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Ticks and acaricide resistance: A major concern

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Abstract
Ticks are blood sucking ectoparasites that are prevalent in tropical and subtropical regions. Ticks not only spread diseases but also reduce sales of meat, dairy products, and skins. Many acaricides are being used to control tick infestations. These comprise organochlorine, organophosphates, fipronil, macrocyclic lactones, amitraz, formamidines, and synthetic pyrethroids. The inattentive and frequent use of the acaricides have developed resistant to these chemicals in different tick species. Acaricide resistance in ticks can occur through a number of processes, including metabolic detoxification and changes in amino acid composition that change the acaricide target's shape. The purpose of this paper is to give a general overview of acaricides and the status of their resistance in tick species, with a focus on Rhipicephalus (Boophilus) microplus ticks.

Keywords: Ticks, acaricides, acaricide resistance, microplus ticks, control tick infestations

Introduction
Ticks are blood sucking ecto-parasites that mostly infect terrestrial and semi-terrestrial vertebrates in tropical and subtropical regions (Guglielmone *et al*., 2020) [21]. Additionally, ticks act as vectors, allowing diseases to spread across their vertebrate hosts. Significant losses occur in the livestock business as a result of host blood depletion, overall pain and irritation, decreased production of dairy and meat, immune function suppression, and hide damage, among other negative effects (Tabor *et al*.,2017) [59].

Acaricides
Acaricides are chemicals used to kill ticks and mites. The term ixodicides is sometimes applied to acaricides used against ticks. Commonly used acaricides include organophosphates (OPs), amitraz, macrocyclic lactones (MLs), synthetic pyrethroids (SPs), Organochlorine (OCs), and fipronil. Through a number of different methods, these acaricides affect the tick central nervous system. The mechanisms include the targets like voltage-gated sodium channels, octopamine tyramine receptors, GABA-gated chloride channels, glutamate gated chloride channels (Glu-Cl), and inhibitors of acetylcholine-esterase (AChEs). Table 1 lists the several kinds of acaricides along with the tick target protein. The existence of different tick species is impacted by the distinct targets and modes of action of various acaricides (Klafke *et al*., 2017) [29]. The many techniques for administering acaricides to host animals include spraying, washing, pouring, and injecting (Food and Agriculture Organisation, 2004) [16].

Table 1: Various Acaricides classes and their target protein in *Rhipicephalus (Boophilus) microplus*

S. No	Class of acaricides	Target protein	Reference
1	Organochlorides	GABA-gated chloride channel gene, Voltage- gated sodium channel gene	Hope *et al*., 2010 [23]
2	Organophosphates	AChE genes	Ghosh *et al*., 2015 [17], Singh *et al*., 2016
3	Formamidines (Amitraz)	Octopamine tyramine gene	Takata *et al*., 2020, Jonsson *et al*., 2018 [26, 60]
4	Synthetic Pyrethroids	Voltage-gated sodium channel gene	Ghosh *et al*., 2015 [17]; Nagar *et al*., 2018 [40]; Janer *et al*., 2021 [24]
5	Macrocyclic lactones	Glu-Cl channel gene	Aguilar-Tipacamú *et al*., 2016 [3]
6	Fipronil	GABA-gated chloride channel gene	Janer *et al*., 2021 [24]

Resistance and its Evolution
World Health Organisation (WHO) has been instrumental in fostering international coordination on resistance by offering standardised approaches to address the problem worldwide. Arthropod resistance was defined by the WHO in 1992 as "an inherited characteristic that imparts an increased tolerance to pesticide(s) allowing resistant individuals to survive a concentration that would typically be lethal to the species." Consequently, we can define the resistance as the selection of particular heritable features within an arthropod population (tick), brought on by chemical exposure, that considerably raises the population's proportion of survivors after a standard dose of that chemical. According to Li *et al*. (2003), cross-resistance is the resistance shown by multiple active chemical components that have similar modes of action.
Ferrari (1996) [15] pointed out three prerequisites that must be met for resistance to evolve:

1. There needs to be genetic diversity in the population.
2. There must be discernible phenotypic variations as a result of these genetic variations.
3. The phenotypic variations need to increase survivorship so that the resistance can be handed down to the following generation.

Therefore, Recombination and mutation are two natural processes that lead to the development of resistance genes (Ferrari, 1996) [15]

Acaricide Resistance in Rhipicephalus (Boophilus) microplus

Abbas *et al*. (2014) [1] identified many key factors that accelerate the development of acaricide resistance in ticks *viz*. improper dilution, wrong administration, chronic use, and overdose. Guerrero *et al*. (2012a) [18] suggested that due to the favourable conditions brought about by widespread distribution and/or unique life cycle features of various tick species, some species are more likely to develop resistance to acaricides. Table 2 shows the timeline of introduction of common pesticides as acaricides and the year of reporting of tick resistance for the first time as per literature available.

Table 2: Timeline of Acaricide: the first report of their introduction and resistance

S. No	Class of Acaricide /common acaricide and Year of introduction	Reference	Report of Resistance in Tick species	Reference
1	Organochlorines (OCs) DDT (Dichloro-diphenyl-trichloroethane) BHC (benzene hexachloride) in 1946	Maunder, 1949 [39]	*R. microplus* from Australia in 1960	Stone and Webber, 1960) [58]
2	Organophosphates (OPs) Dioxathion in 1955	Obaid *et al*., 2022 [45]	*R.decoloratus* and *Amblyomma variegatum* from Zambia in 1987	Luguru *et al*., 1987 [36]
3	Formamidines Amitraz in 1975	Hollingworth, 1976 [22]	*R. microplus* from Brazil in 1995 and also in Mexico in 2001	Martins *et al*., 1995a [37]
4	Synthetic pyrethroids (SPs) in 1977	Obaid *et al*., 2022 [45]	*R. microplus* from Australia in 1979	Nolan *et al*., 1979 [43]
5	Macrocyclic lactones (MLs)in 1981	Obaid *et al*., 2022 [45]	*R. microplus* from Brazil in 2001	Martins and Furlong, 2001 [38]
6	Fipronil in 1987	Obaid *et al*., 2022 [45]	*R. microplus* from Uruguay 2007	Cuore *et al*., 2007) [9]
7	Fluazuron in 1994	Junquera *et al*., 2019 [27]	*R. microplus* from Brazil in 2014	Reck *et al*., 2014

Forms of Acaricide Resistance

WHO (1992) identified three ways to observe and monitor resistance:

- At molecular level–To identify the genes involed. It will also provide evidence of the evolutionary process.
- At Phenotypic level – To measure the susceptibility when exposed to a standard dose
- Third level (disease outbreak) – When the chemical is unable to control vector's transmission of disease

In case of ticks three primary forms of known resistance to acaricides that have been reported so far. Metabolic resistance occurs through the detoxification of acaricides by the metabolic activity of enzymes including glutathione S-transferase (GST), esterases, and cytochrome P-450s (CYP), (Guerrero *et al*., 2012a) [18]. "Target site modification resistance " results from changes in conformation of the target site most likely the enzymes and receptors, thus, impairing the drug's ability to interact with the target (Coles and Dryden, 2014) [8]. Reduced penetration resistance refers to the lower ability of acaricides to enter the interior body environment as a result of changes made to the exoskeleton, the outer layer of ticks (Guerrero *et al*., 2012a) [18]. Comprehensive and advanced insecticide resistance monitoring programs, along with the profound and extensive understanding of the mechanisms behind the development of resistance, are crucial for future tick control efforts. As per the literature avaibale, it is found that the resistance to pyrethroid and organophosphate are extensively characterized among different classes of acaricides. However, little is understood at the molecular level. The resistance mechanisms for other acaricides are less well comprehended. Though the target sites of fipronil and macrocyclic lactones are known, studies on their resistance mechanisms are still in early stages. The target site of amitraz is still unknown, hampering mechanistic research on this acaricide (Guerrero *et al*., 2012a) [18]. Table 3 shows different forms of acaricide resistance reported.

Table 3: Forms of Acaricide Resistance

S. No	Forms of Acaricide Resistance	Enzyme involved	Reference
1	Metabolic resistance	glutathione S-transferase (GST), esterases, and cytochrome P-450s (CYP)	Guerrero *et al*., 2012a [18]
2	Target site modification resistance	neuronal enzymes and receptors	Coles and Dryden, 2014 [8]
3	Reduced penetration resistance	Modifications in the exoskeleton and integument system.	Guerrero *et al*., 2012a [18]

It is crucial and utmost important to monitor acaricide resistance in the tick population for their optimal and strategic utilization with simple, affordable, and effective testing methods (Sabatini *et al*., 2001) [51].

Various Acaricide Monitoring Tools

According to Ferrari (1996) [15] the study of insecticide/ acaricide resistance should follow this sequence:

- Detection of resistance in a population.
- Collection and laboratory colonization of individual arthropods.
- Identification of the mechanisms of resistance.
- Characterization of the genetic control of resistance.

Detection of resistance in tick population is done by various standard bioassays:

1. Larval Packet Test (LPT)

The tick larvae are cultured in acaricide-treated filter paper packets as part of the 1962-developed larval packet test (LPT) (Stone and Haydock, 1962) [57]. Resistance to OPs and SPs has been surveyed and diagnosed using this test (Guerrero *et al*., 2014) [20].

2. Larval Immersion Test (LIT)

Shaw (1966) [54] invented the larval immersion test. Larvae are immersed in the appropriate doses of acaricidal solution in this assay. According to Rodriguez-Vivas *et al*. (2006b)

[48] this test is typically used to characterise resistance against amitraz and macrocyclic lactones.

3. Adult Immersion Tes (AIT)

Engorged female ticks are submerged in acaricide solutions made in accordance with the tested concentrations (Drummond *et al*., 1973) [12]. Although widely applied to test most of the acaricides, but it is not appropriate for testing resistance to amitraz (Jonsson and Hope, 2007) [25].

4. Larval Tarsal Test (LTT)

The first-ever larval tarsal test (LTT) is prompt and sensitive *in vitro* test that has been used to assess resistance in many ixodid tick species (Lovis *et al*., 2011) [35].

Status of resistance in Rhipicephalus (Boophilus) microplus worldwide

Globally, the development of acaricide resistance has accelerated in R. (B.) microplus followed by R. (B.) decoloratus. Both of them are single-host ticks, therefore, their shorter life cycle necessitates frequent acaricide treatment which leads to resistance development (Guerrero *et al*., 2014) [19]. According to Mekonen *et al*. (2002), acaricide resistance is often rare in multi-host ticks because these ticks feed on both domestic and wild animals. High degrees of variation were seen across studies reporting the development of acaricide resistance in worldwide populations of R (B.) microplus. These variations may be related to the location, the financial standing of farmers, the type of cattle, the amount and frequency of acaricide applications, and the sensitivity of the techniques used to identify acaricide resistance (Shyma *et al*., 2015) [55].

R. (B.) microplus has been removed from the United States and Europe, therefore, no reports on the emergence of resistance were reported in North America and Europe (CMPV, 2018) [7]. According to Rodriguez-Vivas *et al*. (2011) [49] the majority of R (B.) microplus populations are resistant to organophosphates, synthetic pyrethroids, amitraz, ivermectin, and fipronil in Asia (India), South America (Brazil), and Central America (Mexico). De Oliveira Souza Higa *et al*. (2015) [11] state that the emergence of acaricide resistance in Brazilian populations of R. (B.) microplus has been linked to the rearing of exotic cattle breeds which are more vulnerable to ticks than native ones. Relatively few publications were found on the development of acaricide resistance in Africa, which may be due to paucity of funds and researchers, as well as the less interest among policymakers. (Ntondini *et al*., 2008) [44].

Status of resistance in Rhipicephalus (Boophilus) microplus in India

There have been many reports of acaricidal resistance in R. (B.) microplus populations in India. The state of Punjab has been the focus of reports of R (B.) microplus, as evidenced by the works of Sharma *et al*. (2012) [53], Kumar *et al*. (2013) [31], Nandi *et al*. (2015) [41], Singh and Rath (2014) [56], Singh *et al*. (2015) and Sagar *et al*. (2020) [52]. In tick populations in India, FAO (2004) expected the emergence of widespread resistance to acaricides. Acaricide resistance has developed in India due to a number of factors, including climate conditions that support tick growth and survival all year round and inadequate infrastructure and management techniques that create easy-to-proliferate tick populations (Kumar *et al*., 2020) [32]. In addition, farmers now have easy access to acaricides due to the veterinary medicine industry's liberalisation, and a lack of oversight has resulted in incorrect dosing, more frequent applications, a restricted amount of acaricide rotation, and disregard for the recommended usage guidelines. The lack of established protocols to control tick infestations and veterinarians' disorganised efforts to track the effectiveness of popular acaricides in real-world settings have also led to the emergence of resistance in India (Kumar *et al*., 2020) [32].

Resistanc status in Rhipicephalus (Boophilus) microplus against various Acaricide

According to Kumar *et al*. (2020) [32], SP has been the most prominent acaricide for tick management in cattle during the last 15 years. According to de Oliveira Souza Higa *et al*. (2015) [11] prolonged use of pyrethroids has encouraged the population selection of resistant ticks, therefore, speeding up the development of resistance. Many nations have recorded cases of pyrethroids resistance, including Mexico (Fernandez-Salas *et al*., 2012b), Brazil (Klafke *et al*., 2017) [29], and India (Kumar *et al*., 2013; Singh and Rath, 2014; Sagar *et al*., 2020) [31, 52, 56]. One of the most often utilised acaricides for control of R (B.) microplus is amitraz in South America, Australia, and southern Africa (Jonsson and Hope, 2007) [25]. Amitraz was first used to suppress OP-resistant tick populations in the 1970s, practically simultaneously with SP but due to its increased cost, its use was curtailed (Jonsson and Hope, 2007). However, amitraz became widely used for tick control in cattle when populations of ticks started to show signs of resistance to SP. Thus, the high rates (>80%) of amitraz resistance in R (B.) microplus populations are likely a result of the constant and careless use of amitraz, particularly at incorrect concentrations. have been documented in numerous nations, such as Mexico (Rosado-Aguilar *et al*., 2008) [50], Argentina (Cutullea *et al*., 2013), Colombia (Lopez-Arias *et al*., 2014) [34], New Caledonia (Barre *et al*., 2008) [5], India (Kumar *et al*., 2014) [33], Benin (Adehan *et al*., 2016) [2], and Brazil (Andreotti *et al*., 2011) [4]. Ivermectin (macrocyclic lactone) is frequently used to manage cattle tick populations and gastrointestinal parasites. In the Mexican veterinary market, for instance, ivermectin is among the most popular antiparasitic medications (Rodriguez-Vivas *et al*., 2014). But during the past 30 years, R (B.) microplus populations has become resistant to it due to its indiscriminate use despite the fact that numerous studies have shown how effective ivermectin is at controlling the ticks. Studies by various scientists worldwide have documented high levels of ivermectin resistance in R. (B.) microplus populations in Mexico, India and Colombia (Perez-Cogollo *et al*., 2010b; (Khangembam *et al*., 2018; Nandi *et al*., 2018; Sagar *et al*., 2020; Villar *et al*., 2016) [28, 42, 46, 52, 61].

The incidence of acaricide resistance in R. microplus species has dramatically increased over the last thirty years. This has mostly been linked to the widespread and careless application of insecticides, which has selected for populations of ticks that are resistant to them (Klafke *et al*., 2017) [29]. Before acaricides were introduced and used, tick populations naturally had minute amounts of the genes that cause resistance to develop. As a result of ongoing selection pressure from acaricide treatments, the frequency of these naturally occurring resistance genes typically rises, decreasing the effectiveness of acaricides (Kumar, 2019) [33].

Conclusion

The majority of acaricide drugs authorised for use against tick species have caused resistance in several populations of ticks, particularly R. (B.) microplus. Therefore, in order to reduce the impacts of acaricide resistance and maintainence of acaricide efficacy, it is imperative to use it strategically. It is also important to monitor the acaricide resistance status of tick populations in different areas. Policymakers and veterinary regulatory agencies should also be familiar with tick management techniques, particularly in nations where tick infestation is a problem.

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