Modelling the effect of climate change on the distribution of *Amblyomma hebraeum* in South Africa

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List of abbreviations

DEA	Department of environmental affairs
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
µm	micrometer
SFG	Spotted fever group
CNS	Central nervous system
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
LDH	Lactate dehydrogenase
GGT	Gamma glutamyl transferase
OmpA	Outer membrane protein A
OmpB	Outer membrane protein B
Tx	Maximum temperature
Tn	Minimum temperature
Hx	Maximum relative humidity
Hn	Minimum relative humidity
CGCM	Coupled Global Climate Model
CCAM	Conformal-Cubic Atmospheric Model
CSIRO	Commonwealth Scientific and Industrial Research Organization
MAXENT	Maximum entropy
PO	Presence-only

DNA	Deoxyribonucleic acid
UV	Ultraviolet
qPCR	Real-time polymerase chain reaction
UDG	Uracil-N-Glycosylase
µM	Micromole
µL	Microliter
°C	Degrees Celsius
AUC	Area under the curve
bp	Base pair
ng	Nano gram

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Thesis summary

It has been postulated that climate change has brought about a change in the distribution of *Amblyomma hebraeum* and subsequently, its potential tick-borne parasites in South Africa and that the effects of climate change may well lead to further future distribution changes. This study demonstrated that *A. hebraeum* may be present in the Western Free State and, although evidence could not be found of *E. ruminantium* or *R. africae* infection in ticks, laboratory results confirmed the presence of the former parasite in brain smears from on-farm mortalities. Habitat suitability modeling using Maxent demonstrated that the distribution of *A. hebraeum* has altered compared to previous scenarios, is still changing, and that by the year 2065, most of the central and eastern parts of South Africa would have a high habitat suitability index for its presence. This indicates that diseases caused by *E. ruminantium* and *R. africae* would also have to be considered in these previously unaffected areas when animals or humans show signs of illness. The economic and health impact of these diseases in the expanded areas could well be quite substantial.

Chapter 1: General introduction

Ticks of the genus *Amblyomma* transmit rickettsial diseases such as *Ehrlichia ruminantium* , the causative organism of Heartwater in animals (Allsopp, 2010), and *Rickettsia africae* , the causative organism of African tick bite fever in humans (Bitam, 2012). The primary vector in South Africa of these disease organisms is *Amblyomma hebraeum*, the South African bont tick (Ledger et al., 2022, Walker and Olwage, 1987). In the southern African region, 65% of all livestock are farmed within *Amblyomma* infested areas, making losses due to tick-transmitted diseases such as Heartwater a significant problem (Chemonics International, Inc, 2001). In South Africa the total estimated annual loss due to Heartwater, including both direct and indirect losses (taken as cost to prevent and treat the disease), is in the region of R1 266 million, of which direct losses contribute 66.47% and indirect costs 33.57% (van den Heever et al., 2022). Little is known about the effect of climate change on the distribution of *A. hebraeum* and as a consequence, of *E. ruminantium* and *R. africae* in South Africa (Leask and Bath, 2020b). Presently, there is an under representation of studies modeling the distribution of *Amblyomma* ticks and the rickettsial diseases that they carry compared to other tick-borne disease in the literature (Lippi et al., 2020). Most of the tick's life cycle is in a non-parasitic condition (Norval, 1973), and therefore the impact of climate change is an essential variable in tick control.

1.1 Aim

Due to the fact that climate change has been proposed as a reason for the wider spread of *A. hebraeum* in South Africa, this study investigated the possible effect of climate change on the distribution and projected distribution of *E. ruminantium* and *R. africae* due to the potential difference in the distribution of the vector, *A. hebraeum* in South Africa.

1.2 Objectives

1. Quantify the climatic conditions suitable for the survival of *A. hebraeum*.

2. Establish the historical and current geographical areas where *A. hebraeum* is found and link these to current and projected future climatic conditions.

3. Investigate the infection rates of *R. africae* and *E. ruminantium* in the more recent distribution areas as compared to in the known established sites.

4. Establish the effect of climate on the modelled distribution of *A. hebraeum* and predict future distribution patterns.

1.3 Benefits arising from the research

The outcome of this project would be a summary of current changes already seen in the distribution of *A. hebraeum* and the parasites it carries due to climate change, as well as a future prediction of areas where these diseases might become established in South Africa. These data would be invaluable to farmers, government departments and veterinarians in planning timeous prophylactic measures and control strategies in order to decrease the debilitating impact of Heartwater in their communities and on their farms. Research and information dissemination efforts may also be increased in the projected areas of distribution. In addition, the well documented direct traumatic effect due to the bites of these ticks may be minimized.

Chapter 2: Literature review

2.1. Other studies on the topic

Previous studies on climate change and suitability for *A. hebraeum* and *A. variegatum* conducted in Zimbabwe found good agreement between field records and modeled spatial range (Estrada-Peña et al., 2008). These studies also concluded that climate change could turn an unfavourable area into a favourable habitat niche for *A. hebraeum* within a few years (Estrada-Peña et al., 2008). It has also been shown that of the limiting variables to the spread of African ticks, climate plays the most important role (Cumming, 2002), after considering a 6x6 degree grid, normalized difference vegetation index, vegetation type, elevation, rainfall, minimum and maximum temperature and political regions. Factors affecting the distribution of *A. hebraeum* have been recorded by Norval (Norval et al., 1994) and Petney (Petney et al., 1987) and included the soil moisture, air temperature, rainfall, altitude and vegetation types. Another potential factor described has been inter-species competition between *A. variegatum* and *A. hebraeum (Bournez, 2015).* These parameters are discussed further under the heading of climatic conditions needed for the survival of *A. hebraeum.* A survey conducted amongst veterinarians and farmers found that there was general agreement that the area in which *E. ruminantium* occurred had changed compared to historical records (Leask and Bath, 2020a).

2.2 Climate change in South Africa

Over the period of 1960 to 2012 the mean average temperature in South Africa has increased by 1.4 degrees Celsius, of which the most marked increase occurred in the Western and Northern parts of the country (DEA: Full technical report on climate trends and scenarios of South Africa). Annual rainfall has stayed relatively consistent but with more dramatic rainfall events and increases in dry or wet periods in some areas have been noted (Kruger, 2006). One change noted in the timeframe of 1960-2010 was a notable decrease in rainfall in the central interior of the country (MackKellar et al., 2014). A historic trend in rainfall measured during the period of 1910-2004 showed a decreased rainfall in northern Limpopo, an area of northeastern Free State, western Kwazulu-Natal and southern Mpumalanga and

another two areas of southeastern Eastern Cape and the South Coast of South Africa (Kruger, 2006)

It is estimated that in the next three decades the temperature will increase by an average of 1.5-2.0 degrees Celsius with most of the Southern Africa region showing a reduced rainfall, except for the central interior and Eastern Cape regions in South Africa that will show a wetter rainfall season. The areas showing the most decrease in rainfall are the eastern part of Limpopo, Mpumalanga, south west- and southern Cape regions (Meissner et al., 2014).

2.3 *A. hebraeum*

Identification of *A. hebraeum:*

The genus Amblyomma may be identified by being large Ixodid ticks with long anterior mouthparts, eyes are present, the presence of rings on the legs and enameled ornamentation on the scutum (females) or conscutum (males). The males and females have festoons, but the festoons are not very visible in fully fed females. A. hebraeum is identified to species level based on its flat, very marginal eyes, the presence of small to medium punctations evenly spaced on the scutum/conscutum and the unique complex enameled ornamentation. The distinctive elaborate enamel on the middle nine of the male festoons range from pink to orange, while the marginal festoons are plain in colour (Walker et al., 2014).

Life cycle of *A. hebraeum*:

A. hebraeum belongs to the family Ixodidae and members of this family have four stages in their life cycle, namely, egg, larva, nymph and adult (Horak et al., 2002b). *A. hebraeum* is a three-host tick meaning that each stage of the lifecycle drops off the host to moult and develop further in the environment before questing to find a blood meal from a new host (Leal et al., 2020). Eggs hatch on the ground and larvae wait on vegetation for a host to pass by. The six-legged larvae will attach and feed for 7-14 days before detaching to moult in the environment. Nymphs and adults are active hunters, activated by CO_2 excretion of the host animal (Norval et al., 1989). Eight legged nymphs will again attach, feed for 7-14 days and detach to

moult (Walker et al., 2014). Adult males secrete a pheromone which attracts adult females and nymphs to attach in their close proximity (Norval et al., 1989). Males of *A. hebraeum* do not complete spermatogenesis until they have had a blood meal, and females have been found not to attach without the presence of males that have been attached for close to a week (Norval, 1973). Females will feed for 7-9 days after mating with the male before dropping to the ground to lay in the region of 20 000 eggs (Walker et al., 2014).

Hosts of *A. hebraeum*

Hosts include domestic ungulates and wild animals and birds, with smaller hosts tending to have more ticks that are immature on them (e.g. guinea fowl, hares, duiker) and the very large hosts (e.g. cattle, eland, buffalo) having more adult stages (Horak et al., 1987). In areas where domestic ungulates are absent or frequently dipped, the role of wild animals as hosts becomes more important (Jensenius et al., 2003b).

Climatic conditions for the survival of *A. hebraeum* in South Africa

As *A. hebraeum* is a three-host tick, it spends 90% of its life cycle in the environment, not attached to a host (Norval, 1973). This makes environmental factors critical for the survival of the species. Environmental factors that affect survival of the tick, especially the larval stages, include relative humidity, temperature, rainfall, seasonality, natural predators, photoperiod and habitat. Most of these environmental factors, if adverse, will affect desiccation of the larvae (Leal et al., 2020).

A. hebraeum is found in wooded areas, and does not occur in treeless grassland. It is predominant in coastal bush, riparian woodland, thornveld and Mopani woodland (Jongejan et al., 2018). The reason for this is most likely the sensitivity of the eggs to desiccation. The temperature and humidity required for survival of the different stages of *A. hebraeum* vary, with later stages being more resistant to temperature and humidity fluctuations. Pre-oviposition females will die at 15 degrees Celsius, with a humidity of 40% after two months. The optimum temperature for oviposition and egg development is 20-30 degrees Celsius, 26-35 degrees Celsius for nymphs, and 20-35 degrees for the pre-moult adult stages. Eggs

are not oviposited in hot open grassland; and in waterlogged areas, the few eggs that are laid, do not hatch (Norval, 1977). *A. hebraeum* also favours areas with a dry period between April to August (Estrada-Peña et al., 2008). An optimal rainfall range of 300-800mm and an altitude of 0-1525 meters above sea level (Petney et al., 1987) has been suggested for this species.

Historical and current geographical distribution of *A. hebraeum* in South Africa

Historically *A. hebraeum* was found in the north-eastern part of South Africa in the coastal belt area, to the Eastern Cape area (Walker et al., 2014). In the last twenty years, the distribution in the Eastern Cape has been noted to be more inland than previously recorded (Horak et al., 2009). There have also been reports of *A. hebraeum* being established in the Free State area of Boshoff (Horak et al., 2015). Goats tested positive for *E. ruminantium* antibodies in the Northern Cape, North West, Limpopo, KwaZulu-Natal and the Eastern Cape. However, some of these animals may have been imported from other provinces or were infected with non-pathogenic strains of *E. ruminantium*, as shown in the Fig.1 below (Mdladla et al., 2016).

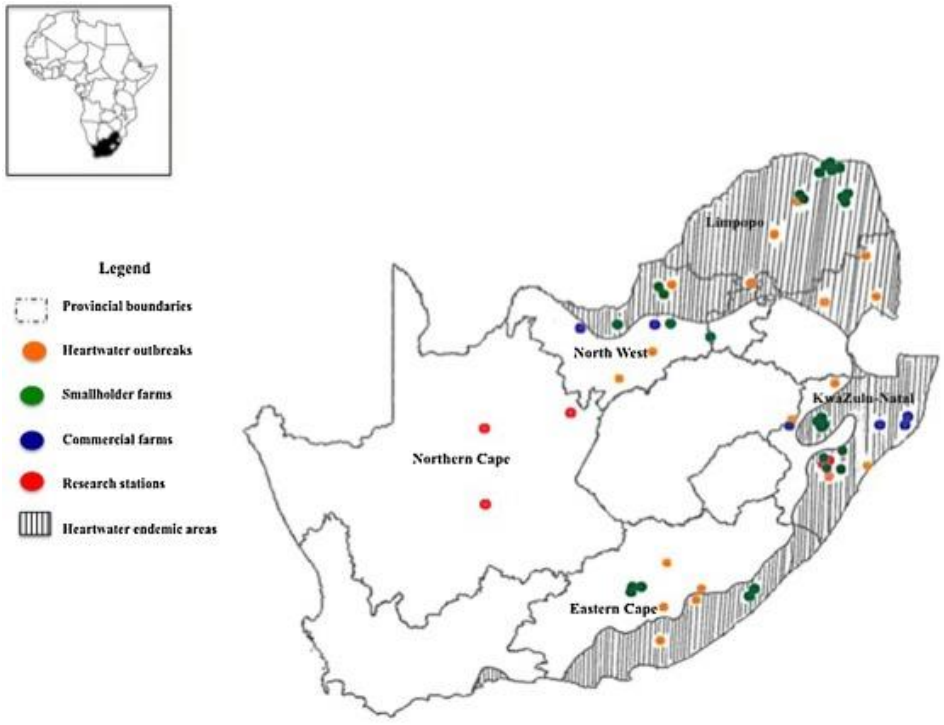

Figure 1. Heartwater distribution and sampling sites for E. ruminantium antibodies (Mdladla et al., 2016)

In addition to literature review distribution points, co-ordinate data from a 2008 study by A.M. Spickett was made available to use as reference for this study. The modelled habitat suitability for *A. hebraeum* is also shown in Fig.2,

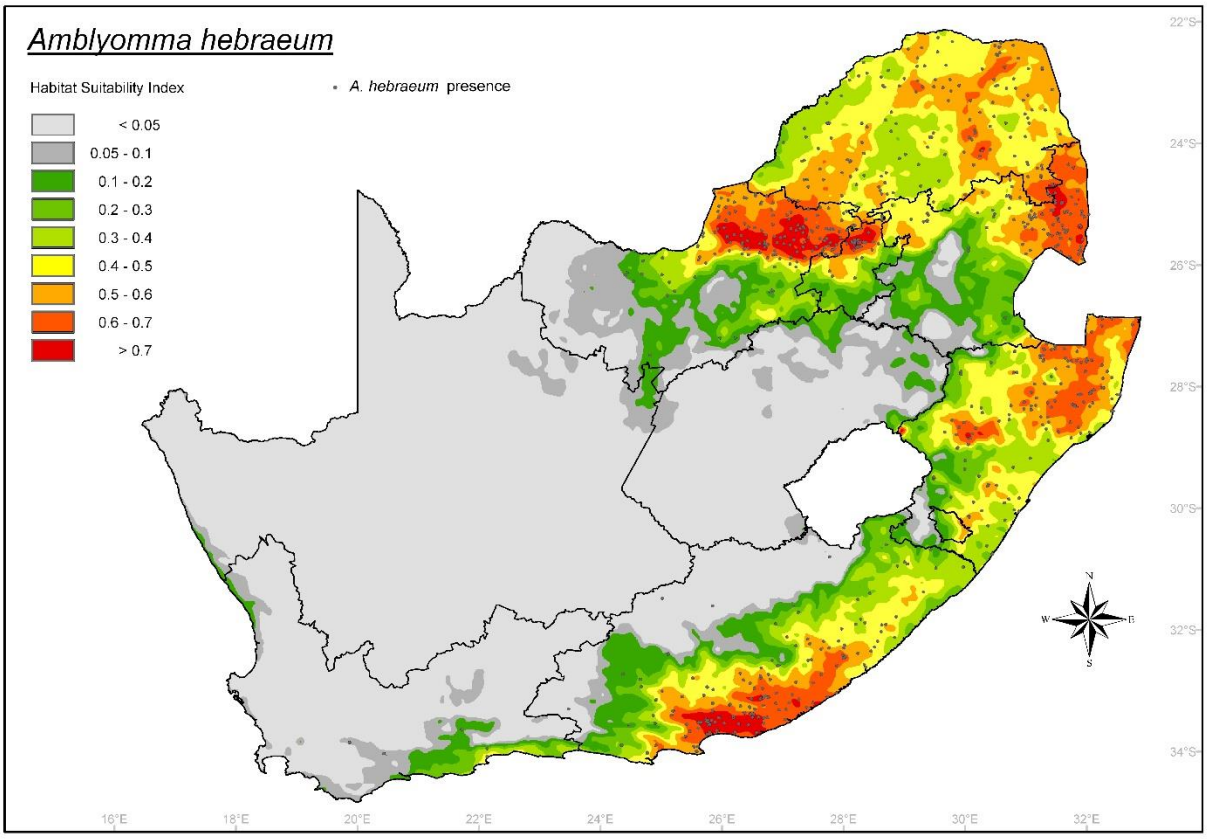

Figure 2. Habitat suitability for A. hebraeum (Spickett et al., 2008)

Changes in land use and its impact on the distribution of *A. hebraeum*

Land use affects the prevalence of ticks as the interaction between the tick and its environment has two requirements, viz. vertebrate hosts for blood meals and a suitable micro environment when they are not on the hosts (Ledger et al., 2019). In some areas such as the Eastern Cape, a change from livestock to game farming has led to an increase in the livestock wildlife interface. This interface has led to an increase in tick prevalence and diversity, with more ticks and tick species found in camps where wildlife and livestock are kept adjacent to each other, than in predominant cattle areas (Smith and Parker, 2010). Vegetation on game farms is normally less controlled than on cattle farms. Grasses are left to grow taller and

bush encroachment is not as controlled. This might lead to a better-suited microclimate that protects ticks from desiccation (Smith and Parker, 2010).

Infection rates of *A. hebraeum* with *E. ruminantium* in historical distribution areas

In the Mnisi area, infection rates of *A. hebraeum* with *E. ruminantium* varied from 17.4% for adult ticks to 28.4% for nymphs (Jongejan et al., 2020). Another study found infection rates in the endemic area to range from 4.7% to 25 % (Bryson et al., 2002). Studies that include the historical distribution of *A. hebraeum* in all provinces showed 25% to 28% infection rates (Mtshali, 2016; Steyn, 2020)

Infection rates of *A. hebraeum* with *R. africae* in historical distribution areas

In the Mnisi area, a study on ticks collected from cattle as well as from vegetation using the drag method showed, infection rates of *A. hebraeum* with *R. africae* of 13.7% for adult ticks and 12.7% for larvae. Transovarial transmission efficacy for the same study also showed a very high 85.7% (Mazhetese et al., 2022). Another study in the same area on ticks collected from goats showed infection rates of adult *A. hebraeum* with *R. africae* of 15.7% and of nymphs of 38.8% (Jongejan et al., 2020). In the Eastern Cape 46.4% of sampled *A. hebraeum* were found positive for *R. africae*; this large difference might be due to the method used for detecting the presence of *R. africae,* which was done by Illumina sequencing that is more sensitive than PCR alone, i.e. using PCR and then sequencing (Kisten et al., 2021).

2.4. *E. ruminantium*

History and classification of *E. ruminantium*

This pathogen was first described by Cowdry, who proposed the name of *Rickettsia ruminantium* when he demonstrated a gram-negative staining bacterium in the endothelial cells of small capillaries (Cowdry, 1925). In honor of Cowdry it was later renamed to *Cowdria ruminantium*. As molecular techniques improved and

phylogenetics was developed, genetic analysis of the 16S rRNA and groESL operon sequences was conducted, re-organizing the order Rickettsiales into two families Rickettsiaceae and Anaplasmataceae and a reclassification of the genera. *C. ruminantium* was found to be more closely related to *Ehrlichia*, and the name was changed to *Ehrlichia ruminantium* (Allsopp, 2010; Dumler, 2001). The type specimen for *E. ruminantium* used for characterizing the species is the Welgevonden strain (Du Plessis, 1985), of which the gene sequence of the 16S rRNA is the same as the Crystal Springs strain deposited in GenBank that can be accessed under accession no. X61659 (Dumler et al., 2001).

Taxonomic tree

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Alphaproteobacteria
- Order: Rickettsiales
- Family: Ehrlichiaceae
- Genus: *Ehrlichia*
- Species: *Ehrlichia ruminantium*

(CABI, 2022)

E. ruminantium shows great genetic diversity accounting for differences in virulence of the different strains. Recombination occurs naturally and can either happen when the vertebrate host is infected with two different isolates, or if two ticks infected with two different isolates feed on the same vertebrate host (Steyn and Pretorius, 2020b). Although the latter is more likely the former was demonstrated as the Kümm isolate from a naturally infected goat (Zweygarth et al., 2002).

Characterization of *E. ruminantium*

E. ruminantium is an obligate intracellular α-proteobacterium causing the disease, Heartwater, in ruminants (Allsopp, 2010). The organism stains purple to blue with May- Grünwald-Giemsa staining depending on its size. Differentiation between

small, medium, large and very large/giant organisms can be made. The size varies from 0.2-2.5 µm. Although mostly found as a coccoid form, pleomorphic forms are common especially in colonies of very large organisms. Shapes found are horseshoe, bacillary and ring shaped. The bacteria can be found in colonies in vacuoles in the cytoplasm of the endothelial cells (Pienaar, 1970). The organism has a wall made up of two membranes and occasionally an extra double membrane is noted. The inner structures has distinct electron-dense and electron-pale areas (Prozesky, 1987).

Developmental cycle in vertebrate host and tick

E. ruminantium multiplies through binary fission (Pienaar, 1970). Jongejan describes a developmental cycle very much like that of Chlamydia where binary fission gives rise to large colonies of reticulate bodies within the vacuoles, these then form smaller intermediate bodies with a more electron-dense core before they are released from the cell as small elementary bodies that invade new cells (Jongejan et al., 1991). This whole cycle lasts 5-6 days but can be as short as 3-4 days (Zweygarth et al., 2002). Initial infection with the organism was demonstrated in lymph nodes 3 days before it becomes apparent in endothelial cells of capillaries of the brain suggesting that initial replication takes place in the reticulo-endothelial cells of the lymph nodes before being released into circulation (Du Plessis, 1970). The released organism is then taken up by the endothelial cells through a process similar to phagocytosis where they touch the cell membrane and are enclosed in the vacuole to start replication. Once the cell ruptures, the organisms are released into the bloodstream and infect more cells. It is most likely at this point that the feeding ticks gets infected with the parasite (Prozesky and Du Plessis, 1987). The presence of *E. ruminantium* organisms in peritoneal macrophages has also been noted but the importance of leucocytes in the lifecycle has not been studied further (Du Plessis, 1985). In the invertebrate host *E. ruminantium* has been found in the mid gut epithelium and in the acini of the salivary glands (Kocan et al., 1987). The stages seen in the salivary glands of the invertebrate host are similar to those used in vaccines suggesting that these stages where highly infective and were the mode of transmission of *E. ruminantium* from the invertebrate host to the vertebrate host, rather than mid gut regurgitation that was previously suggested as a means of transmission (Kocan et al., 1987).

Transmission

Ticks that are infected remain non-infective to other animals for a grace period of 38 hours and 72 hours for nymphs and adults respectively (Bezuidenhout, 1987). Clinically healthy vertebrate hosts can carry *E. ruminantium* parasites at very low levels and still be infective to tick hosts, which becomes a problem in animals moving form infected to non-infected areas as they can carry these pathogens for close to a year (Allsopp, 2010). Transmission of *E. ruminantium* is only transstadial in the tick *A. hebraeum,* which shows complete transstadial transmission i.e. from larval to nymph through to adult stage, while other tick vectors only have partial transstadial transmission; for example *A. sparsum* will only transmit from larval to nymphal stages (Bezuidenhout, 1987). There have been reported cases of vertical transmission in cattle from cow to calf, but the significance of this type of transmission has never been fully investigated (Deem et al., 1996).

Disease symptoms

The severity and incubation period of heartwater depends on the isolate, the route of infection, the amount of infective material and the species affected. (Van de Pypekamp and Prozesky, 1987). Typical signs of the disease are high fever, nervous signs, and death. Post mortem accumulation of fluid in the thoracic cavity, in the pericardial sac, lungs, and brain can be seen (Bezuidenhout, 2009). The disease can range from per-acute to mild. Per-acute infections usually die without any signs of disease, while acutely infected animals can show fever above 40 degrees Celsius, inappetence and various forms of neurological disorders such as incoordination, high stepping gait and convulsions. Petechiae can be seen on conjunctiva of the eyes and on mucus membranes. Respiratory distress is also common (Van de Pypekamp and Prozesky, 1987).

Diagnosis

Veterinarians working in endemic areas of the disease mostly do diagnosis of the disease based on the clinical signs and response to treatment with tetracyclines. Serological tests have not proven useful as cross-reactions have caused false positives and false negatives are frequently found because antibody levels are often too low to detect. Molecular genetic methods have been developed to

detected *E. ruminantium.* PCR targeting of the pCS20 region, the srRNA gene and the *map*1 gene being the currently used methods (Allsopp, 2010). Molecular detection has improved the ability to detect the disease but is often too expensive to justify in a clinical setting. Demonstration of the pathogen in brain smears at post mortem is still the most widely used method of diagnosis confirmation.

Disease control

Heartwater is controlled by various methods and a combination of these methods, such as the use of prophylactic acaricides, vaccination, antibiotic use prophylactically and for treatment of diseased animals, use of resistant breeds of animals and establishment of endemic stability (Allsopp, 2009). Problems that arise with these methods include build-up of resistance against acaricides and antibiotics, the difficulty in storage and administration of the current available vaccine, changes in the genetic diversity of the pathogen leading to altered effectiveness of the vaccine and reduced endemic stability (Allsopp, 2009).

2.5 *R. africae*

History and classification

R. africae, one of the Spotted fever group (SPG) *Rickettsia* and the cause of the disease, African tick bite fever (Bitam, 2012), was officially first described in 1996 in Zimbabwe after isolation from people showing signs of the disease and having a history of being bitten by *Amblyomma* ticks. Although Pijper eluded to its existence as early as 1930, his observations could not be corroborated by later researchers until 1996 (Kelly et al., 1996). The species name was then suggested, with the type strain Z9-Hu for which the 16sRNA gene sequence can be accessed in GenBank library under accession number L36098.

Taxonomic tree

- Domain: Bacteria
- Phylum: Pseudomonadota
- Class: Alphaproteobacteria
- Order: Rickettsiales
- Family: Rickettsiaceae

- Genus: Rickettsia
- Species group: Spotted fever group
- Species: *Rickettsia africae*

(Todar, 2006)

Characterization of *R. africae*

The organism is a rod shaped, obligate intracellular gram-negative staining bacteria with an outer slime layer and a trilaminar cell wall (Kelly et al., 1996; Jensenius et al., 2003a). The size of the rods with electron microscopy is 0.3-0.5µm by 0.9-1.6µm. The cell wall contains lipopolysaccharide antigens that cross react with other Rickettsias of the spotted fever group (Hechemy et al., 1989). Species-specific protein antigens can be found in the outer membrane protein A or outer membrane protein B (Jensenius et al., 2003b). *R. africae* cannot be cultured in cell free media but can be cultured in human embryonic lung cell fibroblasts, Vero cells, L929 or the yolk sacs of chicken embryos (Kelly et al., 1996).

Pathogenicity

The parasite causes diseases in the host 6-7days after being bitten by a tick of the *Amblyomma* species (Raoult et al., 2001). The infection spreads through a process of infecting the endothelial cells before migrating to leukocytes especially the T-cells and macrophages and this perivascular infiltration causes a wide spread lymphohistiocytic vasculitis that is characteristic of all rickettsiosis. This inflammatory reaction and endothelial cell activation is accompanied by an increase in the secretion of mediators including von Willebrand factor and soluble E-selectin (Jensenius et al., 2003c)

Transmission

Between tick transmission of the organism happens transovarially as well as transstadially (Bitam, 2012). Vertical transmission has been proven to be effective for multiple lifecycles at a high rate (Socolovschi et al., 2009). This highly efficient transmission with a low level of human infection has led some researchers to believe that there might be an endosymbiotic relationship between *R. africae* and its tick host (Maina et al., 2014). The tick can transmit the parasites for two generations (Kelly and Mason, 1991).

Disease symptoms

R. africae is the cause of most tick bite rickettsiosis in travelers returning from Southern Africa (Raoult et al., 2001). More than 90% of cases are reported by travelers with most having acquired the infection in South Africa. Reasons for why indigenous population infection rates are so low has been ascribed to the lack of diagnostic testing, the mildness of the disease and the early seroconversion of people living in endemic areas as well as the difficulty in detecting eschars in darker skin (Silva-Ramos and Faccini-Martínez Á, 2021). The disease is often characterized as cluster cases, as *Amblyomma* is not as a species-specific tick (Raoult et al., 2001) and the tick's behavior as an aggressive hunter and its abundance (Horak et al., 2002a) makes multiple bites to one person or bites to a group of people a common occurrence (Ledger et al., 2022). The disease tends to be a mild with rare instances of severe life-threatening effects, however, myocarditis and CNS signs have been noted (Frean and Grayson, 2019). Symptoms can include fevers, neck pain, regional lymphadenitis, and an eschar where the bite occurred (Jensenius et al., 2003a). The eschar is the inoculation site for the bacteria where the tick attaches to the person (Frean and Grayson, 2019). The bites are usually in clusters and a rash is not normally present as with Rocky Mountain spotted fever and Mediterranean spotted fever (Raoult et al., 2001). More recently, a systemic review has shown that clinical signs most commonly found are fever, eschars and headaches followed by lymphadenopathy, myalgia, rash, chills, lymphangitis, arthralgia, fatigue and malaise. Complications are rare at 3.7% with purpuric cellulitis, myocarditis and neurological signs being noted. Only 12.5% of all cases needed hospitalization and the recovery rate was found to be 100% (Silva-Ramos and Faccini-Martínez Á, 2021). Laboratory abnormalities were found in most of the cases with elevation of aspartate aminotransferase (AST) and C-reactive protein being the most recorded abnormalities, followed by elevation in alanine aminotransferase (ALT), leukopenia, thrombocytopenia, elevation in lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) (Silva-Ramos and Faccini-Martínez Á, 2021).

Ruminants have been found with antibodies against *R. africae,* sometimes with no evidence of clinical disease, which might be due to the use of oxytetracyclines used extensively on the ruminant populations, (Maina et al., 2014), or sometimes with mild or no clinical symptoms before seroconversion (Kelly et al., 1991, Parola et al., 1999). The results of this study must also consider the non-specific nature of the

serological tests with cross reaction between SFG Ricketssias, which might mean that the role that ruminants play in the transmission of *R. africae* needs to be further investigated, but with very few studies looking into this role, there is still largely a gap in the knowledge of the disease (Mazhetese et al., 2021).

Diagnosis

Diagnosis is usually based on a combination of tests rather than a single test because sensitivity of tests for the detection of *R. africae* are poor (Raoult et al., 2001).

The most commonly used test worldwide is serology, although most patients only test positive in the convalescent phase of the disease and not in the acute phase (Jensenius et al., 2003a). Various techniques such as micro immunofluorescence, western blotting and cross-adsorption tests have been utilized to confirm clinical diagnosis. Cross reactions between Rickettsias are also problematic with serological testing (Raoult et al., 2001).

Culture and isolation from blood or eschars can be done, but this needs a high biosecurity level laboratory and has to be done within 24hrs. It is also very difficult to culture *R. africae* and some patients do not want to consent to biopsy of the eschar, although sampling from the inoculation site will give the most reliable results (Jensenius et al., 2003b).

Polymerase chain reaction (PCR) gives reliable results from eschar biopsies, especially with the use of “suicide” PCR. A type of nested PCR where the primers are only used once. The areas targeted being sequences in the 16S rRNA gene, the surface membrane proteins OmpA and OmpB and PS120 (Jensenius et al., 2003b).

Control

Although there is no vaccination or preventative medication, control of the disease can be achieved through limiting exposure to infected ticks. This can be done by avoiding areas known to be infested with heavy tick burdens, and the application of repellents and covering of exposed skin. Inspection of skin after being outdoors and showers might also help prevent attachment. If a tick is found to be attached, it should be carefully removed to ensure that the long mouth parts are not left

behind in the skin, which could cause secondary infections. It is unknown how long the tick has to feed on a human to transmit the disease(Jensenius et al., 2003b).

Treatment mainly consists of the tetracycline group of antibiotics, specifically doxycycline and minocycline, which seem very effective in treating the disease. Other antibiotics that can be used include ciprofloxacin, rifampicin and pristinamycin (Silva-Ramos and Faccini-Martínez Á, 2021)

Chapter 3: Materials and methods

3.1 Research design

A qualitative literature review was done to identify the climatic conditions favourable for *A. hebraeum* survival and determine the historical and present geographical distribution of *A. hebraeum*.

Data was collated from the Agricultural Research Council meteorological data sites. A quantitative combination of correlation and survey studies were used to assess if the distribution of *A. hebraeum* to new areas has led to an increase in *E. ruminantium* and *R. africae* infections, and, if climate change could be correlated to this spread. *R. africae* infections served as a control for establishment of the tick population rather than just introduction of new ticks, as the infection is spread transovarially and transstadially, whereas only transstadial spread occurs for *E. ruminantium*. This would mean that it is more likely to see *R. africae* infection compared to *E. ruminantium* if the tick population had established itself in the area, as there are limited hosts for ticks that carry *E. ruminantium* infection.

3.2 Ethical considerations

Ethical clearance was obtained from the Animal Ethics committee of the University of Pretoria Onderstepoort campus. A signed consent from the owner of the cattle from which ticks were collected was also obtained. The process of tick collection was overseen by Dr Wepener (the student), a veterinarian registered with the South African Veterinary Council, Registration nr D09/7589 with 12 years' experience.

3.3 Plotting of Climatic data:

Current data (1991 to 2020)

Information of current weather data was collated from the Agricultural Research Council (ARC) weather stations. The ARC Weather Station Network consists of some

520 active automatic weather stations (Agrometeorology Staff, 2022). In addition, data records of about 1 400 historic stations are hosted in the information system. Hourly data measured at the automatic weather stations include air temperature, rainfall amount, relative humidity, wind direction, wind speed and solar radiation. From these recordings, daily values are calculated for maximum temperature (Tx), minimum temperature (Tn), daily amount of rainfall, maximum relative humidity (Hx) and minimum relative humidity (Hn).

The weather station data was used to prepare monthly interpolated surfaces for the period January 1991 to December 2020 for total monthly rainfall as well as average Hn, Hx, Tn and Tx. Long-term averages were calculated for each month resulting in 12 surfaces for each of the variables.

Climate projections

Six different dynamically downscaled Coupled Global Climate Model (CGCM) projections of future climate change (Engelbrecht et al., 2011) were used. The regional model used is the Conformal-Cubic Atmospheric Model (CCAM), a variable resolution global atmospheric model of the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia (McGregor 2005). The model was applied in stretched-grid mode over southern and tropical Africa, to obtain simulations at a resolution of approximately 0.5° in longitude and latitude. All the CGCM simulations are for the A2 Special Report on Emissions Scenarios (SRES) and were downscaled for the period 1961-2100. The CGCMs that were downscaled are:

1. GFDL-CM2.0 from the National Oceanic and Atmospheric Administration (NOAA)
2. GFDL-CM2.1 of NOAA
3. ECHAM5/MPI-Ocean Model from Germany
4. UKMO-HadCM3 from the United Kingdom
5. MIROC3.2-medres from the Japanese Agency for Marine-Earth Science and Technology (JAMSTEC)
6. CSIRO Mark3.5 from Australia

The daily CGCM data was summarized into monthly rainfall totals, monthly average of maximum temperatures and monthly average of minimum temperatures. The monthly data was in turn averaged over 30-year periods, centred around the

following years: 2005, 2035, and 2065. For example, the average rainfall for January centred around 2035 would be the average of rainfall in January from January 2021 to January 2050 for each of the six CGCMs. Median values of the six CGCMs were used to obtain one set of variables centred around the periods 2005, 2035 and 2065.

Finally, the median values of changes from 2005 to 2035 and from 2005 to 2065 of six different dynamically downscaled Coupled Global Climate Model (CGCM) projections were applied to the long-term average surfaces that were derived from the ARC weather station network, resulting in the following datasets:

- **Dataset1:** Monthly long term average data (Rainfall, Hn, Hx, Tn and Tx) centred around 2005 interpolated from the weather station data (1991-2020)
- **Dataset2:** Monthly long term average data (Rainfall, Hn, Hx, Tn and Tx) centred around 2035 (2021-2050) by applying the median values of changes from 2005 to 2035 of the six CGCMs to dataset1.
- **Dataset3:** Monthly long term average data (Rainfall, Hn, Hx, Tn and Tx) centred around 2065 (2051-2080) by applying the median values of changes from 2005 to 2065 of the six CGCMs to dataset1.

Annual statistics that were derived from the monthly long-term average surfaces (centred around 2005, 2035 and 2065) for usage in Maxent include:

- Average annual rainfall
- Average maximum temperature of the warmest month
- Average minimum temperature of the warmest month
- Average maximum temperature of the coldest month
- Average minimum temperature of the coldest month

The following data is only available for period centred around 2005

- Average annual maximum relative humidity
- Average annual minimum relative humidity
- Elevation
- Nine vegetation biomes
- 35 Bioregions

The elevation was derived from the digital elevation model, as prepared by Weepener et al., (2012).
Mucina and Rutherford (2006) described and mapped 440 zonal and azonal vegetation types, 35 bioregions and nine vegetation biomes for South Africa, Lesotho and Swaziland.
All data were projected to the Albers Equal-Area projection with Central meridian: 24º E, first standard parallel at 24ºS, and second standard parallel at 33ºS. The data were resampled to 1km grid cells with 1647 columns, 1415 rows.

3.3.1 Maxent

Maximum entropy (Maxent) is a machine learning modelling technique to make predictions or inferences. It is used in diverse areas such as astronomy, statistical physics, and signal processing (Phillips et al., 2006). Recently it has been widely used as a general approach to modelling species distribution with presence-only (PO) data points. It estimates the less constrained distribution of training points compared with random background locations, with environmental data layers defining constraints (Baldwin, 2009).The results illustrate how well the model fits the location data compared with random distribution (Phillips et al., 2004; Phillips et al., 2006). This model has been used by Williams et al. (Williams et al., 2016) to identify suitable (optimal) geographical areas for milk production in Holstein herds on pasture with geographical locations of the farms used as PO data points. The same modelling technique may be used to predict the future distribution of ticks, tick-borne diseases and other vectors and associated diseases, as was done in Zimbabwe for *A. hebraeum* and *A. variegatum*. (Estrada-Peña et al., 2008)

3.3.2 Maxent analysis

In order to use Maxent for analysis it was necessary to ensure that the grids were all identical as pertaining to the raw data that was used. To do this the data was first plotted in ArcGIS. GPS co-ordinates of tick occurrence points as supplied by A.M. Spickett, and the new collection sites were used as the current occurrence points for *A. hebraeum.* Environmental layers were then added to include humidity, temperature, elevation, biomes, bioregions and rainfall. Maxent was set to use automatic features, to use 25% of the occurrence points for random testing and 75% for training. The maximum iterations were used as 500, the default prevalence was set at 0.5 and the threshold rule used was minimum training presence. The

replicate run was set to cross validate. Four separate runs were done. In scenario 1 all the environmental layers were added even though no references for future values of humidity, biomes, and bioregions was available. In scenario 2, only the layers for which future data was available were used, viz. temperature and rainfall. In scenario 3, all the layers except for humidity were used as it was inferred that humidity depends on the rainfall and temperature variables and thus already accounted for. In scenario 4, all the layers for which future predictive data was available were used, as well as elevation as that would not change.

3.4 Tick collection

3.4.1 Sample size calculation

A. hebraeum ticks collected from cattle were tested for the presence of *E. ruminantium* and *R. africae* pathogens. Adapting a formula for detecting disease in an infinite population (Thrusfield, 2007) with an expected infection rate of 10% and a confidence level of 95%, 29 samples where needed for one positive result of *E. ruminantium*. As R. africae should have a higher rate of infection. E. ruminantium was taken as the limiting pathogen.

3.4.2 Sampling site

Ticks were sampled from a newly established area in the Free State where the vectors have not historically been found. The site was selected based on the recordings of Horak (Horak et al., 2015). The site was just south of Boshoff in the Free State (Fig. 3). Two camps where cases of heartwater have previously occurred were identified. Co-ordinates for these sites were 28°43’9” S 25°10’54” E and 28°48’30” S; 25°8’38” E. The elevations at these locations were 1 250 meters above sea level and 1 240 meters above sea level respectively.

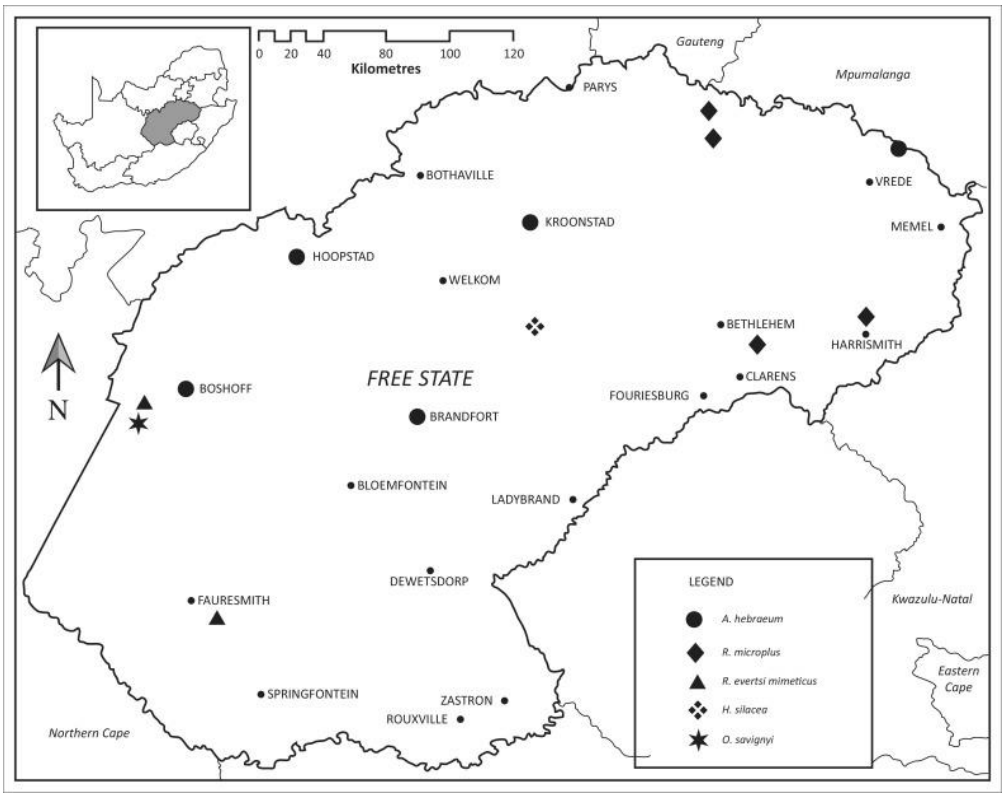

Figure 3: Boshoff, Free State (Horak et al., 2015)

The ticks were placed in 100% Ethanol in plastic specimen jars for transport and DNA extraction was conducted in the research and training laboratories of the Department of Veterinary Tropical Diseases at Onderstepoort.

3.5 Molecular detection of Rickettsial species

3.5.1. DNA extraction:

DNA was extracted using the CHELEX Resin Insect DNA extraction Protocol.

A Preparation of 5% Chelex 100 was made by weighing 5 grams of Chelex® 100 Molecular Biology Grade Resin Sodium Form (Bio-Rad Laboratories) placed in a 100ml falcon tube. The tube was filled to the 100ml mark with UV sterilized water and stirred to mix well.

The method used is a modification of the technique used by Musapa (Musapa et al., 2013). The ticks were cut in half, one half was placed into a 2 ml Eppendorf tube to which 200µL of 5% Chelex® 100 chelating resin was added as well as two Chelex glass beads. The tube was then placed in a homogenizer and run for two cycles of

18seconds each at 5200 rpm, while watching for any spillage of content due to lids of Eppendorf tubes opening. Heating blocks were then used to incubate the sample for 1hour at 56°C, followed by DNA denaturation at 95°C for 30 min. The glass beads were removed at the end of this heating process. The tubes were then centrifuged for 3 min at 12000 x g and frozen until PCR. At the end of the extraction process, DNA can be found in the upper aqueous phase while the Chelex® and the cellular components remain in the lower phase. Before PCR, samples were centrifuged to allow good separation of the phases, this was done because Chelex® beads will inhibit PCR.

3.5.2 PCR detection of *E. ruminantium* and *R. africae*

E. ruminantium

pCS20 Sol1 TaqMan qPCR.

The pCS20 region of the 16s rDNA has been shown to be a highly conserved and specific area in the *E. ruminantium* gene. Cangi developed a pCS20 Sol1 TaqMan qPCR. (Cangi et al., 2017). The protocol followed by them was used as follows:

Real-time PCR is performed on StepOne System thermocycler (Applied Biosystems, Villebon sur Yvette, France), and results were analyzed using 7500 System SDS. Software (Applied Biosystems, Villebon sur Yvette, France). The run included a positive and negative control: *E. ruminantium* from a previous project that was sequenced and verified to contain *E. ruminantium* DNA and distilled water respectively.

The *pCS20* Sol1^{TqM} qPCR assay with a TaqMan probe was performed using the TaqMan® Universal PCR Master Mix (Life Technologies, Courtaboeuf, France). The qPCR master mix contains internal passive reference dye ROX™ and Uracil-N-Glycosylase (UDG). Concentrations used in the reactions was as given in Table 1:

Table 1: Reagents used for pCS20 Sol1 qPCR

Reagents required:	**Final concentration**	**1 Reaction**	**22 Reactions**
Taqman ® Universal master mix	1x	12.5µL	275 µL
Forward primer (10µM) Sol1 F	0.25µM	0.625 µL	13.75µL
Reverse primer (10µM) Sol1 R	0.25µM	0.625µL	13.75µL
Probe (8 µM) Sol1 Probe	0.2µM	0.625 µL	13.75 µL
ddH_2O water		8.625µL	189.75µL
Total reagents		23µL	506µL
DNA template per reaction		2µL	
Total		25µL	

The conditions for the qPCR run was set up as follows:

Table 2: Conditions for pCS20 Sol1 qPCR

Step	**Temp**	**Time**	**Cycles**
UDG activation	50°C	2 min	1
UDG deactivation/AmpliTaq Gold® activation	95°C	10min	1
Denaturation	95°C	15sec	40
Annealing and extension	51°C	60sec	

The primer and probe selection can be found in table 5 below.

***R. africae*:**

***OmpA* cPCR**

OmpA is an outer membrane protein specific to *R. africae.* In order to detect this protein a conventional PCR was used (Kleineman, 2013). The PCR consists of primers Rr190.70F and Rr190.701R as described in Table 3 and the protocol followed was as follows: Each reaction was made up of 2X Phusion Flash mastermix (Thermofisher Scientific South Africa) (made up according to manufactures specifications) 10 µL, Forward primer (10µM) 1µL, Reverse primer (10µM) 1µL, Nuclease free water 6µL and 2µL sample DNA to make a total volume of 20µL. In all the PCR reactions, negative (no template DNA) and positive controls of a

sequence-confirmed *R. africae* DNA sample from a previous study was added (Mandara 2018).

Table 3: reagents for OmpA PCR

Reagents required:	**1 Reaction**	**22 Reactions**
2x Phusion Flash PCR Master Mix	10µL	220µL
Forward primer (10µM) Rr190.70F	1µL	22µL
Reverse primer (10µM) Rr190.701R	1µL	22µL
ddH_2O water	6µL	132µL
Total reagents	18µL	396µL
DNA template per reaction	2µL	
Total	20µL	

The cycling conditions for the PCR machine (Applied Biosystems® MiniAmp® 96-Well Thermal Cycler) was set as previously described by Mazhetese (Mazhetese et al., 2022).

Table 4: Conditions for OmpA PCR

Step	**Temp**	**Time**	**Cycle**
Initial denaturation	98°C	10 sec	1 cycle
Denaturation	98°C	1 sec	30 cycles
Annealing	53°C	5 sec	
Extension	72°C	15 sec	
Final extension	70°C	60 Sec	1 cycle
Final hold	4°C	∞	

Table 5: primer and Probe selection for PCR

Name	Target gene	Sequence 5'-3'	Amplicon Length	Reference
Sol1 F	pCS20	ACA AAT CTG GYC CAG ATC AC	110 bp	Cangi et al 2017
Sol1 R	pCS20	CAG CTT TCT GTT CAG CTA GT	110bp	Cangi et al 2017
Sol1™ probe	pCS20	**6-FAM**-ATC AAT TCA CAT GAA ACA TTA CATG CAA CTG G-**BHQ1**	100bp	Cangi et al 2017
Rr190.70F	OmpA	ATGGCGAATATTTCTCCAAAA	632bp	Kleinerman et al., 2013
Rr190.701R	OmpA	GTTCCGTTAATGGCAGCATCT	632bp	Kleinerman et al., 2013

Electrophoresis was then performed on the PCR product in a 1.5% Agar gel containing a 100bp ladder, and the expected amplicon size was to be around 632bp (Kleinerman et al. 2013), The size can be estimated based on the 100bp ladder that was located on both sides of the target DNA. The result was then visualized by using an ultraviolet trans illuminator system (Bio-Rad).

Qubit® Fluorometer DNA quantification

As Chelex is a crude method of DNA extraction, it was necessary to prove that DNA had been extracted to validate the findings of the PCRs.

The Qubit® 2.0 Fluorometer was used to quantify the DNA. In order to run the test, the protocol in the manual was followed for the machine using the Qubit® dsDNA BR Assay kit, with 0.5 mL tubes used and the lids marked for identification. A Qubit® working solution was made up by adding in 1 µL of Qubit® reagent into 199 µL of Qubit® buffer per reaction that had to be run, after which 190 µL of this working solution was added into the 0.5 mL tube and 10 µL of standard 1 to the first tube and 10 µL of standard 2 to the second tube. These tubes were then vortexed and used to calibrate the Fluorometer. After this 190 µL of the Qubit® working solution was placed in all of the 0.5ml sample tubes remaining and test samples selected at

random to check for the presence of DNA. Ten µL of each test sample was added to the working solution before being vortexed and waiting 2 minutes before reading it on the Fluorometer.

Chapter 4: Results

4.1 Climatic data

The data for current and future *A. hebraeum* distribution as predicted by Maxent is illustrated in the maps below. Occurrence points for *A. hebraeum* is shown on each map. The features chosen by the automation was linear and quadratic features and based on the sample size (occurrence points). The scenarios used for the habitat suitability modelling were as follows:

Scenario 1 (Figure 4 to 6)	All environmental layers used
Scenario 2 (Figure 7 to 9)	Temperature and rainfall
Scenario 3 (Figure 10 to 12)	Humidity was omitted from the layers
Scenario 4 (Figure 13 to 15)	Temperature, rainfall and elevation

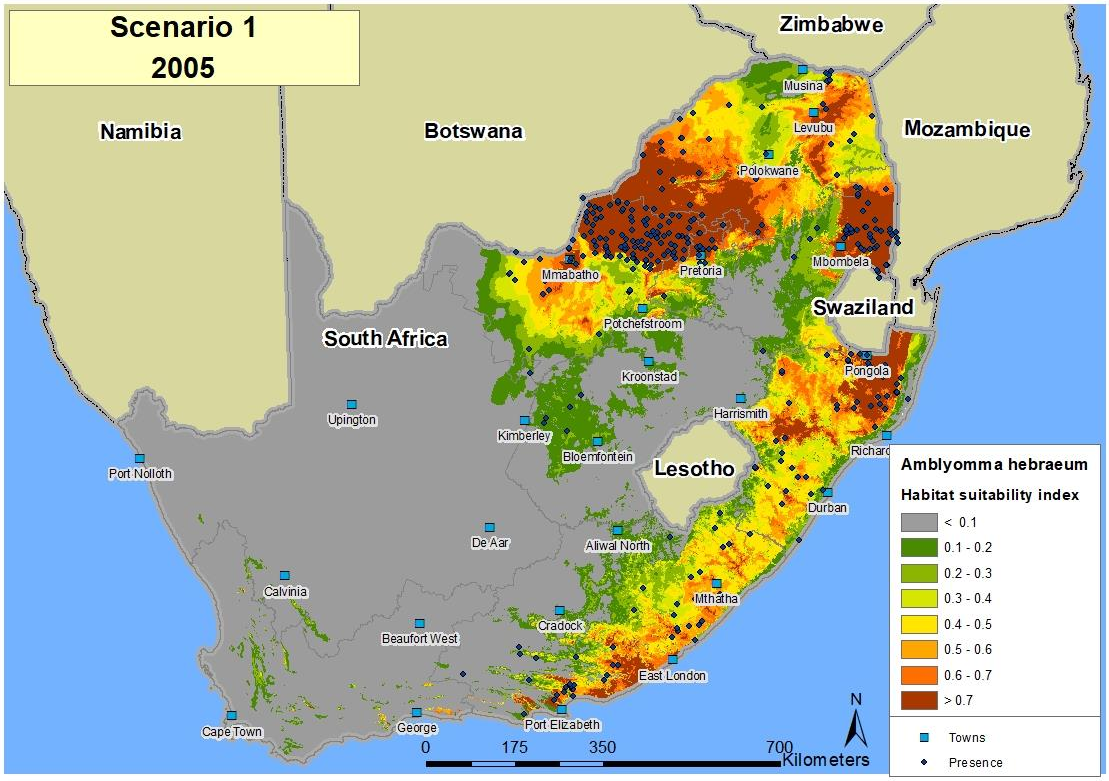

Figure 4: Scenario 1 – 2005: All environmental layers used

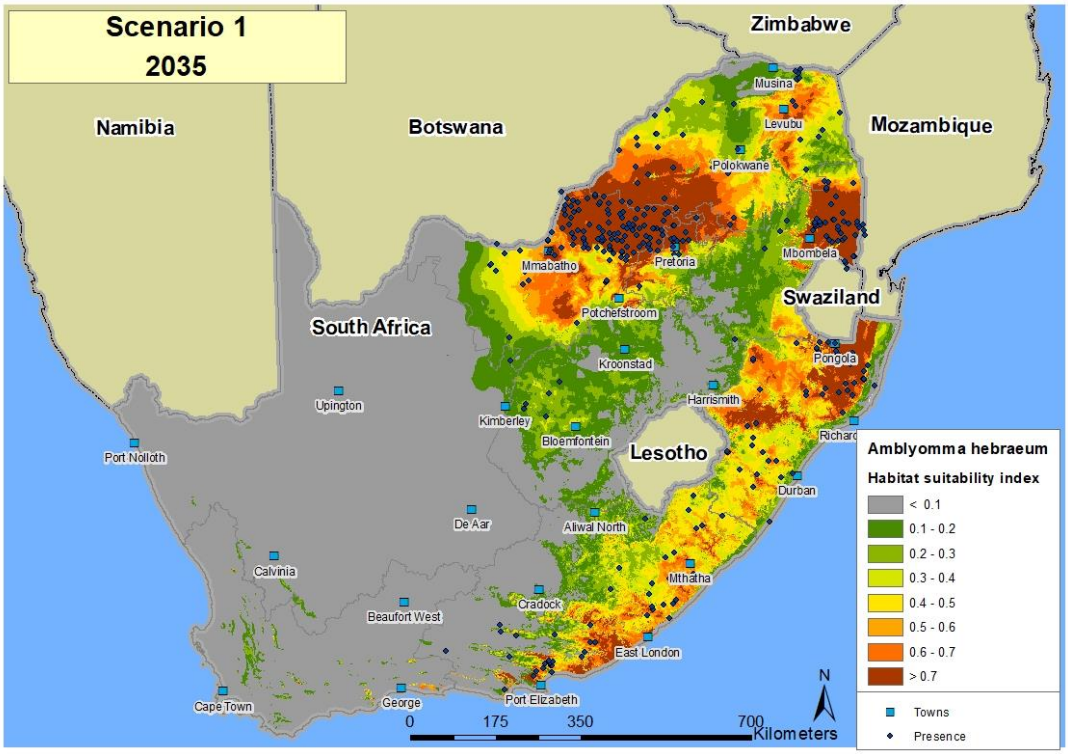

Figure 5: Scenario 1 – 2035: All environmental layers used:

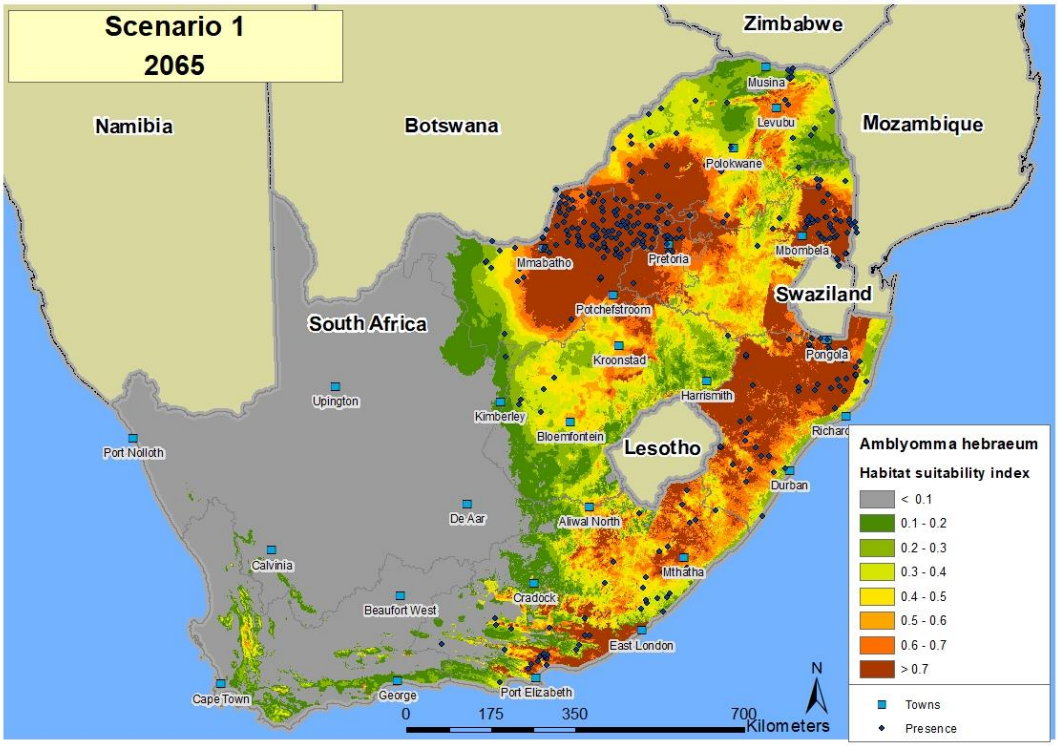

Figure 6: Scenario 1 – 2065: All environmental layers used

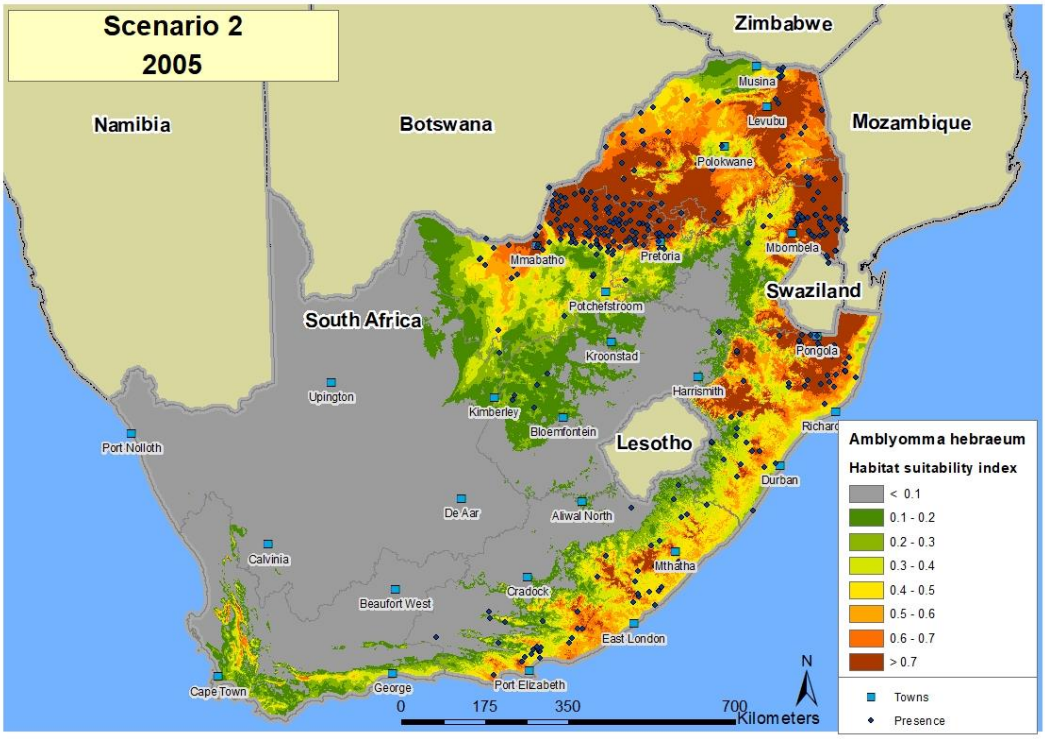

Figure 7: Scenario 2 – 2005: Temperature and rainfall

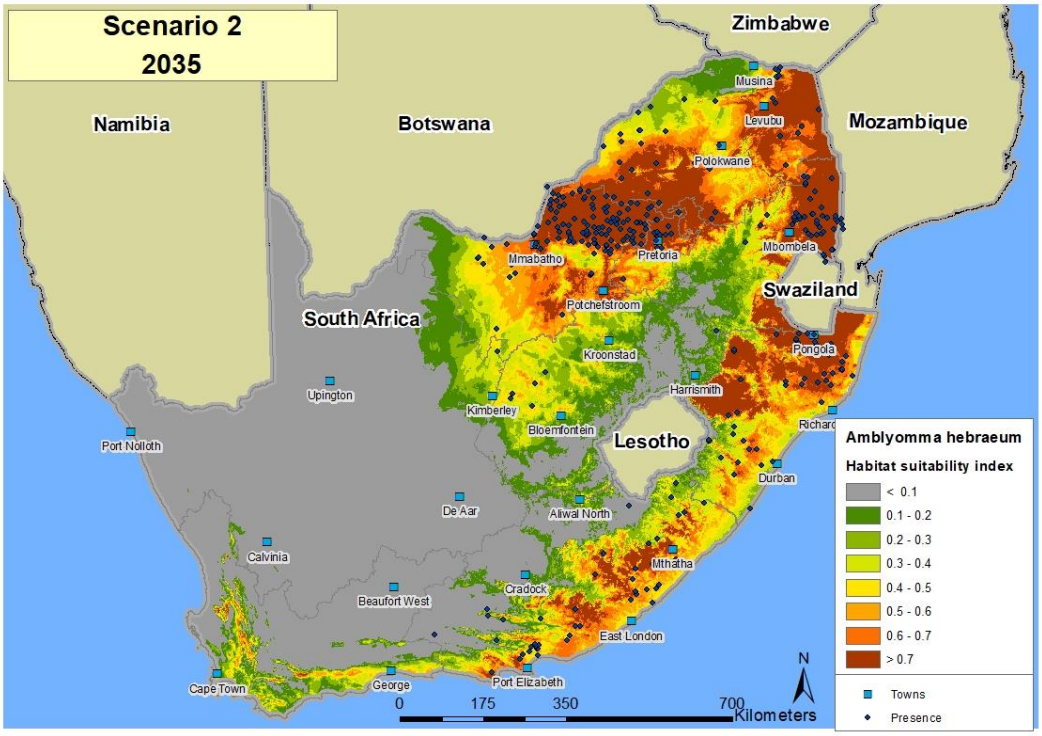

Figure 8: Scenario 2 -2035: Temperature and rainfall

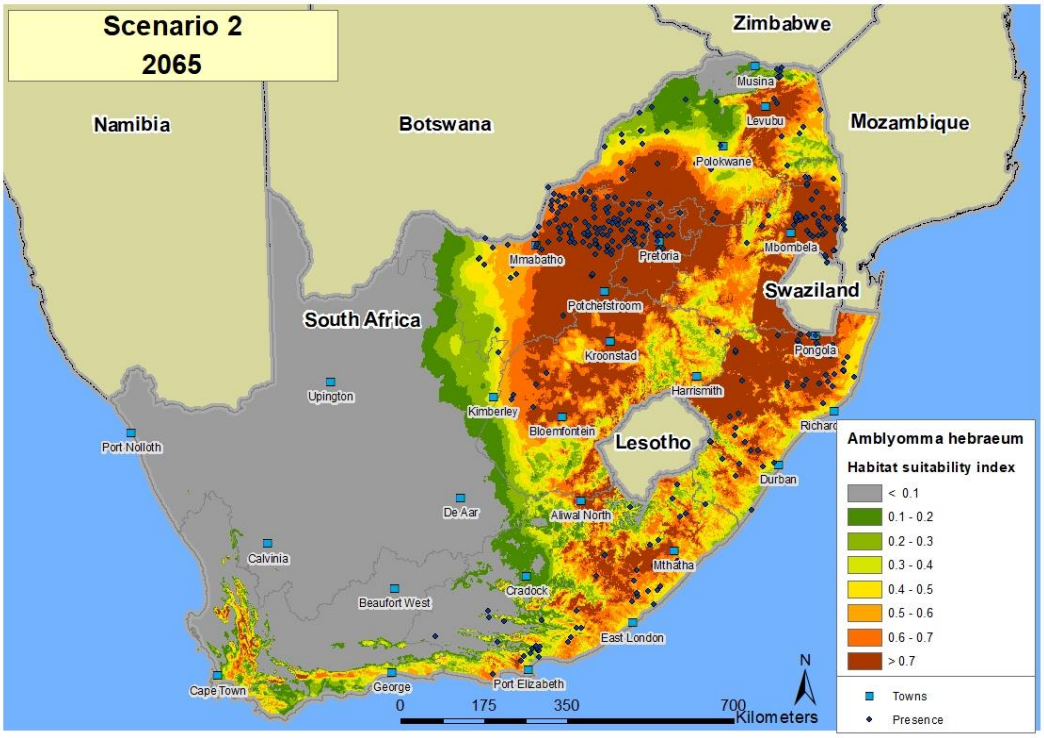

Figure 9: Scenario 2 – 2065: Temperature and rainfall

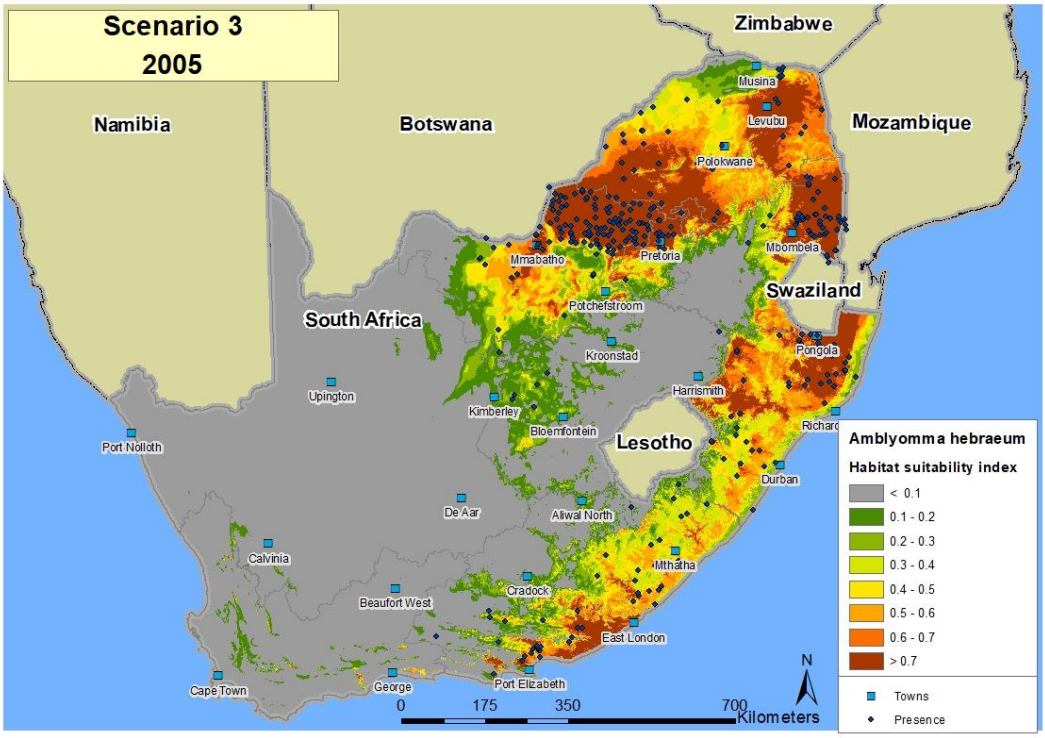

Figure 10: Scenario 3 – 2005: Humidity was omitted from the layers

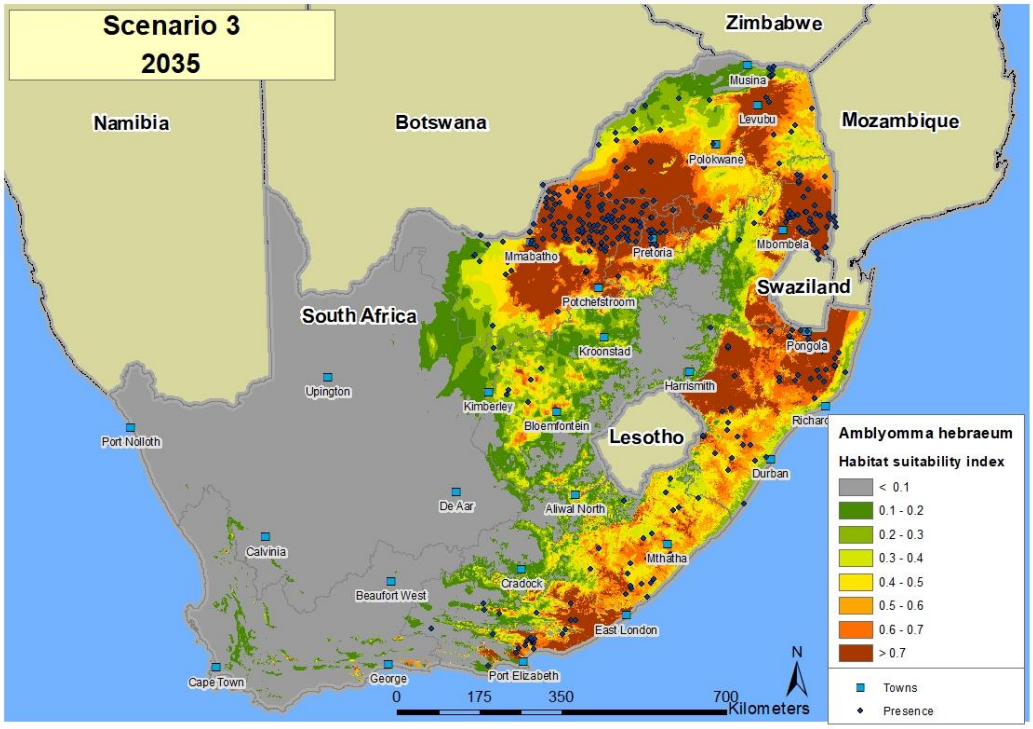

Figure 11: Scenario 3 – 2035: Humidity was omitted from the layers

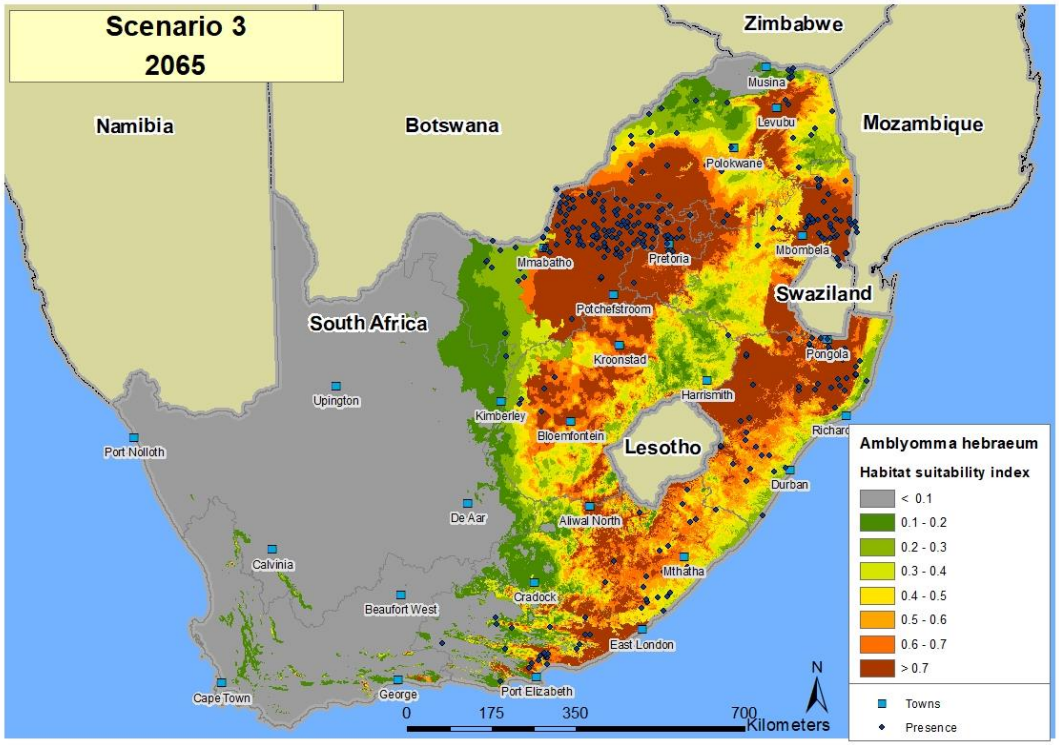

Figure 12: Scenario 3 – 2065: Humidity was omitted from the layers

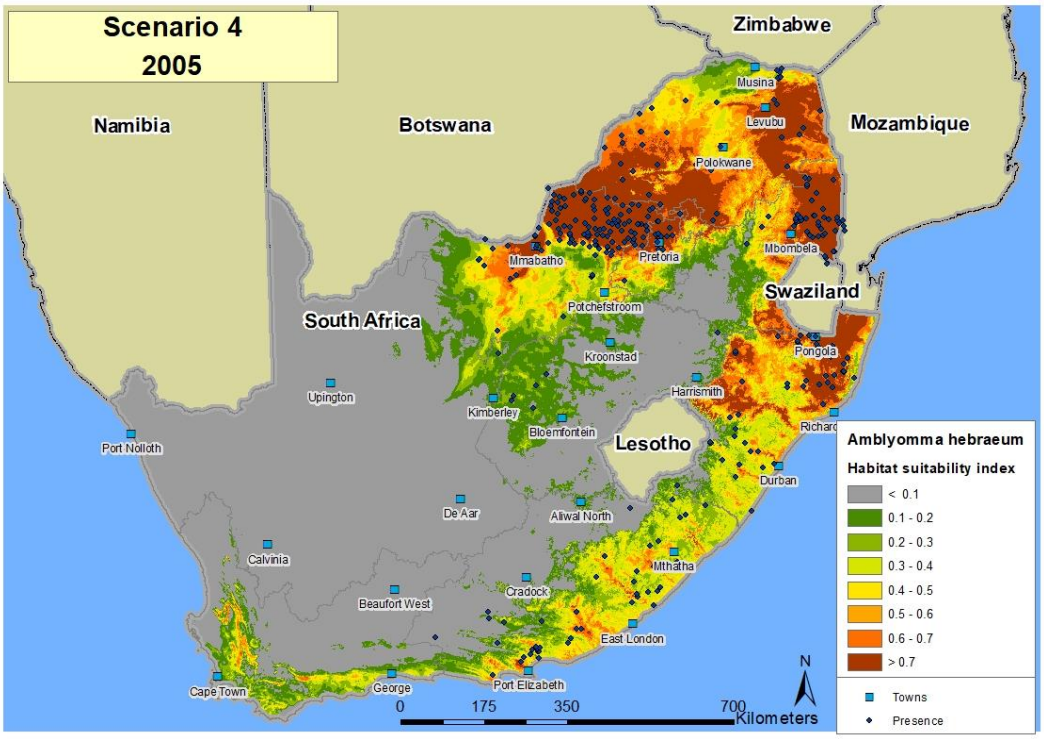

Figure 13: Scenario 4 – 2005: Temperature, rainfall and elevation

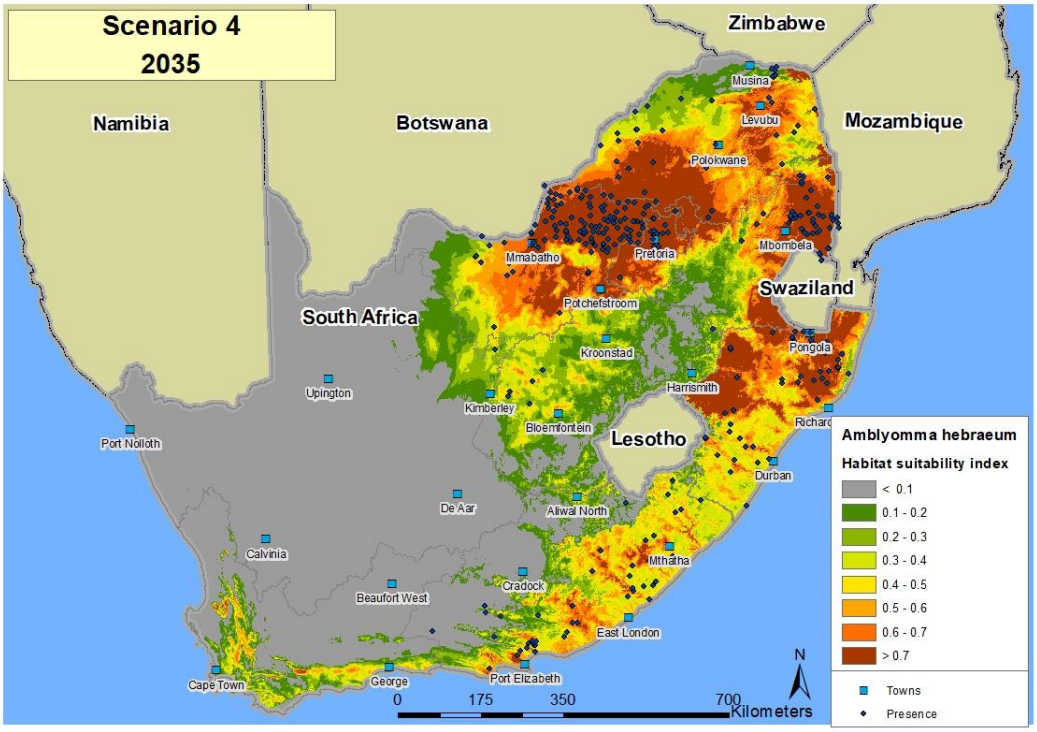

Figure 14: Scenario 4 – 2035: Temperature, rainfall and elevation

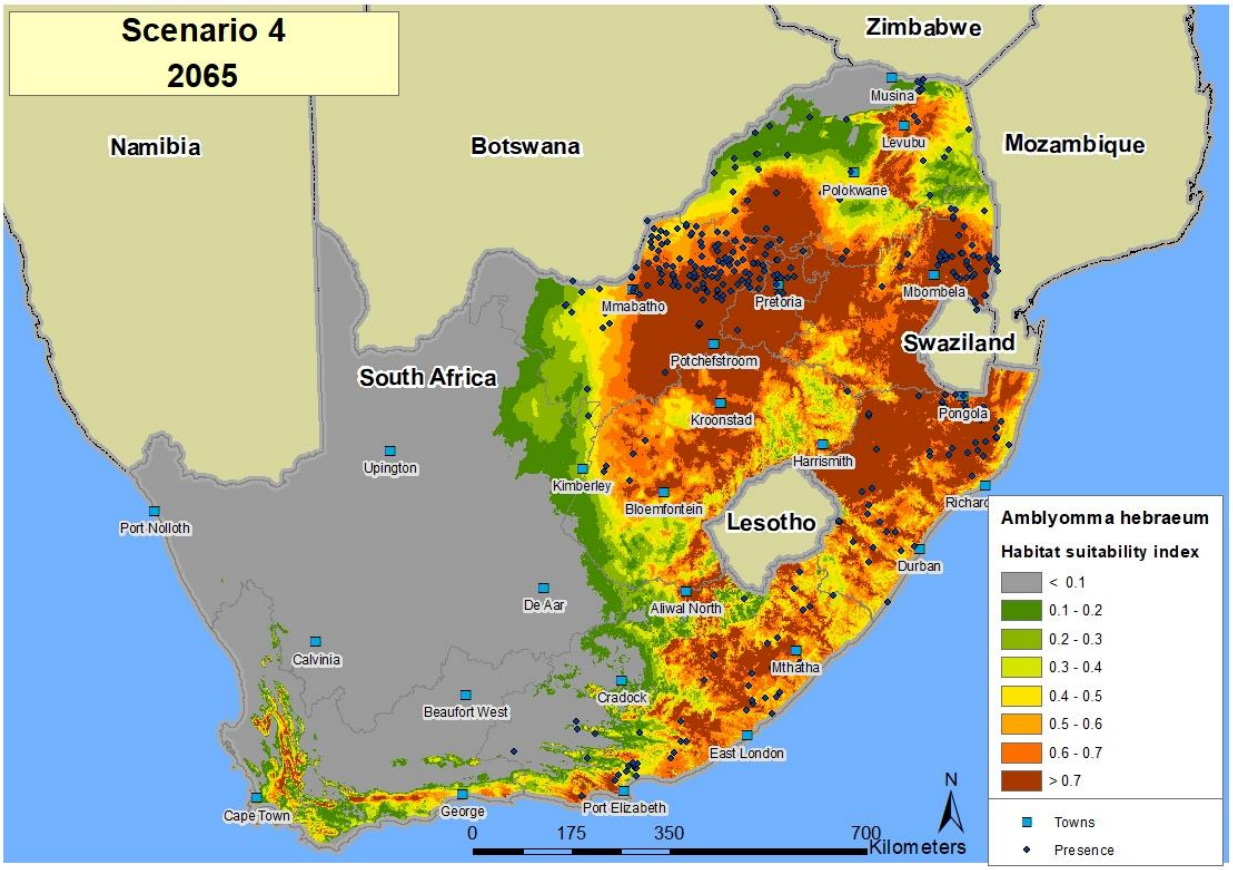

Figure 15: Scenario 4 – 2065: Temperature, rainfall and elevation

Across all the models, the variable (layer) that contributed the most to the distribution of *A. hebraeum* was rainfall, followed by biomes and bioregions when they were included.

To prove the validity of the predictions, the statistical testing of omission rate versus the predicted omission can be used as a function of the cumulative threshold for the threshold-dependent binomial omission test and the Area under the curve (AUC) as the threshold-independent test.

It can see from the 25% of samples that was used for statistical testing that the omission on the test and training samples closely follow the predicted omission line for, scenario 1-4 on the left for the 2035 predictions and on the right the 2065 predictions.

Scenario 1 (Figure 16)	All environmental layers used
Scenario 2 (Figure 17)	Temperature and rainfall
Scenario 3 (Figure 18)	Humidity was omitted from the layers
Scenario 4 (Figure 19)	Temperature, rainfall and elevation

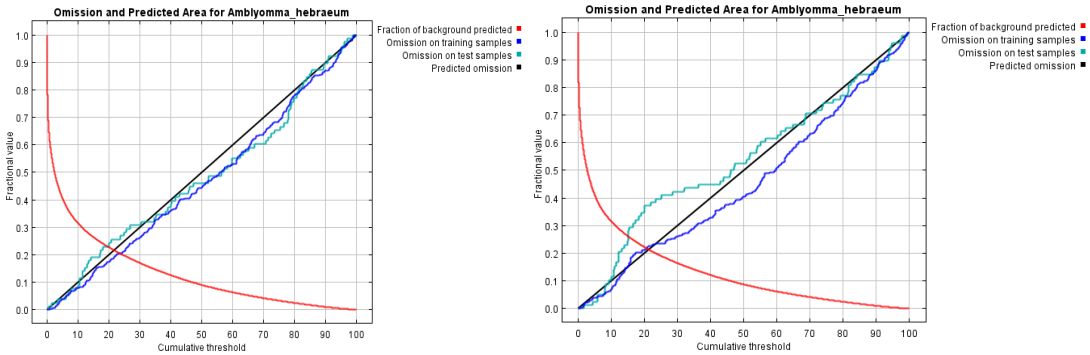

Figure 16: Left-Scenario1-2035, Right- Scenario1-2065

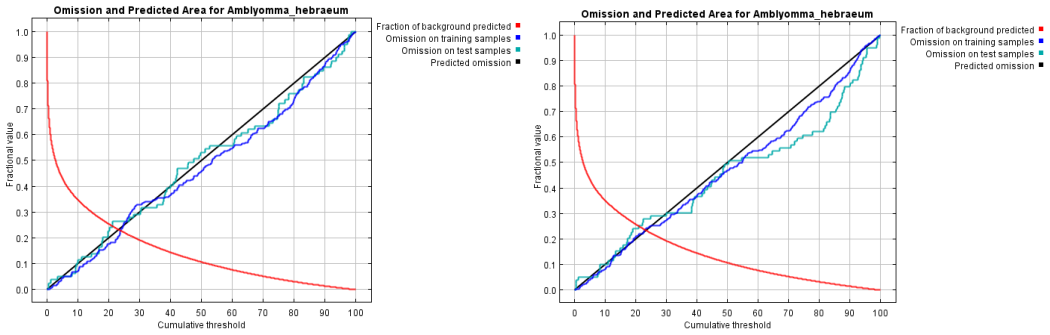

Figure 17: Left- Scenario 2-2035, Right- Scenario2-2065

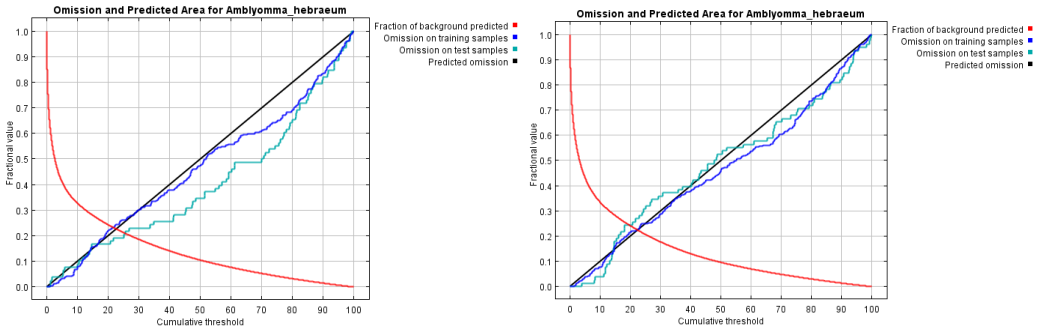

Figure 18: Left-Scenario3-2035, Right-Scenario3-2065

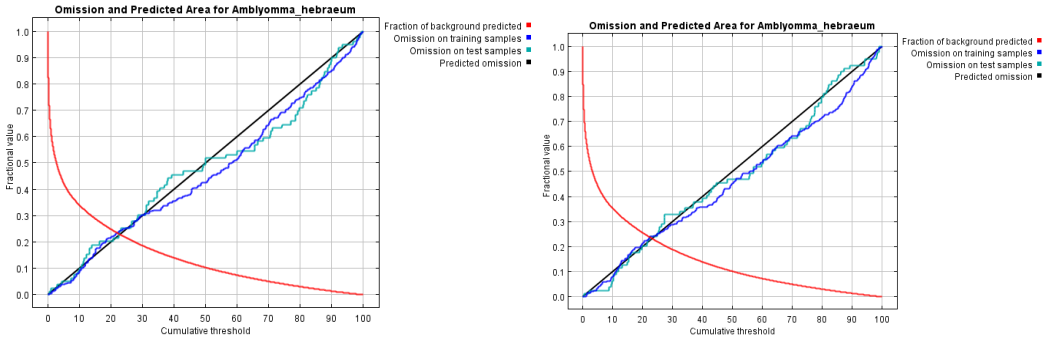

Figure 19: Left-Scenario4-2035, Right-Scenario4-2065

The Receiver operator characteristics curve showed an AUC for the test samples as indicated in Table 6.

Table 6: AUC for all Scenarios

Scenario	**AUC**
Scenario 1-2035	0.868
Scenario1-2065	0.850
Scenario2-2035	0.849
Scenario2-2065	0.853
Scenario3-2035	0.882
Scenario3-2065	0.866
Scenario4-2035	0.855

Scenario4-2065	0.859

4.2 Tick collection

The farmer was asked to report any bont tick (*A. hebraeum)* sightings on cattle as they worked with them. The farmer reported bont ticks from February 2022 and a trip was undertaken to the farm to collect ticks in the beginning of March 2022. Cattle were brought in from the camps and inspected for ticks while standing in a crush. Most ticks were found on the ventral part of the cattle. Unfortunately, only 19 *A. hebraeum* adult males could be found on the cattle and positively identified. These were placed in specimen jars as described earlier for further processing at the Department of Veterinary Tropical Diseases student laboratory.

4.3 Molecular detection of Rickettsial species

E. ruminantium

pCS20 Sol1 TaqMan qPCR.

The samples were run as previously described. Unfortunately, none of the samples was positive for *E. ruminantium.* As there was load shedding the cycler stopped after 20 cycles and had to be re-programmed to continue. The amplification plot shows start from the 21st cycle until the 42nd cycle. As can be seen in the figure the positive control did as was expected and started to exponentially increase on the 22nd cycle, while the negative control remained close to zero. This means that the run can be trusted as being successful. None of the samples showed any reaction.

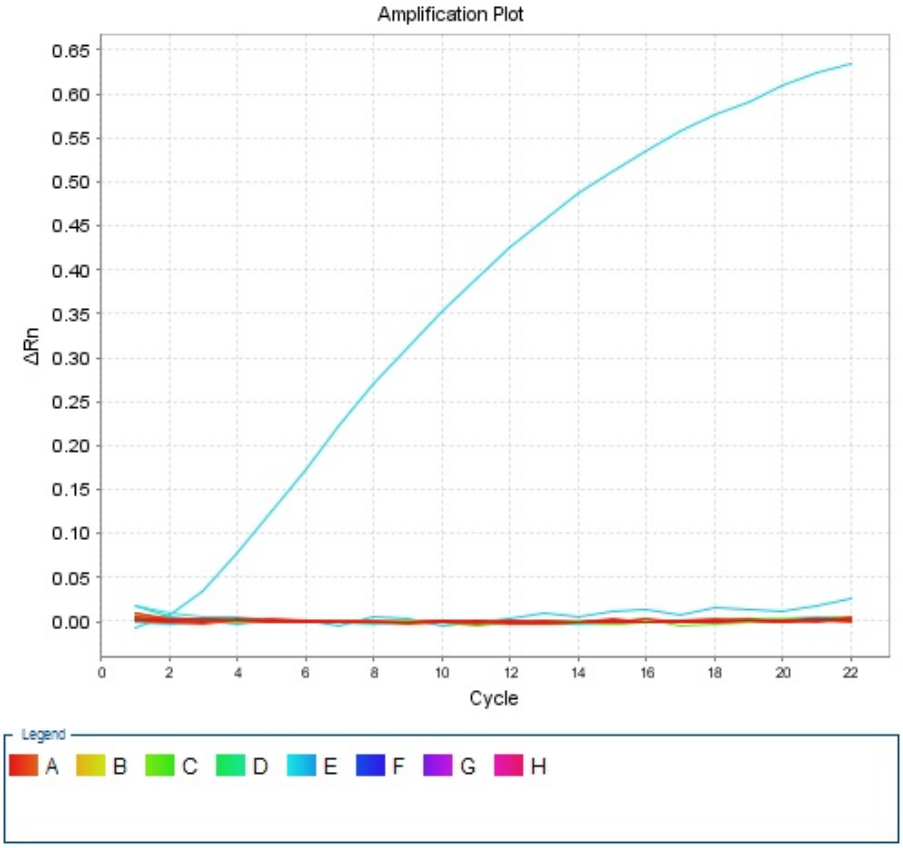

Figure 20: Results qPCR E. ruminantium

R. africae

OmpA cPCR.

The first run was done as described above and a 632bp product was expected. The results show no positive samples although the positive control RE18/30 did show a band at the expected 632bp area as compared to the 100bp ladder meaning that the test was run correctly.

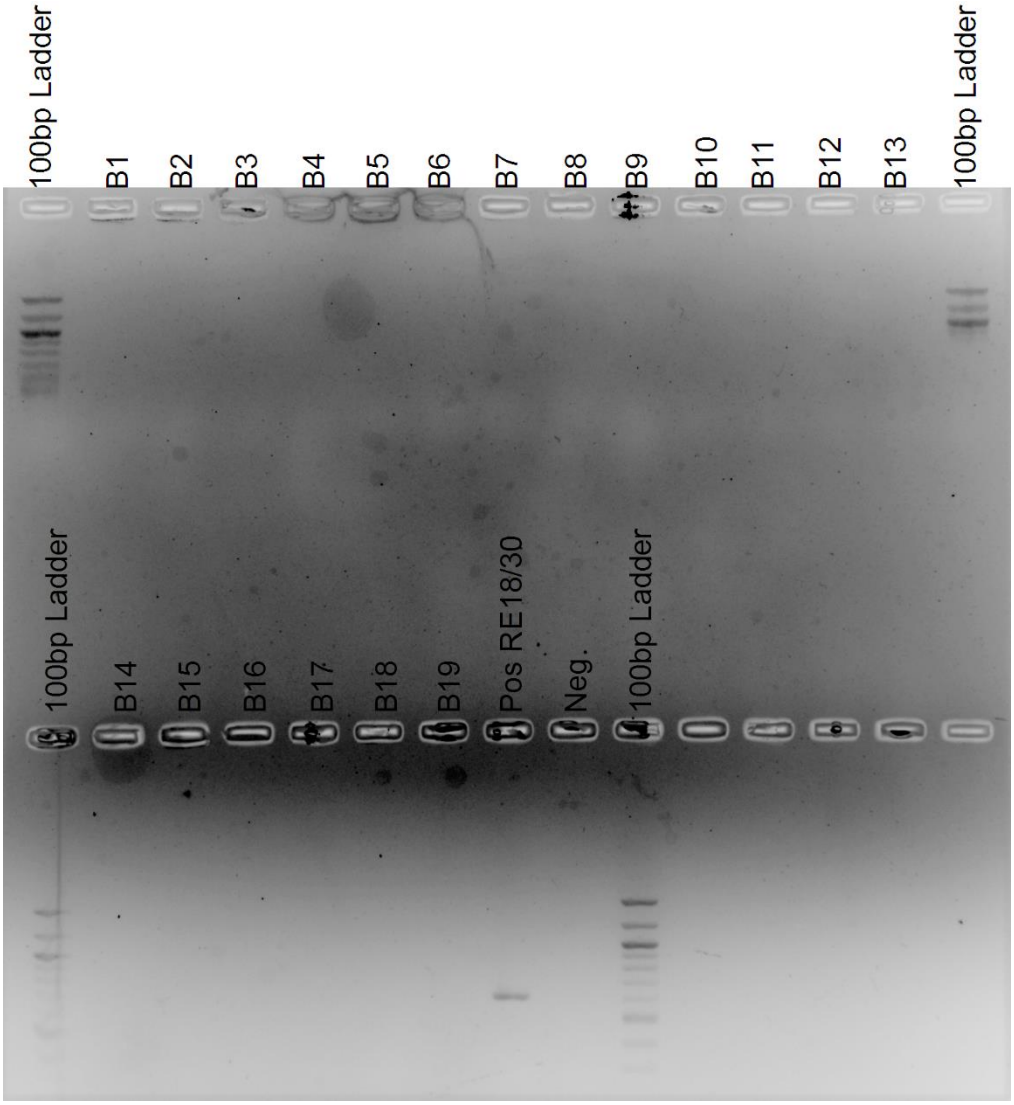

Figure 21: OmpA PCR results R. africae

Qubit®2.0 Fluorometer DNA quantification

As both results did not yield a positive result and the Chelex method is a crude method of extracting DNA, it was necessary to prove that the method was sufficient to extract DNA. As the final volume used was 200 µL, it was not necessary to convert the results.

Table 7: Qubit Results

Sample nr	**DNA amount in ng/ml**
B2	0.0367
B4	0.0134
B6	0.0911
B8	0.0542
B10	0.0342
B12	0.0291
B14	Too low
B16	Too low
B18	0.0108
B3	Too low
B9	0.0418

Chapter 5: Discussion

The climatic data

It can be seen by comparing the current habitat suitability map to the predicted maps, that the area of habitat suitability for *A. hebraeum* has already changed from what was previously predicted.

Areas of increased habitat suitability can especially be seen in the central part of South Africa. It did not matter which of the environmental layers were added or omitted, the prediction for 2035 and 2065 still showed this trend of habitat suitability where the 24.5-degree meridian almost divides the country into an easterly area where *A. hebraeum* occurs and a western area where it is absent. This fact can be corroborated by the increase in *A. hebraeum* location points centered around the Free State area where the ticks for this study were collected. Interestingly the most northern part of South Africa as well as an area just northeast of this shows a decrease in habitat suitability for *A. hebraeum*. It would be interesting to follow this up with actual collection studies in the future. Lesotho seems to remain mostly free of the tick and this might be due to the high elevation of this mountainous kingdom.

According to the models of this study, the variable that will have the most effect on the distribution of *A. hebraeum* is rainfall or the absence thereof, which will affect the desiccation of especially the egg and larval stages of the tick. This can be expected as desiccation of especially the eggs has always been noted as a limiting factor to the survival of the tick (Norval, 1977). As previously discussed, the central interior of South Africa is expected to show an increase in the amount of rainfall in the next 30 years (Meissner et al., 2014), and this relates to the eastern part of the country where the prediction models show an increase in habitat suitability. From meteorological data collected, it is known that there is a northern Limpopo area that has shown decreased rainfall for the period 1910-2004 (Kruger, 2006), and this area also shows a decreased habitat suitability in the current predictions.

The second most important variable was that of biomes, since the effect on the biome would be to give the female ticks an area to lay the eggs that are not too waterlogged or too dry leading to desiccation. It also provides an area for the tick

off the host to survive adverse weather such as cold and drought spells and provide vegetation for the larval and nymph stages to quest on for passing hosts.

The current model was proven to be statistically of good value based on testing using the threshold dependent as well as the threshold independent test.

This study's threshold dependent evaluation is an extrinsic omission rate, meaning how many of current test points fall outside the predicted habitat suitability (Phillips et al., 2006). A low omission rate is necessary for a good model (Anderson et al., 2003). This study's omission line closely followed the predicted omission line and the test omission rate stayed below 2.5%, so it can be concluded that the data is valid.

As a threshold independent evaluation, the Receiver Operator Characteristics (ROC) was used. The ROC curve showed an area under the curve for the test samples all above 0.8, meaning that this study's test results is between 0.8-0.9 and an excellent representation of what is really happening (Mandrekar, 2010).

E. ruminantium

Although this study failed to find a positive *E. ruminantium* sample, two veterinarians that practice in the area of the farm were contacted and both separately indicated that they have been seeing cases of *E. ruminantium* since approximately 2002. These results were validated from brain samples sent for analysis to Pathcare who used the Agricultural Research Council, Onderstepoort veterinary institute to verify the results by direct microscopic evaluation for the presence of the parasite and confirmed with PCR.

Wildlife movement was also indicated as a source of introduction of *A. hebraeum* to this area by these veterinarians.

One possible cause for the negative results could be the inadequate sample size that was collected. The total tick burden on the animals was also extremely low with very few other tick species seen. An improvement on the current collection method to ensure higher sample numbers would be to use the drag method on vegetation to potentially collect immature stages of the ticks as well as include wildlife that are culled by the farmer as sources for tick collection. As well as more frequent collections and a higher proportion of animals inspected.

As discussed previously the increase in game farming can be seen as a major reason for a change in vegetation (e.g., bush encroachment) that would increase the chances of tick survival as it prevents desiccation. Wildlife can also serve as hosts for the survival of these ticks when domestic ungulates are scarce and, as tick control is not generally applied to most wildlife, they can increase the viability and abundance of ticks as well as the diversity of the tick population.

Using the epidemiological model formula in Thrushfield's 2007 book, it can be concluded that the infection rate of *A. hebraeum* with *E. ruminantium* has to be lower than 15%, for at least one positive result to have been observed with the 19 samples collected at a 95% confidence interval (Thrusfield, 2007). Based on the fact that not one positive case was detected it can be assumed that the infection rate is much lower than anticipated, indicating that more ticks should be sampled in order to find a positive case.

Another possibility for the negative result could be due to the low prevalence of the disease because *E. ruminantium* is not transmitted transovarially, but only transstadially and the tick population has simply become non-infective. This hypothesis seems less likely as the ticks also showed no positive results for *R. africae* and we know the *A. hebraeum* transfers the parasite transovarially. The ticks may also have never been infected which seems unlikely due to the fact that most *A. hebraeum* tick studies in South Africa showed some degree of *R. africae* infection (Mazhetese et al., 2022, Jongejan et al., 2020).

The DNA quantification showed that some of the samples had too little genetic material to be read by the fluorometer, meaning that potentially a more refined DNA extraction method could have yielded better results (or that a positive sample might have been missed).

The fact that very few ticks could be found on cattle at the time of examination is indicative of a very low burden suggesting an endemically unstable situation where clinical cases of the disease might be more prevalent than in endemically stable areas where tick burdens are much higher.

R. africae

The PCR was valid but contained no *R. africae* genetic material. As noted with *E. ruminantium,* this may be because the sample size was too small. The rate of infection with *R. africae* would thus have to be low. Improving the collection techniques by dragging or collecting from culled wildlife might improve the sample size.

The negative result can also be due to the fact that the ticks were never infected which seems unlikely due to the fact that most *A. hebraeum* tick studies in South Africa showed some degree of *R. africae* infection (Mazhetese et al., 2022, Jongejan et al., 2020).

It might also be that the ticks had lost their parasite infection due to a low human host prevalence. Where these animals stay there are no people who live in the area and biosecurity is strict where the animals are stocked and they are only herded once a month. Although the parasite is transmitted transovarially it has only been shown to infect two successive generations (Kelly and Mason, 1991).

As noted previously the method of DNA extraction was very crude and this could have led to a false negative result. This can be rectified by using a better method of DNA extraction such as utilizing tissue kits provided by various companies.

Chapter 6: Conclusions and Recommendations

The value of the use of maxent as a model for the prediction of future habitat suitability for *A. hebraeum* shows very good potential. The variable that is shown to be most responsible for the change in its distribution pattern is rainfall, indicative that climate change has a notable effect on the distribution of *A. hebraeum*.

Based on the habitat suitability models, this study has shown that the suitability for A. *hebraeum* to become established has definitely changed, making it possible for the tick to survive in areas in which it has not previously been found and, moreover, that the area of habitat suitability will increase in future especially over the eastern part of the country, spreading westward.

The spread of pathogens can always be related to the spread of their tick vectors, especially with uncontrolled movement of animals from areas where these diseases are present. This study did not prove the presence of *E. ruminantium* or *R. africae* in the eastern Free State although we did find the tick vector *A. hebraeum,* as well as reports by local veterinarians, that at least *E. ruminantium* has been found in the area, and has caused disease in domestic ruminants. We can thus deduce that the spread of *E. ruminantium* and *R. africae* will move over the whole eastern part of the country towards the west following the distribution of *A. hebraeum.*

From the data it can also be concluded that the prevalence of *E. ruminantium* and *R. africae* must be lower than the expected 15% in the sampled area.

A downfall to this study was the limited number of vector ticks that could be sampled for the presence of the pathogens we were looking to find. An attempt to increase tick sampling would potentially give a better picture of the prevalence rates in the area.

It is recommended that farmers should be educated to look out for the ticks as well as to look for symptoms of the diseases caused by these ticks to prevent livestock losses. Good biosecurity and attention to tick control on any animals bought from especially endemic areas needs to be rigorously applied.

More research into the potential adaptation of the tick to survive in different climatic conditions will help to refine the future predictive value of niche habitat suitability modeling.

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Annexure 1: REC approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee 14 January 2022

CONDITIONALLY APPROVAL

Ethics Reference No	**REC167-21**
Protocol Title	**The effect of climate change on the distribution of Amblyomma hebraeum and the pathogens it carries in South Africa**
Principal Investigator	**Dr MP Wepener**
Supervisors	**Prof LCBGD Neves**

Dear Dr MP Wepener,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC167-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals**: FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

NOTES: Conditionally approved pending the following (and to ensure that rerouting to AEC not delayed): (i) Obtaining ALL other relevant approvals. (ii) Upload of a permission letter from the study farm. An alternative would be for the applicant to upload and provide a blank informed consent document that will be signed by the owner once the sampling location has been identified.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS

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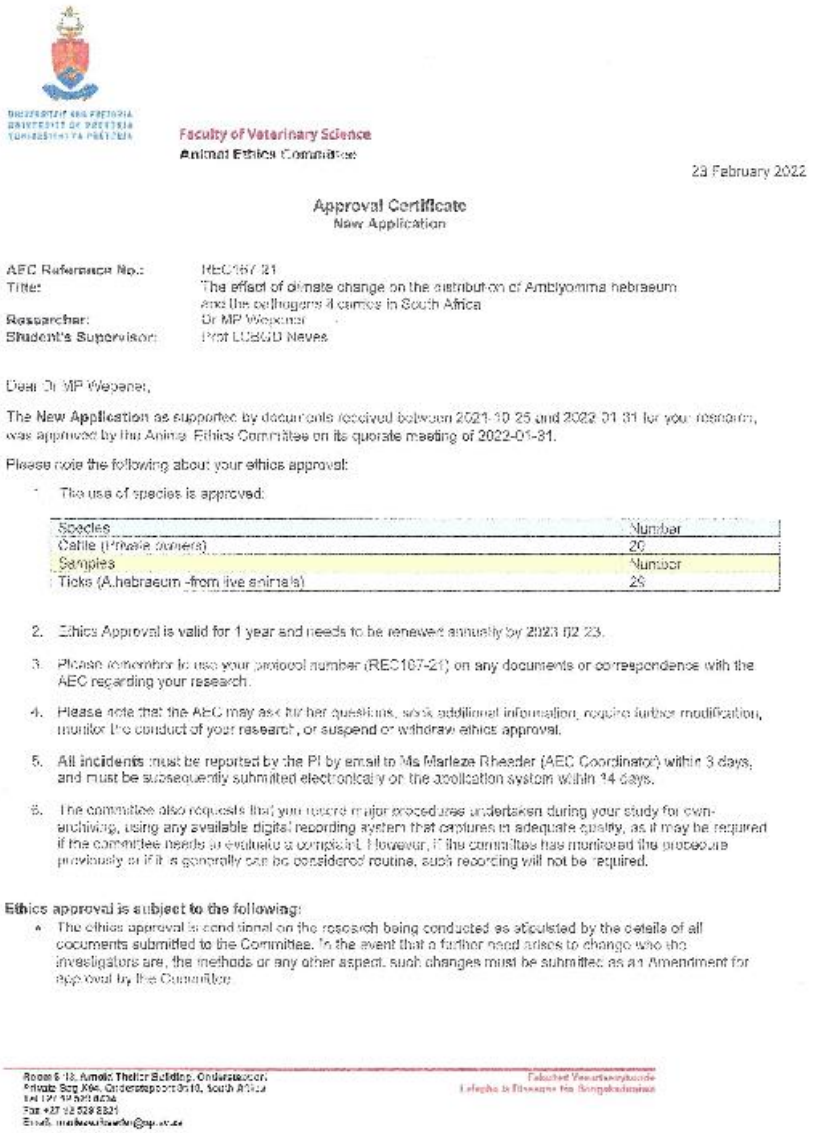
Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Annexure 2: AEC approval



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

23 February 2022

Approval Certificate
New Application

AEC Reference No.:	REC167-21
Title:	The effect of climate change on the distribution of Amblyomma hebraeum and the pathogens it carries in South Africa
Researcher:	Dr MP Wepener
Student's Supervisor:	Prof LDS du Preez

Dear Dr MP Wepener,

The New Application as supported by documents received between 2021-10-25 and 2022-01-31 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-01-31.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Cattle (Female owners)	20
Samples	Number
Ticks (A.hebraeum -from live animals)	20

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-02-23.
3. Please remember to use your protocol number (REC167-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures to adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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