmers in controlling vector infestations and managing disease burden.

The choice of disease management strategies by communal farmers in an effort to handle heartwater disease are presented in Fig. 7. Forty-four percent of farmers reported that they do not take any action in handling the suspected heartwater case while 15% preferred consulting with the veterinary officer and administering antibiotics themselves.

The survey data in Fig. 8 revealed that 92.6% of farmers do not prevent heartwater through use of an available live vaccine. Furthermore, when a follow-up question was asked, 24 out of the 25 farmers (96%) who do not use the heartwater vaccine explained that they were yet to be educated on the live vaccine as a prevention method or could not afford the live vaccine.

Discussion

This study reports on the trends for confirmed heartwater cases, distribution of heartwater vectors, identification of heartwater susceptible breeds kept by Batswana farmers, as well as tick control measures and burden management as practiced in Southern District farms in Botswana. This study submits that the heartwater vector is present across the Southern District of Botswana as a basis of current and potential increased disease burden that could occur in the future. The approach to determining this burden was to study the association of two disease determinants, that is, the frequency of disease and its vector distribution. These determinants were selected as they rank among the most investigated drivers of vector-borne disease. Previous studies in Africa, including Botswana, have reported on the distribution of *Amblyomma* tick vectors [8]–[12]. However, this is the first study to investigate the association of heartwater vector (*Amblyomma hebraeum*) with heartwater clinical cases in Botswana. The current investigation demonstrated that certainly the heartwater endemicity of the area relates with geographic extension of vector species. Additionally, it is important to note that the true burden of heartwater is likely grossly underestimated by the presented clinical heartwater cases as the current diagnostic method relies on post-mortem, microscopic visualization of Ehrlichia ruminantium on Giemsa stained brain smears. All affected and at-risk animals in a single kraal or farm are therefore not included in the presented work.

The heartwater vector as well as clinical heartwater cases were predominantly associated with hardveld Districts (Good Hope, Kanye and Moshupa) than the transition sandveld-hardveld district (Jwaneng). This indicates that *Amblyomma hebraeum* ticks prefer humid, rocky and denser vegetation of hardvelds contrary to the dry, sandy and less dense vegetation of sandvelds [13]. The survival and the distribution of various tick species depend on these humid environments to avoid desiccation and predators. The predominantly collected heartwater vector shares similar ecological preferences with babesiosis, anaplasmosis and theileriosis vectors. These aforementioned vectors are reported to cause the most important tick-borne diseases in Botswana [14].

Eradication of *Amblyomma hebraeum* by acaricide dipping was the most practiced tick control measure used by respondents in this study across the district compared to other control tick methods. In most countries acaricide use is a government initiative [5]. This tick control method in some countries was reported to have been successful, while in some countries like South Africa, acaricide use has been unsuccessful in eradicating some tick species [15]. The exact attribution of the persistence of ticks and resultant heartwater cases across the district in the background of concerted vector control measures is yet to be investigated. The findings however highlight the need for effective tick control measures.

The choice of livestock breeds kept by farmers within the district are shown to vary in host susceptibility to heartwater infections. In this study, heartwater disease was reported to be more frequent in both indigenous and cross breeds than in exotic breeds. This reported data on susceptibility may be attributed either to livestock trade practices that occur from non-endemic to heartwater endemic farms, pathogen transmission to ticks by carrier animals that survived heartwater infections or low immune resistance due to heat-stress.

Livestock trade of various breed types is adopted by farmers with the intention of upgrading local phenotypes that satisfy the demand by local and international consumers. For this reason, exotic livestock are moved to endemic areas in the effort of adapting indigenous breeds through the existing breed development programs in Botswana [16]. Kabi et al (2016) reviewed the effects of phenotypic modification through inter-breeding and noted that disease tolerant traits may be lost due to limitations to skill techniques in matching genotypes during cross breeding practices [17]. As a result, cross breed livestock may lack desired traits such as endemic disease tolerance and adaptation to high temperature in districts. This evaluation may be the reason for heartwater susceptibility of cross breed ovine species in this study. Stringent tick/disease control measures in an attempt to protect naïve exotic breeds traded into endemic areas may also put indigenous breeds at risk. Exotic breeds due to their prized status can be protected with the live vaccine and treated to recovery. In the process of this treatment, carrier exotic breeds may transmit heartwater infection to tick vectors within the endemic areas. Stringent tick control may also inadvertently select for acaricide resistant tick populations. Consequently, indigenous and cross-breeds are at a higher risk of disease transmission. Moreover, asymptomatic carriers in farms may be affected by the disease once the immune resistance is lowered due to the sensitivity to climatic changes or impacted by environmental factors such as drought. The presented findings on heartwater susceptibility of Botswana breeds however are acutely limited and require confirmatory work to elucidate the reasons for higher heartwater disease rates in indigenous breeds of caprine and bovine species.

Conclusion

This study finds validation in endorsing interventional approaches in averting conceivable endemic risks that can occur with growing distribution of *Amblyomma hebraeum* across Botswana. Education of farmers on heartwater disease prevention and management is pivotal. Furthermore, the determinants of the persistence of the vector need to be investigated. These efforts will assist in reducing the burden of heartwater and improving livestock productivity.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Data generated or analysed 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, Botswana*).

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

IR – Performed fieldwork, photography, 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

All authors read and approved the final manuscript.

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Figures

Figure 1

Study area map indicating sample sites within the heartwater-endemic Southern District, Botswana. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Morphographs of various tick species collected during this study: - A) *Rhipicephalus evertsi mimeticus*, B) *Rhipicephalus evertsi evertsi* and C) *Hyalomma truncatum*

Figure 3

Identification features of *A. hebraeum*: 1) ornamentation, 2) sensilla, 3) internal and external spur, 4) bands, 5) festoons & anal groove.

HEARTWATER CASES BY SUB-DISTRICTS (2017-2019)

Figure 4

Relative frequency of heartwater cases in the years 2017, 2018 and 2019 across four Southern sub-districts in Botswana

Figure 5

Number of heartwater cases stratified according to breeds per species within the Southern District, Botswana.

TICK CONTROL METHODS BY FARMERS IN THE SOUTHERN DISTRICT

Figure 6

Tick control methods utilized by farmers in the Southern District, Botswana.

DISEASE MANAGEMENT STRATEGIES USED BY FARMERS

Figure 7

Choice of intervention strategies by subsistence farmers upon suspicion of heartwater.

USE OF THE HEARTWATER LIVE VACCINE BY FARMERS

Figure 8

Use of the live heartwater vaccine by interviewed farmers across the Southern District, Botswana.

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