Potential Causes of Acaricide Resistance in *Rhipicephalus* and *Amblyomma* Ticks (Acari: Ixodidae) in Namwala District, Zambia

Karen Sichibalo*[1], Katendi Changula [2], Chisoni Mumba [3], Natasha Mwila [3], Kennedy Chibesa [1], Benjamin Mubemba [4, 5], King S. Nalubamba [6], Walter Muleya [7], Edgar Simulundu [3, 8], Simbarashe Chitanga * [1, 9, 10]

[1] Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia
[2] Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[3] Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[4] Department of Wildlife Sciences, School of Natural Resources, Copperbelt University, Kitwe, Zambia.
[5] Department of Biomedical Sciences, School of Medicine, Copperbelt University, Ndola, Zambia
[6] Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[7] Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[8] Macha Research Trust, Choma, Zambia
[9] Department of Paraclinical Studies, School of Veterinary Medicine, University of Namibia, Windhoek, Namibia
[10] School of Life Sciences, College of Agriculture, Engineering and Sciences, University of KwaZulu-Natal, Durban, South Africa

*** Corresponding Authors:**
Karen Sichibalo, Department of Biomedical Sciences, University of Zambia, Box 32379, Lusaka, Zambia.
karenschishala@gmail.com
Simbarashe Chitanga, Department of Biomedical Sciences, University of Zambia, Box 32379, Lusaka, Zambia. schitanga@gmail.com

Note: Supplementary data associated with this article
DOI: *https://10.53974/unza.jabs.5.3.668*

Abstract

Acaricide resistance in ticks poses a great threat to livestock production in many parts of the world where ticks are a problem. The objectives of this study were to screen for acaricide resistance in *Rhipicephalus* and *Amblyomma* ticks using phenotypic and molecular assays, and to assess current tick control practices used by cattle farmers in the traditional sector of Namwala District. The larval packet test was used to screen for acaricide resistance in ticks covering concentrations up to twice the discriminatory dose for amitraz, diazinon and cypermethrin. Genetic mutations associated with resistance to amitraz (A22C-T8P and T65C-L22S), and organophosphates/ pyrethroids (G1120A) were screened using polymerase chain reaction and sequencing. Information on tick control practices at the household level was collected using a semi-structured questionnaire. Intermediate resistance (21-50%) to amitraz and cypermethrin was detected in both *Rhipicephalus* and *Amblyomma* ticks, with both tick genera showing susceptibility to diazinon (<10%). None of the ticks in this study had the reported acaricide resistance-conferring molecular markers that were screened for. The findings suggest that the resistance detected in the studied

tick population may be due to other mechanisms yet to be identified. Tick control practices observed amongst the farmers, such as the incorrect use of acaricide concentrations and rotations, could be fuelling the development of acaricide resistance. Considering that acaricide treatment is the mainstay of tick control in the country, it is thus, critical to comprehensively unravel the factors contributing to treatment failure as this would allow for the application of appropriate remedial actions for effective tick control in Zambia.

Keywords: *Acaricide; Resistance; Ticks; Cattle; Zambia*

1.0 Introduction

Ticks and tick-borne diseases (TBDs) impede the productivity of livestock, especially cattle, resulting in severe economic losses [1, 2]. Mixed tick infestations are a common occurrence in Zambia [3], and the genera *Rhipicephalus* and *Amblyomma* are among ticks that transmit TBDs of economic importance, such as East Coast Fever (ECF)/theileriosis, anaplasmosis, ehrlichiosis, and babesiosis [4, 5]. In Zambia, ECF causes up to 10, 000 cattle deaths annually [6] and high mortalities and production losses have continued to be reported, especially in poorly managed traditional cattle herds in Southern Province [7, 8]. Namwala District is the largest cattle farming area in the Southern Province and the country at large [9]. However, the economic viability of the traditional cattle sector is threatened by ticks and TBDs [4].

Globally, vector control using chemical acaricides is the main strategy implemented to control ticks and TBDs [10]. Unfortunately, this vector control method is now under threat from acaricide resistance [11], with widespread reports of phenotypic and genotypic resistance to major acaricide classes (organophosphates, pyrethroids, and formamidines) being documented across the world [10,11, 12]. Acaricide resistance has also been reported in various African countries [1, 13, 14], including the sub-Saharan region [15, 16]. In Zambia, phenotypic resistance to organophosphates and amitraz was previously documented in Namwala and Isoka districts, respectively [17,18], but there is no data on potential molecular markers responsible for this resistance.

Recently, there have been complaints of acaricide treatment failure by farmers in different parts of Zambia, including Namwala District [19]. This has deleterious effects on livestock productivity, especially resource-limited farmers [2]. However, it is not clear whether the reported complaints of acaricide failure are due to drug resistance emergence or improper acaricide usage. Using both phenotypic and molecular assays, this study investigated if the acaricide failure in *Rhipicephalus* and *Amblyomma* ticks was an indication of acaricide resistance. Furthermore, the study sought to determine if the acaricide failure could be attributed to farmer tick control practices.

2.0 Materials and Methods

2.1 Study Site

The study was conducted in five (5) veterinary camps (Maala, Chitongo,

Kabulamwanda, Nakamboma, and Namwala Central) in Namwala District, in the Southern Province of Zambia (15.8222° S, 25.85 22° E) between May 2018 and October 2019 (Fig. 1).

2.2 Tick Collection and Identification

Adult ixodid ticks were collected from cattle in 12 households, following sampling techniques described by Steyn *et al*., [20]. The ticks were collected in crush pens by gently and carefully pulling them out from cattle without destroying the mouthparts and placed in perforated capped tubes, each containing a fresh leaf for the purpose of providing moisture. Ticks were then transported to the laboratory, where they were morphologically identified up to genus level using dichotomous keys [21].

2.3 Bioassay

Fully engorged adult female ticks of the genus *Rhipicephalus* (n = 58) and *Amblyomma* (n = 12) were kept in an incubator at 27ºC ±1 and 85% ±1 relative humidity to allow for oviposition and larval emergence [22]. Fourteen to twenty-one days old unfed larvae hatched from the engorged females were subjected to the larval packet test (LPT) as previously described by FAO [22]. Commercial formulations of amitraz (12.5% *w/v*), diazinon (12.5 % *w/v*) and alpha-cypermethrin (15% *w/v*) were used to represent synthetic amidines, organophosphates and pyrethroids, respectively. Working concentrations covering 0.0001% to 0.4% for each acaricide were made, in duplicates (Supplementary Table 1), the range of which included the manufacturer's recommended dose (MRD) (taken as the discriminatory dose (DD) expected to cause 99.99% mortality) and twice the discriminatory dose (2 X DD) [1, 23].

2.4 Molecular Assays

Deoxyribonucleic Acid (DNA) was extracted from individual ticks (*Rhipicephalus* = 305 and *Amblyomma* = 61) using TRI Reagent® solution (Sigma Aldrich® Life Science, USA) as described by the manufacturer. To screen for amitraz resistance-conferring mutations, A22C-T8P and T65C-L22S, a 417 bp region of the *Octopamine/Tyramine* (*OCT*/*Tyr*) receptor gene was amplified as previously described by Chen *et al*., [24]. In order to screen for the presence of G1120A mutation associated with resistance to pyrethroids and organophosphates, a 372 bp region of the *Carboxylesterase* (*CES*) was amplified as previously described by Hernandez *et al*., and Faza *et al*., [25, 26].

The Wizard® SV Gel Clean-Up System (Promega, Madison, WI, USA) was used to purify amplified samples for each target gene (*OCT/Tyr* and *CES*). The purified DNA was then directly subjected to bidirectional sequencing using Brilliant Dye™ v3.1 Terminator Cycle sequencing kit (NimaGen BV, Nijmegen, Netherlands) on the ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA).

Sequence editing and assembly was done using GENETYX ATGC software version 7.5.1 (GENETYX Corporation, Tokyo, Japan). The BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST) in NCBI GenBank was used to verify sequence identities of the amplified genes with a

percentage similarity range of 90% to 100% to those in the public repository database that are considered as a positive identity. The obtained partial sequences were then deposited in the DDBJ GenBank under the accession numbers LC658951-LC658955 and LC659236-LC659240 for the *CES* and *OCT/Tyr* gene, respectively. The *OCT/Tyr* nucleotide sequences were translated into predicted amino acid sequences using BioEdit software version 7.2.5 (https://bioedit.software.informer.com). Finally, in order to determine the presence of resistance-conferring mutations, ClustalW (https://www.genome.jp/tools-bin/clustalw) was used to align sequences obtained in this study against the respective reference sequences for the *OCT/Tyr* (Gonzalez amitraz susceptible strain, accession number EF490687.1 and Santa Luiza amitraz resistant strain, accession number EF490688.1) and *CES* (Gonzalez pyrethroid/organophosphate susceptible strain, accession number AF182283.1) genes downloaded from GenBank (Supplementary Fig. 1 and Fig. 2).

2.5 Questionnaire Survey

A total of 357 traditional cattle farmers were interviewed at communal grazing sites and main trading places such as abattoirs, following a 20% sampling technique. This was based on the farmer population that is in possession of cattle in Namwala District [27]. The administered semi-structured questionnaire captured information on acaricides currently in use, mode of acaricide delivery on cattle and acaricide rotation (Supplementary Questionnaire). Correct acaricide rotation was considered to be the difference in chemical groups or mechanism of action between the rotated acaricides [11].

2.6 Data Analysis

Probit tool (IBM SPSS v 21.0 for windows. Armonk, NY: IBM Corp) was used to generate lethal dose estimates (LD_{50} and LD_{90}) [22] (Supplementary Table 2 to 7). Resistance levels at doses equal to 2 X DD were categorised into four (4) groups that is high (>51%), intermediate (21-50%), low (11-20%) and susceptible (<10%) [28, 23]. Questionnaire data were entered into Microsoft Excel (2016) spreadsheets, where it was sorted into frequencies and calculated into percentages based on the participant responses.

2.7 Ethical Approval

Ethical approval to conduct the study was obtained from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC) with protocol identity number (ID) 20190217068. Consent to acquire information on tick control methods and tick collection from cattle was obtained from the respondents in the selected study sites. Recommended tick collection methods, as described by Steyn *et al*., [20], were used in order to ensure the safety of animals from which ticks were collected.

3.0 Results and Discussion

Rhipicephalus and *Amblyomma* ticks showed intermediate resistance (21-50%) at 2X DD to both amitraz and cypermethrin, with the lethal dose estimate for *Rhipicephalus* ticks being relatively higher than that of *Amblyomma* ticks. However, both tick genera showed susceptibility (<10%) to diazinon (Table 1).

The level of resistance detected in the field ticks coupled with fitness cost associated with the amitraz resistance allele, and the aspect of refugia in the three-host ticks [16, 11] suggests that ticks in the study area are undergoing high selection pressure towards resistance to amitraz and cypermethrin. This study adds to the growing knowledge of amitraz resistance in the country, with previous reports indicating the presence of resistance in the northern part of the country [18]. Amitraz resistance in ticks has also been documented across the world [10, 11, 23, 29]. Whilst resistance to synthetic pyrethroids is widespread in most parts of the world [10, 12], this is the first time, resistance to cypermethrin has been reported in Zambia. The presence of multi-acaricide resistance in the studied tick population is a cause for concern as it undermines efforts to enhance livestock productivity, especially that the control of ticks and TBDs in the study area relies on chemical acaricides [9]. This finding also confirms the reports by farmers in Namwala District of reduced acaricide effectiveness [19].

Despite the observed level of resistance to amitraz and cypermethrin, sequence analysis of the *OCT/Tyr* and *CES* receptor genes revealed none of the mutations commonly associated with resistance to amitraz (T8P and L22S) and cypermethrin (G1120A) (Supplementary Fig. 1 and Fig. 2). The difference between results from this study and those reported previously [25, 24, 15, 16] could be that ticks in this study may be using different mechanisms of resistance, as other mechanisms have also been proposed to induce resistance against these classes of compounds [30, 31]. Future studies on ticks in this area should go further and screen for all published mutations in order to ascertain the exact mechanisms conferring resistance in these tick species.

Contrary to previous reports of resistance to organophosphates in ticks from Southern Province and other areas of Zambia [17, 32], the ticks in our study showed susceptibility to diazinon, a finding which was also supported by the absence of the G1120A mutation in the *CES* gene (Supplementary Fig 2) that is also linked to organophosphate resistance [26]. The use of organophosphorus compounds was discouraged in the country due to reports of resistance and environmental toxicity [33]. It is also possible that their discontinued use in the area could have resulted in a reduction in acaricidal pressure leading to total loss of the resistance allele and reversion into its wild genotype, especially that the organophosphate resistance gene is semi-dominant [30]. Essentially, this is indicative of the possible future consideration of diazinon for the control of ticks resistant to amitraz and cypermethrin in Namwala District.

Our questionnaire survey revealed that amitraz (84.62%, 302/357) was the most widely used chemical acaricide, followed by cypermethrin (8.72%, 32/357) and fluazuron (3.08%, 11/357). This trend has been observed in previous studies in Zambia [18, 9] and neighbouring Zimbabwe [16]. The popularity of amitraz amongst farmers could be due to its relatively cheaper cost [9, 34] and rapid knockdown effect compared to other acaricides [31].

The frequency of acaricide application was based on tick abundance, with 43.4% (155/357) of the farmers making weekly applications and the rest applying acaricides bi-weekly (53.4%, 191/357) through knapsack spraying. However, dipping has been established as the most effective delivery method for acaricides because it offers full animal body coverage, unlike knapsack spraying, which may not successfully cover areas such as the perineum and inguinal regions, thus, leaving a critical number of ticks unexposed to the acaricide [2]. The shortfall of functional dip tanks in the area and the cost associated with their maintenance [27] could potentially explain farmers' preference for the knapsack spray method in this study.

Amongst the acaricide use practices recorded in this study was incorrect acaricide mixing (50% of respondents), with the majority of farmers using doses higher than those recommended by manufacturers. Also observed was an incorrect rotation of acaricide by farmers (32%, 114/357), with a lack of knowledge on what entails proper rotation being noted as shown by the frequent change of trade names, despite compounds being in the same chemical class. The use of compounds with differences in active molecule and mechanism of action between the acaricide compounds which is being alternated is considered the correct approach [11]. Improper mixing and rotation of acaricides are practices that have also been reported previously [18, 35, 1], and these practices are implicated as drivers of acaricide resistance [11]. Thus, taking these factors into account might possibly explain the resistance observed in this study as evidenced by tick survival at doses higher than MRDs (Table 1 and Supplementary Table 1).

Whilst the study's questionnaire findings could possibly explain the reported inefficacy of acaricides in the area [19], it did not go further to assess the quality of these acaricides. It is noteworthy that failure of commercial preparations has previously been reported [1, 23], and the use of sub-standard chemical acaricides also contributes to resistance [36]. With the liberalisation of the pharmaceutical industry, there is a need to standardise discriminatory doses against MRDs and ascertain the quality of acaricide products on the Zambian market.

This is the first study in Zambia that has attempted to analyse the resistance of ticks to acaricides such as formamidine (amitraz) and organophosphorus/pyrethroid compounds using molecular methods. The major limitation of this study was the lack of a susceptible reference strain that is necessary to determine the degree of resistance in the studied tick population. The study findings highlight the need to strengthen farmer training on the judicious use of chemical acaricides, the substitution of amitraz and cypermethrin with formulations containing chemicals with different mechanisms of action, and the development of acaricide rotation strategies in order to prevent/delay the development of resistance in ticks.

4.0 Author Disclosure Statement

The authors declare that there are no conflicts of interest.

4.1 Acknowledgements

This research work was funded by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under the grant 'Spatial eco-epidemiology of tick-borne rickettsial pathogens' under award number R01AI136035 [PI – Gaff H], through a sub-award to the University of Zambia [PI – Chitanga S]. The content is solely the authors' responsibility and does not necessarily represent the views of the National Institutes of Health.

4.2 Author Contributions

Simbarashe Chitanga: Conceptualisation, fund acquisition, resource, supervision, methodology, and writing-review and editing; **Karen Sichibalo:** Methodology, investigation, writing original script; **Katendi Changula:** Resource, supervision and writing-review and editing; **Chisoni Mumba:** Investigation and writing-review and editing; **Natasha Mwila:** Investigation and writing-review and editing; **Kennedy Chibesa:** Investigation and writing-review and editing; **Benjamin Mubemba:** writing-review and editing; **King S. Nalubamba:** Resource; **Walter Muleya:** Writing-review and editing; **Edgar Simulundu:** Data curation, resource, supervision, writing-review and editing.

5.0 References

1. Vudriko P, Okwee-Acai J, Tayebwa DS, Byaruhanga J, Kakooza S, Wampande E, Omara R, Muhindo JB, Tweyongyere R, Owiny DO, Hatta T. Emergence of multi-acaricide resistant Rhipicephalus ticks and its implication on chemical tick control in Uganda. Parasites and vectors. 2016 Dec;9(1):1-3.
2. Sungirai M. *Ixodid ticks parasitising cattle in Zimbabwe: Ecology and management* (Doctoral dissertation, Ghent University).
3. Speybroeck N, Madder M, Van Den Bossche P, Mtambo J, Berkvens N, Chaka G, Mulumba M, Brandt J, Tirry L, Berkvens D. Distribution and phenology of ixodid ticks in southern Zambia. Medical and veterinary entomology. 2002 Dec; 16(4):430-41.
4. Makala LH, Mangani P, Fujisaki K, Nagasawa H. The current status of major tick-borne diseases in Zambia. Veterinary research. 2003 Jan 1;34(1):27-45.
5. Simuunza M, Weir W, Courcier E, Tait A, Shiels B. Epidemiological analysis of tick-borne diseases in Zambia. Veterinary parasitology. 2011 Feb 10;175(3-4):331-42.
6. Muleya W, Namangala B, Simuunza M, Nakao R, Inoue N, Kimura T, Ito K, Sugimoto C, Sawa H. Population genetic analysis and sub-structuring of Theileria parva in the northern and eastern parts of Zambia. Parasites & vectors. 2012 Dec;5(1):1-1.
7. Inambao CN. *Assessing the factors influencing farmers' decisions in the control of East Coast Fever in Kafue, Zambia* (Doctoral dissertation, Master dissertation, Van Hall Larenstein University of Applied Sciences pp. 1).
8. Laing G, Aragrande M, Canali M, Savic S, De Meneghi D. Control of cattle ticks and tick-borne diseases by acaricide in Southern Province of Zambia: a retrospective evaluation of animal health measures according to

current one health concepts. Frontiers in public health. 2018 Mar 27; 6: 45.

9. Mumba C, Häsler B, Muma JB, Munyeme M, Sitali DC, Skjerve E, Rich KM. Practices of traditional beef farmers in their production and marketing of cattle in Zambia. Tropical animal health and production. 2018 Jan;50(1):49-62.
10. Abbas RZ, Zaman MA, Colwell DD, Gilleard J, Iqbal Z. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Veterinary parasitology. 2014 Jun 16;203(1-2):6-20.
11. Rodriguez-Vivas RI, Jonsson NN, Bhushan C. Strategies for the control of Rhipicephalus microplus ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology research. 2018 Jan 1;117(1):3-29.
12. Klafke G, Webster A, Agnol BD, Pradel E, Silva J, de La Canal LH, Becker M, Osório MF, Mansson M, Barreto R, Scheffer R. Multiple resistance to acaricides in field populations of Rhipicephalus microplus from Rio Grande do Sul state, Southern Brazil. Ticks and tick-borne diseases. 2017 Jan 1;8(1):73-80.
13. Yessinou RE, Akpo Y, Sidick A, Adoligbe C, Karim IY, Akogbeto M, Farougou S. Evidence of multiple mechanisms of alphacypermethrin and deltamethrin resistance in ticks Rhipicephalus microplus in Benin, West Africa. Ticks and tick-borne diseases. 2018 Mar 1;9(3):665-71.
14. Arafa WM, Klafke GM, Tidwell JP, de León AA, Esteve-Gassent M. Detection of single nucleotide polymorphism in the para-sodium channel gene of Rhipicephalus annulatus populations from Egypt resistant to deltamethrin. Ticks and tick-borne diseases. 2020 Sep 1;11(5):101488.
15. Baron S, van der Merwe NA, Madder M, Maritz-Olivier C. SNP analysis infers that recombination is involved in the evolution of amitraz resistance in Rhipicephalus microplus. PloS one. 2015 Jul 9;10(7): e0131341.
16. Sungirai M, Baron S, Moyo DZ, De Clercq P, Maritz-Olivier C, Madder M. Genotyping acaricide resistance profiles of Rhipicephalus microplus tick populations from communal land areas of Zimbabwe. Ticks and tick-borne diseases. 2018 Jan 1;9(1):2-9.
17. Luguru SM, Banda DS, Pegram RG. Susceptibility of ticks to acaricides in Zambia. Tropical animal health and production. 1984 Mar;16(1):21-6.
18. Muyobela J, Nkunika PO, Mwase ET. Resistance status of ticks (Acari; Ixodidae) to amitraz and cypermethrin acaricides in Isoka District, Zambia. Tropical animal health and production. 2015 Dec;47(8):1599-605.
19. LSCS. Livestock Services Cooperative Society. Technical Services Division, 2019 final year report. Lusaka Zambia. 2019, pp.7, unpublished.
20. Steyn HC, McCrindle CM, Du Toit D. Veterinary extension on sampling techniques related to heartwater research. *Journal of the South African Veterinary Association.* 2010 Sep 1;81(3):160-5.

21. Walker AR. Ticks of domestic animals in Africa: A guide to identification of species. Edinburgh: bioscience reports; 2003.
22. [FAO] Food and Agriculture Organisation of the United Nations. Guidelines resistance management and integrated parasite control in ruminants. 2004.
23. Rodríguez-Hidalgo R, Pérez-Otáñez X, Garcés-Carrera S, Vanwambeke SO, Madder M, Benítez-Ortiz W. The current status of resistance to alpha-cypermethrin, ivermectin, and amitraz of the cattle tick (Rhipicephalus microplus) in Ecuador. PloS one. 2017 Apr 7;12(4): e0174652.
24. Chen AC, He H, Davey RB. Mutations in a putative octopamine receptor gene in amitraz-resista Hernandez R, Guerrero FD, George JE, Wagner GG. Allele frequency and gene expression of a putative carboxylesterase-encoding gene in a pyrethroid resistant strain of the tick Boophilus microplus. Insect biochemistry and molecular biology. 2002 Sep 1;32(9):1009-16. nt cattle ticks. Veterinary parasitology. 2007 Sep 30;148(3-4):379-83.
25. Hernandez R, Guerrero FD, George JE, Wagner GG. Allele frequency and gene expression of a putative carboxylesterase-encoding gene in a pyrethroid resistant strain of the tick Boophilus microplus. Insect biochemistry and molecular biology. 2002 Sep 1;32(9):1009-16.
26. Faza AP, Pinto IS, Fonseca I, Antunes GR, de Oliveira Monteiro CM, Daemon E, de Souza Muniz M, Martins MF, Furlong J, de Azevedo Prata MC. A new approach to characterization of the resistance of populations of Rhipicephalus microplus (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. Experimental parasitology. 2013 Aug 1;134(4):519-23.
27. Mpofu, J. 'E-Slip empowers Namwala residents with livestock.' *Ministry of Fisheries and Livestock;* A Smart and Value-Centered Public Service. 17 September. Available at: https://www.mfl.gov.zm/?p=5789#:~:text=According%20to%20the%202010%20Population,to%20that%20of%20human%20population. 2020. (Accessed: 19 September, 2020).
28. Junte RD. *Acaricide resistance in the blue cattle tick in South Africa; A comparison of three assays for determining tick resistance* (Master's thesis). 2009.
29. Makuvadze FT, Hove T, Makaya P, Waniwa E, Nemaungwe T. Resistance of ticks on cattle to amitraz in Zimbabwe. Tropical animal health and production. 2020 Nov;52(6):3323-30.
30. Stone NE, Olafson PU, Davey RB, Buckmeier G, Bodine D, Sidak-Loftis LC, Giles JR, Duhaime R, Miller RJ, Mosqueda J, Scoles GA. Multiple mutations in the para-sodium channel gene are associated with pyrethroid resistance in Rhipicephalus microplus from the United States and Mexico. Parasites and vectors. 2014 Dec;7(1):1-4.
31. Jonsson NN, Klafke G, Corley SW, Tidwell J, Berry CM, Koh-Tan HH. Molecular biology of amitraz

resistance in cattle ticks of the genus Rhipicephalus. Frontiers in bioscience: Landmark. 2018 Jan 1;23(2):796-810.

32. Luguru SM, Chizyuka HG, Musisi FL. A survey for resistance to acaricides in cattle ticks (Acari: Ixodidae) in three major traditional cattle areas in Zambia. Bulletin of entomological research. 1987 Dec;77(4):569-74.
33. De Meneghi D, Stachurski F, Adakal H. Experiences in tick control by acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. Frontiers in public health. 2016 Nov 9;4:239.
34. Chitombo L, Lebani K, Sungirai M. Acaricide resistance in Rhipicephalus appendiculatus ticks collected from different farming systems in Zimbabwe. Tropical Animal Health and Production. 2021 Sep;53(4):1-5.
35. Mekonnen S. *Acaricide resistance profiles of single and multi-host ticks in commercial and communal farming areas in the Eastern Cape and North-West Provinces of South Africa* (Doctoral dissertation, University of Pretoria). 2006.
36. Wambua BN, Muhigirwa E. An Assessment of the causes of tick resistance to acaricides use on cattle in Kazo County Kiruhura District, Uganda. *International Journal of Applied.* 2019 Jun;9(2).
37. Mweemba CE, Nyambe I, Funder M, van Koppen B. Poverty and access to water in Namwala district, Zambia. Report on the results from a household questionnaire survey. DIIS Working Paper; 2011.

Figures and tables

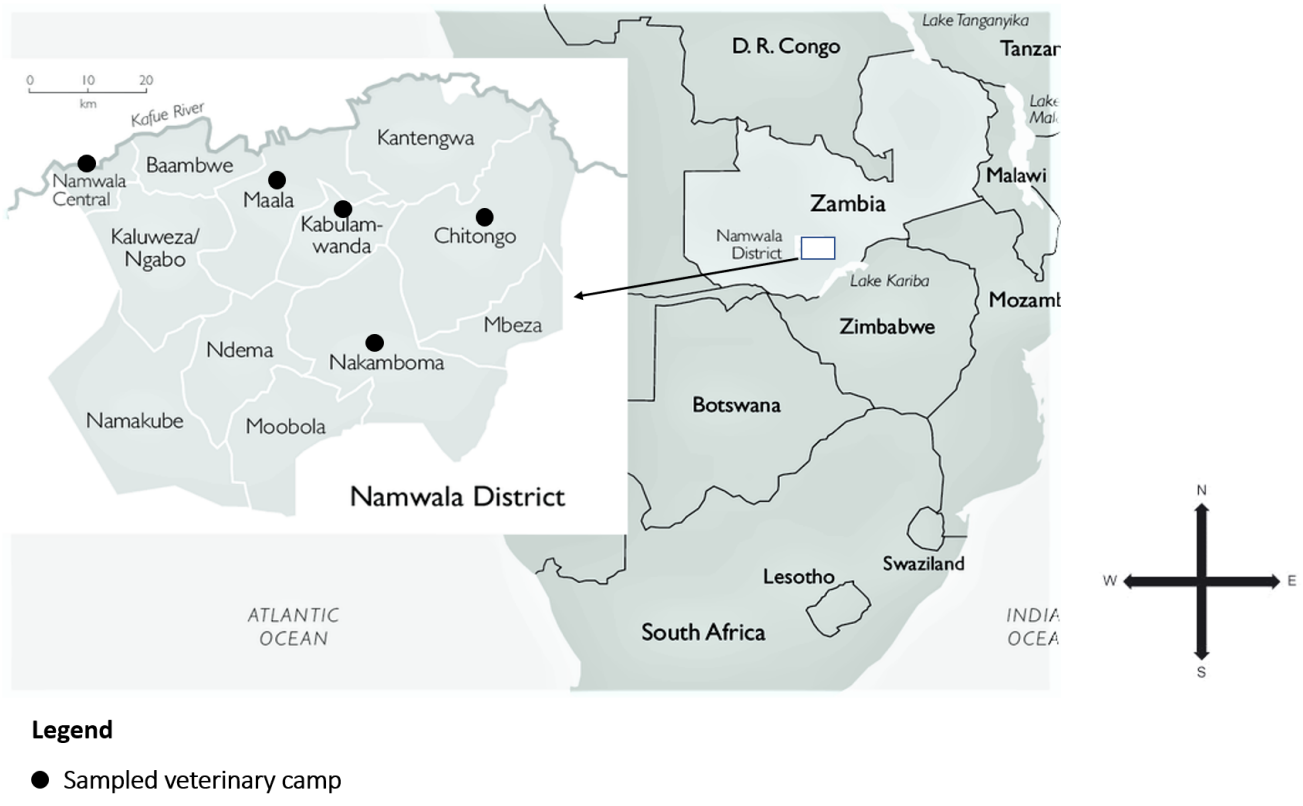

Figure 1: Map of Namwala District showing sampled veterinary camps (black dots). Map adapted from Mweemba *et al*., with slight modifications [37].

Table 1: Percentage resistance at 2 X DD and lethal dose estimates (LD_{50}, LD_{90}) obtained for each acaricide against the respective tick genera.

Genus	Acaricide	% Mortality at MRD	%Resistance at 2 x DD	Slope (SE)	HF	LD 50 (ml/L) (95% CL)	LD90 (ml/L) (95% CL)
Rhipicephalus							
	Amitraz	28.50	46.50	1.530 (±0.24)	1.49*	0.004 (0.003 – 0.005)	0.025 (0.019 – 0.035)
	Cypermethrin	41.00	40.50	0.630 (±0.13)	3.19	0.002 (0.000 – 0.004)	0.109 (0.037 – 0.690)
	Diazinon	74.00	5.50	0.660 (±0.18)	2.40*	0.001 (0.000 – 0.003)	0.044 (0.020– 0.229)
Amblyomma							
	Amitraz	63.50	28.50	0.41 (±0.19)	1.24*	0.000 (0.000 -0.001)	0.261 (0.074 – 2.392)
	Cypermethrin	66.00	21.00	0.70 (±0.19)	1.44*	0.000 (0.000 – 0.001)	0.018 (0.011 – 0.031)
	Diazinon	71.00	0	0.79 (±0.18)	9.71	0.000 (0.000 – 0.001)	0.016 (0.003 – 1.226)

MRD: manufacturer's recommended dose, 2 x DD: 2 x discriminatory dose = 2 x MRD, SE: standard error, HF: heterogeneity factor, LD: Lethal dose, DD: discriminatory dose, 95 per cent CL: 95 per cent confidence limit. *: Data followed probit model ($p<0.05$).