Alternative methods used by small-holder farmers to control ticks and bovine dermatophilosis and the impact of a changing interface of Amblyomma ticks on dermatophilosis in Zimbabwe.

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Declaration

I, Daud Nyosi Ndhlovu, hereby declare that this research is an outcome of my own investigation under the supervision of Prof P. J. Masika; and has not been previously submitted to any university. Where reference to other researchers' work has been made and where assistance was rendered; they have been duly acknowledged in the text.

Daud Nyosi Ndhlovu **Date** **16/09/2014**

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Abstract

Alternative methods used by small-holder farmers to control ticks and bovine dermatophilosis and the impact of a changing interface of Amblyomma ticks on dermatophilosis in Zimbabwe.

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This study was carried out to document the ethno-veterinary plants and non-plant remedies that farmers used to control dermatophilosis and ticks, and validate these *in vitro* and *in vivo*, as well as determine how the interface of *Amblyomma variegatum* and *A. hebraeum* affected the epidemiology of bovine dermatophilosis at selected small-holder areas in North-West Zimbabwe.

A structured questionnaire survey was used to collect information on the control methods used by farmers for the control of bovine dermatophilosis and ticks. A total of 39 plants were used by farmers for various diseases, eleven of these plants were used for the control of dermatophilosis while three were for tick control. Across the study sites, dermatophilosis was controlled using eleven plants. Among these plants; six plants; *Cissus quadrangularis*, *Catunaregan spinosa*, *Pterocarpus angolensis Carica papaya*, *Manihot esculenta* and *Blumea decurrens* which were frequently used were selected for further studies. *In vitro* and *in vivo* studies were conducted to validate the efficacy of these plants. The minimum inhibitory concentration and bactericidal concentration assays were used for the *in vitro* validation of *C. quadrangularis*, *C. spinosa* and *P. angolensis*. *Dermatophilus congolensis* was more sensitive to *P. angolensis* average MIC = 0.63 mg/ml than to *C. quadrangularis* average MIC =

1.25 mg/ml and *C. spinosa* average MIC = 2.08 mg/ml. *Cissus quadrangularis* was selected for *in vivo* studies as this plant was the one most frequently used by farmers. Its therapeutic efficacy was compared to conventional antibiotics that farmers used to treat dermatophilosis infection. In the *in vivo* trials *C. quadrangularis* did not lead to appreciable reduction in clinical disease compared to the conventional drugs.

The larval packet assay was the *in vitro* assay used to validate the efficacy of *Carica papaya, Manihot esculenta* and *Blumea decurrens* against larvae of *A. hebraeum* and *Rhipicephalus appendiculatus*. *Manihot esculenta* at 20 % w/v exhibited the highest larvicidal activity against *R. appendiculatus*. In the *in vivo* study, efficacy of the plants were investigated on naturally tick-infested cattle. *Mannihot esculenta* exhibited the largest tick load reduction compared to the other two plants but its performance was lower than that of conventional acaricides.

The effect of *A. variegatum* and *A. hebraeum* on bovine dermatophilosis was investigated by evaluating how the presence or absence of these ticks and other herd level risk factors predicted clinical dermatophilosis and its prevalence at herd level. A structured questionnaire survey was carried out to collect data on potential risk factors. At the same time, cattle were physically examined for the presence of bovine dermatophilosis, according to a pre-defined case definition, and presence or absence of Amblyomma ticks was also recorded. The multivariable binary logistic model was developed with disease status as outcome, tick presence and infestion and herd level risk factors as predictors. Of the herds examined clinical bovine dermatophilosis was detected in 45% (84/185; 95% CI: 38.2, 52.6%) of them. Herds infested with *Amblyomma variegatum* were associated with higher odds (OR= 6.8;

95% CI: 1.71, 27.10) of clinical dermatophilosis while the association was not significant (P > 0.05) in *A. hebraeum* infested herds. It was concluded that management practices aimed at movement and tick control would help reduce the prevalence of clinical dermatophilosis in herds.

Dedication

I fully dedicate this work to my wife Felistas Ndhlovu, our three children; daughter Phumuzile and sons, Mkhululi and Siphosenkosi, loved brothers and sisters and, the Goromonzi and Ndhlovu families.

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List of abbreviations

CLSI	Clinical and Laboratory Standards Institute
CSO	Central Statistical Office
CFU	Colony Forming Units
DVS	Department of Veterinary Services
FAO	Food and Agriculture Organisation of the United Nations
GLM	General Linear Model
LC_{50}	Lethal Concentration 50
LPT	Larval Packet Assay
ROC	Receiver Operating Curve
TBD	Tick-borne diseases
MBC	Minimum bactericidal concentration
M-H	Mueller-Hinton
MIC	Minimum inhibitory concentration
WHO	World Health Organisation of the United Nations
ZINQAP	Zimbabwe Quality Assuarance Program

Chapter 1 ：Introduction

1.1 Background

Ticks are the most important ectoparasites of cattle and are responsible for the transmission of tick-borne pathogens to cattle and humans (Fuente *et al.,* 2007; Ntondini *et al.,* 2008). Ticks also have been reported to be a major economic constraint to livestock productivity (Swai *et al.,* 2005; Bazarusanga *et al.,* 2007) In Zimbabwe tick infestations, tick-borne diseases and tick associated diseases such as bovine dermatophilosis are important conditions affecting livestock health and productivity (Lawrence *et al.,* 1980; Chatikobo *et al.,* 2009). Damage is also produced indirectly as a result of the association between ticks with some important livestock diseases such as dermatophilosis (Admassu and Alemu 2011).

In Zimbabwe, *A. hebraeum* and *A. variegatum*, are ticks of economic importance as transmitters of heartwater (Peter *et al.,* 1998), association with bovine dermatophilosis (Chatikobo *et al.,* 2009) and also as causes of direct damage to cattle (Meltzer *et al.,* 1995; Walker, 1996). *Amblyomma hebraeum*, *A. variegatum* and the associated disease; bovine dermatophilosis, have in the past been reported to occur only in certain regions of the country, this, as a result of strict cattle movement control and adequate dipping services (Norval, 1983; Chatikobo *et al.,* 2009). *Amblyomma hebraeum* occurs mainly in the south and south east of Zimbabwe (Estrada-Pena *et al.,* 2008) while *A. variegatum* occurs mostly in the north-western parts and in small pockets on the eastern border of the country (Norval, 1983; Peter *et al.,* 1998). Estrada-Pena *et al.,* (2008) reported a northwards spread of *A. hebraeum* and a dispersion of *A. variegatum* outside drier zones to other areas. Chatikobo *et al.,* (2009) reported that the distribution of the equally important bovine dermatophilosis,

which was limited to the Hwange-Lupane area in the northwest of the country, had increased to cover virgin areas.

The Department of Veterinary Services (DVS) in Zimbabwe has faced budgetary constraints; this has affected dipping-service delivery leading to frequent disruptions of dipping activities. There has been an effect on the tick dynamics in the affected areas as happened with disruption of dipping during the liberation war years (Lawrence *et al.,* 1980). Coupled to this, has been the agrarian reform that started in full force in the year 2000. The reform saw the movement of livestock from communal areas into former commercial farms and the breakdown of fences between them. The decrease in the support to small-holder farmer dipping by the government of Zimbabwe, the legal and illegal cattle movements could have dire consequences to the local livestock industry in terms of increased tick associated disease risks and lead to increased interface between the ticks; *A. hebraeum* and *A. variegatum*. These consequences will likely impact the epidemiology of bovine dermatophilosis and affect the small-holder farmers' coping mechanisms with regards to the control of ticks and bovine dermatophilosis. In such a scenario farmers might be forced to rely more on ethno-veterinary practices for the control of ticks and dermatophilosis. Ethno-veterinary practices are the medicinal plants, surgical techniques and traditional management practices used by farmers to prevent and treat a spectrum of livestock diseases (Mathius-Mundy *et al.,* 1989 cited by Mwale *et al.,* 2009). Farmers resort to ethno-veterinary methods of disease control due to the inadequacy of conventional veterinary medicine to meet basic animal health care in a sustainable way (Mathius *et al.,* 1996).

The objective of this research was therefore to determine the ethno-veterinary practices farmers used to treat bovine dermatophilosis and control ticks including the *Amblyomma*

ticks, the interface pattern of *A. variegatum* and *A. hebraeum* at locations where these two ticks interface and its impact on the epidemiology of dermatophilosis.

While research has been conducted on the distribution and some ecological aspects of *A. hebraeum, A. variegatum* and bovine dermatophilosis, however, further research needs to be conducted to characterise how the interface of the two ticks impacts the epidemiology of bovine dermatophilosis and the ethno-veterinary practices that farmers have adopted to manage bovine dermatophilosis infections and tick infestations.

1.2 Justification

Amblyomma variegatum has traditionally been reported in the north-west and from some pockets in the north-eastern border of Zimbabwe. The northward spread of *A. hebraeum* implies an increased interface between it and *A. variegatum*. Investigations of how this zone of interface impacts the epidemiology of bovine dermatophilosis will inform animal health authorities on how to manage the disease and the ticks. Knowledge of the alternative control methods; in the form of ethno-veterinary practices, for the treatment of bovine dermatophilosis and control of ticks including the *Amblyomma* spp. would assist the DVS as it faces challenges in providing a comprehensive dipping service to small-holder farmers. These ethno-veterinary practices can therefore complement government efforts. Currently there is no documented information on the pattern of bovine dermatophilosis at the ticks' interface and on the ethno-veterinary methods small-holder farmers use to treat bovine dermatophilosis; hence the need for such a study.

1.3 Main Objective

The overall objective of this study was to determine ethno-veterinary practices adopted by farmers to manage bovine dermatophilosis and ticks including *Amblyomma* ticks, how the interface of *A. variegatum* and *A. hebraeum* impacts bovine dermatophilosis at selected diptanks where the interface occurs in Njelele, Zhombe and Shamrock small-holder areas of Zimbabwe.

1.4 Specific Objectives

1. To document the ethno-veterinary practices used by small-holder farmers to treat bovine dermatophilosis and control ticks including *Amblyomma* spp.
2. To determine the antimicrobial properties of medicinal plants used by small-holder farmers to treat bovine dermatophilosis.
3. To determine the acaricidal properties of plant remedies used by small-holder farmers to control ticks including *Amblyomma variegatum* and *Amblyomma hebraeum*.
4. To assess the *in vivo* therapeautic properties of medicinal plants used by small-holder farmers for the treatment of bovine dermatophilosis.
5. To determine the point prevalence and risk factors of bovine dermatophilosis at the *A. variegatum* and *A. hebraeum* interface.

1.5 Hypotheses

The following null hypotheses were tested

1. Farmers do not use local remedies and herbs to treat bovine dermatophilosis and for the control of ticks.

2. Local medicinal plants have no antimicrobial effects against *Dermatophilus congolensis*.
3. Local plant remedies have no acaricidal effects on ticks.
4. Local medicinal plants have no therapeutic properties on the disease bovine dermatophilosis.
5. Interface of *A. variegatum* and *A. hebraeum* does not change the epidemiology of bovine dermatophilosis.

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Chapter 2: Literature Review

2.1 Introduction

National tick surveys were conducted in Zimbabwe in 1988-1991 and again in 1996 (Peter *et al.,* 1998b). These surveys and previous work (Norval, 1981; Norval, 1983a; Chatikobo *et al.,* 2001) identified the genera *Amblyomma, Rhipicephalus* and the subgenus *Rhipicephalus (Boophilus)* as the most important ticks associated with major tick-borne diseases (TBDs) and bovine dermatophilosis as a major tick associated disease (Chatikobo *et al.,* 2004; Chatikobo *et al.,* 2009). The most important species in the genus *Amblyomma* include *A. hebraeum* and *A. variegatum*.

2.2 Amblyomma hebraeum

In Zimbabwe the most commonly occurring *Amblyomma* species is *A. hebraeum*, which in the adult stage is parasitic on cattle and other medium to large-sized ungulates, leopards and ostriches; immature stages feed on ungulates, carnivores and tortoises (Norval 1983a; Horak *et al.,* 1987; Dower *et al.,*, 1988). *Amblyomma hebraeum* goes through one generation a year or one every three years depending on the climate (Petney *et al.,* 1987). The distribution of *A. hebraeum* is from central Zimbabwe southwards into South Africa, eastern Swaziland, southern Mozambique and eastern Botswana (Petney *et al.,* 1987). *Amblyomma hebraeum* is the principal vector of heartwater in Zimbabwe. In the northern parts of the country *Amblyomma hebraeum* is replaced by *A. variegatum* (Norval *et al.,* 1994).

2.3 *Amblyomma variegatum*

Amblyomma variegatum is a three-host tick, it is one of the most important and widely distributed of the *Amblyomma* ticks (Walker *et al.,* 1987). All stages of the tick infest cattle, sheep and goats. *Amblyomma variegatum* goes through one generation per year although two to three generations per year are possible (Petney *et al.,* 1987). The distribution of this tick extends from north-western Zimbabwe, the central highveld and on the eastern border of the country, to central and northern Mozambique (Norval 1983a; Peter *et al.,* 1998a). Petney *et al.,* (1987) reported that this tick was absent from drier arid areas. Adults of this tick are present throughout the year on cattle and buffalo although infestations are heavier during the wet warm months of September to May, while nymphs of this tick are only found between June and December (Petney *et al.,* 1987).

Of importance when considering *A. hebraeum* and *A. variegatum* in Zimbabwe, is the fact that these two species have an area of overlap (Peter *et al.,* 1998a). It is reported that in an area of overlap *A. variegatum* completely replaces *A. hebraeum* over a period of three years (Norval *et al.,* 1994). In these areas of overlap there is mating between *A. variegatum* and *A. hebraeum* with the production of sterile hybrids.

2.4 Physical damage and economic losses due to ticks

Ticks are associated with important animal-health problems and cause severe economic losses to the livestock industry worldwide. In addition, as ecto-parasites, they contribute to

reduced productivity in cattle (Young *et al.*, 1997). Ticks cause very high economic losses from damages to skins and hides. Furthermore, there is widespread consensus among animal-health professionals and livestock owners that ticks are major causes of udder damage, a predisposing factor to mastitis (Regassa, 2001). Tick feeding can reduce live mass gain, milk yield and hide quality, provide portals of entry for secondary bacterial infections and for myiasis inducing larvae (Meltzer *et al.,* 1995; Dreyer *et al.,* 1998).

Tick-induced diseases and productivity losses have inflicted large costs on African beef and dairy producers (Samui *et al.,* 1990; Meltzer *et al.,* 1995; Young *et al.,* 1997). Norval (1983b) argued that while it was accepted that ticks caused direct damage to cattle, damage had to be considered rationally in the light of assessing the economic costs of damage compared to the costs of controlling the ticks. In essence the point is that damage should not be looked at in isolation but the economic scenario has to be considered also.

2.5 Bovine dermatophilosis

Bovine dermatophilosis is an important disease of cattle in Africa, it was first reported in Belgian Congo in 1915 (Stewart, 1972; Oppong, 1996). It has been reported in most countries in the continent of Africa.

Bovine dermatophilosis is a tick associated disease caused by an actinomycete bacterium called *Dermatophilus congolensis* (Hadrill *et al.,* 1994). According to Koney *et al.,* (1996) there are multiple predisposing and precipitating factors leading to the development of bovine dermatophilosis chief among them being infestation with *A. variegatum* ticks. Other important predisposing factors are continuous wetting of the skin and intercurrent disease such as

trypanosomiasis, which lower the resistance of animals (Plowright, 1956). The disease can occur in tick-free animals but is more severe in those that are infested by the *A. variegatum* ticks (Ambrose, 1996). Walker (1996), stated that *A. variegatum*'s role in the development of bovine dermatophilosis was through immunosuppression. Dermatophilosis is characterised by an exudative dermatitis, which can be localised or generalised (Koney, 1996), also, the lesions vary in severity. Stewart (1972) described a carrier state in cattle. Economically, bovine dermatophilosis is important due to morbidity and mortality, damage to hides and its effect on draught animal power (Samui *et al.*, 1990). In other parts of Africa it has frustrated the introduction of exotic breeds to improve meat and milk production (Koney, 1996).

2.6 Tick control methods

2.6.1 Chemical control

The traditional control of ticks and consequently the diseases they transmit or are associated with has mainly relied on the use of chemical acaricides (de Castro, 1997; Willadsen, 2006). The use of chemical acaricides has been associated with the development of resistance, residues in meat and milk and environmental contamination (Taylor, 2001; Willadsen 2006). The cost of acaricides both in terms of cost to farmers and the costs associated with research and development are disadvantages in their use (Taylor 2001).

Taylor (2001) reviewed the various ectoparasiticides (and acaricides) in use. Formamidines or amidines; these are exemplified by amitraz whose mode of action is to induce neuronal hyperexcitability and then death in parasites. Interestingly Natala *et al.*, (2005) found that Triatix, a formamidine derivative was poorly effective against all stages of the tick *A.*

variegatum. The macrocyclic lactones group is exemplified by ivermectin and doramectin, their mode of action is on □-amino butyric acid neurotransmission; they block inter-neuronal transmission leading to paralysis. Macrocyclic lactones are characterised by their affinity for fat tissues in the animal body, this leads to residual effects in body tissues and prolonged action. Organophosphates characterised by diazinon and fenthion, inhibit the action of acetylcholinesterase at cholinergic synapses leading to neuromuscular paralysis. Pyrethroids and their synthetic derivatives lead to paralysis. These were initially prepared from extracts of the pyrethrum flower and are made up of a mixture of alkaloids. Pyrethroids have little residual effect and little mammalian toxicity, examples of synthetic pyrethroids are cypermethrin, deltamethrin and flumethrin.

2.6.2 Vaccines

Vaccines have been developed for the control of certain tick species mainly *Rhipicephalus boophilus* spp. (Valle *et al.*, 2004; de la Fuente *et al.*, 2007). Vaccines that have been developed act by reducing; the number of engorging females, their reproductive capacity and leads to a reduced larval infestation in the next generation (Willadsen, 2006). Commercially available vaccines against the *R. boophilus* tick are GAVAC™ and TickGARD™ used in Cuba and Australia respectively (de Castro, 1997). Vaccines have advantages over chemical acaricides in that ticks do not develop resistance and they do not have adverse environmental effects (de la Fuente *et al.*, 2007).

2.6.3 Biological tick control (entomopathogenic fungi)

Several entomopathogenic fungi that are naturally associated with ticks have been found experimentally, to be virulent against the ticks (Fernandes *et al.,* 2008). Of the most studied, *Metarrhizium anisopliae* and *Beauveria bassiana* have demonstrated the highest virulence (Fernandes *et al.,* 2008). Kaaya *et al.,* (2000) reported that the entomogenous fungi *B. bassiana* and *M. anisopliae* induced 100 % mortalities in *R. appediculatus* and *A. variegatum* larvae while this was 80 – 100 % and 80 % - 90 % in nymphs and adults respectively. A mixture of *M. anisopliae* and *B. bassiana* was found to induce higher tick mortalities than the two fungi applied separately; this implied the potential for using the fungi simultaneously for better effect (Maranga *et al.,* 2005).

The fungi kill ticks through penetration of the cuticle, invasion of internal organs, production of mycotoxins (Fernandes *et al.,* 2008). Entomogenous fungi can be applied on pastures to reduce the population of immature stages or on the host (Polar *et al.,* 2008). The advantage of the pasture application is that the fungal infection might become an epizootic and hence infect a large population of immature stages, its disadvantages are that it needs to be applied regularly and on a wide area. According to Polar *et al.,* (2008) entomopathogenic fungi provide an organic-farmer friendly alternative to chemical acaricides.

2.6.4 Ethno-veterinary methods

These are the medicinal plants, surgical techniques and traditional management practices used by farmers to prevent and treat a spectrum of livestock diseases (Mathius-Mundy *et al.,* 1989 cited by Mwale *et al.,*, 2009). Farmers resort to ethno-veterinary methods of disease control due to the inadequacy of conventional veterinary medicine to meet basic animal health care in a sustainable way (Mathius *et al.,* 1996). Also, economically disadvantaged farmers do not have

access to modern veterinary medical care which could be expensive and often not available when needed (Ghotge *et al.,* 2002).

2.6.5 Plants used in ethno-veterinary medicine

Experiments, both *in vitro* and *in vivo* have been conducted to determine the efficacy and safety of a number of plant extracts against endo-, ecto-parasites and bacteria. Bagavan *et al.,* (2009) investigated the adulticidal and larvicidal efficacy of *Annona squamosa* , *Centella asiatica, Gloriosa superba, Mukia maderaspatensi*s, *Pergularia daemia* and *Phylanthus emblica*. All the plant extracts showed toxic effects on ticks, flukes and larvae of malaria mosquitoes on which they were tested. In Zimbabwe *Lippia javanica* was effective against cattle ticks but was not as good as the amitraz-based Tickbuster® (Madzimure *et al.,* 2011).

Aloe Marlothii leaves given orally to cattle had no significant effect on the tick *Boophilus decoloratus* (Spickett *et al.,* 2007). Moyo *et al.,* (2009) found that extracts of *Aloe ferox, Ptaerolyxon obliquum* and *Tagetes minuta* had no significant effects on cattle tick burdens when applied topically. Extracts from *Azadirachta indica* had a significant efficacy against adults of *Boophilus microplus* and significantly inhibited egg laying and hatching (Srivastava *et al.,* 2008). Efficacy and reduction in egg production were significant after use of extracts of *Annona squamosa* seeds; in the same trial *Azaridachta indica* performed better than *A. squamosa* (Magadun *et al.,* 2009). Besides being toxic to ticks, plants can also act as tick attractants a property shown by *Calpurnia aurea* on *Rhipicephalus appendiculatus* and *R. pulchellus* (Nana *et al.,* 2010); this makes them potential components of acaricide-impregnated tags used to control ticks.

2.7 Bovine dermatophilosis control and treatment

2.7.1 Conventional methods

The treatment of bovine dermatophilosis is mainly through the use of penicillin, streptomycin and dihydrostreptomycin (Ilemobade *et al.,* 1979; Coetzer *et al.,* 1994; Awad *et al.,* 2008). Antibiotics can be used alone, in combination with other antibiotics or in combination with ectoparaciticides and local anti-infectives (Arowolo *et al.,* 1987). Self-cure occurs in 20 % of untreated affected animals during the dry season (Ogwu *et al.,* 1981). Hadrill *et al.,* (1996) stated that the use of acaricides was the best option for the control of bovine dermatophilosis. Amitraz based acaricides applied on the predilection sites of *A. variegatum* ticks on cattle reduced the prevalence of bovine dermatophilosis (Morrow *et al.,* 1993; Morrow *et al.,* 1996) as did deltamethrin. Chatikobo *et al.,* (2001), reported that plunge dipping might in fact increase the spread of the disease while hand spraying produced the best results.

2.7.2 Ethno veterinary methods for the treatment of bovine dermatophilosis

High costs of chemotherapeutic drugs for the control of bovine dermatophilosis have led some researchers to investigate the efficacy of local herbs for the treatment of this condition (Imam *et al.,* 2008). Alcoholic leaf extracts of *Sanna lata, Lantana camara* and *Mitracarpus scaber* led to healing of dermatophilosis lesions when applied topically (Ali-Emmanuel *et al.,* 2003). Home remedies have also reportedly been used to treat bovine dermatophilosis Stewart (1972); Arowolo *et al.,* (1987).

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Chapter 3: Ethno-veterinary control of bovine dermatophilosis and ticks in Zhombe, Njelele and Shamrock resettlement in Zimbabwe

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Abstract

A structured questionnaire survey was conducted to determine the ethno-veterinary practices and other control methods used by small-holder farmers for the management of bovine dermatophilosis and ticks. A total of 153 farmers were interviewed from Njelele, Zhombe communal and Shamrock resettlement areas. Crop production contributed most to livelihoods (83.2 %) while livestock contributed 9.0 %. Over 90 % of the respondents had attended school up to primary level, with 11.4 % undergoing animal health and husbandry training. Treatment of livestock diseases was practiced by 96 % of the farmers and 49.7 % of these farmers used ethno-veterinary medicines. Across the study sites, dermatophilosis was controlled using the following plants; *Cissus quadrangularis* (59.7 %), *Catunaregan spinosa* (10.5 %), *Pterocarpus angolensis* (10.5 %), *Kalanchoe lanceolata* (5.3 %), *Aloe chabaudii* (3.5 %), *Cassia abbreviata* (1.8 %), *Dichrostychis cenerea* (1.8 %), *Urginea sangueana* (1.8 %), *Xinemia caffra* (1.8 %) and a plant locally called *umfanawembila* (1.8 %). *Carica papaya, Manihot esculenta* and *Blumea decurrens*, were used for tick control and these were reported once from Njelele communal. Other control methods, besides plants or conventional drugs, were used by 28 % of the farmers for the treatment of dermatophilosis and ticks. Some farmers (14.4 %) claimed that ethno-veterinary medicines performed better than conventional drugs. The study revealed that farmers used ethno-veterinary medical practices and this use was very minimal for tick control.

Keywords: Indigenous knowledge, medicinal plants, practices, demographics

3.1 Introduction

Ticks are the most economically important pests of cattle in the tropics and subtropics as infestation results in reduced growth rates and milk production, paralysis and transmission of tick-borne pathogens (Muhammad *et al.,* 2008). Ticks are responsible for direct damage to livestock due to their feeding behavior (Jongejan *et al.,* 2004) and indirectly as a result of their association with some important livestock diseases such as dermatophilosis (Plowright, 1956). Bovine dermatophilosis is a tick associated disease caused by an actinomycete bacterium called *Dermatophilus congolensis* (Hadrill *et al.,* 1994). In Zimbabwe tick infestations, tick-borne diseases (TBDs) and tick associated diseases such as bovine dermatophilosis are important conditions affecting livestock health and productivity (Norval, 1979; Lawrence *et al.,* 1980; Chatikobo *et al.,* 2009). .

In Zimbabwe, the control of ticks and indirectly, the associated diseases is the responsibility of the Department of Veterinary Services and this has always been one of its major activities (Norval, 1979; Norval *et al.,* 1992). The Department of Veterinary Services (DVS) has faced budgetary constraints; this has affected dipping-service delivery leading to frequent disruptions of dipping activities. The latter has had an effect on the tick dynamics and associated diseases in the affected areas as happened with disruption of dipping during the liberation war years (Norval, 1979; Lawrence *et al.,* 1980). The decrease in the support to small-holder farmer dipping by the government of Zimbabwe and the current economic

challenges faced by the country could have dire consequences to the local livestock industry in terms of increased tick associated disease risks. These consequences will likely affect the small-holder farmers' coping mechanisms with regards to the control of ticks and bovine dermatophilosis. In such a scenario farmers might be forced to rely more on ethno-veterinary practices for the control of ticks and dermatophilosis. Ethno-veterinary practices are the medicinal plants, surgical techniques and traditional management practices used by farmers to prevent and treat a spectrum of livestock diseases (Mathius-Mundy *et al.,* 1989 cited by Mwale *et al.,* 2009). Farmers resort to ethno-veterinary methods of disease control due to the inadequacy of conventional veterinary medicine to meet basic animal health care in a sustainable way (Mathius *et al.,* 1996).

The objective of the study was to document the ethno-veterinary practices farmers used to treat bovine dermatophilosis and ticks in selected small-holder sectors of Zimbabwe.

3.2 Materials and methods

3.2.1 Study sites

Three dip tanks were randomly selected from among 13 dip tanks where *A. variegatum* and *A. hebraeum* had been reported to co-exist (Peter *et al.,* 1998) and from districts where bovine dermatophilosis had been reported in the past. The dip tanks were; Shamrock resettlement in Chegutu (18° 21' S 30° 36' E), Njelele in Gokwe South (18° 9' S 28° 16' E) and Zhombe in Kwekwe (18° 7' S 29° 26' E). The study sites are found in the following agro-ecological regions: region 3 (Shamrock); characterised by intensive farming and

moderate rainfall and region 4 (Njelele and Zhombe); characterised by semi-intensive farming and moderate to erratic rainfall, (Hove *et al.*, 2008).

3.2.2 Data collection

Personal interviews through a structured questionnaire were used to collect data on stock-owner household demographics, livestock figures, common diseases and local control practices which included plants used; how , when, which parts and who applied them, for the control of bovine dermatophilosis and ticks. A total of at least 50 farmers who owned cattle were selected purposively and interviewed.

3.2.3.1 Livestock inventory

The minimum, maximum and median of selected livestock owned per household was computed across all the study sites. The mean (μ) and standard deviation (σ) of cattle owned per household throughout all study sites were computed. These two statistics were used to categorize cattle ownership by herd size as follows; small-sized (< μ- σ), medium-sized (μ± σ) and large-sized herd (> μ+σ). For the other species (i.e. sheep, goats and donkeys), ownership was categorized into whether households kept them or not.

3.2.3.2 Ranking of diseases and classification of disease control methods

Farmers were asked to indicate diseases which they had encountered and rank them according to importance from 1 (most important) to 7 (least important). Disease control methods were classified into two categories; ethno-veterinary and conventional practices. Ethno-veterinary practices included plants or herbs and alternative methods that excluded conventional drugs, chemicals and plants or herbs. Conventional methods were those practices that were ordinarily prescribed and authorized for use in the management of diseases by animal health authorities. The plants used were initially identified by their local names which included Shona and siNdebele names by the respondents; further identification and authentication was performed by botanists at the National Herbarium & Botanical gardens of Zimbabwe in Harare.

3.2.3 Data analysis

The collected data were captured onto Microsoft Access software as a relational database. Descriptive statistics, cross-tabulations, the Two- Sample-Proportion-Test for independent groups in Stata 11 SE (Stata SE, 2009 version 11) was used to measure differences in proportions between categories (age distribution, level of education, animal health training status and herd size). The chi-square test was used to evaluate associations between study site, education and plant use. Kendall's rank correlation tests were used to evaluate important diseases and the the uses to which animals were put using the Statistical package for Social Scientists software version 16 (SPSS, 2007).

3.3 Results

3.3.1 Socio-demography

Zhombe had more older (41 years and above) people (37.1 %) while Shamrock had fewer older people (16.6 %) (Table 3.1). The median household size was 4 (range 1-10). There was a larger population in the younger age group than in the older age group across all study sites (Table 3.1). Over 90 % of the household members had some form of formal education up to secondary level (Table 3.2), education level was associated with study site ($p < 0.001$). Most of the household members (88.7 %) did not have certifiable qualifications in animal husbandry and health with 9.9 % having undergone master farmer training; which entailed basic training on animal health, crop and animal husbandry with the issuance of a certificate of attendance , training obtained was significantly associated with study sites ($p = 0.002$).The major source of income was from crops (83.2 %) followed by livestock (9.0 %) and this did not differ across study sites ($p = 0.162$).

3.1 Demographic distribution of study sites

Factor	Level	Shamrock (N=50)	Njelele (N=50)	Zhombe (N=53)	Total
Age category	=< 25 yrs	135 (49.1 %)[a1]	148 (57.8 %)[a1]	90 (41.7 %)[b1]	373 (49.9 %)
	26 - 40 yrs	95 (34.5 %)[a2]	57 (22.3 %)[b2]	45 (20.8 %)[b2]	197 (26.4 %)
	41 - 55 yrs	22 (8.0 %)[a3]	33 (12.9 %)[a3]	31 (14.4 %)[a2]	86 (11.5 %)
	56 - 70 yrs	18 (6.5 %)[a3]	14 (5.5 %)[a4]	35 (16.2 %)[b2]	67 (9.0 %)
	70 + yrs	5 (1.8 %)[a4]	4 (1,6 %)[a5]	15 (6.9 %)[b3]	24 (3.2 %)

Figures with a different alpha superscript in a row are significantly different at $p<0.05$

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Table 3.2 Education and training background across study sites

Level of education	Level	Shamrock	Njelele	Zhombe	Total
	N/A	28 (10.2 %)[a]	17 (6.6 %)[b]	4 (1.9 %)[c]	49 (6.6 %)
	Never_attended	11 (4.0 %)[a]	2 (0.8 %)[b]	6 (2.8 %)[ab]	19 (2.5 %)
	Primary	103 (37.5 %)[a]	136 (53.1 %)[b]	106 (49.1 %)[a]	345 (46.2 %)
	Secondary	125 (45.5 %)[a]	98 (38.3 %)[b]	96 (44.4 %)[b]	319 (42.7 %)
	Tertiary	7 (2.5 %)[a]	2 (0.8 %)[b]	1 (0.5 %)[b]	10 (1.3 %)
	University	1 (0.4 %)[a]	1 (0.4 %)[a]	3 (1.4 %)[a]	5 (0.7)
Animal Health/Husbandry training	N/A	86 (31.3 %)[a]	90 (35.2 %)[a]	50 (23.1 %)[b]	226 (30.3 %)
	None	170 (61.9)[a]	124 (48.4 %)[b]	143 (66.2 %)[ab]	436 (58.4 %)
	Master_Farmer	16 (5.8 %)[a]	38 (14.8 %)[b]	20 (9.3 %)[a]	74 (9.9 %)
	Certificate_Agric/ Vet	3 (1.1 %)[a]	2 (0.8 %)[a]	1 (0.5 %)[a]	6 (0.8 %)
	Diploma_Agric/Vet	0 (0.0 %)	1 (0.4 %)	0 (0.0 %)	1 (0.1 %)
	Degree_Agric/Vet	0 (0.0 %)	1 (0.4 %)	2 (0.9 %)	3 (0.4 %)

Figures with different superscripts in a row are significantly different at $p<0.05$

3.3.2 Livestock inventory

Of the households interviewed, 128 (83.7 %) indicated that livestock were owned by the father, 15 (9.8 %) claimed ownership by the mother and 3 (2 %) joint ownership. Cattle; herd sizes averaged 9 ± 6.3 (S.D.), median 7 and range 2 - 43, sizes were different across households ($p < 0.001$). Cattle ownership pattern (herd size) did not differ across study sites (Table 3.3). The majority of farmers 106 (69.3 %) kept goats, with a median of 3 and range 0 - 32. Thirteen (8.5 %) farmers kept donkeys and 2 % kept sheep. Cattle were regarded to be of great importance for draught power especially for tillage (Kendall rank - 2.21), while spiritual and sale of hides and horns ranked 9.95 and 10.35 respectively, indicating their less importance.

3.3.3 Diseases

A total of 14 diseases were cited and ranked according to importance by the farmers as indicated in Table 3.4. There was a significant association between disease and ranking ($p < 0.001$). Dermatophilosis was ranked as very important by 36.3 % of the respondents while 30.1 % and 14.0 % of respondents ranked skin diseases and tick infestation as being very important respectively. Conversely, 16.9 %, 1.5 % and 6.2 % ranked dermatophilosis, skin diseases and tick infestations as not important respectively.

Table 3.3 Cattle herd size by study site

Herd size	District			
	Shamrock	**Njelele**	**Zhombe**	**Total**
Low (< μ- σ)	6 (12.0 %)[a]	7 (14.0 %)[a]	3 (5.7 %)[a]	16 (10.5 %)
Medium (μ± σ)	42 (84.0 %)[b]	37 (74.0 %)[b]	45 (84.9 %)[b]	124 (81.0 %)
High (> μ+σ)	2 (4.0 %)[a]	6 (12.0 %)[a]	5 (9.4 %)[a]	13 (8.5 %)

Figures with a different superscript in a column are significantly different at $p<0.05$

Table 3.4 Diseases ranking by farmers

Disease condition	Mean Rank
Skin diseases	2.67
Dermatophilosis	3.33
Tick-borne diseases	3.64
Tick infestation	4.06
Weight loss	4.28
Diarrhoea	5.03
Reproductive problems	9.36
Blackleg	9.51
Behavioural abnormalities	9.54
Opthalmia	9.84
Mastitis	9.88
Respiratory problems	9.91
Anthrax	9.94

3.3.4 Control of dermatophilosis, ticks and other diseases

One hundred and forty seven (96.0 %) respondents indicated that they treated their animals for various diseases and conditions. Of these, 49.7 % used plants and other alternative methods, which varied significantly across study sites ($p < 0.001$). Significantly fewer respondents (24.0 %) from Shamrock resettlement reported use of ethno-veterinary practices compared to those from Njelele (57.8%) and Zhombe (67.3 %) communal lands.

3.3.4.1 Plant use in the treatment of dermatophilosis

Most plants (39.6 %) were used for the control of dermatophilosis followed by tick-borne disease control (23.6 %) while 2.1 % were for tick control (Figure 3.1). Plant use citation was associated with study site ($p = 0.004$). For dermatophilosis, 70 %, 26. 3 % and 3.5 % citations were from Zhombe, Njelele and Shamrock respectively. The only three (2.1 %) citations for tick control were from Njelele. A total of 39 plants were used by farmers for various diseases, eleven of these plants were used for the control of dermatophilosis while three were for tick control; various methods were used to prepare and apply these (Table 3.5).

Figure 3.1 Diseases and conditions for which plants were used

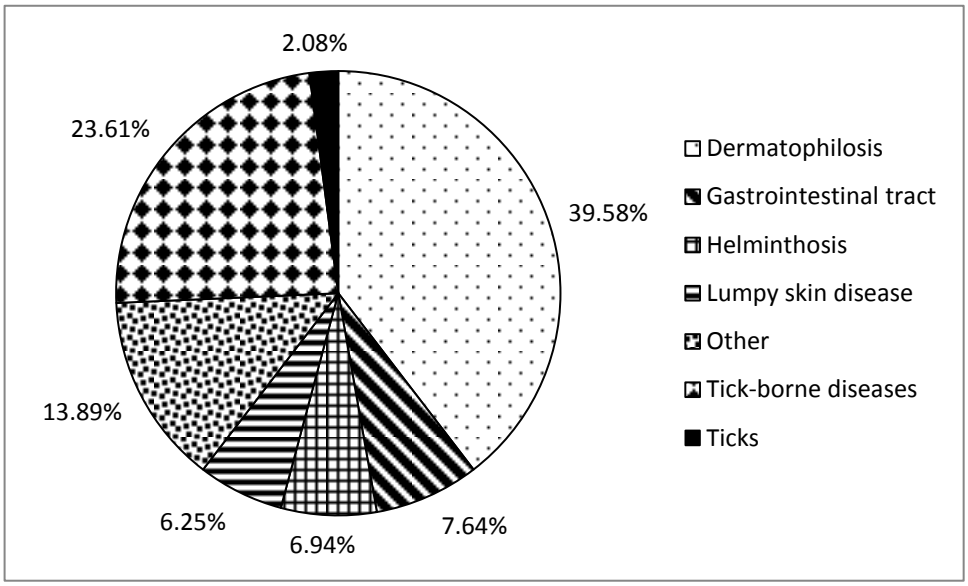

Table 3.5 Preparation and use of plants

Local name	Scientific name	General use	Part used	Preparation	Application	Citations
Murunjurunju (S)	*Cissus quadrangularis*	Dermatophilosis	Stem	Crush or mix with 750 mls of water for thirty minutes	Squeeze and rub onto affected area, drench	36
Chirovaduwuro (S)	*Catunaregan spinosa*	Dermatophilosis	Root, bark	Finely crush and mix with water	Drench	6
Mubvamaropa (S)	*Pterocarpus angolensis*	Dermatophilosis	Bark	Pound and soak in water	Oral	6
Ntelezi (N)	*Kalanchoe lanceolata*	Dermatophilosis	Bulb/stem	Crush	Rub directly into lesions	3
Gavakava (S)	*Aloe chabaudii*	Dermatophilosis	Leaves, stem	Crush and mix with water	Apply directly on affected site,drench	2
Isagenama (N)	*Urginea sangueana*	Dermatophilosis	Bulb	Use bulb	Rub on affected part	1

Mugimbura (S)	Yet to be identified	Ticks	Leaves	mix with water for 12 hours	Spray	1
Mukaya (S)	*Acacia* spp	Dermatophilosis	Bark	Crush and mix with water	Drench	1
Mupangare (S)	*Dichrostychis cenerea*	Dermatophilosis	Fruits	Make powder	Apply on skin	1
Mupopo (S)	*Carica papaya*	Ticks	Leaves	Mix with water for 12 hours	Spray	1
Murumanyama (S)	*Cassia abbreviata*	Dermatophilosis	Bark	Crush and mix with water	Drench, rub on area	1
Tsvanza (S)	*Xinemia caffra*	Dermatophilosis	Leaves	Make powder	Apply on skin	1
Umdungudungu (N)	Yet to be identified	Ticks	Leaves	crush and put in water	Spray	1
Umfanawembila (N)	Yet to be identified	Dermatophilosis	Tubes	Grind and mix with water	Oral	1

N = SiNdebele name, S = Shona name

3.3.4.2 Non-plant alternative materials used for disease and tick control

Forty four (28.8 %) farmers reported use of alternative treatment methods that excluded plants and conventional methods. There were 82 citations for use of alternative treatments. Of the citations, 44 were for the control of dermatophilosis (59.1 %) and ticks (40.1 %) and the remainder being for the management of wounds, anaplasmosis, redwater, screwworm, footrot, sweating sickness and blackleg (Table 3.6).

Table 3.6 Non-plant alternative materials used for dermatophilosis and tick control

Materials	Farmers using(%)		Preparation and application
	Dermatophilosis (N = 26)	Ticks (N = 18)	
Mechanical grease	34.6	5.6	Topically as used paste
Used engine oil	34.6	5.6	Topically as discarded liquid
Paraffin (illuminating)	11.5	44.4	Topically
Ammonium nitrate	7.7	0	Topically as a paste, 3 standard tea cups mixed with 4 litres of water
Soap	7.7	0	Topically, powder or tablet with water
Water	3.8	0	Topically, warm water
Fenkill	0	27.8	Topical spray, 30 ml mixed with 15 – 20 litres of water
Cow dung	0	5.6	Topically as a paste
Ash	0	5.6	Topical as is in dry form
Carbaryl	0	5.6	Topical spray, 30 mg mixed with 4 litres of water

3.3.4.3 Farmer opinions of different control methods

Most (85.6 %) farmers reported that conventional medicines were more effective and disease specific than ethno-veterinary medicines, 14. 4 % reported otherwise. Farmers preferred ethno-veterinary practices as these were claimed to be cheap, locally available and good for first aid treatment. Seventy five respondents indicated that students at agricultural colleges or universities should be taught about ethno-veterinary practices for the control of diseases.

3.4 Discussion

The larger younger population compared to adults in this survey has implications on the adoption and use of ethno-veterinary practices; where younger people will not readily accept these practices leading to a possible loss of this indigenous knowledge by subsequent generations. Yeneger *et al.,* (2008) reported that young people were reluctant to adopt and use ethno-veterinary practices, furthermore the young respond this way since to them there was no demonstrated knowledge that these practices worked. The education levels were high and consistent with those reported by CSO *et al.,* (2007) in which over 90 % of respondents had attended school. A small percentage of farmers underwent some formal training in animal husbandry; this fact could mean that few farmers were trained on the use of conventional treatment methods as such they would easily resort to using ethno-veterinary practices. Consistent with the study areas which were cotton growing areas, the major source of income was crops with livestock playing a major role in tillage but a minor one as a direct source of income this was consistent with reports by Zaibet *et al.,* (2011), and those of Mwale *et al.,* (2005) who indicated that both livestock and crops played a major role in the communities they had worked in.

Zimbabwe is reportedly a patriarchal society (Muchadeyi *et al.*, 2005), ownership of cattle is vested in the male household head as was shown in the current survey. Few women owned cattle in the study; this was consistent with assertions by Mupawaenda *et al.,* (2009) that women mainly owned chickens. The mean cattle herd sizes in the study were slightly higher than those found by Chatikobo *et al.*, (2001) in a neighbouring communal land of Sanyati while it was lower than that reported by Mwale *et al.,* (2005) in the southwestern parts of Zimbabwe. The different herd sizes could be attributed to the different farming systems as the latter study was undertaken in a small scale commercial farming area while the current study combined communal and new resettlement areas. In communal lands due to a higher population density livestock numbers could be limited. Cattle had multiple uses although they were used mainly in crop production as draught power and consumed in the form of milk which was consistent with findings by Mupawaenda *et al.,* (2009).

Dermatophilosis, skin diseases and tick infestations were ranked as some of the most important diseases by farmers; this was similar to findings by Chatikobo *et al.,* (2001) who reported that tick-borne diseases (TBDs), dermatophilosis and parafilariasis, a skin disease, were ranked as most important in the neighbouring Sanyati communal lands. High citations for tick infestations were consistent with the diseases reported such as TBDs and dermatophilosis which are associated with ticks.

Almost 50 % of farmers used ethno-veterinary methods to manage diseases. This finding was in agreement with the findings of Mwale *et al.,* (2005) who, in Zimbabwe found 45.7 % respondents used ethno-veterinary medicines, but lower than the 83.3 % reported by Mwale *et al.,* (2009) in South Africa. The difference could be due to the fact that in South Africa the

farmers were poultry farmers and in Zimbabwe they were cattle farmers. Cattle farmers usually use western medicines to manage diseases due to the high value associated with cattle compared to chickens. Kalawole *et al.,* (2007) in Nigeria reported 5 - 80 % usage of selected ethno-veterinary medicines. The wide range in the Nigerian study could be due to different statuses of the respondents as 10 different communities were interviewed. Although the use of ethno-veterinary methods was relatively widespread, this was mainly for dermatophilosis than for ticks. This could have been due to the fact that the government of Zimbabwe provided subsidized dipping of cattle in the small-holder sector; farmers were therefore not inclined to use alternative methods for tick control. For dermatophilosis and other diseases there was no such "free" facility. The difference between study sites in the use of ethno-veterinary methods could be due to the differences in age structures between study sites. Zhombe and Njelele communal lands had a larger proportion of people in the older age group than Shamrock; as postulated by Yeneger *et al.,* (2008), young people were reportedly reluctant to use ethno-veterinary methods.

Respondents used a variety of plants for the management of bovine dermatophilosis, ticks and other diseases. *Cissus quadrangularis* was the major plant cited for the control of dermatophilosis. *Cissus quadrangularis* is described as a fleshy cactus-like climber distributed in the tropics and also found in Zimbabwe (Mishra *et al.,* 2009; Mullins, 2006). Mohanty *et al.,* (2010); Vijay *et al.,* (2010) reported it to have fungicidal, anti-helmintic, wound healing, antipyretic and anti-inflammatory properties. Its effects on dermatophilosis could be due to its anti-inflammatory and antimicrobial effects as this disease is characterised by cycles of inflammation in the epidermis (Amakiri, 1974; Ambrose, 1996) and *Dermatophilus congolensis*' growth characteristics are similar to those of fungi. *Catunaregan spinosa* the next common plant used has reportedly been used in the management of skeletal

disorders (Rout *et al.,* 2009; Rajakaruna *et al.,* 2002) and was also found to be effective against some gram positive bacteria, both in India. *Pterocarpus angolensis* has been used to manage bleeding, coughs and leg pains by Namibian traditional healers (Cheikhyoussef *et al.,* 2011), it has further been found to have antibacterial and anti-protozoal effects (Samie *et al.,* 2009). The sap extract of *Aloe chabaudii* has been found to be effective against gram positive and gram negative bacteria and fungi (*Candida albicans*) (Mbanga *et al.,* 2010). *Dermatophilus congolensis* is a gram positive fungus-like actinomycete hence could similarly be susceptible to *A. chabaudii*. The shrub *Dichrostachys cinerea* has antibacterial activities (Banso *et al.,* 2007) and analgesic properties (Mishra *et al.,,* 2009). *Cassia abbreviata* has reportedly been used to stop external bleeding, diabetes, diarrhea, sore throats and dysentery (Rao *et al.,* 2004; Setshogo *et al.,* 2011), these diseases can be associated with immunosuppression a situation similar to that of dermatophilosis, as such the plant could boost the immunity of the affected individual. *Carica papaya* which in this case was used against ticks has been reported to have anti-helmintic effects in poultry (Chota *et al.,* 2010).

Alternative materials such as paraffin, used engine oil and mechanical grease were used. This was in agreement with studies by Hlatshwayo *et al.,* (2005); Masika *et al.,* (1997) who reported common use of these materials by resource poor farmers in South Africa particularly for tick control. The danger in using these compounds is the potential toxicity to the animal, environment and consumers as they contain such elements as lead, copper, chromium and zinc (Moyo *et al.,* 2009). Ammonium nitrate use as a paste to manage dermatophilosis could have been due to the availability of this compound which is used as a fertilizer by local farmers. Carbaryl (Carbaryl 85 WP, Agricura, Zimbabwe) is a common garden pesticide and farmers might use it against ticks, when faced with financial challenges. Fenkill (Fenvalerate 20 EC, Zimbabwe Fertilizer Company, Zimbabwe) is a pyrethroid pesticide used to control

arthropod pests (WHO/FAO, 1996) and its availability to those farmers who plant cotton makes it a ready alternative for the control of ticks. Carbaryl and Fenkill are not authorised for use in cattle in Zimbabwe.

A small percentage (14.4 %) of farmers reported that ethno-veterinary practices were more effective than conventional methods; this was lower than the two thirds of farmers reported by Dold *et al.,* (2001) from the Eastern Cape. In other studies farmers have also reported plants to be effective against a variety of diseases (Nfi *et al.,* 2001; Kalawole *et al.,* 2007). Farmers also reportedly preferred ethno-veterinary medicines as these were cheaper and readily available; this was in agreement with findings by Bamikole *et al.,* (2009). Just fewer than 50 % of the farmers indicated that college and university students must be taught about ethno-veterinary practices but Kalawole *et al.,* (2007) found a non-significant association between use of ethno-veterinary medicines and roles of colleges and government.

3.5 Conclusion

Ethno-veterinary practices were used for the control of bovine dermatophilosis, ticks and other diseases by farmers in the areas studied. The relatively high use of certain plants was a measure of the farmers' perception of their effectiveness. In this study, *C. quadrangularis* was the most used plant in the management of dermatophilosis. Further research is required to assess the pharmacological effectiveness of the remedies used by farmers against *D. congolensis* their potential toxicity and safety to livestock.

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Chapter 4: *In vitro* and *in vivo* efficacy of extracts from plants used by small-holder farmers in the treatment of dermatophilosis in cattle

Abstract

Bovine dermatophilosis, an important skin disease of cattle caused by *Dermatophilus congolensis*, negatively impacts the livelihoods of small-holder farmers in Zimbabwe. This impact is through, morbidity, loss of draught animal power, costs incurred to manage the disease, losses associated with devalued damaged hides and the resultant culling of some of the affected cattle. Due to the inaccessibility of conventional drugs to manage bovine dermatophilosis, farmers have been reported to use local medicinal plants to manage the disease. The aim of the study was to evaluate the *in vitro* antimicrobial activities of three plants that small-holder farmers in Zimbabwe used to manage bovine dermatophilosis.

Dried plant materials were ground into powder and extracted individually using, water, 80 % acetone and 80 % methanol. The antimicrobial properties of the plants were evaluated against two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and one Gram-positive (*Staphylococcus aureus*) reference bacterial strains. They were further evaluated against a field isolate of *Dermatophilus congolensis*. The assays used were the disc diffusion, minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Acetone and methanol extracts had superior inhibitory activities than did those of water. *Pterocarpus angolensis* extracts had better inhibitory properties with absolute MIC values of 0.156 – 5 mg/ml, *C. quadrangularis* had MIC values in the range 0.156 – 5 mg/ml while that of *C. spinosa* was 0.156 – 10 mg/ml. *Dermatophilus congolensis* was more sensitive to *P. angolensis* average MIC = 0.63 mg/ml than to *C. quadrangularis* average MIC

= 1.25 mg/ml and *C. spinosa* average MIC = 2.08 mg/ml. These results confirm the potential antibacterial activities of extracts of the three plants and hence farmers are, in a way, justified in using the plants. Better results (lower MIC) could be obtained by extracting and evaluating pure active compounds of the plants. An *in vivo* trial was carried out to test the therapeutic effects of aqueous extracts of *C. quadrangularis* for the treatment of bovine dermatophilosis in Mashona cross-bred cattle in Chegutu Zimbabwe. A total of 16 infected cattle were randomly allocated into three treatment groups, groups 1 and 2 received tetracycline- and penicillin-based antibiotics while group 3 was sprayed with a 20% crude aqueous extract of *C. quadrangularis*. Healing was observed to occur on day 26 in the *C. quadrangularis group*. This healing was characterised by drying of the scabs. Cattle treated with conventional antibiotics were all cured by day 26 while only two in the *C, quadrangularis* group responded to treatment.

Keywords: Zimbabwe, exudative, minimum inhibitory concentration, micro-dilution, ρ-iodonitrotetrazolium

4.1 Introduction

Dermatophilosis is a contagious exudative skin disease caused by a gram-positive actinomycete, *Dermatophilus congolensis* (Dalis *et al.,* 2009; Dalis *et al.,* 2010). The disease affects a wide range of mammals including man, cattle, sheep and goats (Woldemeskal *et al.,* 2010). While affecting a variety of animals, the disease has been reported mainly from cattle in the tropics; where it causes hide damage, prolonged suffering, high morbidity and associated economic losses (Chatikobo *et al.,* 2004; Chatikobo *et al.,* 2009; Woldemeskal *et al.,* 2010; Bayisa *et al.,* 2012). In Zimbabwe, dermatophilosis is reportedly of importance in small-holder farming communities and has steadily been spreading from its traditional foci to other virgin areas (Chatikobo *et al.,* 2004; Chatikobo *et al.,* 2009).

Conventionally, the treatment of bovine dermatophilosis has been through the use of tetracyclines, penicillin and dihydrostreptomycin, separately or in combination (Arowolo *et al.,* 1987; van Tonder *et al.,* 1996; Nath *et al.,* 2010). Gentamycin, given parenteraly has been reported to be more effective than the combination of penicillin and streptomycin (Hamid ***et al.,*** 2009). Topical applications of potassium aluminium hydroxide used with parenteral applications of long acting tetracyclines provided treatment (Nath *et al.,* 2010). Due to the association of bovine dermatophilosis with *Amblyomma variegatum* ticks (Walker, 1996), tick control has been an important adjunct in the control of the disease (Hadrill *et al.,* 1996). Non-conventional or ethno-veterinary preparations have reportedly been used to manage bovine dermatophilosis. Imam *et al.,* (2008) reported that the plant, *Mitracarpus scaber*, had significant inhibitory effects against *Dermatophilus congolensis in vitro*. *Mitracarpus scaber* was also used for the treatment of eczema and other skin conditions in man (Imam *et al.,* 2008). Use of alcoholic extracts of *Sanna lata*, *Lantana camara* and *Mitracarpus scaber* have

resulted in the healing of dermatophilosis skin lesions in cattle (Ali-Emmanuel *et al.,* 2003). Makoshi *et al.,* (2011), reported complete healing of dermatophilosis lesions in 8 weeks after using a cream preparation of the plant *Tephrosia vogelii*. Non-plant alternative remedies, such as quicklime, sulphur, soap, warm water, oil-mixture of potash and torch battery contents have also been used by farmers to manage dermatophilosis (Stewart, 1972; Arowolo *et al.,* 1987).

In a recent study conducted in Zimbabwe (Ndhlovu and Masika, 2013), *C. quadrangularis*, *C. spinosa* and *P. angolensis* were reportedly used for the management of bovine dermatophilosis by smallholder farmers. *Cissus quadrangularis* is a fleshy succulent vine occurring in Asia and Africa including Zimbabwe (Mishra *et al.,* 2009; Mullins, 2006). Mishra *et al.,* (2009) reported *in vitro* antibacterial effectiveness against *Staphylococcus aureus* and *Staphylococcus cerivisiae*. Fungicidal, antihelmintic, wound healing and antiinflamatory properties of *C. quadrangularis* have also been reported (Mohanty *et al.,* 2010; Vijay *et al.,* 2010). *Cissus quadrangularis* has been reported to exhibit analgesic properties (Panthong *et al.,* 2007); such properties would alleviate the pain experienced by a clinically affected animal. Makoshi and Arowolo (2011) reported that *Tephrosia vogelli*, applied as an ointment resulted in treatment on day 3 with complete cure of dermatophilosis after 4 to 8 weeks. *Pterocarpus angolensis* is a small to medium sized deciduous tree (Ali *et al.,* 2008); Namibian traditional healers, have reportedly used it to manage bleeding, coughs and leg pains (Cheikhyoussef *et al.,* 2011) while Samie *et al.,* (2009) reported antibacterial and antiprotozoal activities. *Catunaregam spinosa* has been used for the management of skeletal disorders and was found to be effective against gram positive bacteria (Rajakaruna *et al.,* 2002; Rout *et al.,* 2009). To our knowledge no studies have been conducted to determine the antibacterial activities of the three plants against *D. congolensis*. These studies were

carried out as a follow up to a field survey (Ndhlovu and Masika, 2013) and to investigate antibacterial activities of these plants against *D. congolensis* and selected reference bacterial strains and the *in vivo* therapeutic efficacy of *C. quadrangularis*.

4.2 Materials and methods

4.2.1 Plant collection

Fresh stems of *C. quadrangularis* were collected from the bush in Chegutu while fresh bark and leaves of *P. angolensis* and *C. spinosa* were collected from Zhombe communal lands. Plant specimens were authenticated by Mr. Chris Chapano a botanist at the National herbarium and botanical gardens, Harare, Zimbabwe and stored at the National herbarium and botanical gardens as voucher specimens: DNN01-2012, DNN02-2012 and DNN03-2012 representing *C. quadrangularis, C. spinosa* and *P. angolensis* respectively.

4.2.2 Plant storage and preparation.

The plant specimens (stems and barks) were air dried at room temperature at the Faculty of Veterinary Science Toxicology laboratory and later ground to fine powder using an electric grinding mill (Fritsch Pulverisette, Germany). The resultant powders were stored in air-tight bottles and kept in a dark cool place until further processing.

4.2.3 Extraction procedure

Powdered plant samples were crudely extracted individually using sterile distilled water to prepare the following extract concentrations; 80 % acetone and 80% methanol. The extraction method using acetone and methanol was an adaptation of the methods of other studies (Shale *et al.,* 2005; Mathabe *et al.,* 2006; Konate *et al.,* 2011). Twenty grams of the respective powders were mixed with 200 ml of solvent. The extracts were left overnight on a Variomag magnetic stirrer. The extracts were then filtered through Whatman no.1 filter paper and the filtrate centrifuged in a Centaur 2 centrifuge at 3000 rpm for 10 min. The resultant supernatant was concentrated using a rotary evaporator at 40 °C and later the samples were freeze-dried using a HETO FD3 freeze dryer and kept at 4 °C until use. For the water extract, 20 grams of powder were soaked in 200 ml sterile distilled water for 24 hr. The extract was then filtered using Whatman no. 1 filter paper and later, the filtrate was freeze-dried and stored at 4 °C until use. All the extracts were weighed after freeze drying using a Sartorius electronic balance.

An amount of 3000 grams of *C. quadrangularis* were mixed with 15 l sterile distilled water to prepare 20% (w/v) stock solution for the *in vivo* trial. After stirring the mixtures on a Variomag magnetic stirrer overnight; the mixture was further strained through a muslin cloth and the resultant solution stored at 5 °C until use the following day.

4.2.4 Bacterial strains

Three reference bacterial strains, provided by the Zimbabwe National Quality Assurance Programme (ZINQAP) were used: Gram negative *Pseudomonas aeruginosa* (ATCC 25619) and *Escherichia coli* (ATCC 25922), together with Gram-positive *Staphylococcus aureus*

(ATCC 25923). These bacteria were chosen due to their availability and because they cause disease in livestock. *Pseudomonas aeruginosa* is an opportunistic human pathogen which also causes chronic mastitis in cattle, fleece rot in sheep and suppurative otitis externa in dogs (Kingsford *et al.*, 1997; Tron *et al.*, 2004). *Escherichia coli* is an economically important inhabitant of the gastrointestinal tract of ruminants (Henton *et al.*, 1996) causing diarrhoea in calves and is associated with abscessation. Hides are the most important source of carcass contamination (Arthur *et al.*, 2007; Arthur *et al.*, 2010). Certain strains of *E. coli*, such as the O157:H7 strain, are of zoonotic importance (Fairbrother *et al.*, 2006). *Staphylococcus aureus* is a pyogenic pathogen of economic importance in livestock, associated with mastitis in cattle and small ruminants (Lowy, 2000; Fitzgerald, 2012); infected quarters and the skin of the udder and teats are the main reservoirs of infection (Larsen *et al.*, 2000). Klein *et al.*, (2012) reported that *S. aureus* was a leading cause of mastitis in cattle.

Field isolates of *D. congolensis* were prepared from fresh skin scabs collected from naturally infected cattle from Chegutu. The *D. congolensis* bacteria were identified by direct microscopy, culturing on blood agar and biochemical tests as described by Awad *et al.*, (2008); Mannan *et al.*, (2009).

All the bacteria were maintained on blood agar plates until use, at which time, three colonies of each were transferred into universal bottles containing Brain Heart infusion broth and grown overnight at 37 °C. Before use of broth cultures, their turbidity was adjusted with sterile saline to match 0.5 MacFaland solution; at this turbidity, bacterial count was estimated at 1 - 2 x 10^8 CFU (Clinical and Laboratory Standards Institute (CLSI), 2006).

4.2.5 Antibacterial assays

The antibacterial activities of the extracts were determined using three methods; agar disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Before use, the methanol and acetone extract residues were re-dissolved in 80 % acetone while the water extract residue was re-dissolved in sterile distilled water, each to a final concentration of 10 mg/ml.

4.2.6 Agar disc diffusion assay

The agar disc diffusion method as described by Rout *et al.,* (2009); Tadeg *et al.,* (2005); Mathabe *et al.,* (2006); Ghalem *et al.,* (2009) was used. The inocula of the different bacterial strains were evenly streaked on Mueller-Hinton (M-H) agar using a sterile swab. Sterile Whatman filter paper discs (6 mm diameter); three for each plant extract, were impregnated with reconstituted extracts (10 mg/ml) by soaking them for 15 min. Gentamycin (10 µg) was used as a positive control and discs soaked in distilled water, 80 % acetone, were used as negative controls. Discs were applied on the surface of the dry M-H agar plates and incubated for 18 h at 37 °C. Plates streaked with *D. congolensis* were placed in a candle jar and incubated as for the other bacteria. The plates were observed for zones of inhibition which were measured in millimetres. Absence of a zone of inhibition was interpreted as absence of antibacterial activity. Each extract was tested in triplicate.

4.2.7 Minimum Inhibitory Concentration and Minimum bactericidal concentration

The 96-well micro-plate method for minimum inhibitory concentration (MIC), as described by Eloff, (1998); Masoko *et al.,* (2005); Mathabe *et al.,* (2006); CLSI, (2006) and the

minimum bactericidal concentration (MBC) as described by Moulari *et al.*, (2006) were used. Each reconstituted plant extract; acetone, methanol and water, were two-fold serially diluted starting at a concentration of 10 mg/ml to a final concentration of 0.078 mg/ml. A similar two-fold dilution of gentamycin starting at a concentration of 10 mg/ml was used as a positive control. The negative controls were acetone, methanol and distilled water. An equal volume of 100 µl fresh bacterial cultures were added to the wells. The plates were covered and incubated at 37 °C for 24 h after which, 40 µl of 0.2 mg/ml of ρ-iodonitrotetrazolium violet were added to each well as an indicator and incubated for 30 min. Presence of bacterial growth was indicated by a purple colour whereas non-development of colour indicated where bacterial growth was inhibited. Each test was replicated three times. Absolute lowest MIC and average MIC (±standard deviation) values were computed.

For the determination of MBC, a portion of liquid (collected using a sterile loop) from each well that showed no colour change; was plated on blood agar and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC (Kuete *et al.*, 2008).

4.2.8 Selection of animals for in vivo *trials*

Twenty two Mashona cross cattle that were naturally infected with dermatophilosis were identified. Scab samples were taken for microscopic confirmation of dermatophilosis and cattle with samples that were negative microscopically were left out of the trial.

4.2.9 Treatment

Sixteen cattle that were positive microscopically for *D. congelensis* and exhibited clinical signs were allocated into three treatment groups (1,2 and 3). A weigh band was used to estimate the mass of each animal. Group 1 (n=5) received Alamycin LA® (Norbrook Laboratories Limited, Newry, Ireland) which was an oxytetracycline. Group 2 (n=5) received Synulox®RTU a penicillin-based antibiotic and group 3 (n=6) received 20% w/v crude extract of *C. quadrangularis*. Conventional drugs were administered intramuscularly in the gluteal muscles at the manufacturer's recommended dose. The intramuscular administration were given at the following doses: Alamycin LA®; 1 ml/10kg while Synulox®RTU was given at 1 ml/20kg. *Cissus quadrangularis* was administered as a spray. Treatments were given three times, three days apart until signs of recovery. The observation period for the trial was 30 days.

4.2.10 Determination of efficacy

Cattle were inspected daily by the local veterinary extension assistant and weekly by the principal investigator. The purpose of the inspections was to determine if cattle were responding to treatment. A complete cure was considered when all the scabs dried and fell off the animals.

4.2.11 Statistical analysis

For the agar disc diffusion assays, the effect of extract and bacteria on zone of inhibition was measured by the General Linear Model (GLM) in the Statistical Package for Social Sciences (SPSS) (SPSS, 2007). Extractant had 3 coded categories; (1-water; 2-acetone, 3-methanol), bacteria had four categories (1- *D. congolensis*, 2-*E. coli*, 3-*P. aeruginosa*, 4-S. *aureus*) and

plant had three categories (1- *C. quadrangularis*, 2- *C. spinosa*, 3- *P. angolensis*). Least Significant Differences (LSD) was used as the post-hoc test to measure variances in categories. Values of $P < 0.05$ were considered as significant. The minimum and the mean±standard deviation of the MIC for each extract were computed.

4.3 Results

4.3.1 Extraction yields

The quantities of plant materials extracted by the different solvents are as indicated in Table 4.1. The largest quantity was extracted using methanol from *C. spinosa* (2417 mg) with water extracting the minimum quantity (333 mg) from the same plant. Methanol consistently extracted more material from all the plants while water extracted the least.

Table 4:1 Quantities of materials extracted from plants

Plant	Methanol (% of original)[a]	Acetone (% of original)	Water (% of original)
Cissus quadrangularis	1.739 (8.7%)	1.306 (7.5)	0.730 (3)
Catunaregam spinosa	2.417 (12)	2.055 (10.3)	0.333 (1.7)
Pterocarpus angolensis	2.048 (10.4)	1.733 (8.7)	1.084 (5.4)

[a]Calculated using original plant material of 20 gram

4.3.2 Agar disc diffusion assay

Table 4.2 indicates that extracts from the three plants had some antibacterial activities against mainly Gram-positive bacteria, with diameters of zones of inhibition ranging between 8 and 15 mm. *Dermatophilus congolensis* and *S. aureus* showed apparent resistance to the acetone extracts of *C. quadrangularis* and water extracts of *C. spinosa* respectively. Resistance to, mainly water extracts of *C. quadrangularis* and *C. spinosa*, were seen in the two Gram-negative bacteria, *E. coli* and *P. aeruginosa*. In fact, *E. coli* exhibited resistance to all of the water extracts. Extracts from *P. angolensis* displayed activity against all the bacteria with the exception of *E. coli*. There were significant differences ($P < 0.05$) between the antibacterial activities of the water and methanol extracts of *C. quadrangularis* against *D. congolensis*. Water and acetone extracts of *P. angolensis* exhibited significantly better ($P < 0.05$) activity than methanol extracts against *D. congolensis*. All extracts of *C. spinosa* did not show significant differences ($P > 0.05$), in their activity against *D. congolensis*. There were no significant differences ($P > 0.05$) in activity between acetone and methanol extracts of *C.*

quadrangularis against *E. coli* and *P. aeruginosa*. Water and organic extracts of *P. angolensis* did not differ significantly ($P > 0.05$) in their activity against *P aeruginosa*; whilst the activity differed significantly against *S. aureus*. All bacteria in the study were sensitive to gentamycin with *D. congolensis* and *E coli* being more sensitive than *P. aeruginosa* and *S. aureus*.

Table 4: 2 Antebacterial activities of water, acetone and methanol extracts from plants used by small-holder farmers

Sample	Bacteria[a] tested zone of inhibition (mm)			
	De	*Ec*	*Ps*	*Sa*
Cissus quadrangularis				
Water extract	10.5[a]	R	R	9.5[a]
Acetone	R	8.5[a]	11.5[a]	9.5[a]
Methanol	13.5[b]	11[a]	12[a]	8.5[b]
Catunaregam spinosa				
Water extract	10.5[a]	R	R	R
Acetone	11.5[a]	9.5[a]	R	8.5[a]
Methanol	12.5[a]	7[b]	7[a]	10.5[b]
Pterocarpus angolensis				
Water extract	12.5[a]	R	8[a]	9[a]
Acetone	13[a]	10	9[a]	15[b]
Methanol	11.5[b]	R	7[a]	11[c]
*Gentamycin	25.5	24.5	15	14.5

[a] Bacteria: *De, Dermatophilus congolensis; Ec, Escherichia coli; Ps*, *Pseudomonas aeruginosa*; *Sa*, *Staphylococcus aureus*; R, resistant, *Gentamycin not compared with other discs as it contained a different concentration of active ingredient (10 µg). Figures with the same superscript were not significantly ($P > 0.05$) different.

4.3.3 Minimum inhibitory concentration and minimimum bactericidal concentration

Extracts that did not exhibit activity in the agar disc diffusion assay were still included in the MIC assay. This was done since there was no knowledge on the diffusion characteristics of the extracts through the M-H agar. Becton *et al.*, (2006) reported that zones of inhibition may not be exhibited because discs may lack potency due to storage and preparation. Average MIC values of all the extracts are as shown in Table 4.3. Organic solvent extracts of *P. angolensis* exhibited the lowest absolute MIC of 0.625 mg/ml (3.87±3.5), against *D. congolensis* whilst the water extract of *C. quadrangularis* had the highest MIC of 5 mg/ml against the same organism. Acetone and methanol extracts of *C. quadrangularis* and *C. spinosa* had the lowest absolute MIC of 0.156 mg/ml against *S. aureus*. The highest MIC of 10 mg/ml was exhibited by the water extract of *C. spinosa* against *P. aeruginosa*. The lowest average MIC over the triplicates was 0.21 mg/ml exhibited by the acetone extract of *C. quadrangularis* against *S. aureus*, the water extract of *C. spinosa*, had the highest MIC of 10 mg/ml against *P. aeruginosa* (Table 4.3). Overall, the MIC values, both absolute and average, of all plants indicated that *D. congolensis* and *S. aureus* had higher sensitivity to all the extracts than did the gram negative *E. coli* and *P. aeruginosa*. All organisms were sensitive to gentamycin which exhibited the lowest absolute and average MIC of 0.078 mg/ml (2.5±3.3). There was bacterial growth in the acetone and distilled water negative control while there was none in the methanol control wells.

The MBC assay exhibited a negative response. The response was characterised by regrowth of bacteria after sub culturing of all the samples, as a result, no MBC values were computed in this study.

Table 4.3 Minimum inhibitory concentrations (mg/ml) (mean±standard deviation) of three plants after 24 hour incubation at 37 ºC

	Cissus quadrangularis			*Catunaregam spinosa*			*Pterocarpus angolensis*			Gentamycin
	Water	Acetone	Methanol	Water	Acetone	Methanol	Water	Acetone	Methanol	
Dermatophilus congolensis	7.5±2.74	4.6±3.5	5.7±3.5	6.3±3.3	5.8±3.3	5.4±3.4	5.37±3.4	3.87±3.5	4.4±3.5	2.49±3.3
Escherichia coli	7.5±2.7	5.8±3.3	6.7±3.1	7.5±2.7	5.4±3.4	6.2±3.8	6.7±3.1	4.1±3.5	3.9±3.5	2.49±3.3
Pseudomonas aeruginosa	7.5±2.7	4.6±3.5	2.5±3.4	10±0.0	3.3±3.5	3.9±3.5	7.5±2.7	4.4±3.5	5.0±3.5	2.49±3.3
Staphylococcus aureus	5.3±3.4	2.9±3.4	3.5±3.5	3.87±3.5	3.5±3.5	3.1±3.6	5.8±3.3	3.27±3.5	3.7±3.5	2.5±3.3

4.3.4 Therapeutic effects in vivo

The mean weight of the cattle was 282. 2kg (CI: 271.6, 292.9). Group 1 and 2 started to exhibit a response to treatment on day 2 and all the animals in these two groups responded to treatment by day 26 (Table 4.1). Group 3 animals which were treated with *C. quadrangularis* did not show any improvement except a slight drying off of some of the scabs on two animals on day 19.

Table 4.4 Days taken for cattle to exhibit response to treatment

Group	Proportion respondind to treatment 95% CI			
Group	Day 5	Day 12	Day 19	Day 26
Alamycin	0	0.4; 0.0-.95	1	1
Synulox	0	0.2; 0.0-0.63	0.8; 0.37-1.2	1
C. quadrangularis	0	0	0.33; 0.0-0.75	0.33; 0.0-0.75

4.4 Discussion

Of the three extractants used, methanol was the best compared to acetone and water. Results obtained on the extraction yields of the solvents used in this study seem to agree with those of Masoko *et al.,* (2005) who reported that methanol extracted more material from *Terminalia* spp. than did acetone, hexane or dichloromethane. Suleiman *et al.,* (2010) also reported

comparable results where methanol was shown to extract the most material from seven plants, with the highest yield being from *Loxostylis alata*. The quantities extracted by acetone were greater than those extracted by water from all the plants, this contrasted findings by Abdillahi *et al.,* (2008), who reported that water was a better extractant than acetone. This difference could be as a result of the different extraction procedures used in the two studies. In this study there was no use of sonication and filteration under a vacuum during extraction as was the case with the other study. The other reason for the different results could be that Abdillahi *et al.,* (2008) studied different plant species which belonged to the *Podocarpus* genus. As water yielded the least amount of material from all the plants in this study, this could have negative implications for farmers, since they frequently use water as an important ingredient when preparing remedies from medicinal plants (Shale *et al.,* 2005; Suleiman *et al.,* 2010). Masoko *et al.,* (2005) reported that water was unable to extract non-polar components of plants, as such, for this study, it was possible that the three plants investigated had fewer polar compounds in their structures hence the reduced yield associated with water extraction. On the other hand, despite methanol having been a better extractant in this study, it is not readily accessible to the small-holder farmers. Rios *et al.,* (2005) stressed that it was important, during *in vitro* trials, to use the extractants that the farmers themselves used when they prepared medicinal plants for use. In evaluating the antimicrobial potential of plant extracts, the biological activity of the extractant is more important than the amount of compound extracted.

The agar disc diffusion assay results exhibited a different trend to those of the MIC. Methanol and acetone extracts exhibited better inhibitory activities than water extracts with acetone having lower average MICs than methanol, this was in agreement with Masoko *et al.,* (2005) who reported acetone extracts having the lowest average MIC compared to methanol extracts.

Hamza *et al.,* (2006); Mulaudzi *et al.,* (2011) described a classification of inhibitory activity of extracts according to MIC as follows: strong inhibitor; < 0.5 mg/ml, moderate inhibitor; 0.5 – 1.5 mg/ml and weak inhibitor: > 1.5 mg/ml. According to this classification, methanol and acetone extracts from all the plants had strong inhibitory activity against *S. aureus* while water extracts were weakly inhibitory, this was in agreement with Steenkamp *et al.,* (2004); Mathabe *et al.,* (2006); Abdillahi *et al.,* (2008) who reported that *S. aureus* was susceptible to most plant extracts. Moderate inhibitory activity was exhibited by the acetone extracts of *C. quadrangularis* against *C. congolensis* and *P. aeruginosa*. There was variance between the results of the agar disc diffusion and the MIC assays with regards to the activity of the acetone extract of *C. quadrangularis* against *D. congolensis*. *Dermatophilus congolensis* exhibited resistance in the agar disc diffusion assay while in the MIC it showed moderate activity. The discrepancy between the results of the two assays could be due to failure by the disc-adsorbed *C. quadrangularis*, to diffuse into the agar hence a negative result. Several researchers have reported problems associated with the use of the agar disc diffusion assay. Deviations from the recommended media pH of 7.2 – 7.4 and/or depth of 5 mm, which occur during preparation of the M-H agar media, can negatively affect results of the assay. Murray *et al.,* (1983); Hammer *et al.,* (1999) stated that the disc diffusion assay was of limited use in hydrophobic plant extracts as these did not uniformly diffuse through the agar medium. Water soluble constituents of extracts easily diffuse through agar while the more viscous constituents do not (Hood *et al.,* 2003). Despite these disadvantages, currently, several reseachers (Bharti *et al.,* 2013; Firuzi *et al.,* 2013; Martins *et al.,* 2014) found this method still useful in assessing antibacterial activity of plant extracts. Acetone and methanol extracts of *P. angolensis* performed better (MIC range of 0.63 – 1.04 mg/ml) against *D. congolensis* compared to the other plant extracts. The agar disc diffusion assay results of *P. angolensis* extracts against *D. congolensis* were consistent with those of the MIC, with the micro-

organism exhibiting sensitivity. Steenkamp *et al.,* (2004) reported poor inhibitory effects of *P. angolensis* against both Gram-positive and -negative bacteria with an MIC > 4 mg/ml. The difference in activity of *P. angolensis* between the two studies could be due to the different extract preparation methods. Steenkamp *et al.,* (2004) used maceration and heat drying to prepare the extract, which was not the case in this study. The difference could also be due to the fact that the *P. angolensis* plants used, were collected from different localities and hence could have different active compounds (Shale *et al.,* 2005). In the current study *P. angolensis* had some inhibitory activities against all organisms with MIC that ranged between 0.37 – 1.67 mg/ml. In general, water extracts from all the plants had weak inhibitory activity (MIC > 1.6) against all bacterial strains. The water extract of *C. spinosa* could be considered to have no inhibitory activity against *P. aeruginosa* since its MIC was 10 mg/ml. Mulaudzi *et al.,,* (2011) considered extracts with MIC > 8 mg/ml as having no inhibitory potential. Farmers are therefore faced with two constraints when they use water as an ingredient; (1) poor extraction capacity and (2) weak to very poor inhibitory effects of the resultant extracts. These two constraints may partly explain the apparent lack of efficacy of plant remedies prepared using water. The lack of efficacy may not be intrinsic to the plant remedy, but attributable to the use of water in the preparation process. To overcome these constraints, farmers steeped the plant material in cold water for longer periods or boiled and used the resultant diffusion and decoctions. Alternatively, some farmers prepared and used the plant in the form of a paste as was observed for the management of bovine dermatophilosis in the northern parts of Zimbabwe Ndhlovu and Ndhlovu, (2013).

Gram-positive organisms particularly *S. aureus* were more sensitive to the extracts than were the Gram-negative bacteria. These findings were in agreement with those of Steenkamp *et al.,,* (2004); Mathabe *et al.,* (2006); Suleiman *et al.,* (2010) in which *S. aureus* was reported to be highly sensitive to extracts of *Terminalia sericea*, *Indigofera daleioides* and *Khaya*

anthotheca respectively. The Gram-negative *E. coli* was not sensitive to extracts of *C. quadrangularis* and *C. spinosa* whereas *P. aeruginosa* was sensitive to the acetone and methanol extracts of *C. spinosa*. Acetone and methanol extracts of *P. angolensis* had moderate to weak inhibitory effects on both Gram-positive and Gram-negative bacteria with an average MIC < 2 mg/ml. This was in agreement with Shale *et al.,* (2005) who reported that some forms of *Marvi parviflora* were inhibitory against both Gram-negative and Gram-positive bacteria as did *Podocarpus* species Abdillahi *et al.,* (2008). Luseba *et al.,* (2007) reported that *P. angolensis* was highly effective against the Gram-positive *S. aureus* and the Gram-negative *E. coli*; this was in agreement with the current study. Gram-positive bacteria are reportedly more sensitive to antibiotics due to the lipophilic components of their cell membrane, compared to the Gram-negative bacteria which have hydrophilic components (Abdelkader *et al.,* 2010). Gentamycin had better inhibitory activities than all the plant extracts. Other reseachers (Steenkamp *et al.,* 2004), although working with a different plant extract of *Caesalpima pulcherrima*, reported that the plant extract performed similarly to gentamycin, this could be due to the differing bio-active compounds between plants in our study and the *C. pulcherrima*. In another study gentamycin exhibited superior antimicrobial activity compared to *Nauclea latifolia* a Cameroonian medicinal plant (Tekwu *et al.,* 2012). Generally, conventional drugs have been reported to exhibit superior antimicrobial properties than traditional medicinal plants (Firuzi *et al.,* 2013). The acetone and water negative controls exhibited no inhibitory activities, this was consistent with findings by Eloff, (1998) that acetone is non-toxic to microorganisms. The 80 % methanol negative control inhibited growth of all bacteria. In the current study, this activity of methanol did not affect the results of the assay since it was not used for reconstitution. None of the plant extracts, in the study, exhibited MIC values of less than 0.2 mg/ml, it was however noteworthy that some researchers such as Firuzi *et al.,* (2013) regarded plants with MIC ranging from 0.3 – 5 mg/ml as potential candidates for further analysis of their antimicrobial constituents.

The plant extracts had bacteriostatic rather than bactericidal activity; there was re-growth of organisms for all the ranges of MIC when the minimum bactericidal concentration assay was performed. This meant that farmers needed to repeatedly apply the remedies, daily or every other day until the animal was "cured".

Results from the *in vivo* trial indicated that the crude aqueous extract of *C. quadrangularis* was not potent as a cure for clinical dermatophilosis at 20% concentration. Jainu *et al.,* (2006) reported that *C. quadrangularis* had anti-ulcer effects and as such had potential use for the management of gastric ulcers. Animals suffering from dermatophilosis do at times have cutaneous ulcers which result from traumatic removal of scabs, in this case *C. quadrangularis* application might provide relief through its wound healing properties. The nature of dermatophilosis lesions implies that the animal is on occasion experiencing some pain due to the nature and extent of the lesions. Flavonoids and tannins in *C. quadrangularis* have been postulated to be responsible for the analgesic and anti-inflamatory properties of the plant extracts (Panthong *et al.,* 2007; Bujade *et al.,* 2012). Considering these properties, the extracts as used by the farmers, bring about relief to the animal. The conventional drugs produced appreciable responses in cattle from day 19 and complete healing of all animals on day 26. The performance of the conventional drugs in this study were in agreement with the reports by Awad *et al.,* (2008); Hamid and Musa (2009) who reported efficacy of tetracycline and penicillin derived antibiotics for the cure of dermatophilosis. Nath *et al.,* (2010) reported that administering tetracyclines on dermatophilosis affected animals, daily for seven days produced a cure within 12- 16 days and treated animals went for three years without the disease resurfacing.

4.5 Conclusion

This study confirmed the antibacterial potential of *C. quadrangularis*, *C. spinosa* and *P. angolensis* against *D. congolensis* and the other tested micro-organisms. The inhibitory effects ranged from moderate to weak with acetone extracts performing better than the other extracts. The extracts would probably perform better if they were extracted in pure form as proposed by Suleiman *et al.,* (2010). Other methods such as pounding the fresh plant material and squeezing out the fluid could lead to the production of a more concentrated and hence more effective test material. Since, in terms of the yield and antibacterial activities, water performed poorly when compared to acetone and methanol, more effective methods for the delivery of plant remedies must be devised and investigated. Such systems should be acceptable and affordable to the farmers. Farmers in the field possibly use *C. quadrangularis* for its analgesic and anti-inflamatory properties, further research should be conducted to investigate the therapeutic effects of higher concentrations of *C. quadrangularis*

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Chapter 5 An i*n vitro* and *in vivo* study of the efficacy of ethno-veterinary remedies used to control ticks by small-holder farmers in Zimbabwe.

Abstract

The objective of the study was to determine the *in vitro* and *in vivo* acaricidal efficacy of the plants, *Blumea decurrens, Carica papaya* and *Manihot esculenta* against *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* and also their efficacy on naturally tick-infested cattle. The *in vitro* activity against *R. appendiculatus* and *A. hebraeum* larvae was assessed using the larval packet test assay. In all the experiments the extracts were used at concentrations of 0.156, 0.625, 1.25, 2.5, 5 and 10%. In the *in vivo* trials, naturally tick-infested cattle were sprayed with 20% preparations of the extracts, Carbaryl 85 WP®, Fencure (Fenvelerate) 20 EC® and Triatix®, the latter three prepared according to the farmers and manufacturers recommendation resepectively. The mortalities of *R. appendiculatus* larvae ranged from a minimum of 10.5% to a maximum of 81.7% for *C. papaya* and *M. esculenta* respectively. For *A. hebraeum*, the mortalities were lower, ranging from 1.4% to 26.5% with *B. decurrens* and *M. esculenta* respectively. The LC_{50} values for *M. esculenta, C. papaya* and *B. decurrens* were 3.6, 7.7 and 4.4 mg/ml respectively against *R appendiculatus*. Lethal concentrations values against *A. hebraeum* were 178.3, 148.1 and 354.3 mg/ml for *M. esculenta, C. papaya* and *B. decurrens*. *Manihot. esculenta* exhibited significantly ($P<0.05$) greater acaricidal efficacy than the other two extracts particularly against *R. appendiculatus*. In the *in vivo* trials, *M. esculenta* extract exhibited significantly ($P < 0.05$) greater tick load reduction than the extracts of *B. decurrens* and *C. papaya*. The larval

mortality and the tick reduction associated with the use of *M. esculenta* indicated that it had potential for use as an acaricide for resource-limited farmers in the absence of conventional acaricides.

Keywords: Papain, chymopapain, ectoparasites, larvae, inhibited

5.1 Introduction

Cattle ticks are responsible for large economic losses in livestock production, with *Hyalomma*, *Rhipicephalus*, *Rhipicephalus boophilus* and *Amblyomma* species being the most medically important (Silva *et al.,* 2011; Koc *et al.,* 2013). The traditional control of ticks and consequently the diseases that they transmit or are associated with has mainly relied on the use of chemical acaricides (de Castro 1997; Willadsen 2006), which has been associated with the development of resistance, residues in meat and milk and environmental contamination (Taylor 2001; Willadsen 2006). The cost of acaricides both in terms of cost to farmers and the costs associated with research and development are disadvantages in their use (Taylor 2001).

Other non-conventional tick control methods have been used by stockowners. These methods, collectively called ethno-veterinary practices, are the medicinal plants, surgical techniques and traditional management practices used by farmers to prevent and treat a spectrum of livestock diseases (Mathius-Mundy *et al.,* 1989 cited by Mwale *et al.,* 2009). Farmers resort to ethno-veterinary methods of disease control due to the inadequacy of conventional veterinary medicine to meet basic animal health care in a sustainable way (Mathius *et al.,* 1996). Also, economically

disadvantaged farmers do not have access to modern veterinary medical care which could be expensive and often not available when needed (Ghotge *et al.,* 2002).

Farmers have been reported to use engine oil, Jeyes fluid, paraffin, chickens and plants for the control of cattle ticks and internal parasites (Hlatshwayo and Mbathi 2005; Moyo and Masika 2009; Mwale and Masika 2009). Plant extracts have been used commonly as some have been reported to contain essential oils that have acaricidal, anthelmintic and insecticidal properties (Bagavan *et al.,* 2009).

Experiments, both *in vitro* and *in vivo* have been conducted to determine the efficacy and safety of a number of plant extracts against ecto-parasites. Bagavan *et al.,* (2009) investigated the adulticidal and larvicidal efficacy of *Annona squamosa*, *Centella asiatica*, *Gloriosa superba*, *Mukia maderaspatensis*, *Pergularia daemia* and *Phylanthus emblica*. All the plant extracts showed toxic effects on ticks. In Zimbabwe *Lippia javanica* was reported to be effective against cattle ticks but was not as good as the amitraz-based Tickbuster® (Madzimure *et al.,* 2011). *Aloe marlothii* leaves given orally to cattle had no significant effect on the tick *R. Boophilus decoloratus* (Spickett *et al.,* 2007). Moyo and Masika (2009) reported that extracts of *Aloe ferox*, *Ptaerolyxon obliquum* and *Taegetes minuta* had no significant effects on cattle tick burdens when applied topically. Extracts from *Azadirachta indica* had a significant efficacy against adults of *Boophilus microplus* and significantly inhibited egg laying and hatching (Srivastava *et al.,* 2008). Besides being toxic to ticks, plants can also act as tick attractants a property shown by *Calpurnia aurea* on *Rhipicephalus appendiculatus* and *R. pulchellus* (Nana *et al.,* 2010); this makes them potential components of acaricide-impregnated tags used to control ticks.

Mannihot esculenta (cassava) is an important nutritional crop belonging to the family Euphorbiaceae (Bayoumi *et al.,* 2010). It has reportedly been used as part of a concentrate mixture in broilers where it improved growth and food conversion (Eruvbetine *et al.,* 2003). Cassava has also been reported to improve dry matter feed intake, weight gain and nitrogen retention of West African Dwarf goats when incorporated into supplements at 60% level (Oni *et al.,* 2010). Marie-Magdeleine *et al.,* (2010b) reported anthelmintic activity against *Haemoncus contortus* while Rofaai-Ali *et al.,* (2012) reported high anthelmintic activity against *Teladorsagia circumcincta*. Condensed tannins and terpenoids were postulated to be responsible for the anti-parasitic activity (Marie-Magdeleine *et al.,* 2010b). *Carica papaya* (paw-paw) is a perennial herbaceous plant belonging to the family Caricaceae (Vuong *et al.,* 2013). *Carica papaya* plants produce latex which is a component of the plant's defence mechanism against pathogens (de Oliviera and Vitoria, 2011). Latex is found in the *C. papaya* fruit, seeds and leaves in varying quantities and has been used as a traditional remedy for human ailments (Anuar *et al.,* 2008). Amazu *et al.,* (2010) reported anti-inflammatory effects while Anuar *et al.,* (2008) reported wound healing properties of *C. papaya.* There was a reduction of nematode loads and a resultant weight gain in chickens treated with *C. papaya* latex (Chota *et al.,* 2010). *Blumea (Laggeria) decurrens* is a half-shrub that is reportedly used traditionally, for the management of stomach pains, to relieve foot pain and to treat flu (Damme 1922). Fiori *et al.,* (2000) have indicated that *Blumea decurrens* had biological activity against bacteria and fungi. Information is lacking as regards the use of *B. decurrens, M. esculenta* and *C. papaya*; as tick control agents. The aim of this study was therefore to assess the *in vitro* and *in vivo* efficacy of these plants and remedies that were used by some smallholder farmers from Zimbabwe in the control of cattle ticks (Ndhlovu and Masika 2013).

5.2 Materials and Methods

5.2.1 Collection and preparation of plant extracts

Fresh plants of *Blumea decurrens*, *Carica papaya* and *Mannihot esculenta* were collected from the bush in Njelele communal lands in Gokwe South, Zimbabwe. Plant specimens were authenticated by Mr. Chris Chapano a botanist at the National herbarium and botanical gardens, Harare, Zimbabwe and stored at the herbarium as voucher specimens: DNN04-2013, DNN05-2013 and DNN06-2013; representing *B. decurrens, C. papaya* and *M. esculenta* respectively.

The whole plant of *B. decurrens*, leaves of *C. papaya* and *M. esculenta*, were air dried at room temperature, ground to fine powder using an electric grinding mill (Fritsch Pulverisette, Germany) and stored in powder form in labelled plastic bags at 5°C until further use. Stock solutions of crude aqueous plant extracts were prepared according to the methods of Moyo *et al.,* (2009) and Siddiqui *et al.,* (2009). Forty- five grams of *B. decurrens*, 100 gm of *C. papaya* and 200 gm of *M. esculenta* were separately mixed with 450 ml, 1 000 ml and 2 000 ml sterile distilled water respectively; to prepare 10% (w/v) stock solution for the *in vitro* acaricidal trials. Thirty two-hundred grams of *B. decurrens*, 3 200 gm, of *C. papaya* and 2 468 gm of *M. esculenta* were mixed with 16 l, 16 l and 12.34 l sterile distilled water respectively to prepare 20% (w/v) stock solutions for the *in vivo* trials. After stirring the mixtures on a Variomag magnetic stirrer overnight; stock solutions were prepared by straining the different mixtures through a muslin cloth and the resultant solution stored at 5 °C until use the following day. The non-plant remedies; Carbaryl 85 WP® (Agricura, Zimbabwe) and Fencure (Fenvelerate 20 EC® Zimbabwe Fertilizer Company, Zimbabwe) were prepared to concentrations as prescribed by the farmers (Ndhlovu and Masika 2013).

Carbaryl 85 WP® powder was dissolved at a rate of 37. 5 g per 5 l of distilled water while 6 ml of Fencure (Fenvelerate) 20 EC® was mixed with 5 l of distilled water. Ten ml of Triatrix were mixed with 5 l of distilled water in accordance with the manufacturer's recommendations. The latter three remedies were not included in the *in vitro* trials since these chemicals although regularly used by farmers, they are only authorised to be used for the control of garden pests.

5.2.2 Larval Packet Test (LPT)

The LPT bioassay as described by the Food and Agricultural Organisation of the United Nations (FAO) (2004) was used to determine *in vitro* efficacy of the crude plant extracts. Larvae for the trials were produced from susceptible *A. hebraeum* and *R. appendiculatus* ticks reared at the Central Veterinary Laboratory, Harare. For the LPT, the 10% (w/v) stock solution was used and 50% serially diluted to produce for each extract, concentrations ranging from 10, 5, 2.5, 1.25, 0.625, 0.312 to 0.156% (w/v). Amitraz® at the following concentrations 0.5, 0.25, 0.0625, 0.0312, 0.01562% w/v and distilled water were used as the positive and negative controls respectively. Approximately 70 – 120, 14-day old larvae were placed in filter paper packets (FAO 2004) previously impregnated with the different test solutions. The packets were incubated at 27 – 28 °C and 90 - 95% relative humidity overnight. The tests were conducted in triplicate. The mortality of the larvae was determined according to the methods of Natala *et al.*, (2005); Klafke *et al.*, (2006). Larvae were considered as dead where no motion was observed or when they failed to respond to prodding or being breathed upon.

5.2.3 In-vivo acaricidal trials

In vivo acaricidal efficacy trials for crude aqueous plant extracts and non-plant remedies were conducted at the Henderson Research Station 32 km north of Harare. The vegetation and climate characterising the study site are described elsewhere (Madzimure *et al.,* 2011). The acaricidal efficacy trials were conducted as described by Holdsworth (2006); Moyo *et al.,* (2009). Fifty-five pure-bred Mashona cattle of approximately the same adult age group were randomly allocated to 6 groups, namely: 1,2,3,4,5 and 6; which represented treatments with *B. decurrens*, *C. papaya*, *M. esculenta* at concentrations of 20% w/v, Carbaryl 85 WP®, Fencure (fenvelerate) EC 20® and Triatix® (amitraz-based acaricide) the positive control. Consent to use the cattle for the trial, was granted by the manager of the research station and from the Department of Veterinary Services. The cattle were ethically used in compliance with guidelines of the University of Fort Hare Ethics Committee on Research in animals (clearance certificate no: MAS011SNNDH01).

The crude aqueous solutions of the test materials and the non-plant remedies were applied topically according to the methods of Moyo *et al.,* (2009); Madzimure *et al.,* (2011). This entailed spraying 5 l of 20% concentration of test materials, per group on two occasions; days 0 and 7. The trials were conducted over a period of 10 days. Half-body tick counts were conducted on treated cattle by the same person at the same time of day in the morning between 7 and 8 am. Fully engorged ticks were excluded as these were expected to drop off within 24 h (Pereira and Famadas, 2006). Tick counts were performed on the day of treatment (day 0) before applying test materials, and on days 2, 4, 7 and 10. The efficacy of the test materials were calculated according to the following formula (Holdsworth 2006):

$$Tick\_reduction = 1 - N/No$$

Where:

N_0 = half body tick counts on animal before treatment at day 0 also taken as control.

N = half body tick count on animal after treatment (i.e. days 2, 4, 7 and 10)

5.2.4 Statistical analysis

Larval mortality % was computed as follows: . Larval mortalities as a result of exposure to extracts were evaluated using the Two-sample mean-comparison calculator in Stata SE version 11.2 statistical and data analysis software (StataSE 2012). Differences in larval mortalities were considered significant at $P < 0.05$. The Probit analysis program in SPSS version 16 (SPSS 2007) was used to calculate the lethal dose/concentration (LC_{50}) of test materials that caused 50% larval mortality in the *in vitro* trials. The LC_{50} of the extracts against the larvae were compared using their 95% confidence intervals (CI). If the CIs of the LC_{50} values overlapped, this was interpreted to mean that there was no significant difference between them. Data from the *in vivo* trial were analysed using the generalized linear models (GLM) procedure for Repeated measures analysis of variance in SAS (SAS 2010). Pairwise proportion tick reductions, were compared using Tukey's studentised range test method. The following model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Yij was the response variable, being the proportion reduction of tick population

μ was overall mean common to all observations;

T_i was the effect of the ith Treatment (1, 2, 3, 4, 5, and 6)

ε_{ij} are the random residuals.

5.3 Results

The aqueous extracts of *B. decurrens, C. papaya* and *M. esculenta* exhibited some tick larvicidal effects in the LPT assay as indicated by the larval mortality (Table 5.1) and LC_{50} values (Table 5.2). Extracts exhibited higher mortality to the larvae of *R. appendiculatus* than to those of *A. hebraeum*. The percentage proportion of *A. hebraeum* larval mortality ranged from a minimum of 1.4 ± 1.3% to a maximum of 26.5 ± 2.8% as a result of exposure to *B. decurrens* and *M. esculenta* respectively. The percentage larval mortality was highest against *R. appendiculatus* exposed to *M. esculenta* at 81.7±12.2%. The percentage mortality of larvae increased as the concentration of the extracts did and mortality was significantly different between concentrations (Table 5.1). The LC_{50} of all the crude extracts were significantly higher against the *A. hebraeum* larvae than they were for *R. appendiculatus* (Table 5.2). There were no significant differences ($P > 0.05$) in LC_{50} values due to the different extracts within each tick species as the CIs overlapped. *Carica papaya* exhibited some difference in activity from the other plants against *A. hebraeum* although this was not significant ($P = 0.061$). Amitraz at a concentration of 0.05%, caused 100% larval mortality, it exhibited superior acaricidal activity than the extracts, even when its concentration was 100 fold less than that of the extracts. There was no larval mortality associated with exposure to water which was the negative control.

Table 5.1 Mean mortality (±standard deviation) of unfed larvae of *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* treated with different concentrations of extracts

Treatment (%)	*B. decurrens*		*C. papaya*		*M. esculenta*	
	Amblyomma	Rhipicephalus	Amblyomma	Rhipicephalus	Amblyomma	Rhipicephalus
0.16	1.4 ± 1.3^{a1}	16.9 ± 2.9^{b1}	4.0 ± 2.9^{c1}	10.5 ± 2.5^{d1}	3.5 ± 2.3^{c1}	14.8 ± 3.8^{e1}
0.31	3.6 ± 1.1^{a2}	19.6 ± 4.2^{b2}	5.2 ± 1.5^{c2}	13.5 ± 2.0^{d2}	5.4 ± 1.9^{c2}	18.9 ± 3.9^{b2}
0.63	5.8 ± 1.5^{a3}	22.4 ± 1.9^{b3}	9.6 ± 2.9^{c3}	17.4 ± 2.6^{d3}	8.3 ± 2.1^{e3}	20.8 ± 5.1^{f3}
1.25	7.4 ± 1.8^{a4}	33.2 ± 7.8^{b4}	12.3 ± 2.9^{c4}	20.8 ± 1.4^{d4}	9.7 ± 1.8^{e4}	29.0 ± 5.5^{f4}
2.50	9.1 ± 1.6^{a5}	40.7 ± 4.5^{b5}	14.3 ± 2.1^{c5}	30.0 ± 3.9^{d5}	11.4 ± 1.0^{e5}	41.7 ± 4.9^{b5}
5.0	13.3 ± 3.1^{a6}	49.4 ± 5.5^{b6}	18.4 ± 3.4^{c6}	33.6 ± 7.6^{d6}	17.6 ± 3.8^{c6}	60.4 ± 10.0^{e6}
10.0	19.0 ± 1.2^{a7}	59.3 ± 9.2^{b7}	23.8 ± 1.5^{c7}	46.4 ± 8.6^{d7}	26.5 ± 2.8^{e7}	81.7 ± 12.2^{f7}

Means and standard deviations followed by different superscript letters in a row and different number in a column differ significantly ($p < 0.05$)

Table 5.2 Confidence intervals and LC_{50} values of crude extracts against tick larvae

Test material	*Hebraeum*		*R. appendiculatus*	
	LC_{50}	95 % CI	LC50	95 % CI
B. decurrens	354.3^{a1}	190.2 – 771.1	4.4^{b2}	3.1 – 6.6
C. papaya	148.1^{a1}	87.0 – 288.8	7.7^{b2}	5.1 – 12.7
M. esculenta	178.3^{a1}	103.9 – 350.5	3.6^{b2}	2.6 – 5.3

LC_{50} values followed by different superscript letters in a row and different number in a column differ significantly ($p < 0.05$)

The major tick species infesting the cattle during field trials were *R.* (*Boophilus*) *decoloratus, R. evertsi, R. appendiculatus* and *A hebraeum*. Six treatments were applied in the *in vivo* trial

as opposed to four treatments used in the *in vitro* trials. Proportion tick reduction or efficacy of the crude extracts against the ticks infesting cattle, were as indicated in Table 5.3. The least square means of proportion of tick population reduction ranged from a minimum of 0.35 ± 0.03 associated with *B. decurrens* treatment at day 10 to a maximum of 0.95 ± 0.03 associated with Triatix® treatment at day 10. Generally, there were no significant differences ($P > 0.05$) in tick reductions exhibited between *B. decurrens* and *C. papaya* throughout the days of observations. There was no significant difference ($P > 0.05$) exhibited in tick reduction activities between *M. esculenta* and the non-plant alternative remedies (Carbaryl, Fencure) but significant differences ($P < 0.05$) in reductions were exhibited between these three treatments when compared to both *B. decurrens* and *C. papaya*. The conventional acaricide Triatix®, performed significantly better ($P < 0.05$) than all the other treatments throughout the trials. Overall, the highest tick reductions were associated with day 10 for some treatments with the exception of *B. decurrens*, *C. papaya* and Carbaryl.

Table 5.3 Least square means of proportion reduction in tick populations following treatment

Treatment	Day 2	Day 4	Day 7	Day 10
B. decurrens	0.45 ± 0.02^{a}	0.38 ± 0.02^{a}	0.35 ± 0.03^{a}	0.40 ± 0.03^{a}
C. papaya	0.54 ± 0.02^{ac}	0.46 ± 0.02^{a}	0.47 ± 0.03^{b}	0.50 ± 0.03^{a}
M. esculenta	0.69 ± 0.02^{b}	0.66 ± 0.02^{b}	0.68 ± 0.03^{c}	0.73 ± 0.03^{b}
Carbaryl	0.72 ± 0.02^{b}	0.68 ± 0.02^{b}	0.60 ± 0.03^{c}	0.65 ± 0.03^{b}
Fenkill	0.64 ± 0.02^{bc}	0.56 ± 0.02^{c}	0.61 ± 0.03^{c}	0.74 ± 0.03^{b}
Triatix	0.87 ± 0.02^{d}	0.82 ± 0.02^{d}	0.90 ± 0.03^{d}	0.95 ± 0.03^{c}
LSD	0.10	0.09	0.12	0.12

Least square means in the same column with the different superscript in a column are significantly different.

5.4 Discussion and conclusion

The three extracts exhibited higher mortality against *R. appendiculatus* larvae than they did against *A. hebraeum*. The highest mortality was 81.7% and 26.5% for *R. appendiculatus* and *A. hebraeum* respectively. Ticks differ in their sensitivity to acaricides due to the thickness of the lipid layer of their cuticle. This layer differs between species, and also within species the latter according to developmental stage (Gomes *et al.,* 2014). Therefore, in this study, probably the larvae of *R. appendiculatus* were more sensitive than those of *A. hebraeum* due to the differences in their cuticles. Zaman *et al.,* (2012) stated that there was a wide variation in the time taken for different plant extracts to exert their toxic effects on larvae. In the present study the larvae were exposed to extracts for 24 hrs; perhaps it would have been possible, for the extracts to exhibit higher mortalities (particularly against *A. hebraeum*) had

larvae been exposed for a longer time period. Species differences in sensitivities to other biological products such as for example entomopathogenic fungi, have also been reported (Gindin *et al.,* 2003). The percentage larval mortality and LC_{50} values were comparable to those reported in other studies; although those studies were conducted using different tick species particularly *R.* (*Boophilus*) *microplus*. Most *in vitro* acaricidal efficacy trials use *R. B. microplus* as the test tick (FAO, 2004). Righi *et al.,* (2013) reported comparable larval mortality rates of 2.25 – 99.3% when the dichloromethane extract of *Croton sphaerogynus* was tested against *R. microplus* at extract concentrations similar to those in the current study. The larval mortality at 10% concentration of 83.6% was higher but comparable to the one in our study which was 81.7%. Silva *et al.,* (2011) reported LC_{50} of 2.46 mg/ml when the ethyl acetate extract of *Palicourea marcgravii* was tested on *R. B. microplus* larvae, in the same study, ethanol extracts exhibited LC_{50} = 4.38 and 9.27 mg/ml for the hexane and ethanol extracts of *P. marcgravii* respectively. The latter LC_{50} values, were comparable to the ones calculated in our study for the toxic effects of *B. decurrens, C. papaya* and *M. esculenta* against *R. appendiculatus* larvae, which were 4.4, 7.7 and 3.6 mg/ml respectively. The efficacy values against *A. hebraeum* were low, other workers Giglioti *et al.,* (2011) reported zero larval mortality when *A. indica* seed extract was tested against *R. B. microplus* while it caused mortality in adult ticks and reduced their reproductive capacity.

In the *in vivo* trials, 20% solution of *M. esculenta* extract resulted in tick load reduction that was significantly ($P < 0.05$) higher than the reduction associated with similar concentrations of *B. decurrens* and *C. papaya*. These extracts had a maximum tick reduction of 73, 45 and 54% respectively. These values were lower than those reported by Madzimure *et al.,* (2011) in which a 20% solution of *L. javanica* reduced tick infestation by 89%. Moyo *et al.,* (2009) reported lower tick reduction levels of 58, 9.5, 4.5 and 0% for *Lantana camara, Ptaeroxylon*

obliquum, Tagetes minuta and *Aloe ferox*. Orally administered *A. marlotti* did not have any effect on engorged *R. boophilus decoloratus* (Spickett et. 2007). White and purple varieties of *Tephrosia vogelli* exhibited higher *in vitro* efficacy than our study, exhibiting LC_{50} of 0.83 and 0.81 mg/ml respectively against adult *R. appendiculatus* (Kalume *et al.,* 2012). The differences in activities between these studies could be attributed mainly to the fact that the studies used different plants with different metabolites in the plants and the method of extract application. In Kalume *et al.,* (2012) the adult ticks were immersed in extracts for 15 minutes while in our study exposure was through spraying; as such the former ticks had higher exposure to the extract. *Manihot esculenta* contains a number of metabolites among them are terpenoids, flavonoids and condensed tannins (Marie-Magdeleine *et al.,* 2010b). Terpenoids have been reported to have acaricidal activity (Righi *et al.,* 2013). Condensed tannins in *M. esculenta* have been reported to also be responsible for antihelmintic activity against *Haemoncus contortus* (Marie-Magdeleine *et al.,* 2010a), these tannins may also exert a similar activity on the tick larvae under investigation. The aerial parts of *M. esculenta* contain two cynogenic glycosides; linamarin and lotaustralin (Rivadeneyra-Domínguez *et al.,* 2013), of these two, linamarin has been reported to be neurotoxic to rats and we postulate that it could cause neuronal damage in tick larvae. The hydrogen cyanide released by these glycosides interferes with the function of cytochrome oxidases, consequently the cellular respiratory chain is compromised and tissues suffer energy deprivation leading to death (Hue *et al.,* 2010; Soto-Blanco Górniak 2010). Tick larvae exposed to *M. esculenta* could therefore also suffer from the intoxicating effects of the cyanide.

Kovendan *et al.,* (2012) have reported that chymopapain and papain in *C. papaya* were responsible for the toxicity exhibited against pupa of *Anopheles stephensi* exposed to the latex of *C. papaya*. Benzyl isothiocyanate, another component of *C. papaya* has been

reported to have antihelmintic properties (Kermanshai *et al.*, 2012). We postulate that these toxic compounds, of *C. papaya*, may also exert similar effects on the tick larvae exposed to them. The synthetic amitraz-based acaricides had superior acaricidal activity than the extracts, this was in agreement with Silva *et al.*, (2011) who indicated that no natural products have been reported to have the same acaricidal activities of the synthetic pesticides such as cypermethrin and amitraz. The superiority of Carbaryl 85 WP® and Fencure (Fenvelerate) 20 EC® over the extracts may be due to the fact that they were synthetically developed for use against (garden) pesticides and hence have potent compounds with more acaricidal effects than the extracts. Triatix® performed better than Carbaryl 85 WP® and Fencure (Fenvelerate) 20 EC® because unlike the latter two, it was developed specifically for the control of ticks and hence would be expected to perform better.

In conclusion, the plants and the non-plant remedies exhibited variable efficacy against ticks. *Manihot esculenta* plant extract exhibited better acaricidal activities than did those of *B. decurrens* and *C. papaya* in both the *in vitro* and *in vivo* trials. The larval mortality and the tick reduction associated with the use of the plants and especially *M. esculenta* lends credence to their present use, in the absence of conventional acaricides, by resource-limited farmers. The level of risk of toxicity to farmers and animals as they use the preparations needs to be investigated.

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Chapter 6: Risk factors associated with clinical dermatophilosis in smallholder sector cattle herds of Zimbabwe at the *A. variegatum* and *A. hebraeum* interface

Abstract

A crossectional study was conducted to investigate risk factors for clinical dermatophilosis herd level positivity in smallholder dip tanks from Gokwe (Chemawororo, Gwanyika), Kwekwe (Koronika) and Chegutu (Chivero) Zimbabwe between September 2013 and April 2014. A total of 185 herds were clinically examined for disease and tick infestation. Data on herd and potential herd level risk factors were collected using a structured questionnaire. A herd was classified as clinically positive if an animal satisfied any of the following criteria: small lesions characterised by hairs clumping like a small paint brush, clear exudative circumscribed lesions with scabs of at least 1 cm diameter and confluent progressive exudative scab lesions affecting significant parts of the animal's body. *Amblyomma variegatum* and *A. hebraeum* ticks were identified in situ with further laboratory confirmation. The potential herd-level risk factors for clinical dermatophilosis were tested using multiple logistic regression with herd infection status (positive, negative) being the binomial outcome and risk factors being predictors. Of the herds examined clinical bovine dermatophilosis was detected in 45% (84/185; 95% CI: 38.2, 52.6%) of the herds. The herd prevalence ranged from 6.9% (95% CI: 0.00, 16.7) to 56.7% (95% CI: 43.8, 69.6) with Chivero and Chemawororo dip tanks recording the lowest, and highest prevalences respectively. Herds infested with *A. variegatum* were associated with higher odds (OR= 6.8; 95% CI: 1.71, 27.10) of clinical dermatophilosis while the association was not significant (P > 0.05) in *A. hebraeum* infested herds. A history of having bought cattle (OR=3.5; 95% CI:

1.09, 11.12) compared to not buying was associated with increased herd clinical positivity status. It was concluded that management practices aimed at movement and tick control would help reduce the prevalence of clinical dermatophilosis in cattle herds.

Keywords: acaricides, epidemiology, exudative, livestock hides, ticks

6.1 Introduction

Amblyomma variegatum a three-host tick, is one of the most important and widely distributed of the *Amblyomma* ticks (Stachurski *et al.,* 2010). All stages of the tick infest cattle, sheep and goats. The distribution of this tick extends from north-western Zimbabwe, the central highveld and on the eastern border of the country, to central and northern Mozambique (Norval 1983a; Peter *et al.,* 1998a). Petney *et al.,* (1987) reported that this tick was absent from drier arid areas. Adults of this tick are present throughout the year on cattle and buffalo although infestations are heavier during the wet warm months of September to May, while nymphs of this tick are only found between June and December (Petney *et al.,* 1987). In Zimbabwe the most commonly occurring *Amblyomma* species is *A. hebraeum*, which in the adult stage is parasitic on cattle and other medium to large-sized ungulates, leopards and ostriches (Norval 1983a; Horak *et al.,* 1987; Dower *et al.,* 1988). The distribution of *A. hebraeum* is from central Zimbabwe southwards into South Africa, eastern Swaziland, southern Mozambique and eastern Botswana (Walker *et al.,* 2003). *Amblyomma hebraeum* is the principal vector of heartwater in Zimbabwe, however, it is replaced by *A. variegatum* in the northern parts of the country (Norval *et al.,* 1994). Of importance when considering *A. hebraeum* and *A. variegatum* in Zimbabwe is the fact that these two species have an area of overlap (Peter *et al.,* 1998a). It was reported that in an area of overlap *A. variegatum* completely replaces *A. hebraeum* over a period of three years (Norval *et al.,* 1994).

Bovine dermatophilosis is an important disease of cattle in Africa, it was first reported in Belgian Congo in 1915 (Stewart, 1972; Oppong, 1996). It has been reported in most countries in the continent of Africa (Hamid and Musa 2009). Bovine dermatophilosis is a tick associated disease caused by an actinomycete bacterium, *Dermatophilus congolensis* (Molia *et al.,* 2008;

Gebreyohannes and Gebresselassie 2013) characterised by an exudative acute or chronic dermatitis which could be localised or generalised (Admassu and Alemu 2011). The lesions vary in severity; from small lesions which make hair stand like a small paint brush, to clear circumscribed scabs over 1 cm in diameter and finally to more confluent progressive lesions (Hadrill and Walker 1996). Stewart (1972) described a carrier state in cattle, in such a state the lesions are not easily observed and it was concluded that carrier animals were the chief means of survival for *D. congolensis*. According to Estrada-Peňa *et al.,* (2007) the saliva of *A. variegatum* contributes to the pathogenesis of bovine dermatophilosis. The disease can occur in tick-free animals but it is more severe in those that are infested by the *A. variegatum* ticks (Stachurski *et al.,* 2010). Walker (1996), stated that *A. variegatum*'s role in the development of dermatophilosis was through immunosuppression. The tick was postulated to secrete an immunosuppressing agent in its saliva, or waste metabolites that were toxic to the host. Economically, bovine dermatophilosis is important due to morbidity and mortality, damage to hides and its effect on draught animal power (Samui *et al.*, 1990; Bayisa *et al.,* 2012). In other parts of Africa it has frustrated the introduction of exotic breeds to improve meat and milk production (Koney 1996).

The treatment of bovine dermatophilosis is mainly through the use of penicillin, streptomycin and dihydrostreptomycin given intramuscularly (Hamid and Musa 2009). Awad *et al.,* (2008) indicated that a double dose of long acting tetracyclines given a day apart gave better results than a single dose. Penicillin could be used in combination with streptomycin to produce a cure while gentamycin was reportedly the most effective antibiotic (Hamid and Musa 2009). Acaricides have been reported to be the best option for the control of bovine dermatophilosis (Hadrill *et al.,* 1996). Amitraz based acaricides applied on the predilection sites of *A. variegatum* ticks on cattle, reduced the prevalence of dermatophilosis (Morrow *et al.,*, 1993;

Morrow *et al.,* 1996) as did deltamethrin. The method of tick control is important in the control of bovine dermatophilosis. Chatikobo *et al.,* (2001) reported that plunge dipping could in fact increase the spread of the disease while hand spraying reduced the risk of spread.

In Zimbabwe, research on bovine dermatophilosis has been conducted focusing on its control, prevalence and distribution (Chatikobo *et al.,* 2001, Chatikobo *et al.,* 2004, Chatikobo *et al.,* 2009). The aims of the current study were to identify potential herd level risk factors related to bovine dermatophilosis and its association with *A. variegatum* and *A. hebraeum* ticks at selected dip tanks where the two ticks have been reported to interface. Knowledge of these risk factors will assist animal-health decision makers in the control and management of bovine dermatophilosis.

6.2 Materials and methods

6.2.1 Study sites

The study sites were at four dip tanks where co-existence/interface of the two species of *A. hebraeum* and *A. variegatum* was reported by Peter *et al.,* (1998) (Table 6.1).

Table 6.1 Locations where A. hebraeum and A. variegatum co-exist (Peter *et al.*, 1998)

Province	District	Location	Agro-ecological zone	Latitude	Longitude
Midlands	Gokwe south	Gwenyika	3	-18° 24' S	29° 12'E
Midlands	Gokwe south	Chemawororo (Nyaje)	3	-18° 19' S	28° 47' E
Midlands	Kwekwe	Koronika	3	-18° 07' S	29° 26' E
Mashonaland West	Chegutu	Chivero	2	-18° 21' S	30° 36' E

6.2.2 Study design and selection of individual herds

A cross-sectional study was conducted between September 2013 and April 2014. The study animals (herds) were purposively selected (Dohoo *et al.,* 2003). Samples of thirty to sixty stock-owners were systematically selected per dip tank. This figure was arrived at considering financial and material resources. Stockowners served as proxies for the herds. That is, herds belonging to selected stockowners became the primary sampling units. One hundred and eighty five (185) herds with a total of 1788 cattle were sampled. On the day of sampling, stockowners were interviewed by the principal investigator and by two district veterinarians using a pretested structured questionnaire written in English. The interviews were conducted in the local Shona and siNdebele languages. The questions sought information on herd level factors such as: herd size, keeping of cattle confined in kraals at night, materials that were used to construct the kraals, source of drinking water, how long farmers had been keeping cattle, history of purchase of cattle in the past three years, personal methods of tick control, if farmers treated their cattle for diseases, knowledge of and on the treatment of bovine dermatophilosis, how the stockowners rated dipping service delivery, dipping sessions attended in the past 12 months (verified from stock cards).

6.2.3 Clinical examination of animals, sample collection

All cattle belonging to participating stock-owners were placed in a race and clinically examined. The herd was initially visually inspected for any signs of bovine dermatophilosis. Clinically ill cattle were restrained in a race and a thorough physical examination was conducted. There was a predefined case definition for bovine dermatophilosis. This definition was based on literature (Samui *et al.,* 1990; Hadrill 1994) and also on the experiences of the

investigating veterinarians. Cattle were classified as follows: positive; if their clinical presentation complied with the case definition for bovine dermatophilosis, or negative if, clinical presentation did not conform to the case definition. The case definition embraced an animal whose clinical presentation satisfied any of the following three criteria: small lesions characterised by hairs clumping like a small paint brush, clear exudative circumscribed lesions with scabs of at least 1 cm diameter and confluent progressive exudative scab lesions affecting significant parts of the animal's body. Presence or absence of *A. variegatum* and *A. hebraeum* was also recorded and the ticks were identified in situ using their characteristic ornate markings (Walker *et al.,* 2003). Tick inspections were conducted on at least five animals and tick samples collected and stored in 70 % ethanol for further verification at the University of Zimbabwe Parasitology laboratory. A herd was considered to be dermatophilosis positive if one animal in the herd presented with clinical signs that conformed to the case definition. With regards to tick infestation, a herd was considered to be *A. variegatum* or *A hebraeum* positive if one animal in the herd was infested with either of the ticks.

6.2.4 Statistical analysis

The potential risk factors, stockowner and animal bio-data were captured using the Epi Info software make view questionnaire utility (Epi Info TM version 3.5.3 database and statistics software for public health professionals 2012). Statistical analysis was performed using STATA/SE 11.2 for Windows (StataCorp, College Station, Texas, USA). Fisher's exact test was used in univariate analysis to evaluate the association between the outcome, bovine dermatophilosis (yes or no) and categorical risk factors. Variables with a p-value < 0.25 in univariate analysis were recruited into the binary logistic regression. In the multivariable

logistic regression, the model was built as follows; the outcome was the binomial herd level clinical dermatophilosis positivity status (negative herd = 0, positive herd = 1) and the explanatory variables with p-values < 0.25; identified in the univariate analysis, were fit into the model. The model was manually constructed using forward-selection applying the maximum likelihood estimation procedure and statistical significance contribution of individual predictors (or group of predictors). The logistic regression model was assessed for goodness-of-fit using the Hosmer-Lemeshow test while its predictive ability was determined using the receiver operating characteristic (ROC) curve (Dohoo *et al.*, 2003).

6.3 Results

6.3.1 Herd level clinical bovine dermatophilosis prevalence

Bovine dermatophilosis clinical positivity was detected in 45% (84/185; 95% confidence interval (CI): 38.2, 52.6%) of the herds that were investigated. The herd prevalence ranged from 6.9% (95% CI: 0.00, 16.7) to 56.7% (95% CI: 43.8, 69.6) with Chivero and Chemawororo dip tanks recording the lowest, and highest prevalence respectively (Table 6.2). The proportion of positive herds was significantly lower ($p< 0.05$) at Chivero than at other dip tanks. Prevalence at the other three dip tanks did not differ significantly ($p > 0.05$). Using the Fisher's exact test in univariate analysis (Table 3), *A. variegatum* was significantly ($p < 0.05$) associated with the occurrence of clinical dermatophilosis while association of the latter with *A. hebraeum* was not significant ($p > 0.05$). *Amblyomma variegatum* ticks infested 69. 7% (129/185; 95% CI: 63.0, 76.4) of the herds while 28.1% (52/185; 95% CI: 21.6, 34.6) were infested by *A. hebraeum*.

Table 6.2 Herd sizes, bovine dermatophilosis clinical cases and Amblyomma tick infestation at small-holder diptanks of Gokwe, Kwekwe and Chegutu Zimbabwe

Diptank	Herds sampled	Herd size		Clinical cases		*Amblyomma* tick infestation			
						A.variegatum		*A.hebraeum*	
		Median	Range	Proportion (%)	95% CI	Proportion	95% CI	Proportion	95% CI
Chemawororo	60	7	1, 26	56.7[a]	43.8, 69.6	73.3[c]	61.8, 84.9	16.7[e]	6.96, 26.4
Gwanyika	52	8	2, 50	48.1[a]	34.0, 62.1	82.7[c]	72.1, 93.3	17.3[e]	6.77, 27.9
Koronika	44	8	2, 41	52.3[a]	36.9, 67.6	65.9[c]	51.3, 80.5	52.3[f]	36.9, 67.6
Chivero	29	7	1, 19	6.90[b]	0.0, 16.7	44.8[d]	25.6, 64.1	34.5[e]	16.1, 52.9
Grand Total	185	8	1, 50	45.4[a]	38.2, 52.6	69.7[c]	63.0, 76.4	28.1[e]	21.6, 34.6

Proportions with different superscripts were significantly different at $p < 0.05$, CI = confidence interval

Stockowners served as proxies for the herds

Table 6.3 shows the results of univariate analysis and descriptive statistics of 12 variables from the structured interviewer-administered questionnaires. Eight variables that had a $p < 0.25$ in univariate analysis that is; bought cattle (yes vs no), herd size (small, medium, large), knowledge of dermatophilosis, age category of affected cattle, treating cattle for diseases, *A. variegatum* (present vs absent), dip attendance, purchase of own acaricide and dip tank were, fitted into to the multivariable logistic regression model.

Table 6.3 Distibution of bovine dermatophilosis positive and negative cattle herds (n=185) according to risk factors and results of univariate analysis using Fishers's exact test

Variable	Category	Number	Dermatophilosis		OR[b]	P
			+ve[c]	-ve[d]		
Bought cattle[a]	Yes	92	63	29	7.4	0.00
	No	93	21	72		
Herd size[a]	Small	128	52	76	1.92	0.004
	Medium	40	18	22		
	Large	17	14	3		
Knowledge of dermatophilosis[a]	Yes	95	55	29	1.93	0.001
	No	90	40	61		
Age Category[a]	=<4 years	23	23	0	0.37	0.00
	Greater than 4 years	95	36	59		
Treat cattle for diseases[a]	Yes	63	61	2	0.072	0.00
	No	22	14	8		
Dipping Quality[a]	Poor	84	41	44	0.99	0.336
	Adequate	74	29	45		
	Good	26	14	12		
Dip attendance[a]	Very poor	10	3	7	1.80	0.05
	Poor	39	12	27		
	Good	136	69	67		
Purchase own acaricide[a]	Yes	145	70	14	1.73	0.134
	No	40	75	26		
Amblyomma variegatum[a]	Yes	129	76	53	8.6	0.00
	No	56	8	48		
Amblyomma hebraeum	Yes	52	25	27	1.16	0.743
	No	133	59	74		
Period keeping cattle	5 years and less	23	2	21	1.16	0.423
	6-10 years	24	7	17		
	11-15 years	15	3	12		
	16-20 years	27	7	20		
	More than 20 years	96	25	71		
Dip tank[a]	Chivero	29	2	27	1.6	0.00
	Koronika	44	23	21		
	Gwanyika	52	25	27		
	Chemawororo	60	34	26		
Source of water	Home borehole	10	4	6	1.0	0.970
	Common borehole	64	30	34		
	Dam	10	4	6		
	River	101	46	55		

[a]These variables had Fisher's exact $p < 0.25$ and were presented to the multivariable logistic regression models, [b] OR= odds ratio, [c]+ve= positive, [d] –ve = negative

The multivariable logistic regression model revealed study site, bought cattle, *A. variegatum* (yes vs no) and treatment of cattle for diseases as independently associated with herd dermatophilosis positivity (Table 4). The Hosmer-Lemeshow goodness-of-fit test showed that the model fit the data (X^2 = 4.06, d.f. 8, p = 0.85). The model had a good predictive ability (area under curve = 0.96).

Table 6.4 Final multivariate logistic regression of herd level factors for bovine dermatophilosis positivity in communal area cattle herds of Zimbabwe (2013-2014)[a]

Variable	Level	Multivariable logistic regression[b,c]				
		B	S.E (*B*)	P	OR	95% CI
Constant		-3.88	1.68	0.021	-	-
Study site	Chivero	-	-	-	-	-
	Koronika	3.07	1.47	0.037	21.46	1.21, 380.96
	Gwanyika	1.53	1.46	0.295	4.62	0.26, 80.8
	Chemahororo	2.11	1.48	0.153	8.33	0.46, 152.20
Bought cattle	No	-	-	-	-	-
No	Yes	1.25	0.59	0.035	3.49	1.09, 11.12
Amblyomma variegatum presence	No	-	-	-	-	-
No	Yes	1.91	0.71	0.007	6.80	1.71, 27.10
Treatment of cattle for diseases	No	-	-	-	-	-
	Yes	3.13	0.98	0.002	22.90	3.31, 158.38

[a]Results are given with beta (*B*), standard errors (S.E) and odds ratio (OR) with 95% confidence intervals (CI)

[b]Overall data of the model: Log likelihood – 42.58, LR Chi^2 (7 d.f.) = 169.73, p = 0.00, number of observations = 185.

[c]Dependent variable: herd at least one animal clinically positive (yes/no)

6.4 Discussion

A limitation of this study was the reliance on clinical signs as a proxy for infection with bovine dermatophilosis and the use of purposive sampling. Regardless, the results from the study do provide a picture of risk factors for bovine dermatophilosis in the smallholder sector of Zimbabwe, purposive sampling is a recognised epidemiological sampling method (Dohoo *et al.,* 2003). Further, the investigators had long years of experience encountering bovine dermatophilosis cases in the field which in this case was a positive factor. Other studies have been conducted Nyman *et al.,* (2007); Waage and Vatn (2008); Dippel *et al.,* (2009) where only clinical signs were used as the outcome in efforts to determine potential herd or individual level risk factors for the animal health conditions. These studies were carried out to determine potential herd/individual animal level risk factors for clinical mastitis and lameness in sheep and cattle.

The observed variation in prevalence of dermatophilosis between some dip tanks can be attributed to management factors and agro ecological factors. Chivero dip tank which had the lowest prevalence (6.9%) is in agro-ecological zone 2 while the other three study sites with prevalence ranging from (48.1 – 56.7 %) were in zone 3, for these latter three, the differences in prevalence were not statistically significant. Agro-ecological zone 2 is characterised by intensive farming and moderate rainfall while zone 3 is characterised by semi-intensive farming and moderate to erratic rainfall (Hove *et al.,* 2008). The presence of higher temperatures in zone 3 together with moderate rain could provide an ideal environment for the germination and propagation of *D. congolensis* zoospores as compared to the colder zone 2. The other reason for the difference could be the fact that the proportion of tick infested cattle differed per study site particularly between Chemawororo and Chivero.

Chivero had more cattle infested with *A. hebraeum* than with *A. variegatum* and vice versa for Chemawororo dip tank. Chatikobo *et al.,* (2004; 2009) have reported on the potential association between *A. variegatum* with bovine dermatophilosis as such the difference in herd infestations by these two ticks could explain the differences in dermatophilosis herd prevalence. These findings were also in agreement with those of Admassu and Alemu (2011) who reported that dermatophilosis was more prevalent in *A. variegatum* tick infested cattle. The prevalence values in the current study differed from those of other workers. Dalis *et al.,* (2009) in Nigeria reported a clinical prevalence of dermatophilosis-like cases, of 17%, which dropped down to 8.7% after laboratory examination. In Ethiopia Admassu and Alemu (2011) reported 1.04 % laboratory dermatophilosis positivity. The differences in the prevalences observed between the current study and the other studies could be attributed to cattle management differences and agro-ecological factors. The other reason for the differences could be that in the other studies dermatophilosis positivity was confirmed through laboratory tests which was not the case in the current study. Nath *et al.,* (2010) reported that the isolation of *D. congolensis* was in most cases difficult; this can lead to low prevalences detected at the laboratory. In the current study, the case definition was strictly adhered to so as to exclude cases of lumpy skin disease, sweating sickness, parafilariasis, ringworm and scab.

The history of introducing new cattle into herds through purchases placed those herds at higher odds of clinical dermatophilosis. This introduction increased the odds of clinical dermatophilosis by 3.49 (CI: 1.09, 11.12). The current findings agreed with others Matope *et al.,* (2010); Bamaiyi *et al.,* (2014) who reported that the purchase of animals increased the odds of disease in the new herd. In the other studies the diseases of interest were bovine and ovine brucellosis. Chatikobo *et al.,* (2009) reported that cattle brought from outside were responsible for the occurrence of dermatophilosis in some cattle herds in Kadoma in

Zimbabwe. New introductions to herds could introduce dermatophilosis infection or the tick *A. variegatum* with which it is associated.

Treatment of cattle for various other diseases was associated with an increased odds (OR= 22.9) of dermatophilosis compared to herds where no treatment was provided. This association was most likely due to the fact that, farmers were prompted to treat their cattle if these cattle were showing signs of ill-health with such treatment being of no effect, this was not a causal association. The findings were similar to those of Nyman *et al.,* (2007) who reported preponderance by farmers to provide treatment as soon as their animals showed signs of clinical mastitis, odds ratio (OR) associated with treatment in that study was 12.5. Treatment would be expected to be associated with odds of less than one, as treatment is protective (Dohoo *et al.,* 2003).

The other herd level factors such as source of cattle drinking water which is associated with the communal use of drinking points at rivers and dams were not associated with increased odds of herd dermatophilosis positivity in multivariable logistic regression. This was in agreement with findings by Matope *et al.,* (2010). This finding appeared to be at variance with the epidemiology of diseases, which would be expected to increase when animals congregate. Knowledge of bovine dermatophilosis was associated with increased odds (1.93) of herd positivity. Matope *et al.,* (2010) reported that knowledge of brucellosis was associated with reduced odds of disease. In the current study the increased odds could be the result of increased awareness by farmers of the disease as an important animal health condition. This awareness is possible through colleagues and veterinary extension staff. Herds with a good dipping attendance record had an increased odds (OR = 1.8) in univariate

analysis but this was not significant in multivariable logistic regression, of being clinically positive to dermatophilosis than herds with a poor attendance. Chatikobo *et al.,* (2001) reported that plunge dipping increased the risk of cattle contracting bovine dermatophilosis, this appears to be in agreement with the current study since in this case cattle were plunge-dipped. During plunge dipping cattle congregated and mixed with infected cattle, such herds were likely to pick up infection via scabs that contaminate the dip. The quality of dipping service delivery had no association (OR 0.99) with herd level dermatophilosis positivity. These findings were in contrast to those of Chatikobo *et al.,* (2009) who postulated that poor dipping services were responsible for the spread of dermatophilosis in Zimbabwe. Farmers who purchased their own acaricide had increased odds (OR= 1.73) in univariate analysis, that their herds would be clinically positive than herds belonging to farmers who did not buy acaricides. Farmers usually purchase acaricides that are applied by spraying or pour-on. According to Chatikobo *et al.,* (2001) applying acaricides through these methods reduced the incidence of dermatophilosis, this seems to contradict findings in the current study, in that purchase of acaricide was associated with increased odds of disease. The increased odds associated with purchasing acaricide could be due to the response of farmers to poor dipping, the latter which is reportedly (Chatikobo *et al.,* 2009) associated with dermatophilosis. A larger herd size had increased odds (OR= 1.92) of having dermatophilosis although this was not significant in multivariable logistic regression. Matope *et al.,* (2011) have reported similar findings with this association being significant in the multiple regression models. Large herds increase chances of picking up the disease and these herds are more likely to include brought in or purchased animals. Period of keeping cattle had no association with dermatophilosis as was *A. hebraeum* tick infestation.

In conclusion, the current study showed that clinical dermatophilosis was present in herds at all the four study sites. Area, purchase of cattle, treatment of cattle for diseases and infestation with *A. variegatum* were independently associated with herd level clinical dermatophilosis while *A. hebraeum* was not. The implementation of strict movement control and tick control could reduce the level of clinical dermatophilosis. As such, spraying instead of plunge dipping should be encouraged in high risk areas.

6.5 References

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Chapter 7: General discussion, conclusion and recommendations

7.1 General discussion

Ticks are important parasites of livestock and infestations impact cattle production worldwide (Fuente *et al.,* 2007). The traditional control of ticks and the diseases that they transmit has relied mainly on the use of synthetic products (Willadsen 2006). Bovine dermatophilosis a tick associated disease caused by an actinomycete bacterium, *Dermatophilus congolensis* (Gebreyohannes and Gebresselassie 2013) is characterised by a localised or generalised exudative dermatitis (Admassu and Alemu 2011). Economically, bovine dermatophilosis is important due to morbidity and mortality, damage to hides and its effect on draught animal power (Bayisa *et al.,* 2012). The objective of this study was to determine the ethno-veterinary practices adopted by farmers to manage bovine dermatophilosis and ticks, including, at selected small-holder areas of Zimbabwe how the interface of *A. variegatum* and *A. hebraeum* impacts some epidemiological parameters of bovine dermatophilosis.

In chapter 3 a questionnaire was survey was conducted to determine the ethno-veterinary practices that farmers used to control ticks and dermatophilosis. Economically disadvantaged farmers do not have access to modern veterinary medical care which could be expensive and often not available when needed (Ghotge *et al.,* 2002). High costs of chemotherapeutic drugs for the control of bovine dermatophilosis have led some researchers to investigate the efficacy of local herbs for the treatment of this condition (Imam *et al.,* 2008). Respondents to the questionnaire reported that they mainly used *Cissus quadrangularis*, *Catunaregam spinosa* and *Pterocarpus angolensis* for the control of bovine dermatophilosis. For tick control only a few

respondents reported use of plants. Three plants were used for the control of ticks and these were *Manihot esculenta, Carica papaya* and *Blumea decurrens*.

In order to evaluate the efficacy of the above six plants, *in vitro* and *in vivo* trials were conducted (Chapters 4 and 5). *In vitro* antimicrobial studies demonstrated that *Pterocarpus angolensis* was more effective against *D. congolensis* field strains than were the other three plant extracts. Samie *et al.,* (2009) reported that *P. angolensis* had antibacterial and antiprotozoal activities. In the *in vivo* therapeutic trials *C. quadrangularis* extract led to cure being observed on day 26 of the study. Flavonoids and tannins in *C. quadrangularis* have been postulated to be responsible for the analgesic and anti-inflamatory properties of the plant extracts (Panthong *et al.,* 2007; Bujade *et al.,* 2012). Smallholder farmers possibly use *C. quadrangularis* for its anti-inflammatory and analgesic properties. In the *in vitro* and *in vivo* acaricidal trials (chapter 5), *M. esculenta* (cassava) exhibited superior acaricidal activity than *B. decurrens* and *C. papaya* against *R. appendiculatus,* this was minimal against *A. hebraeum*. *Manihot esculenta* contains a number of metabolites among them are terpenoids, flavonoids and condensed tannins (Marie-Magdeleine *et al.*, 2010). Terpenoids have been reported to have acaricidal activity (Righi *et al.,* 2013). This meant that farmers were justified in their use of some these plants for tick control, in the face acaricide shortage.

The impact of *A. variegatum* and *A. hebraeum,* and that of other potential herd level risk factors on the epidemiology of bovine dermatophilosis were investigated through a questionnaire and cross-sectional survey (Chapter 6). Amblyomma tick infested cattle had increased odds of having dermatophilosis than did those not infested by the tick. *Amblyomma hebraeum* tick infestation had no significant association with herds having dermatophilosis. Chatikobo *et al.,* (2004; 2009) have reported the strong association between *A. variegatum*

and dermatophilosis. Control strategies aimed at either eliminating this tick or reducing its infestation levels would reduce the debilitating effects of dermatophilosis on herds. Purchase of cattle was shown to increase the odds of herds being affected by dermatophilosis, as such implementation of strict movement control would reduce the prevalence of dermatophilosis in smallholder herds.

7.2 . Conclusions

- Smallholder farmers use medicinal plants in the ethno-veterinary control of bovine dermatophilosis and for the control of ticks.
- *In vitro* studies showed that extracts of *C. quadrangularis, Pterocarpus angolensis* and *Catunaregan spinosa* had antimicrobial effects against *Dermatophilus congolensis*. *Cissus quadrangularis* exhibited curative properties against dermatophilosis although not as good as the conventional drugs. Farmers could be benefiting from its analgesic and anti-inflammatory effects
- In the *in vitro* and *in vivo* studies plants exhibited variable acaricidal properties with considerable tick load reductions being exhibited during field trials. *M. esculenta* exhibited superior acaricidal properties compared to *C. papaya* and *B. decurrens.*
- The herd level presence of bovine dermatophilosis was strongly associated with cattle infestation by *A. variegatum* while infestation by *A. hebraeum* did not have a significant association. Other herd level risk factors such as purchase of cattle together with *A. variegatum* had an effect on the epidemiology of bovine dermatophilosis.

7.3 Recommendations

It is recommended that further studies be undertaken in the following areas

1. *In vivo* dermatophilosis therapeutic studies involving different preparations and higher concentrations of *C. quadrangularis* on cattle.
2. Further *in vitro* and *in vivo* acaricide efficacy studies using various extractants on *M. esculenta*

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Appendices

Appendix 1: Questionnaire for ethnoveterinary control of dermatophilosis and ticks

Questionnaire on ethno-veterinary practices in the control of ticks and bovine dermatophilosis in the small-holder sector

This questionnaire will be used to generate information on the various ethno-veterinary practices used by farmers in the small-holder sector of Midlands and Mashonaland West Provinces. Information generated through this questionnaire will be used only for the purposes of this research there are no right or wrong answers.

Household number ☐

Date ☐☐☐☐ ☐☐ ☐☐

Year **Month** **Day**

Province _____ Enumerator name _____

District _____ Respondents name _____

Ward _____ Sex of respondent _____

Village _____

SECTION A: HOUSEHOLD DEMOGRAPHY

1. Who is the head of the household? ☐ 1) Father 2) Mother 3) Children 4) other*
   *Specify _____

2. Table on household demographics

Household member	**Relation with household head[a]**	**Marital status[b]**	**Gender**	**Age[c]**	**Highest level of education[d]**	**Animal husbandry training[e]**	**Occupation[f]**

[a]:(1)- Father (2) mother, (3) son (4) daughter (5) daughter in law (6) son in law (7) wife (8) husband (9) self (10) specify

[b]: (1) single, (2) married, (3) divorced, (4) widowed, (5) separated, (6) other (Specify) (7) N/A

[c]: (1) ≤ 25 years, (2) 26 – 40 years, (3) 41 – 55 years, (4) 56 – 70 years, (5) > 70

[d]: (1) never attended school, (2) Primary level, (3) Secondary, (4) Tertiary level (5) University (5) N/A

e: (1) Master farmer training, (2) Certificate in Agriculture/Veterinary, (3) Diploma in Agric/Vet (4) Degree Agriculture/Veterinary, (5) None (6) N/A

[f]: (1) teacher, (2) student (3) church leader (4) traditional healer (5) nurse (6) police (7) extensionist (8) Farmer (9) other (specify) (10) N/A

3. Sources of income? (1) Crops ☐ (2) livestock ☐ (3) salaries/wages ☐

   (4) other (specify) _____ (5) none ☐ (6) N/A ☐

SECTION B: LIVESTOCK INVENTORY

4. Fill in the table below for the livestock owned

Class	Cattle				Other stock		
Cattle	Cows	Bulls	Calves	Others	Sheep	Goats	Donkeys
Number							

5. Who owns the cattle? (1) Father, (2) Mother, (3) Grandfather (4) Grandmother (5) child (6) other (specify) _____

6. What functions do cattle play in your household? Complete the table below

Role	**Rank according to importance from 1 to 4 which will be the least important**	**Rank subfunctions according to importance within major role** **1= very important, 2= important, 3= least important, 4= not important**		
Relating to crop production		Tillage	Provision of manure	Transport (of inputs, water etc)
Consumption		Milk for domestic	Meat for local	Hides and horns for

		consumption and sale	use or sale	local use and sale
Household finance		Investment from crop income	School fees	Capital storage
Social		Installation of ancestral spirits	Bride wealth	Social status (pleasure in ownership)
Other (specify)				

7. What supplementary feed do you give to your cattle? ☐ (1) None, (2) Stover (3) silage, (4) other (specify) _____

SECTION C: CATTLE DISEASE SITUATION

8. Do you have problems with cattle diseases? (1) Yes ☐ (2) No ☐

9. Of the conditions that affect cattle fill in the following table and rank them according to the importance from 1 (very important) to 7 or greater (least important)

Condition/Disease	**Season of the year**	**Rank**
Ticks		
Skin diseases		
Dermatophilosis		
Diarrhea		
Weight loss		
Behavioural abnormality		
Tick-borne diseases		
Reproductive		
Other		

SECTION D: CATTLE DISEASES AND TICK CONTROL

10. Do you treat any diseases including dermatophilosis or conditions affecting your cattle?
    (1) Yes ☐ (2)No ☐

11. Who performs the treatment; use the key below.
    (1) most often (2) few occasions (3) never

Father ☐ Mother ☐ Extensionist ☐ Vet ☐ Other (specify)

12. Have you ever used alternative methods for control (1) Yes ☐ (2)No ☐

13. For what did you use them for (tick any or all) ticks ☐ dermatophilosis ☐ other diseases ☐

14. How often do you use the following practices for controlling cattle ticks ?; use key below.

Plants ☐ Commercial drugs ☐ non-plant remedies ☐

(1) most often (2) few occasions (3) never

15. How often do you use the following practices for controlling dermatophilosis?; use key below.

Plants ☐ Commercial drugs ☐ non-plant remedies ☐

(2) most often (2) few occasions (3) never

16. How do you rate the effectiveness of the treatment methods used in 14 and 15. Use keys below.

Plants ☐ Commercial drugs ☐ non-plant remedies ☐

(1) very effective (2) moderately effective (3) not effective (4) do not know

17. If for the control of ticks, dermatophilosis and other diseases you have used non-plant remedies complete the table below.

Local practice for treatment	**Rank according to use frequency***	**Preparation method**	**How applied**	**Conditions treated**	**Side effects if any**
Used engine oil					
Mechanical Grease					
Hot water					
Paraffin					
Soap(specify)					
Ash					
Cow dung					
Plants(Herbs)					

Other					

*(1) most frequent (2) moderately used (3) rarely used (4) never

18. Why do you use these alternative control methods

1.
2.
3.
4.

19. If plants are used which are those and how do you rank their importance, complete table below

Disease	**Plant (local name)**	**Part of plant used**	**How prepared**	**How* applied**	**Effective for how long**	**Rank****

*include frequency of use, * *(1) most important (2) moderately important (3) not important

20. If for the control of ticks or diseases you use alternative methods such as plants or non-plant remedies, how did you obtain the knowledge? (Circle the most important source).
    (1) Oral tradition (2) from other farmers (3) read somewhere (4) local elders.
21. Are you allowed to share the knowledge of alternative control methods mentioned above?

    Yes ☐ No ☐

22. Do you think that students should be taught the use of plants in schools, colleges and Universities
    Yes ☐ No ☐
23. Are alternative methods used, better than the use of commercial products, Yes ☐ No ☐

24. What are the advantages and disadvantages of use of alternative methods, such as plants and non-plant remedies, fill table below.

Advantages	**Disadvantages***

*Indicate side effects and precautions taken to minimize these

25. What are the advantages and disadvantages of using commercial drugs for cattle ticks and disease

Advantages	**Disadvantages**

THANK YOU

Appendix 2: Questionnaire risk factors of dermatophilosis

UNIVERSITY OF FORT HARE, DEPARTMENT OF LIVESTOCK AND PASTURE SCIENCE

Questionnaire Survey on: Bovine dermatophilosis and Animal- and Herd-level risk factors in small-holder Cattle herds in Zimbabwe and *Amblyomma hebraeum* and *A. variegatum* Tick infestation and in small-holder Cattle herds in Zimbabwe

GENERAL INFORMATION

Date ☐☐☐☐☐☐☐☐ Herd Number ☐☐☐

Province _____ District _____

Dip tank/Farm _____

Stock-owner name _____ Sex: Male ☐ Female ☐

CATTLE DEMOGRAPHIC INFORMATION

1. Cattle numbers.

Cows ☐☐ Bulls ☐☐ Oxen ☐☐ Steers ☐☐ Heifers ☐☐ Calves ☐☐

2. Do you kraal your cattle at night? Yes ☐ No ☐

3. Type of kraal that you have:

Entirely poles ☐ smooth wire ☐ barbed wire ☐ Other…….

4. Main source of cattle drinking water: Borehole; Home ☐ BH community ☐

River ☐ Dam ☐

5. For how many years have you been keeping cattle:

=<5 ☐ 6-10 ☐ 11-15 ☐ 16-20 ☐ >20 ☐

DISEASE SITUATION

6. Have any diseases affected your heads in the past twelve months

☐ ☐

Yes No

7. Have any of your animals died as a result of diseases in the past twelve months

Yes ☐ No ☐

8. Name and rank (1 – 5) the five most important diseases/conditions that affect your herd

☐ ……..

☐ ……..

☐ ……..

☐ ……..

☐ ……..

9. Did you buy any cattle in the past three years Yes ☐ No ☐

HISTORY AND KNOWLEDGE OF BOVINE DERMATOPHILOSIS

10. Have you ever heard of the disease bovine dermatophilosis? Yes ☐ No ☐
11. Is there a local name for dermatophilosis? Yes ☐ No ☐
12. What is the local name for dermatophilosis if any? _____
13. Do you know the cause(s) of dermatophilosis Yes ☐ No ☐
14. If yes above, what do you think are the causes of dermatophilosis?.....

……..

15. From whom did you get further knowledge about dermatophilosis?…….....
16. Have you ever had bovine dermatophilosis in your herd? Yes ☐ No ☐

17. If yes when was the last time: Current ☐ < 3 mths ☐ 3 – 6 mths ☐

7–9 mths ☐ 10-12 mths ☐ > 12 mths ☐

18. During which time of the year do you get dermatophilosis in your herds

Jan – March ☐ April – June ☐ July – Sept ☐ Oct – Dec ☐

Throughout the year ☐ Do not know ☐

19. When do you get highest cases of bovine dermatophilosis in your herd

Jan – March ☐ April – June ☐ July – Sept ☐ Oct – Dec ☐

Same throughout the year ☐ Do not know ☐

20. Have any of your cattle died/slaughtered/culled due to dermatophilosis? Yes ☐ No ☐

21. Which major sign(s) convince(s) you that your cattle have dermatophilosis……

……

22. Do you think that, bovine dermatophilosis is dangerous to humans? Yes ☐ No ☐
    DontKnow ☐
23. If yes above, what is the danger……..

24. How can you protect yourself from this danger…….

25. Do you consider that bovine dermatophilosis a big problem in your area? Yes ☐ No ☐ DontKnow ☐

26. Do you consider bovine dermatophilosis to be a big problem in your own herd?

Yes ☐ No ☐ DontKnow ☐

CURRENT CASES OF BOVINE DERMATOPHILOSIS

27. Date of onset if known: ☐☐☐☐☐☐☐☐ Unknown ☐

28. Cattle affected

At risk cattle ☐☐ cases ☐☐ Deaths ☐☐

29. Age groups affected < 2 years ☐☐ 2-4 years ☐☐ 5-7 years ☐☐ > 7 years ☐☐

30. Sex (number): Male ☐☐ Female ☐☐

31. Clinical presentation of diseases (tick)

Head	Neck	Dewlap	Brisket	Shoulder	Back	Rump	Belly	Udder	Perineum	Genitalia
☐☐	☐☐	☐☐	☐☐	☐☐	☐☐	☐☐	☐☐	☐☐	☐☐	☐☐

32. Severity of lesions (number of cattle)

Multiple small eruptions ☐☐ Paint brush ☐☐ Scabs > 1 cm ☐☐ Confluent lesions (scabs) ☐☐

33. In the past 12 months how many times has your herd been affected with dermatophilosis?

1-2 ☐ 3-5 ☐ > 6 times ☐ not sure ☐

MANAGEMENT OF DERMATOPHILOSIS

34. Do you know of any treatments for dermatophilosis? Yes ☐ No ☐

35. If yes, above, which ones do you know of……..……..

……

36. Do you treat your cattle for dermatophilosis? Yes ☐ No ☐

37. If yes which drug do you use most often …….

38. Which drug do you think works the best against this disease…….

39. How many days do you give treatment, for the animal to be treated?

1 ☐ 2 - 3 ☐ 4 - 5 ☐ 6 - 7 ☐ >7 ☐

40. How long after first treatment does it take for cattle to be treated?

< 7 days ☐ 8-14 days ☐ 15-21 days ☐ 22- 28 days ☐ 29-35 days ☐ >36 days ☐

41. Who often treats your sick animals?

Father ☐ Mother ☐ Extensionist ☐ Vet ☐ Other (specify) ……

42. Are there other methods you use for treatment besides drugs Yes ☐ No ☐

43. If yes what are they……..

……..

DIPPING SITUATION

44. In the past 12 months how would rate the quality of Government-provided dipping

Very-poor ☐ Poor ☐ Adequate ☐ Very good ☐ DontKnow ☐

45. How many dipping sessions did you miss in the past 12 months*? (check stock card)

None ☐ < 3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐ >12 ☐ do not know ☐

46. When were your cattle last dipped*? (check stock card)

Less than a week ☐ 2-4 weeks ago ☐ 5-8 weeks ago ☐ > 8 weeks ago ☐

47. List the main reasons for missing the dipping sessions…….……

…….

48. In the past year has the dip tank been emptied and cleaned?

Yes ☐ No ☐ DontKnow ☐

49. If no above, how long ago was it last emptied and cleaned

1-2 yrs ☐ 3-4 yrs ☐ 5-6 yrs ☐ >6 yrs ☐ DontKnow ☐

50. Do you at times buy and use your own dipping chemicals? Yes ☐ No ☐

51. How do you mainly apply the dip?

Plunge dip ☐ spray ☐ pour-on ☐ "paste" ☐

52. Do you know if dipping affects dermatophilosis Yes ☐ No ☐

53. If yes above how does dipping treats dermatophilosis……..

54. Any additional comments you wish to make on bovine dermatophilosis

……
……
……

TICK INFESTATION

Tick infested Yes ☐ No ☐

Amblyomma hebraeum Yes ☐ No ☐ ☐ Number ☐

Amblyomma variegatum Yes ☐ No ☐ ☐ Number ☐

THANK YOU