

Acaricide resistance in two tick species of veterinary importance in Zimbabwe. A case study of tick resistance to amitraz in the Mazowe District

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Abstract

*Infestations with ticks have an important economic impact on the cattle industry worldwide and resistance to acaricides has become a widespread phenomenon. To optimize their treatment strategy, farmers need to know if and against which classes of livestock, potential acaricide-resistance does occur. Amitraz is a rapidly acting acaricide that has been in use for the control of cattle ticks for more than 30 years. Resistance against amitraz was first reported in *Boophilus microplus* in Australia in 1980 but has been slow to spread in comparison to resistance against synthetic pyrethroids. There is little reliable information on the prevalence of amitraz resistance in southern Africa. Risk factors have been identified, but the small sample sizes in the studies that have attempted to identify risk factors using survey data suggest caution in their interpretation. Regional variation in prevalence has been reported, as has a positive relationship with frequency of acaricide application. There is evidence to suggest that in Southern Africa, amitraz resistance might have emerged in isolated areas and has been disseminated by cattle movements. The objective of the current study was to assess the susceptibility of field tick populations originating from Mazowe district in Zimbabwe using the Larval Packet Test to Amitraz. The proportions of the ticks collected from the animals revealed that 61% were *Rhipicephalus (Boophilus) microplus* a one host tick. 31% were the *Rhipicephalus appendiculatus*, a three host tick. The resistance ratios at concentrations inducing at least 80% and 99% mortality were used to detect established and emerging resistance. There was no evidence of resistance to amitraz in the *Rhipicephalus appendiculatus* and the *Rhipicephalus B microplus* tick species. The Larval Packet Test proved to be a suitable test to evaluate the susceptibility of *R. microplus* field populations to amitraz.*

Keywords: Acaricide resistance, Larval Packet test, *Rhipicephalus B microplus*

Introduction

Ticks are ecto parasites of animals that feed through sucking of the blood of animals (Sonenshine, 1992). A higher proportion of these ticks parasitize most of the domestic and wild animal species (George, 1990).

FAO (1984) estimated that about 80% of the world's cattle population is exposed to tick infestation. The infestation with ticks can cause significant losses in livestock production, due to tick borne diseases, tick paralysis and physical damage as well as huge financial losses incurred in controlling the ticks (Koney, 2004).

The main tick species of veterinary importance in the tropical and subtropical regions include the *Hyalomma*, *Rhipicephalus*, *Rhipicephalus* sub-genus *Boophilus* and *Amblyomma* species (Nari and Hassen, 1999). These tick species are able to survive under harsh conditions but the correct use of acaricides can reduce their reproductive progression. The main diseases of economic importance transmitted by these ticks include Babesiosis, Anaplasmosis, Heart water and Theileriosis. Global losses are estimated to be between US\$13.09 and US\$18.7 billion annually with over 800 million cattle constantly exposed to the threat of ticks (FAO, 1984). In southern Africa estimated losses caused by the disease theileriosis amount to over US\$168 million per year (Johnson, 2006).

The main method of controlling ticks is through use of chemicals (acaricides). The different compounds that have been used for tick control include: arsenicals, chlorinated hydrocarbon, organophosphates,

carbamates, cyclic amidines, pyrethroids compounds and ivermectines. These are applied to the animals using hand dressing, spraying, systemic and dipping vats at specific intervals that are determined by the ecological region, the species to be controlled and the residual efficacy of the acaricide used.

Dipping vats are used by the majority of communal farmers in Zimbabwe (Norval and Lawrence, 1999). Irrespective of these control measures, ticks still continue to inflict losses of various magnitude in livestock production notably cattle. In Zimbabwe it is estimated that 75% of the livestock diseases occurring annually, are attributed to tick infestations (Norval *et al.* 2004). A possible cause of this could be development of resistance by the ticks to the acaricide(s) in question.

Resistance to a given acaricide can be described as reduction in susceptibility of parasite to the acaricide when it is used at the recommended concentration and according to all the recommendations for its use (Drummond *et al.*, 1973). It is usually first recognized as a failure of treatment to eliminate tick burdens from cattle. Even though failure of treatment is often the result of incorrect preparation and application of acaricide, the persistence of ticks after frequent, correctly applied treatments indicates that acaricide

resistance is likely (Soneshine, 1999). The resistance of ticks to acaricides is an inherent scenario. It results from exposure of populations of ticks to acaricides and the survival and reproduction of ticks that are less affected by the acaricide.

The prolonged and, or incorrect use of acaricides may cause resistance in ticks against acaricides (Whitehead, 1973). When resistant ticks survive they pass on this ability of resistance by genetic material to their offspring. Thus resistance confers ability to survive and continue to cause harm in animal production.

In Zimbabwe, *B. microplus* resistance against arsenicals was documented for the first time in 1963, seven years after they were used periodically and intensively (Martins *et al*, 1995); it was demonstrated that the distribution of the resistance was widespread across the globe. Therefore the authorities in the Animal Health Division in 1947 authorized the use of Cyclodienes and Toxaphane. A year later, in 1948, the first cases of resistance were first recorded in South Africa and then Zimbabwe in the *Rh. evertsi* species (Solomon, 1983). The resistance in the *Rh. appendiculatus* and *Rh. B. decoloratus* to the acaricide was later recorded in 1966 and 1969 respectively. Because of the emergence of co-resistance *B. microplus* ticks to organophosphates and pyrethroids since 1948, the

Animal Health Authorities recommended the use of Amitraz for the control of these resistant strains, and therefore, thirteen years after its use, there is the possibility that in Zimbabwe resistance to Amitraz has developed, as it has occurred in other countries where the same history of intensive use of the acaricide that has been followed by resistance in a relatively short period of time (Martins and Furlong, 2001).

Materials and Methods

Study area

The study was carried in Mazowe communal lands of Mazowe District, located north side of Harare in Mashonaland Central Province of Zimbabwe. Mazowe district lies between latitude 17° 10' 00" S and longitude 31° 00' 00" E in DMS. The District is situated in agro-ecological region II with an altitude of 900m above sea level and receives rainfall ranging from 750 to 1 050 mm per year. Summer starts in October with much of the rainfall being received between December to march. Maximum and minimum daily temperatures range from 24 to 30°C and 15 to 24°C respectively.

Overall Objective

To find whether there is any tick

species, among the tick species of veterinary importance found in the study area that could be showing any resistance to the acaricide(s) in use (Amitraz).

Specific Objective

To determine acaricide resistance in the *Rhipicephalus appendiculatus* and the *Rhipicephalus (Boophilus) microplus* species found in Mazowe Communal lands.

Sampling Design

One dip tank was randomly selected from each of the four dip tanks located at each of the four Animal Health Management Centers (AHMCs) in the study area. Tick samples were collected from between 10 and 20% of randomly selected cattle once every month during the summer period starting January to April according to standard parasitological sampling procedures as reported by Chhabra, (1995). A simple randomized block design was used for the study

Pilot study

Preliminary surveys at two dip-tanks within the study area showed tick infestation being more 10 engorged ticks attached on each selected cattle in 10% of the herd. Under Animal Health Regulations (Cattle Cleansing)

1993 of Zimbabwe, tick infested situation is when:

- In herd one animal has 10 or more engorged ticks attached to it
- In a herd, if 10 or more live ticks are found on at least 10% of the herd
- In herd 5 or more engorged ticks on five or more animals in that herd

It was therefore decided to determine the probable cause of this, of which tick resistance was considered to be a possible cause.

Collection of samples

Tick samples were collected, manually from their predilection sites of attachment on cattle. Collection of sample ticks was done during dipping days. Animals were restrained in a race for easy collection of ticks and to avoid injury to the handler. In cases of temperamental animals, methods like Reuf's and Cris-cross methods of restraining cattle were used to cast the sample animal down in order to safely collect sample ticks (Solomon, 1983). According to the FAO's Larval Packet Test (LPT) method, a sample of 10 to more than 50 fully engorged female ticks is desired and is collected within 6 days of last acaricide application at the dip tank (Martins and Furlong 2000).

The following information was recorded during tick collection in the field; date and time of sample collection, AHMC and dip tank names, and names of tick species collected. Collected live engorged female tick samples were placed in perforated universal screw capped bottles containers to allow ventilation before they were sent for parasitological analysis within two days.)

Laboratory analysis

Resistance was tested using the Larval Packet Test (LPT). The method was developed by Stone and Hydock (1962) in Australia. In Africa it was adapted for use on African ticks by Tatchell in 1973. It was recommended by the FAO for use as a standard tick bioassay (Kemp *et al*, 1998). The test employs papers that are impregnated with acaricide of varying proportions.

In this test the collected engorged female ticks laid eggs. The eggs hatched producing larvae which were tested for resistance. The larvae usually were between 7 to 14 days old from day of hatching. In this test, tick larvae are exposed to chemically impregnated filter papers and their subsequent mortality will be quantified after 24 hours. Results for this larval packet test, for the

diagnosis of resistance in *Rh. B. microplus* took about 6 weeks.

Larval tick mortality counts

Packets were examined in the same order as they were prepared and filled with tick larvae. This was an attempt to reduce variation in the duration of exposure to the test acaricide. The mortality criterion used was that of the inability of the tick to walk. Thus those larvae capable of walking were therefore considered alive. Assessment of walking ability was done through gently breathing directly onto them.

Statistical analysis

The analysis of the results was done using the Genstat Release 14.1 (2013)

Results and Discussion

The patterns of tick susceptibility to amitraz found in the study are similar to those reported around the world (Wharton, 1976; Kemp, *et al* 1998; Martins and Furlong, 2001). At acaricide concentrations of 0.00018%, 0.25% and 0.4% there was no significant variation ($p > 0.05$) in the mortalities of the larvae. This implies that the two tick species are effectively controlled by Amitraz.

Table 1: Mortality percentages obtained by the Discriminative doses test in larvae of the *Rh B. microplus* tick

and the *Rh appendiculutus* species to Amitraz

Table 1: Mortality Rates

<i>Amitraz conc(m/l)</i>	<i>Av, % mortality</i>	<i>Av, % mortality</i>
	Rh. Appendiculutus	Rh. a. Boophilus
Control 0.00018	86	84
0.025	94	92.5
0.4	100	99

All four AHMC sites recorded an average mortality of 98% (*Rh. appendiculutus*) and 94% (*Rhipicephalus (Boophilus) microplus*). At a discriminating dose of 0.4%, the expected mortality was 100% across all the four sites, however two of the sites recorded mortalities of 98% in the one host tick.

Although the differences in mortality in the larvae from all the test centres were statistically insignificant ($p>0.05$) at the three concentrations under study, the ability of some the *Rh B microplus* larvae to survive a discriminating dose of 0.4% at two sites suggested a possible emergence of resistance. Amitraz has been used to control ticks in the area over the last ten years. This can cause the development of mutant genes that will resist the effectiveness of amitraz in controlling the *Rh B microplus* this scenario may be due to the nature to the life cycle of the *Rh B microplus* which is a one host tick (Walker *et al*, 2003). Drummond (1973) also implored that resistance development

in a one host tick is faster when compared to a two or three host tick species, due to exposure of the different generations to the acaride. The sensitivity of *Rh. appendiculutus* may also be explained by its host requirement live cycle (three hosts) which makes it have a reduced selection pressure due to fewer generations per year where the immature larval stages are less exposed to the acaricides.

Although resistance to amitraz has not yet been recorded in Zimbabwe, is not a guarantee that it will not occur in the near future. Resistance to amitraz has been recorded in countries such as Mexico (2002), Colombia (2000), Brazil (1995), South Africa (1999) and Australia (1981) Soberanes *et al*, (2002) in the *Rh B. microplus* tick species.

In Mexico, the presence of resistance to acaricides has had a period of approximately seven years for the different families of acaricides (Soberanes *et al*, 2002). Therefore the implementations of surveillance

systems and preventative programs have to be put in place the country for the early detection of resistance (Johnson, 1997).

Conclusion

Farmers should be encouraged to use selection programmes for tick resistance, by culling their most heavily-infested animals. Such selection programmes would minimize the use of acaricides and thus minimize the development of tick resistance to acaricides (Kalouuw, 2009). Local breeds of cattle are more adapted and resistant to ticks and tick-borne diseases, thus farmers should be encouraged to use these breeds.

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