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Acaricide resistance mechanisms and monitoring tools available for *Rhipicephalus* (*Boophilus*) *microplus*

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Abstract
Parasitic diseases rank among the most prevalent infectious diseases. Availability of antiparasitic drugs is limited due to high cost of development of newer drugs and rapid pace of development of resistance. Literature shows that resistance against all newly launched antiparasitic drugs is noticed within a decade time. Condition is severe and discouraging in tropical countries like India where animals carry substantial parasitic load and there is indiscriminate use of drugs. Chemotherapy being central to the control of parasites, drug resistance is likely to be a major issue in near future. Constant surveillance and monitoring of antiparasitic drug resistance is the need of the hour. The problem of resistance can be circumvented either by delaying its onset or use of alternate strategies in the form of integrated parasite management. During the course of evolution, nature has developed various mechanisms such as entomo-pathogenic soil nematodes, entomo-pathogenic fungi and certain semiochemicals to check and concentrate the tick population on and off the host. In the last 7 years, there is an outburst of acaricide resistance reports (BHC, deltamethrin, cypermethrin, fenvalerate, amitraz, diazinon, malathion) in different states of India. With the advancement in technology, certain interventions such as preparation of anti-tick vaccine, nanoparticle formulations and use of synergistic have been practiced to improve the efficacy of drug formulation and combat the emerging acaricide resistance in India. Investigation of herbal acaricides and exploration of potential lead chemical molecules in proven phyto-formulation is another non-conventional approach considered by researchers as alternate for devising tick control programmes. In the current scenario, an integrated approach consisting of managemental, immunological, chemical and botanicals measures could intervene the emerging resistance problem and develop a holistic approach of tick control.

Keywords: resistance, monitoring tools, *Rhipicephalus* (*Boophilus*) *microplus*

Introduction
Ticks are obligate haematophagous arthropod of vertebrates. There are around 904 species of tick in world, which includes soft ticks, hard ticks and one tick species *Nuttalliella namaqua* which shares some morphological and physiological characters with other families. They are responsible for direct loss in terms of annoyance, blood loss, hide damage and indirect loss as vector of several human and animal infectious agents. In India, since the beginning of modernization and industrialization in agriculture, chemical control forms the mainstay of tick control programme. Since 1914 after first case of insecticide resistance was reported by Melander, acaricide resistance was defined and redefined time to time. WHO [65] defined resistance as the development of an ability to tolerate toxicants which would prove lethal to the majority of individuals in a normal population of the same species. In the year 1965, WHO defined the term as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject. WHO [64] redefined the term as an inherited characteristic that imparts an increased tolerance to a pesticide, or group of pesticides, such that the resistant individuals survive a concentration of the compound(s) that would normally be lethal to the species. Finally, Coles and Dryden [16] defined insecticide/acaricide resistance as the selection of a specific heritable trait (or traits) in a population of arthropods, results in a significant increase in the percentage of the population that will survive a standard dose of that chemical (or a closely related chemical in the case of cross resistance). This review incorporate information on different mechanism of resistance, factors affecting acaricide resistance, tools to estimate acaricide resistance and possible strategies suggested by different tick research group for control of resistant tick population.

Factors that contribute to the development of acaricide resistance

The risk of resistance development varies with acaricide groups and tick species also have significant influence on risk factors. Resistance is particularly high for many of the selective acaricides with specific modes of action. In general, acaricides with a single target site that are applied numerous times will be more vulnerable to resistance development than acaricides that attack several target sites and are used less frequently. In the first situation the selection pressure would be very high; in the latter it would be much lower. However, resistance does not always develop as predicted. The factors that affect resistance development can be grouped into three categories: the tick's genetic make-up (occurrence of resistance gene, number of resistance mechanisms, gene frequency, dominance of resistance gene, cross-resistance, fitness of resistant individuals, etc.); the tick's biology (tick population size, reproductive potential, generation time, host range, acaricide metabolism, number of target sites of the acaricides, etc.), and the operational factors including the acaricide characteristics and application (persistence of acaricide, dose rate, coverage, frequency of application, proportion of population treated, tick life-cycle stages treated, etc.).

Mechanism of Resistance

There is a mixture of resistance mechanisms found for each class of acaricide. These include behavioural changes; cutaneous penetration; metabolic detoxification involving glutathione transferases (GST), monoxygenase (cytP450) and esterases and target sight alterations for every class of acaricide examined. These studies have led to the development of several molecular based techniques that can rapidly detect acaricide resistance.

Physiological/Behavioral changes

Resistant arthropods may detect or recognize a danger and avoid the toxin. There have been several reports in mosquitoes changing their behavior as a result of intensive indoor use of insecticides, but there is currently insufficient data to assess whether these behavioral avoidance traits are genetic or adaptive response. Arthropods may simply stop feeding if they come across certain insecticides, or leave the area where spraying occurred. There is no such report available in ticks. Preliminary studies on tick behavior to correlate with resistance phenomenon could not establish any correlation (Guerin *et al.*, 2000). It has been observed that after *in vitro* acaricidal treatment of ticks, laying occurs in a line rather than heaped in normal condition leading to egg masses with bigger surface area resulting in fast desiccation of eggs and reduced hatching [37].

Cuticular/Penetration resistance

Reduced uptake of insecticide, often referred to as cuticular resistance, is frequently described as a minor resistance mechanism. Schnitzerling *et al.* [50] proved greater penetration of acaricide in susceptible ticks than the resistant tick strains. Certainly for pests where the major route of insecticide action is via contact toxicity, this is likely to be the case. The relative abundance of C16:0, C18:0, C18:1ω9C and C20:0 has proved 2–5 times higher in the extracts from resistant ticks [70]. Villarino *et al.* [61] found increased esterases activity in the inner layers of the integument of OP resistant adult female *R. (B.) microplus* as compared with susceptible one. Three esterases were purified from adult integument, and were in the range of 64 to 66 kDa [60].

Target Site Insensitivity/Altered Target Site Sensitivity (TSR)

In arthropod, the sites where the toxin usually binds become modified to reduce the effects. Several mutations in the sodium channel gene have been associated with resistance to pyrethroids in a variety of arthropods. Pyrethroids target the voltage-gated sodium channel, an integral transmembrane protein consisting of four homologous domains (I–IV), each containing six membrane spanning segments (S1–6) [68]. Mexican isolates found to have nucleotide substitution in domain IIIS6 transmembrane segment of the sodium channel gene [27]. Guerrero [25] developed an allele specific PCR using mutation at domain III S-6 position, however, the same has not been detected in Brazilian SP resistant populations [1,19]. Later, another mutation associated with SP resistance was identified in Australian populations of *R. (B.) microplus* [43] and a PCR assay was developed for genotyping field Habig populations. More recently, an additional point mutation associated with resistance to flumethrin but not to cypermethrin was identified in Australian populations [33]. These two mutations are located in the S4–5 linker of the domain II of the sodium channel gene, leading to leucine to isoleucine (L64I) and glycine to valine (G72V) amino acid substitutions, respectively. In India, Australia and Brazil, mutation at domain II S4-5 linker region of sodium channel gene of pyrethroid resistant *R. (B.) microplus* was detected [35, 43, 19].

Metabolic Resistance (MR)

Metabolic resistance occurs when elevated activities of one or more enzymes results in a sufficient proportion of the insecticide being sequestered or detoxified before it reaches the target site to impair the toxicity of the insecticide. Resistant strains may possess higher levels or more efficient forms of esterase, monoxygenase and glutathione-S-transferase enzymes. In addition to being more efficient, these enzyme systems also may have a broad spectrum of activities viz.,

1) Increase rate of insecticide metabolism;
2) Lowers amount of insecticide reaching target site;
3) Unexplained more complex mechanisms may be involved in reduction of action of insecticides

While metabolic resistance has been generally attributed to the cytochrome P450s, esterases, and glutathione S-transferases (GST), each of these are large gene families present in cattle tick with 115, 81, and 39 individual members, respectively [11]. Enayati *et al.* [21] reported elevated level of esterases (3 fold), monoxygenase (6 fold) and GST (5 fold) activities in SP resistant *R. bursa*.

Esterase linked with resistance

It mainly comprises of six families of proteins belonging to the α/β hydrolase superfamily [31]. Gut, synganglion, integument and hemolymph are the major source of tick esterases [60]. Esterases are a group of enzymes that are reportedly associated with acaricide resistance in *R.*(*B.*) *microplus* which hydrolyze ester linkages present in a wide range of insecticides.

In case of *R.(B.) microplus* the subject has been studied on various reference and field strains such as, in case of Mexican strains, Tuxpan and Tuxla, esterase based acaricide hydrolysis

is found to be contributed in acquiring resistance [14,66]. Jamroz *et al.* [32] showed high level of carboxylesterase, termed Est9, involved in conferring pyrethroid resistance in Coatzacoalcos strain. In a qualitative study, Baffi *et al.* [2] found increased activity of acetylcholinesterase in malathion resistant strain as compared to Mozzo strain.

A comparative qualitative analysis of esterase patterns in malathion and deltamethrin-sensitive, tolerant and resistant tick groups, using non-denaturing polyacrylamide gel electrophoresis revealed four bands proclaimed as EST-1 to EST-4 having esterase activity against α-naphthyl acetate. The EST-3 and EST-4 were detected in all strains and were classified as carboxylesterases (CaEs). The EST-2, classified as an acetylcholinesterase (AChE), was detected in all the groups, but its staining intensity increased from susceptible to resistant groups, indicating an altered production according to the degree of resistance. The EST-1, which was also classified as an AChE, was detected exclusively in tolerant and resistant groups to both acaricides, but displayed greater activity in the malathion-resistant group [2]. Baffi *et al.* [2] for the first time associated OP and pyrethroids resistance in Brazilian tick with higher esterase enzyme activity. Baxter and Barker [7] reported post translational modifications to be responsible for insensitive AChE in Australian strains. Hernandez *et al.* [30] reported increase copy number of esterase in pyrethroids resistant Mexican ticks. Baxter and Barker [7] identified two genes for AChE (AChE1 and AChE2) in synganglia of Australian resistant ticks. Temeyer *et al*. [57] identified AChE3 gene and further established that mutation at position R86Q has definite role in conferring resistance to OP compound [59].

Resistance to carbaryl and the cross-resistance between carbaryl and OPs suggest the involvement of similar resistance mechanisms. Both the carbamate and OP acaricides exert their toxic effect on ticks by inhibiting AChE, a key enzyme critical to the function of the nervous system of invertebrates. Resistance to OP and carbamate pesticides was found to be conferred by insensitive AChE in many pest species [55]. Insensitive AChE has been implicated to play a major role in OP resistance in *R. (B.) microplus* [46]. In addition, a cytochrome P450-mediated oxidative detoxification also has been implicated specifically in resistance to coumaphos but not to diazinon and indicated that resistance to carbaryl was likely to be conferred mainly by insensitive AChE [39].

Glutathione –S-Transferase (GST) linked with Resistance

Glutathione transferases (GSTs) are a diverse family of enzymes found ubiquitously in aerobic organisms. They play a central role in the detoxification of both endogenous and xenobiotic compounds and are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress. Interest in insect GSTs has primarily focused on their role in insecticide resistance. GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione, to produce water-soluble metabolites that are more readily excreted. In addition, they contribute to the removal of toxic oxygen free radical species produced through the action of pesticides. Though the correlation of GST in resistance mechanisms is well established in many arthropods, study on ticks is still at a preliminary stage. Mounsey *et al.* [44] found increased transcription of GST in permethrin resistant mites. GSTs had been reported to play a major role in the organophosphate resistance pathway of the *Musca domestica* [62]. He *et al.* [27] could not correlate GST with OP resistance. da Silva Vaz Jr. *et al.* [17] purified *R.(B.) microplus* rGST and using specific substrates determined that some acaricides (ethion, amitraz, chlorpyrifos, DDT, cypermethrin, diazinon, ivermectin, deltamethrin and flumethrin) inhibited rGST while coumaphos had an activating effect. Saldivar *et al*. [49] for the first time gave strong molecular evidence of correlation between GST and acaricide resistance in ticks.

Cytochrome P450 oxidases linked with resistance

The cytochrome P450 oxidases (also termed oxygenases) metabolize insecticides through O-, S-, and N-alkyl hydroxylation, aliphatic hydroxylation and epoxidation, aromatic hydroxylation, ester oxidation, and nitrogen and thioether oxidation. The cytochrome P450s belong to a vast superfamily. Of the 62 families of P450s recognized in animals and plants, at least four (families 4,6,9,18) have been isolated from insects. The insect P450 oxidases responsible for resistance belonged to family 6. The coumaphos resistant Mexican San Roman strain showed increased expression of a cytochrome P450-like transcript following treatment with low doses of coumaphos [24]. Synergist studies with PBO have indicated that cytochrome P450s plays important role in pyrethroid resistance mechanisms in *R. (B.) microplus* of Mexican isolate [42].

Genetic changes linked with resistance

Bayúgar *et al.* [71] reported increased Phospholipid-hydroperoxide glutathione peroxidase (PHGPx) enzymes in two strains resistant to a single acaricide class (Tuxpan resistant to OP and CEPICH to pyrethroid). Bayugar *et al*. [10] found that carboxylesterase and AChE2 gene expression increased by exposure of acaricide on OP and SP resistant Mora and San Alfonso strains relative to susceptible reference strain in a real time quantification of mRNA. Baffi *et al.* [3] reported overexpression of AChE2 gene at synganglion level in OP resistant and elevated level of carboxylesterase (CzEST9) gene in pyrethroid resistant [32] *R. (B.) microplus*. Saldivar *et al.* [49] studied quantitative gene expression of a number of *R.(B.) microplus* genes of Mexican Pasqueria strain resistant to amitraz and diazinon using microarray and qPCR. A direct correlation between over-expression of GST genes and resistance to both the insecticides was noted and so could not be correlated with a particular acaricide class so far.

Monitoring tools used for characterization of acaricide resistance

Bioassay

Food and Agriculture Organization (FAO) developed Larval Packet Test (LPT) and Adult Immersion Test (AIT) kits have been used to measure tick susceptibility/resistance to acaricides. However, new bioassays were developed as per the requirements and established in different laboratories:

1) The larval packet test (LPT): Developed by Stone and Haydock [56] and was later adopted by FAO [22]. Subsequently, the same test has been standardized in different laboratories using country specific reference tick strain.
2) The adult immersion test (AIT): Drummond *et al.* [20] proposed the protocol in which engorged female ticks which were immersed in technical or commercial acaricides. The is most commonly followed. A number of variations in generalized protocol has been reported. The age and condition of ticks prior to AIT are likely to play

an important role in variability of results [34]. The AIT was standardized with a 14 days oviposition protocol [53] in contrast to the 7 days protocol followed elsewhere [48] and an optimized immersion time of 2 min was validated [36].

3) Larval Immersion Test: First developed by Shaw [54]. The advantage of the Shaw's immersion sandwich technique is that the larvae are immersed in a solution or suspension of the acaricide and this usually increases toxicity.
4) Larval tarsal test [41, 40]: It involves placement of tick eggs into multi-well plates to allow the evaluation of multiple chemicals. The test is under experimental stage and adoption by other laboratories has not been reported.
5) Larval immersion microassay (LIM) [63]: It offers superior sensitivity, flexibility to accommodate multiple formulations, and a robust capability for rapidly screening many compounds with a minimal requirement of test article for evaluation. Using this bioassay speed of kill benchmarks can be obtained for several known acaricides against multiple ixodid ticks. The LIM provided an increase in sensitivity of at least 10-fold over the LPT at measuring EC50 values. Tedious cutting of paper packets and mortality due to sealing material can be avoided.

Enzyme Assay

Quantitative estimation of detoxifying enzymes

1) **Acetylcholinesterase (AChE) Assay**: As described by Hemingway *et al.* [28] and Baxter *et al.* [8] inhibition of AChE activity in the presence of propoxur can be measured and may give information regarding the role of altered acetylcholinesterase in conferring resistance.
2) **Esterase Assay** [28, 32]: Measures esterase activity directly using an esterase substrate (napthyl acetate) and product formed is measured spectroscopically at 570 nm after adding fast blue stain which binds with the product and shows color reaction. In case of enzyme kinetic study, esterase activity can be measured adding p-nitrophenyl acetate (PNPA) as substrate and reading is taken at 405 nm continuously for 2 min. Here non specific activity of esterases is measured, that may be arylesterase, acetylcholinesterase or carboxylesterase. Thus napthyl acetate and PNPA assays need to be supported by synergistic assay to generate specific role of individual enzyme in resistance development.
3) **Monoxygenase Assay** [72]: It is based on measuring the Haem content of tick assuming majority of haem in arthropod is associated with cyt P^{450}. It is a rough means of titrating cyt P^{450}. Tetramethyl benzidine, a chromogenic substrate is added to larval homogenate and heme equivalent of cyt P^{450} is estimated at 650 nm.
4) **Glutathione S-Transferase Assay** [26]: The glutathione - S-transferase (GST) assay utilizes 1-Chloro-2,4-dinitrobenzene (CDNB) which is suitable for the broadest range of GST isozymes. Upon conjugation of the thiol group of glutathione to the CDNB substrate, there is an increase in the absorbance at 340 nm.
5) **Dot blot Assay** [18]: A test was developed to detect the presence of insecticide-resistant acetylcholinesterase (AChE) based on the quasipermanent binding of proteins onto blotting membranes.

a. For elevated esterase: Tick homogenate blotted on a Whatmans filter paper is exposed to napthyl acetate substrate and Fast Garnet GBC stain which provides purple dot in ticks resistant due to elevated esterase.
b. For altered assay: Adult tick homogenate blotted on nitrocellulose membrane on exposure to paroxon and propoxur develop red brown spots in case of altered acetylcholinesterases.

Qualitative assay of enzyme activity

Non dissociating gel electrophoresis was performed mainly to detect esterase based metabolic resistance mechanism [42]. Proteins were separated in separating gel based on their charges and then product-compatible- stains were used to assess and detect the enzyme activity. To confirm the role of specific enzyme, different specific and non-specific inhibitors were used to probe the gels [2,3]. Commonly used inhibitors in inhibition studies have been eserine sulfate, copper sulfate, p-p-chloromercuribenzoate (pCMB), malathion, phenylmethylsulfonyl fluoride (PMSF) and Triphenyl phosphate (TPP) for esterases.

Molecular Assay

Mutation in Sodium channel gene: Sequence analysis of sodium channel gene in pyrethroids resistant tick population reveals mutation at 3 locations of sodium channel gene till now [35, 19, 43, 33, 27]. Based on the data allele specific PCR has been developed by Guerrero *et al.* [25] and Morgan [43] in Mexico and Australia, respectively.

Mutation in Carboxylesterase gene: Villarino [60, 61] identified and purified three carboxylesterases in tick integument. Jamroz *et al.* [32] found an increase in CaEs activity in pyrethroids resistant Mexican tick strain. Hernandez *et al.* [30] identified a point mutation in 372 bp DNA fragment of a putative CaE gene in pyrethroid-resistant Mexican population. This mutation (G to A substitution in the 1120 nucleotide) generates a recognition site for the restriction enzyme EcoRI (CTTAAG). Baffi *et al.* [4] performed PCR-RFLP technique to trace the presence of point mutation in a fragment of a putative carboxylesterase gene in strains of *R.(B.) microplus* and found homozygous mutant genotype (M) in the moderate resistant and highly resistant strains, with higher frequency in the highly resistant strain.

Mutation in acetylcholinesterase gene: Acetylcholinesterase (AChE, EC 3.1.1.7) is a serine esterase in the α/β hydrolase fold enzyme family. The catalytic triad consists of Ser200, His440 and Glu237 and the enzyme is located on the surface of the post synaptic membrane and linked by a GPI anchor [45]. Baxter and Barker [6] characterized *AchE1* gene from Australian isolates of *R (B) microplus* and reported identical pattern of sequences in two susceptible strains, one OP-resistant lab strain (Biarra) and two OP-resistant field strains. They opined that resistance to OPs in Australian *R (B) microplus* was not conferred by a mutation in alleles of the AChE gene. It was postulated that there is a second, undiscovered AChE locus that conferred resistance, or there was post-translational modification of the protein encoded by the AChE locus. Bendele *et al.* [12] identified 72 AChE1 sequence variants, 2 of which are strongly associated with OP-resistant phenotypes. Baxter and Barker [7] further identified, sequenced and compared the *AChE2* of Australian *R.(B.) microplus* OP resistant strains that with susceptible strain (N strain) and found 6-13 nucleotide differences in the coding region of 1689 base pairs. Hernandez *et al.* [29] didn't

find any such difference in Texas OP resistant strain of *R.(B.) microplus* and postulated post-translational modification as possible mechanism to confer OP resistance. Temeyer *et al.* [57] reported, characterized and analyzed *AChE3* gene of *R. (B) microplus.* Temeyer *et al.* [58] demonstrated the presence of the R86Q mutation in a acetylcholinesterase gene of *R (B) microplus* (*BmAChE3*). The highest frequency of the resistant allele (86Q) was found in the Tuxpan (100%), Santa Luiza (96%), Pesqueria (73%), and San Roman (70%) strains of *R.(B.) microplus* and all these strains were characterized as OP-resistant [39]. These results were consistent with the hypothesis of an association between R86Q and OP resistance, possibly highlighting a role for the *BmAChE3* gene in the OP-resistance mechanism.

Quantification of targeted genes: Over expression of detoxifying enzymes are usually discussed by various authors working on resistance in ticks but very few studies using qPCR or microassay for quantification of enzyme mRNA copy number have been reported so far. Acaricides are thought to increase free radical pressure in ticks that can ends fatally. Increase production of free radical scavengers or antioxidants are also one of the proposed mechanisms in insects but rarely studied in ticks. Among available acaricides on the market, organochlorine, organophosphate, and carbamate compounds have proved to stimulate peroxidation of cellular membranes as part of their toxic effect [5, 67, 51, 52]. Lipid peroxidation results from alteration of membrane lipids induced by reactive oxygen species (ROS). The damaging effects of ROS may be counteracted by selenium-dependent peroxidases (SDP), such as glutathione peroxidase (GPx) and phospholipid-hydroperoxide glutathione peroxidase (PHGPx), working in concert with reduced glutathione (GSH). Bayúgar *et al.* [71] found increased PHGPx in two strains (diazinon resistant Tuxpan and deltamethrin resistant CEPICH strain) resistant to a single acaricide class. Strains resistant to two or more classes (San Alfonso and Mora strain) showed a reduction in PHGPx. A statistically significant (3.47±0.302; $p<0.0001$) REU increase on the P-450 gene expression level detected on pyrethroid resistant ticks but not in OP resistant ticks [9]. Bayúgar *et al.* [10] reported a statistically significant increase in cholinesterase and carboxylestrase gene expression level in San Alfonso and Mora strains in comparison to the susceptible strain. A direct correlation between over expression of GST genes and resistance shown via microarray and real time PCR in Pasqueria stain resistant to amitraz and diazinon was reported [49].

Constant surveillance and monitoring of antiparasitic drug resistance in various common parasites of livestock and poultry is the need of the hour. The problem of resistance can be circumvented either by delaying its onset or use of alternate strategies in the form of integrated parasite management. From the last five decades, chemical control forms the mainstay of tick control programme in India [15, 23]. Tick researchers focused upon mechanisms such as entomopathogenic nematodes (EPNs), entomopathogenic fungi and semiochemicals to lure and kill the tick population [38]. Biological control is broadly defined as use of live organism for the control of insect pests, however, there is distinctions from biopesticides. Biopesticides do not multiply in the environment and therefore not expected to have persistent residual effect. In contrast, biological control agents (predators, pathogens, parasites and resistant plants) are expected to establish in environment and have persistent effect on pest population. In past seven years, there is an outburst of acaricide resistance reports from different states of India [47]. Manual interventions such as anti-tick vaccine, nanoparticle formulations and use of synergistic have also been tried to mitigate emerging resistance problem [13, 73]. Investigation of botanicals and deep insight into potential lead molecules in proven phyto-formulation is the need of the hour [74]. Integrated approach entailing acaricide with managemental, immunological and botanicals measures could be a holistic approach for sustainable tick control.

References

1. Andreotti R, Guerrero FD, Soares MA, Barros JC, Miller RJ, Léon AP. Acaricide resistance of *Rhipicephalus (Boophilus) microplus* in State of Mato Grosso do Sul, Brazil. Revista Brasileira de Parasitologia Veterinária. 2011; 20(2):127-33.
2. Baffi MA, de Souza GR, de Sousa CS, Ceron CR, Bonetti AM. Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of *Riphicephallus (Boophilus) microplus* (Acari, Ixodidae). Molecular and biochemical parasitology. 2008; 160(1):70-3.
3. Baffi MA, de Souza GR, Vieira CU, de Sousa CS, Gourlart LR, Bonetti AM. Identification of point mutations in a putative carboxylesterase and their association with acaricide resistance in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). Veterinary parasitology. 2007; 148(3-4):301-9.
4. Baffi MA, Pereira CD, Souza GR, Bonetti AM, Ceron CR, Gourlart LR. Esterase profile in a pyrethroid-resistant Brazilian strain of the cattle tick *Boophilus microplus* (Acari, Ixodidae). Genetics and molecular biology. 2005; 28(4):749-53.
5. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology letters. 1999; 107(1-3):33-47.
6. Baxter GD, Barker SC. Acetylcholinesterase cDNA of the cattle tick, *Boophilus microplus*: characterisation and role in organophosphate resistance. Insect Biochemistry and Molecular Biology. 1998; 28(8):581-9.
7. Baxter GD, Barker SC. Analysis of the sequence and expression of a second putative acetylcholinesterase cDNA from organophosphate-susceptible and organophosphate-resistant cattle ticks. Insect biochemistry and molecular biology. 2002; 32(7):815-20.
8. Baxter GD, Green P, Stuttgen M, Barker SC. Detecting resistance to organophosphates and carbamates in the cattle tick *Boophilus microplus*, with a propoxur-based biochemical test. Experimental & applied acarology. 1999; 23(11):907-14.
9. Bayugar CR, Miranda-Miranda E, Ortiz-Najera A, Neri-Orantes S, Olvera-Valencia F. Cytochrome P-450 monooxygenase gene expression supports a multifactorial origin for acaricide resistance in *Ripicephalus microplus*. Res. J Parasitol. 2008; 3:59-66.
10. Bayugar CR, Miranda-Miranda E, Portilla-Salgado D, Osorio-Miranda J. Quantitative PCR detection of cholinesterase and carboxylesterase expression levels in acaricide resistant *Rhipicephalus (Boophilus) microplus*. Journal of Entomology. 2009; 6(2):117-23.
11. Bellgard MI, Moolhuijzen PM, Guerrero FD, Schibeci D, Rodriguez-Valle M, Peterson DG *et al*. CattleTickBase:

An integrated Internet-based bioinformatics resource for *Rhipicephalus (Boophilus) microplus*. International journal for parasitology. 2012; 42(2):161-9.
12. Bendele KG, Guerrero FD, Miller RJ, Li AY, Barrero RA, Moolhuijzen PM *et al*. Acetylcholinesterase 1 in populations of organophosphate-resistant North American strains of the cattle tick, *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology research. 2015; 114(8):3027-40.
13. Benelli G, Maggi F, Romano D, Stefanini C, Vaseeharan B, Kumar S *et al*. Nanoparticles as effective acaricides against ticks-a review. Ticks and tick-borne diseases. 2017; 8(6):821-6.
14. Bull DL, Ahrens EH. Metabolism of coumaphos in susceptible and resistant strains of *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology. 1988; 25(2):94-8.
15. Chaudhury RP, Naithani RC. Resistance to BHC in the cattle tick *Boophilus microplus* (Can.) in India. Bulletin of Entomological Research, 1964, 55-405.
16. Coles TB, Dryden MW. Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. Parasites & vectors. 2014; 7(1):8.
17. da Silva Vaz Jr I, Lermen TT, Michelon A, Ferreira CA, de Freitas DR, Termignoni C *et al*. Effect of acaricides on the activity of a *Boophilus microplus* glutathione S-transferase. Veterinary parasitology. 2004; 119(2-3):237-45.
18. Dary O, Georghiou GP, Parsons E, Pasteur N. Dot-blot test for identification of insecticide-resistant acetylcholinesterase in single insects. Journal of economic entomology. 1991; 84(1):28-33.
19. Domingues LN, Brasil BD, de Paiva Bello AC, da Cunha AP, de Barros AT, Leite RC *et al*. Survey of pyrethroid and organophosphate resistance in Brazilian field populations of *Rhipicephalus (Boophilus) microplus*: Detection of C190A mutation in domain II of the para-type sodium channel gene. Veterinary parasitology. 2012; 189(2-4):327-32.
20. Drummond RE, Ernst SE, Trevino JL, Gladney WJ, Graham OH. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. Journal of economic entomology. 1973; 66(1):130-3.
21. Enayati AA, Asgarian F, Amouei A, Sharif M, Mortazavi H, Boujhmehrani H *et al*. Pyrethroid insecticide resistance in *Rhipicephalus bursa* (Acari, Ixodidae). Pesticide biochemistry and physiology. 2010; 97(3):243-8.
22. FAO. Ticks and Tick-borne Disease Control. A Practical Field Manual. Food and Agriculture Organization, Rome. 1984; I:246-99.
23. Ghosh S, Gupta S, Kumar KA, Sharma AK, Kumar S, Nagar G *et al*. Characterization and establishment of a reference deltamethrin and cypermethrin resistant tick line (IVRI-IV) of *Rhipicephalus (Boophilus) microplus*. Pesticide biochemistry and physiology. 2017; 138:66-70.
24. Guerrero FD, Bendele KG, Chen AC, Li AY, Miller RJ, Pleasance E *et al*. Serial analysis of gene expression in the southern cattle tick following acaricide treatment of larvae from organophosphate resistant and susceptible strains. Insect molecular biology. 2007; 16(1):49-60.
25. Guerrero FD, Davey RB, Miller RJ. Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology. 2001; 38(1):44-50.
26. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of biological Chemistry. 1974; 249(22):7130-9.
27. He H, Chen AC, Davey RB, Ivie GW, George JE. Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. Biochemical and biophysical research communications. 1999; 261(3):558-61.
28. Hemingway J, Brogdon W. Techniques to detect insecticide resistance mechanisms. Document *WHO/CTD/ CPC/MAL/98.6*. World Health Organization, Geneva, 1998.
29. Hernandez R, He H, Chen AC, Ivie GW, George JE, Wagner GG. Cloning and sequencing of a putative acetylcholinesterase cDNA from *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology. 1999; 36(6):764-70.
30. Hernandez R, He H, Chen AC, Waghela SD, Ivie GW, George JE *et al*. Identification of a point mutation in an esterase gene in different populations of the southern cattle tick, *Boophilus microplus*. Insect Biochemistry and Molecular Biology. 2000; 30(10):969-77.
31. Hotelier T, Renault L, Cousin X, Negre V, Marchot P, Chatonnet A. ESTHER, the database of the α/β-hydrolase fold superfamily of proteins. Nucleic acids research. 2004; 32:D145-7.
32. Jamroz RC, Guerrero FD, Pruett JH, Oehler DD, Miller RJ. Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican strains of the southern cattle tick, *Boophilus microplus*. Journal of Insect Physiology. 2000; 46(5):685-95.
33. Jonsson NN, Cutullè C, Corley SW, Seddon JM. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus microplus* associated with resistance to flumethrin but not to cypermethrin. International journal for parasitology. 2010; 40(14):1659-64.
34. Jonsson NN, Hope M. Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick *Boophilus microplus*. Veterinary parasitology. 2007; 146(3-4):193-8.
35. Kumar R, Nagar G, Sharma AK, Kumar S, Ray DD, Chaudhuri P *et al*. Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus (Boophilus) microplus*: identification of C190A mutation in the domain II of the para-sodium channel gene. Acta tropica. 2013; 125(2):237-45.
36. Kumar S, Paul S, Sharma AK, Kumar R, Tewari SS, Chaudhuri P *et al*. Diazinon resistant status in *Rhipicephalus (Boophilus) microplus* collected from different agro-climatic regions of India. Veterinary Parasitology. 2011; 181(2-4):274-81.
37. Kurup SP, Azhahianambi P, Ghosh S. Effect of surface area to weight ratio of egg masses on the hatchability of *Boophilus microplus* eggs. Journal of vector borne diseases. 2008; 45(2):164.
38. Latha BR. Semiochemical – A novel thought for tick control. Veterinary and Animal Science, 2012; 43:1-10.
39. Li AY, Davey RB, Miller RJ, George JE. Resistance to coumaphos and diazinon in *Boophilus microplus* (Acari: Ixodidae) and evidence for the involvement of an

oxidative detoxification mechanism. Journal of medical entomology. 2003; 40(4):482-90.
40. Lovis L, Mendes MC, Perret JL, Martins JR, Bouvier J, Betschart B *et al*. Use of the Larval Tarsal Test to determine acaricide resistance in *Rhipicephalus (Boophilus) microplus* Brazilian field populations. Veterinary parasitology. 2013; 191(3-4):323-31.
41. Lovis L, Perret JL, Bouvier J, Fellay JM, Kaminsky R, Betschart B. A new in vitro test to evaluate the resistance level against acaricides of the cattle tick, *Rhipicephalus (Boophilus) microplus*. Veterinary parasitology. 2011; 182(2-4):269-80.
42. Miller RJ, Davey RB, George JE. Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology. 1999; 36(5):533-8.
43. Morgan JA, Corley SW, Jackson LA, Lew-Tabor AE, Moolhuijzen PM, Jonsson NN. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus (Boophilus) microplus* associated with resistance to synthetic pyrethroid acaricides. International journal for parasitology. 2009; 39(7):775-9.
44. Mounsey KE, Pasay CJ, Arlian LG, Morgan MS, Holt DC, Currie BJ *et al*. Increased transcription of Glutathione S-transferases in acaricide exposed scabies mites. Parasites & vectors. 2010; 3(1):43.
45. Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM *et al*. The alpha/beta hydrolase fold. Protein Eng. 1992; 5(3):197-211.
46. Pruett JH. Comparative inhibition kinetics for acetylcholinesterases extracted from organophosphate resistant and susceptible strains of *Boophilus microplus* (Acari: Ixodidae). Journal of economic entomology. 2002; 95(6):1239-44.
47. 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, 2017, 1-27.
48. Sabatini GA, Kemp DH, Hughes S, Nari A, Hansen J. Tests to determine LC50 and discriminating doses for macrocyclic lactones against the cattle tick, *Boophilus microplus*. Veterinary Parasitology. 2001; 95(1):53-62.
49. Saldivar L, Guerrero FD, Miller RJ, Bendele KG, Gondro C, Brayton KA. Microarray analysis of acaricide-inducible gene expression in the southern cattle tick, *Rhipicephalus (Boophilus) microplus*. Insect molecular biology. 2008; 17(6):597-606.
50. Schnitzerling HJ, Schuntner CA, Roulston WJ, Wilson JT. Characterization of the Organophosphoms-resistant Mt Alford, Gracemere and Silkwood Strains of the Cattle Tick, *Boophilus microplus*. Australian journal of biological sciences. 1974; 27(4):397-408.
51. Seth V, Banerjee BD, Bhattacharya A, Chakravorty AK. Lipid peroxidation, antioxidant enzymes, and glutathione redox system in blood of human poisoning with propoxur. Clinical biochemistry. 2000; 33(8):683-5.
52. Seth V, Banerjee BD, Chakravorty AK. Lipid peroxidation, free radical scavenging enzymes, and glutathione redox system in blood of rats exposed to propoxur. Pesticide Biochemistry and physiology. 2001; 71(3):133-9.
53. Sharma AK, Kumar R, Kumar S, Nagar G, Singh NK, Rawat SS *et al*. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. Veterinary Parasitology. 2012; 188(3-4):337-45.
54. Shaw RD. Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. Bulletin of Entomological Research. 1966; 56(3):389-405.
55. Siegfried BD, Scharf ME. Mechanisms of organophosphate resistance in insects. InBiochemical sites of insecticide action and resistance. Springer, Berlin, Heidelberg, 2001, 269-291.
56. Stone BF, Haydock KP. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). Bulletin of Entomological Research. 1962; 53(3):563-78.
57. Temeyer KB, Davey RB, Chen AC. Identification of a third *Boophilus microplus* (Acari: Ixodidae) cDNA presumptively encoding an acetylcholinesterase. Journal of medical entomology. 2004; 41(3):259-68.
58. Temeyer KB, Pruett JH, Olafson PU, Chen AC. R86Q, a mutation in BmAChE3 yielding a *Rhipicephalus microplus* organophosphate - insensitive acetylcholinesterase. Journal of medical entomology 2007; 44(6):1013-8.
59. Temeyer KB, Pruett JH, Olafson PU. Baculovirus expression, biochemical characterization and organophosphate sensitivity of rBmAChE1, rBmAChE2, and rBmAChE3 of *Rhipicephalus (Boophilus) microplus*. Veterinary parasitology. 2010; 172(1-2):114-21.
60. Villarino MA, Waghela SD, Wagner GG. Biochemical detection of esterases in the adult female integument of organophosphate-resistant *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology. 2003; 40(1):52-7.
61. Villarino MA, Waghela SD, Wagner GG. Histochemical localization of esterases in the integument of the female *Boophilus microplus* (Acari: Ixodidae) tick. Journal of medical entomology. 2001; 38(6):780-2.
62. Wei SH, Clark AG, Syvanen M. Identification and cloning of a key insecticide-metabolizing glutathione S-transferase (MdGST-6A) from a hyper insecticide-resistant strain of the housefly *Musca domestica*. Insect biochemistry and molecular biology. 2001; 31(12):1145-53.
63. White WH, Plummer PR, Kemper CJ, Miller RJ, Davey RB, Kemp DH *et al*. An in vitro larval immersion microassay for identifying and characterizing candidate acaricides. Journal of medical entomology. 2004; 41(6):1034-42.
64. World Health Organization. Vector resistance to pesticides. Fifteenth report of the WHO expert committee on vector biology and control, 1992.
65. World Health Organization. Seventh report of the WHO expert committee on insecticides. World Health Organ Tech Rep. 1957; 125:1-32.
66. Wright FC, Ahrens EH. Cholinesterase insensitivity: a mechanism of resistance in Mexican strains of *Boophilus microplus* (Acari: Ixodidae) against coumaphos. Journal of medical entomology. 1988; 25(4):234-9.
67. Yarsan E, Tanyuksel M, Celik S, Aydin A. Effects of aldicarb and malathion on lipid peroxidation. Bulletin of environmental contamination and toxicology. 1999; 63(5):575-81.
68. Dong K. Insect sodium channels and insecticide resistance. Invertebrate Neuroscience. 2007; 7(1):17.

69. Guerin M, Patrick, Kro. Thomas & Ber, Chemosensory and Behavioural Adaptations of Ectoparasitic Arthropods. Nova acta Leopoldina: Abhandlungen der Kaiserlich Leopoldinisch-Carolinisch Deutschen Akademie der Naturforscher. 2000; 83:213-229.
70. Dana M, Gindina G, Rotc A, Eshela D, Teper-Bamnolkera P, Ben-Ze'evd I *et al*. Role of cuticular lipids and water-soluble compounds in tick susceptibility to Metarhizium infection. Biocontrol Science and Technology. 2013; 23:956.
71. Bayúgar CR, Miranda E, Holman PJ. Molecular cloning of a phospholipid-hydroperoxide glutathione peroxidase gene from the tick, *Boophilus microplus* (Acari: Ixodidae). Insect biochemistry and molecular biology. 2005; 35(12):1378-87.
72. Brogdon WG, McAllister JC, Vulule J. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. Journal of the American Mosquito Control Association. 1997; 13(3):233-7.
73. Vatsya S, Yadav CL. Evaluation of acaricide resistance mechanisms in field populations of *Rhipicephalus (Boophilus) microplus* collected from India. International journal of acarology. 2011; 37(5):405-10.
74. Banumathi B, Vaseeharan B, Rajasekar P, Prabhu NM, Ramasamy P, Murugan K *et al*. Exploitation of chemical, herbal and nanoformulated acaricides to control the cattle tick, *Rhipicephalus (Boophilus) microplus*–a review. Veterinary parasitology. 2017; 244:102-10.