AN INVESTIGATION TO DETERMINE THE RESISTANCE OF THE *Boophilus* TICK (BLUE TICK) TO AMITRAZ IN SELECTED AREAS OF ZIMBABWE.

By

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Abstract

The purpose of this study was to determine if the *Boophilus* species spectrum of ticks infesting cattle owned by resource limited farmers in the state owned communal land areas of Zimbabwe, are resistant to Amitraz. The study was also aimed at finding a concentration of the acaricide that the tick species are susceptible to, and can give at least 99.9% mortality. Five districts were selected from the Matabeleland South province and five farms were randomly selected from each district. The study was carried out from December 2012 to February 2013. The most numerous ticks collected were the *Rh. B. microplus* and *Rh. B. decoloratus* and these were the ticks of interest as they cause the major Tick Borne Diseases (TBDs) that have a great impact in the economy. Fully engorged female ticks were collected at random from the cattle in all the selected farms. Sample collection was repeated after every month within the duration of the study. More than 100 fully engorged female ticks were collected from Insiza, Mzingwane, Mberengwa, Nkayi and Tsholotsho and these were identified, cultured and larval ticks were produced in the laboratory. The laboratory work was carried out at Central Veterinary Laboratories in Harare, following their standard operating procedures. Using the Larval packet test, pieces of Whatmann 541 filter papers were impregnated with oil solution containing the acaricide, Amitraz. The papers were formed into packets where tick larvae were put and the response was determined after 24 hours. The packets were opened and the tick larvae examined under a lude light. All moving larvae were counted and removed by sucking them up a vacuum pump. Only those larvae capable of walking were considered alive. The remaining dead ticks were counted and results recorded. The data obtained were expressed as percentage mortality at each concentration level. A two way ANOVA was conducted to examine the effect of concentration and species on tick mortality. The dependant variable (mortality) was normally distributed for the groups formed by the combination levels of concentration and species as assessed by the Shapiro-Wilk test. There was homogeneity of variance as assessed by Levine's test for equality of error variances. Data was organized and represented in the form of descriptive tables, histogram and line graphs to assist in the analysis of data. Three districts showed about 99.5% elimination of the ticks using Amitraz whereas the other two showed 79% and 47%. The results implied that there is no acaricide resistance in the selected districts and that tick control failure is due to factors other than resistance.

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Dedications

This project is dedicated to my late mother Mrs S. Yiwombe for the love and support she always gave me and my sister Wendy Yiwombe.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cattle are a source of food for household consumption (Sansoucy, 1995) and they provide draught power for crop production, hides, manure and cash through sales (Chimonyo, Kusina, Hamudikuwanda and Nyoni, 1999; Palmer and Ainslie, 2006). Cattle owned by resource-poor farmers are kept on communal rangelands where they are grazed extensively (Masika and Mafu, 2004). Communal grazing is characterized by poor management of cattle and low productivity. Communal farmers rarely use drugs to treat their animals. Consequently, diseases and parasitism are rife and major threats to cattle production in communal areas (Kaewthamasorn and Wongsamee, 2006; Rajput, Hu, Chen, Arijo and Xiao, 2006). Surveys have indicated that communal farmers perceive ticks as the most important health constraint to their cattle (Dreyer, Fourie and Kok, 1998; Dold and Cocks, 2001).

Ticks cause substantial losses in cattle production, in terms of diseases, reduced productivity and fertility and often death, and are economically the most important ecto-parasites of cattle (Rajput *et al.*, 2006). Ticks suck blood; damage hides and skins, introduce toxins and predispose cattle to myiasis and dermatophilosis (Gates and Wescott, 2000; Mtshali, de Waal and Mbati, 2004). Furthermore, they reduce body weight gains and milk yield, in addition to creating sites for secondary invasion by pathogenic organisms (Gates and Wescott, 2000; Turton, 2001; Kaufman, Koehler and Butler, 2006). More significantly, ticks transmit diseases from infected cattle to healthy ones. Ticks transmit a greater variety of pathogenic micro-organisms than any other arthropod vector group, and are among the most important vectors of diseases affecting animals

(Jongejan, 2007). The most economically important genera of tick-borne prokaryotic and eukaryotic haemoparasites infecting cattle in communal areas of Zimbabwe are the rickettsiae *Anaplasma* and *Ehrlichia* (*Cowdria*), and the protozoan parasites *Babesia* and *Theileria* (Bell-Sakyi, Koney, Dogbey and Walker, 2004).

Anaplasmosis, heartwater and babesiosis are the most important constraints to the health and improved productivity of cattle in Zimbabwe. They cause high morbidity and mortality, decreased meat and milk production and loss of draught power, manure and financial resources. Most indigenous cattle in areas where tick-borne diseases (TBDs) occur possess a natural resistance to these diseases (d'Ieteren and Kimani, 2007). These cattle are exposed to the diseases early in life and thus do not usually develop the clinical disease and are subsequently immune (Latif, 1992). In Zimbabwe, tick-related problems are mostly seen during the rainy season while isolated cases may be recorded in winter months. This has prompted farmers to put more attention to tick control during the summer months from November to May, with little attention during the rest of the year (Chimonyo *et al.,* 1999). Tick control programmes implemented on the farm must take cognisance of tick developmental stages and formulate a continuous integrated approach year round.

An acaricide is a pesticide designed to control harmful species of mites (Acari). Mites (subclass Acari), are a morphologically and ecologically very diverse assemblage of tiny invertebrates, belonging to class Arachnida (together with spiders and scorpions), subphylum Chelicerata and phylum Arthropoda. The arthropods also include insects, from which mites differ, besides being eight-legged animals (insects are hexapods) by the lack of true head and conspicuous body

segmentation. There are some 50 000 mite species known today, but it is estimated that the true number is 20 times higher. Besides agricultural pests and their natural enemies (predators), mites include species of medical and veterinary importance (house dust mites, scabies mites, ticks), while the species living in soil and water are important environmental indicators (Dekeyser, 2005).

The use of acaricides has increased substantially over the past half of the 20th century. Since the first serious and widespread outbreaks of spider mites populations, during the 1950s, organophosphorous and other neuroactive insecticides were replaced by specific acaricides (compounds exclusively or primarily effective against mites). Several generations of structurally diverse synthetic acaricides, directed against various biochemical and physiological targets, have been commercialized until now. Besides specific acaricides, a number of insecticides with considerable acaricidal activity (pyrethroids, avermectins, and benzoylureas) have also been used, while some older neuroactive compounds are still available for the control of phytophagous mites (Jeppson, Keifer and Baker, 1975; Knowles, 1997; Dekeyser, 2005; Van Leeuwen, Witters, Nauen, Duso, and Tirry, 2010).

The *Boophilus* tick commonly known as the blue tick is one of the commonest, most widespread tick in this country, that is, *Rhipicephalus B. microplus. Rh. B. decoloratus*, *Rh. B. annulatus, Rh. B. geigyi* and *Rh. B. kohlsi* in this case (Rajput *et al.*, 2006). Consistently heavy infestations of *Boophilus* species cause hides to be downgraded. Generally all hides from a region are downgraded, so that losses can be prevented only by eradication (de Castro, 1997). Ticks of other genera are of lesser significance in respect of hide damage as they have predilection sites

for feeding that are either of no value (ears, perianal) or small value (axilla, groin, udder, scrotum), in this case, damaged areas are removed during trimming (de Castro, 1997).

If the losses in cattle production from ticks and the diseases they transmit are to be prevented or eliminated, it is necessary to control or eradicate ticks. The most widely used effective method is the treatment of animals by dipping them or spraying them with chemicals that kill ticks called acaricides or ixodicides or tickicides (de Castro, 1997).

Resistance of ticks is known to occur in all or almost all areas where cattle have been treated with acaricides to control tick infestations (Allen, and Uilenberg**,** 1994). Where the 1-host ticks *Rh. B. microplus* and *Rh. B. decoloratus* are important parasites, it has been necessary to change to new classes of acaricides at frequent intervals because of resistance (Angus, 1996; Bruce and Mazhowu, 1992). Resistance must be suspected when cattle, having been treated in the same way for years, are observed to be more heavily infested than expected (Allen, and Uilenberg**,** 1994).

When a control failure is reported, the first reaction must be to check whether the acaricide is being applied correctly and at the correct concentration. In such instances the larval packet test can be used to detect resistance of ticks at different concentrations of the acaricide (de Castro and Newson, 1993).

1.2 Justification

The purpose of the study was to determine the susceptibility of *Rh. Boophilus decoloratus* and *microplus* ticks to Amitraz. The main reasons why ticks are controlled is that they act as vectors for certain live stock diseases and they also cause tick paralysis or toxicosis and physical damage to the skin. More and more chemical acaricides have been synthesized and tick resistance to those chemical pesticides poses a serious threat to most farmers worldwide. This has caused farmers to spend a large percentage of their time and money on the management of ticks and tick-borne diseases. Because of the above mentioned reasons it is necessary for research to look at how susceptible these ticks are to Amitraz as it is among the widely used acaricides throughout the country. Given this challenge there is a need for a testing method that can truly determine if there is resistance or not and also give us the effective concentration of Amitraz that will give a 99.5% mortality kill. Therefore the Larval Packet Test is specific, sensitive and cheaper than other methods.

Therefore, there is need to sample ticks from around the country as communal farming is an important activity that contributes to the country's economy. Information on the susceptibility of these ticks to Amitraz facilitates the development of sustainable control strategies to enable communal farmers to reduce the burden of these parasites on their stock. For the farmers to fully benefit from the research, there is need for their active participation during data collection.

1.3 Objectives

1.3.1 Main objective:

- to determine if the *Rh. Boophilus* ticks have developed resistance to Amitraz.

1.3.2 Specific objectives:

- to determine the effective concentration of Amitraz,
- to investigate the potential acaricidal activity of Amitraz, and
- to test for resistance in the specified areas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Life cycle of *Rhipicephalus Boophilus*.

Rhipicephalus Boophilus is a one host tick, all stages are spent on one animal (Walker, 1991). The eggs hatch in the environment and the larvae crawl up grass or other plants to find a host. They may also be blown by the wind (Sonenshine, 1991). In summer, *Rh. Boophilus* can survive for as long as three to four months without feeding (Hoogstraal, 1978). In cooler temperatures, they may live without food for up to six months. Ticks that do not find a host eventually die of starvation (Sonenshine, 1991).

Newly attached seed ticks (larvae) are usually found on the softer skin inside the thigh, flanks, and the forelegs. They may also be seen on the abdomen and brisket. After feeding, the larvae moult twice, to become nymphs and then adults. Each developmental stage (larva, nymph and adult) feeds only once, but the feeding takes place over several days (Figure 1). Adult male ticks become sexually mature after feeding, and mate with feeding females. An adult female tick that has fed and mated detaches from the host and deposits a single batch of many eggs in the environment (Kemp, Stone, and Binnington, 1982). Typically, these eggs are placed in crevices or debris, or under stones. The female tick dies after ovipositing. Ticks in the subgenus *Boophilus* complete their life cycle in three to four weeks; this characteristic can result in a heavy tick burden on animals (Hoogstraal, 1978).

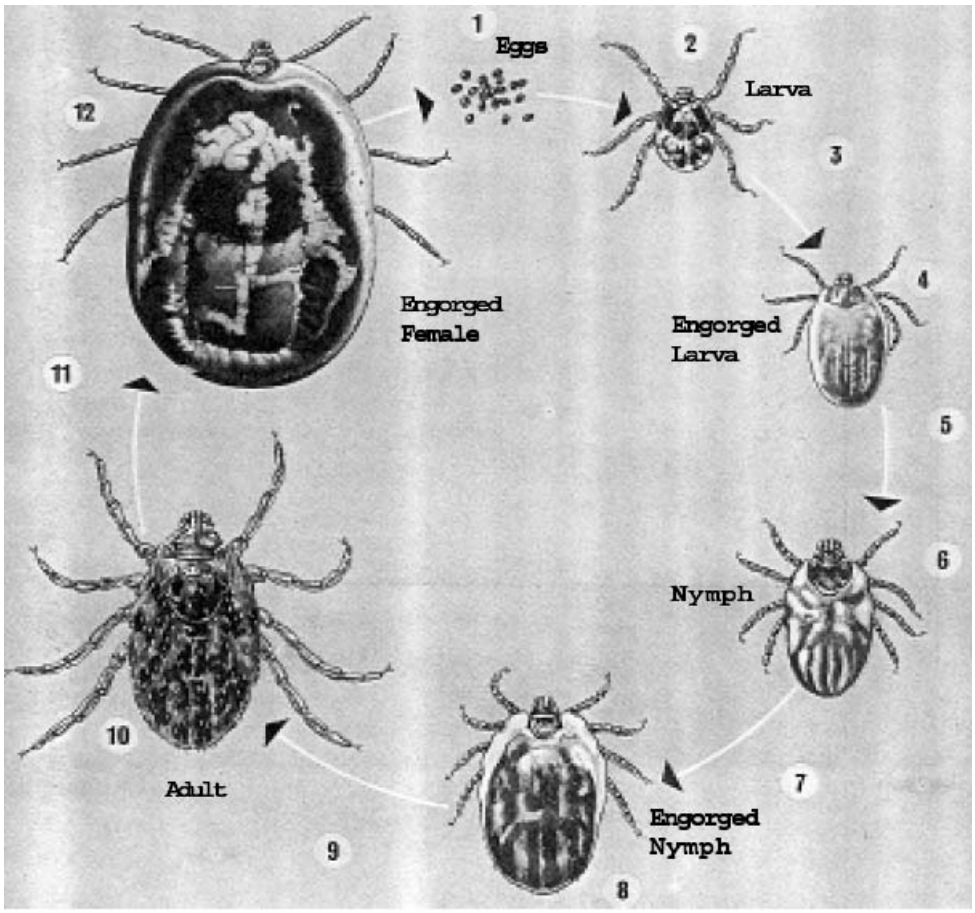

Figure 1: life cycle of *Rhipicephalus Boophilus* ticks

2.2 Taxonomy, distribution and host name

Ticks are ectoparasites of livestock, which are classified (together with mites) in the order Acari. All ticks are obligate ectoparasites of vertebrates. They have four pairs of legs as nymphs and adults, and the body is divided into the capitulum (which bears the mouthparts) and the opisthosoma. There are at least 840 tick species in two major families, namely the Ixodidae or 'hard' ticks (so called by virtue of their hard dorsal shield) and the Argasidae or 'soft' ticks (due to their flexible leathery cuticle). The family Ixodidae comprises approximately 80% of all tick species, including the species of greatest economic importance. However, Argasid ticks also play a significant role as vectors of diseases, especially in poultry.

2.2.1 Ixodidae

There are three active stages in the life cycle of a hard tick: larvae, nymphs and adult ticks. Each instar takes a blood meal only once and long periods are spent on vegetation between blood meals (Balashov, 1972). Most ticks require three different hosts to complete one full cycle. These three-host ticks detach on completion of feeding, drop from the host, moult and wait for another host. However, in some tick species, the engorged larvae remain on the host, where they moult rapidly to become nymphs, continue to feed and then drop as engorged nymphs (Balashov, 1972). These two-host ticks include *Rhipicephalus evertsi* and some *Hyalomma* species. In one-host ticks, the nymphs also remain on the same host and continue to feed as adults (Hoogstraal, 1956). *Boophilus* species are typical one-host ticks. After the female drops from the host, she seeks a sheltered place for oviposition, where she lays a single batch of several thousand eggs and then dies (Balashov, 1972). Balashov (1972) also stated that males usually remain much longer on the host, where they may mate repeatedly. As long periods often elapse between the different feeding periods, ticks are well adapted for long term survival, maintaining their water balance by taking up moisture from the atmosphere (Balashov, 1972).

2.2.2 Argasidae

The life cycle and feeding pattern of the soft ticks are different from those of the hard ticks. The Argasidae (multi-host ticks) have several nymphal stages and the adults also feed repeatedly (Sonenshine, 1991). Feeding can last from a few minutes to hours, or even days for the larvae of some species (Uilenberg, 1992). Most Argasid ticks live in nests or burrows, although there are exceptions. Adults usually mate in the nest or burrow. Mated females take small, repeated blood meals to support the production of small batches of eggs (Sonenshine, 1991). Sonenshine (1991) also stated that the occurrence of several nymphal instars and frequent adult blood meals

contributes to an unusually long life span (several years) and high resistance to starvation. These species are extremely hardy and can survive in hot, dry conditions for long periods without a blood meal. Argasid ticks also concentrate their blood meal by eliminating excess water via the coxal apparatus, which is located in the proximal part of the front pair of legs (Uilenberg (1992). There are approximately 170 species of soft ticks. Species of medical or veterinary importance belong to the genera *Argas, Ornithodoros* and *Otobius* (Sonenshine, 1991).

2.2.3 *Rhipicephalus Boophilus*

The genus *Rhipicephalus Boophilus,* formerly known as *Boophilus,* contains only five species of small ticks, all of which are one host ticks and take approximately three weeks to complete their blood meal, preferably on cattle. The *Boophilus* spp. which have now been classified as a subgenus of the genus *Rhipicephalus* (Horak *et al*., 2002), have short palps which are ridged dorsally and laterally, and they possess eyes which are sometimes difficult to discern (Hoogstraal, 1978). *Rh. Boophilus* ticks have short mouthparts which are unable to penetrate very deeply into the skin (Kemp, Stone, and Binnington, 1982). However, damage to hides is considerable as the preferred feeding sites are often of good leather potential. *Rh. B. microplus* and *Rh. B. decoloratus* are the most important species (Hoogstraal, 1956).

Rh. B. microplus is mainly a tropical cattle tick (Jonsson, 2006). This tick occurs in savanna climates with wooded grasslands which are used as cattle pastures. It is found in Australia, West Indies, Mexico, Central America, South America, Asia, and South Africa (Walker and Olwage, 1987). It has been suggested that *Rh. B. microplus* was introduced into East and South Africa from Madagascar, where it had originally arrived with cattle from southern Asia (Walker and Olwage, 1987). It is a one-host tick and eggs hatch on the ground. Females lay up to 4400 eggs.

The ticks cause irritation and loss of condition, loss of blood in severe infestations and can lead to death. Hosts are mainly cattle, though the tick can be found in sheep, goats and horses (Kemp, Stone, and Binnington, 1982). In cattle, the tick causes *Babesia bigemina* (red water fever), *Anaplasma marginale* (gall fever) and *Babesia berbera* (Kocan, de la Fuente, Blouin and Garcia-Garcia, 2004; Krause, Telford, Ryan, Conrad, Wilson, Thomford, and Spielman, 1994). There is evidence that where favourable humid and warm climatic conditions exist, it competes with and is able to replace the indigenous *Rh. B. decoloratus* (Walker and Olwage, 1987).

Figure 1: Rhipicephalus (B.) microplus - Fed Female **Rhipicephalus (B.) microplus - Male (left) Female (right)**

Rh. B. decoloratus is found in continental Africa, south of Sahara, India, and Yemen (Walker and Olwage, 1987). It is a one-host tick and eggs hatch on the ground. Eggs to adult takes three to four weeks. A toxic protein has been identified in eggs (Hoogstraal, 1978). The tick causes irritation and loss of condition, loss of blood in severe infestation can lead to death. Bites to humans can result to severe inflammation (Kemp *et al*., 1982). Hosts are mainly cattle but can be found in sheep, pigs and goats. In cattle *Rh. B. decoloratus* causes *Babesia bovis, Babesia*

bigemina (red water fever), *Anaplasma marginale, Borrelia theileri, Pseudomonas aeruginosa* (Balashov, 1972).

2.3 Tick control in Zimbabwe

Intensive dipping of cattle for tick control was introduced in Zimbabwe about 80 years ago as a control measure for East Coast Fever caused by *Theileria parva* (Young, Groocock and Kariuki, 1988). The dipping policy was strictly enforced and by the mid 1950s had resulted in apparent eradication of the more virulent forms of *T. parva* and the effective control of other TBDs. However, dipping was disrupted during the pre-independence war in the 1970s and large numbers of susceptible cattle died following exposure to the TBDs. Since independence, the costs of dipping have escalated considerably (Young *et al.,* 1988).

The epidemiological implication of and high costs associated with intensive dipping have led to a re-evaluation of Zimbabwe's policy on control of ticks and TBDs (Norval, 1979). Intensive dipping proved extremely effective in the control of *Amblyomma hebraeum*, the main vector of heartwater, and by the early 1970s, the tick had been eradicated from large areas of the country (Norval and Lawrence, 1979). Control of *Rhipicephalus appendiculatus*, the vector of Theileiriosis, by means of regular dipping, was the only control measure available against January disease (Lawrence and Norval, 1978). Babesiosis transmitted by *Boophilus decoloratus* and Anaplasmosis, were also considered to be effectively controlled by intensive dipping (Matson, 1966).

Ticks are difficult to control because they use multiple hosts and exhibit four developmental stages, making it difficult to thwart them with one method at once (Young *et al.,* 1988). This

means that they can either be on the ground as eggs, on the grass waiting for a host or attached to one of the multiple hosts. Tick control programmes normally target the accessible host (cattle). This has its own challenges from drug selection to spraying and dipping techniques. Farmers usually have a preferred drug which is used again and again (sometimes wrongly) until the types of ticks on the farm become resistant to the drugs (Ndavambi, 2012).

Ndavambi (2012) states that as a rule of thumb, farmers dip cattle weekly in summer and fortnightly in winter, and that this regime is effective when the choice and use of drug is correct. He also stated that with high infestations on the farm, reducing the population of ticks is not as easy as following this routine dipping programme. The tendency is to skip or delay dipping in winter because there are no "visible" signs of infestation (Ndavambi, 2012). During this period ticks will be on the nymphal and larval stages and isolated cases of tick-borne diseases may be diagnosed on the farm. According to Ndavambi (2012), this is because animals are presumed to be "tick free" as the early stages of tick development cannot be seen easily by a naked eye. To reduce tick numbers on the farm, it may be worthwhile to run a full winter season on weekly dipping and try to reduce tick levels before reverting to the conventional fortnightly dipping in the following year. This is expensive in the first year but when this approach is implemented together with other management practices of controlling animal movement it proves to be worthwhile (Ndavambi, 2012).

Major advances have been made in the development of novel methods and strategies for the control of ticks in the recent years (Rajput *et al*., 2006). New and easier methods of applying acaricides are available, ear tags, neck bands, tail bands and pour-on. A mechanical applicator

has also been developed (Pegram, Tatchell, de Castro, Chizyuka, Creek, McCosker, Moran, and Nigarura, 1993). Tick repellents to use on livestock are limited (Pegram *et al.*, 1993), however, several studies have indicated the potential benefits of using tick repellent grasses and plants such as *Melinis minutiflora, Stylosanthus* species and *Cassia absus* (Pegram *et al.*, 1993).

2.4 Resistance development in ticks

The resistance of ticks to acaricides is an inherited phenomenon. It results from exposure of populations of ticks to chemical parasiticides (acaricides) and survival and reproduction of ticks that are less affected by the acaricide. The higher reproductive rate of ticks that have heritable resistance factors and the resulting increase in the proportion of the population of ticks that carry genes for these factors are known as selection (Morales *et al*., 1999).

According to Nolan and Schnitzerling (1986) resistance to a given acaricide can be described as a reduction in susceptibility of a parasite to the acaricide when it is used at the recommended concentration and according to all of the recommendations for its use. In most cases, it is likely that genes that confer resistance are already present at very low levels in the tick population before the introduction of a new acaricide. The rate at which a resistant allele becomes established in the population and the time it takes for the control of ticks to break down is dependent upon many factors. These include the frequency of the original mutation in the population before treatment, the mode of inheritance of the resistant allele (dominant, co-dominant or recessive), the frequency of acaricide treatment, the concentration gradient of the acaricide and the proportion of the total tick population that is not exposed to the acaricide (refugia) (Nolan and Schnitzerling, 1986).

Although the frequency of resistant genes initially only increases slowly, by the time declining efficiency of dipping or treatment is noticed, the rate of increasing frequency of resistance genes is usually high (Nolan and Schnitzerling, 1986). In the initial phase, the frequency of heterozygous resistant individuals (single allele mutation) within the population is low and the rate of increase in the frequency of the resistant allele is low. In the next, emerging phase, given continued exposure to a drug, the frequency of heterozygous resistant individuals within the population increases. Finally, the sustained selection pressure results in increasing numbers of homozygous resistant individuals, which ultimately predominate in the population (Nolan and Schnitzerling, 1986).

It is increasingly common for livestock producers to experience multispecies resistance in parasite populations exposed to acaricides (Morales *et al*., 1999). This sometimes involves multiple tick species (e.g. *Amblyomma variegatum* and *Rh. B. microplus*) or multiple taxa (e.g. *B. microplus* and *H. irritans*). The increasingly frequent use of endectocides in livestock production systems may accelerate this trend inducing resistance in ticks (Nolan and Schnitzerling, 1986).

2.5 Acaricide resistance in Zimbabwe

In many parts of the world the development of acaricide resistant tick strains has with time rendered one chemical agent after another ineffective (Bell-Sakyi *et al*., 2004). To date, the only tick investigated for acaricide resistance in Zimbabwe is the predominant species infesting cattle, *A. variegatum* (Bell-Sakyi *et al*., 2004).

2.6 Diagnosis of resistance in ticks

In selecting a suitable laboratory test for acaricide resistance, the following requirements must be satisfied. The test should be sensitive enough to identify resistance early in its emergence (Kemp *et al*., 1999). It should also cover the full range of chemical groups that are in use, including the most recently developed active ingredients. The diagnostic test should be simple and inexpensive. It should provide a rapid and reliable result, and be suitable for standardization among laboratories in many countries (Kemp *et al*., 1999).

The fact that there are several tests in use for the diagnosis of acaricide resistance in ticks should serve to indicate that none of the tests is perfect in all circumstances (Kemp *et al*., 1999). The larval packet test is considered to be the most repeatable, although it is limited by the length of time that it takes. Hence, it remains the test of choice for surveys and for definitive confirmation of a diagnosis of resistance (Bagherwal, Sisodia, Sharma, Dhanotiya, Ghosal and Ashok, 1995).

2.7 The larval packet test (LPT)

Results for this larval bioassay for the diagnosis of resistance in *Rh. B*. *microplus* takes about six weeks and is based on protocols used for many years by the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Queensland Department of Primary Industries (DPI) in Australia. However, it can be used for other species of Ixodid ticks and has also been widely employed in Latin America and Africa. Following the adoption of this test by the FAO as the preferred means of diagnosis of resistance in ticks, it was promoted in the form of the FAO Acaricide Resistance Testing Kit. It is anticipated that it will continue to be prepared and distributed by the RRLs in Latin America and possibly elsewhere (FAO. 1984, Bagherwal *et al.,* 1995, Baxter, Green, Stutten, and Barker, 1999).

Because of the technically exacting requirements of the bioassays for resistance, very specific requirements exist with regard to the number, stage and age of the ticks and will vary according to the purpose of the test.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Central Veterinary Laboratories, Harare, from December 2012 to February 2013.

3.2 Collection of ticks

The tick samples were collected from five districts in the Matabeleland south province and from each district, five farms were randomly selected. At each farm cattle were restrained and fully engorged female *Boophilus* ticks were collected with a maximum of twenty five ticks from any one animal. Most engorged ticks drop off in the early morning thus the ticks were collected from the cattle and from the ground early in the morning. The ticks were put in small boxes made of cardboard with a few small holes to allow air to circulate. Freshly cut, green grass was placed in the boxes to keep the environment humid and to protect the ticks from damage. Immediately upon arrival at the laboratory, the engorged female ticks were washed with distilled water to remove any eggs that were laid during transportation. The ticks were identified in the laboratory under a microscope to ensure that the specified ticks were the ones collected, that is, the *Rhipicephalus B .microplus* and *Rhipicephalus B. decoloratus.* The project was carried out in a period of three months (December 2012 to February 2013) during the rainy season as there was more infestation of ticks at that time. Sample collection was repeated after a month with all farms in the three month period.

3.3 Culturing of the ticks

The fully engorged female ticks were incubated at 27^{o}C ± 1 for egg laying in labelled lunch boxes. The eggs were separated from depleted females, and three lots of 0.5g of eggs were weighed into tick culture vials. A single layer of organza fabric was put and a lid was placed on top. The samples were incubated at 27^{o}C ± 1 for egg hatching. The eggs were carefully monitored from the start of egg hatch to the finish of egg hatch. The hatched larvae were collected and placed in labelled test tubes.

3.4 Acaricide preparation

Two solvents were used; olive oil was of BP quality and technical grade Trichloroethylene (Trilene) in the ratio 1:2 to produce olive oil/trilene mixture (O/T). Stock solutions of the chemicals (1% concentration of acaricide) were prepared, that is, a mixture of 20 ml of O/T and a calculated acaricide quantity. From the stock solution, other concentrations were prepared by serial dilutions with O/T, each concentration being 50% of the former as shown in figure 3. These preparations were done in a fume hood cabinet. Tubes were tightly closed during dilutions to avoid evaporation of trilene which is used to obtain a uniform layer of acaricide on the filter paper.

Figure 3: The preparation of the acaricide.

3.5 Filter paper preparation and impregnation with acaricide

Whatmann 541 filter papers (each 8.5cm ×7.5cm) were labelled with a pencil indicating the tick species, strain, acaricide and concentration and the date of test. The filter papers were closed on nails fixed on a board with pointed ends facing down. 0.67ml of each acaricide concentration was added to the filter papers in duplicate using a calibrated Gilson pipetman. Control papers treated with O/Trilene were also prepared in duplicate as shown in figure 4. The papers impregnated with low concentrations of the acaricide were made before those of high concentrations to avoid contamination between chemicals. The papers were hung on a rail for an hour for trilene to evaporate. The filter papers were folded into packets by clipping the two extreme ends with bulldog clips leaving the centre open for inoculation of the larvae.

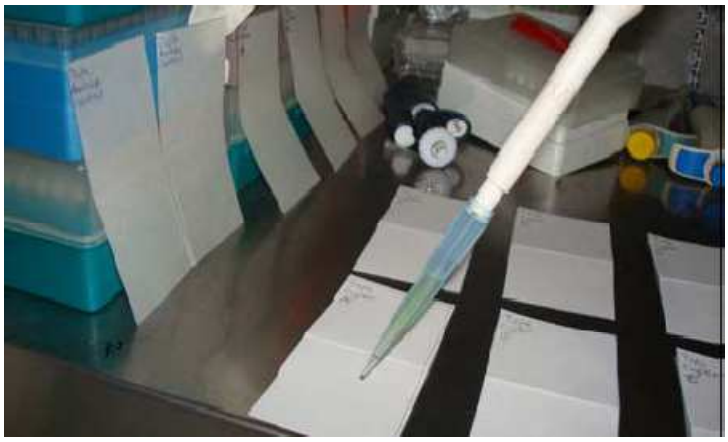

Figure 4: Filter paper impregnation with the acaricide.

3.6 Exposure of ticks to acaricide

Tubes containing unfed larvae (7-14 days old) were placed and attached in petri dishes with a double sided cello tape. Diluted detergent solution was added into the petri dishes to surround the tube. The tubes with the larvae were opened for 10-15 minutes to allow the active larvae to climb to the rim of the tube. Using a small paint brush the larvae (about 100) were transferred from the rim of the tube into the packets. Papers impregnated with low concentrations of the acaricide

were inoculated first before those of high concentration to avoid contamination between chemicals.

3.7 Exposure conditions

The packets with the ticks were incubated for 24 hours at 27^0C and 87-100% RH.

3.8 Tick larval mortality counts

The packets were examined in the same order as they were prepared and filled with tick larvae. This was done to minimise variation in duration of exposure to test acaricide. The recommended mortality criterion was the inability of tick larvae to walk. Only those larvae capable of walking were considered to be alive. For assessment of walking ability, a magnifying glass and lamp were used. Ticks were stimulated by gently breathing directly onto them. All other larvae, including those that moved their appendages but did not walk, were counted as dead.

A control packet was opened by holding it by one side and laying it on the polystyrene block with the top opening to one side of the block, the top clip was removed and the bottom side of the paper packet secured to the block with a pin. The remaining clips were removed and the packet secured to the block in the open position with the other pin. The live larvae were removed with the paint brush and immobilized on cotton wool moistened with a wetting agent (detergent) in water. The dead larvae remaining were then counted and recorded, followed by the living larvae that were trapped in the cotton wool.

Counting of larvae was not necessary if it was evident that they were all or very predominantly alive (i.e. considered being zero percent mortality). The second control packet was opened and

its tick larvae examined and counted. If control mortality was greater than 10%, then the test conditions may have been faulty. The test method was checked carefully and the entire test was repeated correctly. If control mortality was less than 10%, the experimental tick packets were opened one by one**,** in ascending order of acaricide concentration in which they were prepared with larvae for incubation**.** In each lot, live larvae were removed and trapped in moistened cotton wool for counting as before. The dead larvae were counted *in situ* on the paper of the opened packet. Detailed counting was not necessary if the larvae in a packet were clearly all dead; such lots were automatically considered to be of 100% mortality (Baxter, 1999). Mortality figures were recorded on worksheets and mortality percentages were calculated.

3.9 Analysis of results

A two way ANOVA was conducted that examined the effect of concentration and species on tick mortality. The Shapiro-Wilk test was used to assess for normality in the dependant variable (mortality) and homogeneity of variance was assessed by Levine's test. Data was organized and represented in the form of descriptive tables, histogram and line graphs to assist in the analysis of data.

CHAPTER FOUR

4.0 RESULTS

4.1 Mortality of the *Rh. Boophilus* ticks at different concentrations of Amitraz.

All experimental treatments induced mortality. Dead ticks were distinguished from live ones by their inability to move. For all tick species, every extract concentration level and replicate induced mortality (Table 1). No mortality was recorded for all individuals in the control treatments.

Table1: Mortalities induced by different concentrations of Amitraz on tick larvae from the different districts.

Tick	**Concentration (%)**				
species/district	**0**	**25**	**50**	**75**	**100**
A1	14,3	30,19	63,77	98,80	74,84
A2	0,2	25,50	85,90	100,99	100,100
B1	16,16	14,14	22,10	37,33	47,47
B2	1,0	33,50	89,80	98,100	99,100
B3	4,2	33,60	67,75	87,95	100,99

Table 2 Mean mortality figures for tick larvae.

	Concentration (%)				
Tick species	**0**	**25**	**50**	**75**	**100**
A1	9	25	70	89	79
A2	2	37.5	87.5	100	100
B1	2	15	16	35	47
B2	1	42	84.5	99	99.5
B3	3	46.65	71	91	99.5

The highest mortality of 100% was achieved for *Rh. B. microplus* larvae and the least mortality of 47% was recorded for *Rh. B. decoloratus* (Table 2). The susceptibility trend for each of the species shows a rise in mortality with increase in concentration. A small difference was recorded between the 75% and 50 % concentration. The acaricide was more effective against *Rh. B. decoloratus* species while slightly less potent against *Rh. B. microplus.*

There was a general increase in mortality with increasing acaricide concentration. From the zero concentration to the 25% mark, a small count of mortality was observed for all the species from all the districts but there was a remarkable difference noted between the 25% and 50% concentrations. A slight increase in mortality was observed after increasing the acaricide concentration from 50 to 75%.

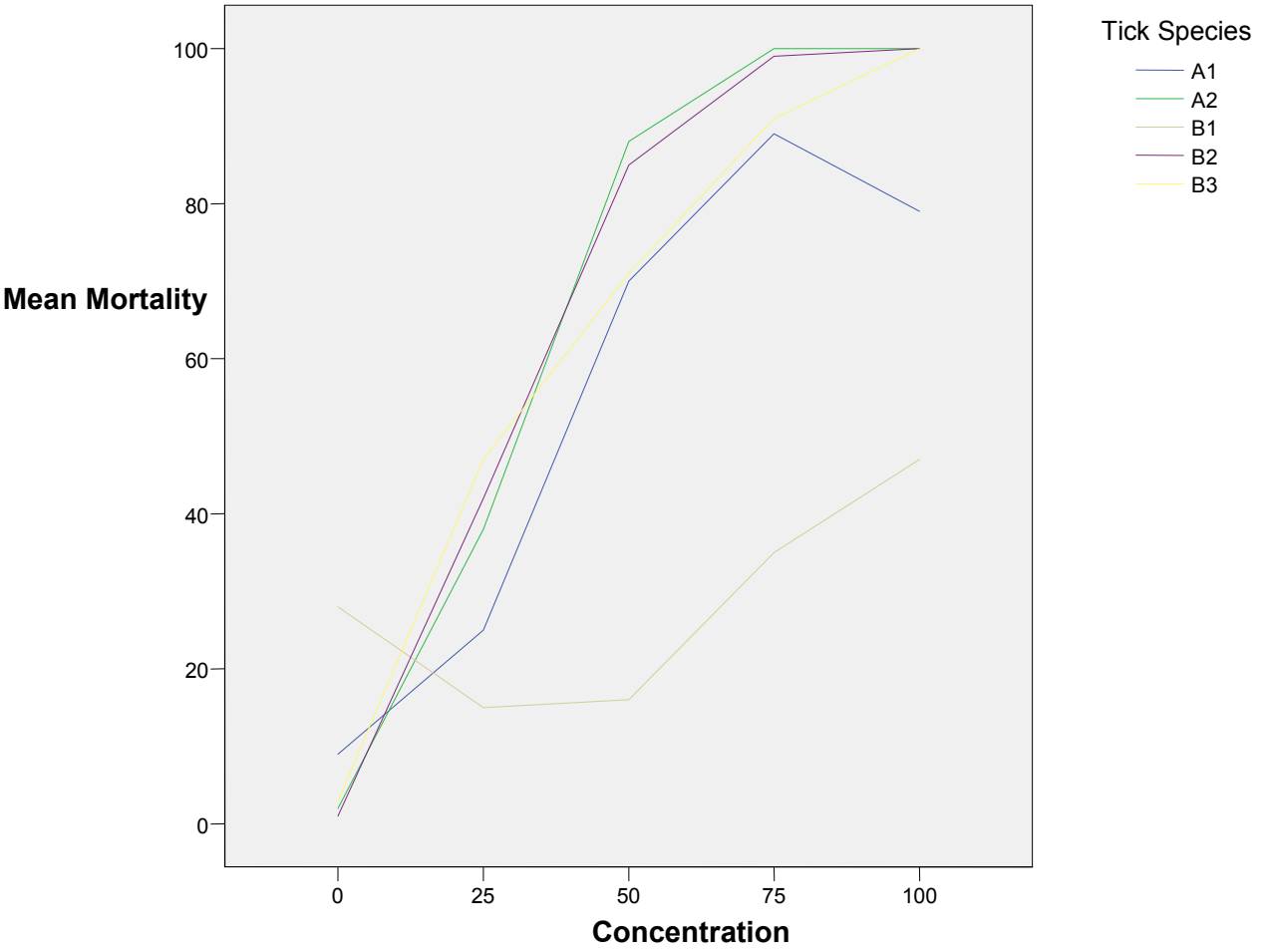

Figure 5: A comparative line plots for the two ticks' mortality figures

A1- *Rh. B. microplus* from Insiza

A2- *Rh. B. microplus* from Tsholotsho

B1- *Rh. B. decoloratus* from Mzingwane

B2- *Rh. B. decoloratus* from Nkayi

B3- *Rh. B. decoloratus* from Mberengwa

The data summarised for each of the tick species shows that an increase in concentration from 25 to 50% resulted in a significant rise in mortality ($p>0.05$). There were no significant difference

between the mean mortality for the 50 and 75% concentration ($p<0.05$). There were, however, significant differences between the mean mortalities observed at 75% and that of 100% for all two species ($p>0.05$).

There were significant differences between the mean mortalities observed for A1 ($p=0.317$), A2 ($p=0.154$), B1 ($p=0.561$), B2 ($p=0.210$) and B3 ($p=0.588$).

CHAPTER FIVE

5.0 DISCUSSION

Roughly 5 000 tick larvae were collected and examined for their susceptibility to Amitraz with the larval packet test (L.P.T). Amitraz induced mortality in all experimental treatments. There is enough evidence to suggest that Amitraz is a potential acaricide against *Rh. B. decoloratus*, and *Rh. B. microplus*, therefore the experimental treatments showed that there is no acaricide resistance in the selected districts. Susceptibility of the two species to the acaricide differed though the trend for each of the species showed a rise in mortality with increase in concentration. The L.P.T is a contact method of pesticide control and thus the mode of action of Amitraz was through contact. Active ingredients on the larval packets had contact with the larval ticks during the 24 hours of incubation.

Toxicological studies on Amitraz by other scholars show that the acaricide has identified adverse responses in human and animal studies (Arnold, 1988). Its effectiveness is traced back on alpha-adrenergic agonist activity, interaction with octopamine receptors of the central nervous system and inhibition of monoamine oxidases and prostaglandin synthesis (Bonsall and Turnbull, 1983). Therefore it leads to over excitation and consequently paralysis and death in ticks (Bonsall and Turnbull, 1983).

It is mostly likely that one of its active compounds had a toxic effect on the tick larvae on contact with the treated larval packets. There was an increase in mortality with increase in acaricide concentration in all the experimental treatments. This could have been attributed to the increasing concentration of active ingredient and thus indicating that toxicity is dosage-

dependant. There was a similar noted trend with *Rh. B. decoloratus* larvae but with a significantly higher susceptibility level, mortalities of above 50% being recorded in all three replicates. For *Rh. B. microplus* the general trend of increase in mortality with increasing acaricide concentration was evident but at a lesser level of susceptibility. It was apparent that mortality was species and concentration dependant and differences in mortality arose as a result. Differential susceptibility showed differential resistances between the species of the same genus.

The resistance of *Rh. B. microplus* was lower than that of *Rh. B. decoloratus*. Slight differences were seen in the highest mortalities recorded for *Rhipicephalus* species. This might have been due to closely related mechanisms of resistance. Differences in mortality might also have been due to differences in mode of nutrition and tolerance levels. Some larval ticks might not have been able to tolerate adverse conditions in the larval packets during the incubation period.

Researchers have shown that resistance development in pest populations is influenced by many biological, ecological, genetic, and operational factors. Biological and ecological factors include: characteristics of the pest, such as the rate of reproduction, the number of generations per year and mobility of the species, characteristics of the orchard, such as proximity to untreated areas, suitability of alternate hosts for pest development, immigration of susceptible pests and effectiveness of biological control (Dekeyser, 2005).

Genetic factors include: the number of genes conferring resistance, the frequency and intensity of resistance genes in the population, the ability of resistant individuals to grow and reproduce relative to susceptible pests. Operational factors include: characteristics of the chemical,

treatment thresholds, application methodology and equipment, chemical use strategies such as chemical rotations or mixtures (Knowles, 1997).

In practice, many of these factors are not readily manipulated by farmers. From the practical standpoint, farmers wishing to manage resistance should give attention to the following factors:

- How effectively you employ methods of integrated pest management.
- How often you use pesticides.
- How you select and apply the pesticides you use.

Farmers who use pesticides the least have the most effective resistance management programmes. Resistance cannot be managed in situations where a pesticide is used many times each season (Latif and Jongejan, 2002).

Resistance to acaricides in ticks is a result of multiple factors. The strength of acaricide used as well as the frequency of application (Sutherst and Comins, 1979). A very effective acaricide applied frequently will result in rapid elimination of susceptible ticks. This will result in higher selection rates and as a result higher incidence of interbreeding among resistant members giving rise to genetically resistant strains (Sutherst and Comins, 1979). Ticks with a shorter life cycle will also have a higher resistance (Spickett, 1998). This is because of the selective elimination of a large number of the susceptible individuals in that population. A number of problems affecting the course of the study and the results were identified during the experiment.

During the period that the research was done (December to February), it was not possible to collect *Boophilus* ticks from around the whole country, therefore, it was necessary to search for them from selected districts. Also during the preparation of the filter papers, the papers were impregnated with the different concentrations of acaricides. Every time the same concentrations were placed on the same working place and after every assay of one acaricide, the working place was cleaned very well. Despite all these measures contamination could still be possible. As written in the protocol of the University of the Free State, the forceps and rods used to introduce the larvae into the packets were cleaned by dipping in an acetone solution. After dipping the instruments were dried by wiping with a tissue, but many larvae died directly after contacting the acetone cleaned materials. Without cleaning the chance of causing contamination would have been high.

5.1 CONCLUSION

There is enough evidence at the 5% significance level to support the hypothesis that Amitraz is a potential acaricide against the tick larvae of *Rh. B. decoloratus* and *Rh. B. microplus.* This is supported by the mortality data obtained when tests were run exposing ticks to Amitraz. The research also showed that with increasing concentration, mortality also increased indicating that toxicity of the acaricide is dosage-dependant.

In conclusion, the results strongly suggest that there is no acaricide resistance in the selected districts and that the tick control failure is due to other factors other than resistance.

5.2 RECOMMENDATIONS

Though the results show that there is no resistance in the selected districts, this does not conclude that there is no resistance in some areas around the country, hence the following recommendations were made to prevent it;

- Rotation or alternation of different groups of acaricides that have no cross resistance may help reduce the selection pressure for resistance to any one acaricide group.
- The use of mixtures of acaricides is another strategy that has the potential to slow the emergence of resistance.
- Having an internationally harmonised system for acaricide registration, this would help national authorities resist pressure from chemical manufacturers.
- Educating farmers on tick resistance to acaricides and the control measures to reduce it.

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7.0: APPENDICES

Appendix 1

Tests of Normality

	Tick Species	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Mortality	A1	.271	5	.200(*)	.882	5	.317
	A2	.295	5	.177	.836	5	.154
	B1	.218	5	.200(*)	.925	5	.561
	B2	.276	5	.200(*)	.855	5	.210
	B3	.187	5	.200(*)	.929	5	.588

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

Appendix 2

Tests of Normality

	Conc	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Mortality	0	.290	5	.196	.757	5	.035
	25	.237	5	.200(*)	.934	5	.624
	50	.355	5	.039	.783	5	.058
	75	.390	5	.012	.706	5	.011
	100	.338	5	.063	.754	5	.032

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

Appendix 3

Interactive graphs