Assessment of knowledge, attitude and practices of small household farmers towards heartwater disease and molecular characterization of *Ehrlichia ruminantium in* Limpopo province, South Africa

A dissertation submitted in the partial fulfilment of Master of Science Degree in Microbiology

by

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DECLARATION

I, Doris Mathebula, student number 11595368 hereby declare that this dissertation submitted under the Department of Biochemistry and Microbiology, University of Venda, is my original work and has not been submitted for any degree at any other university or institution. The dissertation does not contain other person's writing unless referenced accordingly.

Signature ……Mathebula D…… Date …02/05/2023……..

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DEDICATION

I dedicate this work to my loving parents and all my siblings. I am grateful for your support and guidance you have offered me all the way to the success of this dissertation. To my lovely son Karabo, may you follow my footsteps without any hesitation.

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LIST OF ABBREVIATIONS

Abbreviations	Definition
bp	Base pairs
°C	Degrees Celsius
et al	Et alia (and others)
µl	Micro liter
KAP	Knowledge, Attitude and Practices
%	Percentage
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
RT PCR	Real-time reverse transcription–polymerase chain reaction
KDa	Kilodalton
g	Grams
DNA	Deoxyribonucleic acid

WHO	World Health Organization
CIRAD	French Agricultural Research Centre for International Development
ARC-OVI	Agricultural Research Council-Onderstepoort Veterinary Institute
UP	University of Pretoria
MAP1	Major Antigen Protein
SrRNA	small regulatory Ribonucleic Acid
SA	South Africa

Abstract

Background: Heartwater is a disease spread by ticks that is brought on by the obligatory intracellular bacterium *Ehrlichia ruminantium.* Heartwater disease is one of the major obstacles to livestock production as it affects many livestock animals including domesticated animals such as goats, cattle, and sheep as well as wild ruminants. In Southern Africa, it is spread by *Amblyomma hebraeum* ticks, and in the rest of Sub-Saharan Africa, it is spread by *A. variegatum* ticks. In this study, epidemiological and molecular features of *Ehrlichia ruminantium* in Mopani and Vhembe Districts, from ticks isolated from cattle, goats and sheep were investigated.

Method: A total of 121 small household farmers from different villages in the Vhembe and Mopani Districts were recruited in the study after they have signed an informed consent. The participants were interviewed using a questionnaire to collect data related to their knowledge, attitude and practices towards heartwater disease. Ticks were collected from cattle, goats, and sheep belonging to 48 households, yielding a total of 244 ticks. Genomic Deoxyribonucleic acid was extracted from the ticks and analysed using real-time qPCR assay targeting a 226bp fragment of the PCS20 gene. Finally, a number of samples were sent for sequencing to identify different strains circulating in the region. Neighbor-joining method was used to infer phylogenetic positions on the basis of 16S rRNA gene.

Results: According to the findings of the questionnaire evaluation, only about 23.1% of the participants had some knowledge of heartwater disease. Furthermore, the highest proportion of the study population (76%) associated heartwater with air-borne transmission and 77.7% of the

participants failed to identify the season in which heartwater commonly occurs. About 69% of the respondents associated ticks with animal diseases while 49.6 % correctly highlighted that, ticks are disease carriers. Farmers had a positive attitude towards control and treatment of heartwater by stating that they would use prescribed medicine 23.9%, vaccines 7.4% or consulting a specialist 2.5%. Few farmers indicated that they use homemade mixtures (0.8%), dipping and spraying (0.8%) to manage animal diseases. The most preferred method of tick control used by farmers was spraying of acaricide treatment 63.6%. On account of the poor animal services reported among the visited rural communities, some farmers opted for removing ticks by hand 28.9% as their supplementary tick control method. Majority of the farmers fed their livestock at the bush 68.6% which was the most contributing factor to increased tick infestation as reported by farmers. Several plants used as medicine to treat various animal diseases were mentioned. These included: *Melia azedarach, Albizia adianthifolia, Gymnosporia senegalensis, Dicerocarryum senecioides* etc. The type of disease affecting the livestock mentioned by the participants was diarrhoea (43.0%) which is among the list of heartwater symptoms. The study demonstrates that respondents had inadequate knowledge about heartwater disease. Animal services need to be upgraded in order to help the farmers improve the quality of their produce.

The results of the PCR test showed that 56.2% of the household farms had *E. ruminantium* infection. The distribution of *E. ruminantium* by source of ticks and animal type revealed that cattle are more prone to tick distribution 43.5% as compared to other animal sources of ticks. Nzhelele municipality had the highest prevalence of *E. ruminantium* (37.0%) compared to the other municipalities. Prevalence of *E. ruminantium* by gender of farmers was found to be high from males 36.7% than females 25.0%. Farmers with household income > R20000 had the highest

prevalence of *E. ruminantium* (50.0%) than farmers with household income < R500, (14.3%). The age of the farmers 15 – 20 years old revealed the highest prevalence 46.7 % of *E. ruminantium* infection. However, there were no infection among the ticks obtained from animals belonging to farmers above 60 years old. Farmers who had tertiary level of education showed the highest prevalence 46.8% probably because of limited time to attend to their livestock. Many clades were identified by phylogenetic analysis of the *E. ruminantium* PCS20 genotypes.

Conclusion: This study showed that there is need to further educate small farmers on heartwater disease. It also showed that indigenous knowledge still contributes significantly to the management of animal diseases in most rural communities. The application of real time PCR showed a high prevalence of *E. ruminantium* infected *A. hebraeum* ticks from livestock and should be considered in the continuous monitoring of the animal population in order to avoid heartwater disease outbreak in the communities which could be detrimental for the local economy. Furthermore, future vaccine development against *E. ruminantium* should consider the diversity observed in the present study. That could be helpful in managing heartwater disease in the areas that were investigated.

Key words: Heartwater disease, knowledge, attitude, practice, cattle, sheep, goats, *Ehrlichia ruminantium, Amblyomma hebraeum,* PCS20 gene, tick.

Chapter 1: Introduction

1.1 Background

Heartwater disease is caused by a type of bacterium called *Ehrlichia ruminantium,* which is an intracellular organism that cannot survive outside of its host cells, and it affects cattle, goats, sheep, and wild ruminants (Gofton *et al.,* 2017). Heartwater is a significant challenge to maintaining a high animal productivity in Africa. It is transmitted by *Amblyomma hebraeum* ticks in Southern Africa and *A. variegatum* ticks transmit the disease to the rest of Sub-Saharan Africa and to Islands within the Indian Ocean and the Caribbean (Allsopp *et al.,* 2007).

E. ruminantium persists in wildlife reservoir hosts and circulate in mammal-tick-mammal transmission cycles wherein ticks act only as vectors and not as reservoirs (Bonnet *et al.,* 2018). In mammals, *Ehrlichia ruminantium* invade the endothelial cells wherein they form and multiply within intracytoplasmic vacuoles (Gofton *et al.,* 2017). Asymptomatic *Ehrlichia ruminantium* infections can persist in numerous wildlife reservoirs. Domestic animals that have been exposed to the disease by a tick bite can get major illnesses like nausea, anaemia, muscle pains, fever, rash, headaches, and severe cases could even result in death (Pfeffer *et al.,* 2018).

Vaccines capable of managing the infection are required of which test vaccines which include recombinant, weakened and inactivated antigens have not been especially effective, due to the antigenic variability of the infectious agent responsible for the disease (Cangi *et al.,* 2017). The only commercially available immunization for disease control is the *E. ruminantium* Ball3 strain blood vaccine. It uses virulent Ball3 strain-infected sheep blood infection, followed by antibiotic

therapy, and this approach provides only modest protection against a few prevalent virulent genotypes (Nefefe *et al.,* 2017).

There has been very few research done considering knowledge, attitude and practices of small household farmers towards heartwater disease. However, the perception of farmers on young goat deaths in communal farming settings in South Africa has been conducted wherein the ticks were considered a major cause of deaths in livestock animals (Slayi *et al.,* 2014).

Recently, the only members of *Amblyomma* reported in the Northern part of Vhembe region are *A. hebraeum* ticks of which the male was found to be the most common compared to the female (Ramashia, 2018). This can be introduced to other areas of Vhembe during the process where domestic animals are transported from one area to another. Introduction of heartwater presents a major concern to certain areas including Tshikonelo, Khalavha, Bungeni, Mititi and Matsa since the areas fall under Vhembe and Mopani regions.

The most accurate and sensitive technique for detecting *E. ruminantium* available today is the PCS20 PCR assay (Cangi *et al.,* 2017). The PCR probe-based assays require a great deal of work. Hence, they are time consuming which requires handling of radioactive materials and can take a period of 5 days (Ambrasiene *et al*., 2004). Real-time PCR assays are more sensitive, does not require a lot of effort and can detect parasite DNA within 70 minutes (Espy *et al.,* 2006).

To track the establishment of cell culture vaccines, the MAP1 gene of *E. ruminantium* was initially detected using quantitative real-time PCR (Pfeffer *et al.*, 2018). The MAP1 gene is known to be polymorphic and field experiments may not always be successful at discovering heartwater

isolates. Nevertheless, the PCS20 region is conserved, it is perfect for diagnostic and epidemiological studies (Allsopp and Allsopp, 2001).

1.2. Study rationale

Heartwater disease prevents livestock farmers from producing high-yielding breeds in their herds. This is proved by the fact that farmers in places where heartwater disease is prevalent find it difficult to evaluate the disease's economic impact and are frequently unable to pay for conclusive diagnostics (Allsopp, 2015). In general, the most reliable diagnosis of heartwater is achieved through examination of capillaries in the brain smears. Additionally, heartwater diagnosis can also be achieved through xenodiagnostic, although it is expensive, inconvenient and time consuming (Peter *et al.,* 2000). However, these two methods are not suitable for studies involving epidemiology of heartwater in a wide scale. Consequently, more sensitive polymerase chain reaction-based assay methods for the detection of *Erhlichia ruminantium* from both ticks and blood sample have been advanced and received worldwide application (Sayler *et al.,* 2016). Amplification of *E. ruminantium* PCS20 gene in *Amblyomma* ticks may be more suitable to determine *E. ruminantium* carrier status of livestock animals (Anifowose *et al.,* 2020).

In a study conducted in Maputo province, Mozambique, the prevalence of *E. ruminantium* in cattle blood samples was 15%. The main aim of their study was to look at the prevalence and genetic diversity of *E. ruminantium* in cattle blood samples. DNA blood samples were initially used in PCR assays that looked for *E. ruminantium* PCS20 gene fragments. The *E. ruminantium* MAP1 gene was targeted by PCR test on the positive samples (Matos *et al.*, 2019). Furthermore, a study conducted in provinces of South Africa, KwaZulu-Natal, Eastern Cape, Free State and

Mpumalanga revealed that *E. ruminantium* is prevalent with about 28% of domestic animals being infected. They performed DNA extraction and samples were further subjected to amplification by PCR and sequencing (Mtshali *et al.,* 2015). Hence, with the high prevalence that has previously been reported in Limpopo province (91.8%), it is quite clear that *E. ruminantium* is a threat to the lives of cattle, goats and sheep in this part of the world (Collins *et al*., 2003). Another study by Ndou in 2017 (unpublished) showed that *E. ruminantium* is still prevalent in Limpopo (21.48 %). Similarly, in a study by Ramashia in 2018 showed that the overall prevalence of *E. ruminantium* in Vhembe was 23% (24/106) in the *Amblyomma ticks*. However, the number of samples was quite small and there was molecular characterization of the strains obtained.

There have been a lot of research conducted with the aim of producing a powerful vaccine for heartwater disease. An experimental attenuated vaccine has been prepared, the use of the Senegal isolates of *E. ruminantium,* and it is effective for protecting animals against homologous challenge (Jongejan *et al*., 1991). Unfortunately, it does not provide cross-protection in comparison with other virulent isolates, and these have commonly been resistant to in-vitro attenuation.

However, they have had much less success in field trials. Experimental inactivated vaccines have successfully offered protection against homologous laboratory challenge. It has been demonstrated that a nucleic acid vaccine containing the MAP1 gene of *E. ruminantium* protects mice from a fatal homologous challenge when given either alone or in combination with MAP1 protein as a booster (Collins *et al*., 2005). Unfortunately, the degree of protection was quite variable, and additional *E. ruminantium* genes have been tried in comparable model systems with inconsistent outcomes.

The infection and treatment approach currently employed for heartwater vaccination have significant issues. Cryopreserved blood from sheep infected with the virulent *E. ruminantium* organisms of the Ball 3 isolate serves as the only currently available vaccine, but it is costly to prepare, store, and use (Collins *et al*., 2003).

There has not been much research to evaluate the KAP of small household farmers towards heartwater disease in Mopani and Vhembe regions of South Africa. Therefore, there is limited information on the KAP studies of heartwater, specifically in Limpopo province. Seeking to understand KAP of small household farmers towards heartwater disease is vital in the implementation and the design of integrated disease control strategies. In the present study, we conducted the assessment of KAP of small household farmers towards heartwater disease and molecular characterization of *E. ruminantium* in ticks in Limpopo province, South Africa.

1.3. Objectives

1.3.1. Primary objective

- The main objective of this study is to assess the knowledge, attitude and practices of small household farmers towards heartwater disease and characterize *E. ruminantium* from ticks in the Mopani and Vhembe regions in Limpopo Province.

1.3.2. Secondary objectives

- Assessment of knowledge, attitude and practices of small farm holders in the Mopani and Vhembe regions concerning heartwater disease using a questionnaire

- Detection of *E. ruminantium* from ticks in the Mopani and Vhembe regions of Limpopo province using genomic DNA extraction and polymerase chain reaction
- Genetic characterisation of *E. ruminantium* from ticks using Sanger sequencing

1.4. Research questions and hypothesis

1.4.1. Research questions

- Do small household farmers have good and clear knowledge about heartwater disease?
- What is the attitude of small household farmers towards heartwater disease?
- What are the practices of small household farmers towards heartwater disease?
- What is the distribution of *E. ruminantium* in ticks in the region?
- What are the factors that might influence the distribution of *E. ruminantium* in ticks in the region?
- What are the different variants of *E. ruminantium* in the Mopani and Vhembe regions?

1.4.2. Hypothesis

- There are possibly new genotypes of *E. ruminantium* circulating in Mopani and Vhembe districts.

Chapter 2: Literature review

2.1. Introduction

Louis Trichardt made the first documented reference of what was later considered to be heartwater in 1838. (Neitz and Alexander, 1945). Many of his sheep perished when he was hiking through South Africa's Limpopo province three weeks after they had experienced a serious tick infection. Sixty years later, Dixon and Edington (1898) demonstrated that heartwater could be transmitted via the exchange of infected animal blood with susceptible ones, and Lounsbury (1900) established that *Amblyomma hebraeum* was the vector in South Africa **(**Allsopp, 2009).

Heartwater is a life-threatening bacterial disease caused by the intracellular bacterial organism *Ehrlichia ruminantium*, affecting wild and domestic ruminant animals such as sheep, goats and cattle. It is also known as cowdriosis (Nefefe *et al.*, 2017). The disease is transmitted by ticks of the genus *Amblyomma*. Wild ruminants may not show symptoms serving as *E. ruminantium* reservoirs in endemic regions (Allsopp, 2010). The disease has long been known for having a significant economic impact on the development and production of cattle in Sub-Saharan Africa and on a number of Caribbean Islands. When it comes to the epidemiology of heartwater, ticks are quite important. Yet planning for effective tick control techniques is essential for avoiding sickness (Merrill *et al.*, 2018).

Heartwater disease normally occurs during the rainy season (Kerario *et al.,* 2018). It is present in the Caribbean and in almost the whole sub-Saharan Africa. Although, there is a limited number of available vaccines because of high genetic diversity of *E. ruminantium* in a specific geographical

area of study. This is due to limited cross protection between field and vaccinal strains (Vachiery *et al.,* 2013). The production of the most effective vaccine that will act against heartwater disease requires knowledge about the diversity of circulating strains of *E. ruminantium* within specific regions, the origin of introduction as well as their evolution. Currently, the genetic diversity of *E. ruminantium* has been documented through conserved and polymorphic genes including MAP1, 16S rRNA for a limited number of strains (Cangi *et al.,* 2016).

2.2. Vectors of heartwater disease

Amblyomma hebraeum ticks are the most excellent vectors for heartwater disease transmission, and they are placed in the second position only to mosquitoes as vectors of malaria. They can carry and transmit a wide array of pathogens which include bacteria, protozoa, viruses, nematodes, and toxins (Crowder *et al.,* 2010). Ticks are obligate hematophagous arthropods that parasitize every class of vertebrates except for fish in almost every region of the world (Parola and Raoult, 2001). The Argasidae and Ixodidae are the two largest tick families. Hard ticks belonging to the genus *Amblyomm*a are primarily found in tropical and subtropical areas of Africa and South America. All varieties of domestic and wild animals are affected by *Amblyomma* ticks (cattle, sheep, goats, pigs, horses, dogs and cats, as well as birds and reptiles). *Amblyomma* ticks, like all ticks, are obligatory parasites because they are unable to survive without sucking blood from their hosts. Because they represent the actual prevalence of *E. ruminantium* in the field population, estimates of the prevalence of infection rates in *Amblyomma* species are crucial (Ringo *et al*., 2018).

There are two main vectors of heartwater disease of the genus *Amblyomma.* This include *Amblyomma variegatum and A. hebraeum* (Allsopp, 2010). *Amblyomma variegatum* is found widespread in eastern Caribbean and Sub-Saharan Africa with no occurrence found in most Southern African countries. In most cases, *Amblyomma hebraeum* (Figure 2.1) is found to be more responsible for the transmission of the disease (Pintore *et al*., 2021). Figure 2.2 shows male and female *Amblyomma variegatum* ticks that are responsible for causing heartwater disease. Despite the fact that at least one genotype of *E. ruminantium* is known to exist and is not likely to be the source of heartwater where it is found, this genotype has been around for a while (Allsopp *et al.,* 2007).

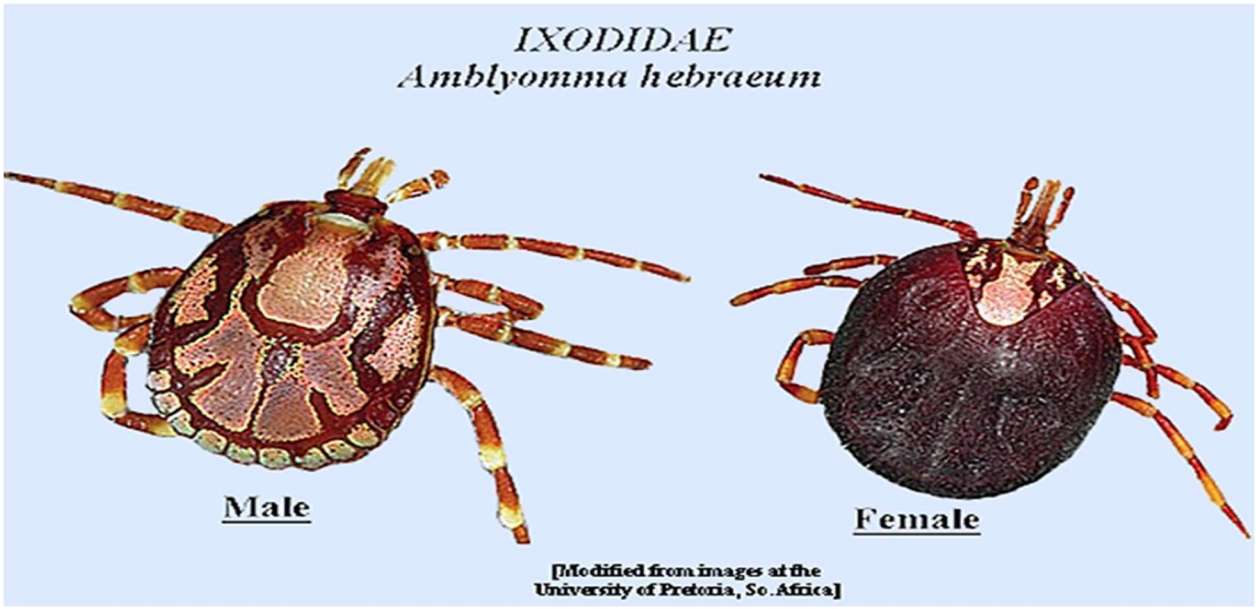

Figure 2.1: Male and female *Amblyomma hebraeum* ticks (https://faculty.ucr.edu/~legneref/medical/jpg/Amblyomma%20hebraeummale%20&%20female.jpg).

Male *Amblyomma variegatum*

Female *Amblyomma variegatum*

Figure 2.2: *Amblyomma variegatum* male and female. Photo: Alan Walker

2.3. Biology of *E. ruminantium*

2.3.1. Taxonomic classification of *E. ruminantium*

The taxonomic classification had been based on behavioural and morphological grounds. The first use of genetic analysis was when the *16S rRNA* gene of *Cowdria ruminantium* was sequenced. The organism was discovered to be phylogenetically closely associated to several members of what was then known as the tribe *Ehrlichieae* (Adelabu *et al.,* 2020). The clustering of *C. ruminantium* with some *Ehrlichia* species suggested that the tribe *Ehrlichieae* required adjustments (Van Vliet *et al*., 1992). Dumler *et al.* (2001) suggested a new classification of the Rickettsiales on the basis of the phylogenetic analysis of the *groESL* operon and the sequences of *16Sr*RNA gene. *Ehrlichia* (*Cowdria*) *ruminantium comb. nov*. together with *E. muris, E. canis*, *E.*

ewingii and *E. chaffeensis* and were assigned to the genus *Ehrlichia*. This analysis categorizes *E. ruminantium* in the following manner:

Kingdom: Bacteria

Phylum: Pseudomonadota

Class: Alphaproteobacteria

Order: Rickettsiales

Family: Ehrlichiaceae

Genus: *Ehrlichia*

Species: *Ehrlichia ruminantium* (King, 1907).

2.3.2. MAP1 gene of *E. ruminantium*

MAP1 is immunodominant, major outer membrane protein expressed by *E. ruminantium* in the mammalian host (Van Heerden *et al.,* 2004). The MAP1 gene of *E ruminantium* has a multigene family known as MAP1 which consists of 16 copies of homologous genes encoding 2830 KDa of outer membrane proteins (Figure 2.3). *E. ruminantium* strains are highly diverse among MAP1 related genes (Steyn and Pretorius, 2020). MAP1 sequences constitute a gold marker to characterize the genetic diversity of *E. ruminantium* (Mnisi *et al.,* 2022). *E. ruminantium* map genes are organized in an operon whereby genes are differentially regulated. This depends on vector and host cell environment (Bekker *et al.,* 2005).

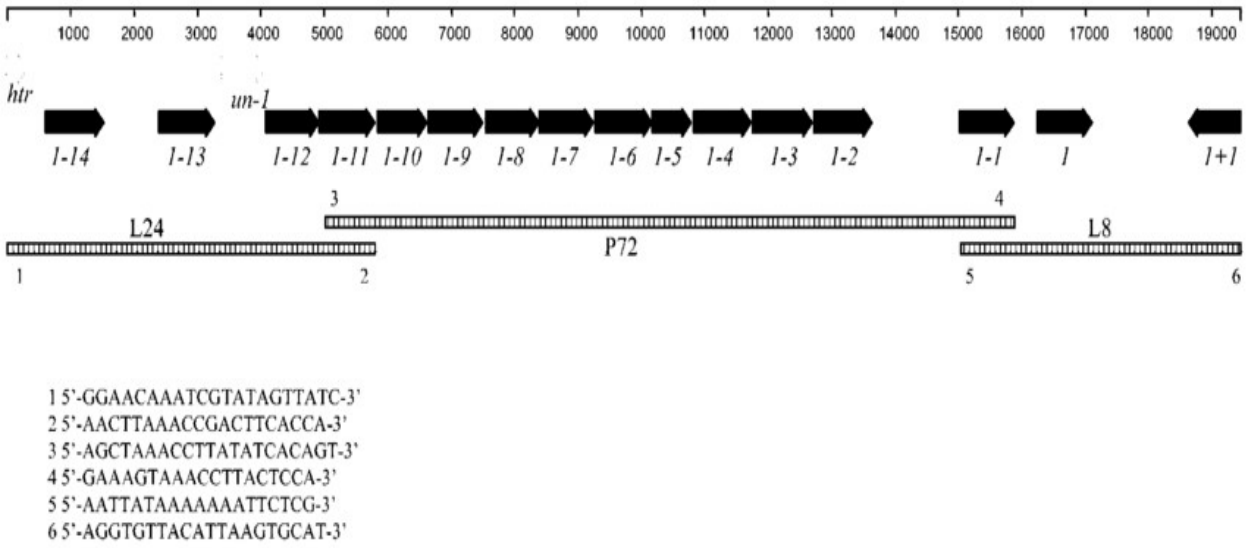

Figure 2.3: Schematic representation of the *E. ruminantium* Senegal MAP1 multigene family. The arrows represent the genes and their orientations. The three clones that were constructed with long-template PCR are indicated by the bars. The MAP1 paralog names are indicated below the solid arrows and the other gene names below the open arrows. *htr*, hypothetical transcriptional regulator; *un-1*, unknown gene (Bekker *et al.,* 2005).

2.3.3. *E. ruminantium* shows specific genomic features.

E. ruminantium genome is circular and approximately 1.5 megabases in size (De Villiers *et al.,* 2000). Its genome shows a low G+C content of 28 % with 920 to 957 of protein-coding sequences depending on the strain (Frutos *et al.,* 2006). Normally, a small genome size best characterizes an intracellular parasite. However, this is the greatest genome of all the sequenced Rickettsiales genomes (Williams *et al.,* 2022). *E. ruminantium* is classified under the smallest coding ratio

bacterial genomes (Figure 2.4). The mean size of the protein-coding sequences is identical to that of other archaebacterial and bacterial genomes which is 1 kb. Because of the unusual long intergenic regions, *E. ruminantium* has the low coding ratio. The presence of numerous tandem repeats is another specific and striking feature of *E. ruminantium* (Collins *et al.*, 2005).

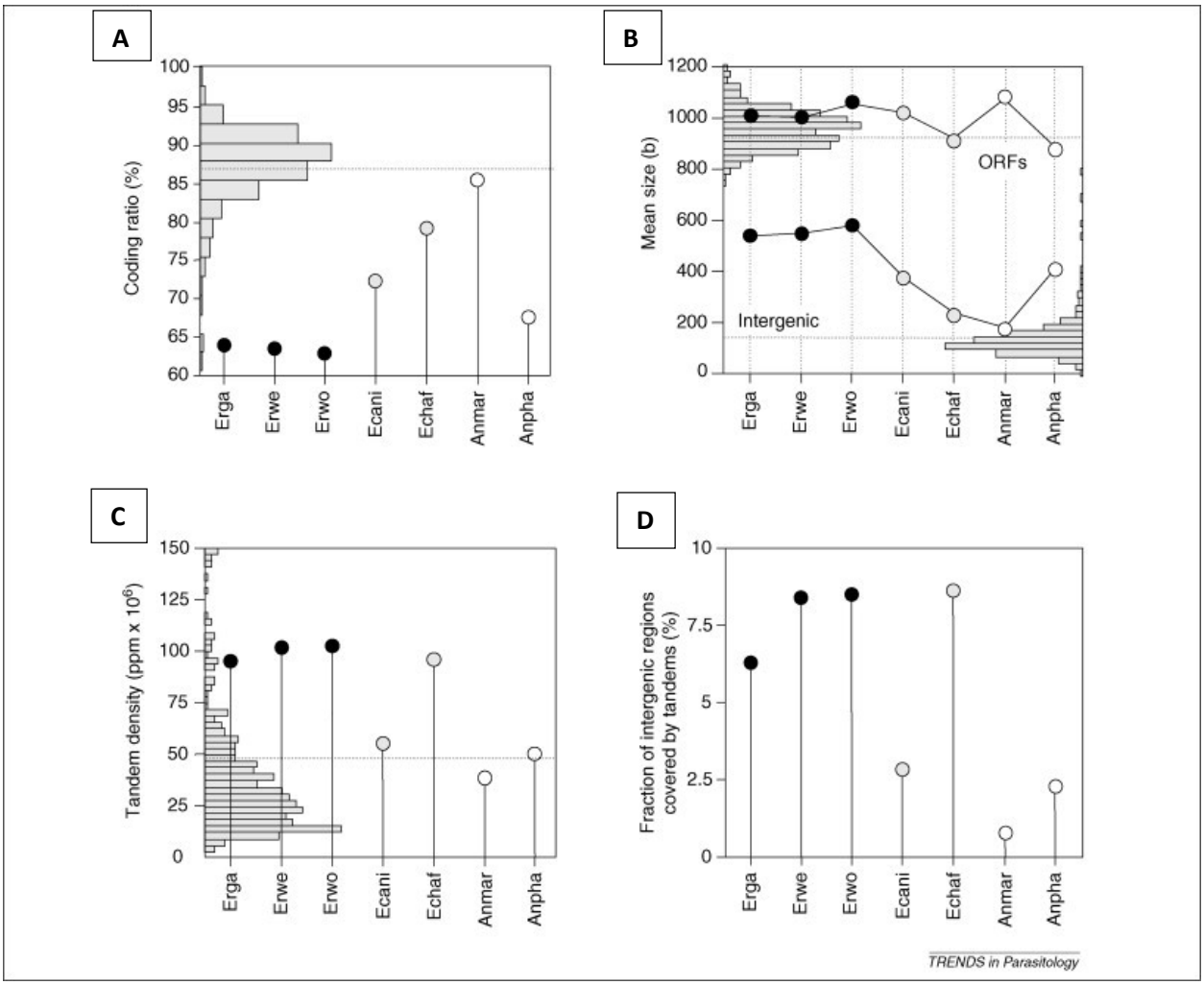

Figure 2.4: Representation of some features of *Anaplasma and Ehrlichia* genomes by means of graphs (Frutos *et al.,* 2006).

The X-axis represent abbreviations of the genomes which are: Erga, *Ehrlichia ruminantium* strain Gardel; Erwe, *Ehrlichia ruminantium* strain Welgevonden (CIRAD); Erwe, *Ehrlichia ruminantium* strain Welgevonden (ARC-OVI) (all *E. ruminantium* species are represented by black circles); Ecani, *Ehrlichia cani* strain Jake; Echaf, *Ehrlichia chaffeensis* strain Arkansas (both indicated by grey circles); Anmar, *Anaplasma marginale* strain St Maries; Anpha, *Anaplasma phagocytophilum* HZ (both indicated by white circles). **(A–D)** The circles represent the property indicated for each of the species shown on the X-axis (**A-C**) the histograms give the distribution of the same property for 394 fully sequenced bacteria and archaebacteria; the horizontal dotted line is the mean of this distribution. (**A**) Coding ratio (the percentage of the genome that encodes proteins).

Ehrlichia and particularly *E. ruminantium*, show a very low coding ratio. (**B**) Mean size of histogram and top circles with intergenic regions (histogram and bottom circles). The unusually long intergenic regions are the best characteristics for *Ehrlichia species*. (**C**) Tandem repeat density is defined as the number of bases covered by tandem repeats divided by the total genome size. High density of tandem repeats best characterizes *Ehrlichia.* (**D**) Percentage of intergenic regions covered by tandem repeats. Although tandem repeats of *E. ruminantium* cover up to <10% of intergenic regions, they account for almost the whole of the differences in genome size observed between the three strains (*Erga–Erwe* 13 kb; *Erwe–Erwo* 3 kb).

2.4. Transmission route of *E. ruminantium*

Normally, *E. ruminantium* is transmitted to susceptible ruminant animals from reservoir wildlife hosts by *Amblyomma* ticks, the natural vectors of heartwater disease. Mostly the widespread vector of heartwater is *Amblyomma variegatum*, distributed in the Sub-Saharan Africa and the Caribbean (Faburay *et al.,* 2007). *Amblyomma* ticks can inhibit itching and pain, homeostasis, wound healing and inflammation when biting. Moreover, this also results in modulation of the host adaptive and innate immune responses which further favour the transmission of *E. ruminantium* (Šimo *et al.,* 2017). This results in hydropericardium, pyrexia and nervous signs due to severe damage produced to the vascular endothelium (Blowey and Weaver*,* 2011). In Caribbean islands, heartwater began with the introduction of cattle infected by *A. variegatum*. Domestic ruminants that are more susceptible to heartwater disease include goats, sheep and cattle as well as other kinds of domesticated animals like dogs (Saito and Walker, 2016).

The route of transmission can either be through transovarial or transstadial means. Infected *Amblyomma* ticks transmit *E. ruminantium* when feeding on the susceptible host animal. Transmission normally takes 4 days for adult ticks and 2- 3 days for nymphs (Provost and Bezuidenhout, 1987). Vertical transmission may occur when calves ingest leukocytes in colostrum infected with *E. ruminantium* (Deem *et al.,* 1996).

2.5. Pathogenesis of heartwater disease

Because of its intracellular nature, *E. ruminantium* requires a set of specific virulence factors, such as type IV secretion system and the outer membrane proteins (Map proteins) to evade and deteriorate the immune system of the host cell. However, its expression of some virulence determinants can be linked to the fact that bacterial pathogenesis is highly associated with the mechanisms of iron uptake (Pinarello *et al.,* 2022). Although *Ehrlichia ruminantium* life cycle is still not clearly understood in both ruminants as well as in ticks. In ruminants, local multiplication occurs initially in macrophages and reticulo-endothelial cells. This is where the inoculation of the bacterium occurs in the infected tick. Immunomodulation action of the numerous salivary molecules inoculated during the blood meal of the tick makes easier the infection of the cells (Rodrigues *et al*., 2018).

Once *E. ruminantium* is introduced to the host cell, it adapts inside the intracytoplasmic inclusions in the endothelial cells and neutrophils. *E. ruminantium* has a complex life cycle within host endothelial cells. It is comprised of two forms called intracellular and extracellular, in which the intracellular form is known to have metabolically active reticulate bodies but non-infectious whereas the extracellular form is comprised of infectious elementary bodies (Marcelino *et al.,* 2021). Although some prevention and control strategies against heartwater have been produced but with limited efficiency. Ever since 1990, heartwater research pace has rapidly accelerated because of important advances in phylogeny, diagnosis, immunology and epidemiology. The present use of omics methods contributes to the identification of virulence factors for *E. ruminantium* providing new insights on the interactions between vector and pathogen with their

host. This knowledge provides clues for the development of improved vaccines against heartwater (Pinarello *et al.,* 2022). Antigenic diversity causes failure of different strains of *E. ruminantium* to induce heterologous cross protection (Dumler, 2005). Furthermore, genetic diversity also has impact on the rate of pathogenicity of different strains of *E. ruminantium* whereas some strains are non-pathogenic in nature while others appear to be highly virulent (Faburay *et al.,* 2017).

2.6. Four clinical forms of heartwater disease

This includes subclinical, acute, subacute and hyperacute form. The subclinical form may be unrecognized in field conditions, being the most insidious one. Rapid breathing, fever and mild apathy may go unnoticed until recovery, that occurs after a few days. *E. ruminantium* may be detected in the bloodstream only during the febrile period. It is commonly known as the parasite of endothelial cells with lesions being recorded in various systems and organs (Some *et al*., 2022). The most striking changes in most fatal cases include severe hydrothorax, hydropericardium, and in some cases, ascites. Congestion of the interlobular and parenchyma secta and Lung oedema are often observed. The bronchi and trachea often contain a fibrinous froth. The mucosa is congested and covered with petechial hemorrhages (Camus *et al.,* 1996b).

Hemorrhages and degeneration on myocardium may be common in the subacute and acute forms. Pathological changes on the digestive system are regular findings in cattle but, they are not common in goats and sheep. Lymph nodes are frequently edematous with petechial hemorrhages. In angora goats, kidneys may be highly congested. Oedema of the meninges and the brain

commonly occurs in ruminants suffering from acute and hyperacute forms. Normally, the entire brain is noticeably swollen, with the result that there is a partial herniation of the cerebellum through the foramen magnum (Thomas, 2016).

The acute form has 2-6 days incubation period been most the common in sheep and goats. The most typical symptoms of this form include depression, shortness of breath, presence of froth from the nose and dyspnea. Basically, diarrhea is present when cattle approach death with a temperature rise that exceeds 40°C. this temperature reaction may last for the entire disease period till the death of the animal (Gutiérrez and Simões, 2017). Furthermore, subacute form is characterized by the incubation period of 10 days. Mortality is lower, clinical signs are less severe, and death is sometimes due to secondary digestive or respiratory complications. The hyperacute form is likely to affect exotic goat breeds such as agora and Boer goat. It is characterized by a temperature reaction in which after some few hours, sudden death occurs (Camus *et al.,* 1996c).

2.7. Replication cycle of *E. ruminantium*

Replication begins in the mononuclear phagocytic system cells particularly in the regional lymph nodes wherein dissemination takes place through the blood stream with invasion of vascular endothelial cells. Following transmission, the organism adapts in the regional lymph nodes where replication occurs. Organisms are now located in granulocytes particularly in the neutrophils and plasma cells (Pinarello *et al*., 2022). This is referred to as the febrile stage which normally develops between 3 and 10 days after transmission. Although the cause of the disease seems to be

uncontrollable by antibodies. However, genetic factors play a crucial rule in the development of immunity against heartwater (Figure 2.5) (Saito and Walker, 2016).

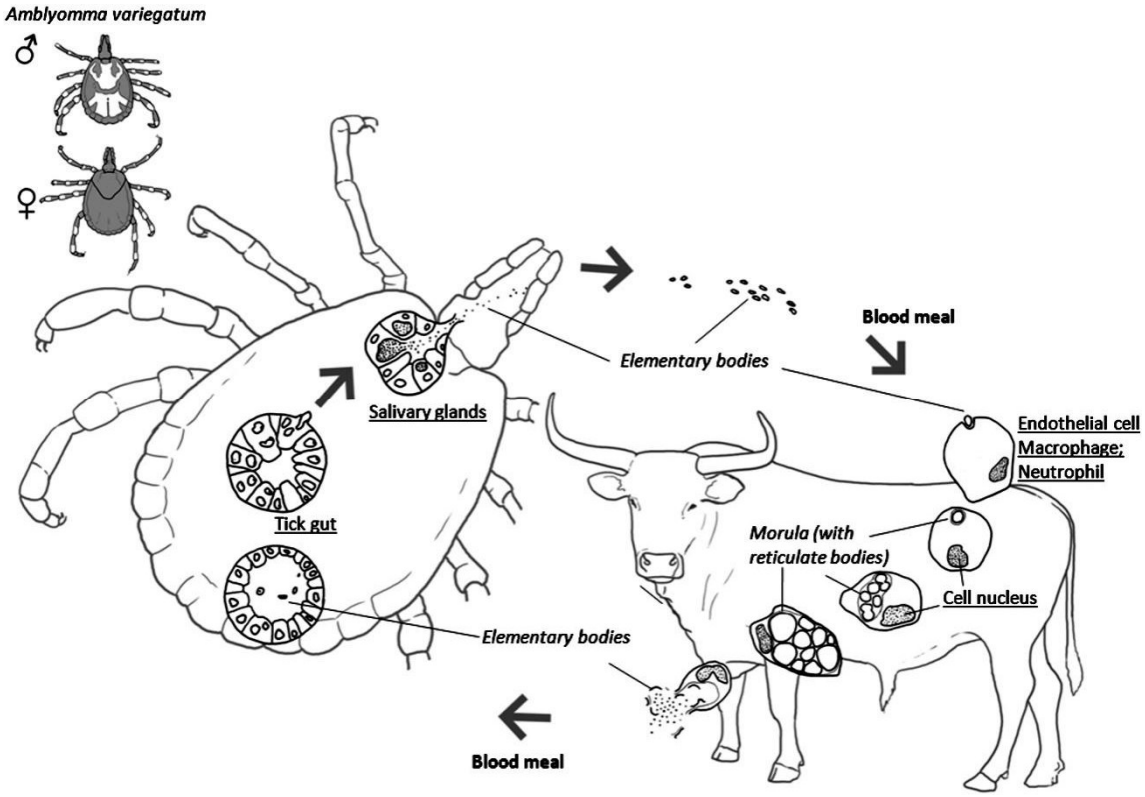

Figure 2.5: Replication cycle of *Ehrlichia ruminantium* (Marcelino *et al.*, 2012).

2.8. Clinical signs

Heartwater typically takes 18 days in cattle and 14 days in sheep and goats to incubate. There are many different clinical symptoms, and the disease severity ranges from mild to clinically inapparent. The age and breed of the afflicted animal have an impact on how the infection develops. For instance, the per acute form is frequently seen in Angora and Boer goats and affected animals may collapse abruptly and fade away in convulsions without exhibiting any other symptoms (Nair *et al*., 2021).

The severity of the tick challenge and the virulence of the genotype of the *E. ruminantium* strain involved are two additional crucial variables. Clinical diagnosis of heartwater in live animals is challenging due to the range of symptoms, particularly because many of the clinical symptoms lack definite diagnosis (Anifowose *et al*., 2020) Elevated fever, loss of appetite, heavy breathing, drooping head, stiff walk, depression, excessive blinking and chewing movements, anorexia, hyperaesthesia, lacrimation, convulsions, recumbency, and death are among the usual indications, which are listed in order of increasing severity. Most of these symptoms would not alone constitute a conclusive diagnosis, which ultimately rests on the post-mortem examination and laboratory diagnosis's recognition of *E. ruminantium* (Xuan *et al*., 2019).

2.9. Diagnosis

Normally, when *E. ruminantium* colonies are found in the brain capillaries of animals with clinically suspected heartwater during the disease's acute stage, a precise diagnosis can be made Figure 2.6 (Camus *et al*., 1996a). However, *E. ruminantium* can be difficult to be recovered from dead animals six hours after death once decomposition has begun and it is very weak in the environment (Latif *et al*., 2020).

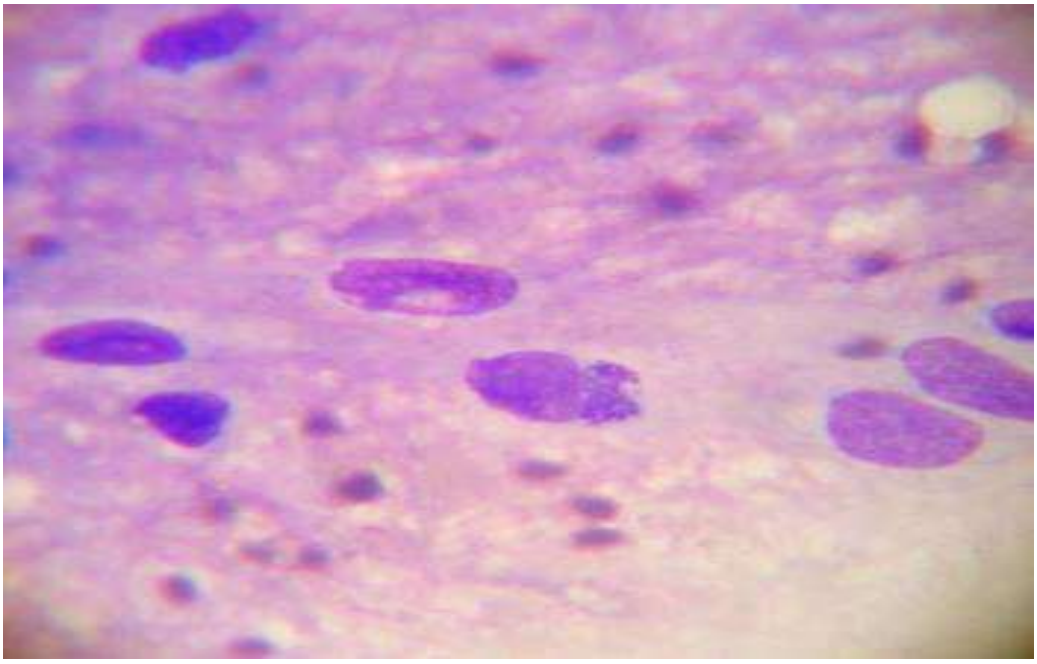

Figure 2.6: Brain smear of a naturally *E. ruminantium*–infected animal (https://www.merckvetmanual.com//media/manual/veterinary/images/e_ruminantium_high.jpg?la=en&thn=0&mw=350)

Several serological tests have been extensively employed for epidemiological studies, although they do not differentiate among different *Ehrlichia* species because of similarity considering antigenic organization (Dreher *et al.*, 2005). To increase diagnostic capacity for heartwater, PCR must be performed by testing tissues from dead animals. These methods could improve the understanding of the epidemiology of heartwater by their ability to detect low levels of *E. ruminantium* infection in *Amblyomma* ticks (Matos *et al.,* 2019).

The only accurate methods for diagnosing *E. ruminantium* have been made available by the PCR-based molecular genetic revolution in diagnostic procedures. The first genetic target found specifically for *E. ruminantium* diagnostics was the PCS20 genomic region (Tumwebaze *et al*., 2020) and it has demonstrated *E. ruminantium* specificity by showing no cross-reactions with other Ehrlichia species (Matos *et al*., 2019). It has been widely utilized to find the organism in pets, wild game, and ticks. It is the most sensitive of the *E. ruminantium* detection probes that are available (Peter *et al*., 2000). The PCS20 test has also been adapted to a quantitative real-time polymerase chain reaction format (Steyn *et al.*, 2008).

The MAP1 gene is extensively polymorphic and has been used as a diagnostic target for *E. ruminantium* in order to characterize different antigenic variants of the parasite (Allsopp and Allsopp, 2001). MAP1 gene has been found as a good tool to characterize the genetic diversity among Madagascar, Caribbean islands and African strains including new emerging isolates of *E. ruminantium*. Also, different MAP1 paralogs define different genotypes showing divergent evolution. Additionally, there is no correlation between the geographic origins of the strains and all MAP genotypes (Raliniaina *et al*., 2010).

2.10. Control and treatment methods

Basically, the methods implemented for the control of heartwater disease rely on chemical approaches. However, the methods require regular and frequent treatments which are expensive. Moreover, contamination of the environment, meat and milk with the chemical residues is the biggest disadvantage in the livestock production sectors (Gondard *et al.,* 2020).

Basically, control is primarily based on the use of chemicals such as acaricides. Although this control strategy is likely to result in chemical residues in milk, hides, meat and the environment, accumulating the cost of production (Pfeffer *et al.,* 2018). Understanding knowledge, attitude and practices of small household farmers is vital in future planning and fulfillment of effective control strategies. Tetracycline (i.e. oxytetracycline and doxycycline) is usually administrated at a dose of 10 mg/kg during the early febrile stage. This method is likely to result in recovery. However, in the advanced stages of the disease there is a serious need for proper consideration of additional supportive therapy even though it is not always successful. Nevertheless, antibiotics of the tetracycline family are efficient if given properly and rapidly, as soon as fever is noticed (Kerario *et al.,* 2018).

Normally, farmers also practice tick control making use of plants that possess pharmacologically active substances. *A. ferox* is commonly used in the treatment of heartwater disease. It has laxative effect because of a glycoside aloin. *Elephantorrhiza elephantina* is also used to treat heartwater (McGaw *et al*., 2020; Sanhokwe *et al.,* 2016).

In South Africa, there are limited number of vaccines available for the control of heartwater disease. In the past years, the only accessible vaccine was driven from infected blood of sheep.

The vaccine is given to the animals intravenously, followed by inoculation and treatment using antibiotics while monitoring body temperature. Basically, this is an infection and treatment method (Bezuidenhout *et al*., 1985).

Since new open reading frames for *E. ruminantium* can be discovered through a combination of proteomics and bioinformatics. This data is important for the investigation of new effective vaccines against heartwater disease (McBride *et al*., 2022). In general, the type of immune responses likely to mediate protection guides approaches used for identification of vaccine candidates specifically for recombinant vaccine development (Thema *et al.,* 2016). Recently, bioinformatics tools are being utilized successfully in search for available vaccine candidates. Genes expressed in the elementary bodies might be important for infection of tick host and mammalian cells. However, those strictly expressed in the red blood cells may be crucial. Alternatively, those completely expressed in the red blood cells may be vital for survival in the host cells. Secreted or expressed proteins completely expressed at this stage are excellent targets for the stimulant of within host cytotoxic T-lymphocyte immune responses (Tjale *et al.,* 2018).

Molecular characterization of *E. ruminantium* will contribute to better understanding its biology and possibly to the future production of new effective vaccines. Although, the control of heartwater disease is likely to depend on chemical treatment and vaccination (Latif *et al.,* 2020). However, there are arguments in the agreement of utilizing an attenuated vaccine for control of heartwater disease. The effectiveness of the vaccine on the bases of Welgevonden stock should be under evaluation in the field trials. Prior to utilization of this vaccine outside of South Africa, a study on the possibility of reversal virulence must be conducted to eliminate it before new genotypes can

be introduced where it might not already occur. Although, this vaccine grants cross-protection upon most isolates present in South Africa (Zweygarth *et al.,* 2008).

2.10.1. Vaccine development

Antibiotics were first introduced in 1945, which sparked the creation of a still-used infection and treatment control strategy (Neitz and Alexander, 1945). After at least 50 years of work, a method for the continuous in vitro cultivation of the organism was introduced in 1985 (Jongejan, 1991). The *E. ruminantium* genome has been fully sequenced and annotated (Collins *et al*., 2005), and it appears to be the first whole genome discovered in Africa. Additionally, in recent years, two brand-new experimental vaccines have been created: a recombinant vaccine in 2003 (Collins *et al*., 2003) and an attenuated vaccine in 2005 (Zweygarth *et al*., 2008).

Developing a new effective and improved subunit vaccine would provide great social, ethical and economic benefits. Generally, advances employed to determine vaccine candidates for recombinant vaccine development are influenced by the type of immune response that are more likely to mediate protection (Thema *et al.*, 2019). Recently, there is no reliable vaccine for heartwater disease (Faburay *et al*., 2017). In a study conducted by Van Kleef *et al.* (2000) purified proteins of *E. ruminantium* were demonstrated in vitro to stimulate lymphocytes to proliferate and produce IFN-γ from infected goats, cattle and sheep inoculated with inactivated organisms (Van Kleef *et al.*, 2000; Esteves *at al*., 2004).

2.11. Molecular epidemiology of heartwater disease

Heartwater affects both domestic and wild ruminant animals because of the presence of tick vectors, particularly in Africa (Allsopp, 2010). The distribution of heartwater varies significantly across the globe. Heartwater has been reported in the islands of Antigua, Marie-Galante, and Guadeloupe. *Ehrlichia ruminantium* prevalence in these regions during the years 2003 to 2005 in *A. variegatum* ticks reached 6% in Antigua, 36% in Marie-Galante and 37% in Guadeloupe (Stachurski *et al.,* 2019). Serologically, evidence for *E. ruminantium* was reported in Montserrat and Martinique in asymptomatic sheep. However, results showed cross reactions with closely related *Ehrlichia* spp. (Torina *et al.,* 2020). Whereas *E. ruminantium* presently seems to be deprived to three islands, there is possibility of pathogen introduction in present *E. ruminantium*-free areas because of the wide distribution of the *A. variegatum* vector throughout the West Indies (Gondard *et al*., 2017).

In Africa, heartwater disease is one of the major obstacles in the livestock production of Cameroon for example, particularly in the cattle industry (Pamo, 2008). Currently, there is limited knowledge for the presence of heartwater in cattle in many parts of the continent. This encourages the requirement for more studies to be conducted focusing on the determination of the role and extent of heartwater in the cattle industry (Esemu *et al.,* 2018). This is a serious challenge to the department of food and nutrient security (Allsopp, 2015). In central and dry south areas of Namibia, heartwater is not available since *Amblyomma* ticks cannot survive. However, the vector survives in the northeast areas characterized by average rainfall of 650 mm per year (Pascucci *et al.,* 2014).

South Africa started to recognize heartwater in the 19th century. It was determined to be a tick-borne disease in the 1900s. The seasonal occurrence of the tick vector, *A. hebraeum* is dependent on climate changes. There are three stages of the life cycle of the host. This includes nymphs, larvae and adults feeding on separate hosts. Basically, nymphs survive in large numbers during winter and spring months, whereas larvae during late autumn, colder, dry and winter months. Adults tend to survive during wet and warm summer months. Varying numbers of all developmental stages can be found on hosts all over the year. This appears to be the major obstacle that prevents the upgrade of local livestock production (Jongejan *et al.,* 2020).

Ehrlichia ruminantium was identified in 1925 and cultured in-vitro in 1985. This finding encouraged scientists to conduct more research on *E. ruminantium*. Since biology was emerging into the molecular genetic age. Over the past years, there have been more improvements in our understanding of *E. ruminantium.* This has yielded more improvements in genetic characterization, immunology, phylogeny, epidemiology, diagnosis and vaccine developments (Allsopp, 2015).

In South Africa, *E. ruminantium* is found endemic mostly in dry and hot areas of Limpopo, Eastern Cape and KwaZulu-Natal provinces. *A. hebraeum* is found widespread in these areas as well. Traditionally, the survival of tick vectors is influenced by environmental conditions that drives the distribution of Heartwater disease (Mdladla *et al.,* 2016). The disease is among the tick-borne diseases that cause economic loses resulting in high rates of disease and death rates among the infected animals (Mapholi *et al.,* 2016). Although the control of ticks remains a serious challenge in the livestock production industries particularly in the subtropical and tropical areas of the world. This is because there are still ongoing reports on reduced wellbeing and economic loses of

ruminant animals (Rajput *et al.,* 2006). Conducting PCR-based tests in comparison appears to be highly specific and sensitive. This could improve our understanding of the epidemiology of heartwater by their ability to detect low levels of *E. ruminantium* infection in *Amblyomma* ticks (Faburay *et al.,* 2007).

2.12. New insights for epidemiology and control of heartwater disease

Animals respond in different ways whenever they are exposed to heartwater. However, this depends on several factors including stock of *E. ruminantium,* breed, species, immune status and age of the animal. In 2005, heartwater losses in Southern Africa were approximately R189.6 million (Spickett *et al.,* 2011). Exotic goats and sheep breeds are of high mortalities and susceptibility of 50% or greater because heartwater have been seen in cattle and sheep imported into sub-Suharan Africa. Agora goats are the more receptive animals to heartwater compared to all the domestic ruminants, with mortality rates exceeding 90% in imported stock. Merino sheep are classified as more susceptible ruminants to heartwater disease. Principally, it is not always possible to judge if resistance has been acquired, through natural selection that favours survival of the least susceptible animals upon prolonged resistance, exposure or a characteristic of a breed (Garcia *et al*., 2022) Although, the impact of goats breeds and systems of production on each ecological zone on the occurrence of heartwater disease has not yet been investigated. This kind of interactions could be important to the development of sustainable and effective disease control strategies (Mdladla *et al.,* 2016).

2.13. Distribution and prevalence of *E. ruminantium*

Since *E. ruminantium* is transmitted by ticks in the genus *Amblyomma*. Its spread is determined by the distribution of *Amblyomma* species, especially *A. variegatum* and *A. hebraeum,* which is the main vector in southern Africa. As a result, the majority of sub-Saharan Africa as well as its adjacent islands in east and west Africa are home to *E. ruminantium*. The primary tick vector *A. variegatum* and *E. ruminantium* were established into the Lesser Antilles from Senegal during the slave trade in the 18th or 19th century (Sili *et al.,* 2021).

E. ruminantium is found in eastern and northern parts of south Africa, 54%, 35% and 12% of total goat, cattle and sheep are estimated to be at high risk of contracting heartwater (Spickett *et al*., 2009). Nearly all sub-Saharan African nations, as well as islands in the Indian Ocean, Atlantic Ocean, and Caribbean, have documented cases of *E. ruminantium*. In southern Africa, it is one of the most commercially significant pathogens of livestock illnesses (Molepo *et al*., 2022; Allsopp *et al*., 2005). It is confined to the north-eastern regions of South Africa, starting in the province of Northwest, moving through Limpopo and the north-eastern regions of Mpumalanga, along the coastal region of KwaZulu-Natal, and ending in the Eastern Cape (Salim *et al*., 2019). Figure 2.7 shows the potential distribution of *E. ruminantium* based on habitat stability.

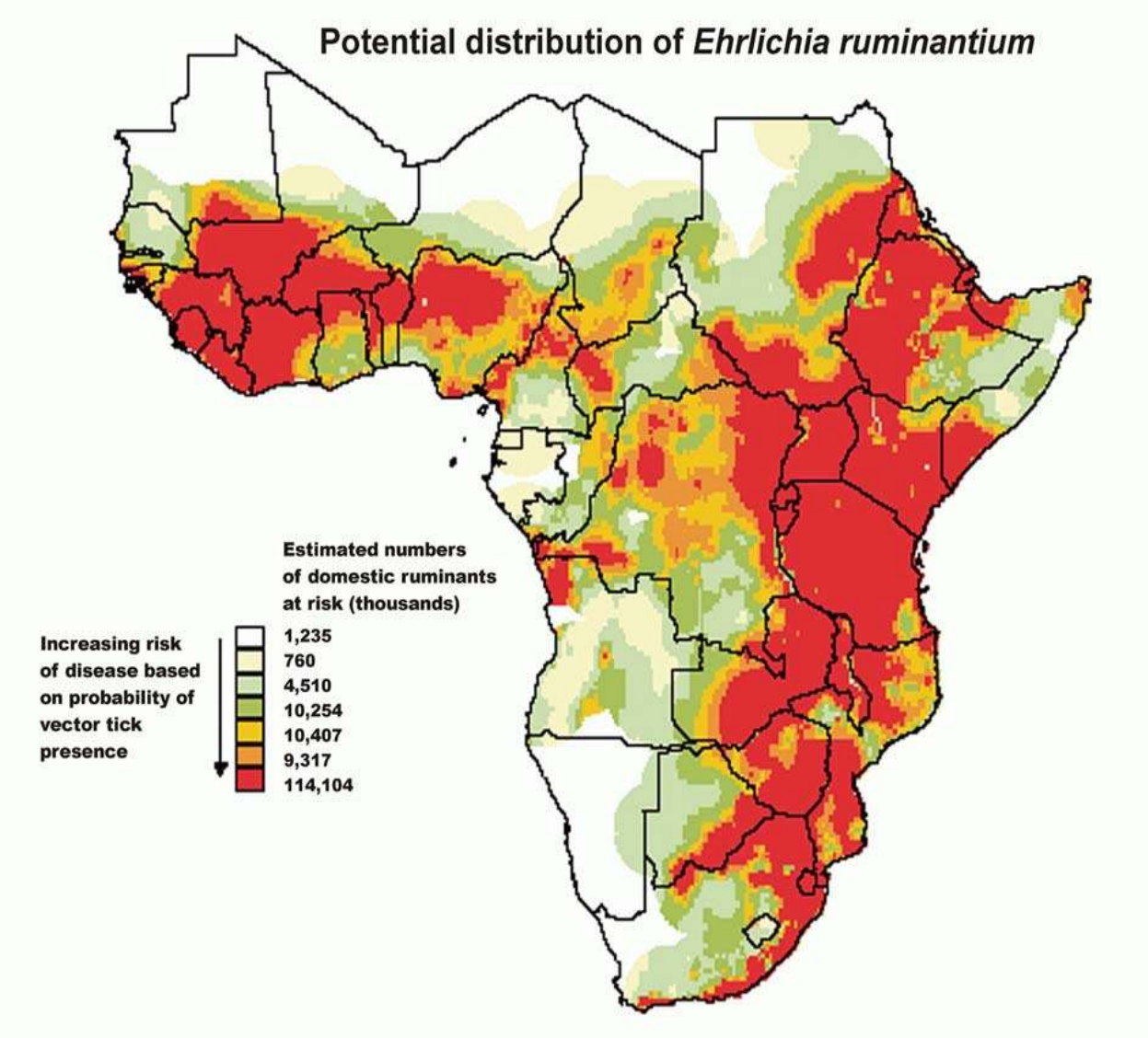

Figure 2.7: Potential distribution of *E. ruminantium* based on habitat stability (Allsopp, 2010).

2.14. *E. ruminantium* virulent genotype strains

Genome sequences from the Sankat 430 strain from Ghana, the Kerr Seringe strain from the Gambia, and the Crystal Springs strain from Zimbabwe have all been revealed. Because the Welgevonden strain has been sequenced by two distinct groups, there are ten genome sequences for *E. ruminantium* that collectively represent nine different strains. Welgevonden and Senegal are the two strains that have been isolated the longest, having done so since 1981. Ker Seringe, the "youngest" sequenced strain, was isolated in 2001 (Nakao *et al*., 2016). Table 2.1 shows the *E. ruminantium* genomes available at GENBANK.

Table 2.1: *E. ruminantium* genomes available in the GENBANK.

Country of origin	Strain name	Year1	Year2	Accession number	Size	#of contigs	Submitter	Reference
Ghana	Pokoase	1996	2016	BDDM00000000	1.47	390	Hokkaido	Bell-sakyi *et al.*, 1997
Ghana	Sankat 430	1996	2016	BDDN00000000	1.46	183	Hokkaido	Bell-sakyi *et al.*, 1997
Senegal	Senegal (vir)	1981	2017	MQUJ00000000	1.46	8	CIRAD	Jongejan *et al.*, 1988
Senegal	Senegal (p63)	N/A	2017	MRDC00000000	1.46	8	CIRAD	Jongejan, 1991
The Gambia	Ker Seringe	2001	2016	BDDL00000000	1.45	118	Hokkaido	Faburay *et al.*, 2005
Caribbean	Gardel	1982	2006	CR925677	1.50	1	CIRAD	Uilenberg *et al.*, 1985
Zimbabwe	Crystal Springs	1990	2016	BDDK00000000	1.48	34	Hokkaido	Byrom *et al.*, 1991
Zimbabwe	Palm River	1989	2016	LUFS00000000	1.49	368	µFORGE	Byrom *et al.*, 1991
South Africa	Welgevonden	1981	2005	CR767821	1.52	1	UP	Du Plessis, 1985
South Africa	Welgevonden	1981	2006	CR925678	.51	1	CIRAD	Du Plessis, 1985

Year1= isolated from the field

Year2 =sequence published

#of contigs = Number of contigs

2.15. Genotypes of ribosomal RNA

It is important to attempt and determine the specific genotype of *E. ruminantium* due to the notable variations in the biological properties of the organism that have been observed. According to a recent analysis of average nucleotide identity among fully sequenced prokaryotic genomes, a level of 70% DNA-DNA reassociation correlates, on average, to 93-94% and 99% sequence identity for the srRNA gene (Rodriguez-R *et al*., 2018). A widely used phylogenetic and taxonomic tool for classifying bacteria is the srRNA gene (Edwards *et al*., 2017).

A variety of virulent genotype strains, including Ball3, Welgevonden, Gardel, and Mara 87/7, to name a few, characterize the *E. ruminantium*. Among the different genotypes of *E. ruminantium*, there appears to be extensive gene recombination, which suggests that lately in the field, recombined strains are rapidly developing. This is probably going to occur when before intracellular infection develops, pathogens are already extracellular in the tick following a blood meal (Mnisi *et al*., 2022). There are eight different srRNA genotypes of *E. ruminantium*. They are each representing a sequence identity greater than 99.4% in consideration of the others (Allsopp, 2010). Below is the table with a summary of essential reference data of the eight genotypes of *E. ruminantium* (Table 2.2).

Table 2.2: Important information of eight different srRNA genotypes of *E. ruminantium (*Allsopp, 2010)

Genotype	**Habitat**		**Pathogenicity**			**Accession number**	**References**
	Geographical	**Biological**	**Mice**	**Cattle**	**Sheep goats**		
Pretoria North	South Africa	Dog	N/A	N/A	N/A	AF325175	Allsopp and Allsopp, 2001
Welgevonden	South Africa	*A. hebraeum*	P	P	P	U49843	Du Plessis, 1985
Senegal	Senegal	Bovine	MP	P	P	X74250	Jongejan *et al*., 1988
Mara 87/7	South Africa	*A. hebraeum*	P	P	P	AF368008	Marcelino *et al*., 2021
Omatjenne	Namibia	*H. truncatum*	NP	NP	MP	AF368012	Du Plessis *et al*., 1990
Gardel	Guadeloupe	*A. hebraeum*	NI	P	P	U50832	Matos *et al*., 2019
Ball3	South Africa	Bovine	NP	P	P	AF355200	Bell-Sakyi *et al*., 2018
Kiswani	Kenya	Bovine	N/A	P	P	None	Raliniaina *et al.,* 2010

P= pathogenic, NP= Non Pathogenic, MP= Middle pathogenic, NI= Non infective, N/A= Not available (Adapted from Allsopp, 2010)

2.16. Prevalence of *E. ruminantium* from various countries

Several studies have been conducted in South Africa with the aim of determining the distribution of *E. ruminantium* (Adelabu *et al*., 2020; Mnisi *et al*., 2022; Steyn and Pretorius, 2020). A study conducted in Pakistan has documented *E. ruminantium* prevalence of 11.98% from bovine (Basit *et al*., 2022). Another study reported a 14% prevalence in bovines by PCR (Baticados *et al*., 2010). A 3.6% *E. ruminantium* prevalence in cattle from China have been documented. Additionally, a very low 1.7% prevalence of *E. ruminantium* has been reported from cattle in China (Guo *et al*., 2018). Below is a table highlighting prevalence of *E. ruminantium* from various countries Table 2.3.

Table 2.3: Prevalence of *E. ruminantium* as documented from various countries.

Region	**Country**	**Sample type (Target gene)**	**Source of sample**	**Number of samples**	**Prevalence (%)**	**Reference**
Somali region (Eastern)	Ethiopia	*Amblyomma gemma* (PCS20 and 16S rRNA)	Camels and cattle	104	4.8	Tomassone, *et al.,* 2012
Free State and KwaZulu-Natal	South Africa	Blood (PCS20)	Goats and sheep	91	14.3	Ringo *et al*., 2018
North and South west regions	Cameroon	*Amblyomma variegatum* (PCS20)	Cattle	500	28.4	Esemu *et al.,* 2013
Khartoum and East Darfur State	Sudan	*Amblyomma variegatum* (PCS20)	Cattle	536	10.07	Mossaad *et al.*, 2021
Ogun	Nigeria	*Amblyomma variegatum* (PCS20)	Cattle	252	50	Anifowose *et al.,* 2020
Pemba Island	Tanzania	Blood (PCS20)	Cattle	245	7.4	Ringo *et al*., 2019
Southern region	Malawi	Blood (16S ribosomal RNA)	Sheep and goats	107	4	Chatanga *et al.,* 2021
Maputo province	Mozambique	Blood (PCS20)	Cattle	210	15	Matos *et al.*, 2019

Chapter 3: Knowledge, attitude and practices of small household farmers towards heartwater disease in rural areas of Mopani and Vhembe Districts, South Africa

Abstract

Background: In most African countries, heartwater is responsible for the majority of the economic losses in cattle, goats and sheep (livestock) production. Community involvement and awareness through conducting knowledge, attitude and practices (KAP) studies are regarded as one of the important strategies for the sustainability and success of any programme for disease control. The aim of the study was to investigate small household farmers' knowledge, attitude and practices towards heartwater disease in rural areas of Mopani and Vhembe districts, South Africa.

Methodology: A total of 121 small holder farmers were recruited in the study after informed consent was obtained. A semi-structured questionnaire consisting of 47 questions translated to Xitsonga and Tshivenda was used for data collection conducted between September and December 2020. Collected data was entered in the excel spreadsheet and analysed using SPSS. Chi-square ($\chi 2$) test was used to determine the potential association between different characteristics of the cattle, sheep, goats and heartwater infection as well as other parameters such as tick control methods, feeding habits etc. The differences among the villages were considered significant at $P \leq 0.05$.

Results: Our study showed that the general knowledge of heartwater disease was fairly low among the farmers (23.1%). A fairly high proportion (76%) of the respondents associated heartwater disease with air-borne transmission. Overall, 13.2% of respondents had heard of the available vaccine for heartwater. A high proportion of 94.2% respondents had a favourable opinion of having knowledge about heartwater disease. Additionally, a total of 68.6% of the respondents correctly associated ticks with animal diseases. A high proportion (74.4%) indicated that they are not in contact with a state veterinarian office while 65.3% highlighted that the government is not helping enough in their livestock farming.

Conclusion: In our sampled group, adherence to preventive measures such as heartwater vaccination was inadequate. This is because respondents have limited knowledge towards heartwater transmission, symptoms and treatment. At the same time provision of livestock services in rural communities is inadequate. The findings of the present study may assist in supplementing the already available information in the implementation of future heartwater control strategies.

3.1. Introduction

Heartwater disease affects both domestic and wild ruminants (Pascucci *et al.,* 2007). Heartwater disease is responsible for more than 60% of all cattle death in South Africa (Sungirai *et al.*, 2016). The disease poses a negative impact to the development of livestock breeding in Africa as a whole. Although, livestock farming is important to 70% of people living under disadvantaged backgrounds whose majority are residing in rural areas little research is being done to understand the knowledge and attitude of the farmers toward certain important diseases (Moegi, 2022).

Universal economic losses due to tick-borne diseases have been put at 18.7 billion of US dollars per year (De Clercq *et al.,* 2012).

The losses are caused by effects of ticks as they suck blood from the animals. The vectors also lead to fertility problems, reduced growth rates, reduced value of hides, increases in livestock mortality rates and decline in milk production. Additionally, farmers fail to adapt fully to the costs associated with control and treatment of the disease thereby increasing number of endemic areas (Van den Heever *et al*., 2022). The best control of heartwater is achieved through the control of the *Amblyomma* ticks (Mnisi *et al*., 2022).

Amblyomma hebraeum and *variegatum* ticks are emerging arthropod of veterinary and economic importance. There is little information in South Africa with regards to KAP study of heartwater disease among small household farmers. In other parts of the world, knowledge, attitude and practices studies are commonly utilized to document missing information on KAP of small household farmers towards veterinary important diseases like heartwater (Pike, 2016).

Ruminants such as cattle, sheep and goats contribute to reduce poverty in rural communities (Seketeme *et al*., 2022). Approximately 80% of cattle population in the world are at high risk of heartwater. Ticks pose a number of effects in the livestock population. This includes opening skin wounds making the animal more prone to secondary infection, cause toxicosis, severe skin damage and paralysis in most instances (Benelli *et al.,* 2016).

There is a need for new products that will aid in the control of ticks that causes heartwater. This is as a result of tick resistance evolution to acaricides (Itenge *et al.,* 2020). There are several factors associated with acaricide use including contamination of milk and meat of livestock and

environmental pollution all over the world. These factors have promoted the search for alternative methods of control that present some level of correspondence with principles of sustainable agriculture. Apart from utilizing acaricides, plastering all surfaces with smooth cement to block cracks and crevices may avoid tick infestation of livestock sheds. Nevertheless, this can be performed by the farmers who have a separate shed for keeping their livestock (Ghosh *et al.,* 2007). However, many farmers cannot afford using modern drugs as they are not affordable. Hence, small household farmers rely on their indigenous knowledge, practices, and accessible plants in the management of diseases of their livestock animals (Yineger *et al.,* 2008). Ticks are the major breeding, health and productivity concerns in livestock farming. The cost of acaricides together with residues in food, loss of livestock, undesirable effects on the environment and development of resistance by ticks, are some of the challenges in relation to the use of acaricides (Solomon and Tanga, 2020).

Approximately three quarters of the livestock animals are bred under small households. Normally, children and women are the most caretakers of livestock animals. In South Africa, over 60% of people live in rural communities where in most instances small household farming is practiced. However, the internationally agreed standard set of recommendations on how to compile measures of economic activity underestimate the contribution of small household farming communities to national economies. As a result, many important non-food outputs are not included in the calculations (Marandure *et al.,* 2020). Upgrading the adaptability and productibility of livestock farming is therefore important to assist in sustainable food security and agricultural development in rural communities (Leonard, 2022).

Improper livestock management, poor sanitation, and introduction of new animals into a herd promote the spread of heartwater disease. The prevalence of heartwater agent leads to low growth rate and high mortality rates. Hence, *E. ruminantium* becomes one of the major obstacles in the industry of livestock production worldwide. It becomes more important to investigate the farmer's knowledge, attitude and practices towards heartwater disease (Msimang *et al*., 2022). This is achievable through conducting epidemiological interviews where farmers are involved in defining heartwater, impact of ticks on livestock animals and developing solutions to those problems.

Knowledge, attitude and practices studies provide a good platform to the initiation of more effective management of livestock diseases. It is also important to provide an accurate definition of the distribution of heartwater disease risk and prevalence of infection in the vector population. As a result, this will promote an urge to upgrade the livestock industry and prioritize future research on the development of improved control measures (Faburay *et al.,* 2007). Community involvement and awareness through conducting KAP studies are regarded as one of the important tools for the sustainability and success of any programme for disease control (Govere *et al.,* 2000). Therefore, the current study concentrates on assessing knowledge, attitude and practices of small household farmers towards heartwater disease in different villages in the Mopani and Vhembe regions.

3.2. Materials and Methods

3.2.1. Ethical clearance

Ethical clearance certificate was obtained from the University of Venda Health and ethics committee. The objectives of the study were clearly explained and accepted by the owners of livestock who participated by giving their information and allowing access to their livestock. All participated farmers were requested to sign a consent form (refer to appendix: B) and to complete a questionnaire (appendix: A).

3.2.2. Study sites

This study was conducted at villages including, Bungeni, Khalavha, Kutama, Matsa, Mitititi, Tshikonelo, Tshivhilidulu and Xikukwani of Mopani and Vhembe regions in Limpopo Province. Mitititi and Xikukwani fall under Mopani region which is dominated by Xitsonga-speaking people. Bungeni, Khalavha, Kutama, Matsa, Tshikonelo and Tshivhilidulu fall under Vhembe region which is dominated by Tshivenda-speaking people. From the selected villages the most domesticated livestock animals are cattle, goats and sheep (Figure 3.1).

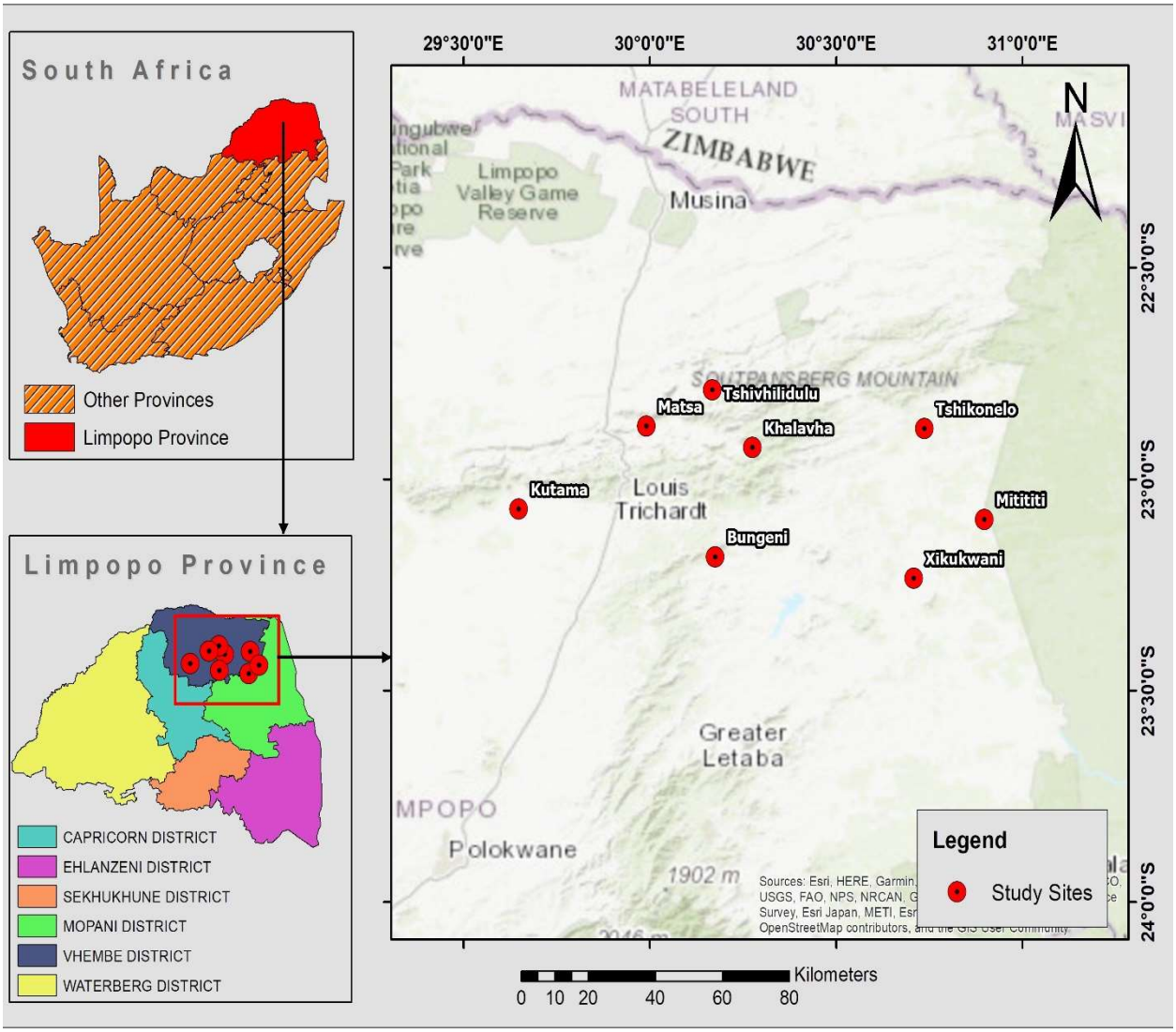

Figure 3.1: Map of Limpopo province showing sampled areas indicated by the red circles.

3.2.3. Inclusion and exclusion criteria

This is a cross-sectional study conducted in Mopani and Vhembe regions with interest on ruminants of any age arranged into categories (< than 1, 1-2, 3-4 and 5 or more years old). Permission for sample collection was requested in a form of a formal letter that was given to the chiefs of the villages. All farmers with interest of allowing their animals to enrol in the study were given the chance to participate.

Farmers were chosen irrespective of their economic background. Those who use chemical tick control strategies and those who are not practicing it were given a chance to allow their animals to participate. Prior to sampling, the researcher asked the farmer for assistance in identifying the animals according to the categorized ages. Four to five ticks were considered for the study per animal in a farm. However, 5 animals were randomly sampled in a farm of more than 10 animals. For farmers with less than 6 animals, 2 to 3 animals were selected in a farm for sample collection.

3.2.4. Data collection technique

Trained project assistants from the university of Venda, Department of Microbiology: Molecular Parasitology and Opportunistic Infections Programme were employed during data collection. This promoted our idea to recruit small household farmers and conduct the interviews. Prior to using the questionnaire, piloting was conducted to ensure every question is clear, comprehensive and appropriate. The objectives and concepts of the study were clearly explained to the farmers before an animal was enrolled in the study. Each farmer signed a consent form (Appendix B) in agreement of participation in the study. Trained project assistants administered questionnaire to the small

household farmers that were 18 years and older. Each completed questionnaire was assigned a code in relation to the farmer's details. The questionnaire was in English, Tshivenda (Appendix B1) and Xitsonga (Appendix B2) as these are the local spoken languages of the villages in Vhembe and Mopani regions.

The survey questionnaire comprised of the following sections: participant's demographics; knowledge, attitude and practices (KAP) of small household farmers towards heartwater disease. A questionnaire and conducting interviews with the small household farmers were chosen as the data collection technique to secure appropriate methods of data collection. Researchers who are not associated with the current study, however having relevant expertise and knowledge, checked the questionnaire to certify that questions were phrased correctly and that all the important information needed for the study was captured.

Data collection was conducted through a purposive sampling technique and the sampling frame was districts, villages and finally households. The purposive sampling was used to select the districts and villages which are situated in heartwater disease risk areas. This was driven by the findings from the previous studies that have been conducted in 2016 and 2017. In each of the two districts, villages practicing livestock farming were selected. This forms a total of nine villages wherein a village with households keeping cattle, sheep or goats were selected randomly. Ten to thirty households per village were sampled, giving a questionnaire sample size of 121 in total. The heads of the households were the main respondents. Although, other members of the household were given an opportunity to provide supplementary information.

The questionnaire was developed to collect detailed demographic information and KAP related data of livestock farmers about heartwater disease. The demographic information collected included gender, household income, age of farmer, number of people staying in the household and level of education. The livestock farm associated details included different types and number of animals domesticated in the household. However, only breeds of animals sampled (cattle, goats and sheep) were recorded. For knowledge assessment of the livestock farmers, the basic questions were asked about heartwater disease. Initially, the farmers were asked if they have heard about heartwater disease and were later asked what they know most about the disease. They were also asked to select the mode of transmission from the options provided. The answer was only considered 'correct' if the farmer was able to tick the correct option from the list. Farmers were also asked to mention few animals that can be affected by heartwater disease. However, the answer was considered 'correct' if the farmer managed to correctly identify at least one of the animal hosts. Furthermore, additional selection of an animal host unrelated to heartwater disease was considered as an incorrect answer.

Attitude related information about services provided in the area about animal farming was also asked. This was conducted by asking farmers if they are in contact with a state veterinarian in their area and their opinions for including a state veterinarian in their farming. Moreover, information about how farmers are producing in their livestock farming was also requested. Farmers were also asked to highlight on the association of any diseases experienced on the animals with ticks. Any concern related to ticks for livestock farming was also mentioned.

Information related to practices included the period by which a farmer has been practicing livestock farming, availability of the farmer to look after the livestock, record of animals who died

in the past years as a result of diseases, number of deaths. Furthermore, farmers were also asked if they practice tick control methods (spraying/dipping/spot treatment). The habit of using supplementary tick control method, medicinal plants and if they know of any medicinal plants that can be used to treat heartwater and to control ticks. Farmers were asked of information related to how their animals feed considering where and at what time do they start feeding. Additionally, farmers were asked if they once experienced other kind of illnesses as a means of finding out if their animals are affected by heartwater disease or not. Furthermore, other diseases animals suffered from were also mentioned.

3.2.5. Focus group discussion.

Following individually conducted interviews, focus group discussion (FGD) was conducted in a respondent moderator format. The researcher recruited a participant in the group to take a role of a moderator. The main aim of the FGD was to gather more information that confirms what was already obtained from the questionnaires. Moreover, FGD assisted us to see whether some new ideas emerged or not. This discussion consisted of 9 randomly selected individuals who managed to honour the invitation. Six individuals from the study site who were already participants (one of which served as the moderator), one investigator and two assistants. The moderator served to facilitate the discussion ensuring a smooth flow of the discussion, also ensuring that participants did not diverge from the topic under discussion, as well as ensuring participation by all members of the FGD (Moyo *et al.,* 2017). The two assistants served to keep record of the participants' responses following each consent. Each participants' responses were collected with a tape record for correlation with what was written in the questionnaires.

3.2.6. Data analysis

The KAP related information collected were coded and recorded into excel spreadsheets. The data obtained from this study were analysed using the Statistical Package for Social Sciences (SPSS) version 22.0. Chi-square ($\chi 2$) test was used to determine the potential association between different characteristics of the cattle, sheep, goats and heartwater infection as well as other parameters such as tick control methods, feeding habits etc. The percentage of famers' response for a particular variable such as age and education of the farmers, experience in keeping livestock, other diseases experienced on animals, tick control method used, whether they practiced dipping/spraying/ spot treatment and types of animals kept in the household were compared among the villages using a chi–square test to determine whether the proportions in the four municipalities were different from each other. The differences among the villages were considered significant at $P \leq 0.05$.

3.3. Results

3.3.1. Demographic information of the farmers interviewed.

The demographic data of the household farmers is shown in Table 3.1. Majority of the respondents were males (67.8%). Most of the respondents had secondary education (33.6%). Household income within 121 small household farmers varied from < 500 to > 20000. However, livestock keeping happened to be the main source of livelihood among all (100%) of the respondents.

Table 3.1: Demographic information of the farmers interviewed.

Category	Characteristics	Frequency	Percentage (%)
Gender of farmer	Male	82	67.8
	Female	39	32.2
Age of farmer	15-20	3	2.5
	21-30	5	4.1
	31-40	12	9.9
	41-50	17	14.0
	51-60	23	19.0
	Above 60	61	50.4
Household income	<500	20	16.5
	500-1500	26	21.5
	1500-7500	61	50.4
	7500-20000	3	2.5
	>20000	1	0.8
	No income	10	8.3
Level of education	No formal education	29	24.4
	Primary education	32	26.9
	Secondary education	40	33.6
	Tertiary education	18	15.1
	No answer	2	1.7

3.3.2. The different types of livestock kept per household.

The different types of livestock kept per household are shown in Figure 3.2. Majority of the respondents had goats 81.0% (98/121). Followed by cattle 42.1% (51/121). However, chicken also happened to be common amongst most respondents 35.5% (43/121). Dogs are found in the high proportion among small household farmers with a total of 32.2% (39/121). However, pigs are rare types of livestock found within visited small household farmers 14.0% (17/121). Sheep 7.4% (9/121), Donkeys 6.6% (8/121) were also found in lower proportion as compared to other types of livestock animals found among small household farmers. Few small household farmers mentioned other types of livestock 6.6% (8/121).

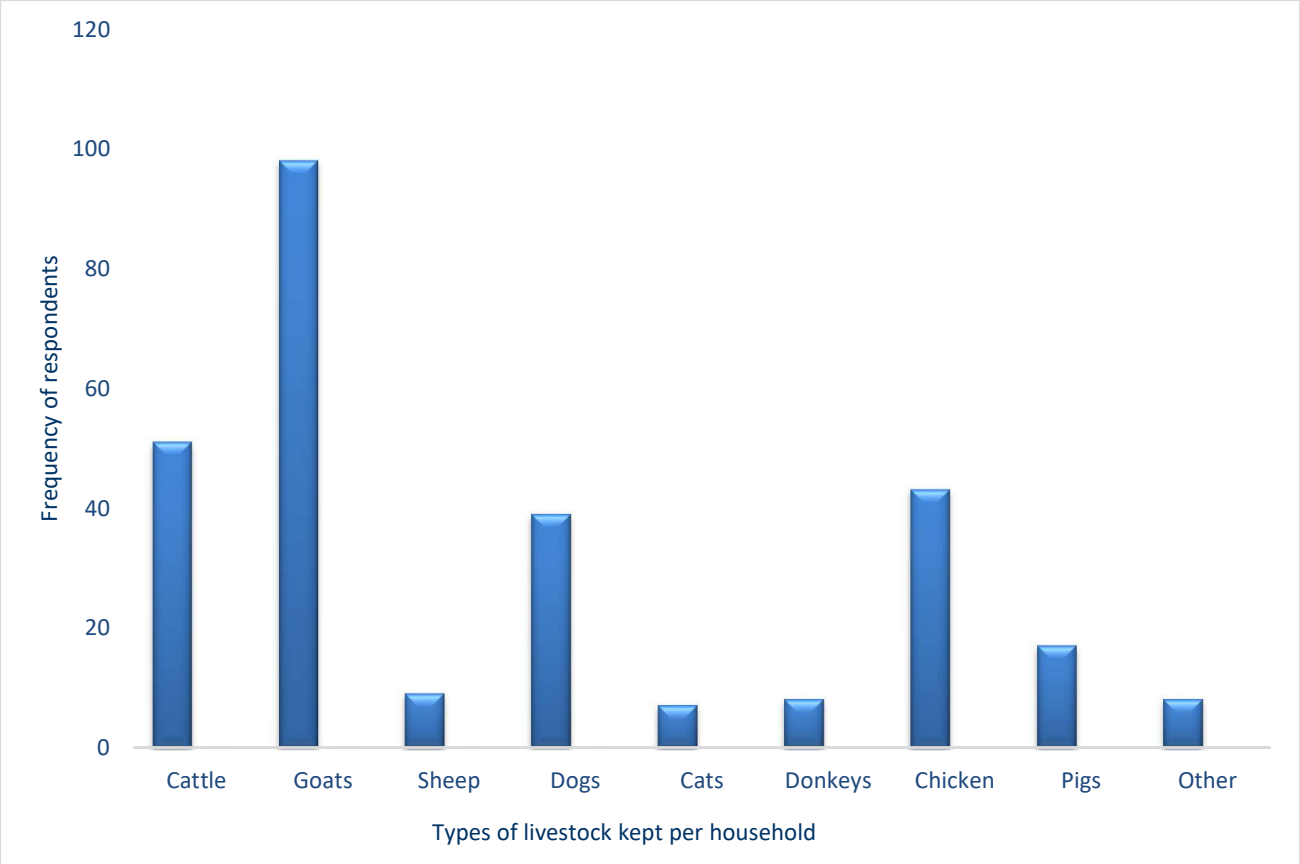

Figure 3.2: The different types of livestock kept by household farmers in the Vhembe and Mopani Districts.

3.3.3. Knowledge of small household farmers towards heartwater disease

The different characteristics showing knowledge of small household farmers are summarised in Table 3.2. A total of 28 (23.1%) of the participants had knowledge towards heartwater. However, a highest proportion of respondents failed to explain what they know about heartwater disease, 97 (80.4%). Other respondents managed to mention some of the correct characteristics of heartwater. Among these, the highest proportion of respondents mentioned fever, loss of appetite, diarrhoea and shivering 9 (7.4%), high mortality rates in livestock 8 (6.6%). Very few respondents highlighted ticks as the vectors of heartwater 4 (3.3%). Some participants indicated that meat produced from heartwater affected animals is slippery and produces foam when cooking 2 (1.7%) and heartwater is a seasonal infection 1 (0.8%) were the least represented.

Table 3.2: Knowledge of small household farmers on heartwater disease.

Category	Characteristics	Frequency	Percentage
Do you know heartwater disease?	Yes	28	23.1
	No	89	73.6
	No answer	4	3.3
What do you know about heartwater disease?	Fever, loss of appetite, diarrhoea and shivering	9	7.4
	High mortality rates in livestock animals	8	6.6
	It is caused by ticks	4	3.3
	Heartwater is a seasonal infection	1	0.8
	Meat produced from heartwater affected animals is slippery and produces foam when cooking	2	1.7
	No answer	97	80.2

3.3.4. Knowledge on transmission and the control of heartwater

Some of the characteristics showing knowledge of small household farmers on the transmission and control of heartwater are shown in Table 3.3. Of the 121 small household farmers, 12.4% (15/121) associated heartwater with tick vectors. However, a high number of the participants had no knowledge about the mode of transmission of heartwater. This proportion differed according to categories: Air-borne 76.0% (92/121), Water-borne 1.7% (2/121), Food-borne 3.3% (4/121) and No answer 6.6% (8/121).

When asked of the types of animals heartwater affects, the highest proportion of those who had knowledge about heartwater, further demonstrated their knowledge of heartwater by mentioning that heartwater affects only domestic animals (livestock) and both wild and domestic animals. However, the highest proportion mentioned only livestock animals 21.5% (26/121) and only one respondent mentioned both wild and domestic animals 0.8% (1/121).

When asked if there is a way of controlling heartwater, 13.2% (16/121) agreed. The very same proportion had also heard of an available vaccine for controlling heartwater 13.2% (16/121). When asked, in which season of the year does heartwater commonly occur, 14.0 % (17/121) respondents correctly identified the season. However, majority had no idea 77.7% (94/121). Other respondents failed to select the correct answer. They selected dry season 4.1% (5/121) and All seasons 4.1% (5/121).

Table 3.3: Small household farmers' knowledge on transmission and control of heartwater.

Categories	Characteristics	Frequency	Percentage
Mode of transmission	Air-borne	92	76.0
	Water-borne	2	1.7
	Tick-borne	15	12.4
	Food-borne	4	3.3
	No answer	8	6.6
What type of animals does it affect?	Livestock animals	26	21.5
	Livestock and wild animals	1	0.8
	No answer	94	77.7
Is there a way of controlling it?	Yes	16	13.2
	No	11	9.1
	No answer	94	77.7
Have you ever heard of any vaccine available for heartwater?	Yes	16	13.2
	No	11	9.1
	No answer	94	77.7
In which season of the year does heartwater commonly occur?	Dry season	5	4.1
	Rainy season	17	14.0
	All season	5	4.1
	No answer	94	77.7

3.3.5. Farmers' knowledge on the symptoms of heartwater

A total of 5.0 % (6/121) of the participants correctly identified all the symptoms of heartwater. However, a total of 1.8 %, (2/121) respondents did not know of any symptoms of heartwater

disease. Furthermore, a total of 22.3 % (27/121) had no idea of the heartwater symptoms (Figure 3.3).

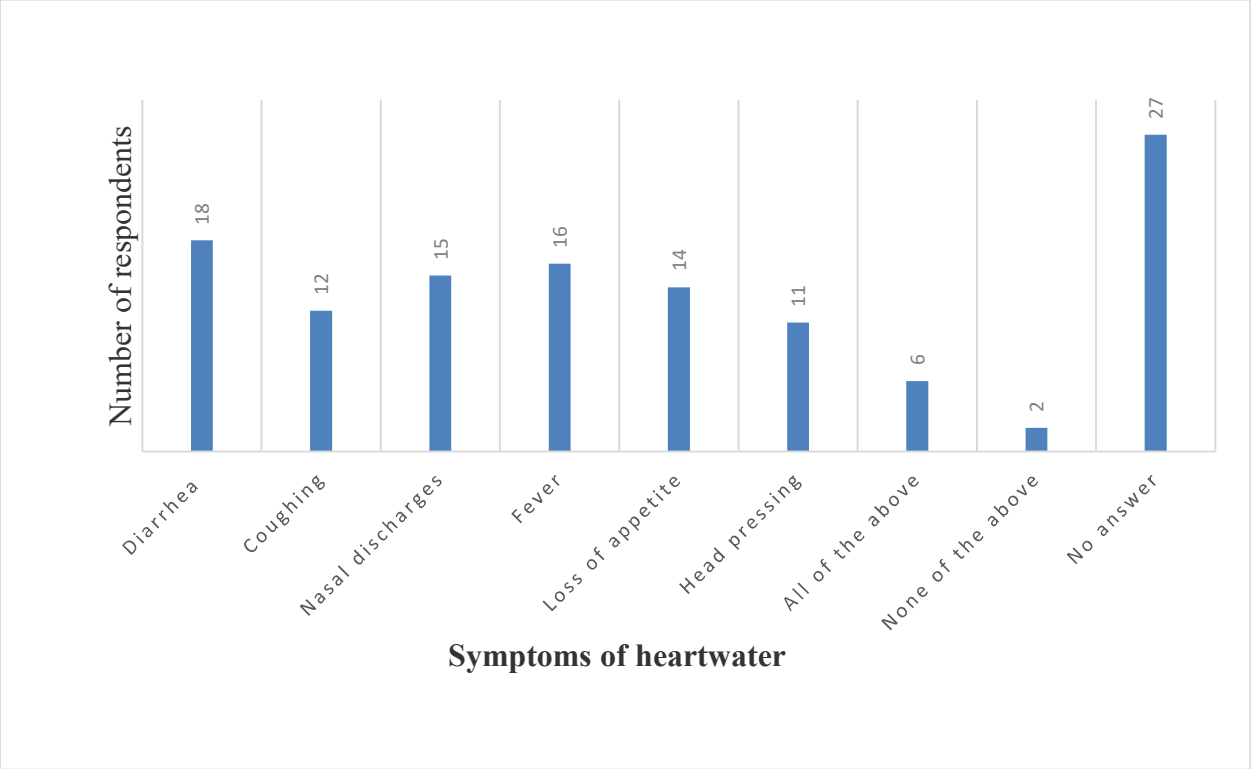

Figure 3.3: Household farmers' knowledge of heartwater symptoms.

3.3.6. The different characteristics of attitude of small household farmers towards heartwater

A summary of some characteristics of farmers' attitude towards heartwater disease is shown in Table 3.4. The highest proportion of respondents are not satisfied at all 43.0% (52/121) when coming to the animal services provided by the government (Department of Agriculture/State Veterinarian) in their area. However, 20.7% (25/121) of the respondents were completely satisfied.

Some farmers 19.8% (24/121) indicated that there are no livestock education in their area. Some stated that sometimes they feel satisfied 11.6% (14/121).

When asked if they think it is important to have knowledge about heartwater, it was clear that even those who had no knowledge of heartwater in the first place demonstrated a good attitude towards having knowledge about the disease. The highest proportion 94.2% (114/121) agreed and very few 4.1% (5/121) thought it is not important to have knowledge about heartwater. When asked, do they think heartwater is a dangerous disease, 83.5% (101/121) stated that it is a dangerous disease while 0.8% (1/121) thought it is not dangerous.

A total of 23.9% (29/121) of the participants had a positive attitude regarding treating heartwater by stating that they would use prescribed medicine whenever their livestock start showing symptoms of heartwater. Some farmers in a proportion of 7.4% (9/121) highlighted that making use of vaccines would aid in the treatment of heartwater. However, (1/121) 0.8% thought heartwater can be treated by making use of homemade mixtures while a total of (3/121) 2.5% mentioned that heartwater can be treated by consulting a specialist for assistance. Only (1/121) 0.8% of the respondents mentioned dipping and spraying could be the better way of treating heartwater disease.

Table 3.4: Attitude of small household farmers towards heartwater disease.

Category	Characteristics	Frequency	Percentage
Are you satisfied with the animal services provided in your area?	Very much	25	20.7
	Sometimes	14	11.6
	Not at all	52	43.0
	There are no livestock education available in my area	24	19.8
	No answer	6	4.9
Do you think it is important to have knowledge about heartwater disease?	Yes	114	94.2
	No	5	4.1
	No answer	2	1.7
Do you think heartwater is a dangerous disease?	Yes	101	83.5
	No	1	0.8
	No answer	19	15.7
How do you think heartwater disease is treated?	Prescribed medicine	29	23.9
	Using indigenous plants	2	1.7
	Vaccine	9	7.4
	Home mixtures	1	0.8
	Consulting specialists	3	2.5
	Dipping and spraying	1	0.8
	No answer	76	62.8

3.3.7. The different characteristics of attitude in response to the association of ticks with animal diseases by farmers

The different characteristics of attitude in response to the association of ticks with animal diseases by farmers are shown in Table 3.5. A total of 68.6% (83/121) of the respondents had positive attitude towards the association of ticks with animal diseases. A small proportion 2.5% (3/121) stated that livestock animals get more ticks while grazing. However 49.6% (60/121) correctly highlighted that, ticks are vectors. Some respondents stated they never thought ticks would cause diseases 9.1% (11/121). This was further supported by a highest proportion 67.8% (82/121) who indicated their concern of ticks in livestock farming as a serious problem.

Table 3.5: Attitude of small household farmers on the association of ticks with animal diseases by farmers.

Categories	**Characteristics**	**Frequency**	**Percentage**
Per your own point of view, do you associate any diseases experienced on your animals with ticks?	Yes	83	68.6
	No	28	23.1
	No answer	10	8.3
Comments	Livestock animals get more ticks when grazing	3	2.5
	Ticks are vectors	60	49.6
	Causes high mortality	7	5.8
	Never thought about it	11	9.1
	No answer	40	33.0
Are ticks an issue of concern for your livestock farming?	Not a problem	9	7.4
	A moderate problem	8	6.6
	A serious problem	82	67.8
	Somewhat a problem	7	5.8
	Never thought about it	7	5.8
	No answer	8	6.6

3.3.8. The different characteristics describing the farmers attitude towards government services.

Some of the characteristics showing attitude of small household farmers to government services are shown in Table 3.6. A highest proportion 74.4% (90/121) indicated that they are not in contact with the state veterinarian office, while 19.8% (24/121) indicated that they are in contact with the state veterinarian office. When asked of their opinion to include a state veterinarian in their livestock farming, a high proportion 23.1% (28/121) stated that, including a state veterinarian in animal farming will promote good health to aid in producing many livestock. At least 1.7% (2/121) stated that, it reduces mortality rates. Some 10.7% (13/121) stated that it improves their health education. However, 21.5% (26/121) never thought about it. When asked, their feeling if they think the government is helping enough in their livestock farming, a highest proportion 65.3% (79/121) disagreed while a small proportion 28.1% (34/121) agreed that the government is helping enough in their livestock farming.

Table 3.6: The attitude of small household farmers to government services.

Categories	**Characteristics**	**Frequency**	**Percentage**
Are you in contact with the state veterinarian office?	Yes	24	19.8
	No	90	74.4
	No answer	7	5.8
What is your opinion for including a state veterinarian in your animal farming?	Promotes good health to aid in producing many livestock	28	23.1
	I never thought about it	26	21.5
	It reduces mortality rates	2	1.7
	It is expensive	8	6.6
	Improves health education	13	10.7
	No answer	44	36.4
Do you think the government is helping enough in your livestock farming?	Yes	34	28.1
	No	79	65.3
	No answer	8	6.6

3.3.9. The different characteristics of the feelings of farmers on the number of livestock animals they wish to produce.

The different characteristics of the feelings of farmers on the number of livestock animals they wish to produce, are shown in Table 3.7. A total of 61.2% (74/121) had a positive attitude towards how their livestock farming is going. However, a proportion of 25.6% (31/121) stated they are not satisfied at all. Many farmers have no limit on the number of livestock they wish to have. However, 0.8% (1/121) wish to produce more than 1000 livestock. A total of 76.0% (92/121) agreed that they are producing enough livestock while a total of 14.0% (17/121) said they are not producing enough.

Table 3.7: Feelings of farmers on the number of livestock animals they wish to produce.

Category	Characteristics	Frequency	Percentage
Are you satisfied with how your livestock farming is going?	Yes	74	61.2
	No	41	33.9
	No answer	6	4.9
Comments	Livestock always come back home safe	5	4.1
	Because I make enough profit	10	8.3
	Mortality rate is very high	11	9.1
	Because they are breeding very well	19	15.7
	I am happy because I learn more about animal diseases	2	1.7
	I just started practicing livestock farming	1	0.8
	Not satisfied at all	31	25.6
	No answer	42	34.7
What are your feelings on the number of livestock would you wish to have?	Wishing to produce >/ =100	5	4.1
	Satisfied with less than/= 10	20	16.5
	Less than /= 1000	1	0.8
	Satisfied with less than /=50	27	22.3
	No limit	56	46.3
	No answer	12	9.9
Do you think you are producing enough livestock?	Yes	92	76.0
	No	17	14.0
	No answer	12	9.9

3.3.10. Practices of small household farmers towards heartwater disease

Some of the practices of small household farmers towards heartwater disease are shown in Table 3.8. Most of the farmers had been practicing livestock farming for greater than 10 years with a proportion of 63.6% (77/121). A very high proportion of 87.6% (106/121) is available on the full-time basis to look after their livestock. Only 32.2% (39/121) employed someone to take care of their livestock. Among the farmers, 36.4 % (44/121) had record of animals who died in the past years because of heartwater. A highest proportion mentioned less than 10 animals affected by heartwater resulting in mortality. However, 12.4% (15/121) indicated greater than 10 animals died because of heartwater disease.

Table 3.8: Practices of small household farmers.

Category	Characteristics	Frequency	Percentage
For how long you have been doing livestock farming?	≤5 years	29	24.0
	>10 years	77	63.6
	1 year	11	9.1
	No answer	4	3.3
Are you available on a full- time basis to look after your livestock?	Yes	106	87.6
	No	12	9.9
	No answer	3	2.5
Do you have someone employed to take care of your animals?	Yes	39	32.2
	No	79	65.3
	No answer	3	2.5
Do you have record of animals who died in the past years because of heartwater disease?	Yes	44	36.4
	No	55	45.5
	No answer	22	18.1
Number of animals affected by heartwater resulting in mortality/death	< 10	30	24.8
	> 10	15	12.4
	No answer	76	62.8

3.3.11. Practices of small household farmers in response to the use of medicinal plants and diseases experienced on animals.

The different characteristics of farmers' practices in response to the use of medicinal plants and diseases experienced on animals are shown in Table 3.9. A total of 24.0% (29/121) farmers used medicinal plants to cure animals for heartwater or other diseases. A proportion of 28.9% (35/121) agreed that they know of the medicinal plants used against ticks. 23.1% (28/121) mentioned other diseases apart from heartwater disease experienced by their livestock. However, a very high proportion 76.9% (93/121) never noticed any other diseases.

Table 3.9: Practices of small household farmers in response to the use of medicinal plants and diseases experienced on animals.

Category	Characteristics	Frequency	Percentage
Do you use any medicinal plants to cure animals for heartwater?	Yes	29	24.0
	No	85	70.2
	No answer	7	5.8
Do you know any medicinal plants used against ticks?	Yes	35	28.9
	No	81	66.9
	No answer	5	4.1
Other diseases animals suffer from	Mentioned other diseases	28	23.1
	No other diseases	93	76.9

3.3.12. Tick control methods

The tick control methods are shown in Table 3.10. A highest proportion of 63.6% (77/121) of the farmers used spraying of medicine to prevent their livestock from ticks. Some 24.8% (30/121) use dipping while 23.1% (28/121) treated the spot left by ticks on the animal skin surface. When considering supplementary tick control methods, a high proportion 28.9% (35/121) of small household farmers removed by hand. Some farmers used tick grease 9.1% (11/121). However, some farmers made use of homemade mixtures 16.5% (20/121). Other farmers used engine oil 26.4% (32/121). At least 0.8% (1/121) indicated that they use all the mentioned supplementary tick control methods.

Table 3.10: Tick control methods used by small household farmers

Category	Characteristics	Frequency	Percentage
Which tick control method do you use?	Spraying	77	63.6
	Dipping	30	24.8
	Spot treatment	28	23.1
Which supplementary tick control method do you use?	Removing by hand	35	28.9
	Tick grease	11	9.1
	Homemade mixtures	20	16.5
	Engine oil	32	26.4
	All of the above	1	0.8

3.3.13. Practices of small household farmers in maintaining the livelihood of their animals

Some of the different characteristics of the farmers' practices in maintaining the livelihood of their animals are shown in Table 3.11. Most of the cattle, sheep and goats, fed in the bush close to home 68.6% (83/121) followed by those that fed at home 11.6% (14/121). Those that fed from both home and bush were 10.7% (13/121) while those that fed on the mountain within 5 km distance away from home were 5.0% (6/121). Only 0.8% (1/121) fed at the farm.

A high proportion of farmers takes out their livestock for feeding 56.2% (68/121). However, farmers assistant who take livestock out for feeding were 15.7% (19/121). Followed by those that are taken out by children at home 11.6% (14/121). In some cases, either the farmer or their children take out the livestock for feeding 6.6% (8/121) while some farmers work hand in hand with their assistants 4.1% (5/121).

Most cattle sheep and goats drink water at home 69.4% (84/121) while others get their water from extra sources such as river 22.3% (27/121) and farm 0.8% (1/121). However, other livestock get water from both home and river 4.9% (6/121).

Table 3.11: Practices of small household farmers in maintaining the livelihood of their animals.

Category	Characteristics	Frequency	Percentage
Feeding places	Bush	83	68.6
	Farm	1	0.8
	Home	14	11.6
	Home/bush	13	10.7
	Mountain	6	5.0
	No answer	4	3.3
Who takes animals out for feeding?	Assistant	19	15.7
	Children	14	11.6
	Farmer	68	56.2
	Farmer /assistant	5	4.1
	Farmer/children	8	6.6
	No answer	7	5.8
Water source	Home	84	69.4
	River	27	22.3
	Home/river	6	4.9
	Farm	1	0.8
	No answer	3	2.5

3.3.14. Reported times for animals to return home after feeding

The reported times for animals to return home after feeding, varied from 06:00 to 18:00. Most animals returned home between 16:00 - 17:00, 41.3% (50/121) followed by those that returned home between 17:00 - 18:00 23.1% (28/121). Some returned home as between 15:00 - 16:00, 15.7% (19/121) while few returned home between 06:00 - 08:00, 4.1% (5/121) and 14:00 - 15:00, 4.1% (5/121). However, some animals had no specific time of returning home after feeding 11.6% (14/121). The feeding period for cattle varied from 05:00 - 17:00. Few cattle started feeding between 05:00-06:00 2.5% (3/121) and 10:00 - 17:00 2.5% (3/121). However, the highest proportion 62.8% (76/121) of cattle had no specific time for feeding. Furthermore, some cattle started feeding between 07:00 - 08:00, 18.2% (22/121) followed by those that started feeding between the times 08:00 - 09:00, 8.3% (10/121) and 09:00 - 10:00, 5.8% (7/121). Most goats started feeding between 11:00 - 15:00, 39.7% (48/12) followed by those that fed between 07:00 - 11:00 in a proportion of 28.9% (35/121). Few goats fed between 15:00 - 18:00 in a proportion of 5.8% (7/121) while others had no specific time. Some sheep 2.5% (3/121) fed between 12:00 - 14:00 while others 1.7% (2/121) fed between 10:00 - 12:00 (Table 3.12).

Table 3.12: Reported time intervals for feeding livestock

Category	Characteristic	Frequency	Percentage
Reported times of animals to return home after feeding.	06:00 - 08:00	5	4.1
	14:00 - 15:00	5	4.1
	15:00 - 16:00	19	15.7
	16:00 - 17:00	50	41.3
	17:00 - 18:00	28	23.1
	No specific time	14	11.6
Time for feeding: cattle	05:00 - 06:00	3	2.5
	07:00 - 8:00	22	18.2
	08:00 - 09:00	10	8.3
	09:00 - 10:00	7	5.8
	10:00 - 17:00	3	2.5
	No specific time	76	62.8
Time for feeding: goats	07:00 - 11:00	35	28.9
	11:00 - 15:00	48	39.7
	15:00 - 18:00	7	5.8
	No specific time	31	25.6
Time for feeding: Sheep	10:00 - 12:00	2	1.7
	12:00 - 14:00	3	2.5
	No answer	116	95.8

3.3.15. Medicinal plants that are used by the farmers to cure different kinds of animal diseases.

The different kinds of medicinal plants used by small household farmers to treat different kinds of animal diseases are shown in (Table 3.13).

Table 3.13: Medicinal plants and plant parts used by farmers to treat different kinds of livestock diseases.

Local common name	English common name	Scientific name	Part used	Route of administration
Muserenga: Venda	Chinaberry and Umbrella tree	*Melia azedarach*	Shrub infusion	Oral
Muelela: Venda	flat-crown	*Albizia adianthifolia*	Bark and roots	Topical
Tshipandwa: Venda	Confetti tree	*Gymnosporia senegalensis*	Sterm and leaves infusion	Oral
Museto: Venda	Devil's Thorn	*Dicerocarryum senecioides*	Sterm and leaves infusion	Oral
Murumbulambudzana: Venda	Bushman's grape	*Rhoicissus tridentata*	Leaves, tubbers and roots infusion	Oral
Mugwiti: Venda	Velvet bushwillow	*Combretum mole*	Leaves Infusion	Oral
Vhangazi: Tsonga, Mutondo: Venda	Transvaal teak	*Pterocarpus angolensis*	Bark infusion	Oral
Dinda- Tsonga	Devil thorn	*Dicerocaryum Eriocarpum*	Shoots infusion	Oral and topical
Xidomeja: Tsonga Mafuredonga: Venda	Castor-oil tree	*Jatropha zeyheri*	Roots infusion	Oral
Mulongekanye: Venda	Devil's backbone	*Kalanchoe daigremontiana*	Branch infusion	Oral

Muboma: Venda	Long pod cassia	*Eucalyptus camaldulensis*	Stem bark infusion	Oral
Tshikhopha: Venda Mhangani: Tsonga	Mountain Aloe	*Aloe marlothii*	Leaf infusion	Oral
Muswoswo: Venda	Dead-man's tree	*Synadenium cupulare*	Stems	Topical
Phathane: Venda	Star-flowered bitter-tea	*V. colorata*	Roots infusion	Oral
Tshifhure: Venda	Red ironwood	*Onchna holstii*	Shoots infusion	Oral

3.3.16. The different kinds of diseases experienced by animals.

The different kinds of diseases experienced by animals are shown in Table 3.14. The highest proportion of animals experienced diarrhoea 43.0% (52/121) followed by coughing and nasal discharges 40.5% (49/121). Some animals experienced fever and loss of appetite 29.8% (36/121). Head pressing was experienced in a proportion of 26.4% (32/121). Some animals experienced running around in a proportion of 19.8% (24/121) while some experience rolling of eyes in a proportion of 19.0% (23/121). The lowest number of animals showed symptoms of being noisy 15.7% (19/121).

Table 3.14: Different kinds of diseases experienced by animals.

Category	**Characteristics**	**Frequency**	**Percentage**
Illnesses experienced on animals by farmers	Diarrhoea	52	43.0
	Rolling eyes	23	19.0
	Noisy	19	15.7
	Coughing and nasal discharges	49	40.5
	Running around	24	19.8
	Fever and loss of appetite	36	29.8
	Head pressing	32	26.4

3.4. Discussion

The present study explored knowledge, attitude, and practices among small household farmers towards heartwater disease in Mopani and Vhembe districts of Limpopo province. Demographic data of livestock keepers are also reported in this study. Our study showed that, livestock farming is mostly dominated by males. Most of the famers involved in the present study were above the age of 60. Our findings agree with that reported by Nxumalo and Oladele in (2013) although most of the respondents involved in their study, had no formal education.

Household income and level of education of the study participants in our study agree with those reported by others (Evans and Ngau, 1991). Our study shows that most of the farmers had household income ranging from R1500 - 7500 and they attained secondary education. This is an implication that the farmers who took part in the survey had a fair understanding of livestock illnesses and could convey relevant information on how to manage and control them. According to Nkonya (2004), education is a vital instrument for socioeconomic development in every rural community since it improves access to knowledge, goods, and services while also enabling farmers to take appropriate decisions (Nkonya, 2004).

The large number of goats per family reflects the importance of goats to improving livelihoods amongst farmers' households. This is supported by a study conducted by Kaumbata *et al,* (2020), which proved that goats contribute towards the reduction of poverty because of its profitability and economic viability (Kaumbata *et al.,* (2020).

Knowledge, attitudes, and practices surveys have been widely utilized worldwide for the different applications in public health. This is based on the principles that increasing the level of knowledge

may result in changing attitudes and practices to minimize the spread of diseases (Sambo *et al.,* 2014). However, to improve the strategies used for the control of heartwater, local farmers must be involved in research. The present study showed that only 23.1% of the participants had knowledge towards heartwater. Very few respondents managed to mention what they know about heartwater disease. Among this fever, loss of appetite, diarrhoea and shivering were the most reported signals of heartwater disease followed by high mortality rates in livestock animals. Nevertheless, a very high proportion 80.2% of the respondents did not have an idea about the disease. To our knowledge there are very few or no studies that gave been conducted on knowledge attitude and practice of farmers toward heartwater disease in the country.

This study revealed poor knowledge of heartwater disease as most of the respondents failed to highlight the mode of transmission. Furthermore, most of the respondents failed to mention the types of animals heartwater commonly affect. Most of the respondents further showed that there is no existing way of controlling heartwater disease, and they have not heard of any vaccine available for heartwater. Furthermore, a high proportion of the respondents mentioned that heartwater disease occur during the rainy season. This agrees with the findings of a study conducted in Tanzania (Kerario *et al.,* 2018). However, literature showed there is presently no scientific evidence that current climate or other epidemiological variables are influencing the prevalence of heartwater in South Africa (Bath and Leask, 2020).

The knowledge of heartwater symptoms is usually common among farmers who are aware of the clinical signs and symptoms of the disease. In traditional communities, farmers typically pass on their knowledge and expertise in livestock management to their children when they are still very young so that when they get older, they can take care of their herd (Laisser *et al.,* 2015). This is

the reason some farmers managed to highlight symptoms of heartwater. From the available literature, it is highlighted that diarrhea is present when animals approach death (Gutiérrez and Simões, 2017). This further supports the findings of the present study as some farmers mentioned they are not producing enough livestock due to high mortality rates. Furthermore, a high proportion of farmers mentioned diarrhoea as the most common symptom of heartwater disease. Hence, diarrhoea is the possible cause of the high mortality rates reported by farmers.

Most of the respondents considered heartwater as a dangerous disease. However, their common belief in indigenous knowledge, allowed them to use prescribed medicine and vaccines. A small proportion of respondents opted for dipping and spraying. This is an implication that the use of acaricides was not common among farmers. It should be noted that acaricides are inexpensive and effective in reducing tick populations. This is the common method of tick control and eradication efforts (Basit *et al.,* 2022). Furthermore, a high number of respondents associated any of the diseases experienced by their animals with ticks. A high proportion of respondents highlighted that ticks are common vectors. However, the highest proportion indicated their concern of ticks in livestock farming as a serious problem. This is a direct implication of no proper use of acaricides. This finding contradicts with Vudriko *et al,* (2018) who discovered acaricide application was the most common method of tick control.

In the present study, majority of the respondents reported they are not satisfied at all with the animal services provided in their communities. However, minority indicated they are sometimes satisfied with the animal services provided in their communities. This is an implication that respondents did not have enough access to services of extension officers or the state veterinarian. This agrees with a study conducted in semi-arid rural areas of South Africa of Limpopo province

(Akpalu, 2013). Similar situation was reported in a study conducted by Bath *et al,* (2016). Although the impact of including a state veterinarian in animal farming was known among most of the respondents. The different advantages of including a state veterinarian in animal farming were mentioned. The majority mentioned including a state veterinarian in animal farming, promotes good health to aid in producing many livestock and some mentioned it improves their level of health education needed in livestock farming.

However, according to the available literature, poor access to veterinarians is linked to lack of information and counsel offered to farmers. Furthermore, access to legal veterinary medicinal products through an established supply infrastructure and access to education and counsel from trained professionals for farmers are both crucial elements of efficient animal health services and are of utmost significance for farmer education and decision-making (Oladele *et al.,* 2013).

Majority of the respondents highlighted the fact that the government is not helping enough in livestock farming. Although there is a need for the government to hold organized seminars for all farmers to help them learn about livestock management and control (Khapayi and Celliers, 2016). Despite the absence of government intervention in their livestock farming, majority were satisfied with how their livestock is going. Among this, some mentioned their animals are producing very well. Additionally, respondents had learned about animal diseases, and they are making enough profit by selling their animals. Some respondents mentioned their animals always come back home safe. This is a direct implication that livestock farmers are situated in areas where wild animals are monitored enough not to cause harm to the domestic animals.

The respondents mentioned they experience high mortality rates in their animals. However, the cause of high mortality was because of the diseases experienced by the animals. Although, a high proportion of farmers indicated their animals are producing very well. To eradicate loss of animals because of drought, small household farmers should be trained to make reservations such as silage and hay so they can conserve surplus forage in rainy seasons. They should also be advised to plant fodder to reduce pressure on the natural veld (Kelio, 2022).

Majority of the respondents had no limit on the number of livestock they wish to produce. This is because, in rural communities of Vhembe and Mopani regions, there is sufficient land available for livestock farming. This contradicts the findings of a study conducted in Eastern cape province. The study revealed that insufficient land availability in South Africa is still a challenge that many emerging farmers face. This has negative implications for farm income and sustainability, especially for small household livestock emerging farmers who depend on the availability of land for expansion and grazing of livestock production (Khapayi and Celliers, 2016).

Some respondents were specific to certain limits because they thought of livestock management. Some stayed next to the mountains where animals are at risk of being attacked by the wild animals. This prevented many farmers from keeping many livestock in their backyard. This is supported by a study conducted by Bath and Leask (2020) on the perceptions and observations of farmers and veterinarians on heartwater occurrence, distribution and associated factors in South Africa. Their study revealed the movement of livestock and wildlife is a significant aspect that should be taken into consideration in farming since it contributes to unnecessary spread of heartwater disease. Besides all the challenges, many farmers thought they are producing enough livestock animals.

A high number of respondents reported they have been doing livestock farming for more than 10 years ago. Similarly, in a study conducted by Kelio (2022), majority of farmers had been practicing livestock farming for more than 10 years and more. This implies the farmers included in this study, had enough experience in livestock farming. Furthermore, many farmers were available on a full-time basis to look after their livestock. In most cases farmers in rural areas prefer looking after their livestock animals on their own. Although some preferred employing someone for assistance where applicable. This is because many farmers experienced a high economic pressure. Therefore, better extension services, investments in infrastructure and livestock education programs should be implemented to assist farmers improve their farming practices (Radolf *et al.,* 2022).

Minority indicated they had record of animals who died in the past years because of diseases. However, a high proportion indicated less than ten animal counting from the beginning of their farming practice. Some farmers indicated use of medicinal plants to cure animals for heartwater. Among this, farmers reported they know of the specific medicinal plants used against ticks. The present study revealed the knowledge of medicinal plants used to treat animal diseases, is confined to very few farmers in our study area. The similar scenario was discovered in a study conducted in the Eastern cape province of South Africa (Sanhokwe *et al.,* 2016). Furthermore, other diseases animals suffer from were mentioned. The ethnoveterinary practices used for the treatment of heartwater disease reported in the present study is similar to the scenario reported in a study conducted in the Eastern cape province of South Africa (Mthi *et al.,* 2020).

Our study revealed that the common tick control method used by farmers was spraying. This method normally involved the use of acaricides. The similar finding was reported in a study conducted in Uganda (Vudriko *et al.,* 2018). However, a high proportion of farmers indicated a

use of supplementary tick control method which involved removing by hand. Similar incidence was reported in a study conducted by Amenu *et al.* (2017). Most of the farmers who participated in the study chose to feed their animals at the bush. Additionally, there were few opportunities for farmers to hire an assistant because they were always involved in moving their animals outside for feeding. Farmers used water sources at home to allow their animals drink water after feeding.

In the present study, farmers reported using 15 different medicinal plants to treat livestock diseases. Medicinal plants were reported in the respondent's local language, and we report here the English common name, scientific names, part used and the route of administration with the diseases treated. Some medicinal plants treat just one condition, whereas others treat a variety of disorders. Our findings agree with the study conducted in Pakistan by Khan *et al.* (2019). Farmers involved in the study mentioned different kinds of diseases experienced by animals which included diarrhoea, rolling of eyes, noisy, coughing, and nasal discharges, running around, head pressing, fever and loss of appetite. From the available literature the symptoms of heartwater disease involves all the mentioned diseases experienced by animals (Dharani *et al.,* 2015). This is a signal that livestock animals involved in the present study are at high risk of heartwater disease.

3.5. Conclusion

The present study reveals lack of knowledge of heartwater disease among small holder farmers in rural areas of the Vhembe and mopani Districts. This calls for a change in outreach programs. It is possible to develop communication plans that are appropriate for the local communities that will lead to better awareness among farmers and improvements in the farmers' attitude and practices toward heartwater disease. The government should assist the small household farmers through the

provision of subsidized livestock inputs such as the purchase of high-yielding cattle, sheep and goats, shed construction materials, fodder and feed, and marketing and farm equipment. This will encourage many farmers to adapt to the modern market-based farming. Hence, farmers may gain access to interact with livestock officials. The knowledge of small household farmers towards heartwater disease would have been improved through these interactions. This should lower the risks of market- and trade-associated disease spread, improving livestock health and smallholder farmers' livelihoods in the Mopani and Vhembe regions.

Chapter 4: Distribution and molecular characterization of *E. ruminantium* in ticks from cattle, sheep and goats in Limpopo province, South Africa

Abstract

Background: *E. ruminantium* is an intracellular bacterium widely known to cause heartwater disease in livestock and wild animals. This disease is fatal to animals, threatening food security in endemic areas. However, very few studies have been conducted on this disease in the Limpopo Province, South Africa. The objective of the study was to determine the distribution and molecular characterization of *E. ruminantium* in ticks from cattle, sheep and goats.

Methodology: A total of 244 *Amblyomma* ticks were collected from cattle, sheep and goats from 48 small households in Vhembe and Mopani Districts. Following sample collection, genomic DNA was extracted from the ticks and analysed using real-time PCR assay targeting a 226bp PCS20 gene fragment. Samples that showed clear bands were sent for sequencing to identify different genotypes of *E. ruminantium* circulating in Mopani and Vhembe Districts. Neighbor-joining method was used to infer phylogenetic position based on 16S rRNA genes using MEGA11.

Results: Out of 244 ticks collected, 56.2% of the households had *E. ruminantium* infection. Nzhelele municipality had the highest *E. ruminantium* distribution (37.0%) compared to the other municipalities. Among these, the distribution of *E. ruminantium* by source of ticks and animal type revealed cattle are more prone to tick distribution 43.5% as compared to other animal sources of ticks. Moreover, animals aged between 3-4 years had the highest tick distribution 45.2%.

Distribution of *E. ruminantium* by gender was found to be high from males 36.7% than females 25.0%. Moreover, farmers with household income > R20000 had the highest prevalence 50.0 % than farmers with household income < R500, 14.3%. The age of the farmers 15 – 20 years old revealed the highest prevalence 46.7% of *E. ruminantium* infection. However, there was no prevalence found among farmers above 60 years old. The distribution of *E. ruminantium* was greater in farms whose owners had tertiary education 47.6%. Phylogenetic analysis of *E. ruminantium* PCS20 genotypes were positioned into multiple clades. The isolates from the present study showed a wide genetic diversity when compared with reference sequences from other regions from South Africa and other African countries. Isolates of the present study CP040119.1 and CP040118.1 clustered with CP040113.1 and CP040115.1. MK371030.1 was closely related to CR925678.1. 212er was the far away from all the isolates of the present study though they shared a common ancestor.

Conclusion: The present study revealed high prevalence of *E. ruminantium* among the ticks that are vectors of this pathogen in areas of Mopani and Vhembe regions are endemic to heartwater disease. Future vaccine development against *E. ruminantium* should consider the diversity observed in the present study that will assist in the control of heartwater disease in the present study areas.

4.1. Introduction

Heartwater is widely known as a disease that is associated with *Amblyomma hebraeum* ticks. It is known to affect livestock animals in South Africa (Guo *et al.,* 2019). The disease poses a serious economic threat to livestock production in sub-Saharan Africa. It affects both wild and domestic animals. It is normally caused by *Ehrlichia ruminantium*. This proteobacterium is transmitted by *Amblyomma* ticks. The most widespread vector being *Amblyomma variegatum*, which is distributed in the Caribbean and sub-Saharan Africa (Faburay *et al.,* 2007). In South Africa, the most widely distributed heartwater vector is *Amblyomma hebraeum* (Horak *et al.,* 2006).

E. ruminantium has MAP1 multigene family. It comprises of 16 paralogs tandemly arranged in the genome (Yu *et al.,* 2007). The *E. ruminantium* strains sequenced so far serve to maintain paralogs in the same order in the genomes (Nakao *et al.,* 2016). For this reason, the genetic diversity amongst *E. ruminantium* isolates has been confirmed in previous epidemiological studies using the MAP1 gene family with known strains and field isolates in infected cattle and sheep from different heartwater endemic origins in Africa (Mnisi *et al.,* 2022). A few of these isolates were from South Africa (Welgevonden), Senegal (Senegal), Zimbabwe (Crystal Springs, Kwekwe, Palm River and Plumtree), Botswana (Sunnyside), Sudan (Um-Banein), Caribbean (Antiqua, Gardel) and Nigeria (Nigeria D225) (Allsopp *et al*., 2004; Faburay *et al*., 2008).

Many methods have been utilized in the past for screening ticks and ruminants for *E. ruminantium*. These included serological tests, electron microscopy and fluorescence microscopy (Jongejan *et al*., 1991). These older screening techniques have been replaced by the developed *E. ruminantium* specific DNA probe designated PCS20. These were implemented to overcome the challenges of

unknown specificity and sensitivity that resulted from the older techniques (Van Heerden *et al.*, 2004). The probe used in the PCS20 assay does not detect DNA from other closely related *Ehrlichia* species. It is the whole 1306 bp insert purified from a plasmid clone of the PCS20 region (Peter *et al.*, 2020).

Livestock farming of cattle, sheep and goats, play an essential role in the economy of South Africa (Gwaze *et al.,* 2010). In most instances, cattle farming produces more profit than small livestock farming in rural communities. However, farmers in rural communities of South Africa, are aware of the high mortality rates caused by heartwater disease among livestock animals (Slayi *et al.,* 2014). Although the methods of control are readily available, some farmers still fail to reach out to the state vets for help. To initiate new effective control measures against heartwater, it is important to understand the distribution of *E. ruminantium* and the origin of their introduction in regions. In this study, we used real-time PCR to screen for the presence of *E. ruminantium* PCS20 gene in ticks collected from cattle, sheep and goats from Vhembe and Mopani regions of Limpopo province and sequence analysis was used to compare the strains with those in the gene bank.

4.2 Materials and methods

4.2.1. Ethical approval, study sites, inclusion, exclusion criteria and sample collection

Ethical approval, study sites, inclusion, exclusion criteria and sample collection were completed with reference to Chapter 3 and a total of 244 ticks were collected individually from cattle, goats and sheep into 2ml tubes containing 70% ethanol. The samples were transported to the

Parasitology laboratory, Department of Microbiology at the University of Venda. Samples were kept at room temperature until DNA extraction was performed.

4.2.2. DNA extraction

Genomic DNA was extracted from ticks using Zymo Quick-gDNA Miniprep (Inqaba biotec). Briefly, the entire tick was placed in a ZR Bashing bead Lysis/Filtration tube using forceps and 800µl of Genomic Lysis buffer was added. Samples were vortexed for 4-6 seconds, then allowed to stand at room temperature for 5-10 minutes. The mixture was transferred to Zymo-Spin Column in a collection tube then centrifuged for 1 minute. The collection tube was discarded with the flow through. The Zymo-Spin Column was transferred to a new collection tube and 200µl of DNA Pre-Wash Buffer was added to the spin column and then centrifuged for 1 minute. A total amount of 500µl g-DNA Wash Buffer was added to the spin column and centrifuged for 1 minute. The spin column was transferred to a clean microcentrifuge tube and 50µl of DNA Elution Buffer was added. The solution was incubated for 2-5 minutes at room temperature and then centrifuged at maximum speed for 30 seconds to elute the DNA. The eluted DNA was stored at -20˚C until further use. DNA concentration and absorbance ratio were measured using a spectrophotometer (Nanodrop, Term Scientific, Madison, WI).

4.2.3. PCS20 quantitative Real-time PCR (qPCR)

Real-time PCR was performed to detect positive samples using a set of primers shown in Table 4.1 below: -

Table 4.1: Primers targeting 226bp fragment of the PCS20 region of *E. ruminantium.*

Primer name	Primer sequence	Reference
CowF	5'-CAA-AAC-TAG-TAG-AAA-TTG-CAC A-3'	Steyn *et al*. (2008)
CowR	5'-TGC-ATC-TTG-TGG-TGG-TAC-3'	

Reactions were performed in a final volume of 20 µl containing Luna universal qPCR master mix (Iqaba biotec) of 10 µl, 0.3 µl of each primer, 4.4 µl of nuclease-free water and 5 µl of DNA as a template. Nuclease-free water was used as a negative control. The reaction was performed in the LightCycler® 480 (Roche Diagnostics).

Two samples that gave clear bands were identified, amplified and used as a positive control in future tests. Initial activation was performed at 40°C for 10 minutes followed by activation of the Fast Start DNA polymerase at 95°C for 10 minutes. This was followed by 40 cycles of denaturing at 95°C, 60s with a 20°C/s slope, annealing at 60°C for 1 minute with a 20°C/s slope and elongation at 60°C, 30s with a 20°C/s slope, and a final cooling step to 40°C. Fluorescence data at 520 nm was acquired at the end of the extension step of each cycle. The results were analyzed with a threshold defined for the user of 200 PCR baseline sub structured curve-fit relative fluorescence units (CP RFU). The level of positivity of the samples was indicated by the cycle threshold (Ct) values which represent the number of cycles necessary for the samples to cross the threshold, in which the lowest small number represents more availability of amplified DNA.

4.2.4. Gel electrophoresis for purification of the amplicons

Following real-time PCR, 1% agarose gel was prepared by adding 50ml of TAE buffer in a water bottle. An amount of 0.5g agarose powder was weighed and placed inside the water bottle containing TAE buffer. The solution was mixed well by shaking and placed in the microwave to heat for 3 minutes. The solution was removed from the microwave, and it was allowed to cool at room temperature without solidifying. An amount of 3µl Ethidium bromide was added to the solution and mixed well without causing bubbles. Prior to solidification, the gel was poured into the casting tray and left at room temperature to solidify. After the gel has solidified, the combs were removed. A tray containing a solid gel was placed inside the electrophoresis chamber filled with TAE buffer with wells facing the negative terminal. 1µl of ladder was added to the first well followed by a negative control (nuclease-free water) in the second well and the positive control in the third well. Each PCR product (5µl) was loaded to each well respectively. After loading, the lid was closed properly, and the machine was operated for 45 minutes at 100V. After 45 minutes, the tray was removed from the chamber and the results were read in a Gel Doc machine.

4.2.5. Sequencing and phylogenetic analysis

Ehrlichia ruminantium positive PCR products of some amplicons were sent to Inqaba Technologies (Inqaba, Pretoria, South Africa) for sequencing. The obtained sequences were analysed by BLASTn tool in NCBI GenBank and the computer program ClustalX 1.83. The phylogenetic trees were constructed using the neighbor-joining method with MEGA11 Software (Tamura *et al.,* 2021).

4.3. Results

4.3.1. Demographic characteristics of the animals sampled.

The different characteristics showing the demographic data of animals sampled are shown in Table 4.2. A total of 244 ticks were collected from goats, cattle, and sheep from 7 different villages. Five of which are found in Vhembe region while two are found in the Mopani region. The youngest animals from which samples were collected was 1 year old whereas the oldest animal was 10 years old. The number of ticks collected from each household varied between 1 and 30. The animal sex was dominated by females (63.5%). Nutritional status of ticks was classified as fed (full of blood) and unfed (without blood). Amongst the owners, 80.3% of the animals belonged to the males.

Table 4.2: Demographic characteristics of the animals from which the ticks were collected.

Category	Characteristics	Frequency	Percentage (%)
Animal source of tick	Goat	185	75.8
	Cattle	23	9.4
	Sheep	36	14.8
Sex of animal	Female	155	63.5
	Male	89	36.5
Age of animals	Less than 1 year	43	17.6
	1-2	67	27.5
	3-4	84	34.4
	5 or more	50	20.5
Tick sex	Female	208	85.2
	Male	36	14.8
Nutritional status of tick	Fed	38	15.6
	Unfed	206	84.4

4.3.2. Demographic data of small household farmers from whom animals were sampled.

The different characteristics of the demographic data of farmers who's animals were sampled are described in Table 4.3. Majority of the respondents from where samples were obtained were males 80.3%. Most of the respondents were aged between 51 and 60 years (45.1%). The level of education was most dominated by those who attended secondary education 34.8%. Majority survived on salary ranging between 7500 and 20000 (38.1%). Farmers who had 5-6 people in the household were the most represented (28.7%).

Table 4.3: Demographic information of farmers whose animals were sampled.

Category	Characteristics	Frequency	Percentage (%)
Gender of farmer	Female	48	19.7
	Male	196	80.3
Age of farmer	15-20	45	18.4
	21-30	41	16.8
	31-40	22	9.0
	41-50	18	7.4
	51-60	110	45.1
	Above 60	8	3.3
Level of education	No formal education	63	25.8
	Primary education	34	13.9
	Secondary education	85	34.8
	Tertiary education	62	25.4
Household income	<500	7	2.9
	500-1500	75	30.7
	1500-7500	53	21.7
	7500-20000	93	38.1
	>20000	16	6.6
People in the household	1-2	39	16
	3-4	53	21.7
	5-6	70	28.7
	7-8	38	15.6
	9 or more	44	18.0

4.3.3. General characteristics of ticks collected.

Of the 244 *Amblyomma* ticks collected, 208 were female *A. hebraeum* and 36 were male *A. hebraeum* shown in Figure 4.1. From these, Xikukwani had the highest number of *Amblyomma* ticks (92), followed by Ha-Matsa (60), Khalavha (25), Bungeni (27), Tshikonelo (24), Mititi (9) and the lowest Tshivhilidulu (7) as shown in the Figure 4.2.

Figure 4.1: Two types of ticks found during sample collection. Adult female *Amblyomma hebraeum* (A) and Adult male *Amblyomma hebraeum* (B).

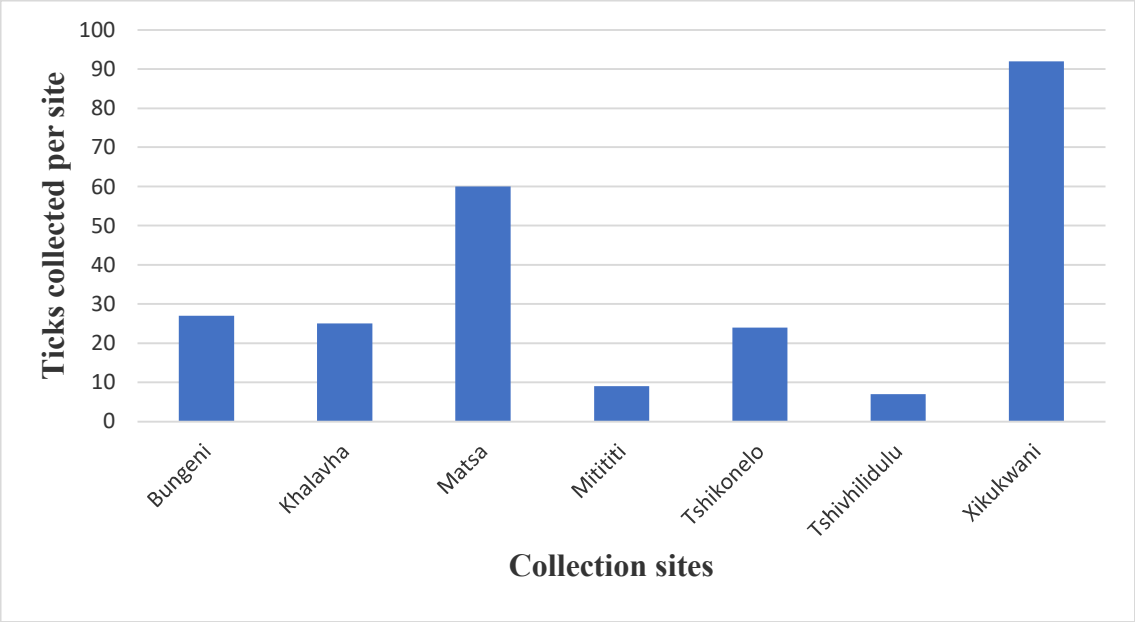

Figure 4.2: Number of *Amblyomma hebraeum* ticks collected per site

4.3.4. Detection of *E. ruminantium*

4.3.4.1. PCS20 quantitative Real-time PCR (qPCR)

Real-time PCR was run twice, samples that retained good amplification curves were re-amplified and used as a positive control. Each PCR round contained positive and negative controls. Pure PCR water was used as a negative control. Amplification curves are shown in Figure 4.3.

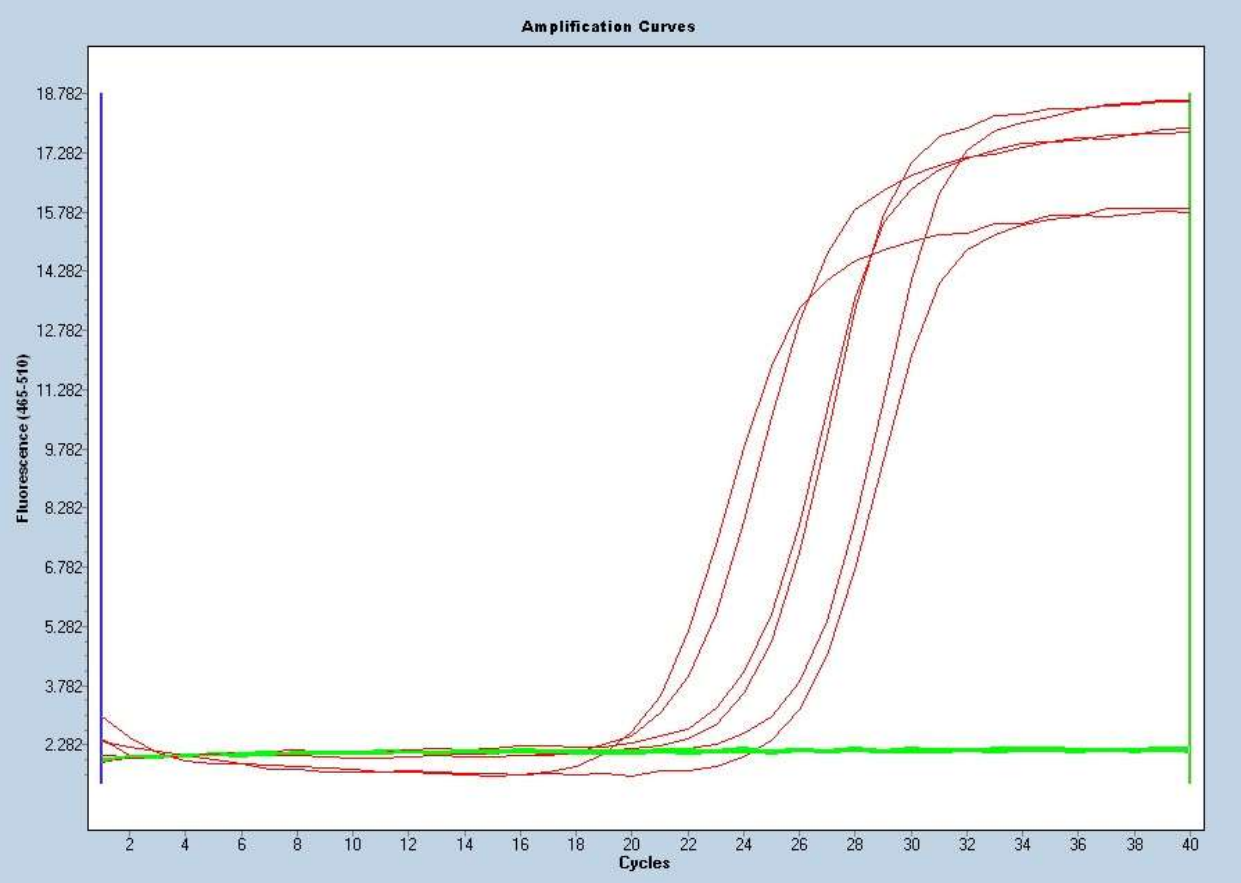

Figure 4.3: Amplification graphs obtained from the Light Cycler® 480 (Roche diagnostics) software, the picture shows the positive samples in pink that have good curves showing DNA amplifications. The negative samples are shown in green lines as they do not have curves as the DNA was not amplified.

4.3.4.2. Detection of *E. ruminantium* in the study population

Amplification of *E. ruminantium* was also detected by agarose gel electrophoresis as shown in Figure 4.4.

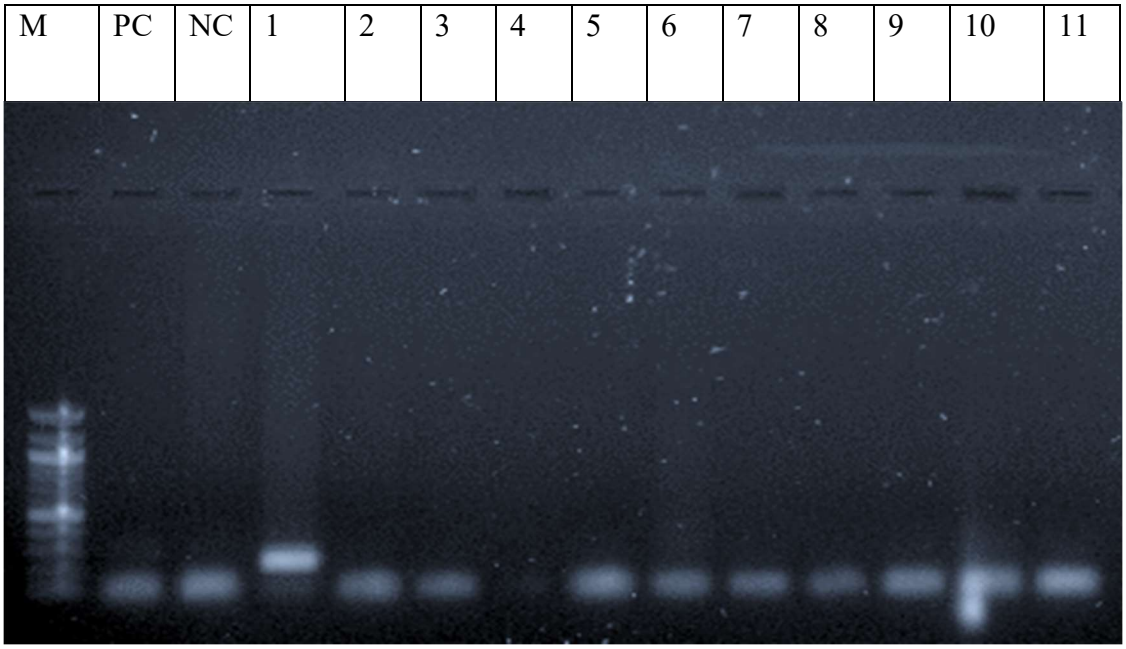

Figure 4.4: 1**%** Agarose Gel photograph for real-time qPCR confirmation of *E. ruminantium* positive samples from Mopani and Vhembe isolates. Lane M is 1Kb molecular ladder, Lane PC is positive control, Lane NC is a negative control and lane 1 is the positive *E. ruminantium* sample and lane 2-11 are the negative *E. ruminantium* samples.

4.3.4.3. Distribution of *E. ruminantium* in the study population

The distribution of *E. ruminantium* in the study population is highlighted in Table 4.4. Of these, the highest distribution of *E. ruminantium* in municipalities was found in Nzhelele 37.0% and Giyani 36.6%. The lowest prevalence was found from Thulamela 25.0% and Vuwani 25.9%. Furthermore, the significant high distribution of *E. ruminantium* in villages was found from Mitititi 44.4%, Khalavha 44.0%, Matsa 36.7% and Xikukwani 35.9%. Low distribution was found from Tshivhilidulu 14.3%, Tshikonelo 25.0% and Bungeni 25.9% villages. However, the results were not statistically significant in the study populations.

Table 4.4: Distribution of *E. ruminantium* by municipalities

Categories	Characteristics	F (Distribution %)	Total	Chi square	P value
Municipalities	Giyani	37 (36.6)	101	2.288	0.515
	Nzhelele	34 (37.0)	92		
	Thulamela	6 (25.0)	24		
	Vuwani	7 (25.9)	27		
Villages	Bungeni	7 (25.9)	27	4.776	0.687
	Khalavha	11 (44.0)	25		
	Matsa	22 (36.7)	60		
	Mitititi	4 (44.4)	9		
	Tshikonelo	6 (25.0)	24		
	Tshivhilidulu	1 (14.3)	7		
	Xikukwani	33 (35.9)	92		

4.3.4.4. Distribution of *E. ruminantium* by gender, household income, age, people per household and level of study

Distribution of *E. ruminantium* by gender of the farmer was found to be high in males 36.7%. Females had the lowest prevalence. Moreover, small household farmers with household income > R20000 had the highest prevalence 50.0% with the lowest prevalence found among farmers with household income < R500 (14.3%). The age of the farmers 15 – 20 years old revealed the highest prevalence 46.7% of infection. However, there was no infection found among the ticks collected from farmers above 60 years old. The number of people living in the household also influenced the level of infections in the ticks. In household with between 3 – 4 people the prevalence was 37.7% while in the household with more than 6 people, the prevalence was 28.9%. Farmers who had tertiary level of education showed the highest prevalence. Overall, the results were not statistically significant from the above-mentioned categories (Table 4.5).

Table 4.5: Table showing the distribution of *E. ruminantium* according to household characteristics.

Category	Characteristics	Distribution	Total	Chi square	P value
Gender of farmer	Female	12(25.0)	48	2.352	0.125
	Male	72(36.7)	196		
Household income (R)	<500	1 (14.3)	7	3.696	0.449
	500-1500	28(37.3)	75		
	1500-7500	18(34.0)	53		
	7500-20000	29(31.2)	93		
	>20000	8(50.0)	16		
Age of farmer	15-20	21 (46.7)	45	7.920	0.161
	21-30	15 (36.6)	41		
	31-40	6 (27.3)	22		
	41-50	6 (33.3)	18		
	51-60	36 (32.7)	110		
	Above 60	0 (0.0)	8		
People per household	1-2	14 (35.9)	39	3.825	0.975
	3-4	20 (37.7)	53		
	5-6	26 (37.1)	70		
	7-8	11 (28.9)	38		
	9 or more	13 (29.5)	44		
Level of education	No formal education	19 (30.2)	63	7.583	0.055
	Primary education	7 (20.6)	34		
	Secondary education	29 (34.1)	85		
	Tertiary education	29 (46.8)	62		

4.3.4.5. Distribution of *E. ruminantium* in relation to the different breeds found within the household of the farmers.

The distribution of *E. ruminantium* in relation to the different breeds found within households is shown in Table 4.6. The highest prevalence was found among the Nguni cattle breed 66.7%.

Moreover, Boer/indigenous goats breeds were found with the highest proportion 83.3% of infection. Among the sheep breeds, the most presented was indigenous black and white breed 50.0%. Overall, the results of different breeds sampled were not statistically significant.

Table 4.6: Different breeds of sampled animals found within households.

Category	Characteristics	F (%)	Total	Chi square	P value
Cattle breed	7 tala	1 (33.3)	3	3.502	0.623
	Nguni	2 (66.7)	3		
	Indigenous	15 (40.5)	37		
	Red breed	2 (50.0)	4		
	None	64 (32.5)	197		
Goats breed	Angora	0 (0.0)	1	9.813	0.366
	Boer/indigenous	4(83.3)	13		
	Brown/black	0 (0.0)	1		
	Indigenous	65 (71.1)	194		
	Pedi/indigenous	2 (66.7)	3		
	None	13 (40.6)	32		
Sheep breed	Indigenous Black/white	4 (50.0)	8	1.266	0.867
	Indigenous Brown/ white	2 (40.0)	5		
	Indigenous	2 (40.0)	5		
	Indigenous/ Symbok	8 (38.1)	21		
	None	68 (33.2)	205		

4.3.4.6. Distribution of *E. ruminantium* by source of ticks and animal type

The different characteristics showing the distribution of *E. ruminantium* by source of ticks and animal type are shown in Table 4.7. Cattle are more prone to *E. ruminantium* distribution shown by high prevalence 43.5% as compared to other animal source of ticks although the results were not showing some level of statistical significance. From these, female animal hosts 42.2% appeared with a high level of *E. ruminantium* distribution. Male animal source of ticks presented the lowest level of *E. ruminantium* distribution. These results were statistically significant. The age of the animal showed statistical significance with the highest level of *E. ruminantium* distribution found between the animals of 3 - 4 years old, 45.2%.

Table 4.7: Distribution of *E. ruminantium* by source of ticks and animal types.

Category	Characteristics	Distribution	Total	Chi square	P value
Animal source of tick	Goat	65 (35.1)	185		
	Cattle	10 (43.5)	23		
	Sheep	9 (25.0)	36	2.293	0.318
Sex of animal host	Female	70 (42.2)	155		
	Male	14 (15.7)	89	21.693	0.000
Age of animal (years)	<1	10 (23.3)	43		
	1-2	17 (25.4)	67		
	3-4	38 (45.2)	84		
	≥5 years	19 (38.0)	50	9.442	0.024

4.3.4.7. Distribution of *E. ruminantium* in ticks in response to household farmers keeping different types of animals

The different characteristics showing the distribution of *E. ruminantium* in ticks in relation to household farmers keeping different types of animals are shown in Table 4.8. Cattle are more prone to tick distribution shown by high prevalence 43.5% as compared to other animal source of ticks although the results were not showing some level of statistical significance. From these, female animal hosts 42.2% appeared with a high level of tick distribution. Male animal source of ticks presented the lowest level of tick distribution. These results were statistically significant. The age of the animal showed statistical significance with the highest level of tick distribution found between the animals of 3 - 4 years old, 45.2%.

Table 4.8: Distribution of *E. ruminantium* in ticks in relation to household farmers keeping different types of animals.

Category	Characteristics	Distribution	Total	Chi square	P value
Types of animals found per household	Cattle	20 (40.8)	49	1.109	0.292
	Goats	6 (26.1)	84	0.621	0.431
	Sheep	10 (47.6)	21	1.772	0.183
	Dogs	13 (27.1)	48	1.427	0.232
	Cats	6 (26.1)	23	0.782	0.376
	Donkeys	9 (31.0)	29	0.168	0.682
	Chicken	29 (33.7)	86	0.029	0.864
	Pigs	15 (36.6)	41	0.102	0.750

4.3.5. Molecular characterization of *E. ruminantium* in ticks in Limpopo province, South Africa

4.3.5.1. Sequence alignment

Sequence alignment was conducted on the PCS20 gene *E. ruminantium* isolates detected from goats, cattle and sheep in areas of Mopani and Vhembe region. Nucleotide sequences of *E.*

ruminantium had appeared to be conserved with multiple nucleotide sequence polymorphisms (Figure 4.5).

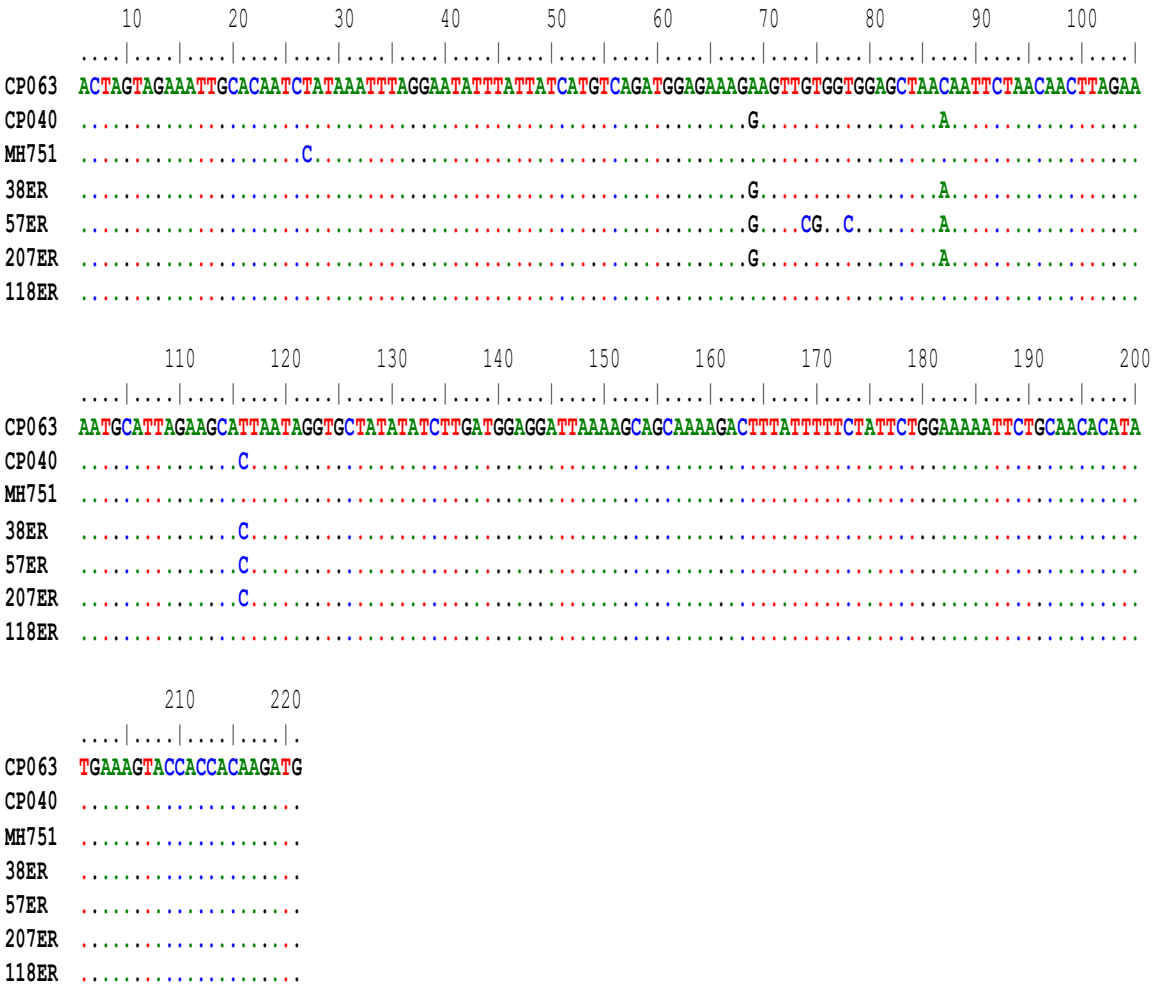

Figure 4.5: Sequence alignment of PSC20 gene of *E. ruminantium* detected in *Amblyomma hebraeum* ticks in Mopani and Vhembe regions of Limpopo province. Sequences of the present study included CP040. The reference sequences from the gene bank are 38ER, 57ER, 207ER, and 118ER, CP063 and MH751.

4.3.5.2. Phylogenetic tree

E. ruminantium PCS20 sequences obtained from the present study were positioned in three different clusters (Figure 4.6). Two PCS20 nucleotide sequences from this study (CP040119.1 and CP040118.1) were positioned in the top cluster and grouped with sequences from Zambia (CP040115.1), Uganda (CP040113.1) and sub- Saharan Africa (207ERKWA and 44ERKWA). Moreover, PCS20 sequence (MK371030.1) was grouped with sequences from South Africa (CR9256.78.1), Senegal (AY236061.1) finally 118ERGROOT (data on country of origin not found).

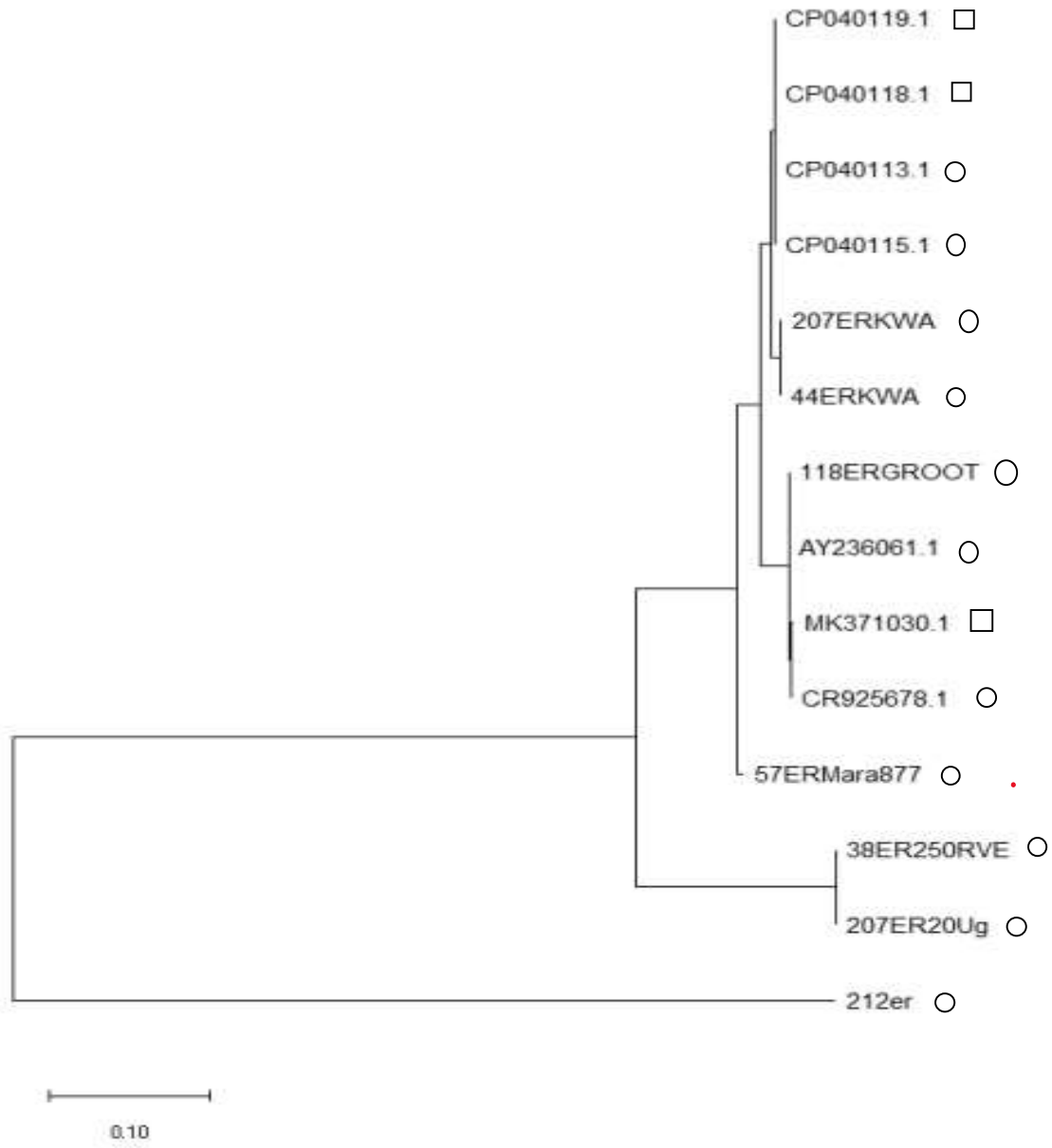

Figure 4.6: Phylogenetic analysis of *E. ruminantium* identified in this study based on PCS20 gene sequences using the neighbor-joining method. The squares indicate the sequences from this study. The circles indicate the sequences from the gene bank. The tree was constructed using the MEGA11 software program.

4.4. Discussion

The current study investigated the distribution, associated risk factors and molecular characteristics of *E. ruminantium* from tick obtained from cattle, sheep and goats in Mopani and Vhembe regions, Limpopo province, South Africa. Our results show a high prevalence of *E. ruminantium* in the study area. Samples were collected in a season where tick burden appeared to be very high. The current climatic conditions or other epidemiological factors that might affect the occurrence of heartwater in South Africa are not supported by any scientific research at this time (Bath and Leask, 2020).

In the present study, we tested 244 tick samples collected from 48 households. Of the 48 households, 56.2% of households had *E. ruminantium* infection. This prevalence is higher than the rate of infection in Mpumalanga Mnisi community which the prevalence was 17.4% (Jongejan *et al.,* 2020).

Nguni cattle breeds appeared to be more susceptible to infection than other breeds. Furthermore, Breebok goats breeds are the recently introduced breeds in Mopani and Vhembe regions. This further supports their significantly higher prevalence than other breeds. However, Mdladla *et al.* (2016) found the higher prevalence in non-indigenous animals and Savanna. Moreover, our study showed resistance to infection of the indigenous brown and black goats. The indigenous black and white sheep breeds showed more susceptibility to infection.

The differences in distribution of *E. ruminantium* have been reported in blood samples of cattle, goat, sheep and dog (Sili *et al.,* 2021). Alternatively, the distribution of *E. ruminantium* had been reported in cattle and sheep by Guo *et al*. (2019). The study revealed a higher prevalence of the

parasite in cattle than in sheep. Similarly, the present study presents the highest prevalence of *E. ruminantium* in cattle than the other small ruminants (sheep and goats). Moreover, the sex of the animal host appeared to be the significant factor wherein females present the highest level of *E. ruminantium* prevalence than males. Furthermore, the prevalence of the parasite by the age of the animal host showed the highest level at the age of 3-4 years and the lowest distribution was showed by animals that are less than 1 year.

Heartwater in small ruminants is a widespread problem that has a negative impact on the wellbeing and productivity of sheep and goats (Basit *et al.,* 2022). In the present study, the differences in the prevalence of *E. ruminantium* in response to farmers keeping different types of animals, was higher in farmers who had sheep in their household. This is an important point to note that sheep in the Vhembe and Mopani region might be more prone to heartwater. However, household farmers keeping goats had a lower prevalence as compared to household found with sheep.

BLASTn analysis revealed that *E. ruminantium* PCS20 nucleotide sequences obtained from *A. hebraeum* ticks of cattle, sheep and goats from Limpopo province were highly conserved (98-100%). According to Anifowose *et al*. (2020), the amplification that targets the PCS20 gene in engorged ticks may be more suitable to determine the *E. ruminantium* carrier status of cattle, sheep and goats. Furthermore, PCS20 gene have proved to be specific for *E. ruminantium,* giving no cross- reactions with other *Ehrlichia* species (Matos *et al.*, 2019).

In the present study, the investigation of the genetic diversity of *E. ruminantium* was conducted based on the PCS20 sequences detected in cattle, sheep and goats from the four municipalities of

Limpopo province. High degree of sequence polymorphism was observed in accordance with previous reports (Raliniaina *et al.,* 2010).

Given that our study did not evaluate the clinical/health state of the sampled cattle, sheep and goats. There is little alternative to farmer diagnosis for assessing heartwater. This is because there are no quick and accurate field tests for heartwater. This study was conducted during the Covid-19 pandemic era, there was also a low response rate during the sampling period. Some farmers were sensitive to allow their animals in the study. Disclosure of financial status was met with some reluctance from the respondents. Furthermore, access to the university laboratory was very limited. This has delayed the completion of DNA extraction, PCR and sending samples for sequencing. Further research may be necessary to determine the pathogenicity of the strains observed. Although the animals appeared healthy, this may indicate that the indigenous breeds, particularly exotic types, may operate as indicator species for *E. ruminantium*, spreading it to other susceptible ruminants.

The *E. ruminantium* PCS20 partial sequences from this study separated into two distinct clades, in the phylogenetic tree. This demonstrates that the parasite may exist in more than one strain in the studied area. As a result, it is critical to implement the necessary measures to prevent the spread of *E. ruminantium* and the development of new strains in the Vhembe and Mopani regions.

Chapter 5: Conclusion and Recommendations

5.1. Conclusion

Our study shows that the level of knowledge from the farmers is not adequate to assist in the control and prevention of heartwater disease. Although attitude and practices by farmers towards heartwater disease are directly influenced by the lack of access to government services. Moreover, the present study examined the distribution of *E. ruminantium* in cattle, sheep and goats from Mopani and Vhembe regions of Limpopo province, South Africa. The *A. hebraeum* ticks collected from cattle, sheep and goats from the study area had a greater prevalence 48.8% of *E. ruminantium*. It is quite clear that the present study areas are endemic to heartwater disease. The findings of the present study may assist to better understand how common is *E. ruminantium* in cattle, sheep and goats around Mopani and Vhembe regions. The *E. ruminantium* strains observed in the present study areas have been reported to be endemic from other parts of South Africa. Future vaccine development against *E. ruminantium* should consider the diversity observed in the present study. That will assist in the control of heartwater disease in the present study areas.

5.2. Recommendations

- To further understand the distribution of *E. ruminantium* in Limpopo province, we recommend that studies covering a wider area should be conducted.

- Further studies on heartwater disease in cattle, sheep and goats in Limpopo province are highly recommended in order to accurately determine the geographic distribution, prevalence and genetic diversity of *E. ruminantium* species in the Province.
- Livestock farming of indigenous African breeds should be given more preference in areas lacking veterinary services and adequate tick control measures in order to provide a more sustainable approach to the control of heartwater disease.
- Further studies on transmission and pathogenicity of the different strains of *E. ruminantium* must be carried out particularly with the aim of manufacturing of heartwater diagnostic kit.
- The development of a more effective, practical, affordable and safe vaccine should be of the highest priority and concern.

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Appendix A

UNIVERSITY OF VENDA

DEPARTMENT OF MICROBIOLOGY

Research questionnaire

Research topic: Molecular characterization of *Ehrlichia ruminantium* in Limpopo province

Objective: Knowledge, attitude and practices of small farm holders in Vhembe and Mopani region concerning heartwater disease

SECTION A: PARTICIPANTS DEMOGRAPHICS

Collection details

Date : ……..……. Time: ……..

Collection site : ……...

1. PID code : ……...

Collectors : ……...

Consultation details

Important note	Kindly make your selection with a tick where required
Farmer's details	
Farmer's name	
2. Gender	Male ☐ Female ☐
3. Household income	< 500 ☐ 500-1500 ☐ 1500-7500 ☐ 7500-20000 ☐ > 20000 ☐
4. Age of farmer	15-20 ☐ 21-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ Above 60 ☐
5. How many people staying in the household?	
6. Level of education	No formal education ☐ Primary education ☐ Secondary education ☐ Tertiary education ☐
7. Types of animals in the household (Indicate number in the appropriate box)	Cattle ☐ Goats ☐ Sheep ☐ Dogs ☐ Cats ☐ Donkeys ☐ Chicken ☐ Pigs ☐ Other ☐
8. Breed of animals sampled	Cattle …… Goats…….. Sheep……..

NOTE: Kindly select the correct answer with a mark

SECTION B: KNOWLEDGE

9. Do you know about heartwater disease?	Yes ☐ No ☐
10. What do you know most about heartwater disease?	
11. What is the mode of its transmission?	Air-borne ☐ Water-borne ☐ Tick-borne ☐ Food-borne ☐
12. What type of animals does it affect?	
13. What are the symptoms of heartwater disease?	Diarrhea ☐ Coughing ☐ Nasal discharges ☐ Fever ☐ Loss of appetite ☐ Head pressing ☐ All of them ☐ None of the above ☐
14. Is there a way of controlling it?	Yes ☐ No ☐
15. Have you ever heard of any vaccine available for heartwater?	Yes ☐ No ☐
16. In which season of the year does heartwater commonly occur?	Dry season ☐ Rainy season ☐ All season ☐
SECTION C: ATTITUDE	
17. Are you satisfied with the animal services provided in your area?	Very much ☐ Sometimes ☐ Not at all ☐ There are no livestock education available in my area ☐
18. Do you think it is important to have knowledge about heartwater disease?	Yes ☐ No ☐
19. Do you think heartwater is a dangerous disease?	Yes ☐ No ☐
20. How do you think heartwater disease is treated?	
21. Per your own point of view, do you associate any diseases experienced on your animals with ticks?	Yes ☐ No ☐
22. Please explain	
23. Are ticks an issue of concern for your livestock farming?	Not a problem ☐ A moderate problem ☐ A serious problem ☐ Somewhat a problem ☐ Never thought about it ☐
24. Are you in contact with the state veterinarian office?	Yes ☐ No ☐

25. What is your opinion for including a state veterinarian in your animal farming?	
26. Do you think the government is helping enough in your livestock farming?	Yes ☐ No ☐
27. Are you satisfied with how your livestock farming is going?	Yes ☐ No ☐
28. Please explain	
29. What are your feelings on the number of livestock would you wish to have?	
30. Do you think you are producing enough livestock?	
SECTION C: PRACTICES	
31. For how long you have been doing livestock farming?	≤5 years ☐ >10 years ☐ 1 year ☐
32. Are you available on a full-time basis to look after your livestock?	Yes ☐ No ☐
33. Do you have someone employed to take care of your animals?	Yes ☐ No ☐
34. Do you have record of animals who died in the past years because of heartwater disease?	Yes ☐ No ☐
35. Number of animals affected by heartwater resulting in mortality/death	< 10 ☐ > 10 ☐
36. Which tick control method do you use?	Spraying ☐ Dipping ☐ Spot treatment ☐
37. Which supplementary tick control method do you use?	Removing by hand ☐ Tick grease ☐ Homemade mixtures ☐ Engine oil ☐ All of them above ☐
38. Do you use any medicinal plants to cure animals for heartwater?	Yes ☐ No ☐
39. Do you know any medicinal plants used against ticks?	Yes ☐ No ☐
40. Mention any medicinal plants you use to treat animal diseases	
41. Where do your animals feed?	
42. Who takes them out for feeding?	

43. What time do they come back?	
44. At what time, do you take your animals for feeding?	
45. Where do your animals drink water?	
46. Which illnesses have you once experienced on your animals amongst the following?	Diarrhea ☐ Rolling eyes ☐ Noisy ☐ Coughing and nasal discharge ☐ Running around ☐ Fever and loss of appetite ☐ Head pressing ☐
47. What other diseases do your animals suffer from?	

Appendix B

RESEARCH ETHICS COMMITTEE

UNIVEN Informed Consent

LETTER OF INFORMATION

Title of the Research Study : Molecular characterization of *Ehrlichia ruminantium* in Limpopo province, South Africa

Principal Investigator/s/ researcher : Ms Mathebula D, BSc Honors in Microbiology

Co-Investigator/s/supervisor/s : Prof A. Samie, PHD and Dr MT Sigidi, PHD

Brief Introduction and Purpose of the Study:

Outline of the Procedures : The main aim and objectives of the study were clearly explained to the household farmers who allow their animals to participate in the study. They were given consent forms to sign completed a questionnaire during sample collection. The completed questionnaires were stored in an office that is locked. The study concentrate on animals of any age. Laboratory analysis was achieved through DNA extraction, PCR and sending positive samples to Iqaba biotech for sequencing. Feedback will be given to each household farmer who allowed their animals to participate in the study, orally and by writing.

Risks or Discomforts to the Participant : None

Benefits : None
Reason/s why the Participant May Be Withdrawn from the Study: (*Non-compliance, illness, adverse reactions, etc. Need to state that there will be no adverse consequences for the participant should they choose to withdraw)*

Remuneration : None
Costs of the Study : None
Confidentiality : The questionnaire and consent forms will be kept confidential
Research-related Injury : There are no research-related injuries involved in this project

Persons to Contact in the Event of Any Problems or Queries:

Supervisor: Prof Amidou Samie, Please contact the researcher Mathebula Doris at (072 912 8838), my supervisor (060 504 4384) or the University Research Ethics Committee Secretariat on 015 962 9058. Complaints can be reported to the Director: Research and Innovation, Prof GE Ekosse on 015 962 8313 or Georges Ivo.Ekosse@univen.ac.za
General:
Potential participants must be assured that participation is voluntary and the approximate number of participants to be included should be disclosed. A copy of the information letter should be issued to participants. The information letter and consent form must be translated and provided in the primary spoken language of the research population

CONSENT

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, (*Ms Mathebula Doris*), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _,
- I have also received, read and understood the above written information (*Participant Letter of Information*) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

Full Name of Participant Date Time Signature

I, ……. …… …… ……..

(*Name of researcher*) herewith confirm that the above participant has been fully

Informed about the nature, conduct and risks of the above study.

Full Name of Researcher

Mathebula D

……. Date……. Signature……

Full Name of Witness (If applicable)

……. Date ……. Signature……..

Full Name of Legal Guardian (If applicable)

…… Date…… Signature…….

Please note the following:

Research details must be provided in a clear, simple and culturally appropriate manner and prospective participants should be helped to arrive at an informed decision by use of appropriate language (grade 10 level- use Flesch Reading Ease Scores on Microsoft Word), selecting of a non-threatening environment for interaction and the availability of peer counseling (Department of Health, 2004)

If the potential participant is unable to read/illiterate, then a right thumb print is required and an impartial witness, who is literate and knows the participant e.g. parent, sibling, friend, pastor, etc. should verify in writing, duly signed that informed verbal consent was obtained (Department of Health, 2004).

If anyone makes a mistake completing this document e.g. a wrong date or spelling mistake, a new document has to be completed. The incomplete original document has to be kept in the participant's file and not thrown away, and copies thereof must be issued to the participant.

References:

Department of Health: 2004. *Ethics in Health Research: Principles, Structures and Processes*

http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

Department of Health. 2006. *South African Good Clinical Practice Guidelines*. 2nd Ed. Available at:

http://www.nhrec.org.za/?page_id=14

Appendix B1

RESEARCH ETHICS COMMITTEE

Consente ya UNIVEN

VHURIFHI HA THENDELANO

Thoho ya thoduluso : Molecular characterization of *Ehrlichia ruminantium* in Limpopo province, South Africa

Muranga-panda/ mutodulusisi : Ms Mathebula D, BSc ya science kha Microbiology

Vhanwe vha vhatodulusisi/ Varhanga-panda va thoduluso : Prof A. Samie, PHD na Dr MT Sigidi, PHD

THALUTSHEDZO YA THANDELA YA THODULUSO NA FOMO YA THENDELO VHARANGAPHANDA VHA THODULUSO EDZI.

Ri a vha ramba na u vha humbela u dzhenelela thoduluso dza thandela ya vhulwadze hu dinaho na u vhulaya zwifuwo (Heartwater). Tshipikwa tshihulwane tsha thoduluso iyi tshi do talutshedzwa nga vhudalo na nga u dodombedza kha vhane vha zwifuwo. Ngauralo mushumo wa thoduluso iyi wo disendeka kha u todulusa tshi khokhonono tshi vhidzwaho upfi *Ehrlichia ruminantium*. Tshikhokhonono itshi tshisa vhonaliho nga mato a nama tshi vhanga vhulwadze vhu dinaho vhukuma kha zwifuwo lune mafheleloni azwo tsha fhedza tshikho vhulaya tshifuwo zwa minwaha yothe u katela na zwifuwo zwi ne zwa kha di tobva u begwa. Ngauralo, thandela iyi i katela zwifuwo zwa minwaha yothe hu sa londwi uri tshifuwo tsho vha hone shangoni nga nwaha u fhio. Mushumo wa thoduluso wa tshikhokhonono itshi tshi sa vhonaliho tshino vhanga vhulwadze kha zwifuwo zwashu udo itelwa University of Venda kha dzi laborothari dza

vhadivhalea vhahulwane vhano shuma u todulusa zwithu zwi vhangaho malwadze kha zwifuwo vhane vhavha vho Prof A Samie na mutikedzi wavho ane avha vho Dr MT Sigidi. U sumbedza vhundeme na matshilisano kha vho razwifuwo vhashu, musi thandela iyi yono fhela, bvelelo dza thandela iyi dzido nwalwa dza dovha hafhu dza talutshedziwa vhane vha zwifuwo tshipikwa tshihulwane hu uri ri shumisane rothe u kona u thivhela malwadze aya a dinaho zwifuwo zwashu.

Ahunga dovha na zwithu zwine zwanga vha si a vhakhomboni kana zwa sa vha fare zwavhudi.

Malamba: vhane vhazwifuwo vhado divhadzwa nga ha mvelelo dza thandela iyi fhedziha ahunga dovha na malamba a masheleni a ne a do newa vhane vhazwifuwo

Confidentiality: Ri vha fulufhedzisa uri zwidodombedzwa zwine vhado ri nea zwone zwido vhulungwa lwa tshiphiri na hone azwinga divhiwi nga vhathu.

Khombo kha thandela iyi: a hu nga dovha na khombo dzine dza nga I te musi thandela iyi I vhukati.

Vhasa farea zwavhudi vhanga kwama muhulwane vha university kana vhara ngaphanda vha thoduluso idzi vhane vha vha Vho-Prof A Samie kha 060504 4384, Vho Mathebula Doris kha 072912 8838 kana mabalani wa University Research Ethics Committee kha 015 962 9058. Zwisolo zwo tanganezea kha Dayirekitara wa Research and Innovation Vho-Prof GE Ekosse kha nomboro heyi, 015 962 8313 kana nga email kha GeorgesIvo.Ekosse@univen.ac.za

General

U dzhenelela havho kha thandela iyi zwi fanela u vha zwitshibva mbiluni yavho na hone avha kombetshedziwi u vha tshipida tsha thandela iyi. Na hone vhathu vho dzenelelaho kha thandela iyi vha do tea u bviselwa khagala. Muthu ane a do dzhenelela kha thandela iyi udo newa vhurifhi vhu sumbedzaho zwidodombedzwa zwa thandela iyi hu u itela uri apfe o tsireledzea kha u vha tshipida tsha thandela iyi. Zwidodombedzwa zwothe zwa thandela iyi zwido nwalwa nga luambo lwa damuni lwa vhathu vhano do dzhenelela kha thandela iyi.

THENDELO YA U VHA TSHIPIDA TSHA THODULUSO EDZI.

- Nne…(dzina la mufuwi) …….ndi kho tenda uri ndo vhalelwa nga vhudalo nga ha thoduluso eyi nahone nda dovha hafhu nda talutshedzwa zwothe zwikatelaho iyi thoduluso
- Ndo newa bambiri li talutshedzaho thandela iyi nahone nmdo vhala nda pfesesa nda dovha hafhu nda vhudzisa dzi mbudziso fhethu he nda vha ndi sa kho fhirisea zwavhudi
- Ndi a divha uri zwidodombedzwa zwazwifuwo zwikatelaho minwaha, mbeu, maduvha a mabebo zwido shumiswa kha thandela iyi nahone zwido vhewa na u shumiwa nga khomphiyutha.
- Nahone ndo tendelwa usa tsha vha tshipida tsha ino thandela tshifhinga tshinwe na tshinwe tshine nda toda
- Ndo newa tshipida tsha u vhudzisa tshi thu tshinwe na tshinwe tshe ndavha ndisa kho fhirisea khatsho nahone nda fhiwa phindulo ngaha zwe nda vhudzisa

- Nahone ndi a pfesesa uri mvelelo dza thandela iyi ndi do dzi newa hu sa londwi uri ho waniwa zwithu zwiswa nga kha zwifuwo zwanga.

Madzina a mufuwi Duvha Tshifhinga Signature
Nne, ……. …… …… ……..

Nne…… ndi kho tenda uri zwidodombedzwa zwothe zwire kha fomo iyi zwo talutshedzwa muthu oyu ano kho dzhenelela kha thandela iyi.
Madzina a mutodulusisi**:** Mathebula D
……. Date**…….** Signature**……**
Madzina a thanzi (If applicable)
……. Date **…….** Signature**……..**

Madzina a Mubebi (If applicable)
…… Date**……** Signature**……**

Kha vha zwi jiyele thohoni uri :
Thoduluso I tea u vha yo nwalwa nga ndila I pfeseseaho, isa kondi nahone ya dovha hafhu yavha I sumbedzaho mikwa na u thonifha sialala la vha dzheneleli vha thandela. Nahone vhadzheneleli vha thandela iyi vha tea u talutshedzwa nga ndila I sa kondiho hu u itela uri vhavhe na pfaneleo ya u tenda kana u landula uvha tshipida tsha thoduluso idzi nahone nga luambo lu pfeseseaho na hone lu sa kondi (Grade 10 level-use Flesch Reading Ease Scores on Microsoft word), selecting of non-threatening environment and the availability of peer counselling(Department of Health, 2004)

Arali mune wa zwifuwo asa koni u vhala kana u wala, gunwe la tshanda tsha ula li tea u shumiswa hu tshiga tsha u sumbedza uri thendelano yovha hone nahone u tea u vha na muthusi musi atshi ita ezwo. Muthusi wawe angavha shaka, mufunzi kana khonani nahone muthusi un fanela u saina fomo u sumbedza uri o thusedza khau sainiwa ha fomom ya zwidodombedzwa.(Department of Health, 2004).

Arali hangavha na vhukhakhi musi hu tshi kho dadziwa fomo, hungavha vhukhakhi ha duvha, kana vhu vhum khakhi vhufhio, fomo ntswa I tea u shumiswa. Yeneyo fomo yo khakheaho ite u lata na hone mudzheneleli wa thandela u tea u newa fomo ya khophi nae.

References

Department of Health: 2004. ***Ethics in Health Research: Principles, Structures and Processes***
http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/
Department of Health. 2006. ***South African Good Clinical Practice Guidelines*****. 2nd Ed. Available at:**
http://www.nhrec.org.za/?page_id=14

Appendix B2

RESEARCH ETHICS COMMITTEE

Consente ya UNIVEN

PAPILA RA VUTHALA

Hloko-mhaka ya vulavisisi : Molecular characterization of *Ehrlichia ruminantium* in Limpopo province, South Africa

Mulavisisi nkulu/ mulavisisi : Ms Mathebula D, Ntokoto wa dyondzo ya science eka ndzawulo ya Microbiology

Valavisisi kuloni/ Varhangeri va ntlawa : Prof A. Samie, PHD na Dr MT Sigidi, PHD

Vuxoko-xoko na xikongomelo xa ndzavisiso:

Pfapfarhuto wa maghenelo : Xikongomelo na swiyenge swa ndzavisiso lowu swi ta hlamuseriwa hi vuenti eka vafuwi vale makaya lava pfumelelaka swifuwo swa vona eka ndzavisiso lowu. Va ta nyikiwa ti fomo to tata ku tiyisisa. Fomo ya swivutiso yi ta hlayisiwa ku hlonipha vuxoko-xoko bya vafuwi. A swi khathaleki kuri xifuwo xi dyuhale ku fika kwihi kuva xiyenge xa ndzavisiso. Dzavisiso wuta endliwa e Laboratory hiku tirhisa DNA extraction, PCR ku katsa no rhumela ti sample eIqaba biotech ku checkisisa mixaka ya switsongotsongwani. Mbuyelo wuta nyiketiwa mufuwi wunwana na wunwana loyi a nghenelerisaka xifuwo xa yena eka ndzavisiso lowu hi mbulavulo kumbe hi ku tsala.

Leswi nga mi kanganyisaka ku nghenelela : Ku hava

Mbuyelo : Swi va swiri ka nwina ku tekela nhlokweni vuvabyi bya swiharhi

Leswi nga endlaka leswaku mi vekiwa tlhelo eka ndzavisiso lowu: a ku na swita-ndzaku ehenhleni ka ku tshika ku nghenelela eka ndzavisiso lowu

Mahakelelo : ku hava
Costs of the Study : None
Ku hlayiseka ka vuxoko-xoko bya nwina : Tinhlamulo na fomo ya consete swi ta vekiwa hi vurhonwani ku hlayisa vumbhoni bya nwina
Ku vaviseka hikokwalaho ka ku van a xiave eka ndzavisiso lowu : ku hava ku vaviseka ehenhleni ka swifuwo swa nwina

Vanhu lava mi nga ti hlanganisaka na vona loko mi hlangana na swikanganyiso kumbe ku va na swivutiso:

Muofiseri: Prof Amidou Samie, Tihlanganiseni na mulavisisi yena Mathebula Doris eka 072 912 8838, Muofiseri eka 060 504 4384 kumbe Matsalani wa University Research Ethics Committee eka 015 962 9058. Swisolo na swibumabumelo swi nga kongomisiwa eka Dayirekitara wa Research and Innovation yena Prof GE Ekosse eka nomboro leyi, 015 962 8313 kumbe eka GeorgesIvo.Ekosse@univen.ac.za

General:
Vafuwi va fanele ku twisisa leswaku ku nghenelela eka ndzavisiso lowu I ku tinyiketela nakona hlayo ya swifuwo leswi lavekaka yi ta paluxiwa. Papila ra vuxoko-xoko ri ta humelerisiwa eka vafuwi. Papila ra vuthala na fomo ya consente swi fanele ku tsariwa hi ririmi leri vafuwi va tirhisaka rona ekaya

CONSENTE

Xitatimende xo pfumela ku van a xiave eka ndzavisiso lowu

- Ndza pfumela leswaku ndzi tivisiwile hi mulavisisi (Vito ra mulavisisi), Mayelana na pfapfarhuto, maghenelo, mbuyelo na khombo ra dyondzo leyi, Research Ethics Clearance Number: ……. ,
- Ndzi amukerile, ku hlaya no twisisa mahungu lawa nga tsariwa laha henhla (*Papila ra vuthala*) mayelana na dyondzo leyi.
- Ndzi tivisiwile leswaku mbuyelo wa dzavisiso lowu, xikanwe na voxoko-xoko bya rimbewu, malembe na diagnosisi swi ta tsariwa eka papilla ra mbuyelo hikwawo.
- Hi ma langutelo ya swilaveko swa dzavisiso lowu, ndza pfumela leswaku vuxoko-xoko lebyi nga kumiwa eka dzavisiso lowu byita pfapfarhutiwa hi ndlela ya kahle eka ti compuyuta hi mulavisisi
- Ndza pfumeleriwa e handleni ka ku tshikeleriwa ku tshika ku nghenelelala eka dyondzo leyi
- Ndzi kumile nkarhi wo ringanela ku vutisa swivutiso naswona ndzi tshembha ndzi nga kanakani ku nghenelerisa swifuwo swa nga enga ndzavisiso lowu.
- Ndza swi twisisa leswaku mbuyelo lowu kumekaka eka dyondzo ya dzavisiso lowu, wu nga va na xiave eka ku nghenelela ka mina, ndzi ta hlamuseriwa hi wona.

Mavito ya mufuwi siku nkarhi Sayino

Mina, …… …… …… ……..

(*Vito ra mulavisisi*) Ndza pfumela leswaku mufuwi loyi u tivisiwile hi manghenelo, ku ti nyiketela na swita-ndzaku swa dzavisiso lowu.

Mavito ya mulavisisi

Mathebula D

…… Siku…… Sayino……

Vito ra mbhoni (loko swi koteka)
…… Siku …….. Sayino……..

Mavito ya mutswari (Loko swi koteka)
…… Siku…… Sayino…….

Tekelani hlokweni leswi landzelaka:

Dzavisiso wu fanele ku humelerisiwa hi ndlela yo hlonipha, ku twisiseka na ku ka wu nga paluxi marito ya vusopfa. Vafuwi va fanele ku pfuniwa ku twisisa hikwaswo leswi va swi sayinelaka h ririmi ra manana (Department of Health, 2004)

Ku ngava mufuwi anga koti ku hlaya kumbe ku tsala, u fanele ku ba xihambano kumbe ku kombela ku pfuniwa hi va rixaka kwala kaya, la swi kotaka nakona a tiva mufuwi e.g. mughana, mutswari, mufudhisi etc.. u fanele ku sayina leswaku consete yi amukeriwile (Department of Health, 2004).

Ku ng ava na wunwana loyi a endlaka xihoxo eka fomo e.g. ku hoxisa matsalele, u fanele ku kuma fomo yintshwa a tata yona. Fomo leyi nga tshikiwa e pfhukeni yi fanele ku vekiwa eka file ya mufuwi ku ngari ku cukumetiwa naswona ti copy ti fanele ku nyikiwa mufuwi.

References:

Department of Health: 2004. *Ethics in Health Research: Principles, Structures and Processes*

http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

Department of Health. 2006. *South African Good Clinical Practice Guidelines*. 2nd Ed. Available at: http://www.nhrec.org.za/?page_id=14

Appendix C

ETHICS APPROVAL CERTIFICATE

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Ms D Mathebula

STUDENT NO:
11595368

PROJECT TITLE: **Molecular characterization of *Ehrlichia ruminantium* in Limpopo Province, South Africa.**

PROJECT NO: SMNS/20/MBY/03/0707

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof A Samie	University of Venda	Supervisor
Dr MT Sigidi	University of Venda	Co - Supervisor
Ms D Mathebula	University of Venda	Investigator – Student

Type: **Masters Research**
Risk: **Minimal Risk to humans, animals and environment**
Approval Period: **July 2020 – July 2022**

The Animal, Environmental and Biosafety Research Ethics Committee (AEBREC) hereby approves your project as indicated above.

General Conditions
While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following.

- The project leader (principal investigator) must report in the prescribed format to the REC:
  - Annually (or as otherwise requested) on the progress of the project, and upon completion of the project
  - Within 48hrs in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project. The project leader must apply for approval of these changes at the REC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date; a new application must be made to the REC and new approval received before or on the expiry date.
- In the interest of ethical responsibility, the RECs retains the right to:
  - Request access to any information or data at any time during the course or after completion of the project.
  - To ask further questions; Seek additional information; Require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
  - Any unethical principles or practices of the project are revealed or suspected.
  - It becomes apparent that any relevant information was withheld from the REC or that information has been false or misrepresented.
  - The required annual report and reporting of adverse events was not done timely and accurately.
  - New institutional rules, national legislation or international conventions deem it necessary

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE
Date Considered: July 2020

Name of the AEBREC Chairperson of the Committee: Prof Irene Barnhoorn

Signature:

Director Research and Innovation

Signature: .....

UNIVERSITY OF VENDA
OFFICE OF THE DIRECTOR
RESEARCH AND INNOVATION
2020 -07- 2 0
Private Bag X5050
Thohoyandou 0950

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