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Navigating the resistance: Current perspectives on ectoparasite control in veterinary medicine

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ABSTRACT

Ectoparasites present a global challenge to animal health, affecting a wide range of species. These parasites can infect both animals and humans, leading to significant health issues. The impact of ectoparasites extends beyond health, as they can also reduce the productivity of livestock. Moreover, many ectoparasite species act as vectors for bacterial, viral, or parasitic pathogens, thereby posing significant risks to human and animal health. Historically, agents such as organochlorides, organophosphates, carbamates, insect growth regulators, neonicotinoids, spinosad, fipronil, avermectins, isoxazolines, and synthetic pyrethroids have been extensively used to treat and prevent ectoparasitic infestations. However, the extensive use of these chemicals has resulted in the development of resistance among many target species, potentially reducing the effectiveness of these treatments. This article aims to survey the current status of resistance in ectoparasites to active pharmaceutical ingredients used in veterinary medicine. It also seeks to update the understanding of resistance mechanisms and explore measures that can be implemented to prevent the development of resistance.

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Introduction

The issue of ectoparasitic drug resistance has been progressively increasing and has become a problem over the last three decades (McNair, 2015). Ectoparasitic drug resistance is characterized as “the capacity of a parasite strain to survive and/or proliferate despite the administration and absorption of a drug at doses equal to or higher than those typically recommended but within the subject's tolerance” (WHO, 2001). Ectoparasitic drug resistance refers to the selection of specific heritable traits within a population of ectoparasites in response to exposure to an active pharmaceutical ingredient. Ectoparasites develop resistance to drugs by different mechanisms, including receptor modifications that prevent the binding of drugs to their targets or metabolic changes that lead to enzymatic degradation and rapid elimination of the drug. As a result of resistance, the recommended standard dosage of the active pharmaceutical ingredient may lead to a significant increase in the proportion of the population that does not respond to the active pharmaceutical ingredient used (Coles and Dryden, 2014). Resistance to one ectoparasiticide may develop towards another ectoparasiticide, either via side or cross-resistance. Side resistance describes a reduced susceptibility to multiple ectoparasiticides within the same chemical class, whereas

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cross-resistance refers to a decreased susceptibility to multiple ectoparasites from distinct chemical groups with a similar mode of action (Abbas et al., 2014).

The development of resistance reduces drug efficacy and leads to increase in parasite populations, which increases treatment costs in companion and livestock animals and also economic losses due to decreased productivity in food animals. The aim of this article is to describe the current status of ectoparasite resistance and perspectives on ectoparasite control. The scope of this article is to provide information on the status of ectoparasiticide resistance in ticks, mites, lice, and fleas, possible resistance mechanisms, methods of detection, strategies to delay the resistance, and monitoring programs. Mites, arthropods, mosquitoes that are non-habitable on animals, and endoparasites are beyond the scope of this article.

Current status of ectoparasite resistance

Ticks

The majority of reports on tick resistance mainly focus on *Rhipicephalus* (formerly *Boophilus*) *microplus* which is a single-host tick that prefers cattle and buffalo. The fact that this tick completes its complete life cycle on a single species of animal, makes it more susceptible to post-treatment resistance selection compared to ticks that feed on multiple hosts. At present *Rhipicephalus microplus* is not endemic to Europe, but it is prevalent in subtropical and tropical regions worldwide. Notably, this species has been eradicated in the United States. Various studies have examined the global resistance status of this tick (FAO, 2004; Abbas et al., 2014). Research carried out in Brazil has revealed the resistance of *R. microplus* to all existing classes of systemic-acting and contact acaricides, organophosphates, and combinations of pyrethroids (Valsoni et al., 2020). In addition, *R. microplus*, collected from the Indian states of Madhya Pradesh, Punjab, and Uttar Pradesh, was found to be resistant to both deltamethrin and cypermethrin (Sagar et al., 2019). Reports of resistance to avermectins in this tick species have emerged from Brazil (Martins and Furlong, 2001; Klafke et al., 2006; Cruz et al., 2015) and Mexico (Perez-Cogollo et al., 2010). Ivermectin resistance was first reported in ten brown dog tick (*Rhipicephalus sanguineus*) populations in Mexico (Rodriguez-Vivas et al., 2017). An *in vitro* study of dogs, conducted in Spain, reported a high prevalence of resistance to deltamethrin and a variable susceptibility to propoxur in *R. sanguineus* ticks. Notwithstanding, all tested *R. sanguineus* have been shown to remain sensitive to amitraz (Estrada-Pena, 2005). Permethrin resistance was investigated in the United States. All thirty-one of *R. sanguineus* were identified as resistant to permethrin, and nine of these were highly resistant to permethrin (Eiden et al., 2015). Ticks were collected from Porto Alegre, Brazil, to research acaricide resistance in *R. sanguineus* s.s. populations, seven samples with resistance ratios (RR) ranging from 2.32 to 5.67 were considered to be resistant to deltamethrin. Three of the five analyzed tick populations were resistant to fipronil, with resistance rates ranging from 2.56 to 13.83, whereas ivermectin resistance rates ranged from 1.54 to 2.14. This is the first study to document the presence of acaricide-resistant *R. sanguineus* s.s. populations in Brazil (Becker et al., 2019).

Mites

Dermanyssus gallinae: In poultry farming worldwide, especially in layers, *Dermanyssus* (*D.*) *gallinae* reduces productivity by sucking the blood of animals and poses a threat to public health as vector. The poultry mite *Dermanyssus gallinae* was found to be resistant to synthetic carbamates and pyrethroids for the first report in Italy (Genchi et al., 1984). In a study conducted in former Czechoslovakia, the resistance of *D. gallinae* to trichlorfon, tetramethrin, and permethrin was observed on several farms. Meanwhile, resistance to DDT is common even though the use has been banned (Zeman, 1987). It has been demonstrated that *D. gallinae* is resistant to synthetic pyrethroids in France (Beugnet et al., 1997), Sweden (Nordenfors et al., 2001), Italy (Marangi et al., 2009; Schiavone et al., 2023), China (Wang et al., 2021), and Türkiye (Koç et al., 2022). The presence of permethrin, cypermethrin, bendiocarb, and malathion resistance in field-isolated *D. gallinae* was demonstrated through comparisons with lab-grown sensitive mites in the United Kingdom (Fiddes et al., 2005). *D. gallinae* mites demonstrated resistance to all acaricides and insecticides examined in Japan, it showed resistance against carbaryl, trichlorfon, and a combination of fenitrothion, permethrin, and phthalathrin, but weak growth was observed than other acaricides and insecticides. 19 (13.7 %) of 139 poultry farms from 2007 to 2010 and 22 (18.5%) of 119 poultry farms from 2011 to 2013, the emergence of resistance to all commercial ectoparasiticides targeting the control of *D. gallinae* mites has been confirmed in Japan (Murano et al., 2015). It was reported exceptionally elevated levels of pyrethroid resistance in Greece, in addition to the identification and geographic distribution of pyrethroid resistance mutations in poultry red mite populations in Europe (Kastavou et al., 2019). Similarly, the mechanism of acaricide resistance of *D. gallinae* populations in Türkiye was investigated. Results obtained from this study show that a high level of resistance has been demonstrated for alpha-cypermethrin and phoxim (Koç et al., 2022).

Varroa destructor: One of the most destructive honeybee disease is varroosis, caused by the ectoparasitic mite *Varroa destructor*. Four populations of *Varroa destructor* were examined for their susceptibility to the organophosphorus acaricide coumaphos in Italy. This is the first study to report *V. destructo*r's resistance to the coumaphos (Spreafico et al., 2001). In a six-hour exposure study conducted in the United States to determine coumaphos resistance in *V. destructor*, it was determined that resistance existed based on the results (Elzen and Westervelt, 2002). A study involving amitraz revealed significant LC_{50} differences between susceptible and resistant mites in Argentina. The LC_{50} was found to be 35-39 times greater than its initial value. These results indicate that the mites in Argentina have resistant to amitraz (Maggi et al., 2010). *V. destructor* mites from colonies in the Czech

Republic were recently tested *in vitro* for resistance to formamidine amitraz and, the synthetic pyrethroids acrinatrin and tau-fluvalinate (Kamler et al., 2016). In addition, an update was provided on the actual spread of target site resistance to tau-fluvalinate in *V. destructor* samples collected from the Lombardy region in Italy (Panini et al., 2019). Resistance to coumaphos, amitraz, and tau-fluvalinate was investigated in Spain. In the analyzed samples, coumaphos mortality ranged from 2% to 89%, while tau-fluvalinate mortality ranged from 5% to 96%. In contrast, amitraz caused 100 percent mortality in every case. These outcomes indicate the presence of fluvalinate- and coumaphos-resistant *Varroa* mites in the majority of sampled apiaries, regardless of the reality that these active constituents have not been used over the span of years (Higes et al., 2020).

Psoroptes ovis: Flumethrin-resistant *Psoroptes* spp. populations have already shown side resistance to high cis-cypermethrin (Bates, 1998). Furthermore, moxidectin, ivermectin, and doramectin resistances were observed in *Psoroptes* mites in the United Kingdom (Doherty et al., 2018; Sturgess-Osborne et al., 2019). The presence of macrocyclic lactone resistance was detected in Belgian Blue cattle on sixteen cattle farms in Belgium and the Netherlands (van Mol et al., 2020). It was determined *in vitro* whether ivermectin and two other macrocyclic lactones (doramectin and moxidectin) were effective against *P. ovis* in sheep in Argentina. In light of this study, the presence of resistance to these active substances was determined (Soler et al., 2022).

Sarcoptes scabiei: Two dogs treated with 300 µg⁄kg bw ivermectin with case reports indicated that *S. scabiei* was clinically resistant to treatment in these dogs (Terada et al., 2010). Moreover, increased transcription of mu-1, delta-1, and delta-3 Glutathione S-transferase molecules, indicating permethrin resistance, was observed (Mounsey et al., 2010).

Lice

A study conducted in South Australia investigated cypermethrin resistance in sheep lice from 71 flocks infested with lice between 1990 and 1991, 16 flocks with suspected ectoparasitic resistance, and 31 flocks from Kangaroo Island. The frequency of resistance was determined to be 34% in lice collected from flocks with lice infestations, 50% in lice collected from flocks with suspected resistance, and 68% in lice collected from flocks on Kangaroo Island (James et al., 1993). A retrospective study in Australia examined the treatment history and response to cypermethrin of a strain resistant to pyrethroids of sheep lice over an 18-year period. In the study, the resistance of lice strains resistant to cypermethrin decreased when cypermethrin was not used but highly increased when pyrethroid was used again (Levot 2012).

Due to reports of decreased efficacy against triflumuron and diflubenzuron, the Insect Growth Regulator group of *Bovicola (B) ovis* ectoparasites, a resistance study was conducted in Australia. In order to evaluate sensitivity to active pharmaceutical ingredients, a laboratory test based on the moulting inhibition test was devised and used in the study. The lice used in the experiment were collected from sheep treated ineffectively with triflumuron and diflubenzuron. This resulted in the confirmation of the emergence of resistance in lice (James et al., 2008).

Simultaneously, a study utilizing the louse hatch test was conducted based on the inability of treated with diflubenzuron *B. ovis* to produce nymphs. The test is able to detect populations resistant to benzoylphenyl urea. The results of the test demonstrated the development of resistance. (Levot and Sales, 2008). In Scotland, it was suspected that sheep colonies were infested with *B. ovis* resistant to synthetic pyrethroids. Laboratory data and dependable field data have been shown to be effective methods for testing for potential deltamethrin resistance (Bates, 2001). In 16 (94%) of the 17 colonies examined during a pilot study in Ireland, lice were found. Lice species *Bovicola ovis* and *Linognathus vituli* were identified. Four farms' *B. ovis* samples exhibited tolerance to deltamethrin, as determined by *in vitro* contact biological tests. In addition, repeated evaluations of lice infestations in farm-treated animals confirmed this (Mckiernan et al., 2021). In Ethiopia, it has also been shown that *B. ovis* is resistant to 1% ivermectin (Legesse et al., 2020).

Fleas

Ctenocephalides (C.) felis and *C. canis*

Ctenocephalides (C.) *felis*, known as the cat flea exhibits resistance to various insecticides, including organophosphates, carbamates, pyrethroids, organochlorine, and pyrethrins. In general, resistance ratio, which is the LC50 of field isolates divided by the LC50 of a susceptible strain and indicates the level of insecticide resistance, is less than 20, and cross-resistance between carbaryl and organophosphate insecticides has been observed (Coles and Dryden, 2014). Due to the distinct mechanisms of action of the substances, one strain resistant to fipronil, the phenylpyrazole insecticide, was susceptible to nitenpyram, a neonicotinoid (Schenker et al., 2001). Bioassays for fipronil and imidacloprid were conducted on both adult and larval *C. felis* in a study. Adult and larval bioassays for fipronil and imidacloprid yielded comparable results. Both laboratory strains and field isolates have been found susceptible to fipronil treatment. However, the majority of strains and isolates were substantially more sensitive to imidacloprid (Rust et al., 2014). In a separate study, resistance to certain ectoparasites was investigated via topical application of 12 cat flea isolates collected from the field and 4 laboratory strains. No resistance to fipronil or imidacloprid was detected in the course of the experiments conducted. All isolates and strains exhibited pyrethroid resistance to permethrin and deltamethrin. Although the Rdl mutation, which confers site-specific cyclodiene insecticide resistance, is present in the majority of isolates and strains, the response to fipronil, which affects the identical receptor protein as cyclodiene insecticides, has not been changed (Rust et al., 2015). In a study involving Germany, France, England, the United States, and Australia, a bioassay was conducted on 1687 of 2307 cat flea isolates collected between 2002 and 2013 to determine their

resistance to imidacloprid. The results showed that the cat flea is still susceptible to imidacloprid despite decades of widespread use (Kopp et al., 2013).

The presence of T929V and L1014F mutations in the *para* gene of cat fleas, which correspond to pyrethroid resistance, was examined in flea samples collected from goats on seven farms in Türkiye where cypermethrin was widely used. According to the results, all collected fleas were *C. felis* (Alak et al., 2020).

From five localities in the northwest and west of Iran, 67 *C. canis* fleas were collected. Adult fleas were exposed to cypermethrin at a concentration of 0.75 percent for 1 and 8 hours, and mutation sites in the VGSC gene were analyzed. The mortality of this dose for *C. canis* was 33.33%, 41.17%, and 66.66%, 80.33% after 1 and 8 hours of exposure, respectively. Sequence analysis of VGSC revealed one mutation site in susceptible fleas and two mutation sites in resistant fleas (Seidy et al., 2022).

Mechanisms of resistance

Research on the mechanisms of resistance in ectoparasites is of concern for the development of new drugs and control strategies. Although these mechanisms have not been fully explored, some resistance mechanisms are outlined below. Due to a similar mode of action, parasitic resistance can develop within the same chemical class (Stafford and Coles, 2009).

Two main mechanisms of resistance have been identified:

1. Metabolic resistance: Ectoparasiticides cannot reach their target sites due to detoxification enzyme-based resistance, such as esterases, oxidases, and glutathione S-transferases (GST). This may be the result of the amplification of a single amino acid, which modulates the catalytic center activity of the enzyme, or multiple gene copies in resistant ectoparasites.

2. *Point mutation:* Point mutations inhibit the migration of ectoparasiticide within the target area. These mutations may occur at a single point in the DNA sequence or in the protein structure targeted by the ectoparasite. Consequently, the drug may not bind effectively to its target and may lead to reduced lethality in the parasite. Over time, the frequency of these mutations in the population may increase, and resistant parasites may transfer the mutations as they continue their genetic lineage, leading to an increase in ectoparasitic resistance in the population. Therefore, understanding and monitoring point mutations is important for the development of new drugs. In a study, it was found that resistance development was faster in the case of resistance due to only one gene mutation, especially if this single gene mutation constitutes a dominant allele (Jonsson and Hope, 2007). When multiple genes are involved in resistance formation, the propagation of the resistance rate within the population will decrease.

Pyrethroids: Pyrethroid resistance mechanism of *Rhipicephalus* (formerly *Boophilus*) *microplus* was investigated. Tick genotypes known to be resistant to pyrethroids were utilized for this purpose. These DNAs were sequenced after obtaining partial *para*-homologous sodium channel cDNA from these ticks. In the extremely preserved domain IIIS6 of the homologous sodium channel of ticks that are extremely resistant to pyrethroids, a point mutation that results in an amino acid change from Phe to Ile was identified (He et al., 1999).

In a study involving cat fleas from the United States and the United Kingdom, the mechanism of pyrethroid resistance was investigated. This study involved the cloning of domain II sequences from the cat flea *para* gene and the identification of two mutations, L1014F and T929V, pyrethroid resistance in residues previously implicated (Bass et al., 2004).

A study was conducted to ascertain the pyrethroid resistance of *Varroa destructor*. In this study, valid new methodologies for determining DNA methylation in resistant and susceptible strains were also identified. In the case of five mitochondrial gene fragments, polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) was used to identify nucleotide substitutions in the DNA of pyrethroid-resistant, pyrethroid-susceptible, and control mites. The drug-resistant strains contained more bands than the other two categories. In pyrethroid-resistant mites, global DNA methylation levels were observed to be lower (Stracheka et al., 2015). TaqMan analyses performed on tau-fluvalinate-resistant *V. destructor* mites in the Lombardy region of Italy demonstrated the presence of the L925V mutation. (Lupi, 2019).

Organophosphates: Organophosphates (OP) are derived from one of the phosphorus acids and contain phosphorus. Organophosphates are specific for the acetylcholinesterase (AChE) protein. Due to the complexity and multifactorial nature of organophosphate resistance, an extensive investigation has been conducted to identify the mechanisms underlying organophosphate resistance, however, there has been little progress at the molecular level (Guerrero et al., 2012). The existence of OP resistance is supported by biochemical and bioanalytical data (Jamroz et al., 2000).

Biochemical, bioassay, and molecular assays were used to determine the OP resistance status of *R. microplus* ticks collected in the Indian state of Punjab. As a measure, the Adult Immersion Test (AIT) was utilized. Malathion resistance was detected in 12 isolates following the test. The gene sequences of AChE3 and AChE activity were analyzed to determine the potential resistance mechanism. A significantly higher level of uninhibited AChE activity was observed in all collected ticks compared to the susceptible population. While V71A mutation was observed in seven isolates collected from the field, R86Q mutation was found in all isolates collected from the field (Singh et al., 2016).

Macrocyclic lactones: In a study involving two tick strains, one resistant and one susceptible, a lethal time bioassay was conducted. The significance of the ivermectin detoxification enzymes cytochrome P450, esterases, glutathione-S-transferase, and ATP Binding Cassette Transporters in resistance was investigated. As a consequence, it was determined that the most important

detoxification mechanisms in resistant strains are those mediated by ABC transporters. It was discovered that esterases, glutathione-S-transferases, and cytochrome-oxidases perform a lesser role in detoxification (Le Gall et al., 2018).

Carbamates: Carbamates are derived from carbamic acid. Inhibiting acetylcholinesterase (AChE) is their mode of action. Ticks that are resistant to organophosphates may also be resistant to carbamates due to their analogous mode of action (Li et al., 2005).

The emergence of resistance to propoxur, permethrin, DDT, and malathion in *R. microplus* was examined using the Larval Packet Test in Sri Lanka. The 30% insensitivity of tick populations' acetylcholinesterases, the target site of carbamates and organophosphates, to propoxur inhibition suggests that altered acetylcholinesterases are partially responsible for the carbamate and organophosphate resistance observed in tick populations (Bandara and Karunaratne, 2017).

Phenylpyrazoles: Fipronil is the only phenylpyrazole compound used to treat and/or control animal ectoparasite infestations. Very little is known about fipronil resistance in ectoparasites. In 100% of field populations of German cockroaches, A302S target site mutations of the GABA-gated chloride channel associated with fipronil resistance were identified (Gonzales-Morales et al., 2022).

Amitraz: It has been hypothesized that the target of amitraz activity is one of the biogenic amine receptors, obviously the adrenergic or octopaminergic receptors. Two nucleotide substitutions were discovered in the octopamine receptor sequence of resistant tick strains, causing amino acids that are distinct from those of all susceptible strains (Chen et al., 2007; Corley et al., 2013). These mutations provided the initial proof of a modified target site as a tick amitraz resistance mechanism. In addition, because the target site of amitraz has not been conclusively identified, the precise mechanism of resistance to amitraz remains unknown (Leeuwen et al., 2010; Guerrero et al., 2012; Pohl et al., 2012).

Methods of detecting resistance

In vivo studies are conducted by applying the product directly to animals in accordance with the recommended route of administration and dosage, and the number of parasites before and after treatment (Abolins et al., 2007).

In vitro studies vary depending on the specific chemical and ectoparasite being studied. The majority of the tests require laboratory conditions. A number of these tests can be conducted in the field, such as the CDC bottle test (CDC, 2012) and the Fly Box mobile testing device (Jandowsky et al., 2010). Thresholds (e.g. dose discrimination) differ between ectoparasite species and ectoparasiticides with different modes of action. When defined reference strains of susceptible or resistant ectoparasites are used to assess the validity of these methods, they are all valid.

Exposure of larvae or adults to treated surfaces

Adults: This strategy typically relies on direct contact of ectoparasites with a chemically treated surface under research. It involves exposing ectoparasites to surfaces treated with different subjected to various dilutions of the researched chemical for a specified time period. Mortality of ectoparasites is assessed at defined diagnostic time points. Various materials may be used for these surfaces, e.g. glass, fabric, or paper, although the principle has not changed (Thompson et al., 2002 Jandowsky et al., 2010; Sternberg et al., 2014; Rust et al., 2014).

Larvae: The larval pack test (LPT) is a test that tests that assesses the susceptibility of tick larvae to treated surfaces (FAO, 2004). It is recommended that this test can be used in conjunction with the concept of discriminating concentration as a low-cost and rapid resistance diagnostic technique (Eiden et al., 2015). The discriminating concentration consists of a single ectoparasitic concentration that will kill the most susceptible genotype while leaving the resistant genotypes remain viable.

This category of tests is not suitable to test the resistance of Insect Growth Regulators (IGR) acting by interfering with the moulting process and/or hatching of eggs. To test IGR resistance in transient pests such as flies, fly eggs are commonly incubated in raring media with increasing IGR concentrations (Jandowsky et al., 2010). Specific test conditions may be required for ectoparasites that remain consistently in the host, eg; the use of scrapings of the host's wool or skin is considered mandatory for the lice hatching (James et al., 2008; Levot and Sales, 2008).

Topical application to adults or larvae

Adults: Topical application to a specific location on the surface of the body of ectoparasites is a frequently used method. Using different dilutions, the chemical under investigation is administered in small droplets by micro-syringe to ectoparasites immobilized, e.g. by carbon dioxide or cooling. The mortality rate of ectoparasites is evaluated at the conclusion of the test (Pessoa et al., 2015).

Immersion testing is the second form of topical application. At the time of this test, ectoparasites are immersed in various dilutions of the researched ectoparasiticide (Castro-Janer et al., 2009).

Larvae: Larval Immersion Test (LIT) is a comparable evaluation for larvae (Shaw, 1966). This test is not extensively utilized and does not receive recommendations from FAO.

Feeding tests with treated rearing media

The tested chemical is added at varying concentrations to the culture medium for the larval stages of the ectoparasite, according to the fundamental principle. Such biological assays can be used to evaluate larvicide activity (Kelly et al., 1987; Rust et al., 2014).

Biochemical and molecular assays

These experiments have the potential to investigate the mechanisms of resistance in specific ectoparasite interference, thereby validating resistance. Nevertheless, it is currently used only for research purposes. WHO (1998) has described a number of biochemical and immunological assays to test for an increase or change in ectoparasite enzymes that are associated with higher tolerance to ectoparasites. Biochemical microtiter plate assays, for

experiments to test enzyme activity, e.g. to detect modified acetylcholinesterase, increased esterase, and glutathione-S-transferase. The enzyme activities are measured either visually or by spectrophotometer. Moreover, It should be emphasized that biochemical tests are not available for all identified resistance mechanisms and thus cannot replace conventional susceptibility testing.

Monitoring programs for resistance

Currently, there are no systematic surveillance programs for resistance to ectoparasites. Multiple initiatives monitor the environment and health status of honeybee colonies, including the national distribution of Varroa mite infestation in countries such as Spain, Germany, and Italy. On the other hand, they do not examine resistance levels specifically, and there is no global surveillance initiative that uniformly collects evidence on Varroa resistance.

System of pharmacovigilance

The World Health Organization (WHO) defines pharmacovigilance as " the science and activities concerned with the detection, evaluation, comprehension, and prevention of adverse effects and other drug-related issues".

The anticipated dearth of efficacy must be notified within the system of pharmacovigilance. These reports may provide evidence of the potential development of resistance to a particular active ingredient.

It is arduous to detect resistance in the field, and the expected lack of effectiveness is frequently underreported. Consequently, it is probable that the true incidence of inefficacy is underreported. Therefore, the current pharmacovigilance system's capacity for detection and monitoring of resistance is limited.

Management strategies to delay the development of resistance

Locating: Regular resistance monitoring is recommended before selecting a suitable ectoparasitic drug for administration (FAO, 2004; Abbas et al., 2014; Karakus et al., 2017). Monitoring requires a defined standard methodology, including a recognized laboratory in charge of resistance testing, a susceptible reference strain, and if required, a known resistant strain (FAO, 2004).

Usage of ectoparasites:

Reducing the number of applications: Reducing selection pressure for resistance in the field can delay the emergence of resistance, according to a consensus (FAO, 2004; Thullner et al., 2007). Additionally, reducing ectoparasitic use (e.g., treatment timing based on epidemiology) and avoiding treatment of non-infested animals have been suggested (FAO, 2004; Thullner et al., 2007; Heath and Levot, 2015). A case-control study supports this assertion conducted on farms in Australia where territorial variations in the prevalence of acaricide resistance in *R.* (*Boophilus*) *microplus* were observed. Certain sites and frequencies of ectoparasiticide treatment have been consistently linked to resistance; for instance, The risk of resistance to synthetic pyrethroids and amitraz has been observed to increase if more than five acaricides were administered the year before, the number of acaricide-resistant insects will increase (Jonsson et al., 2000).

Application technique of the veterinary medicinal product: FAO (2004) and Jonsson et al. (2000) cite the method of administration as an additional strategy for preventing the emergence of resistance. For tick eradication programs, topical application by dip or pulverizer is more effective in terms of efficacy than application by spray apparatus, because application by spray apparatus results in poor dispersion and/or poor wetting of animals, exposing ticks to non-lethal concentrations. According to Jonsson et al. (2000) and the World Health Organization (WHO) (2014), ineffective application methods may contribute to the emergence of resistance.

Rotation of distinct ectoparasite classes: Alternate or alternative use of various ectoparasiticide groups without cross-resistance has also been argued to prevent resistance (Kunz and Kemp, 1994; Abbas et al., 2014). This strategy demonstrates that, in a population of ectoparasites, the frequency of individuals resistant to each previously used chemical will decrease as alternative agents are administered (Kunz and Kemp, 1994). In this context, methods for susceptible external parasites to maintain their refugium in order to dilute resistant alleles have been considered (Kunz and Kemp, 1994; FAO, 2004; WHO report, 2014); however, a study indicates that this is challenging to implement in practice (Heath and Levot, 2015). Neither strategy has been sufficiently demonstrated to effectively reduce resistance (Cloyd, 2010).

Rotating pyrethroids (deltamethrin) and organophosphates (coumaphos) may postpone the emergence of pyrethroid resistance, according to a laboratory study involving identified *R. microplus* tick strains. To validate such a strategy, however, field trials are required (Thullner et al., 2007).

Products with more than one active pharmaceutical ingredient: The use of products comprising two or more ectoparasitic agents (multi-drug-containing products) with various mechanisms of action against the identical ectoparasite is another strategy under consideration for delaying resistance. According to the premise that it is unlikely for a single parasite to contain resistant alleles for two or more insecticides or acaricides with distinct modes of action (Kunz and Kemp, 1994; Abbas et al., 2014), this approach entails the use of a single acaricide or insecticide. This strategy necessitates that the active pharmaceutical ingredients in a multi-drug product be used in compatible, equivalent persistence, and at prescribed concentrations (to prevent non-lethal concentrations of an ingredient from selecting resistant heterozygotes). However, the potential risk of developing multiple resistance cannot be ruled out entirely, and further clarification of this approach appears necessary prior to drawing definitive conclusions about its utility.

Synergists

Piperonyl butoxide: Piperonyl butoxide (PBO) is frequently employed as a synergist with a number of ectoparasites

(e.g., carbamates, pyrethroids) for insect control. PBO has no inherent insecticidal properties and is virtually nontoxic to mammals and birds (NPIC, 2017). PBO inhibits many enzymes capable of degrading the active substance in insects prior to its activation. PBO impedes the detoxification of ectoparasites via binding to Mixed Function Oxidases (MFO), which contribute to the degradation of active pharmaceutical ingredients (Wexler et al., 2005). By incorporating PBO into a product, resistance according to the increased activity of insect MFOs can be partially overcome, protecting the product from the deleterious effects of synthetic pyrethroids and carbamates.

Environmental control measures

Increased measures that could reduce the infestation burden and, consequently, the incidence of ectoparasiticide administration in accordance to postpone the emergence of resistance have been discussed in the literature. Pasture management (e.g., adequate aeration, complete manure removal, optimum animal density, and minimal stress) (Jonsson et al., 2000; Abbas et al., 2014) has been shown to improve animal health (Jonsson et al., 2000; Abbas et al., 2014). As with fleas, it is common practice to treat the environment to reduce or eliminate re-infestations in order to reduce infestation pressure. Mosquito traps, horsefly traps, and fly traps (lights, adhesive strips) are examples of control methods (Heath and Levot, 2015). In addition, quarantine of newly-acquired livestock can be considered a preventative measure against possible infestation and the subsequent need to treat the entire herd (FAO, 2004). This is practiced in South America and Africa to prevent the transmission of *Ampblyomma*, *B. ovis*, or *Sarcoptes* (an obligate non-flying ectoparasite), and is recommended for ticks, lice, and mites with a single host.

Alternative management strategies

Alternative methods of ectoparasite control include the utilization of natural enemies and vaccinations:

Natural enemies: In poultry production, predatory mites such as *Androlaelaps casalis*, which consume *D. gallinae,* are utilized. Even though predatory mites are commercially available, additional research is necessary before they can be used in the field (Sparagano et al., 2014). In separate research, it was determined that *Cheyletus malaccensis* is the most potent natural enemy for *Dermanyssus gallinae* and *Megninia ginglymura*, which have been identified as a global economic threat to the poultry industry (Faleiro et al., 2015).

Vaccination: Vaccines against parasites have benefits such as preventing the development of resistance against ectoparasites and preventing environmental contamination (de La Fuente et al., 2017). Numerous years have been spent on the emergence of a vaccine against the single-host tick *R. microplus*, which has a significant negative effect on animal productivity (de La Fuente et al., 2007; Vargas et al., 2010; McNair, 2015; Schetters et al., 2016). Currently, only one vaccine containing the intestinal antigen Bm86 of *R. microplus* is commercially available (Guerrero et al., 2012b). Nonetheless, according to Guerrero et al. (2012 b), the effectiveness of this vaccine varies from strain to strain, and its adoption is limited. Advanced bovine tick vaccines are the subject of continuous research in order to develop them.

Similar strategies are believed to be effective for other infestations, such as sheep scabies and sea lice (McNair, 2015). Nonetheless, the determination of appropriate antigens as vaccine candidates is frequently a main limitation (Smith et al., 2001; Smith and Pettit, 2004), and no vaccines against ectoparasites are presently available.

Conclusion and Recommendations

Increasing global resistance to ectoparasitic agents found in veterinary medicinal products is a serious concern for animal welfare, animal productivity, and to some extent human health. There have been reports of site-specific ectoparasite resistance due to differences in reproduction and environmental conditions, as well as the life cycle and incidence of the ectoparasite. The global resistance status of ectoparasite species appears to have been investigated extra thoroughly.

Resistance mechanism

It is known that the host, the parasite, the frequency of use of antiparasitic products, and the environment/breeding system influence the development of antiparasitic resistance. In external parasites, i) point mutations and ii) enzyme-based detoxification mechanisms have been identified as the two most prevalent mechanisms of resistance. For a number of ectoparasite species, resistance mechanisms against a number of the relevant ectoparasiticide classes have been identified. Ticks have shown clinically significant resistance to amitraz and macrocyclic lactone compounds, for which the underlying mechanism of resistance is currently unknown. Consider the potential that resistance to an ectoparasiticide or class of ectoparasiticides may be caused by multiple mechanisms. In conclusion, this subject requires more information, including the inheritance of resistance genes, so that resistance management programs can be established. Therefore, continued inquiry into the extremely complex process of resistance development is required.

Determination of resistance

In general, resistance is suspected due to a lack of clinical efficacy. Ineffectiveness may also result from improper application of a product, such as insufficient dosing, inappropriate dosing frequency, inappropriate treatment timing, or poor administration procedures. However, these inappropriate practices can also result in the emergence of ectoparasite species with resistance. In a study conducted on fleas, it was determined that the lack of ectoparasitic activity was likely not due to the development of resistance, but rather to the absence of environmental flea control (Dryden and Rust, 1994).

According to the conducted studies, the prolonged usage of ectoparasites of the same class over an extended period of time is a risk factor for the emergence of ectoparasitic resistance in Australia (Levot et al., 1995;

Wilson et al. 1997; Jonsson et al., 2000). It can also be an issue when identical products are used to control various ectoparasites and the epidemiology of different types of infestations, such as resistance in external parasites when macrocyclic lactone anthelmintics are used (FAO, 2004). In order to determine the cause of an observed lack of efficacy in an ectoparasitic, it may be beneficial to be aware of previous treatment methods.

Complicating the situation is the fact that it is frequently challenging to corroborate that the observed ineffectiveness in the field is the result of resistance to the veterinary medicinal product. Presently, the majority of available methods to corroborate suspected resistance require lengthy laboratory conditions. Moreover, prior to resistance testing, laboratory propagation of ectoparasite populations requires specialized knowledge. Therefore, it is necessary to develop resistance detection methods that can provide timely results regarding the susceptibility/resistance conditions of an ectoparasite population and can be conducted routinely in the field.

Monitoring the resistance

The published information on resistance in external parasites is inconsistent and primarily focuses on mites, marine lice, lice, and flies, with a lesser emphasis on ticks, fleas, and mosquitoes.

Only a few countries offer structured resistance surveillance programs and for exclusive to particular ectoparasites. Aside from that, there is a severe lack of data regarding the resistance status and probable temporal trends of the majority of ectoparasite species in relation to currently employed ectoparasites. Therefore, systematic monitoring is requhas on a global scale. Countries such as Australia and New Zealand have demonstrated that such knowledge is essential for managing the development of resistance. (Jonsson et al., 2000; FAO, 2004; Abbas et al., 2014; Karakuş et al., 2017).

Strategies to delay resistance development

In addition to the correct administration of the veterinary medicinal product, a properly affirmed diagnosis is the most natural method for minimizing the risk of developing resistance and achieving the anticipated therapeutic effect.
To ensure that an appropriate ectoparasite is chosen, it is beneficial to regularly monitor the development of resistance to the various ectoparasiticide classes in the region.

On the assumption that increased exposure increases the risk of emerging resistance, it is also necessary to reduce the superfluous routine use of chemical controls as a preservative, according to the prevalent belief. the concept of targeted selective therapy, such as the concept of refugia, which has been demonstrated to delay the emergence of anthelmintic resistance. A portion of the ectoparasite population is left as a control group, in accordance with the refugia concept, in order to decrease the selective pressure on resistance-conferring alleles. The information currently available is insufficient to draw definitive conclusions regarding the utility of this concept for ectoparasites; nevertheless, refugia may be useful for delaying resistance to ectoparasites such as fleas, lice, and mites (Kunz and Kemp, 1995; Cloyd, 2010; Abbas et al., 2014; McNair, 2015). Still, additional clinical research is considered necessary (Cloyd, 2010).

Evaluation of ectoparasite product applications

The prospective emergence of clinically significant resistant arthropod species is a prerequisite for marketing authorization applications. Marketing authorization holders are also required to supply information on the known resistance mechanism. Also beneficial would be the presentation of scientifically supported risk reduction measures to reduce the risk of resistance development. However, due to a dearth of surveillance and efficient detection methods, it is acknowledged that the capacity to provide information on resistance is limited. Although the scientific literature contains some information on resistance mechanisms, the database is presently narrow, particularly in terms of information on inheritance patterns. However, marketing authorization holders should be encouraged to support all data regarding the development of resistance to the active pharmaceutical ingredient in the to-be-approved product. A sufficient quantity of various presentations should be made available in order to treat varying numbers of animals without leaving behind residues that could be misused.

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