Distribution and acaricide resistance status of the theileriosis vector, *Rhipicephalus appendiculatus* Neumann (Acarina:Ixodidae) in response to changing climate in Zimbabwe

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Student Declaration

I, Tinotenda M. Nemaungwe declare that the Master of Science (MSc) research dissertation that I herewith submit at the University of the Free State, is my own original and independent work and that I have not previously submitted or will submit it for qualification at another institution of higher education.

24 October 2022

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Abstract

Tick control is a key aspect of managing Tick-Borne Diseases (TBDs) with chemical control being the widely applied method. However, continued application of chemicals can lead to the development of resistance. A recent increase in theileriosis in Zimbabwe coupled with indications of shifts in outbreaks from seasonal to spontaneous, raised possibilities of acaricide resistance by the vector, *Rhipicephalus appendiculatus*. Factors behind changes in the spatio-temporal distribution of the tick and the disease it transmits are poorly understood. This study evaluated the distribution of the theileriosis vector, *R. appendiculatus,* its response to frequently applied acaricides and the implications of current management practices on acaricide resistance development in Zimbabwe. Initially, the thermal tolerance of larvae, nymphs and adult *R. appendiculatus* ticks were assessed followed by modeling the current and future distribution of suitable habitats using MaxEnt. Bioassays were then conducted to determine the tick's resistance status to frequently applied acaricides namely amitraz and deltamethrin. Furthermore, a survey was undertaken to understand the relation between tick management strategies and acaricide resistance development in ticks of economic importance.Results indicated that suitable habitats for *R. appendiculatus* may expand into previously non-endemic areas between 2040 and 2060 if temperatures rise by 2 °C. This expansion may be aided by the ability of the species to tolerate higher temperatures during assays than those recorded in the environment. Results further showed that *R. appendiculatus* populations are still highly susceptible to amitraz and deltamethrin (Resistance ratios<4). In contrast, the indicator species *R. decoloratus* which was used for comparison had 50% of populations moderately resistant to amitraz and two dip tanks resistant to deltamethrin (4<RR<10). Farmers noted a high prevalence of TBDs which they attributed to a breakdown in the national tick management program. However, there were indications of practices by farmers which might lead to the development of resistance even in the three-host ticks in future. These include, indiscriminate use of acaricides and poor rotation of acaricide molecules. Overall, this study suggests that current theileriosis outbreaks may be driven by the spread of *R. appendiculatus* into non-endemic areas rather than acaricide resistance development. Therefore, there is a need for national programs to expand surveillance and control measures into areas previously non-endemic, but predicted to be suitable for the theileriosis vector.

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Abbreviations and acronyms

AIT	Adult Immersion Test
ANOVA	Analysis of Variance
CI	Confidence Intervals
AUC	Area Under the Curve
CVL	Central Veterinary Laboratory
CCRT	Chill Comma Recovery Time
CTLs	Critical Thermal Limits
CT_{max}	Critical Thermal Maxima
CT_{min}	Critical Thermal Minima
DNA	Deoxyribonucleic acid
DVS	Department of Veterinary Services
DDT	Dichlorodiphenyltrichloroethane
DEM	Digital Elevation Model
FAO	Food and Agricultural Organization of the United Nations
HCT	Heat Comma Temperature
HKDT	Heat Knock Down Time
LC_{50}	Effective $dose_{50}$
LIT	Larval Immersion Test
LPT	Larval Packet Test
LLT	Lower Lethal Temperature
MaxEnt	Maximum Entropy
OP	Organophosphate
PCR	Polymerase Chain Reaction
ROC	Receiver Operated Characteristics
RF	Resistance Factor
RR	Resistance Ratio
SSP	Shared Socioeconomic Pathway
SDMs	Species Distribution Models

SP	Synthetic Pyrethroid
TBDs	Tick-borne Diseases
ULT	Upper Lethal Temperature
WHO	World Health Organization

CHAPTER 1

GENERAL INTRODUCTION

Overview

Ticks and tick-borne diseases (TBDs) are amongst the major factors affecting livestock production in tropical and subtropical regions of the world (Graf *et al.,* 2004; Abbas *et al.,* 2014). Globally, economic losses resulting from tick and tick-borne diseases are estimated to be between US$22 billion and US$ 30 billion annually (Lew-Tabor and Rodriguez Valle, 2016). These costs arise from losses in milk production, hide damage, reduced meat production as well as costs of tick and TBDs control (Lovis *et al*., 2012). A number of tick species are vectors of diseases of socio-economic importance in livestock (Horak *et al*., 2009; Spickett *et al.,* 2011). The most common TBDs responsible for high livestock mortalities worldwide are babesiosis, anaplasmosis, theileriosis and ehrlichiosis. Generally, the prevalence of TBDs is dictated by the distribution of the vector as well as the hosts (Hudson *et al*., 2001; Bolzoni *et al.,* 2012). Hence, tick management remains one of the major strategies to reduce morbidity and mortality from TBDs as it influences the epidemiology of the pathogen transmitted.

Vector management remains one of the most viable and effective strategies adopted globally to control vector borne diseases (FAO, 2004). Although a number of vector management strategies are available, chemical control remains the major technique applied. As such, there has been an evolution in the chemical formulations used in vector control over the years. Reasons for the continuous change in chemicals used range from environmental concerns regarding the safety of these chemicals to resistance development of ticks to the effect of these chemicals. For example, Dichlorodiphenyltrichloroethane (DDT), was the major pesticide with varied applications including livestock pest control during the 1940s. Its bioaccumulation properties and development of resistance by some pests, led to its gradual out phasing (Graham and Hourrigan, 1977). The World Health Organization (WHO) (1992) and Krostad (1996) also reported insecticide resistance that threatened insecticide residual spraying in the control of mosquitoes as vectors of malaria.

Resistance of ticks to acaricides may be a result of one or a combination of factors. These include overuse of acaricides (Castro-Janer *et al.,* 2010; Li *et al.,* 2015), poor rotation of available chemicals (Klafke *et al.,* 2006), incorrect and inconsistent use of the acaricides as well as lack of resistance monitoring (Abbas *et al.,* 2014). Indiscriminate and unregulated sell of acaricides coupled with poor farmer knowledge on the judicious application of acaricides also plays a significant role in tick resistance development (Mendes *et al.,* 2013). Although the rate of emergence of resistance to any one acaricide varies among tick species in most

countries, one host ticks such as *Rhipicehalus decoloratus* and *Rhipicephalus microplus* species are considered as 'indicator species' because they are subject to the greatest selection pressure and the most frequent exposure to acaricides (Arthur and Londt, 1973). In Zimbabwe there is an increasing limitation in the number of available hosts, leaving cattle as the major tick host especially in farming areas. This increases the chances of multi-host ticks getting more exposure to acaricides with the subsequent possibility of resistance development.

To effectively manage vectors and vector-borne diseases, knowledge of their ecology, physiology and distribution is vital. As ectotherms, the distribution of arthropods is mainly shaped by climatic variables. In addition, their life history traits such as survival, evolution and development, reproduction and fecundity are also influenced by climatic variables such as temperature, photoperiod, and relative humidity (Nguyen *et al.,* 2014). Climate change may alter these traits and in effect the ticks distribution. Such is the case with ticks which were found to establish beyond their previous ranges in Zimbabwe due to shifts in these abiotic factors (Gambiza and Nyama, 2000). Knowledge of arthropod thermal tolerance levels, such as the critical thermal limits, and upper and lower lethal temperatures, assist in understanding the distribution and activity of arthropods in a particular area.

Justification of the study

There has been an increase in the outbreak of theileriosis, a disease transmitted by *Rhipicephalus appendiculatus,* in Zimbabwe (Fig. 1.1). These outbreaks have been linked to more than 60% of livestock mortalities between 2014 and 2019 (Department of Veterinary Services, 2019). The occurrence of the disease has changed from the pattern known since the 1930s, of being seasonal with localized distribution, slow spreading rate and low mortality levels, to being a non-seasonal with wide spread distribution, rapid spreading rate and high mortality levels. While factors behind this spike in theileriosis are unclear, there are possibilities of climate change mediated distributional shifts in the host, *R. appendiculatus,* and its resistance to commonly applied acaricides. In addition, there are farmer reports of chemicals used in the control of ticks, gradually decreasing in efficacy. Sungirai *et al*. (2016) reported high mean tick burdens on cattle despite consistent dipping by farmers (weekly in summer and fortnightly in winter), further indicating possibilities of acaricide resistance.

While cases of resistance have been reported in one-host ticks (eg. *Rhipicephalus decoloratus* and *R. microplus*), very few cases are documented for three-host ticks such as *R.*

appendiculatus (Gono *et al*., 2014; Vudriko *et al*., 2016; Makuvadze *et al*., 2020; Chitombo *et al*., 2021). However, most of these assays were limited to amitraz, although several compounds are readily available on the market and are being utilized by farmers. Furthermore, resistance of *R. appendiculatus* to synthetic pyrethroids was reported in South Africa and Uganda (Ntondini *et al*., 2008; Vudriko *et al*., 2016).

To fully understand the drivers behind the recent spike in cases of theileriosis, the ecophysiology of the vector, *R. appendiculatus*, needs to be studied. It is necessary to evaluate among other factors, the distribution (current and future) of the vector, *R. appendiculatus,* its thermal tolerance levels as well its response to frequently applied acaricides. Understanding current tick management practices helps in identifying strategic gaps for intervention. Early detection of resistance offers an opportunity to anticipate and manage resistance before the diffusion of resistance genes in wider populations (Abbas *et al*., 2014; Ghosh *et al*., 2015).

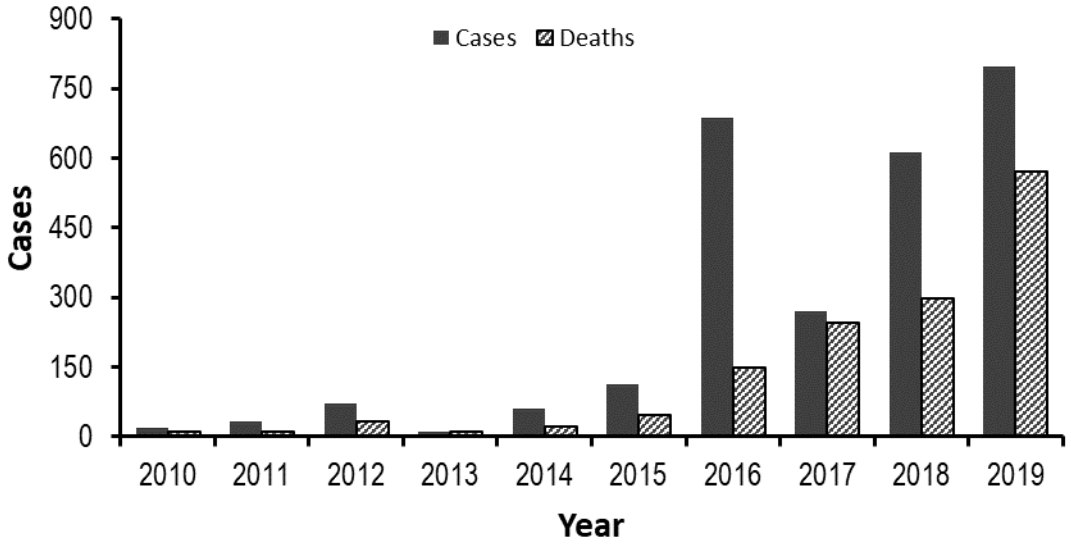

Figure 1.1: Cases of theileriosis and related mortalities recorded in Zimbabwe between 2010 and 2019. (Adapted from Department of Veterinary Services 2019 Annual Report).

Objectives

Main objective

The aim of this study was to determine the current and predicted future distribution of *R. appendiculatus*, its responses to commonly used acaricides and determine potential drivers of resistance development in Zimbabwe.

Specific objectives

1. To determine the current and future distribution of suitable habitats and the basal thermal tolerance for *R. appendiculatus*.
2. To determine the resistance status of *R. appendiculatus* to the commonly applied acaricides and how this relates to current tick management practices.

Hypotheses

1. The distribution of *R. appendiculatus* has shifted from historical areas and will continue to expand due to predicted climate change.
2. *Rhipicephalus appendiculatus* is developing resistance to frequently applied acaricides due to current management practices.

CHAPTER 2

LITERATURE REVIEW

Tick distribution in Zimbabwe

The geographical distribution of ticks in Zimbabwe was updated by Sungirai *et al.* (2015) following the last known published national tick survey carried out in 1996 (Peter *et al.*, 1998). This survey showed that eight tick species namely *Amblyomma hebraeum* (Koch, 1844), *R. microplus* (Canestrini, 1888), *R. decoloratus* (Koch, 1844), *Hyalomma truncatum* (Koch, 1844), *Hyalomma rufipes* (Koch, 1844), *Rhipicephalus* (near) *punctatus* (Warburton, 1912), *R. appendiculatus* (Neumann, 1901), *and Rhipicephalus evertsi evertsi* (Neumann, 1897) are distributed across all ecological zones of Zimbabwe. In support of this observation, Shekede *et al.* (2021) also indicated shifts in tick distribution and spatial overlaps in ticks' realized niches.

The north and north-eastern districts of the country are now dominated by *Amblyomma variegatum*, *R. decoloratus*, *R. microplus* and *Rhipicephalus compostus* whilst *A. hebraeum*, *H. rufipes*, *H. truncatum* and *R. evertsi* are prevalent in the southern districts. Sungirai *et al.* (2015) indicated *A. hebraeum* to have the highest prevalence countrywide, across all ecological regions, followed by *R. microplus*, whilst *R. appendiculatus* is mainly dominant in cooler and wetter parts of the country which offers adequate vegetation cover needed for its survival. The realised niche of *R. microplus* has also expanded from the eastern highveld to as far as the south-eastern lowveld, northern lowveld and northern highveld where it was not previously found (Gambiza and Nyama, 2000). This species encroached into areas commonly occupied by *R. decoloratus* but has not displaced the latter as reported in other countries in Africa (Tønnesen *et al*., 2004; Lynen et al., 2008; Adakal et al., 2013; Nyangiwe et al., 2013).

Factors influencing tick and tick-borne disease distribution

The distribution of ticks continuously changes due to fluctuating climatic conditions as well as the sensitive nature of ticks to abiotic factors such as humidity, photoperiod, and temperature (Springer *et al.*, 2015). Each tick species responds differently to these factors as different species may have different preferences (Estarada-Peña *et al.*, 2006). Changes in climate (temperature, rainfall, and relative humidity) both directly and indirectly affect the population dynamics and biogeography of many organisms including ticks. Their life history traits such as survival, seasonal activity, questing behavior, reproduction, fecundity, and development are dependent on the availability of favorable conditions (Nguyen *et al*., 2014).

In Zimbabwe, the distribution of ticks and TBDs may also be influenced by anthropogenic factors. These include changes in the land tenure system following the agrarian reform in 2000 which resulted in the mass movement of livestock across the country as well as relaxation of previously strict animal control measures. Several tick species are now detected in previously non-endemic areas. However, their survival and persistence appear to be shaped by climatic and environmental conditions in their introduced ranges.

Warmer climates increase tick survival, shorten life cycles, and lengthen tick activity (Gray *et al.,* 2009; Estrada-Peña *et al.,* 2012; Berger *et al*., 2014). But, extreme conditions may cause lethal or sub lethal damages. This has a bearing on the tick abundance and distribution patterns except in rare cases where anomalous features are displayed when ticks are found in areas exhibiting less favorable conditions for their survival. Other factors significant in determining tick distribution include host species availability and their movements (Bolzoni *et al.,* 2012), vegetation type and cover (Tack *et al*., 2011; Daniel *et al*., 2015), tick control strategies and topographical features such as elevation and slope.

The major determinant of tick-borne disease (TBD) incidence is vector abundance and pathogen prevalence in the tick vectors (Hudson *et al*., 2001). Moreover, behavioral changes in ticks driven by changing abiotic factors such as temperature, humidity and photoperiod regulate host-tick interactions thereby affecting the dynamics of TBDs (Gilbert, 2010).

Tick thermal biology

Climate change modifies the environment, and organisms living in this altered environment need to adapt to the new conditions or go extinct. Variations in climatic conditions play a direct influential role on the proliferation of ticks by affecting their survival, duration of development, host seeking activities and reproduction capacity (Estrada-Peña and Salmon, 2013). Apart from these direct effects, changes in abiotic factors like temperature and humidity affect evolutionary changes of ticks by altering the environmental determinants of fitness (Rodgers *et al.,* 2007). Temperature plays an important role in regulating the abundance and geographical range of most tick species by having direct and indirect effects on tick behavior, activity, physiology, and development (Petzoldt and Seaman, 2006). Most tick species fail to acclimate to extremely low and high temperatures (Sonenshine, 1991; Holmes *et al*., 2018). This makes them prone to freezing in winter and desiccation in summer (Rosendale *et al.,* 2016); unlike insects with very long-life cycles which are able to moderate temperature inconsistencies over their entire life history. Any positive or negative shift from the optimal temperature affects tick activities such

as oviposition, moulting, egg incubation and questing behavior (Dautel *et al.*, 2008). Temperature variations between seasons are therefore marked by a decrease in tick activity. For instance, the Southern African tick, *R. appendiculatus* becomes inactive and undergoes behavioral diapause when temperatures drop in winter and resume activity when temperatures increase with the onset of summer (Short and Norval, 1981; Madder *et al*., 2005). Exposing ticks to temperatures below or above their optimum temperature also negatively affects their metabolism and energy reserves (Rosendale *et al*., 2016).

Performance curves can be used to illustrate arthropod responses to temperature (Fig. 2.1) (Mutamiswa, 2017). Quantifiable thermal traits include the upper lethal temperature (ULT), lower lethal temperature (LLT) and an array of other non-lethal attributes such the critical thermal limits (CTLs) and time taken to recover from heat knockdown (heat knock down time (HKDT)) or cold-induced coma (chill coma recovery time (CCRT)) (Chown and Nicholson, 2004; Castaneda *et al*., 2005). Thermal tolerance limits and preference vary among tick species. Fieler *et al.* (2021) indicated variations in thermal tolerance among six tick species with the ULT having a range from 41 °C to 47 °C and the LLT ranging from -15 °C to -24 °C. According to Loeschecke and Hoffmann (2007), the survival of any organism under adverse conditions is a sign of fitness. Information on thermal tolerance limits is key in understanding organism ecology, physiology, and evolution; important traits in determining species range distribution (Hazell *et al*., 2008) and how climate change influences them (Walther *et al.*, 2002). Thermal tolerance traits also determines the likelihood of a tick species successfully proliferating when introduced in a new environment (Nyamukondiwa *et al*., 2010).

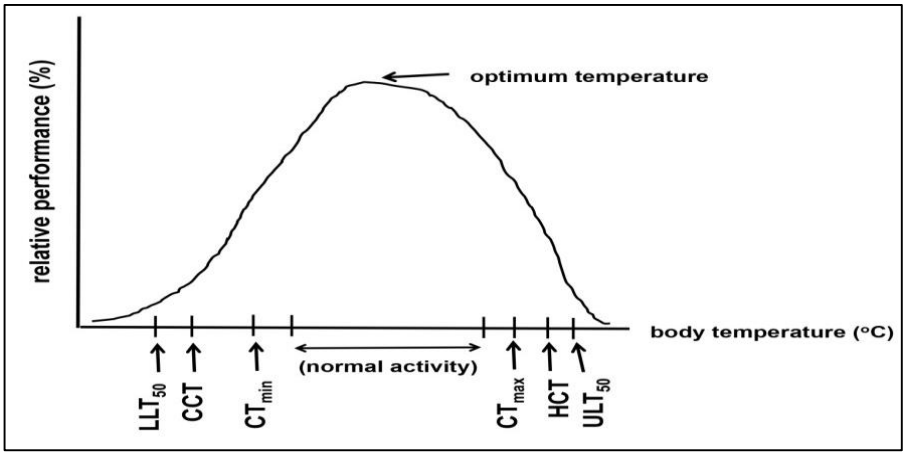

Figure 2.1: Responses to temperature in a hypothetical organism (Adapted from Mutamiswa, 2017). CT_{max} = critical thermal maxima; CT_{min}= critical thermal minima; CCT = chill coma

temperature; HCT = heat coma temperature; ULT = upper lethal temperature; LLT = lower lethal temperature**.**

Tick control strategies

Vector management remains one of the most viable and effective strategies to control both the vector and vector-borne diseases (FAO, 1984). The principle behind successful tick control is based on the knowledge of the ecology of the tick and tick-host interactions in a specific environment or management system (Ginsberg, 2014). Traditional, chemical, ecological, and biological control strategies among others are available and have often been used in integration for sustainable tick control.

Chemical control

Chemical control of ticks using acaricides, applied singly or as an integrated approach with other complimentary methods, is the most widely used tick control method worldwide. In light of this high demand for chemical control of ticks, the need to preserve the effectiveness of available acaricides was highlighted by Abbas *et al.* (2014). Unlike other control strategies, chemical control can be successfully implemented in the absence of knowledge on tick ecology or epidemiology (Mendes *et al*., 2013). However, this strategy has limitations such as high cost, residual effects on non-targeted species and environment, selection of resistant populations that may cause the disappearance of the favorable enzootic stability (George *et al*., 2004). Using chemical control as a strategy therefore requires periodic monitoring of efficacy to rule out resistance development. Various acaricide formulations with different application methods have therefore been developed in a bid to reduce the cost but improving the efficiency of the acaricide. Dipping vats, hand sprays, spray races and pour-ons are the major application methods. Other less common methods include the use of intra ruminal boluses, injectable formulations, impregnated ear tags, tail tags, foot baths and automatic devices such as Duncan applicators (for tick control in wildlife).

Ecological tick control/ Environmental management

The principle behind this control strategy is the modification of the environment (biotype) to hinder tick/host encounter or disrupt completion of the tick life cycle (Ginsberg, 2014). Knowledge of the tick ecology is therefore a prerequisite for this strategy to be effective. Ecological control uses strategies like pasture spelling, zero grazing, rotation of crops with

livestock, avoidance of heavily infested pastures and encouraging cattle grazing when tick activity is reduced (Ginsberg, 2014). Alternatively, the use of repulsive or tick toxic plants and bush fires can be employed (George, 2006; Lizzi *et al*., 2014). The use of bush fires is however not effective for ticks hidden in ground crevices and result in more ticks being disseminated as animals graze more on the re-growth (Minshull and Norval, 1982).

Biological control

Biological control involves the use of agents such as predators, pathogens, parasitoids, nematodes, and fungi naturally available in the environment to destroy the parasitic free stages of the ticks (Samish *et al*., 2004; Ginsberg, 2014). This technique is widely accepted globally due to low cost and minimal impact to the environment compared to other methods. Although many biological control agents are known, only a few biocontrol programs have been developed for ticks, unlike in plant pest control where numerous studies have been carried out (Samish & Rehacek, 1999). Butler *et al.* (1979) found that the fire ant (*Solenopsis invicta*) caused the disappearance of *Amblyomma americanum* in Florida pastures which were full of ants' nests. Research by Stutterheim *et al. (*1988) has also indicated that predators such *as Buphagus erythrorhynchus* and *Buphagus africanus* can consume up to 15g of ticks daily. Studies in Europe (Kalsbeek *et al*., 1995) indicated mortalities of up to 50% of *Dermacentor* and *Ixodes* ticks due to fungal infections. The use of biological control agents however requires thorough research to select an appropriate, non-generalist and efficient species, followed by production of sufficient quantities for practical use (Samish *et al*., 2004; Ginsberg, 2014).

Traditional control of ticks

These are indigenous control mechanisms such as manual tick removal and the oral removal of ticks attached to the udder by suckling calves. The former is time consuming therefore not effective for large cattle breeders whilst the latter only targets ticks attached to the udder leaving other predilection sites unattended (Ginsberg, 2014).

The use of vaccines

Vaccines provide an effective and environmentally friendly approach for tick control (Merino *et al.,* 2013) and were commercially made available in the early 1990's (de la Fuente *et al*., 2005). Vaccines work by inducing the production of antibodies which damage the tick gut, reduce survival of ticks, egg production and hatchability, reducing the tick population in an

area over time (Rodriguez *et al*., 1995). With this option, treatment frequency is reduced hence also reducing the risk of resistance development. Vaccines can be used in conjunction with other chemical and non-chemical strategies.

Herbal remedies

This involves the use of plants or plant extracts with acaricidal or tick repellent properties. Plants like *Melinis minutiflora* and *Brachiaria brizantha* were found to have larvicidal properties (Wilson and Sutherst, 1990). The use of herbal remedies however needs thorough research and assessment before adoption.

Resistance of ticks to acaricides

The use of chemicals to control arthropod vectors of diseases is limited by unavailability of suitable pesticides, lack of connection between risk and applied treatment as well as incorrect and inconsistent application regimes. These factors often lead to the development of resistance in a number of disease vectors (Hurwitz *et al.,* 1981; Rosario-Cruz *et al*., 2009a; Raynal *et al*., 2013). Resistance is generally defined as a shift in the susceptibility of a target parasite species to a specific drug (Sangster, 2001; Corley *et al*., 2013). The parasite therefore continues to multiply because the administered drug will be within its limits of tolerance and needs to be applied in higher than recommended doses (WHO, 1965). FAO (2004) defined tick resistance as a phenomenon whereby an acaricide fails to eliminate ticks despite it being used at the recommended concentration and according to all the recommendations for its use by the manufacturer.

Origin and occurrence of resistance

Tick resistance to acaricides was recorded as early as 1935 when *R. microplus* became resistant to arsenic after being used for 40 years in Australia (George *et al.,* 2004). Arsenic resistance later extended to other countries like South America, Jamaica, and several African countries. In the mid-1940s, organochlorines were introduced that were able to eliminate the arsenic resistant ticks but were later withdrawn because of persistence in the environment (Graham and Hourrigan, 1977; Ware, 2000). The extent of acaricide resistance continued to expand in terms of geographic distribution, number of chemicals and tick species involved. Diverse groups of acaricides were successively introduced for tick control due to progressive evolution of resistance by ticks to the acaricides. Organophosphates (1955), carbamates (1955),

formamidines (1975), pyrethroids (1977) and macrocyclic lactones (1981) were introduced in succession to solve the ever-evolving tick resistance problem against each preceding acaricide group (Abbas *et al.,* 2014). Nowadays, tick resistance is widely distributed though it varies on the type of chemical and tick species from place to place.

In Zimbabwe, acaricide resistance has been detected since the 1960s (FAO, 1983). The single host tick *R. decoloratus*, has been reported to be resistant to all major compounds used in the country over the years. Between 1963 and 1975 some *R. decoloratus* populations were found to be resistant to arsenic, dieldrin and the organophosphate-carbamates group. For this species, resistance continues to be reported even for chemical compounds currently in use. These include synthetic pyrethroids such as flumethrin, cypermethrin and deltamethrin as well as amidines (Mazhowu, 1995; Rodriguez-Vivas *et al*., 2018; Sungirai *et al.*, 2018a; Makuvadze *et al.,* 2020). Although *R. decoloratus* has been termed the indicator species for acaricide resistance, some multi-host tick species have also been reported to develop resistance on a smaller scale. For example, *R. appendiculatus*, *R. evertsi* and *A. hebraeum* were found to have developed resistance to arsenic (FAO, 1983). Recently, emerging resistance to amitraz and chlorfenvinphos was also reported in *R. appendiculatus* collected from small scale farming systems in Mashonaland West Province, Zimbabwe (Chitombo *et al.*, 2021).

Acaricide resistance development in ticks

The mere use of an acaricide does not create resistant ticks but permits the selection of individuals with genes that have the ability to confer resistance to the progeny. This begins with a few heterozygous resistant individuals who upon sustained selection pressure from continued exposure to the same acaricide will ultimately, after the completion of many generations, produce homozygous resistant individuals in the population (Abbas *et al*., 2014). Resistance is an inherited phenomenon, and many factors play a role in its establishment. These factors range from the frequency of the original mutation in the population before treatment, mode of inheritance of the resistant allele, frequency of acaricide treatment to the proportion of a population that is not exposed to the acaricide (refugia). The influence of each of these factors will determine the rate at which a resistant allele becomes established in the population and the time it takes for the control of the tick population to be lost (Sangster, 2001; FAO, 2004).

Mechanisms that enable resistance development are a reduction in the rate of penetration of the acaricide into the tick, changes in tick metabolism, storage or excretion of the pesticide and

changes in the site of action, which render the tick less sensitive to the effects of the acaricide (Van Leeuwen *et al*., 2010). Once established in ticks, resistance appears to be stable even in the absence of selection pressure. High incidences of *R. microplus* resistance to chlorinated hydrocarbons were witnessed in Australia despite not using the acaricide for 20 years (Li *et al*., 2003).

Cross resistance

This occurs when acaricides with the same mode of action share resistance. For instance, when carbamates were introduced to eliminate organophosphate resistant *R. microplus* ticks, the ticks also developed resistance to the carbamates group (Li *et al*., 2005; Madder *et al.*, 2011) because they are both acetylcholinesterase inhibitors resulting in malfunctioning of the nervous system (Jensen *et al.*, 2011)

Acquired resistance

Acquired resistance emanates from a decrease in sensitivity to drugs with time (Meyer *et al*., 2012). For instance, Lin *et al.* (2009) and Feng *et al.* (2011) reported 8.7- and 28.7-fold resistance of *Tetranychus cinnabarinus* to abamectin and fenpropethrin after 42 and 20 generations of selection, respectively. Exposing a tick population to continued selection pressure by using the same acaricide constantly removes susceptible individuals in the population and consequently increase the number of resistant individuals (Lees *et al.,* 2013).

Multiple resistance

This is when an organism develops resistance to more than one drug despite the drugs having different modes of action. This is typical of *R. microplus* resistance to pyrethroids, chlorinated hydrocarbons, organophosphates and formamidines in Mexico (Foil *et al.,* 2004).

Factors influencing tick resistance development

The development of resistance by ticks to acaricides has often been linked to genetic, biological and several operational and intrinsic factors. According to Sutherst and Comins (1979), the genesis of resistance has three steps namely, establishment, development and finally emergence. Genes responsible for initial resistance genesis are present at low levels in a population before the use of a new acaricide (Alonso-Diaz *et al.,* 2013) and they are increased gradually by continued acaricide selection pressure (Rodriguez-Vivas *et al.,* 2011). Early

detection of resistance offers an opportunity to anticipate and manage resistance before the diffusion of these resistance genes into the wider population (Abbas *et al.*, 2014; Ghosh *et al*., 2015). Although the rate of emergence of resistance to any one acaricide varies among tick species, one host ticks such as *R. decoloratus* and *R. microplus* are considered as 'indicator species' because they are subject to the greatest selection pressure and frequent exposure to acaricides (Li *et al*., 2015)

Operational factors

Operational factors enable the selection of mutants, which under normal conditions will not survive acaricide treatment. These include drug application factors such as overuse or under dosage of acaricides (Castro-Janer *et al*., 2010; Li *et al*., 2015), poor rotation of available chemicals (Klafke *et al*., 2006) and lack of resistance monitoring (Abbas *et al*., 2014). Other operational factors include the chemical nature of the drug, drug clearance kinetics, possibility of cross resistance, mode of drug application, timing, and frequency of treatment (Abbas *et al*., 2014). Resistance emanating from operational factors can be rectified by proper management practices by acaricide users.

Biological factors

Biotic and behavioural factors constitute biological factors. Biotic factors include tick breeding patterns, generation time and offspring per generation. Behavioural factors are factors influencing gene flow such as mobility, isolation, host range and migration among others (Abdullah *et al.*, 2012).

Genetic factors

The relative fitness of resistant organisms, diversity of a population, initial frequency of resistant genes and dominance of resistant alleles, are among other genetic factors influencing resistance development (Mulchandani *et al.*, 1998). Alleles for resistance in heterozygous individuals present in low quantities in a population and gradually increase with continuous exposure to a drug until homozygous resistant individuals predominate the population. Chevillon *et al.* (2013) highlighted the difficulty in managing these factors since they are not under human influence.

Acaricide resistance diagnosis

In order to extend the useful life of new and existing acaricides, new and improved approaches to the diagnosis and mitigation of tick resistance must be employed. According to FAO (2004), a simple, cheap, rapid, and sensitive method which is able to detect resistance at its early stage of emergence and covering all chemical groups is most desirable. Various diagnostic methods have been developed to ascertain whether tick control failures will have emanated from acaricide resistance although none of them meet FAO's criteria. Bioassays such as the Larval packet test (LPT) (FAO, 1984), Larval immersion test (LIT) (Shaw, 1966), Adult immersion test (AIT) (Drummond *et al.,* 1973) among others are useful tools for the diagnosis of acaricide resistance in tick populations. The characterization of resistance patterns is usually based on probit analysis of the dose-mortality responses of susceptible and resistant tick strains.

According to George (2000), bioassays have their own limitations despite widespread use. Drawbacks include lack of information about genetic mechanisms, low sensitivity to detect resistance emergence at an early stage and the long duration of time needed to obtain results (George, 2000). For instance, *R. decoloratus* requires a minimum of 35 days from collection of engorged females to produce larvae ready for testing using the Larval packet test. Nowadays, there are biochemical assays which can produce results early and provide information on resistance mechanisms. These include the use of antisera specific for an enzyme to identify the presence of enzymes that confer resistance and the use of molecular assays such as the Polymerase Chain Reaction (PCR) to detect specific DNA sequences related to a resistant genotype (Brown and Brodgon, 1987).

Management of tick acaricide resistance

Laboratory confirmation of resistance and its documentation may not automatically result in the withdrawal of a particular acaricide from use or from the market, since resistance is usually not ubiquitous (Abbas *et al*., 2014). The level of resistance guides the course of action to be taken. Resistance management ensures that resistance development is either prevented, eradicated or that the rate of development is slowed down over successive tick generations (Abbas *et al*., 2014). Prevention of resistance development may not be possible whilst eradication of resistance is only achievable if the distribution of the resistant strain is limited to a small area or if the tick species is highly host specific as in the case of *R. decoloratus*. Unlike one-host ticks, resistance of three-host ticks is very difficult to eradicate once they have become well established (Rodriguez-Vivas, 2008).

Various management approaches have been developed to mitigate the effects of tick resistance. Georghiou and Taylor (1986) pointed out the need to treat a population when the numbers of resistant individuals are still low. This however is in contrast to their previous view that a reservoir (refugia) of untreated individuals should be maintained to compete with the resistant species, thus delaying the selection of the genotypes with resistance (Georghiou and Taylor 1977).

According to Norval *et al.* (1988), indigenous cattle breeds such as the Zebu and Sanga require little or no tick control and tick-borne disease associated mortalities are generally insignificant due to enzootic stability. Breeding of these tick resistant breeds is therefore a viable resistance management option. However, Seifert (1984), highlighted the difficulties associated with advising farmers to breed less sensitive or tick resistant animal breeds to manage resistance; farmers seem to prefer more productive European breeds, combined with the use of chemical tick control. With technological advancement, tick resistant European breeds have also been bred that require less treatments per season than the susceptible breeds (Rodriguez-Valle *et al.*, 2012). Resistance monitoring is another good option of resistance management which ensures early detection of resistance before it results in field control failure. Sun *et al*. (2011) suggested laboratory confirmation of resistance and correlation with the management practices for successful resistance management.

Another management option is the rotation of acaricides, not belonging to the same chemical group or having the same mode of action. This reduces the selection pressure for resistance to any one acaricide group. Thullner *et al.* (2007) proved that the resistance ratio of *R. microplus* selected with deltamethrin for 11 generations was very high (RF =756) compared to that of the same species selected with deltamethrin in rotation with coumaphos for 10 generations (RF = 1.6). Adakal *et al.* (2013), however, stated that the rotation of acaricides has no confirmed beneficial effects and it is costly and difficult to practice as there is no stipulated time frame for acaricide change. To delay emergence of resistance, acaricides with compatible chemicals can be used in mixtures. This practice can be based on the fact that the probability of having an individual with alleles resistant to acaricides with different modes of action is very low (Lovis *et al.*, 2013).

Other management strategies include vaccination, nutritional management, preventing dispersion of resistant ticks by controlling animal movement and quarantining newly

purchased, or returning animals. All the above strategies can be integrated for effective resistance management.

The study species: Rhipicephalus appendiculatus (Neumann)

General description and life cycle

Rhipicephalus appendiculatus (Ixodidae) is commonly known as the brown ear tick because it prefers ears of host animals as the attachment and feeding predilection site. It is a small to medium sized tick (ranging from 1.8 − 4.4 mm in length) and brownish, reddish-brown or very dark in color (Fig. 2.2). It is distinguished from other Rhipicephalids by the presence of festoons, short mouth parts, hexagonal basis capitulum and small to medium punctations on the scutum (Walker *et al.,* 2003). Distinctions between this tick and *R. zambeziensies* are however minute and difficult to observe. *Rhipicephalus zambeziensies* is more robust, darker in colour, and more heavily punctated than *R. appendiculatus.*

Rhipicephalus appendiculatus is a three- host tick, meaning each developmental stage (larvae, nymph, adult) feeds on a separate host (Fig. 2.3). It spends approximately 10% of its life cycle, feeding on the animal host and 90% in the environment, where its proliferation depends on climate and micro-climatic conditions (Walker *et al.,* 2000). Under optimum climatic conditions, more than one generation can be attained per year since it takes from 3 months to a year to complete a single life cycle. In tropical areas with sufficient rainfall, up to three generations can be completed each year (Walker *et al.,* 2003) whilst in subtropical regions, only a single life cycle is completed per year (Madder *et al.,* 2005).

Figure 2.2: Adult male (right) and female (left) *Rhipicephalus appendiculatus* ticks. (Picture by Nemaungwe T.M).

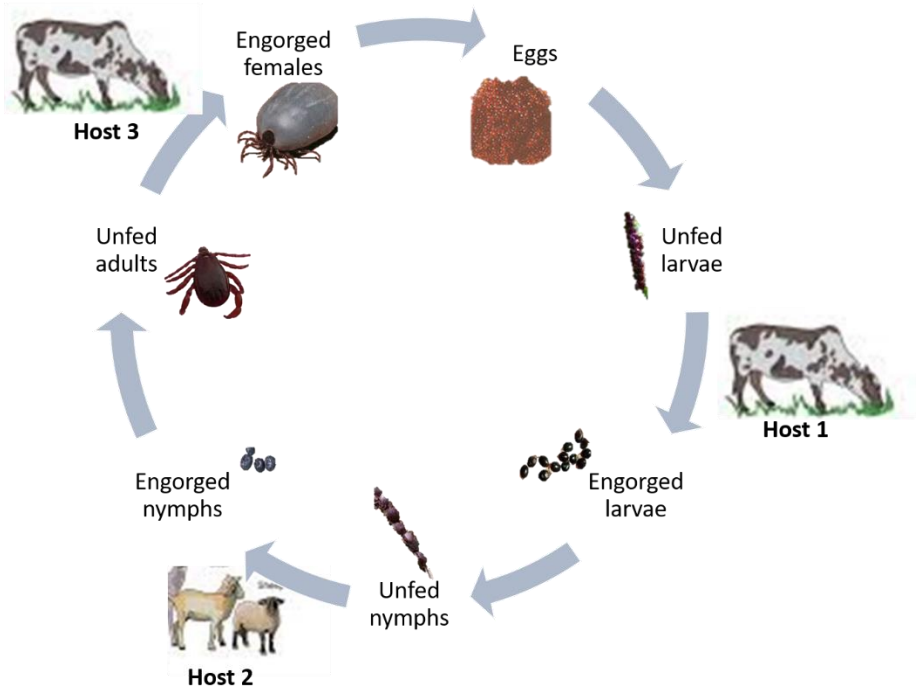

Figure 2.3: Life cycle of a three-host tick. (Adapted from Walker *et al*.,2003).

Hosts and economic importance

The brown ear tick prefers cattle as the main host, but it also parasitizes goats, sheep, dogs and wild ungulates such as buffaloes, elands, sable antelopes, waterbucks and greater kudus (Walker *et al.*, 2003). Nymphs and larvae also parasitize smaller antelopes and hares. *Rhipichepalus appendiculatus* is well known as a vector of different strains or subspecies of the protozoan *Theileria parva* causing diseases such as east coast fever, corridor disease, and theileriosis (Little, 2008). Theileriosis is one of the major tick-borne diseases in sub-Saharan Africa, and it is prevalent in eastern, central, and southern Africa where it causes significant livestock mortalities (Walker *et al.*, 2003). In addition, *R. appendiculatus* is also a vector of other bacterial, viral and rickettsial diseases such as bovine ehrlichiosis, nairobi sheep disease, and tick typhus in humans. Heavy infestation by the tick leads to severe physical damage to ears, anemia and toxicosis which may cause animals to succumb to other tick-borne infections (Rajput *et al.*, 2006).

Habitat, distribution and seasonal activity

Several factors such as host availability, animal movements, variations in climatic conditions, tick control strategies among others play an influential role in the distribution of most ixodid tick species (Tønnesen *et al.*, 2004). Their establishment is also highly dependent on the

continued presence and density of host animals coupled with favorable climatic conditions (Lèger *et al*., 2013). *Rhipicephalus appendiculatus* is dominant in countries located in central, eastern, and south-eastern Africa situated in savanna and temperate climatic regions and receiving a minimum rainfall of 600 mm annually. In Zimbabwe, it is highly prevalent in the high veld (>600m above sea level) where its presence is influenced by high rainfall (≥ 650mm per year), cooler temperatures (10 ºC − 30 ºC), adequate vegetation cover and bovid host availability (Hove *et al.,* 2008; Sungirai *et al*., 2015). Recent surveys, however, have indicated possibilities of a shift in the distribution of this tick. This shift might be mediated by climate change as well as an increase in cattle movements across the country (Tønnesen *et al.,* 2004; Barre and Uilenberg, 2010). According to the Department of Veterinary Services annual report (2019), theileriosis outbreaks also indicate possibilities of this shift as cases were recorded in traditionally non-endemic locations.

Fluctuations in abiotic factors such as temperature and humidity results in seasonal occurrence of *R. appendiculatus* (Short and Norval, 1981; Rechav, 1981). This is governed by the response of unfed adults to weather patterns. According to Madder *et al.* (2005), adult activity is influenced by temperature, humidity, and photo phase (day length) whilst these elements have no direct effect on larvae and nymphs. Adults become inactive and enter into behavioral diapause during the cold-dry season when they are prone to desiccation. Activity resumes at the beginning of the rain season when warm wet conditions with longer day lengths occur. Generally, adults are found during the wet season (December-March) whilst the occurrence of larvae and nymphs overlap from the cooler late summer season, into winter and early spring (Walker *et al.,* 2003).

Theileriosis in Zimbabwe

Theileriosis is an acute, frequently fatal disease of cattle, sheep, goats and wildlife. In Zimbabwe, the disease is caused by *Theileria* parva infection which is transmitted by the brown ear tick (*R. appendiculatus*). The disease was first recognized and distinguished from classical East coast fever in 1936, the former being characterized by mild fever and lower virulence as compared to the latter (Lawrence *et al*., 2017). Symptoms of the disease include swelling of lymph nodes, running eyes, rough skin and loss of appetite and ultimately death especially in older, weaker animals. The highest number of cases of theileriosis tends to be encountered in January when traditionally rainfall activity will be high, hence the common name January disease although occasional outbreaks also occurred in winter months (Latif and Hove, 2011).

Between 2014 and 2019, 60% of cattle losses in Zimbabwe were attributed to theileriosis which has impacted hugely on people's livelihoods and their ability to farm (Department of Veterinary Services, 2019). In previous years, theileriosis was known to be seasonal with localized distribution, slow spreading rate, low mortality levels and widely distributed only in the highveld areas. This has since changed to having non-seasonal disease incidences, with wide spread distribution, rapid spreading rate and high mortality levels. Affected districts spontaneously increased with nearly all parts of the country being affected including areas in the notionally drier, non- endemic areas (Moyo *et al*., 2017). In recent years, the government instituted national dipping program appears to fail to contain the vector and subsequently the disease.

CHAPTER 3

Biogeography of the theileriosis vector, *Rhipicephalus appendiculatus* under current and future climate scenarios of Zimbabwe*

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Abstract

Climate has a direct influence on the epidemiology of vector-borne diseases at various spatial and temporal scales as implied by the geographic ranges of vectors. In Zimbabwe the recent increased incidences of theileriosis, a disease mainly transmitted by *Rhipicephalus appendiculatus*, caused the investigation of the thermal tolerance of adult, nymphal and larval stages of the tick species to project their current and possible future distribution using the machine learning algorithm, Maxent. *Rhipicephalus appendiculatus* larvae had an Upper Lethal Temperature (ULT) of 48 ± 0.5 °C, and marginally higher for nymphs and adults at 49 ± 0.5 °C. Environmental temperatures recorded in selected zonal tick habitats were below the determined lethal limits, indicating the ability of the tick to survive in this region. The resultant model under current climatic conditions showed confinement of areas, with high suitability indices to the eastern, north-eastern, and south-eastern parts of the country mainly in Masvingo, Manicaland and Mashonaland Central Provinces. Future predictions as determined by 2041 – 2060 climatic conditions indicate a 73 069km^2 range expansion of the species habitats into non-endemic areas in regions IV and V if future temperatures increase by approximately 2 °C. Cattle densities had the highest contribution towards the model (44.5%) while maximum temperature of the warmest month (bio 5) had the highest gain (>0.22), thus contributing more information on species habitat suitability when used independently. Overall, the study showed the importance of principal host (cattle) population dynamics and climate parameters on the biogeography of this parasite of economic importance. These results indicate the need to expand surveillance and management techniques against the species even in traditionally non-endemic areas of Zimbabwe.

Keywords: Climate change, Microhabitats, Species Distribution Modelling, Thermal tolerance.

Introduction

Ticks and tick-borne diseases (TBDs) are major causes of livestock mortalities and subsequent economic losses globally (Abbas *et al*., 2014). A number of tick species are disease vectors of medical and veterinary importance and inflict huge annual costs for management of the ticks and reduction of the burden of the diseases they transmit (Horak *et al.,* 2009; Spickett *et al.*, 2011; Vudriko *et al*., 2016). Direct and indirect production losses due to TBDs in sub-Saharan Africa are estimated to be in the range of 10–80% (Chepkony *et al.*, 2021). Therefore, there is a need to constantly monitor tick distributions in order to anticipate future changes and craft appropriate mitigation strategies.

Given the economic importance of ticks, constant surveillance to monitor their current and also project future geographic distribution is needed. This necessitates the need for knowledge of tick ecology, physiology, and geographic distribution. For ectotherms, the distribution is highly dynamic and is heavily influenced by environmental perturbations particularly temperature (Chidawanyika *et al*., 2020; Zannou *et al*., 2021). Body temperature among these ectotherms depends on ambient environmental conditions as well as behavioral mechanisms, for thermoregulation (Chown and Nicholson, 2004; Kearney *et al.,* 2009). Hence, arthropod distribution is mainly dictated by thermal margins with a presumptively higher abundance in areas offering most suitable conditions (Peterson *et al*., 1999; Estrada-Peña *et al*., 2016).

In recent years, climate change mediated shifts in the distribution of organisms have become evident. Longbottom *et al*. (2020) predicted a decline in tsetse abundance in traditional endemic low-lying areas while previously cooler high-altitude areas are expected to provide suitable habitats in Zimbabwe. These predictions are premised on projected temperature rises which are expected to range between 3 – 4 ºC by 2100 (Engelbrecht *et al*., 2015). While some arthropods may counter these changes through mechanisms such as phenotypic plasticity and other adaptive evolutionary responses (Nyamukondiwa *et al*., 2013; Sgro *et al*., 2016; Mutamiswa *et al*., 2017a), biogeographical changes are expected for several species, which fail to adapt to changing environments (Parmesan *et al*., 1999; Forsman *et al*., 2016). Changes in climatic factors may alter arthropod life history traits such as their survival, reproduction, fecundity and impact the encounter of hosts and pathogen- carrying vectors, thereby affecting disease transmission dynamics and ultimately population dynamics (Nguyen *et al*., 2014). Pfaffle *et al*. (2013) reported that temperatures below 7 °C led to continuous tick inactivity which negatively affected their questing behaviour. Such individual responses to both diurnal and seasonal

thermal fluctuations mediate population level effects where severe unfavourable conditions can lead to either migration or extinction.

Even though climatic factors such as temperature and humidity are key determinants of the distribution of animal disease vectors (Tønnesen *et al*., 2004), host distribution, vegetation cover and control efforts also modulate observed distributions (Estrada-Pena, 2008). Absence of ticks in some areas may not be an indicator of non-conducive eco-physiological requirements as host animal movement is key in the dispersal of ticks into novel environments regardless of the prevailing climatic conditions (Barre and Uilenberg, 2010; Sungirai *et al*., 2018b). The survival and proliferation of the ticks' free-living stages in these environments is mainly determined by their adaptability to microclimates, availability of suitable hosts and vegetation cover among other key factors (Greenfield, 2011). The presence of *R. microplus* both in the southern and northern lowveld of Zimbabwe was attributed to animal movement during the land reform programme (Mavedzenge *et al*., 2008). Therefore, in the absence of habitat suitability models, it may be difficult to explain whether the current distribution is linked to changing habitat conditions or animal movement mediated dispersal into already suitable habitats.

Species distribution models (SDMs) have often been used to predict suitable habitats or ecological niches and model potential habitats for ticks based on presence data and environmental covariates (Hahn *et al*., 2016; Sungirai *et al*., 2018b; Pascoe *et al*., 2019; Namgyal *et al*., 2021a). Elsewhere, these models have been used to optimize the control of animal disease vectors. Dicko *et al*. (2014) used a MaxEnt derived habitat suitability model to delimit target areas for the implementation of an area-wide integrated tsetse control program in Senegal. Thus, predictive models fill in gaps of a known distribution (Estrada-Pena *et al*., 2016) and address issues of accessibility and costs which prohibit large-scale intensive surveys (Chikowore *et al*., 2017). Dobson and Randolph (2011) described this as a 'top-down' approach which can be implemented rapidly. Furthermore, the availability of spatially explicit species occurrence records and remotely sensed datasets allows species specific microenvironments to be identified (Ozdenerol *et al*., 2008). Putting the predicted potential risk areas under surveillance is an effective way of managing the vector and its associated diseases (Hahn *et al*., 2016).

In Zimbabwe, the distribution of most tick species previously followed altitude delimited temperature zones with high prevalence on the highveld (>600m above sea level) (Sungirai *et al*., 2015). Recent studies have shown shifts for several species, with some becoming

established beyond their previous geographical ranges (Gambiza and Nyama, 2000). Sungirai *et al*. (2017) reported shifts in the distribution of *Amblyomma* species and *R. microplus* ticks in Zimbabwe. The former is reported to have expanded its range from the lowveld to some parts of the highveld. The latter shifted from occupying only the eastern highveld to reach as far as the south-eastern lowveld, northern highveld and northern lowveld of the country offering low temperatures and high rainfall suitable for its survival. Studies by Shekede *et al*., (2021) have also confirmed these shifts by reporting spatial clustering of *R. microplus* in the north and north-eastern districts of the country.

The brown ear tick, *Rhipicephalus appendiculatus* is highly prevalent in the highveld (>600m above sea level). These areas are characterized by high rainfall (≥ 650mm per year), cooler temperatures (10 ºC – 30 ºC) and adequate vegetation cover (Hove *et al*., 2008; Sungirai *et al*., 2015). The tick transmits the parasite *Theileria parva,* which causes theileriosis in bovids. Norval *et al*. (1991) previously reported that *R. appendiculatus* diapause broke the transmission of the parasite in Zimbabwe. However, the Department of Veterinary Services in Zimbabwe reported an increase in the incidence of the disease and related cattle mortalities from 2014 to 2019. Some of the incidences were reported in traditionally non-endemic areas and cases also changed from being seasonal to all year round. Therefore, questions arise on the distribution of the disease vector, *R. appendiculatus*. Answers were sought in this study by modelling the current and future distribution of habitats suitable for *R. appendiculatus*. The study specifically aimed to answer the following questions: (1) Has the distribution of *R. appendiculatus* shifted from historical areas and will it continue to expand due to climate change? (2) Is the thermal tolerance of *R. appendiculatus* determining its current distribution? The study hypothesized that the distribution of *R. appendiculatus* has shifted from historical areas and will continue to expand due to predicted climate change.

Materials and Methods

The study area

The study was conducted in Zimbabwe, which lies in the sub-tropical region and is predominantly savanna. The country is divided into five agro-ecological regions based on temperature and rainfall where regions I, II and III lie in the highveld whilst region V and part of IV are in the lowveld (Norval *et al*., 1994; Sungirai *et al*., 2018b). Rainfall received per annum generally decreases from region I to V while temperature increases. Despite the differences in rainfall and temperature patterns, agricultural activities are carried out across all

regions. Specialized and diversified crop-livestock farming is practiced in region I. Region II and III are intensive and semi-intensive farming regions while regions IV and V receive extremely low rainfall (less than 500mm) per annum and are therefore occupied by semi-intensive and intensive beef and game ranching.

Influence of temperature on survival and distribution of Rhipicephalus appendiculatus

Hygrochron iButton data loggers (DS1923-F5, ±0.5 °C, Cold chain Technologies) were deployed in tick habitats in selected districts to record microhabitat temperature fluctuations between April and November 2021 at hourly intervals. This period encompassed both the coldest and warmest seasons in Zimbabwe. After the prescribed period, the mean, maximum and minimum temperatures of the tick habitats were calculated. To determine the impact of thermal extremes on the survival of *R. appendiculatus*, upper lethal temperatures (ULTs) were assayed using the direct plunge protocol (Chidawanyika *et al*., 2020; Fieler *et al*., 2021). Higher temperatures were considered due to heatwaves experienced in the country recently as well as the absence of sub-zero temperatures from environmental data collected during the study. Since *R. appendiculatus* is a three-host species, larval, nymphal, and adult stages were assayed. For each developmental stage, 10 unfed ticks were placed in five 60 ml screw top polypropylene vials to yield a sample size of N=50 for each treatment. Lids of the vials were perforated for ventilation and a piece of moistened cotton wool was attached in each vial to maintain relative humidity above 80%. Vials were then placed in Ziploc bags and immersed in a programmable Nuve water bath (SANAYI MALZEMELERI, Turkey) for 2-hour durations at temperatures ranging from 38 – 50 °C ±0.5 that elicit 0–100% mortality. After the treatment, the vials were incubated in a POL-EKO humidity chamber (POL-EKO –Aparatura sp.j, Poland) at 27 °C ± 1 and 80-90% humidity for 24 hours before scoring survival. Survival was defined as the ability to coordinate normal locomotory response to stimuli such as gentle prodding.

Tick presence data

Tick presence data was collected during a national tick survey conducted across all agro-ecological zones of Zimbabwe by the Department of Veterinary services in 2013. This survey was carried out using a stratified sampling approach where agro-ecological zones of Zimbabwe were the strata (Fig. 3.1). Five districts were randomly selected from each stratum except the stratum representing agro ecological zone I, with three districts which were all sampled. A total of 23 districts were selected and 10 dip tanks, which constituted the sampling units, were sampled from each of the selected districts. At each dip tank, ten animals were randomly

sampled (maximum of ten ticks per predilection site). Targeted sites were the head, neck and dewlap, ears, body and belly, legs and tail switch, udder, and scrotum. Samples were collected in universal bottles and preserved in 70% alcohol, 5% glycerol and 1 % chloroform. Ticks were collected on a monthly basis throughout the year and were identified at the Central Veterinary Laboratory (CVL) using morphological keys by Walker *et al*. (2003). The data was used to define the presence locations, which are the pixels where ticks were observed, while MaxEnt generated pseudo-absences, formed the background data. Tick presence locations were cleaned in ArcGIS to ensure coordinate precision, remove duplicates, and remove wrong coordinates.

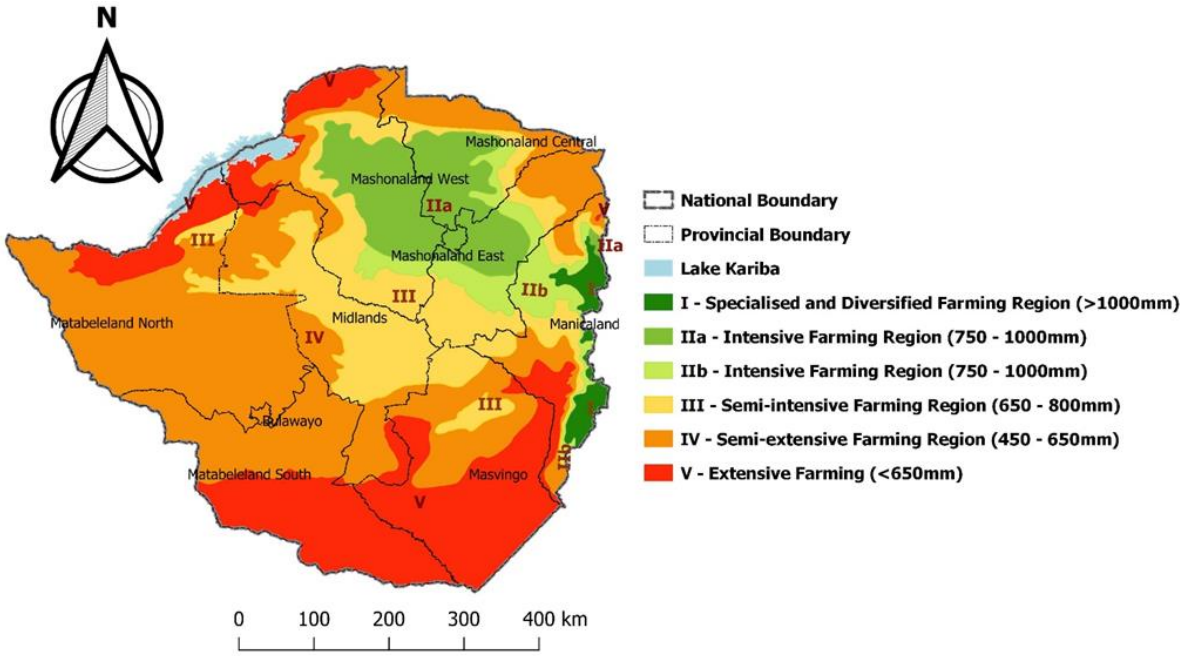

Figure 3.1: Study area showing the ecological regions of Zimbabwe (Adapted from Vincent and Thomas, 1961).

Environmental covariates

Climatic variables to build the model were downloaded from the Worldclim 2.0 database and the Food and Agricultural Organization (FAO) gridded livestock of the world data (Hijmans *et al*., 2005; Wint and Robinson, 2007). The 19 bioclimatic variables and the Digital Elevation Model (DEM) were downloaded and subjected to multi-collinearity analysis using Pearson's correlation in R (R Core Team, 2021). Only those variables which best describe the ecology of ixodid ticks were selected from pairs with a coefficient (r) greater than 0.9 to avoid model

overfitting and improve the interpretability of the model (Merow *et al*., 2013). As a result, a total of 9 bioclimatic variables (Table 3.1), the Digital Elevation Model (DEM) and the FAO gridded livestock data were used to construct the model. The raster data sets were clipped for Zimbabwe and converted to binary ASCII format compatible with the MaxEnt software using ArcGIS 10.2 (ESRI). The contribution of each independent bioclimatic variable, when used in isolation, was assessed using the jackknife test (Rong *et al*., 2019).

The same bioclimatic variables were also downloaded from Worldclim 2.0 for the Shared Socioeconomic Pathway 2 (SSP2-4.5) 2041 – 2060 climate scenario (Fick and Hijmans, 2017; Meinshausen *et al*., 2020). These were used to predict habitats suitable for *R. appendiculatus* in the future.

Table 3.1: Predictor variables used to model suitable habitats for *Rhipicephalus appendiculatus* in Zimbabwe.

Variable	Code	Source
Cattle densities	catda2	FAO
Annual Rainfall	bio12	Worldclim
Elevation		Worldclim
Precipitation of the Driest Month	bio14	Worldclim
Mean Temperature Diurnal Range	bio2	Worldclim
Maximum temperature of the warmest month	bio5	Worldclim
Precipitation of Wettest Month	bio14	Worldclim
Isothermality	bio3	Worldclim
Min Temperature of Coldest Month	bio6	Worldclim

Data Analyses

Microclimatic data

To visualize temperature and humidity in tick habitats, readings were plotted against sampling time in excel (Microsoft Office 2016). Their differences were then compared using one-way analysis of variance (ANOVA) in STATISTICA version 7 (TIBCO Software) while Tukey-Kramer *post hoc* tests were used to separate statistically significant groups.

Lethal Temperature Limits

The response of *R. appendiculatus* larvae, nymphs, and adults to increasing temperatures were modelled using the R statistical software version 3.3.0 (R core team, 2021). The 'drc' package (Ritz *et al*., 2015) was used to construct 2-parameter log-logistic models with the lower and upper limits fixed at 0 and 1 respectively. The temperature where 50% of the tick stage dies was inferred from the model using the 'ED' function of the package.

Habitat suitability modelling

Current and future habitats suitable for *R. appendiculatus* were modelled using the maximum entropy technique (MaxEnt) (Phillips *et al*., 2006). MaxEnt is a logarithmic technique which uses mathematical principles to determine habitat suitability by comparing conditional density of presence sites with marginal density of an object (Hijmans and Graham, 2006). The model uses presence data, randomly selected pseudo-absences as background points and environmental variables to generate a probability distribution across a landscape (Philips *et al*., 2006; Elith and Leathwick, 2009). The MaxEnt model was trained using 75% of the dataset whilst the remaining 25% was reserved for model validation (Zhao *et al*., 2021). A bias file was used for the sampling of background points since the tick sampling survey was stratified. Habitat suitability maps were produced in ArcGIS showing indices ranging from 0 (low suitability) to 1 (high suitability). The predictive power of the model was evaluated using the threshold- independent Receiver Operated Characteristics (ROC) curve and Area under the Curve (AUC) metric (Elith *et al*., 2006; Phillips *et al*., 2006). An AUC closer to 1 indicated a good predictive model with better correlation between environmental variables and the projected geographical distribution of the tick species. The current and future models were compared to determine any changes in the suitable habitats.

Results

Environmental temperatures in Rhipicephalus appendiculatus habitats

Bindura in Mashonaland Central recorded the highest mean temperatures in tick habitats (Fig. 3.2a). Post-hoc tests also indicated that mean temperatures were significantly higher at this site while Murombedzi in Mashonaland West had the lowest temperatures (Fig. 3.2b). There were no significant differences in mean temperatures for Nyazura and Tsanzaguru tick habitats in Manicaland province compared to Masiyarwa in Mashonaland West province. When

853 temperatures were decoupled into minimum and maximum, Masiyarwa in Mashonaland West
854 had the highest maximum temperatures (Fig. 3.2c) and Murombedzi had the lowest minimum
855 temperatures (Fig. 3.2d).

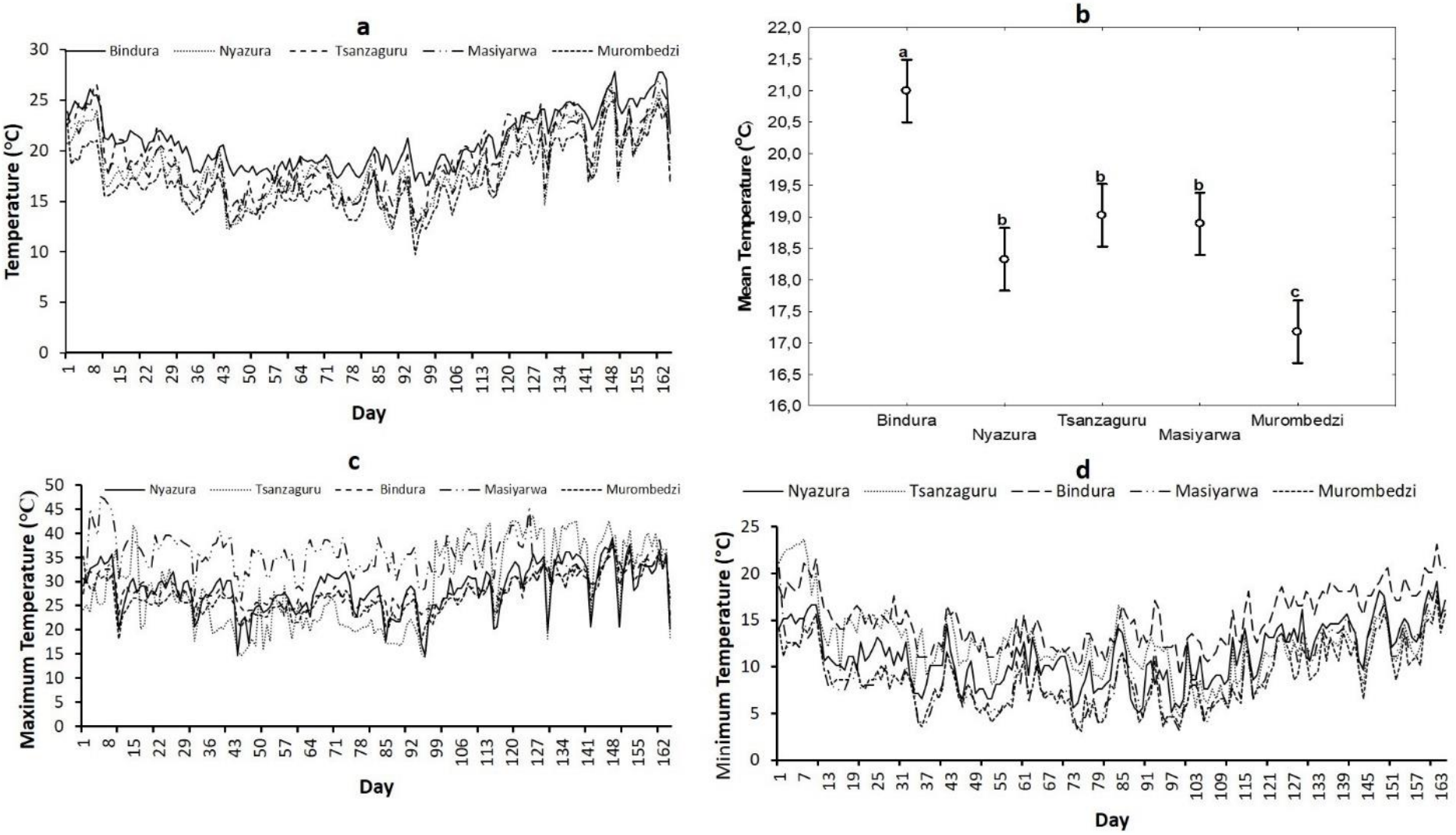

Figure 3.2: Mean temperature fluctuations (a), differences in mean temperatures (mean ± 95% confidence interval) (b), Maximum temperature fluctuations (c) and Minimum temperature fluctuations (d) in selected districts in Zimbabwe.

Upper lethal temperature assays

The Upper lethal temperature (ULT) for *R. appendiculatus* larvae was 48 ± 0.5 °C. However, there was an increase of 1 °C for both nymphs and adults to 49 ± 0.5 °C. The 2-parameter log-logistic model predicted the lethal temperature at which 50% of the larvae dies (ULT_{50}) at 44.23 ±0.27 °C (Mean ± SE) while those of nymphs and adults were 46.81 ±0.09 °C and 46.8 ±0.04 °C respectively (Fig. 3.3). Thermal tolerance results show that the mortality of *R. appendiculatus* was directly proportional to temperature with an increase in temperature severity resulting in a corresponding increase in tick mortalities.

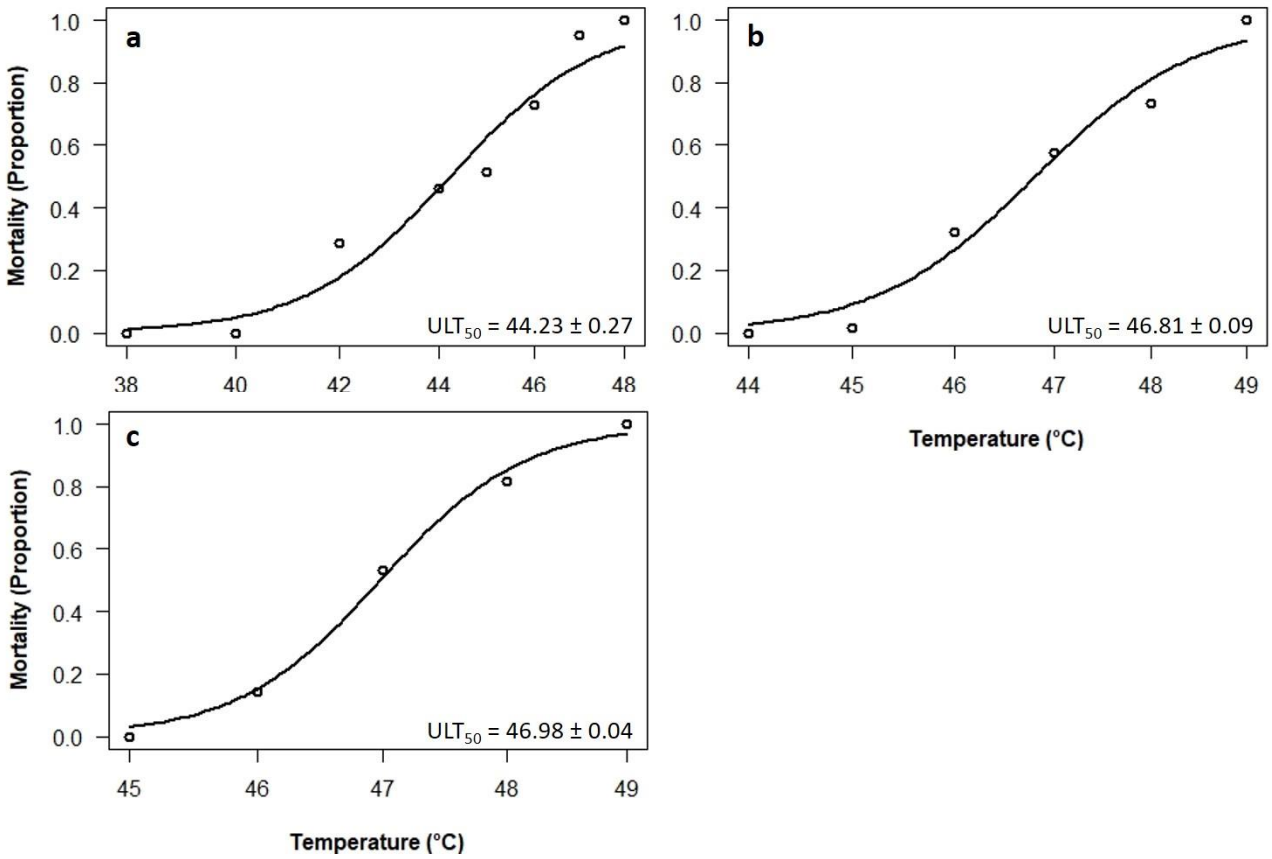

Figure 3.3: The response of *Rhipicephalus appendiculatus* larvae (a), nymphs (b) and adults (c) to increasing temperature on a 2-parameter log-logistic model (with lower limit at 0 and upper limit at 1) with predicted ULT_{50} ± Standard errors.

Distribution of R. appendiculatus

The distribution of *R. appendiculatus* based on 2013 presence data is mainly confined to the high elevation regions (>600m above sea level) (Fig. 3.4). These constitute the interior and some exterior regions of Zimbabwe. Although the tick was found across all ecological regions,

it was rarely found in low lying areas (<400m above sea level) and distribution also decreased towards the extreme southern and western parts of the country characterized by hot-dry weather conditions. Highest densities of the tick were found in Mashonaland Central to the northern side of the country (Fig. 3.4).

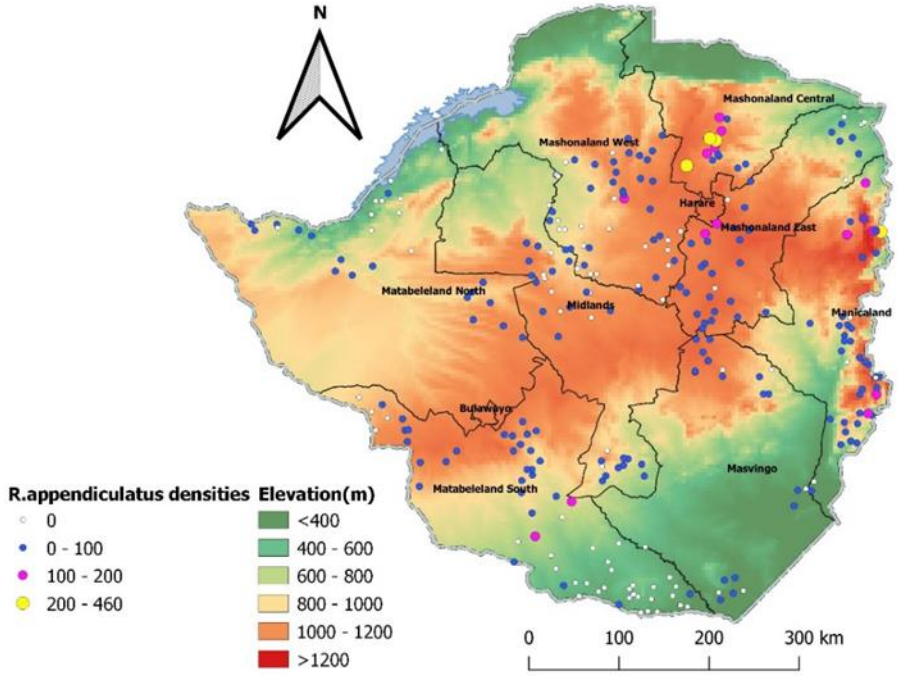

Figure 3.4: Distribution and presence locations of *Rhipicephalus appendiculatus* collected in Zimbabwe in 2013.

Current and future distribution of suitable habitats for <u>R. appendiculatus</u>

The habitat suitability map for *R. appendiculatus* based on 2013 National tick survey data and as determined by current climatic conditions, indicate that the suitable range of the tick is very wide despite spatial differences in suitability indexes. Areas with high suitability indices are confined to the eastern, north-eastern, and south-eastern parts of the country mainly in Masvingo, Manicaland and Mashonaland central provinces (Fig. 3.5a). Most least suitable habitats (habitat suitability index 0.25) in the southern parts of the country were characterized by zero tick presence during the survey while clusters were found in areas with suitability index of 0.5 and above. Compared with the current predicted suitable habitats, future predictions as determined by 2041 – 2060 climatic conditions, indicate a countrywide expansion of suitable

habitats even into previously known non-endemic areas such as the lowveld areas (Fig. 3.5b). Future predictions indicate a marginal habitat loss of 1 868 km^2 and a gain of 73 069 km^2 into natural regions IV and V which are key livestock production areas. The habitat suitability index of some areas like Matabeleland South will remain constant but others like Matabeleland North will have a significant increase in suitability index from 0.25 to 0.75. Generally, areas with suitability indices from 0.75 to 1 have a widespread increase in future predictions. The AUC for training data was 0.76 whilst for test data was 0.68 (Fig. 3.6) indicating that the model has good predictive power.

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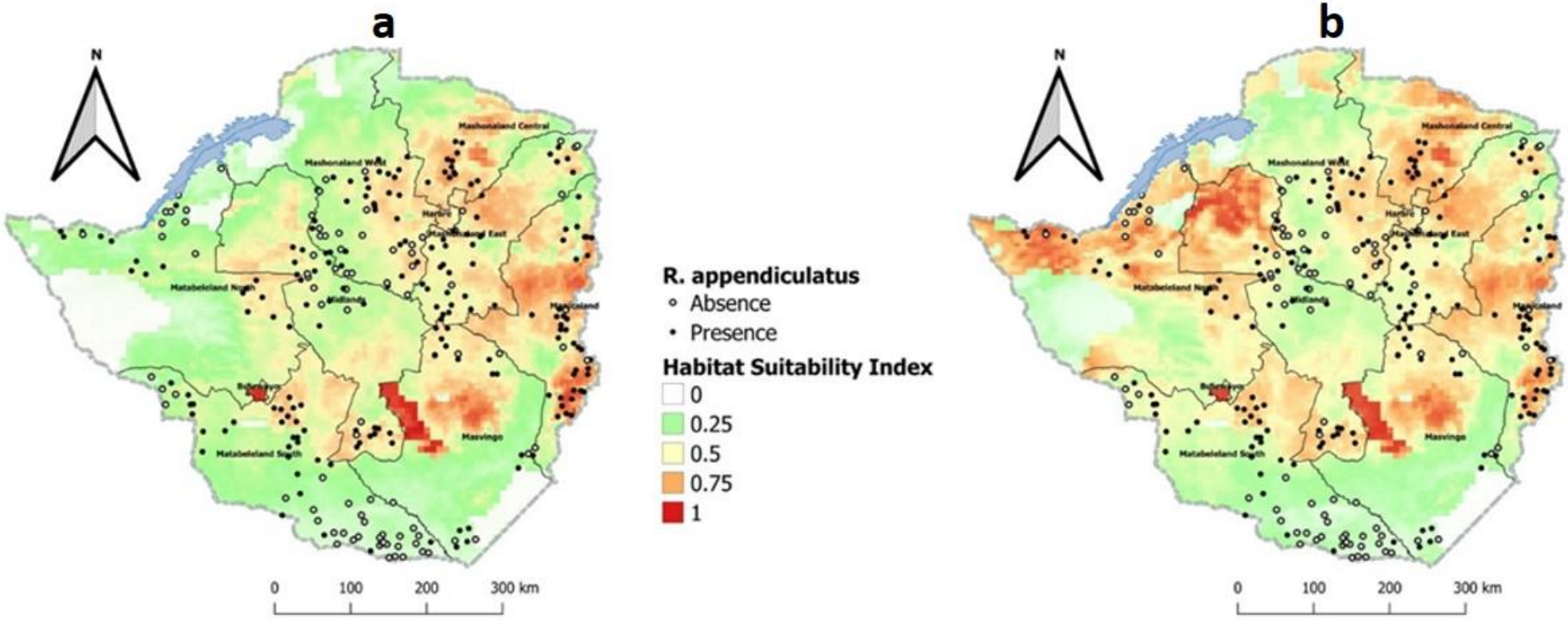

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902 **Figure 3.5**: Suitable habitats for *Rhipicephalus appendiculatus* for Zimbabwe under current environmental conditions (a) and the Shared
903 Socioeconomic Pathway 2 (SSP2-4.5) 2041 – 2060 climate scenario (b).

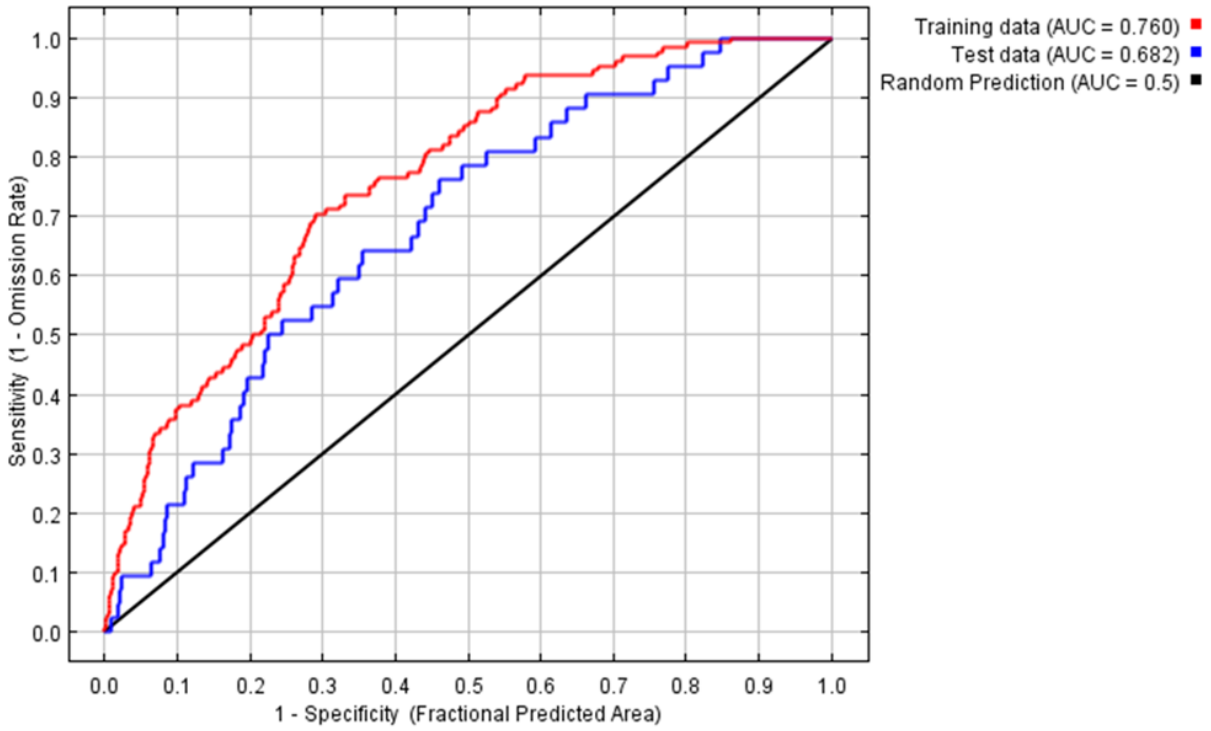


Figure 3.6: The Receiver Operated Characteristics (ROC) Area Under the Curve (AUC) for *Rhipicephalus appendiculatus* habitat suitability model.

The Jack-knife test (Fig. 3.7a) showed that maximum temperature of the warmest month (bio5) had the highest gain (>0.22) when used independently followed by mean temperature diurnal range (bio2). Minimum temperature of the coldest month (bio6) had the least gain when used in isolation, meaning it contained little information on the species habitat distribution. Cattle densities had the overall highest contribution (44.5%) while for climatic variables, annual rainfall had the highest contribution (15.3%) (Fig. 3.7b). Minimum temperature of the coldest month (0.4%) had the lowest contribution towards the model.

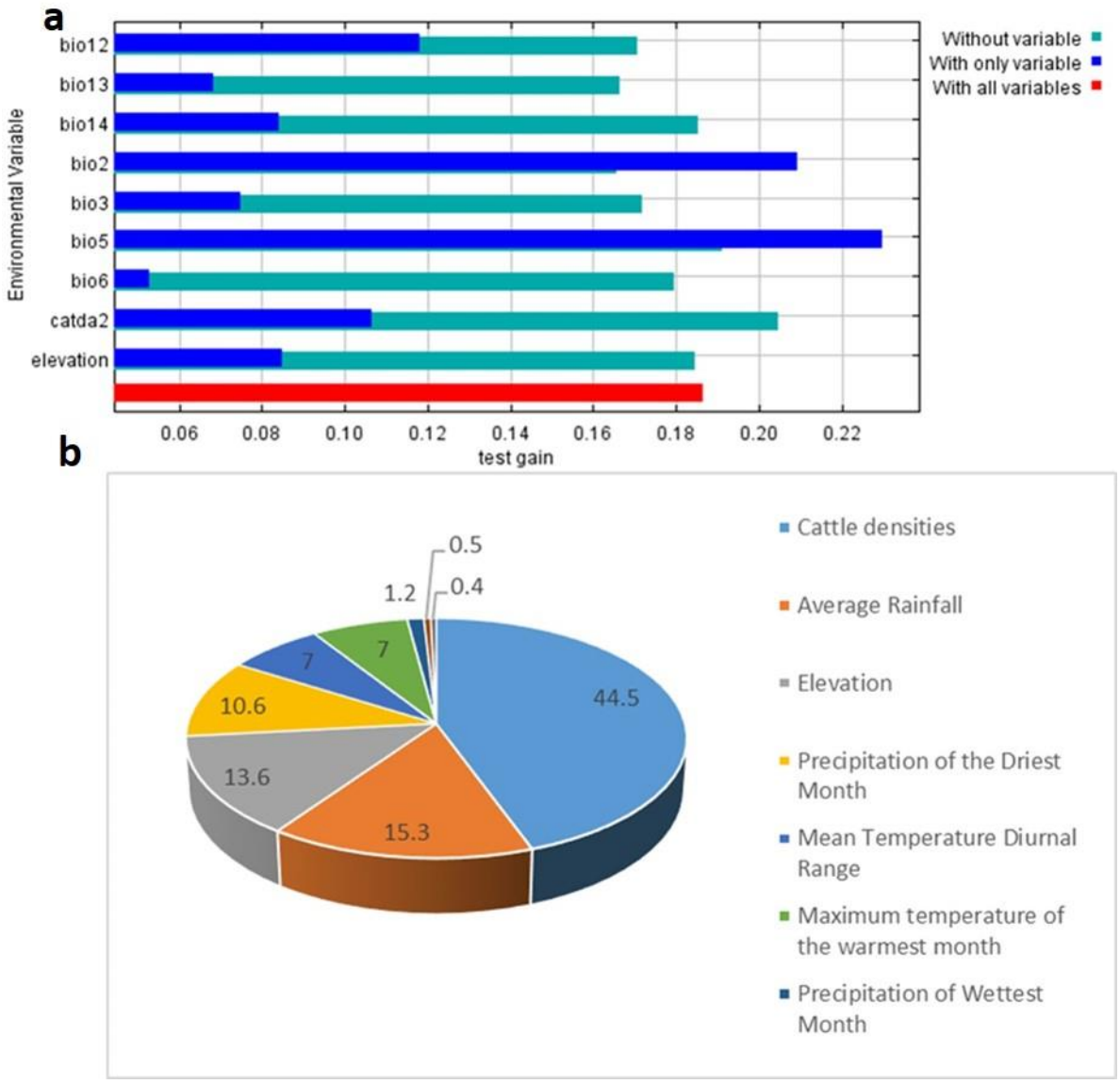


Figure 3.7: Jack knife test results (top) and percentage contributions (bottom) of predictor variables in the Maxent model for *Rhipicephalus appendiculatus* collected in Zimbabwe.

Discussion

This study predicted a wide geographic range for *R. appendiculatus* in Zimbabwe, which is expected to expand despite a predicted temperature rise of about 2 °C in the future 2040 to 2061 climate scenario. The model indicates the importance of climatic conditions in shaping the distribution of ectotherms. Several authors have noted the importance of climate on tick survival, development, behavior, activity and pathogen incubation and transmission (Hunter, 2003; Dantas-Torres and Otranto, 2011; Bellard *et al*., 2012). Few countries have records on the influence of climate on tick and tick-borne disease incidences due to absence of long-term replicated data on their abundance, distribution, and prevalence (Nuttal, 2021). This study

showed the utility of species distribution models in understanding the potential distribution of organisms. The model predicted low habitat suitability for *R. appendiculatus* (suitability index of 0.25) in areas in the southern parts of the country covering the lowveld. This is supported by the absence of the tick in some sampled sites during the 2013 National tick survey which is also in agreement with Shekede *et al*. (2021), who reported cold spots of the tick in the same region. Furthermore, the model had an AUC of 0.76 which is higher than 0.5 (random prediction threshold) indicating its usefulness in decision making. This is in line with recommended model performance matrices where an AUC value >0.75 indicates good performance by the model (Ferrier, 2000; Elith *et al*., 2006).

This study also showed an expansion of 73 069 km^2 in the range of *R. appendiculatus*. This is in agreement with Leighton *et al*. (2012) whose analysis, using 20 years' surveillance data, highlighted the rapid spread of *Ixodes scapularis* ticks during warmest years regardless of other potential contributing factors. Likewise, predictions by the model in this study indicated that global warming would result in the expansion of the suitable habitats of *R. appendiculatus* mainly into previously known non-endemic regions IV and V. Changing climate thereby necessitate changes in the distribution of both the tick and the associated tick-borne diseases.

The dataset used in modelling areas, suitable for *R. appendiculatus*, was collected almost a decade ago and this data produced a model that showed a wider distribution of the tick species than observed then. Judging from the recent occurrence of cases of theileriosis, a disease transmitted by *R. appendiculatus*, the model is valid in predicting an expansion in the range of the species even under future climate scenarios. In support of this observation, Danielova *et al*. (2010) suggested that rising temperatures may expand both the altitudinal and latitudinal ranges of tick species. This was due to an observation they made on the occurrence of *Ixodes ricinus*, from 750m to >1000m above sea level when temperatures rose by 1.4 ºC over two decades. Thomas *et al*. (2004) indicated that global warming would impact ecosystem operation as well as survival of some species as temperatures are expected to rise by approximately 1.5-3 ºC by 2050 (Gemeda and Sima, 2015). In this regard, Olwoch *et al*. (2009) suggested a temperature fluctuation mediated increase of tick prevalence in endemic regions which would ultimately affect tick infestation and TBD incidence. Moreover, the displacement of *R. decoloratus* by *R. microplus* in South Africa was linked with increases in environmental temperatures. Increased temperatures favor the proliferation of the more heat tolerant *R. microplus* (Nyangiwe *et al*., 2013). Dergousoff *et al*. (2013) also reported that range expansion of ticks in Canada beyond their geographic limits coincided with climate warming.

Although *R. appendiculatus* is well known to favor areas with cooler temperatures (10-30 °C) (Hove *et al*., 2008), this study shows that the tick is tolerant to much higher temperatures. Larvae, nymphs, and adult ticks were subjected to temperatures between 38-50 °C and had ULTs ≥ 48 °C. Tolerance of such high temperatures by even larvae, a vulnerable stage of the tick's life cycle, has implications on the survival of the tick both on and off the host during the late summer to winter months (April to October), the seasonal period when the larval stage occurs. Such tolerance is an attribute of the tick's vectorial competence and is in line with Gilbert *et al*. (2014) and Rosendale *et al*. (2016) who highlighted that the success of disease vectors such as ticks is often determined by the ability to survive in unfavorable conditions such as temperature fluctuations that directly affect their physiology and behavior. Although thermal tolerance, physiological mechanisms, and thresholds in relation to survival may vary from one developmental stage to another (Holmes *et al*., 2018; Mutamiswa *et al*., 2019), this study showed that all the developmental stages of *R. appendiculatus* are tolerant to high temperatures increasing the probability of survival of the tick as a disease vector in Zimbabwe. Different mechanisms are also employed as a means for survival. Adults tend to enter into diapause in winter to combat low temperatures whilst questing larvae, nymphs or adults withdraw into refugia as a survival mechanism against high temperatures (Hove *et al*., 2008).

The microclimatic data recorded in Manicaland, Mashonaland west and Mashonaland central tick habitats in the current study, indicate that the mean and maximum temperatures in all habitats were lower than the tick's observed ULTs. Although thermal tolerance of poikilotherms is dynamic and influenced by a number of factors (Mutamiswa *et al*., 2017b), the results showed that the tick has high chances of surviving the predicted future temperature increases due to global warming since the observed ULTs of the tick were higher than the microclimatic data recorded in tick habitats.

In conclusion, this study has shown that there will be a future expansion in habitats suitable for *R. appendiculatus* in Zimbabwe from the endemic eastern, north-eastern, and south -eastern parts of the country to the extreme southern parts which were previously non-endemic as predicted by the model. In addition, there is also high probability of dispersal and proliferation of the tick into these habitats thus increasing the probability of occurrence of the associated tick-borne diseases. A review of the national tick control strategies is therefore needed to consider the possible impacts of predicted changes in tick and TBD distributions.

CHAPTER 4

Acaricide resistance profiles of *Rhipicephalus appendiculatus* and *Rhipicephalus decoloratus* in relation to management practices of Zimbabwe

Abstract

Resistance of ticks to acaricides is a growing threat to livestock production globally. Continuous exposure of ticks to the same chemicals as well as application of sub-lethal doses are amongst the major drivers of resistance to the detriment of livestock production. In this study, the larval packet test was used to evaluate the resistance of one and three-host ticks, (*R. decoloratus and R. appendiculatus*) to commonly applied acaricides in Zimbabwe following increased incidences of theileriosis. In addition, the study also assessed if the current tick management practices might lead to the development of resistance. Results confirmed moderate resistance of *R. decoloratus* populations at 50% of the sampled dip tanks (4<RR<10) while two dip tanks also had moderate resistance to deltamethrin. But, tested *R. appendiculatus* populations were susceptible to both amitraz and deltamethrin despite an indication that management practices by farmers may subsequently lead to resistance development in the future. These results suggest that increases in theileriosis incidences may be linked to other factors rather than acaricide resistance of the vector *R. appendiculatus.* These findings therefore support the need for a holistic tick- and tick-borne disease management approach incorporating the correct and consistent application of available acaricides, acaricide rotation, acaricide resistance monitoring as well as vaccination against tick-borne diseases (TBDs).

Key Words: Acaricide resistance, Tick management practices, Amitraz, Deltamethrin

Introduction

The resistance of disease pathogens and their vectors to drugs and pesticides is a growing global concern (Pocquet *et al*., 2014; Vanaerschot *et al*., 2014). The World Health Organisation (WHO) previously singled out antimicrobial resistance as a major threat to the effective control of human diseases (WHO, 2014). In agricultural systems the evolution of pesticide resistance increases the vulnerability of the sector to pest and disease outbreaks (Nauen, 2007) including livestock systems. In the management of vector-borne diseases, vector control remains the most widely accepted method worldwide (FAO, 1984; Wilson *et al*., 2020). While advances have been made in the adoption of an integrated vector management approach, chemical control remains the most frequently used technique (Hawkins *et al*., 2019; van den Berg *et al*., 2021). However, continuous exposure of organisms to the same chemicals as well as application of sub-lethal doses are some of the major drivers of resistance development (Hurwitz *et al.,* 1981; Raynal *et al*., 2013; Abbas *et al*., 2014). This is also the case for the major human disease vector, mosquitoes, hampering efforts to combat malaria (WHO, 1992; Krostad, 1996). Thus, resistance is a major impediment in effective vector control because of the use of pesticides (Barre *et al*., 2008).

The response of organisms to pesticides may be influenced by ambient environmental conditions, and possibly the interplay between physiology, environment, and pesticide tolerance (Rodriguez-Vivas *et al*., 2018). For example, climate change increased voltinism in the red-legged earth mite, *Halotydeus destructor*, thus accelerating evolutionary responses to selection pressures (Maino *et al*., 2017). This has also been the case with several disease vectors where the interaction of genetic, biological, operational, and intrinsic factors has often resulted in the development of resistance to the detriment of disease management programmes (Hurwitz *et al.,* 1981; Rosario-Cruz *et al*., 2009b; Raynal *et al*., 2013; Abbas *et al*., 2014).

The ecology of disease vectors plays a role in their response to pesticides. For example, tick resistance to acaricides develops more easily in one-host compared to two- and three-host ticks due to the former's shorter generation time, causing a more frequent exposure to acaricides (Rodriguez-Vivas, 2008). This has been the case in Zimbabwe where acaricide resistance has been frequently detected in *Rhipicephalus decoloratus*, a one-host tick (Mazhowu, 1995; Gono *et al.,* 2014; Makuvadze *et al.,* 2020). *R. decoloratus* Kock (Acari: Ixodidae) (blue tick) is thus treated as the 'indicator species' in monitoring acaricide resistance development as cases of resistance of this tick species have been reported for almost all acaricides (Yawa *et al*., 2022).

However, in recent years, cases of resistance in the three-host tick, *Rhipicephalus Appendiculatus* Neumann (Acari: Ixodidae) (brown ear tick), have been reported locally and in other countries (Vudriko *et al*., 2016; Chitombo *et al*., 2021).

Rhipicephalus appendiculatus, is a widely distributed species endemic to sub-Saharan Africa (Perry *et al*., 1991). While cattle serve as the main host, the tick also parasitizes goats, sheep, and wild ungulates such as buffaloes, elands, sable antelopes, waterbucks, and greater kudus (Walker *et al.,* 2003). The tick is a vector of a species complex of *Theileria parva*, an obligate intracellular protozoan parasite of bovids. This parasite causes acute and frequently fatal diseases such as east coast fever, corridor disease and theileriosis (Ganaie *et al.,* 2019). In addition, infestation of hosts by *R. appendiculatus* reduces their economic value through direct production losses such as weight loss and retarded growth (Peter *et al*., 2005; Ghosh *et al*., 2007; Sajid *et al*., 2007). Heavy infestations may also lead to clinical and sub-clinical anaemia and otoacariasis (FAO, 1998; Kemp *et al.*, 1998).

Theileriosis currently accounts for approximately 60% of cattle mortalities in Zimbabwe, with an increasing trend that has been observed over the past five years (DVS, 2019). Several factors could be driving the prevalence of this disease, among them the development of resistance to currently applied acaricides and possible changes in the transmission dynamics of the disease due to climate change. Farmers have previously reported a decrease in the efficacy of chemicals used in the control of ticks. Sungirai *et al.* (2016) highlighted the emergence of resistance as a possible reason behind increasing tick burden on cattle despite consistent dipping. However, these reports were not specific to *R. appendiculatus*, the theileriosis vector. This study sought to determine the resistance status of *R. appendiculatus* as a possible explanation for the increased prevalence of theileriosis in Zimbabwe. This assessment was also conducted on *R. decoloratus*, a species previously reported to be resistant to multiple acaricides in Zimbabwe for comparative purposes. In addition, the study investigated the current tick management practices and assessed if they might lead to the development of resistance. Specific questions asked were: (1) What are the current tick management strategies and perceptions of farmers regarding acaricide resistance? (2) What is the resistance status of *R. appendiculatus* and *R. decoloratus* to amitraz- and deltamethin-based acaricides?

Materials and Methods

Study area

The study was conducted in six districts namely, Bindura, Chikomba, Makoni, Marondera, Wedza and Zvimba situated in Mashonaland West, Mashonaland East, Mashonaland Central and Manicaland Provinces (Fig. 4.1). The six districts are located within the highveld of Zimbabwe (Altitude 1200 -1700m) and agro-ecological zone II. This zone is characterized by moderately high annual rainfall (750- 1000mm) with temperatures ranging between 19 – 23 °C (Chikodzi *et al*., 2013). Agricultural activities in these areas include intensive farming of crops like tobacco and maize as well as livestock production. Animal health services are provided through public sector management centres while cattle dipping is done communally at plunge dips. The most commonly applied acaricide is amitraz which is applied fortnightly during the dry season and weekly in summer.

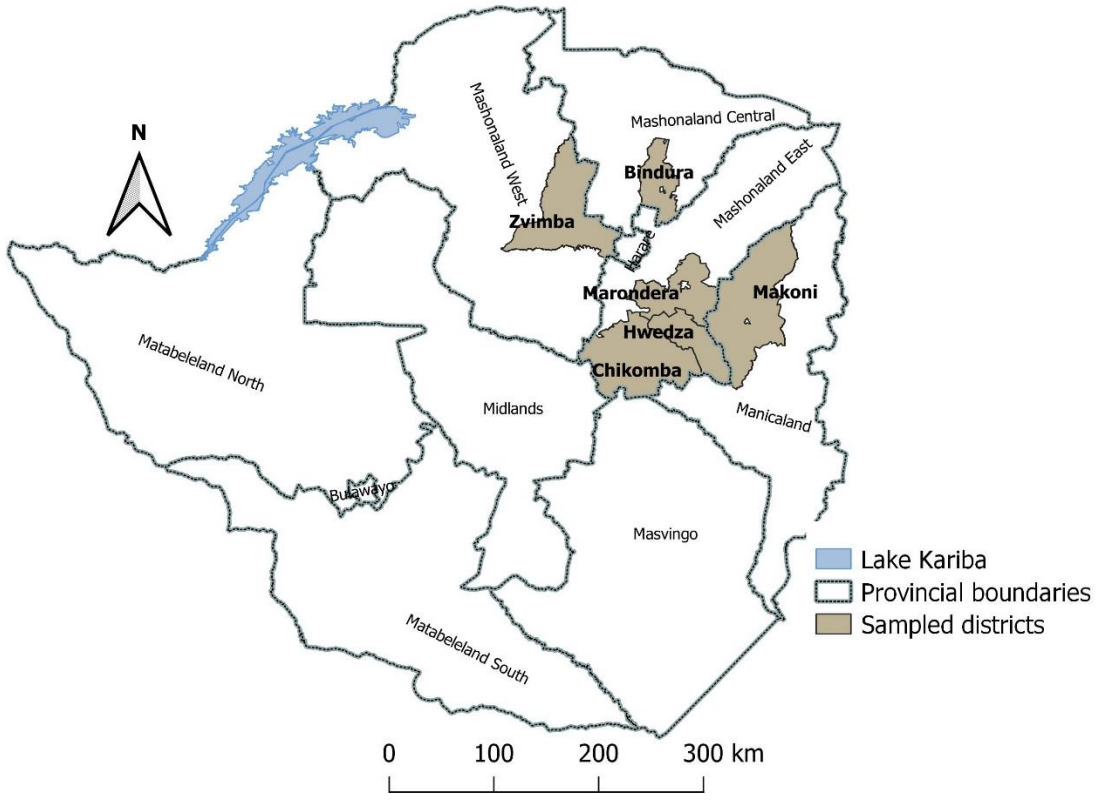


Figure 4.1: Districts sampled for *Rhipicephalus appendiculatus* and *Rhipicephalus decoloratus* to determine acaricide resistance in Zimbabwe.

Field tick sample collection, identification and rearing of larvae

The current studies were granted ethical clearances by the National Animal Research Ethics Committee in Zimbabwe (001/2021), the Animal Research Ethics Committee of the University of the Free State (UFS-AED2021/0032/21), the Environment & Biosafety Research Ethics Committee (UFS-ESD2021/0181/21) and the General/Human Research Ethics Committee (GHREC) (UFS-HSD2021/0418) (Appendices 3, 5, 6 and 4 respectively). Tick collections were conducted 3-8 days post-acaricide treatment at 15 out of the targeted 20 communal dip tanks in the six districts. Sampling was done during the wet season months between April 2021 and March 2022 when tick prevalence was high. Sampled districts and dip tanks were purposively selected based on current and previous reports of high prevalence of tick and tick-borne diseases, particularly theileriosis. *Rhipicephalus decoloratus* ticks were collected from 11 dip tanks while collections for *R. appendiculatus* were extended to an additional four dip-tanks as the initial survey failed to yield adequate samples for bioassays. The additional districts were sampled following reports of theileriosis outbreaks. From each dipping center, at least 25 engorged female ticks were collected from heavily infested cattle according to protocols developed by FAO (2004). The ticks were placed in labeled plastic jars with perforated lids for ventilation and transported to the Central Veterinary Laboratory in Harare, Zimbabwe. At the laboratory, ticks were identified into morpho-species using keys by Walker *et al.* (2003) after which *R. decoloratus* and *R. appendiculatus* were set aside for bioassays. These were placed separately in perforated empty vials and cultured in a Memmert humidity chamber (HCP 246 Memmert, USA,) at a temperature of 26 − 28 °C and relative humidity of 90 − 95% (Lovis *et al*., 2013) until oviposition. Eggs were collected four days post-oviposition, weighed (0.5g per tube) and also incubated under the same conditions till hatching. After hatching, scutums of larvae were allowed to harden for 14 days prior to bioassays (Lovis *et al*., 2013). The number of ticks collected for each dip-tank determined the number of tests that could be conducted.

Reference tick strains

Reference specimens of *R. decoloratus* and *R. appendiculatus* were collected in 2018 from Charara and Chivero National Parks respectively. These were reared for four generations at the Central Veterinary Laboratory in Harare to maximize susceptibility to acaricides. Both national parks have no history of chemical tick management hence the reference specimens are considered highly susceptible.

Test Chemicals

Two technical chemical compounds (>99.5% potency) formamidine (amitraz) and synthetic pyrethroid (deltamethrin) commonly used in Zimbabwe were obtained from a private company, Coopers Zimbabwe (https://www.shumbacoopers.co.zw/). For each chemical compound, the acaricide was mixed with two parts trichloroethylene and one part of olive oil to give a stock solution with a concentration of 1% (Miller *et al*., 2002). The following formula was used to determine the amount of pure acaricide to be used for the preparation of the stock solution:

$$M = \left(\frac{X}{\%\ of\ acaricide\ potency}\right) \times \left(\frac{20}{3}\right)$$

where M = Amount of acaricide, X= % of highest concentration, 20 = final volume and 3 = a constant.

The 1% stock solution was further diluted into seven concentrations ranging from 0.0078125% to 0.5%, with each concentration being half of the former. Whatman number 541 filter papers (8.5cm x 7.5cm) were impregnated in duplicate with 0.67ml of each concentration of the acaricide using a Gilson micropipette and dried in a fume hood for two hours. For the controls, the filter papers were immersed in a mixture of trichloroethylene and olive oil. Dried filter papers were wrapped in aluminum foil and stored at 4 °C prior to use.

Acaricide resistance testing

The Larval Packet Test (LPT) (FAO, 1984) was used to assess the resistance status of the two tick species, *R. decoloratus* and *R. appendiculatus.* Acaricide impregnated filter papers were folded into envelopes leaving one end open and approximately 100 larvae were put in each folded packet using a camel hairbrush. The envelopes were then closed using paper clips and incubated in a Memmert humidity chamber at a temperature of 26 − 28 °C and relative humidity of 90 − 95% for 24 hours. After 24 hours, the envelopes were opened, and the number of dead larvae was counted under a magnifying glass (Fig. 4.2). Larvae were considered dead if they failed to coordinate normal locomotory response to stimuli such as gentle prodding.

Figure 4.2: Insertion of tick larvae into acaricide-impregnated filter paper (left), incubation (middle) and mortality counting (right) during acaricide resistance testing at Central Veterinary laboratory of Zimbabwe.

Farmer perceptions and tick management practices

To understand the perceptions of farmers towards current tick management practices and the response of ticks to chemicals, structured and unstructured interviews were conducted at 20 dip-tanks (Fig. 4.3; Appendix 1). Informed consent was obtained, and participation was voluntary while confidentiality was maintained (Appendix 2). Forty farmers were interviewed per district and respondents were randomly selected per dip-tank during dipping sessions. The interviews were focused on five key areas *viz*, 1) farmer demographics, 2) tick identification, 3) tick management practices, 4) acaricide efficacy and 5) disease incidence.

Figure 4.3: Farmer interviews at a selected dip tank in Makoni district of Zimbabwe.

Statistical analyses

All data analyses were carried out using the Microsoft Excel package and R software (version 3.2.1) (R Development Core Team., 2021). The LPT data were first corrected for natural mortality using Abbot's formula (Abbot, 1987). The corrected data was then subjected to dose response modeling in R using the '*drc'* package (Ritz *et al*., 2015). The '*drm'* function was used to model the tick mortality as a function of acaricide concentration using a two-parameter log- logistic model. The model had the lower and upper asymptotes fixed at 0 and 1 respectively while the slope, Effective Dose 50 (hereafter LC_{50}) and the 95% confidence intervals (95% CI) were computed from the model. Resistance ratios (RR_{50}) between reference and field strains were then computed using the '*EDcomp'* function. A population was defined as susceptible when $RR_{50}<4$, moderately resistant when $4< RR_{50}<10$ and super-resistant when $RR_{50}>10$ as determined by Jonsson and Hope (2007).

Since deductive coding was used in the design of the questionnaire, data was captured and organized into its respective codes. These codes were demographics, tick identification, tick management practices, acaricide efficacy and disease incidence. The frequency of responses to particular thematic areas was then compared while Chi-square goodness of fit tests were used to determine the differences in some responses across districts.

Results

Tick collections

From the 15 dip-tanks where ticks were collected, *R. decoloratus* were obtained from 11 dip tanks while only five dip tanks produced *R. appendiculatus* specimens (Table 4.1).

Acaricide resistance

Moderate resistance to amitraz ($4< RR_{50}<10$) was detected in *R. decoloratus* from five dip-tanks in three districts (Fig. 4.4, Table 4.1). The resistance ratio (RR_{50}) was markedly higher for Madzivire dip-tank (9.216) in Chikomba district followed by Brockely dip tank in Bindura district (6.47). Similarly, *R. decoloratus* from two dip-tanks in Bindura district had moderate resistance to deltamethrin (Fig. 4.5, Table 4.1). Generally, all dip-tanks had large resistance ratios for both amitraz and deltamethrin.

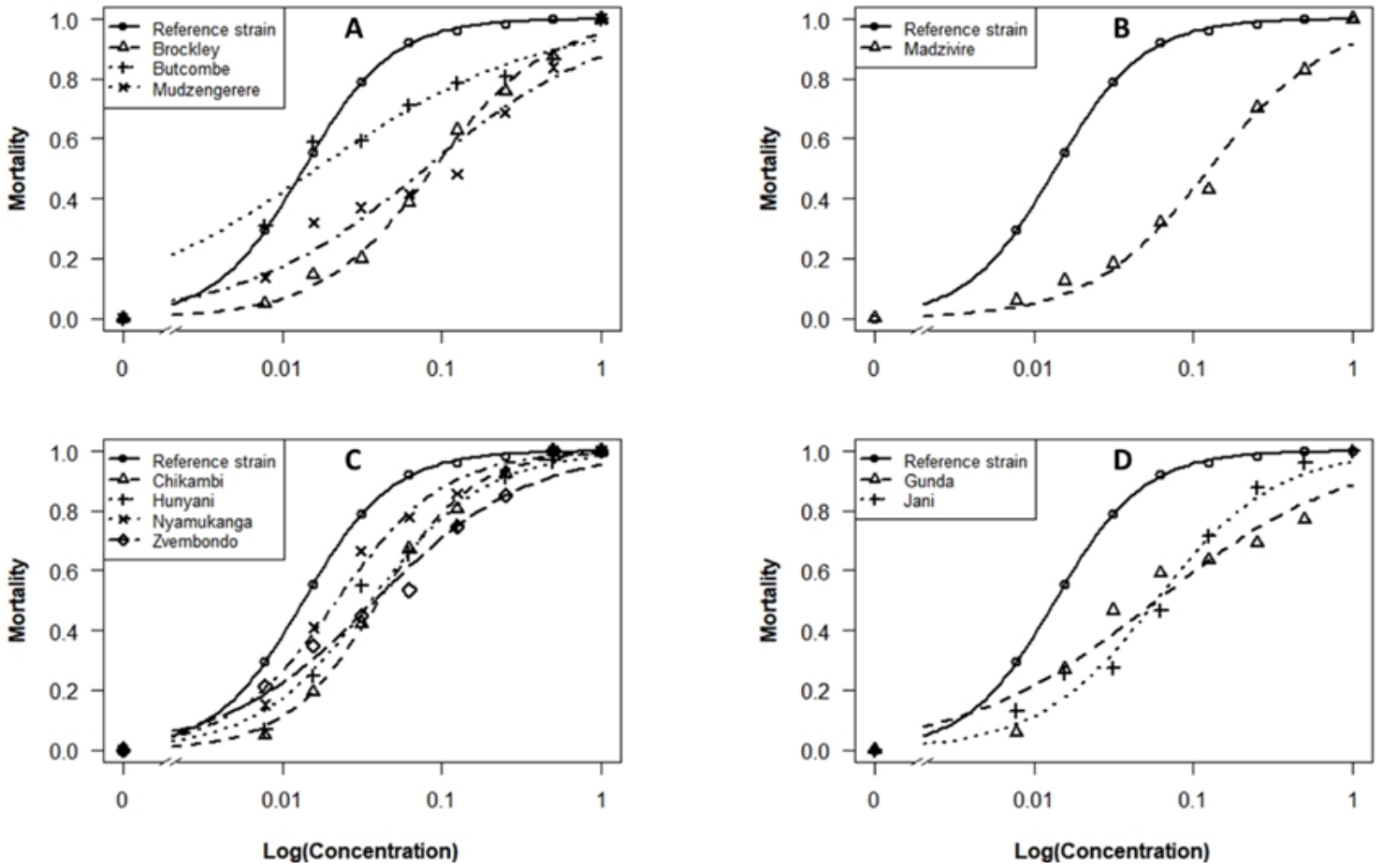


Figure 4.4: Dose-response curves used in the determination of LC_{50}s for *Rhipicephalus decoloratus* strains obtained from Bindura (A), Chikomba (B), Makoni (C) and Zvimba (D) districts of Zimbabwe against amitraz. Solid lines represent the curves for the reference strain.

Table 4.1: Resistance status of *Rhipicephalus decoloratus* and *Rhipicephalus appendiculatus*, sampled from the different dip tanks in the chosen districts of Zimbabwe, to the two acaricides amitraz and deltamethrin.

District	Dip tank	Species collected	Amitraz resistance			Deltamethrin resistance		
			LC_{50}(95%CI)	RR_{50}(95%CI)	Resistance	LC_{50}(95%CI)	RR_{50}(95%CI)	Resistance
			Rhipicephalus decoloratus					
	Reference strain		0.01(0.01-0.02)			0.01(0.01-0.01)		
Bindura	Brockely	x	0.09(0.08-0.10)	6.47(5.16-7.79)	Moderate	0.06(0.05-0.07)	5.71(4.50-6.92)	Moderate
	Butcombe	x	0.02(0.01-0.02)	1.21(0.65-1.77)	Susceptible	0.05(0.04-0.07)	4.97(3.58-6.36)	Moderate
	Mudzengerere	x	0.08(0.06-0.10)	5.72(3.71-7.73)	Moderate	0.04(0.03-0.04)	3.47(2.70-4.23)	Susceptible
Chikomba	Madzivire	x	0.13(0.10-0.15)	9.216.64-11.78)	Moderate	0.03(0.03-0.03)	2.78(2.18-3.38)	Susceptible
	Northstratsque	x	-	-	-	0.03(0.02-0.03)	2.54(1.68-3.40)	Susceptible
Hwedza	Chikurumadziva	-	-	-	-	-	-	-
Makoni	Jani	x	0.06(0.05-0.07)	4.29(2.98-5.60)	Moderate	-	-	-
	Gunda	x	0.06(0.04-0.08)	4.30(2.44-6.16)	Moderate	0.04(0.03-0.05)	3.78(2.93-4.63)	Susceptible
	Arnoldine	-	-	-	-	-	-	-
Marondera	Rockery	-	-	-	-	-	-	-
Zvimba	Chikambi	x	0.04(0.03-0.05)	2.96(2.32-3.60)	Susceptible	0.02(0.02-0.03)	2.24(1.90-2.58)	Susceptible
	Nyamukanga	x	0.02(0.02-0.03)	1.61(1.23-1.99)	Susceptible	0.03(0.02-0.03)	2.40(1.93-2.87)	Susceptible
	Zvembondo	x	0.04(0.03-0.05)	2.80(1.98-3.61)	Susceptible	0.03(0.03-0.03)	2.64(2.18-3.09)	Susceptible
	Hunyani	x	0.04(0.03-0.04)	2.64(1.92-3.34)	Susceptible	-	-	-
	Gwebi	-	-	-	-	-	-	-
			Rhipicephalus appendiculatus					
	Reference strain		0.03(0.03-0.04)			0.02(0.02-0.02)		
Makoni	Gunda	x	0.02(0.01-0.02)	0.50(0.50-0.6)	Susceptible	0.02(0.02-0.02)	0.96(0.78-1.14)	Susceptible
	Arnoldine	x	0.04(0.004-0.07)	1.18(-0.33-2.69)	Susceptible	0.03(0.01-0.05)	1.46(-0.2-3.11)	Susceptible
Hwedza	Chikurumadziva	x	0.08(0.07-0.09)	2.54(2.18-2.89)	Susceptible	0.04(0.03-0.04)	1.88(1.64-2.12)	Susceptible
Marondera	Rockery	x	0.01(0.012-0.014)	0.41(0.36-0.47)	Susceptible	-	-	-
Zvimba	Gwebi	x	0.03(0.03-0.033)	0.97(0.88-1.07)	Susceptible	-	-	-

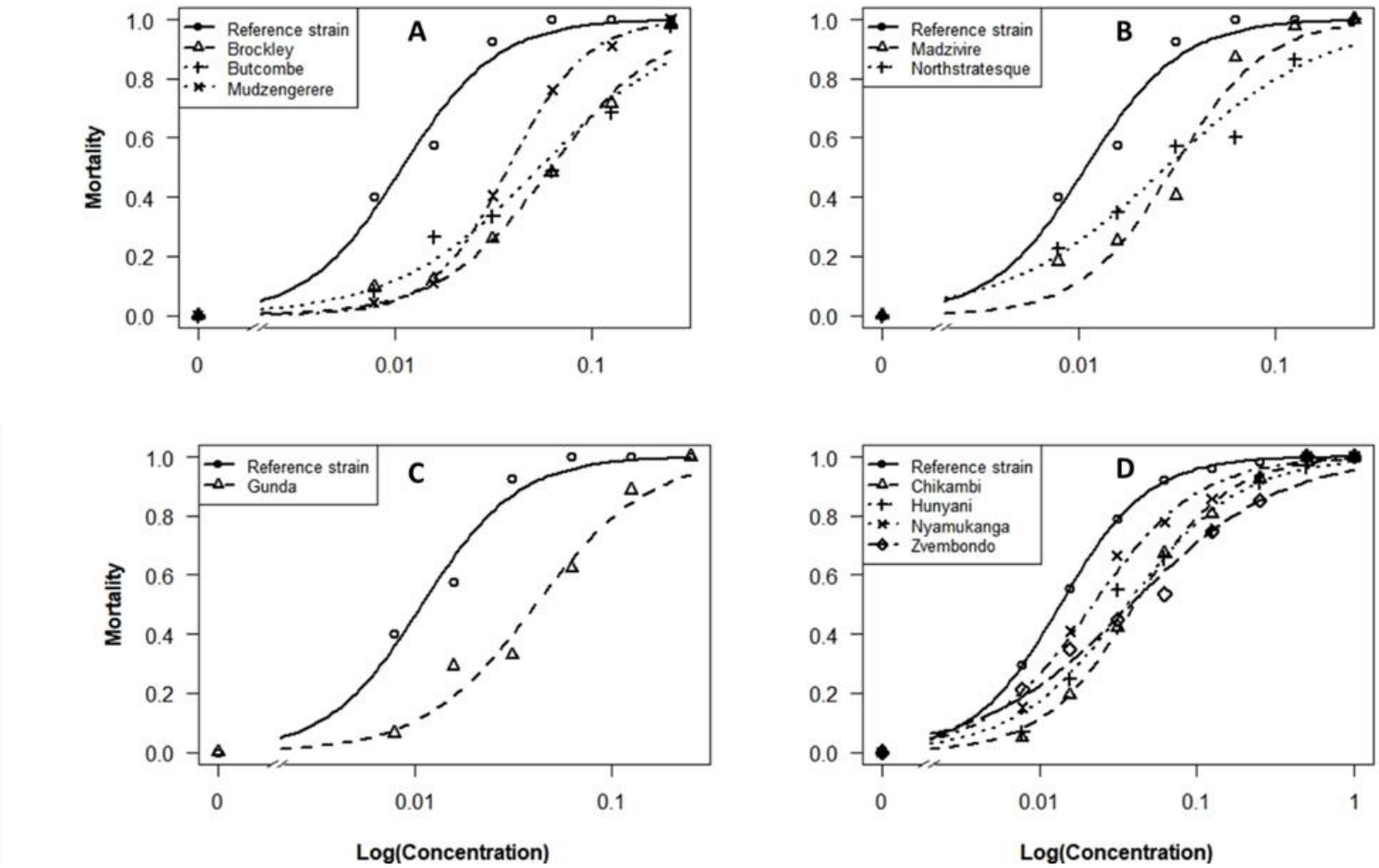


Figure 4.5: Dose-response curves used in the determination of LC_{50}s for *Rhipicephalus decoloratus* strains obtained from Bindura (A), Chikomba (B), Makoni (C) and Zvimba (D) districts of Zimbabwe against deltamethrin. Solid lines represent the curves for the reference strain.

All the dipping centers with the exception of Gunda in Makoni district did not yield *R. appendiculatus* during the initial sampling period. The ticks obtained from Gunda were highly susceptible to both amitraz and deltamethrin, RR_{50} = 0.5 and 0.96 respectively. *Rhipicephalus appendiculatus* from the additional four dip-tanks were also susceptible to amitraz (Fig. 4.6, Table 4.1). However, the resistance ratio (RR_{50}) against amitraz was markedly higher for Arnoldine dip-tank (1.18) in Makoni district and Chikurumadziva in Wedza district (2.54). Rockery and Gwebi dip tanks in Marondera and Zvimba districts did not yield enough larvae to test for deltamethrin resistance while the other two dip tanks were all susceptible to the acaricide (Fig. 4.7, Table 4.1).

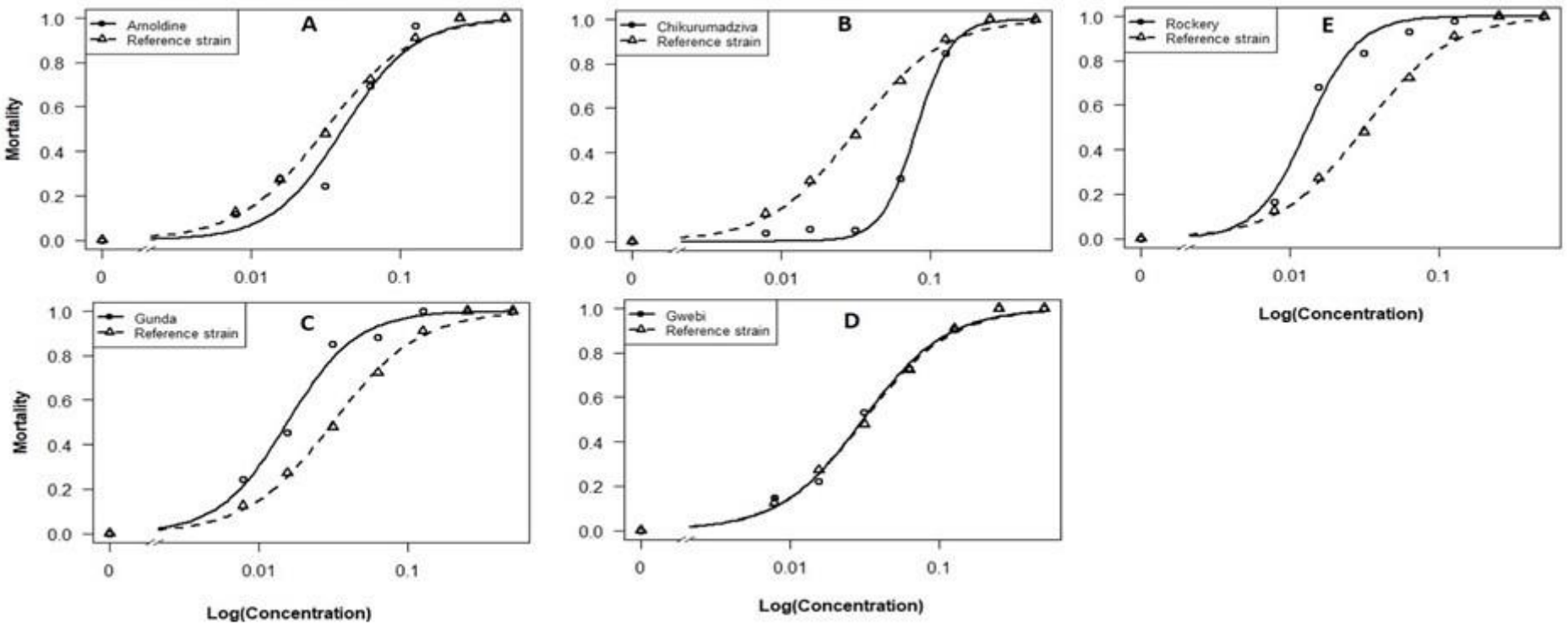


Figure 4.6: Dose-response curves used in the determination of LC_{50}s for *Rhipicephalus appendiculatus* strains obtained from Makoni (A and C), Hwedza (B), Zvimba (D) and Marondera (E) districts of Zimbabwe against amitraz. Broken lines represent the curves for the reference strain**.**

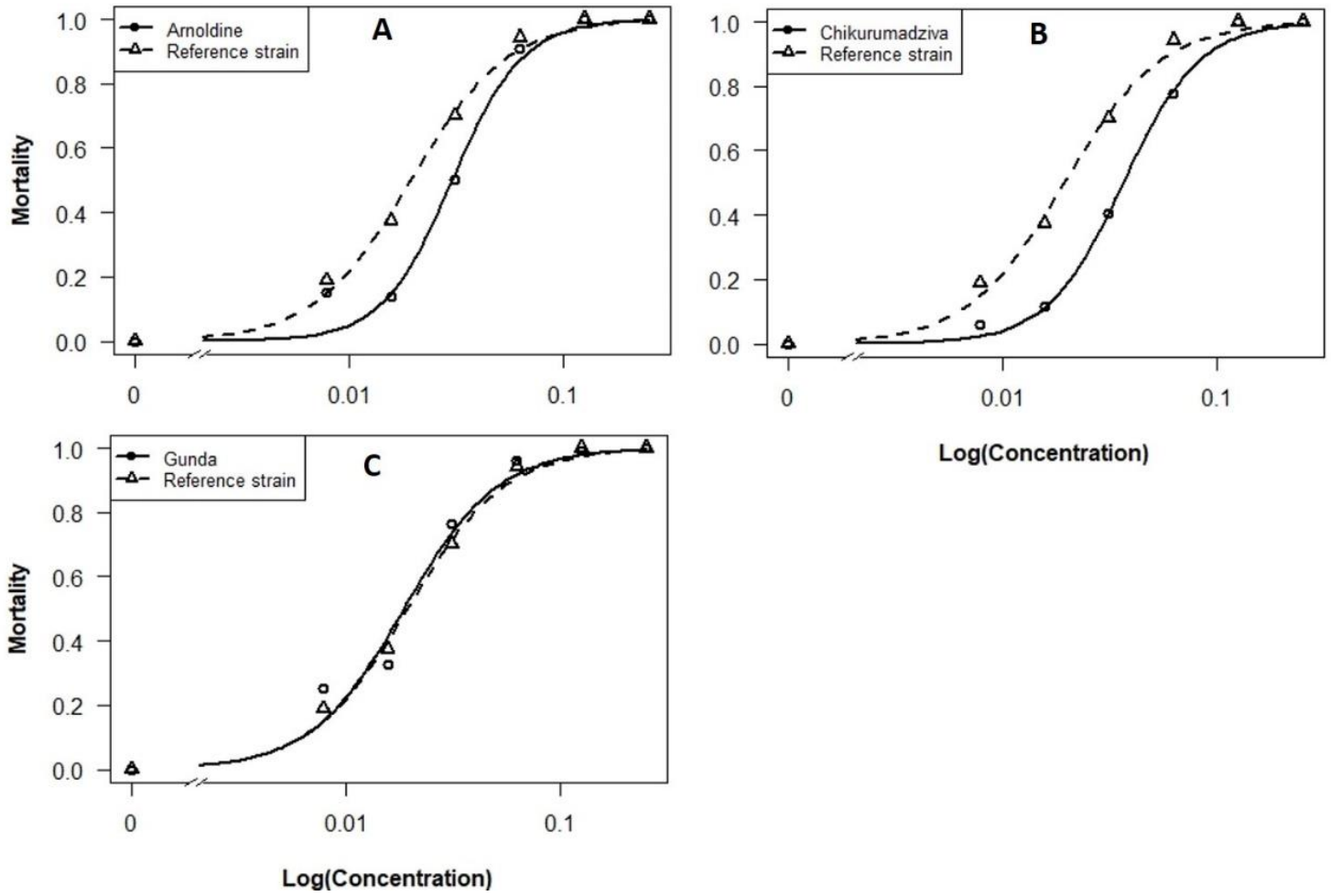


Figure 4.7: Dose-response curves used in the determination of LC_{50}s for *Rhipicephalus appendiculatus* strains obtained from Makoni (A and C) and Hwedza (B) districts of Zimbabwe against deltamethrin. Broken lines represent the curves for the reference strain.

Farmer perceptions

Location and demographics

All farmers interviewed use government infrastructure for animal health services. The majority of farmers, across all districts, own between five and ten cattle and live within a 5 km radius of a dip-tank (Fig. 4.8). However, Chikomba district in Mashonaland East province, had the highest number (20%) of farmers who travel more than 5km to access dipping facilities.

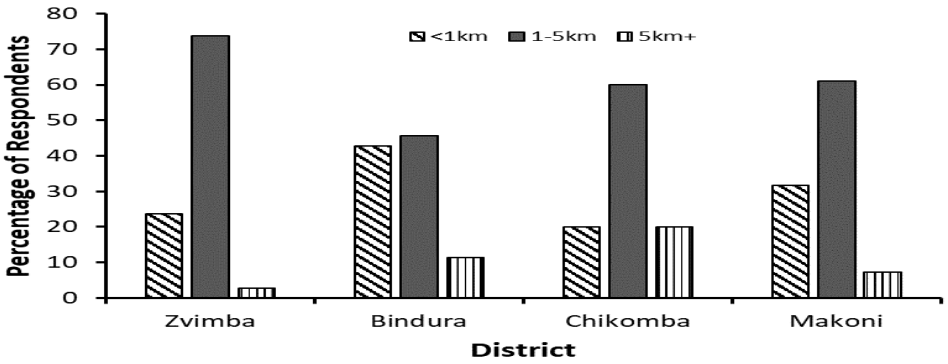


Figure 4.8: Distance travelled by farmers to access communal dipping facilities in Zvimba, Chikomba, Bindura and Makoni districts of Zimbabwe

Tick identification

All tick specimens presented to respondents were reportedly present across all districts. The most common tick species was *R. decoloratus* followed by *R. appendiculatus* (Fig. 4.9). Tick prevalence was reportedly high during the rainy season with cattle as the main hosts followed by goats. Although all specimens of tick species presented to farmers were reportedly common in all sampled districts, a few respondents (7.5 - 25%) identified some as new in their areas (Fig. 4.10). *Rhipicephalus decoloratus* was the only tick species reported to be endemic to all districts while some respondents from Makoni district also identified *R. appendiculatus* as endemic. There were mixed responses as to whether new ticks were being observed in the districts although a few respondents were of the view that they have observed new ticks. *Rhipicephalus evertsi* was indicated as a new tick species by 5% of farmers in three of the districts (Fig. 4.10).

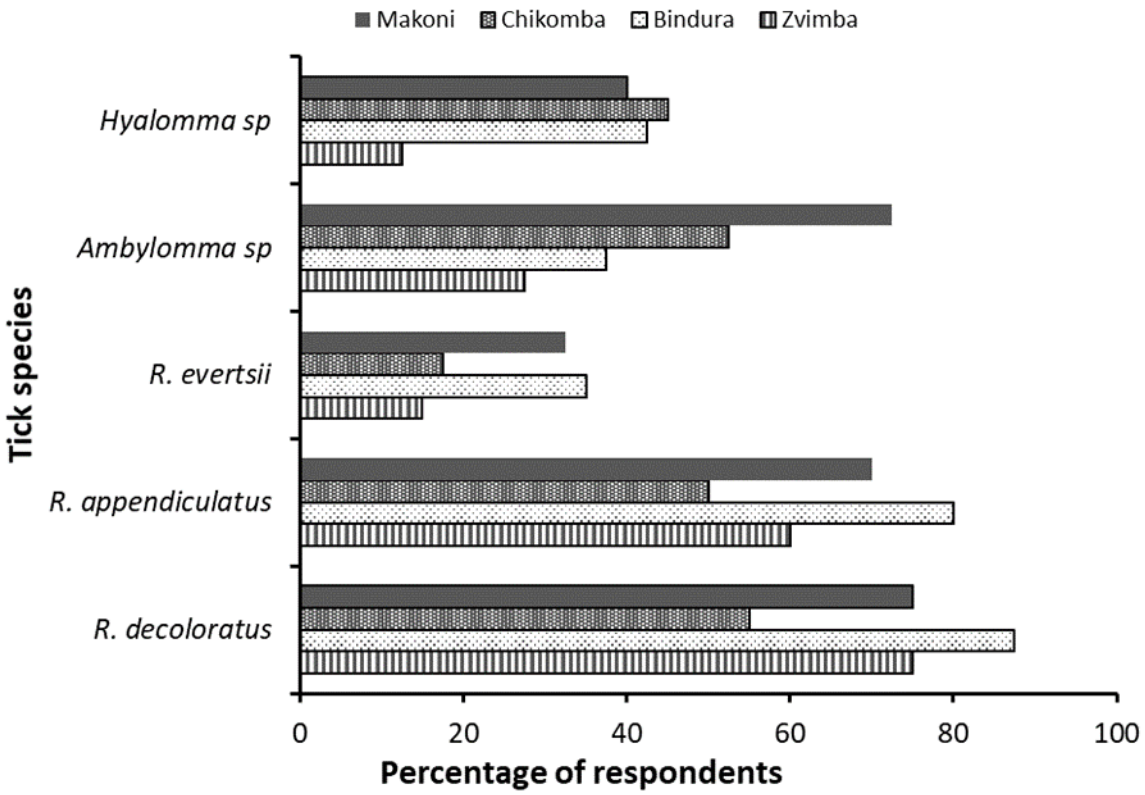


Figure 4.9: Tick species commonly identified by farmers in the sampled districts of Zimbabwe.

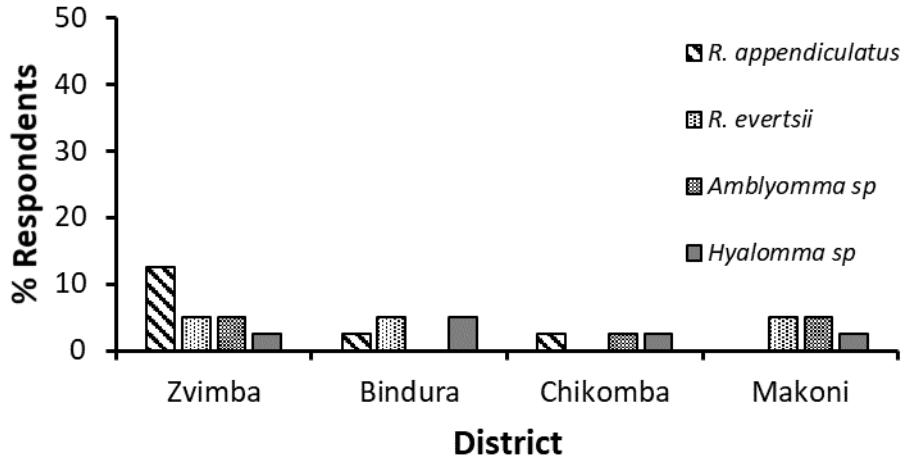


Figure 4.10: Tick species identified as new by farmers in selected districts of Zimbabwe.

Tick management practices

All farmers use government dipping facilities and the majority (>50%) consistently follow the dipping regimes of weekly in summer and fortnightly in winter (Table 4.2). There was a significant difference in adherence to the stipulated dipping regimes across districts in summer ($p<0.05$) and in winter ($p = 0.001$). Respondents mentioned acaricide availability as the determinant of these dipping regimes. The most common chemical compounds supplied by the government in all districts were reported to be amitraz (formamidine) or deltamethrin (synthetic pyrethroid).

In addition to government provided plunge dipping, a large proportion of farmers (>50%) across all districts supplemented dipping through hand spraying and hand dressing (Table 4.2). This practice varied significantly across all districts although it was found to be higher in Zvimba district ($p<0.05$). The most common chemical compounds used to supplement dipping were formamidine and synthetic pyrethroid groups of acaricides applied at weekly or fortnightly intervals (Fig. 4.11). Some respondents applied own chemicals whenever ticks appeared on animals and this practice was viewed equally effective across all districts ($p>0.05$) (Table 4.2). Very few respondents were aware of the availability of vaccines against tick-borne diseases although more than 50% across all districts were willing to embrace them. The minority who was not willing to use them, cited unaffordability and possibilities of side effects.

Table 4.2: Additional tick and tick-borne disease management strategies used by farmers and frequency of dipping and chemical compounds supplied by the government to communal dipping facilities in the four districts of Zimbabwe. Letters on the question marked by an asterisk stand for amitraz (A) and deltamethrin (D).

	% Respondents (Yes)						
Question	**Zvimba**	**Bindura**	**Chikomba**	**Makoni**	χ^2	**DF**	***p*-value**
1. Do you dip your cattle at the government communal dip tank?	100	100	100	100	-	-	-
2. Is routine weekly dipping followed in summer?	70	92.5	55	67.5	10.26	3	0.016
3. Is the routine fortnightly dipping followed in winter?	67.5	95	52.5	90	15.49	3	0.001
***4.** Which dipping chemical (s) is supplied by government at the dip tank?	A	A/D	D	A/D	-	-	-
5. Do you supplement government dipping by own dipping chemical purchases?	87.5	67.5	55	77.5	8.07	3	0.045
6. Are they effective?	85	62.5	57.5	70	6.27	3	0.1
7. Have you heard about vaccines for TBDs?	15	47.5	52.5	32.5	23.18	3	<0.001
8. If available would consider using them on your animals?	80	95	52.5	95	14.94	3	0.002

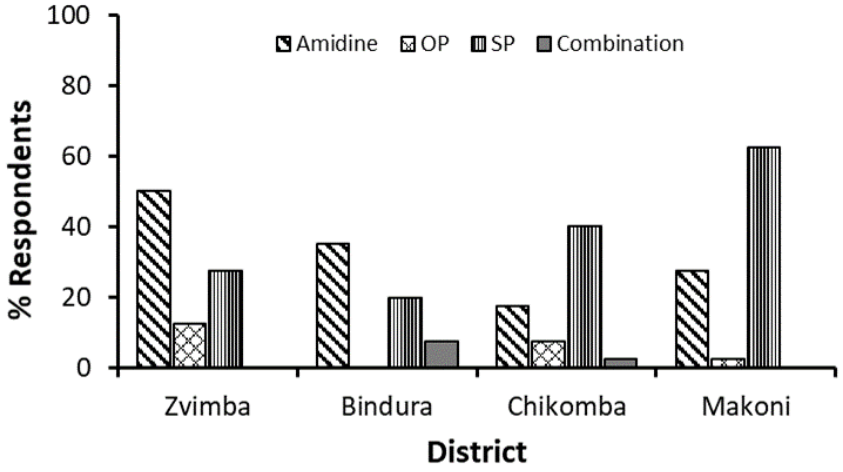


Figure 4.11: Chemical compounds used to supplement government dipping in the selected districts of Zimbabwe. Note: OP and SP stands for chemicals in the organophosphate and synthetic pyrethroid groups respectively.

Acaricide efficacy

Generally, a large proportion of farmers judged the efficacy of the acaricide supplied by the government for dipping to be good across all districts (Fig. 4.12). However, 50,7% of the respondents admitted to seeing mainly immature and flat adult stages of ticks one to three days post dipping. The most observed tick species post dipping were *R. decoloratus* and *R. appendiculatus* and occasionally *Amblyomma* species especially in Makoni district (Fig. 4.13).

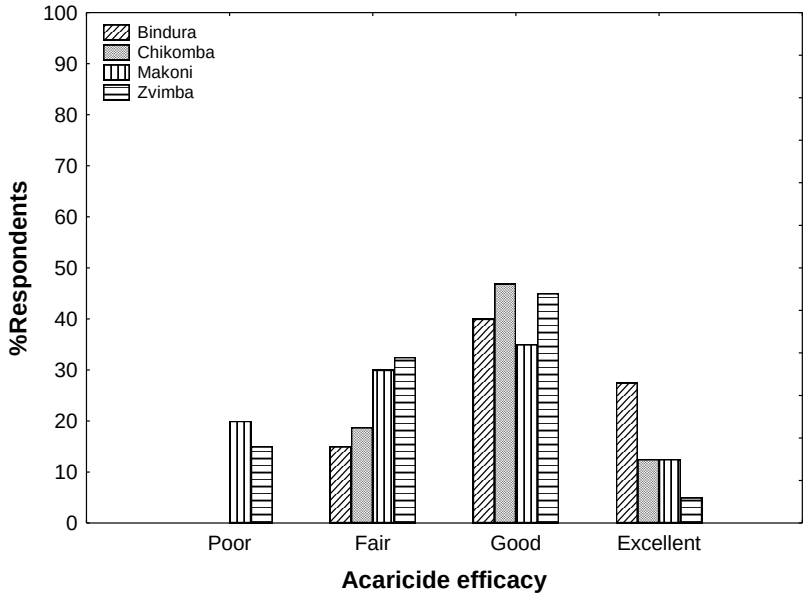


Figure 4.12: Farmers' perceptions on the effectiveness of commonly applied acaricides in selected districts of Zimbabwe.

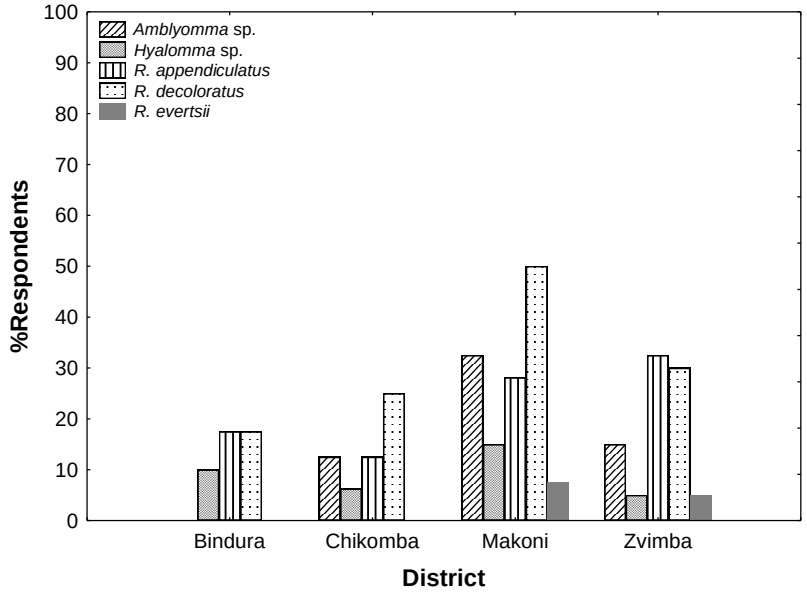
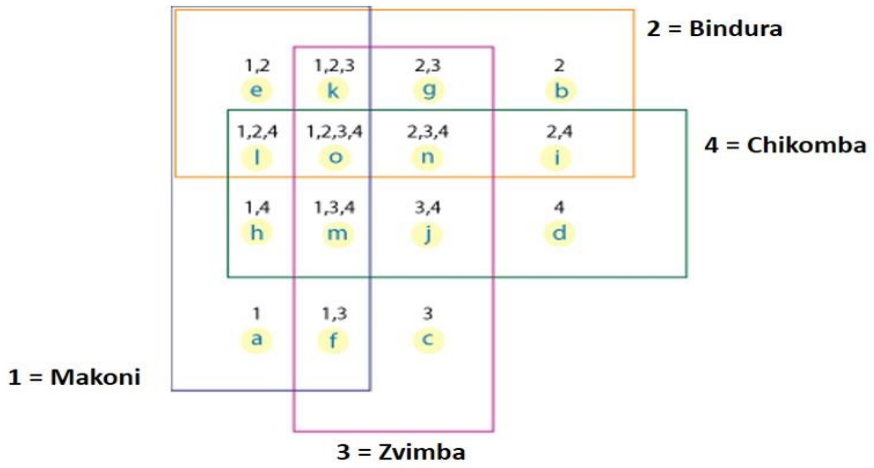


Figure 4.13: Tick species observed post-dipping by respondents in the four districts of Zimbabwe.

Disease incidence

Respondents mentioned a total of 24 diseases as the most common and economically important in all the districts surveyed. Of these, five were common in all districts, four of these were tick-borne diseases (Fig. 4.14). The four tick-borne diseases were January disease (theileriosis), red water, gall sickness, and heart water. Bindura district in Mashonaland Central province had the highest number of other diseases not mentioned in other districts. More than 50% of farmers lost between one and five animals due to tick and tick-borne diseases over the previous year. These farmers sought assistance from local government authorities while a few relied on traditional remedies to manage the diseases.



Key	Diseases at the intersection					
a	Goat diahorrea	Joint illness	Liver cirrhosis			
b	Abscesses	Bottle jaw	Calf sores	Foot and Mouth Disease	Helminthosis	Pink eye
c	Babesiosis	Rabies				
d	Botulism	Fasciolitis	Mastitis			
e	-					
f	-					
g	Anthrax	Dermatophilosis				
h	-					
i	-					
j	-					
k	Foot rot					
l	-					
m	Sweating sickness					
n	Blackleg					
o	Gall sickness	Heartwater	Lumpyskin	Redwater	Theileriosis	

Figure 4.14: Common animal diseases reported to be associated with cattle mortalities in the selected districts of Zimbabwe

Discussion

This study showed that *R. appendiculatus* is still susceptible to both amitraz and deltamethrin. Since *R. appendiculatus* is a three-host tick species, its selection for resistance when compared to an one-host tick species like *R. decoloratus,* is less intense. *Rhipicephalus decoloratus* has a short generation time and spends three weeks on the same host, causing it to be exposed to the treatment acaricide more frequently and for longer periods than *R. appendiculatus* (Arthur and Londt, 1973). These results are also supported by evidence of increased difficulty encountered in finding specimen even eight days post-dipping when compared to *R. decoloratus*. This might mean that the acaricides in use are effective in controlling *R. appendiculatus* compared to the one-host *R. decoloratus*. Thus, it is plausible that a breakdown in public dipping services rather than resistance development might be the reason behind the spike in theileriosis, a disease transmitted by *R. appendiculatus*. This is also the perception of farmers across all districts surveyed, as they observed increased tick loads during periods of inconsistent dipping regimes. However, the development of resistance by *R. appendiculatus* cannot be completely ruled out as Chitombo *et al*. (2021) reported emerging resistance by the tick to amitraz and chlorfenvinphos in small scale farming systems in Mashonaland West province.

As expected, resistance was detected in *R. decoloratus* which is a single-host species. Resistance ratios for the species were consistently higher indicating the possibility of developing resistance in the populations sampled. This is consistent with findings by Sungirai *et al*. (2017) who observed complete resistant genotypes in all provinces indicating that the resistance allele is spreading in the country. Resistance to both synthetic pyrethroids and formamidines has been repeatedly recorded for *R. decoloratus* over the years (Mazhowu, 1995; Rodriguez-Vivas *et al*., 2018; Sungirai *et al*., 2018a; Makuvadze *et al*., 2020). In Uganda, Vudriko *et al*. (2016) also reported multi-acaricide resistance of the tick species. Thus, this makes it an ideal indicator species for acaricide resistance as it is subject to the greatest selection pressure and frequent exposure to acaricides (Arthur and Londt, 1973).

In the context of this study resistance of *R. decoloratus* might indicate a lack in tick management practices to control ticks which might be driving the development of resistance. It further validates findings that *R. appendiculatus* is still susceptible in the country and its reported abundance and related disease incidence might be a management issue. Farmers highlighted that in the years preceding 2020, acaricide supplies from government were inconsistent resulting in increased tick loads. This corroborates with Sungirai *et al*. (2016) who

also found that farmers would skip dipping for more than a month due to delays in acaricide supply.

Inadequate animal health facilities may affect farmer compliance on mandatory requirements such as dipping. However, this study showed that most farmers have access to dipping facilities and do comply with seasonal dipping requirements. Despite this, high incidences of theileriosis have been reported over the years. In contrast, previous research described the disease to be problematic only in livestock-wildlife interface areas where buffaloes were in close contact with livestock (De Garine-Wichatitsky *et al*., 2013; Sungirai *et al*., 2016). However, current trends might possibly be due to climate induced changes in the distribution of the vector, *R. appendiculatus* or host-parasite interactions as reported in Chapter 3 of this study.

Although no resistance by *R. appendiculatus* was detected, management practices by farmers pose a real threat to its development in future. This study showed that farmers are indiscriminately applying acaricides obtained over the counter, posing two problems. Firstly, there is a danger of applying sub-lethal doses and secondly there are increased chances of frequently exposing even multi-host ticks to acaricides due to the shortened application intervals. Both these factors have been cited as the reason behind acaricide resistance development (Sungirai *et al*., 2016; Makuvadze *et al*., 2020). This study also gave indications of developing resistance in *R. appendiculatus* as signified by widening resistance ratios in some of the populations tested. Emerging resistance by the tick species has already been reported in the country (Chitombo *et al*., 2020).

Most respondents reported ticks to be more prevalent in summer than in other seasons. Biguezoton *et al*. (2016) and Debbarma *et al*. (2018) reported similar prevalence results in Burkina Faso and the Indian state of West Bengal, respectively. The low prevalence in the winter season is linked to the presence of low temperatures which negatively affects processes such as oviposition, moulting and questing (Estrada-Peña, 2015; Namgyal *et al*., 2021b). In addition, the study observed that chances of spreading of ticks and TBDs were very high since most communities share grazing land and watering holes. This is not the case with farms which have their own separate facilities, typical of farms in Pakistan and Bhutan in West Asia where stall-feeding is practiced (Rehman *et al*., 2017; Namgyal *et al*., 2021b).

Unlike the majority of farmers in the southern lowveld of Zimbabwe who managed to describe TBDs with names or clinical and post-mortem signs (Sungirai *et al*., 2016), a large proportion of interviewed farmers in this study only described the clinical signs and symptoms of a disease

1417 but failed to name the disease. This might be as a result of the absence of tick and TBD
1418 awareness programs to farmers due to no previous major outbreaks. In this regard, Sungirai *et*
1419 *al*. (2016) highlighted that accuracy in the knowledge of tick and tick-borne diseases by
1420 communal farmers in Zimbabwe, usually increased with the level of education and attendance
1421 of training courses. The same was reported for farmers living in rural areas of Morocco, South
1422 Africa's Eastern Cape province and Bhutan in West Asia (Sahibi and Rhalem, 2007; Moyo and
1423 Ferguson, 2010; Namgyal *et al*., 2021b).

1424 In conclusion, the brown ear tick is still susceptible to acaricides commonly used in Zimbabwe.
1425 Thus, increase in the incidence of theileriosis may need to be investigated in light of other
1426 epidemiological parameters other than resistance of the vector. There are however indications
1427 of reduced efficacy of acaricides which we could not definitively ascertain. As such, the role
1428 of acaricide resistance may not be dismissed in future transmission dynamics of the disease.

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CHAPTER 5

General discussion

The dissertation has shown that the increasing incidence of theileriosis in Zimbabwe is mainly driven by an expansion in the habitat of the vector, *R. appendiculatus* coupled with a breakdown in tick management prior to the upsurge of the disease. This was also the case in Malawi where interruptions in dipping coupled with cattle movements was followed by an outbreak of tick-borne diseases (Lawrence *et al*., 1995). This is the first study to comprehensively investigate environmental, social, and physiological factors behind the distribution of the tick species and the transmission dynamics of theileriosis in Zimbabwe. Thus, it provides key evidence on priority management areas in the containment of the disease.

Chapter 3 of this study demonstrated that *R. appendiculatus* tolerates high temperatures, hence it is not surprising that the predicted distribution is expected to expand with a 2 °C rise in temperatures between 2040 and 2060. Although the mechanism behind temperature tolerance in ticks is not well known, some researchers attribute tolerance of high temperatures to the presence of shock and stress proteins (Villar *et al*., 2010). The anticipated changes in the distribution of suitable habitats for *R. appendiculatus* could thus significantly alter the range and distribution of the tick species together with the associated tick-borne diseases. Although tick-borne diseases can be eradicated, the vectors are usually difficult to eradicate especially where the ecology is suitable for their survival and where there are numerous alternative hosts not subjected to tick control (Wilson, 1996).

This study eliminated resistance development as the reason for the increase in the prevalence of *R. appendiculatus*, but resistancedevelopment must not be ruled out in future investigations due to the practice of unregulated acaricide applications by farmers. In addition, moderate resistance by the indicator species, *R. decoloratus* against both amitraz and deltamethrin gives an indication that commonly used acaricides gradually lose their efficacy if there is no effective monitoring. Striking a balance between effective tick control and endemic stability is also one of the contentious issues in tick control. However, the control of ticks can be strategized (by reducing dipping intervals when tick loads decrease) and only limited to combat physical effects if endemic stability is maintained (Wilson, 1996).

Since theileriosis remains a challenge in livestock production systems in Zimbabwe, several recommendations can be made as outlined below:

- There is need to further investigate the host-parasite interactions between *R. appendiculatus* and *T. parva* and the role of climatic factors in the transmission dynamics as a possible factor behind increased disease incidence in the country.

- The country adopted an intensive dipping regime to try and contain the disease. However, there is the risk of resistance development as this increases the exposure of the ticks to acaricides. It is recommended that consistent dipping be adhered to without necessarily increasing the frequency of dipping. Additional measures may include proper acaricide application intervals, recommended doses and taking proper consideration before purchasing supplementary acaricides to avoid using acaricides with similar mode of actions as the ones used in the communal dip tanks. It is therefore evident that execution of farm awareness on the above-mentioned factors among others is key in the containment of both resistant tick populations and tick-borne infections.
- The country could also consider adopting an integrated approach in the management of tick-borne diseases *viz* dipping and vaccinations.
- Testing for acaricide resistance should be expanded and conducted on an on-going/continuous basis since there are indications of resistance genes developing.

These interventions will help in reviewing tick control strategies considering the possible impacts of predicted changes in ticks and TBD distributions as well possible influence of acaricide resistance in future transmission dynamics of theileriosis in Zimbabwe. Future studies on other thermal traits, not covered by this study, will also provide valuable insights to compliment the available information and can be used for better prediction of the potential distribution of both the tick and the associated tick-borne diseases. A holistic approach including quarantining of affected areas, controlling movement of animals and stringent and diligent dipping regulations are among viable solutions to control both the tick and associated tick-borne diseases.

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Appendices

Appendix 1

Drivers of resistance of ticks to acaricides in Zimbabwe

The Division of Veterinary Technical Services in collaboration with the Agricultural Research Council of Zimbabwe and the University of the Free State, South Africa, is researching possibilities of resistance of the brown ear tick, *Rhipicephalus appendiculatus* to commonly applied acaricides in Zimbabwe. The tick is the vector for January Disease (Theileriosis) which is responsible for high cattle mortalities in the country. The purpose of the questionnaire, therefore, is to understand current tick management practices and their efficacy in communal cattle herds and determine any possible drivers of resistance.

This questionnaire consists of 38 questions in 5 sections.

A) Location and demographics

1. Dip tank.....
2. District.....
3. Province.....
4. GPS Coordinates: Long ……; Lat ……..
5. Number of cattle owned by farmer.....
6. Distance to the diptank
   ☐less than 1km ☐1km-5km ☐more than 5km

B) Vector knowledge

7. Are ticks a problem in your area? ☐Yes ☐No
8. Which ticks are common?

☐Blue ticks ☐Brown ear ticks ☐Red-legged ticks

☐Bont ticks ☐Bont- legged ticks ☐Others(specify).....

9. Which season of the year are the ticks most prevalent?
   ☐Rain season ☐Dry season

10. Which animals are mostly affected?
    ☐Cattle ☐sheep ☐goats ☐others (specify).....

11. Which sites are the ticks mostly found?
    ☐Ears ☐head, neck and dewlap ☐udder and scrotum
    ☐Body and belly ☐legs and tail switch ☐others (specify).....

12. When do your cattle mix with cattle from other areas/farms?
    ☐During grazing ☐during dipping ☐during watering ☐at no time at all

13. Have you observed any new ticks that were not common in your area? ☐ Yes
    ☐No

14. If yes, which ticks have you observed?

☐Blue ticks ☐Brown ear ticks ☐red legged ticks

☐Bont ticks ☐Bont- legged ticks ☐Others (specify).....

C) Tick management practices

15. Do you dip your animals at the government communal dip tank? ☐Yes
    ☐No

16. Which dipping chemical (s) is supplied by government at the dip tank?……..

17. Is routine weekly dipping followed in summer? ☐Yes ☐No

18. Is the routine fortnightly dipping followed in winter? ☐Yes ☐No

19. Do you supplement government dipping by own dipping chemical purchases?
☐Yes ☐No

20. If yes, where do you buy the dipping chemicals?.....

21. What quantities do you buy per year?.....

22. How much (in USD) do you spend on dipping chemicals per year?.....

23. Which dipping chemicals have you used to supplement government dipping for the past 4 years?

.....
.....

24. How do you apply them?
☐Spray-race ☐Hand spray ☐Hand dressing (Pour-on or tick grease)
☐Other methods
(specify).....

25. At what interval do you apply the dipping chemicals?.....

26. Are they effective? ☐Yes ☐No

27. Have you heard about vaccines for TBDs? ☐Yes ☐No

28. If available would consider using them on your animals? ☐Yes ☐No

29. If no, state any reasons.....

.....
.....

.....
.....

D) Acaricide efficacy

30. From your judgement, the performance of the dipping chemical currently in use at the communal dip tank is:

☐Poor ☐Fair ☐Good ☐Excellent

31. Do you observe ticks on animals post dipping? ☐Yes ☐No

32. If yes, how many days after dipping?.....

33. Where do you mostly find the ticks attached to after dipping?

☐Ears ☐head, neck and dewlap ☐udder and scrotum ☐Body and belly ☐legs and tail switch ☐others (specify).....

34. Which tick types usually remain attached post dipping?

☐Blue ticks ☐Brown ear ticks ☐red legged ticks

☐Bont ticks ☐Bont- legged ticks ☐Others (specify).....

35. Stages of ticks found on animals post dipping

☐Immatures ☐Flat adults ☐Semi-engorged ☐Engorged

E) **Diseases and mortalities**

36. In order of importance, name any 5 diseases which cause the highest animal mortalities in your area:
    1:.....
    2:.....
    3:.....
    4:.....
    5:.....

37. How many animals do you lose to diseases per year?.....

38. How are the diseases managed?

……
……

……
……

……
……

F) Conclusion

Your assistance, time, and input are greatly appreciated.

If there is anything further that you would like to comment, note, suggest, raise, or otherwise divulge please feel free to include it in the "Additional Notes" section at the end of this booklet.

The information recorded on this form will only be used for this study and will not be disclosed to anyone without your prior consent

If you have any issues, queries or otherwise wish to get hold of me my email address is 2019878616@ufs4life.ac.za and I am available on 0778081743

Thank you for your time

Additional Notes:

Appendix 2

PROJECT INFORMATION

Informed consent will be obtained from all individual participants included in the study after explanation of project information.

RESEARCH STUDY INFORMATION LEAFLET AND CONSENT FORM

DATE _____*2021*

TITLE OF THE RESEARCH PROJECT
Drivers of resistance of ticks to acaricides in Zimbabwe

PRINCIPLE INVESTIGATOR / RESEARCHER(S) NAME(S) AND CONTACT NUMBER(S):
Tinotenda M. Nemaungwe *Student number: 2019878616* *0242705885-6*

Sign: _____

FACULTY AND DEPARTMENT:
Natural and Agricultural Sciences
Zoology and Entomology

STUDYLEADER(S) NAME AND CONTACT NUMBER:
Ellie van Dalen (UFS staff member)
+27 (0) 51 401 2371

Sign:_____

WHAT IS THE AIM / PURPOSE OF THE STUDY?
To understand how current tick management practices, increase the risk of acaricide resistance development in communal cattle herds.

WHO IS DOING THE RESEARCH?
This research is being done by Tinotenda M. Nemaungwe as part of her MSc studies at the University of the Free State, Bloemfontein Campus

HAS THE STUDY RECEIVED ETHICAL APPROVAL?
Approval number: 001/2021

WHY ARE YOU INVITED TO TAKE PART IN THIS RESEARCH PROJECT?
You have been chosen to participate in this research as you live in one of the districts where cattle mortalities due to Theileriosis (January disease) have been reported. The disease and the associated brown ear tick (Rhipicephalus appendiculatus) might also have affected your livestock. You are part of a group of 40 individual participants who will be interviewed to understand how animal losses due to tick-borne diseases might be linked to tick management practices, dipping regimes, acaricides in use and their effectiveness. The study will help to explore the management practices behind the development of acaricide resistance and come up with mitigation strategies.

WHAT IS THE NATURE OF PARTICIPATION IN THIS STUDY?

You will be asked to respond to a questionnaire which consists of 38 questions in 5 sections. This process is expected to take almost 25 minutes.

CAN THE PARTICIPANT WITHDRAW FROM THE STUDY?

Your participation in this study is entirely voluntary and should you, at any stage, wish to withdraw from participating further, you may do so without any negative consequences. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a written consent form. However, please note that it will not be possible to withdraw from the study once you have submitted your questionnaire as your material may be unidentifiable from others.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART IN THIS STUDY?

The study will help to determine if the recent surge in Theileriosis is linked to resistance development to commonly used acaricides by the tick vector. Your participation will be kept confidential. However, information about you may be given to the study sponsor who is obliged to maintain confidentiality.

WHAT IS THE ANTICIPATED INCONVENIENCE OF TAKING PART IN THIS STUDY?

There are no unforeseen inconveniences arising from taking part in this study.

WILL WHAT I SAY BE KEPT CONFIDENTIAL?

Confidentiality and anonymity of records will be maintained and your name and identity will not be revealed to anyone who has not been involved in the conduct of this study. Your answers will be used only for the purpose of this study and may be reviewed by people responsible for making sure that research is done properly, including the transcriber, external coder, and members of the Research Ethics Committee. Records will only be kept to indicate your participation in the study but will not be linked to your responses. Results from this study will be used to compile an MSc thesis for the researcher and might also be used to compile journal articles and presented in conferences. However, your responses will remain anonymous to protect your privacy.

HOW WILL THE INFORMATION BE STORED AND ULTIMATELY DESTROYED?

Hard copies of your answers will be stored by the researcher for a period of five years in a locked cupboard/filing cabinet at the Central Veterinary Laboratory of Zimbabwe for future research or academic purposes; electronic information will be stored on a password protected computer. Future use of the stored data will be subject to further Research Ethics Review and approval if applicable. Once 5 years lapse, all hardcopies will be shredded according to the laboratory's document disposal policy. There are no anticipated risks or inconveniences hence there is no indemnity or insurance coverage for your participation.

WILL I RECEIVE PAYMENT OR ANY INCENTIVES FOR PARTICPATING IN THIS STUDY?

There are no financial or any other reward for participating in this study.

HOW WILL THE PARTICIPANT BE INFORMED OF THE FINDINGS / RESULTS OF THE STUDY?

If you would like to be informed of the final research findings, please contact Tinotenda Nemaungwe on 0242 705885-6 or email: 2019878616@ufs4life.ac.za or website www.ufs.ac.za. The findings are accessible for 3 years. Should you require any further information or want to contact the researcher about any aspect of this study, please contact Ellie van Dalen on +27(0)51 401 2371or email: vanDalenEM@ufs.ac.za

Thank you for taking time to read this information sheet and for participating in this study.

Appendix 2: PARTICIPATION CONSENT

I, _____ (participant name), confirm that the person asking my consent to take part in this research has told me about the nature, procedure, potential benefits and anticipated inconvenience of participation.

I have read (or had explained to me) and understood the study as explained in the information sheet. I have had sufficient opportunity to ask questions and I am prepared to participate in the study. I understand that my participation is voluntary and that I am free to withdraw at any time without penalty (if applicable). I am aware that the findings of this study will be anonymously processed into a research report, journal publications and/or conference proceedings.

I agree to the recording of my responses on the questionnaire.

I have received a signed copy of the informed consent agreement.

Full Name of Participant: _____-____

Signature of Participant: _____ Date: _____

Full Name(s) of Researcher(s): _____

Signature of Researcher: _____ Date: _____

Appendix 3

National Animal Research Ethics Committee
All communications should be addressed to:
The Chairman
National Animal Research Ethics Committee
18A Borrowdale Road
P.O Box CY 551, Causeway
Harare
Vetlabs@dvts.org.zw
263-24-703753, 705886/7

07 April 2021

To: Tinotenda Nemaungwe

Ref: Distribution and acaricide resistance status of the Theileriosis vector, Rhipicephalus appendiculatus Neumann. (Ixodidae) in response to changing climate in Zimbabwe (001/2021).

We have the pleasure to inform you that the National Animal Research Ethics Committee (NAREC) has granted the approval to conduct the above research.

Project Name: "Distribution and Acaricide resistance status of the Theileriosis vector, Rhipicephalus appendiculatus Neumann. (Ixodidae) in response to changing climate in Zimbabwe."

Institution: University of Free State

Principal investigator: Tinotenda Nemaungwe

DIRECTOR
DIVISION OF VETERINARY TECHNICAL SERVICES
07 APR 2021
BOX CY 551 CAUSEWAY
TEL: 0242-705885/7

07/04/21

Dr P.V. Makaya
Chairman
National Animal Research Ethics Committee (NAREC)

Appendix 4

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

GENERAL/HUMAN RESEARCH ETHICS COMMITTEE (GHREC)

28-Jun-2021

Dear Mrs Tinotenda Nemaungwe

Application Approved

Research Project Title:
Drivers of resistance of ticks to acaricides in Zimbabwe

Ethical Clearance number:
UFS-HSD2021/0418

We are pleased to inform you that your application for ethical clearance has been approved. Your ethical clearance is valid for twelve (12) months from the date of issue. We request that any changes that may take place during the course of your study/research project be submitted to the ethics office to ensure ethical transparency. furthermore, you are requested to submit the final report of your study/research project to the ethics office. Should you require more time to complete this research, please apply for an extension. Thank you for submitting your proposal for ethical clearance; we wish you the best of luck and success with your research.

Outcome: Approved

The study is approved – however, please take note of the following recommendations:

If a social (human) study will be conducted in conjunction with the scientific study, then the student should add information regarding the social study into the proposal as well as its not clear in the proposal how the social data will be collected and analysed and no information regarding ethics of the participants has been indicated. The student should also add the objectives of the social study into the proposal and justify the purpose/aim of collecting the social data.

Under 'Risk Mitigation' – would the participants not experience 'Loss of work time/study time' to complete the questionnaire, and how will the researcher mitigate this.

Yours sincerely

Dr Adri Du Plessis
Chairperson: General/Human Research Ethics Committee

Adri du Plessis
Digitally signed by Adri du Plessis
Date: 2021.06.29 08:37:44 +02'00'

205 Nelson Mandela Drive
Park West
Bloemfontein 9301
South Africa

P.O. Box 339
Bloemfontein 9300
Tel: +27 (0)51 401 9337
duplessisA@ufs.ac.za
www.ufs.ac.za

Appendix 5

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

Animal Research Ethics

16-Aug-2021

Dear Mrs Tinotenda Mercy Nemaungwe

Student Project Number: UFS-AED2021/0032/21

Project Title: Distribution and acaricide resistance status of the Theileriosis vector, Rhipicephalus appendiculatus Neumann. (Ixodidae) in response to changing climate in Zimbabwe.

Department: Zoology and Entomology Department (Bloemfontein Campus)

You are hereby kindly informed that, at the meeting held on 01-Aug-2021, the Interfaculty Animal Ethics Committee approved the above project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.

2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.

3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Yours Sincerely

Mr. Gerhard Johannes van Zyl

Approved

Appendix 6

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
RESEARCH DEVELOPMENT
NAVORSINGSONTWIKKELING

Environment & Biosafety Research Ethics Committee

15-Jul-2021

Dear **Mrs Tinotenda Nemaungwe**

Project Title: **Distribution and acaricide resistance status of the Theileriosis vector, Rhipicephalus appendiculatus Neumann. (Ixodidae) in response to changing climate in Zimbabwe.**

Department: **Zoology and Entomology Department (Bloemfontein Campus)**

APPLICATION APPROVED

This letter confirms that this research proposal was given ethical clearance by the Environment & Biosafety Research Ethics Committee of the University of the Free State.

Your ethical clearance number, to be used in all correspondence is: **UFS-ESD2021/0181/21**

Please note the following:

1. **This ethical clearance is valid for two years from the issuance of this letter.**
2. **If the research takes longer than two years to complete, please submit a Continuation Report to the Ethics Committee before ethical clearance expires.**
3. **If any changes are made during the research process (including a change in investigators), please inform the Ethics Committee by submitting an Amendment.**
4. **When the research is concluded, please submit a Final Report to the Ethics Committee.**

Approved

Thank you for your application and we wish you well in all of your research endeavours.

Yours Sincerely

Prof. RR (Robert) Bragg
Chairperson: Environment & Biosafety Research Ethics Committee
University of the Free State

Directorate: Research Development
T: +27 (0)51 401 9398 | +27 (0)51 401 2075 | E: smitham@ufs.ac.za
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205 Nelson Mandela Drive | Park West, Bloemfontein 9301 | South Africa
P.O. Box 339 | Bloemfontein 9300 | South Africa | www.ufs.ac.za

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