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SNPs and other polymorhisms associated with acaricide resistance in *Rhipicephalus microplus*

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1. ABSTRACT

Ixodicides resistance of ticks is one of the most important problems for the livestock industry in tropical and subtropical regions, mainly due to the increase in cases of multiple resistance in all families of the ixodicides used. Molecular markers such as single nucleotide polymorphisms (SNPs) has been proposed to identify the resistance to ixodicides in *Rhipicephalus microplus*. Many studies have recently been conducted using SNPs and other types of molecular markers to determine if they are associated with resistance to different products in many parts of the world. Knowing these changes at the molecular level, will allow to establish mechanisms and control strategies for the use of ixodicides. In this review, we will discuss and describe the different SNPs and other polymorphisms associated with resistance in *R. microplus.*

2. INTRODUCTION

Tick resistance to acaricides is one of the most important problems facing the livestock industry of the tropical and subtropical regions, due to the multiple resistances to the ixodicides used today. The resistance is like a change in the genetic code of an organism in response to selection due to toxic substances (1). In some populations this can develop quickly, while in others it can be relatively slow (2). The emergence of tick populations with this type of resistance is an evolutionary adaptation due to constant pressure of selection because of the constant use of pesticides (3). This resistance is due to the genetic variability caused by random mutations or genetic rearrangements (4).

Cross resistance is the resistance to different acaricides that have similar mechanisms of action and it has been reported with two Organophosphate (coumaphos and diazinon) and a carbamate (carbaryl) in different strains of *Rhipicephalus microplus* (5, 6). Multiple resistances have also been described in strains of *R. microplus* with different ixodicides (organochlorates, pyrethroids, organophosphates and formamidines) (7). The use of molecular markers such as single nucleotide polymorphisms (SNPs) has been proposed for the identification of the resistance to ixodicides in *R. microplus*. At the molecular level, mutations in the III domain of the 6th segment of the voltage dependent sodium channel are associated with the resistance to pyrethroids (8).

The molecular aspects of the metabolic resistance are not well defined in *R. microplus*, but it has generally been attributed to enzymes such as cytochrome P450, esterases and glutathione S transferase (9). Studies of bioassays and synergists have been done in order to provide evidence regarding the mechanisms of resistance to organophosphates and carbamates, but have been unsuccessful in the identification of specific mechanisms, so it has been hypothesized that the resistance to these compounds is complex and multigenic (10). Little is known about the resistance to amitraz, fipronil and ivermectines. Given the importance of the resistance to ixodicides, this review aims to discuss the recent advances in the knowledge of molecular mechanisms of action that confer resistance to different ixodicides used today as control methods for *R. microplus*.

3. ORGANOCHLORATES

3.1 Mechanism of action

Organochlorates were comercialized in 1945 to control different populations of insects. The chemical structure corresponds to that of chlorinated hydrocarbons, which are insoluble in water, non-volatile and highly soluble in organic solvents (11). Organochlorates include the ethane-derived chlorates, cyclodienes and the compounds related to hexachlorocyclohexane (HCH). Among the ethane chlorates we can find dichlorodiphenyltrichloroethane (DDT); the chlorinated derivatives of cyclodienes include chlordane, aldrin, dieldrin, endrin, heptachlor, and toxaphene; and the HCH-related compounds such as lindane (12).

The most widely researched mechanism of action for these organochlorates has been the one for chlorinated ethanes, mainly for DDT (13). It has a toxic effect on the axon´s Na+ channels, causing the deactivation or closing of the channel after the membrane has been depolarized, leading to a persistent leak of Na+ ions through the neural membrane, bringing about a negative destabilization after the membrane potential, keeping the channels open for a longer period of time than normal. The hyperexcitability of the nerve results in repetitive charges after only one stimulus (14–16). However the cyclodienes and the HCH also affect the nervous system, mainly blocking the neuromuscular transmission because of the effect on the chlorine channels dependent on gamma amino butyric acid (GABA) of the nerve membrane (13). This interrupts the ion transfer and the nervous impulses between the cells (17), causing the insect to respond to external stimuli with violent tremors (18).

3.2 Resistance mechanism

The first hints of resistance in *R. microplus* to organochlorates were described in Brazil in the 1950s, in the Alegrete strain, and in the 1970s the first cases of organophosphate resistance were reported (19). For the control of *R. microplus* in Mexico, aspersion baths with organochlorates and organophosphates have been performed historically. Nonetheless, in 1981, resistance to organophosphates in the tick (Tuxpan strain) was first reported due to failures in control in the Gulf of Mexico, in the region of Tuxpan, Veracruz (20). Likewise, another strain (Tempoal) was reported multiple resistance to both organochlorates and organophosphates, which was distributed widely within the Huastecas of the country and Yucatan (21–23). Later, different strains of *R. microplus* were reported with multiple resistances to organochlorates, organophosphates and other ixodicides in Mexico (22). The mechanisms of resistance can occur either because the insecticide does not bind to the target site or due to an increase of the detoxifying enzymes (esterases, oxidases and glutathione S transferases), keeping the insecticide from reaching its target site. In the case of DDT, its target is the sodium channels of the axons and the resistance can emerge due to a change in an amino acid of the binding site of the insecticide (24). Similarly, the resistance to cyclodienes is due to a point mutation in the same gene that codes for the

GABA receptors (25). The arthropods can synthesize enzymes that belong to the alfa/beta hydrolase superfamily (esterases, oxidases and glutathione S transferases) for the detoxifications of xenobiotics, which are transcribed by sever multigenes (24, 26).

The first reported resistance to organochlorates was in 1947 in *Aedes tritaeniorhynchus*, *Aedes solicitans* and more than 100 species of mosquitoes have been reported resistant, where the majority is anophelines (27, 28). This resistance is caused by the presence of DDR dehydrochlorinase, which was first recognized as a glutathione S transferase in the *Musca domestica* (29, 30). The glutathion S transferases are dimeric multifunctional enzymes that play an important role in detoxification, catalyze the nucleophilic attack of the reduced glutathione (GSH) in the electrophilic centers of the lipophilic compounds (31). They are also present as groups of genes that have been mixed throughout the genome by recombination (32). In *R. microplus*, the mechanisms of resistance that have been reported against organochlorates are due mainly to mutations in the active cycle of the chlorine channel (33) and the sodium channel (34).

3.3 Description of the mutations and polymorphisms

Associations between inversion polymorphisms and resistance to insecticides such as organochlorates, have been described in the mosquito *Anopheles gambiae*. These inversion polymorphisms include three on the right side of the chromosome (2Rb, 2Rc, and 2Rd) and one (2La) on the left side, and these have been associated when there are changes in the weather (35, 36). The 2Rb inversion has been proven to cause resistance to DDT (37), as well as the 2La investment which causes resistance to dieldrin (38, 39). Also, cross resistance to cyclodienes and DDT, as well as fipronil, has been shown in *Blatella germanica*, *Musca domestica* and *Anopheles gambiae* (40–42). Brooke *et al.* (40) found that there are two inversion polymorphisms in *Anopheles gambiae,* 2La and 2Rb, which confer resistance, the first is associated to resistance to dieldrin, and the second one to DDT. In *R. microplus*, two mutations have been described in the gene that codes for the chloride channels dependent on GABA, at the 868–9 position, where a threonine is changed into a lysine in strains resistant to dieldrin (33). Castro *et al.* (43) showed that there is double resistant to fipronil and lindane in *R. microplus* in strains from Brazil and Uruguay.

4. ORGANOPHOSPHATES

4.1 Mechanism of action

The organophosphate compounds are esters, amides and thioles derived from phosphoric, phosphonic and phosphortic acid (11). The organophosphates have as target site the enzyme acetylcholinesterase (AChES), which is a serine esterase enzyme (44); that participates in nervous impulses at the cholinergic synapses, as it catalyzes hydrolysis of the neurotransmitter acetylcholine (45). The organophosphates have an analogue conformation to that of acetylcholine, and when they bind to AChES the enzyme suffers transphosphorilation (46), that is to say, there is a phosphorilation of the hydroxyl group of a serine in the active site of the enzyme. The transphosphorilated AChES enzyme then is inhibited and cannot divide the acetylcholine; this causes an increment of acetylcholine in the post-synaptic membranes, which then leads to the contraction of the muscle and paralysis of the tick (4, 22, 47).

The organophosphates can be mixed with water, their toxicity varies considerably, but the majority is highly toxic for mammals (11), and generally has been used to control larvae, flies, ticks and lice in livestock, as well as ticks in dogs and cats (48).

4.2 Resistance mechanism

The resistance to organophosphates is due mainly to metabolic changes, and it is also associated to a change in the conformation of the AChES that turns it insensitive to organophosphates (4), such mutated forms of AChES have been characterized biochemically and show a wide spectrum of sensibility among species and between compounds within the species (49, 50).

The insensibility of AChES to organophosphates has been reported in insects such as the migratory locust, flies, mosquitoes and other dipterans (45, 49, 51, 52), as well as arachnids such as *R. microplus* and *Tetranychus urticae* (53, 54). Furthermore, an overproduction of carboxyl esterases has been reported against the organophosphatesin arachnids, mosquitoes, aphids and cockroaches (28). In the mosquito, mainly the genus *Culex*, it has been studied that these enzymes are B esterases that have an active site with are a serine residue and these catch the organophosphates, protecting the AChES (28, 55). In the tick *R. microplus*, it has been reported an over expression of esterases when it is the larva stage, and an increase in the metabolism of esterases inside the layers of tegument in the tick (56). Lastly, it has also been reported a reduction of the penetration of the organophosphates in the cuticle (57).

4.3 Description of the mutations and polymorphisms

The resistance to organophosphates is related mainly to mutations in the gene *ace* that codes for the AChES (4, 51, 58, 59), which can be duplicated,

as is the case for nematodes and arachnids which have multiple loci for *ace*, but insects only have two loci for *ace* (*ace1* and *ace2*), that can code for two different AChES; however, there are some insects and arachnids that only have one locus (60, 61). Dipterans of the suborder of Cyclorrapha such as *Drosophila melanogaster, Musca domestica, Lucilia cuprina, Bactrocera oleae* and *Bactrocera dorsalis*, only have one locus for *ace*, known as *ace2* and all of the mutations for resistance against organophosphates have been associated to this gene. However, in *Anopheles gambiae* and *Culex pipiens* it has been reported the existence of another *ace2* gene that gives resistance to the organophosphates (58, 60).

The majority of the mutations align in the inlet of the cavity of the active site, mainly the oxyanion hole, a pocket in the binding site for acyl and the anionic catalytic sites that are critical for the catalytic activity of the enzyme (59). These mutations alter the hydrolysis of the substrate, decreasing the speed of enzymatic deacetylation, as well as the stability of the enzyme. Furthermore, each point mutation confers resistance to an insecticide but it can increase the sensibility to another (51). In *Drosophila melanogaster* five resistance mutations have been reported against organophosphates (F115S, I199V, I119Y, G303A and F368Y), causing a substitution in amino acids that alters the conformation of AChES (46). Similarly, in *Musca domestica* there are five described mutations (V180L, G262V, F327Y and G365A), which are alone or in combination, and these confer different spectrums of resistance (45). In *Bactrocera oleae* a point mutation (G488S) has been reported to be associated to increased resistance to organophosphates (62). However, in *Chilo supressalis* five mutations have been reported, A314S, H668P, E101D, F402V and R667Q (63). Nonetheless, point mutations are not a synonym for resistance since in some insects and arthropods (*Aphis gossypii, Nephotettix cincticeps, R. microplus*) mutations have been found in both susceptible strains and resistant strains (5, 64, 65). However, resistance of insects to the organophosphates are not only due to the insensibility of the AChES, but also other mechanisms such as an increase in detoxification due to the increase in esterases and the glutathione S transferase, as it has been reported for *L. migratoria manilensis* and *Culex tritaeniorhynchus* (66, 67). In arachnids, the insensibility of AChES has been described mainly for *Tetranychus urticae*, which has only one gene (*Tuace*) similar to *ace*1 in insects, in which have been detected five point mutations confer resistance to organophosphates (G119S, A201S, T280A, G328A and F331W) (68, 69).

In *R. microplus* the insensibility to AChE and the metabolic detoxification by the non specific carboxyl esterases (CaE) have been considered to be the principal mechanism of resistance to organophosphates. The mechanism of insensibility of AChES to organophosphates was first suggested by Lee and Bathman (70), and later reported in the Tuxpan strain of *R. microplus* in Mexico (71). Pruett (53) confirmed the mechanism of insensibility of AChES in different Mexican strains, including the San Roman strain. Later, three cDNAs that code for the AChES in *R. microplus* (BmAChE1, BmAChE2 and BmAChE3) were identified (64). Temeyer *et al.* (72) identified multiple mutations in the cDNA sequence that codes for BmAChEs in the San Roman strain, these are 48L, I54V, R86Q, V137I, I492M and T548A. The most common mutation is R86Q, which results in the change of a glutamine for an arginine at position 86 of BmAChEs. Moreover, it has been shown that this mutation confers insensibility to paraoxon organophosphate (parasympatomimetic); however, this mutation was also found in susceptible strains, suggesting that the mutation contributes to resistance, although it isn't the only factor that has influence on it. Later, in another study, the other five mutations were genotyped and their frequence was evaluated, finding that these mutations were also in susceptible strains, showing that none of the mutations by themselves are directly responsible for the insensibility of the AChE to organophosphates (73). Temeyer *et al.* (74) analyzed the sequence of the genes AChE1, AChE2 and AChE3 from susceptible and resistant strains of *R. microplus*, detecting substitutions of amino acids in these genes in different strains. Gosh *et al.* (75) found four new substitutions in the amino acids (HQ184947, HQ184946, HQ184944, HQ184943) in AChE2 in *R. microplus* line IVRI-III. These substitutions replace a valine for an isoleucine in position 297, a serine for a threonine in position 364, histidine for a tyrosine in 412 and a lysine for an arginine in position 468. Recently, Singh *et al.* (76) reported six point mutations in the gene AChE3 in strains of *R. microplus* from the state of Punjab in India (I48L, I54V, R86Q, V71A, I77M and S79P), in which the first three were previously associated to resistance against organophosphates in the Mexican San Roman strain (72), and the other three were reported for the first time. However, these mutations must be evaluated, as Li and Han (5) demonstrated that the mechanism of resistance of *R. microplus* San Roman strain has two different forms: the insensibility of the AChES and the metabolic detoxification due to the increase in cytochrome P450. However, different studies have reported that certain Mexican strains of *R. microplus* increase the esterases as a resistance mechanism against the organophosphates (8, 77–80). Saldivar *et al.* (81) reported resistance to organophosphates through the glutathione S transferase mechanism in strains of *R. microplus.* Lastly, cross resistance between organophosphates and other acaricides such as carbamates, has been reported in different strains of *R. microplus* (5, 82).

5. PYRETHROIDS

5.1 Mechanism of action

Among the neurotoxins that alter the properties of the sodium channels are the pyrethrins, which are natural insecticides derived from the plant *Chrysanthemum cinerariafolium*, which possesses an excellent ability to cause sudden death in insects and low toxicity in mammals, has been very useful in products such as ectoparasiticides; however, the discovery of other products similar to pyrethrins have replaced them, such as is the case for synthetic pyrethroids, which are synthetic analogues to pyrethrins, that have the advantage of possessing molecules which are more stable in sunlight, dissolve better in water and have a residual effect greater that pyrethrins (83–86).

Pyrethroids are chlorinated or brominated halogenated esters of one of the isomeric forms of chrysanthemic acid and a molecule of synthetic alcohol. The structure may vary because they possess numerous asymmetric carbon atoms, but the characteristic that gives them the insecticide activity and toxicity consists of isomers of 1RαS and generally the more toxic ones are 3-cis in comparison to the 3-trans (87).

Pyrethroids can be classified as type I and II, depending on the presence or absence of a cyano group at the alcohol part. These two types of pyrethroids have a neurophysiologic mode of action and different target sites (88).

The type I pyrethroids act upon the peripheric nerves, causing repetitive discharges in the nervous fibers. This induction of multiple spikes are the result of the prolongued entrance of sodium ions; however, this depolarization of the membrane blocks the conduction of the nervous impulse (85, 89). In the case of type II pyrethroids act at the central level and delay the closing of the sodium channels (inactivation) for a more prolongued period of time than the type I. This delay in the closing of the channel causes depolarization of the membrane potential, blocking the conduction of the nervous impulse (88, 89).

In studies performed to discover the way in which pyrethroids act, it has been reported that these bind to the alfa subunits of the sodium channels and present a greater affinity for the opened state of the channel to become fixed and act (90, 91). Once the pyrethroids bind to the receptor site of the channel, they stabilize the open state (90), causing a delay in the closing of the channel after the nervous impulse has passed; this prolongued entry of sodium into the internal part causes repetitive discharges in the nerves and hyperexcitibility, causing paralisis and death in the insects (83, 89, 90, 92, 93).

Pyrethroids can penetrate the insect´s organism through the tegument and spread throughout the whole organism in solution or diluted in lipid particles. Its penetration will depend on the characteristics of the insecticide such as the formulation, physical and chemical properties, and the nature of the solvent (84, 85). Pyrethroids have two types of effects on insects: an initial sudden effect of abatement known as Knockdown (Kd), loss of movement and a lethal subsequent effect (94).

5.2 Resistance mechanism

In *R. microplus* two mechanisms of resistance to pyrethroids have been described: the increase in the metabolic activity mediated by enzymes that include mainly esterases, and the insensibility at the target site (sodium channel) (78, 95–97). However, the most common mechanism seen in populations of ticks with resistance to pyrethroids in the field are mutations present in the gene for the sodium channel (8).

Voltage dependent sodium channels are the target site for pyrethroids and the resistance to these products is associated to mutations that cause insensibility to these compounds. The Knockdown resistance (*Kdr*) to DDT and pyrethrines was first identified in the domestic fly (89, 98–100) and has been widely studied at the molecular level in numerous insects that are resistant to pyrethroids (99, 100).

5.3 Description of the mutations and polymorphisms

Mutations at the sodium channel have been reported for *Blatella germanica*, *Myzus persicae*, *Plutella xylostella*, *Anopheles gambiae*, *Haematobia irritans* (99, 101, 102). The most common cause for resistance is the presence of point mutations at the target site of the pyrethroids and they have been reported for several populations of insects (103). In insects and arthropods a mechanism has been discovered that confers them resistance to pyrethroids and DDT, which is denominated “Knockdown resistance”, and this type of resistance causes a reduction in the sensibility to these compounds because of the mutations present at the target site of the sodium channels (89, 98, 99, 102, 104).

One of the peculiarities that this type of resistance presents is that it limits the effectiveness of all pyrethroids and DDT, which is of great importance out in the field due to the fact that once it is detected, it is difficult to keep using these compounds as chemical control methods against insects (99, 104). Knockdown resistance has been widely researched, and since 1951 it has been identified and characterized in the domestic fly, but in recent years a few mutations have been identified in the genes for the sodium channels which are responsible for resistance in insects (99).

Two putative genes have been identified in *Drosophila melanogaster*, *DSC1* and *Para* (105). Primers have been made for the gene "*Para*" which codes for the sodium channels in order to isolate segments of genes for the sodium channel of other species of insects (99). In the fly *Haematobia irritans*, two mutations have been reported in the channel, which have been denominated as: *Kdr*, which causes a substitution of a leucine to a phenylalanine at the amino acid residue 1014 (L1014F) and super-*kdr*, which causes a substitution of a methionine to a threonine at the residue 918 (M918T), and the role that these substitutions play in the resistance to pyrethroids (101, 106).

The mutations M918T and L1014F reported for the domestic fly have been found next to a third site (T929) in a wide range of insects (101). The L1014F mutation has been found in the domain II of segment 6 (DIIS6), and M918T is found in the loop that connects segment 4 (S4) and segment 5 (S5). It is important to mention that the mutation known as super-*kdr* has not been identified in the absence of mutation L1014F (99, 106). Mutation L1014F has been identified and reported in other species, such as *Anopheles gambiae, Blatella germanica, Culex pipiens*, *Musca domestica*, *Leptinotarsa decemlineata*, *Myzus persicae, Plutella xylostella* (102, 106–110). Also, when the mutations present in DIIS6 of the sodium channel are analyzed it is possible to observe a reduction in the sensibility to pyrethroids (78, 101, 102, 110–112). In addition, a *kdr* mutation has been reported in the sodium channel of the German cockroach in the domain III of segment 6, a substitution of phenylalanine to isoleucine at residue 1519. This mutation has also been identified in the tick that infects cattle, *R. microplus*, conferring it resistance to pyrethroids (90, 99, 104).

The site F1519 in the sequence for the sodium channel of insects is important for the binding of the pyrethroids, as it is in this position that an aromatic residue of phenylalanine, tryptophane or tyrosine (F, W or Y) is necessary for the pyrethroids to act, however when there is a mutation at this site the insects become less sensitive towards acaricides (90).

In numerous sodium channels of insects the L993F mutation present in the IIS6 domain is also associated with the binding of the pyrethroids. The specific amino acid residues involved shape the receptor site of the pyrethroids in the sodium channels of insects (90, 113). The inactivation of the sodium channel is also affected when there is a substitution of the phenylalanine for several hydrophilic residues (114).

At present, some *in vitro* expression studies have been performed using cloned genes and mutagenesis of the site directly in the sodium channels of vertebrates and invertebrates. One of the findings from these studies has been that one amino acid affects the selectivity to the toxicity of pyrethroids, and vertebrates are less sensitive to pyrethroids. This in part is due to the fact that vertebrates possess a residue, which confers resistance to acaricides in insects. The study performed to find this difference in the amino acid consisted in comparing sequences of sodium channels of mammals and insects, and the results showed that the sites L1014 and T929 are highly conserved sites, but they are not associated to the selectivity to the pyrethroids. Nonetheless, at position 918 of the channels of mammals, if a methione replaces a leucine, there is an increase of 100 times more sensibility to pyrethroids. These results show the importance this amino acid has at that position for selectivity, and for a strong interaction to be made with the pyrethroid molecule (83, 115).

In arthropods, the resistance to pyrethroids is associated to mutations in the domains I, II, III, and IV of genes for the voltage dependent sodium channel. In *R. microplus*, known mutations that confer resistance are in domain II (C190A) in populations from Australia, Africa and South America, and a mutation in domain III (T2134A) that only occurs in Mexico and the United States (U.S.) (116). In field strains of *R. microplus* from the U.S. and Mexico, SNPs were detected and associated to resistance to pyrethroids in domain III (T2134A); a SNP C190A in the domain II of strains from the U.S. and a new SNP was detected in domain II (T170C) in both strains, which correlates to resistance in other insects (116). In addition, Hernandez *et al.* (97) in a study of a pyrethroid-resistant strain of *R. microplus* coatzacoalcos (CZ), identified by PCR assays a mutant allele G→A substitution at nucleotide 1120 either on individual tick larvae or hemolymph from adults.

6. AMITRAZ

6.1. Mechanism of action

The amidines (amitraz) belong to the group of the formamidines, which have a vast biologic activity as a bactericide and antiparasitic activity against helminths, they also possess activity against fitophagous and/or parasitic mites and ticks from cattle (117). One of the main advantages of this group is the low toxicity when it is used at adequate concentrations for insects such as bees and spiders, birds, fish and mammals (118, 119). Amitraz is one of the most important acaricides for the control of the tick *R. microplus*, and it was introduced to Australia in the 1970s (120).

Octopamine acts exciting the neurons in the abdominal ganglion of larvae, the activity of these neurons is related to the increase in motor activity

(121). Amitraz is an antagonist of octopamine, and it competes for the binding to the receptor of octopamine (ROA), causing uncoordinated motor activity or hyperexcitability in the larvae using sublethal doses that cause paralisis and death of the larvae. The hyperexcitability prevents the tick from anchoring to its host and so it prevents the tick from biting the host in order to feed off its blood. Amitraz is metabolized into N^2-(2,4-dimethylphenyl)-N^1-methyformamidine (DPMF) and other polar metabolites such as 2,4-dimethylaniline in the larvae of the tick *R. microplus* (119). Previous studies have shown that amitraz and DPMF act upon the ROA, increasing the levels of cAMP through the activation of cyclase adenylate (122).

6.2 Resistance mechanism

As it was previously mentioned, the mechanisms of resistance to ixodicides are common and they occur in response to a survival mechanism of ticks in order to detoxify, inactivate and/or kidnap the ixodicides. These mechanisms of resistance are subject to the inactivation of metabolizing enzymes, among which is found the receptor for octopamine. The first cases of resistance to amitraz were reported in Australia (120). In Mexico, the use of amitraz emerged as an ixodicide option due to the presence of ticks resistant to organophosphates (20, 21, 123). However, three years later, the first case of ticks resistant to amitraz was reported at the Emiliano Zapata Ranch in the state of Tabasco, Mexico (124). In other countries like Colombia, Brazil, Australia and South Africa, there have also been reported strains resistant to amitraz (101, 125, 126).

Different studies have proposed that the resistance to amitraz developed because it is used as a prophylactic or repellent, preventing the tick from infecting treated animals, although this resistance has also been suggested to be due to an inherent recessive mode for the development of amitraz (127). On the other hand, the octopamine receptor has been widely studied as a pharmacological target of the group of formamidines, of which amidine or amitraz are a part of octopamine, which is a biogenic amine that acts as a neurotransmitter and it is found in the central nervous system and the periphery of ticks, interacting with receptors that are bound to the G protein, that transmit signals via second messengers (121).

6.3 Description of the mutations and polymorphisms

In the genome of some insects such as ticks, the gene for octopamine is a member of a superfamily of genes that code for proteins with membrane domains. In mammals, the gene for octopamine is not present, which is why this protein is a pharmaceutical target to be used as an ixodicide (121, 128). One of the first proposals that was established in reference to the existence of resistant strains was that these developed due to the existence of point mutations, but a series of molecular studies among resistant and non resistant *R. microplus* did not show convincing evidence to establish that this resistance to amidines was caused by point mutations in the gene that codes for octopamine (129). Recent studies have identified receptors bound to G protein, more specifically the β-adrenergic receptor for octopamine (*Rmβ AORs*). Interestingly, polymorphisms have been found in this gene in a certain population of resistant *R. microplus* denominated I61F (130). Additionally, the presence of a non synonymous SNP was reported on the first membrane domain of *RmβAORs,* and this SNP has been proposed as a marker to detect resistant *R. microplus*. The amino acid isoleucine in the codon where the SNP is found is highly conserved in *βAORs,* which suggests that the presence of this amino acid is of relevant importance for the structure and function of *βAOR* (130).

7. MACROCYCLIC LACTONES

7.1 Mechanism of action

Glutamate dependent chloride channels are the target site for macrocyclic lactones such as ivermectins, milbemycin and moxidectin (131). The mechanism of action for ivermectins has been studied in nematodes and arthropods, focusing on the α subunit of the selective chloride channels, which act as agonists (83, 131).

In ticks, the mechanism of action of macrocyclic lactones is possible due to the interaction and high affinity for the glutamate or gamma amino acid receptors present in muscle and nerve cells that control the entry of ions into the chloride channels (132, 133), giving way to an irreversible increase of the membrane´s conductance, which leads to muscular paralisis and subsequently death (134).

7.2 Resistance mechanism

At present, several states of Mexico use MLs (ivermectin, doramectin, and moxidectin) as a control method for NGI and ticks. Recently, two long-action MLs were introduced (ivermectin 3.1.5% and moxidectin 10%). When these are inoculated in bovines, they remain more than 70 days in the bloodstream, skin and hair, exposing the parasites to therapeutic and subtherapeutic doses for a prolonged period of time (133).

Resistance against macrocyclic lactones was first described in the state of Rio Grande do Sul, Brazil, at the beginning of the 21st century. Cross resistance to doramectin, ivermectin and moxidectin in *R. microplus*

was first reported in Brazil by Martins and Furlong (135). Later, Klafke *et al.* reported phenotypic resistance against ivermectine in Sau Paulo, Brazil (136, 137). Similarly, other authors have found ivermectine resistence in Uruguay (138), Colombia (139) and India (140). In Mexico, Perez-Cogollo *et al.* (141), reported resistance against ivermectin for the first time in field strains in the state of Yucatan. Another mechanism involves the ATP-binding cassette transporter efflux pump is a defense mechanism against ivermectin in *R. microplus* (142, 143). However, the molecular mechanisms of resistance are not yet clear or characterized.

7.3 Description of the mutations and polymorphisms

There have been reports about the resistance to macrocyclic lactones in *Blatella germanica, Plutella xylostella* and *Leptinotarsa decentineata*, which is due to an increase in the oxidative metabolism of citocromo P450, conjugated by glutation S tranferasa and esterases (10). In Mexico, a resistant and susceptible strain of *R. microplus* to ivermectin presented a substitution at position 546 and 575 in the nucleotide sequence of the glutamate-gated chloride channels reported in GenBank (Mozo strain) (144).

8. FIPRONIL

8.1 Mechanism of action

Fipronil is a phenylpyrazolic insecticide used mainly for the control of plagues in agriculture (145), and it is also used for tick control (*R. microplus*) since the 1990s. Fipronil acts as an inhibitor of the chloride ion channels that are regulated by the GABA neurotransmitter, which participates in the transmission of signals in the central nervous system of insects (146–149). Aditionally, fipronil and its metabolite, fipronil sulfone, block two types of ionic chloride channels that activate glutamate in insects (150, 151).

8.2 Resistance mechanism

In Brazil and Uruguay reports have been shown of strains resistant to fipronil shortly after its introduction (152–154). In Mexico, the first report of resistant strains to fipronil was in Tamaulipas in 2013 (155). Additionally, cross resistance has been reported when using fipronil and lindane, which are believed to have the same target site, so there have been reports of cases of cross resistance when using either of these two insecticides (43). Furthermore, there are reports of cross resistance with cyclodienes (115, 156). Until today, there have been no reports that show the mechanism of action by which this resistance to fipronil develops. This can be due to the fact that this acaricide is relatively new.

9. CONCLUSIONS

Two main mechanisms of ixodicides resistance in *R. microplus* have been described: 1) the increase of metabolic activity mediated by enzymes that mainly comprise the esterases and 2) the insensitivity of the site of action. The insensitivity in the binding site of the enzymes is the most common ixodicide resistance mechanism and principally it is caused by the presence of point mutations. In order for these mutations to be favorably selected, it is important that amino acid substitution decreases binding with ixodicide, without causing loss of primary site-of-action function. Thus, amino acid substitutions are limited and identical mutations are associated with ixodicide resistance in other insects.

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Abbreviations: *R. microplus, Rhipicephalus microplus;* GABA, gamma amino butyric acid; SNPs, single nucleotide polymorphisms; AChES, enzyme acetylcholinesterase; *Kdr*, Knockdown resistance; *RmβAORs,* β-adrenergic receptor for octopamine; MLs, Macrocyclic lactones.

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