1 13 September 2018
2 EMA/CVMP/EWP/310225/2014
3 Committee for Medicinal Products for Veterinary Use (CVMP)

4 Reflection paper on resistance in ectoparasites

5 Draft

Draft agreed by Efficacy Working Party (EWP-V)	May 2018
Adopted by CVMP for release for consultation	September 2018
Start of public consultation	21 September 2018
End of consultation (deadline for comments)	31 August 2019

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Keywords	Ectoparasites, resistance to ectoparasiticides

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Reflection paper on resistance in ectoparasites

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1. Introduction

A wide variety of ectoparasite species of importance to animal health is found in Europe. Ectoparasite infestation is seen in both food-producing and companion animals. It may be associated with a significant decline in animal health and may result in production losses within farming systems. Infested animals may act as a source of infection to both animals and, in the case of ectoparasites of zoonotic importance, humans. Furthermore, some ectoparasite species act as important vectors of bacterial, viral, helminth or protozoan pathogens, some of which pose a serious threat to animal and public health.

The scope of this reflection paper is to give an overview of the currently known resistance situation in ectoparasites to active substances used in both veterinary medicinal products (and also biocides) with a special focus on Europe, and to provide a review of the current knowledge on resistance mechanisms. This information might be useful for guidance on prudent use or for future applications.

2. Definition of resistance

Resistance to ectoparasiticides is the selection of a specific heritable trait (or traits) in an ectoparasite population as a result of exposure of that population to an active substance, resulting in a significant increase in the percentage of the population that will fail to respond to a standard dose of that chemical when used as recommended (slightly modified from Coles and Dryden, 2014 ; WHO, 2010).

Resistance to one ectoparasiticide may also confer resistance to another ectoparasiticide through side- or cross-resistance. Side-resistance is decreased susceptibility to more than one ectoparasiticide within the same chemical class, e.g. resistance to two synthetic pyrethroids. Cross-resistance is decreased susceptibility to more than one ectoparasiticide within different chemical classes with a similar mode of action (Abbas *et al.,* 2014). The WHO (2016) has defined thresholds when testing resistance in malaria vector mosquitoes: i.e. a mortality >98% is considered susceptible, <90% is considered resistant and between 97–90% mortality requires additional testing. For other ectoparasites, the mortality rate of the strain under investigation is usually compared with the mortality rate of a known susceptible strain (sometimes available from the WHO) under laboratory conditions using various concentrations of an ectoparasiticide (see methods) normally expressed either as resistance factor (RF) or resistance ratio (RR).

3. Current state of ectoparasite resistance

Reports of resistance or susceptibility status in a number of ectoparasite species of veterinary importance have been published worldwide. However, literature on ectoparasite resistance in Europe is currently not very comprehensive.

3.1. Ticks

Global: Most reports on resistance in ticks refer to *Rhipicephalus* (formerly *Boophilus*) *microplus*, also known as the southern or Australian cattle tick, which is a one-host tick preferring cattle and buffalo. All stages spend their life cycle on one animal species only, which makes them more sensitive to selection of resistance after treatment compared to multi-host ticks. At present, the tick is endemic in subtropical and tropical regions wordwide, but not in continental Europe. It is meanwhile eradicated in the USA. An overview on the global resistance situation of this tick is given by FAO (2004) and Abbas *et al.* (2014). Resistance of the cattle tick, *Rhipicephalus (R.) microplus,* against avermectins has been

reported from Brazil (Martins and Furlong, 2001, Klafke *et al.,* 2006) and from Mexico (Perez-Cogollo *et al.,* 2010). The first documentation of ivermectin resistance in 10 brown dog tick populations (*R. sanguineus*) has been described in Mexico by Rodriguez-Vivas *et al.* (2017).

Europe: There are currently no reports on tick resistance in cattle. In dogs, an *in vitro* study in Spain reported a high resistance rate in *R. sanguineus* ticks to deltamethrin and variable sensitivity to propoxur. However, all tested *R. sanguineus* strains still appeared to be sensitive to amitraz (Estrada-Pena, 2005). Currently, there is no documented evidence of resistance in *Ixodes ricinus* or *Dermacentor reticulatus* to ectoparasiticides.

3.2. Mites

Dermanyssus gallinae

Europe: First evidence of tolerance of the red poultry mite *Dermanyssus (D.) gallinae* to synthetic pyrethroids and carbamates was reported from Italy in the 80s of the last century (Genchi *et al.*, 1984). A survey in the former Czechoslovakia indicated resistance of *D. gallinae* to the synthetic pyrethroids permethrin and tetramethrin as well as to the organophosphate trichlorfon at few farms. Resistance to the banned DDT was, however, widespread (Zeman, 1987). Resistance to synthetic pyrethroids in *D. gallinae* has also been reported from France (Beugnet *et al*., 1997), Sweden (Nordenfors *et al.,* 2001) and Italy (Marangi *et al*., 2009).

In UK, comparisons with laboratory-reared susceptible mites suggested the presence of resistance to malathion, bendiocarb, cypermethrin and permethrin in field isolated mites (Fiddes *et al*., 2005). A first comprehensive testing of *D. gallinae* from 10 big laying hen companies in Germany in 1999 to 2000 ascertained partial or nearly complete resistance to organophosphates, synthetic pyrethroids and carbamates. The synthetic pyrethroid group revealed the highest degrees of resistance (Liebisch and Liebisch, 2003).

Varroa destructor

Europe: Resistance of the *Varroa (V.) destructor* mite to synthetic pyrethroids, i.e. flumethrin and tau-fluvalinate, has been reported since the early 1990s in the Lombardy region in Italy, spreading quickly to Switzerland, Slovenia, France, Belgium, and Austria (Faucon *et al*.1995, Lodesani *et al*, 1995, Troullier, 1998). From there, it continued its spread throughout Europe following the established colony trade routes in France, reaching Germany in 1997 and Finland in 1998 via possible bee movement from Italy (Martin, 2004). In 2001, the resistance surveillance programme of the UK's National Bee Unit confirmed the first cases of pyrethroid resistance in the UK (Thompson *et al*., 2002, 2003, Martin, 2004, Lea, 2015). Pyrethroid resistance is now considered widespread in UK (Lea, 2015). More recently, resistance to the synthetic pyrethroids acrinathrin and tau-fluvalinate as well as to the formamidine amitraz was detected in *V. destructor* mites from hives in the Czech Republic, using *in vitro* test methods (Kamler *et al.,* 2016).

In 2001, the first laboratory and field detection of *V. destructor* resistance to the organophosphate coumaphos was reported in Italy (Spreafico *et al.*, 2001).

Psoroptes ovis

Europe: Resistance in sheep scab to the organophosphate propetamphos and the synthetic pyrethroid flumethrin has been reported in the UK (Synge *et al.,* 1995; Clark *et al.,* 1996; Coles, 1998; Bates, 1998). Populations of *Psoroptes spp.* that were resistant to flumethrin already showed side-resistance to high cis cypermethrin (HCC) (Bates, 1998). In addition, there is evidence of moxidectin resistance in *Psoroptes* mites in the UK (Doherty *et al.,* 2018).

Sarcoptes scabiei

Global: Case reports on two dogs treated with 300 µg/kg bw ivermectin suggested that *S. scabiei* in these dogs was clinically refractory to the treatment (Terada *et al.,* 2010).

3.3. Lice

Biting/chewing lice:

Global: Most scientific reports on the development of resistance in the biting louse *Bovicola (B.) ovis* in the last decades stem from Australia and New Zealand. Reduced efficacy was first reported after only a few years following the introduction of synthetic pyrethroid formulations in 1981 in Australia (Boray *et al*., 1988). Resistance factors (RF) of 26 x to the synthetic pyrethroid cypermethrin were calculated being sufficient to prevent adequate efficacy (Levot *et al.,* 1995). At nearly the same time in New Zealand low to moderate cypermethrin-RF ranging up to 12 x in the *B. ovis* field populations were identified (Wilson *et al.,* 1997). In 1991, a population of *B. ovis* in New South Wales of Australia was found to have a high RF of 642 to cypermethrin, with side-resistance conferred to other synthetic pyrethroids (Levot *et al.,* 1995). It has also been observed that an Australian strain of *B. ovis* that had originally been highly resistant to pyrethroids appeared susceptible after having been left untreated for several years. When challenged by cypermethrin backline treatment (pour-on); however, high level resistance was again selected rapidly (Levot, 2012). Resistance against the more recently introduced insect growth regulators (IGR), the benzoyl urea derivatives triflumuron and diflubenzuron, was confirmed in lice populations using both a moulting inhibition test (James *et al.*, 2008) and a louse egg hatch test (Levot and Sales, 2008).

Europe: In northern England, resistance to y-benzene hexachloride (y-BHC), aldrin and dieldrin, used in plunge dips, developed in populations of sheep lice in the mid 1960s (Barr and Hamilton, 1965; Page *et al.,* 1965). In Scotland, a sheep flock was suspected of being infested with a synthetic pyrethroid resistant population of *B. ovis*. A bioassay demonstrated a deltamethrin RF of 14.1, which is greater than a resistant reference strain (Devon isolate) that showed a RF of 10.4. Laboratory data and reliable field data, thus, indicated possible resistance to deltamethrin (Bates, 2001). Recently, a population of *Bovicola ocellatus*, collected from donkeys in UK, displayed a high level of pyrethroid tolerance which is likely to reflect development of resistance (Ellse *et al.,* 2012). Based on data from a survey of OIE member countries and FAO questionnaires in Europe lice insecticide resistance has been mapped for UK and France (FAO, 2004).

Sucking lice:

Europe: Information on insecticide resistance in the sucking louse *Haematopinus suis* is rare. In Germany, a population resistant to the organophosphate insecticide dichlorvos was described by Müller and Bülow in 1988.

3.4. Fleas

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

Global: Against the banned synthetic organochlorine methoxychlor, only a single case of cat flea resistance was reported from Europe (Denmark) in 1986. Other cases were reported from outside Europe with a total of 28 and 12 documented cases for *Ct. felis* and *Ct. canis*, respectively (Mota-Sanchez and Wise, 2017).

There are also reports of *Ct. felis* resistances to carbamates, organochlorine, organophosphates, pyrethrins and pyrethroids. Resistance ratios (RR_{50}), were typically less than 20 and some cross-resistance between carbaryl and organophosphate insecticides were observed (Coles and Dryden,

2014). A strain with resistance to the phenylpyrazole insecticide fipronil was found susceptible to the neonicotinoid nitenpyram owing to the different modes of action of both compounds (Schenker *et al.,* 2001).

The susceptibility of 12 field isolates from cats and dogs and four laboratory reference strains of the cat flea *Ct. felis* collected throughout Australia, the United States and Europe was determined following the topical application of insecticides to adult fleas. In the field isolates, the LD_{50} values in fleas following fipronil and imidacloprid administration (i.e. 0.09 to 0.35 ng/flea and 0.02 to 0.18 ng/flea, respectively) were consistent with published baseline figures. Results for the synthetic pyrethroids permethrin and deltamethrin, however, suggested a level of resistance in all isolates, whilst for tetrachlorvinphos only one field-collected isolate from Australia showed a 21-fold resistance at LD_{50} compared to the reference strains (Rust et. al., 2015).

Large-scale monitoring of the imidacloprid resistance status in *Ct. felis* has been carried out in Australia, Germany, France, the UK and the USA. Between 2002 and 2012, 770 isolates from dogs and 1516 isolates obtained from cats were collected. Results confirmed sustained susceptibility of *Ct. felis* to imidacloprid, despite its extensive use for almost 20 years (Rust *et al.,* 2011; Kopp *et al.,* 2013).

3.5. Flies

Insecticide resistance in the house fly *Musca (M.) domestica* is widespread with reports about resistance from a huge number of countries around the world. The following overview is limited to Europe.

Europe*: M. domestica* strains resistant to organophosphates and synthetic pyrethroids have been identified on German farms (Pospischil *et al.*, 1996). In a more recent study, 58 out of 60 *M. domestica* field populations from dairy farms in Germany showed varying degree of resistance towards the pyrethroid deltamethrin using the "Fly Box" test method (Jandowsky *et al.,* 2010). Pyrethroid resistance could be confirmed in the laboratory by topical application of the discriminating dose of 2.5 ng cyhalothrin/fly to 15 isolates selected from these field populations.

In Denmark pyrethroid resistance in *M. domestica* has also been observed. Four out of 21 field populations showed more than 100-fold resistance at the LD_{95} of bioresmethrin synergised by piperonyl butoxide. These farms had a history of heavy pyrethroid use. In addition, resistance to the organophosphate azamethiphos was found to be widespread (Kristensen *et al.,* 2001). Furthermore, neonicotinoid-resistant houseflies are present at a detectable level in Danish field populations from livestock farms. The field populations were 6–76-fold resistant to the neonicotinoid thiamethoxam. The cross-resistance seen between the neonicotinoids thiamethoxam and imidacloprid let the authors conclude that their use as replacements for each other should be avoided (Kristensen and Jespersen, 2008).

In the UK, low-level resistance of fly eggs (RF 2.9) and larvae L1 (RF 2.4) to the insect growth regulator (IGR) cyromazine was reported in a field strain of house flies from a pig farm (Bell *et al.,* 2010). In Denmark, resistance toward the benzoylurea IGR diflubenzuron was observed. Two out of 21 populations had larvae surviving 6.1 times the LC_{95} of diflubenzuron. They also found field populations with some resistance to the IGR cyromazine. Eight out of the 21 field populations had larvae surviving 2.2 times the LC_{95} of a susceptible strain, and one population had larvae surviving 4.4 times the LC_{95} (Kristensen and Jespersen, 2003).

In another study, the susceptibility of 31 Danish field populations of *M. domestica* from live stock farms to spinosad, a compound of the spinosyn class, varied from RF (LC_{50}) 2.2 to 7.5-fold compared to the susceptible WHO reference strain in a feeding assay at 72 h. Based on the steep slope determined and

the limited variation of spinosad activity against the field populations, it was considered that overall these field populations are still susceptible at the proposed discriminating dose of 12 µg spinosad/g sugar (Kristensen and Jespersen, 2004). In Turkey, field strains of *M. domestica* collected between 2004 – 2006 from cow farms in the Antalya and Izmir area, revealed year to year variable resistance levels against synthetic pyrethroids. Very high resistance levels against cypermethrin were reported for the Antalya strain (Akiner and Çağlar*,* 2012).

In France, a *Stomoxys (S.) calcitrans* strain, collected from cattle commonly treated with synthetic pyrethroid, showed an LD_{90} for blood-engorged flies that was 7.1 and 22.6 times over the recommended dose of both deltamethrin and fenvalerate, respectively (Salem *et al.,* 2012). In Germany, 95 % of *S. calcitran*s populations tested on 40 dairy farms were suspected to be resistant against deltamethrin when using the FlyBox test method. The on-farm observations were confirmed in the laboratory, demonstrating that 24 hrs after topical application of the LD_{95} of deltamethrin (2.3 ng/fly) the mortality rate was below 80 %. At the LD_{95} of azamethiphos (4.9 ng/fly) all stable fly colonies also turned out to be resistant (Reissert *et al.,* 2017).

3.6. Mosquitoes and sand flies (Nematocera)

Studies regarding the examination of resistance or susceptibility of products with insecticidal efficacy focus on vector control programs. There are numerous reports from different areas of the world, that describe the occurrence of resistance in mosquitos and sandflies against commonly used chemical classes (Alexander and Maroli, 2003; Dhiman and Yadav, 2016; Fawaz *et al.,* 2016; Salim-Abadi *et al.,* 2016). It can be assumed that such data also has relevance for the efficacy of veterinary medicinal products if these contain insecticidal substances of the same class as used for vector control programs or in agriculture. However, there is only limited information on the resistance situation in Europe (incl. the Mediterranean region).

Culex spp.

Following a bioassay examination of the resistance status of 13 *Culex (C.) pipiens* populations from 5 regions in Greece (Attika, Phthiotis, Thessaloniki, Serres, Evros) (according to the standard methodology of WHO) over a three year period, susceptibility to deltamethrin could be demonstrated in 12 populations; one population in the Attika region was found to be resistant (Kioulos *et al.,* 2013). In another study conducted in Greece using the CDC bottle bioassay according to the guideline for evaluating insecticide resistance in vectors (CDC, 2012), resistance of *C. pipiens* to deltamethrin was shown for the Evros and the Thessaloniki region (Fotakis *et al.,* 2017).

Aedes albopictus

In *Aedes (Ae.) albopictus* the level of resistance is assumed to be relatively low as reviewed by Vontas *et al.* (2012). Concerning pyrethroids, the data indicated that deltamethrin and permethrin seemed to be effective against *Ae. albopictus* adults as all populations that had been tested from a wide geographical area over a range of years remained susceptible. The data collection included bioassay results from *Ae. albopictus* populations from Greece and Italy of the year 2009, which showed clear susceptibility to deltamethrin (Vontas *et al.,* 2012).

Phlebotomus spp.

In the eastern Mediterranean region, resistance against deltamethrin and permethrin was detected in the west of Turkey where both insecticides have been applied for a long time. However, no resistance was found in a neighbouring province without insecticide use. Susceptibility tests and determination of the resistance status were performed according to current WHO standards (Karakus *et al.,* 2017).

Likewise, two Italian sandfly populations (*Phlebotomus (P.) perniciosus* and *P. papatasi*) were found to be susceptible to 3 different insecticides including permethrin compared to a known susceptible laboratory reference strain, based on bioassay tests according to the WHO standard protocols (Maroli *et al.,* 2002).

3.7. Sea lice (Copepods)

Europe: Reduced sensitivity of *Lepeophtheirus salmonis* (the salmon louse) to organophosphates, pyrethroids and emamectin benzoate has been documented (Ljungfeld *et al.,* 2014, Sevatdal *et al., 2005*, Espedal *et al., 2013).*

Surveillance in Norway also revealed reduced sensitivity of *L. salmonis* to azamethiphos and deltamethrin (Grontvedt *et al.*, 2014).

4. Mechanisms of resistance

Resistance can occur within the same chemical class due to a common mode of action (Stafford and Coles, 2009; IRAC). Different classes of ectoparasiticides might have a mutual target site, e.g. sodium channel gate for DDT and pyrethroids (Vijverberg *et al.,* 1982).

Two major resistance mechanisms have been identified:

1. Detoxification enzyme-based resistance occurs when enhanced activity levels of e.g. esterases, oxidases, or glutathione S-transferases (GST) prevent the ectoparasiticide from reaching their target site. This could be caused by a change in a single amino acid altering the catalytic centre activity of the enzyme, or by amplification of multiple gene copies in resistant ectoparasites.
2. Point mutations prevent the ectoparasiticide from acting at the target site. Jonsson and Hope (2007) concluded that the development of resistance will occur faster if resistance is dependent on only a single gene mutation, especially if this single gene mutation forms a dominant allele. If multiple genes play a role in causing resistance, the spread of resistance will be slower within the population

4.1. Pyrethroids

Resistance mechanisms to pyrethroids in many ectoparasites are extensively described in the literature and are generally based on point mutations at the target site. As an example, a specific sodium channel gene mutation has been shown to be associated with resistance to permethrin in *R. microplus* (Foil *et al.,* 2004). Also, point mutations in a sodium channel gene confer tau-fluvalinate (pyrethroid) resistance in *Varroa destructor* (Hubert *et al.,* 2014, Gonzales-Cabrera *et al.,* 2013). A molecular study identified sodium channel gene mutations that could lead to knock down resistance (kdr) phenotypes to pyrethroids in several insect species, including the housefly (Martinez-Torres *et al.,* 1997). Resistance to both pyrethroids and DDT has been observed in *Aedes aegypti*, and was suggested to be caused by the (kdr)-type resistance mechanism (Brengues *et al.,* 2003). An overview of the position of resistance-associated point mutations in the sodium channel genes is given by Rinkevich *et al.* (2013). Detoxification enzyme based resistance to pyrethroids is also known. A specific metabolic esterase with permethrin-hydrolyzing activity, CzEst9, has been purified and its gene coding region cloned. This esterase has been associated with high resistance to permethrin in *R. microplus* (Foil *et al.,* 2004).

4.2. Organophosphates

Pruett (2002) showed that an insensitive acetylcholinesterase, i.e. target site, was involved in organophosphate resistance in two strains of *R. microplus*. It is suggested that point mutations within the AChE gene may be the molecular basis for target site insensitivity as shown by studies with *Drosophila melanogaster* (Mutero *et al.,* 1994).

In salmon lice across the North Atlantic a Phe362Tyr mutation was found to be strongly linked to lice survival following chemical treatment with azamethiphos, demonstrating that this mutation represents the primary mechanism for organophosphate resistance. It was observed that the Phe362Tyr mutation is not a *de novo* mutation but probably existed in salmon lice before the introduction of organophosphates in commercial aquaculture (Kaur *et al.,* 2017).

4.3. Neonicotinoids

Kavi *et al.* (2014) investigated the mechanisms underlying imidacloprid resistance in house flies. Their results suggested that resistance is not due to detoxification changes by cytochrome P_{450}s, in contrast to earlier findings (Markussen and Kristensen, 2010) but results from a different resistance mechanism that could be linked to autosomes 3 and 4 of the house fly.

4.4. Macrocyclic lactones

There is evidence for the participation of ATP-binding cassette (ABC) transporters in ivermectin resistance in the cattle tick *R. microplus*. ABC transporters are known as efflux transporters, and found in all organisms reducing cellular concentrations of toxic compounds (Pohl *et al.,* 2011). However, presently, the exact mechanism of resistance is still unknown (Abbas *et al.,* 2014).

4.5. IGRs

Juvenile hormone analogues (JHA):

Microsomal cytochrome P450 monooxygenases were found to play an important role in the pyriproxyfen resistance of houseflies. Cytochrome (Cyt) P450 and Cyt b5 were investigated in microsomal enzymes of houseflies (*M. domestica*) from the gut and fat body of 3rd instar larvae of both pyriproxyfen susceptible (WHO) and resistant (established in Japan) strains. Microsomes of the pyriproxyfen-resistant housefly strain had higher levels of total Cyt P450s in both the gut and fat body in comparison to the susceptible strain. *In vitro* metabolism studies of pyriproxyfen indicated that the metabolic rates were much higher in both the gut and fat body of resistant compared to susceptible larvae (Zhang *et al.* 1998).

An *Ae. albopictus* population from Florida showed significant resistance against two juvenile hormone analogues methoprene and pyriproxyfen. The population presented over-expressed Cyt P450s, esterases (ESTs), and glutathione-S transferase (GSTs), suggesting that the global overexpression of the detoxification enzyme families may cause the reduced susceptibility towards IGRs (Marcombe *et al.,* 2014).

Reduced susceptibility of the JHA-carbamate fenoxycarb on diapausing and non diapausing 5th instar larvae of the codling moth *Cydia pomonella* in Greek orchards was correlated with elevated Cyt P450 monooxygenases activity, followed by elevated glutathione-S-transferase activity and reduced carboxylesterases activity (Voudouris *et al.,* 2011). Although this effect was observed in the codling moth, the same mechanism could be expected in other insects of veterinary interest.

Chitin Synthesis inhibitors:

A study from Douris *et al.* (2016) provided compelling evidence that benzoyl urea insecticides (BPUs), etoxazole and buprofezin share the same molecular mode of action by direct interaction with chitin synthase 1 (CHS1). They detected a mutation (I1042M) in the CHS1 gene of a BPU-resistant *Plutella xylostella* (diamondback moth) at the same position as the I1017F mutation reported in spider mites that confers etoxazole resistance. Using a genome-editing CRISPR/Cas9 approach, homozygous lines of *Drosophila melanogaster* bearing either of these mutations were highly resistant to etoxazole and all tested BPUs (diflubenzuron, lufenuron, triflumuron). These findings have immediate effects on resistance management strategies of major agricultural pests but also on mosquito vectors of serious human diseases (e.g. Dengue, Zika), as diflubenzuron, the standard BPU, is one of the few effective larvicides in use.

4.6. Carbamates

Carbamate insecticide resistance in *Anopheles (An.) gambiae* s.l. was mainly considered due to target–site insensitivity arising from a single point mutation (Ace-1^R) since the mean Ace-1^R mutation frequency had increased significantly after a two years campaign of indoor residual spraying using the carbamate insecticide bendiocarb in Benin [Aïkpon *et al,* 2014 a, b]. However, a low Ace-1^R mutation frequency in *An. gambiae* populations, associated with the resistance to carbamate and organophosphate detected in a further study (Aïkpon *et al.,* 2014c), strongly supported the involvement of metabolic resistance based on the high activities of non-specific esterases, Glutathione-S-transferases and mixed function oxidases. Similar findings have been reported in *Culex (C.) quinquefasciatus* and *An. gambiae,* where greater oxidase and esterase activities were observed in resistant *C. quinquefasciatus and An. gambiae*, when Ace-1^R was absent (Corbel *et al.,* 2007). The likely implication of metabolic mechanisms in bendiocarb resistance in *An. gambiae* populations from Cameroon was also stressed by Antonio-Nkondjio *et al.* (2016). Sanil and Shetty (2010) studied the genetic basis of propoxur resistance in *An. stephensi* and showed that the resistance gene *pr* is autosomal, monofactorial, and incompletely dominant. According to the authors information on the inheritance mode of the resistant gene is considered relevant for a better understanding of the rate of resistance development.

4.7. Amitraz

The target of amitraz activity has been proposed to be one of the biogenic amine receptors, most likely the adrenergic or octopaminergic receptors. In resistant tick strains two nucleotide substitutions in the octopamine receptor sequence have been detected resulting in amino acids that differ from all the susceptible strains (Chen *et al.,* 2007; Corley *et al.,* 2013). These mutations provided the first evidence for an altered target site as a mechanism of amitraz resistance in ticks. However, since the target site of amitraz has not been definitively identified the exact mechanism of resistance to amitraz is still not completely understood (Leeuwen *et al.,* 2010; Guerrero *et al.,* 2012 a; Pohl *et al.,* 2012).

5. Methods of detecting resistance

In vivo trials are carried out directly on animals by means of administering the product according to the recommended dose-rate and application mode, and the number of arthropods pre- and post-treatment is subsequently compared.

In vitro trials are numerous and vary according to the specific chemical and arthropod being investigated. Some approved test methods are given by FAO (2004), CDC (2012), WHO (2005, 2016)

and IRAC (consulted 2017). Most but not all of the tests require laboratory conditions. Tests which can be performed under field conditions are e.g. the CDC bottle test (CDC, 2012) or the "Fly Box" mobile test kit (Jandowsky *et al.,* 2010). Threshold values (e.g. discriminating doses) vary among different arthropod species and different ectoparasiticides with various modes of action. The validity of any of these methods is evaluated by using defined reference strains of arthropods (either susceptible or resistant).

5.1. Exposing adults or larvae to treated surfaces

Adults: This approach usually requires the direct contact between a surface treated with the chemical under investigation and the arthropods. It involves exposing arthropods to surfaces treated with different dilutions of the chemical under investigation for a predetermined period of time. At defined diagnostic time points the mortality of the arthropods is evaluated. Materials used for these surfaces may vary, e.g. paper, fabric or glas, but the principle remains the same (Thompson *et al.,* 2002, Jandowsky *et al.,* 2010; Rust *et al.,* 2014; Sternberg *et al.,* 2014).

Larvae: A method for testing the susceptibility of tick larvae on treated surfaces is the larval packet test (LPT) promoted by the FAO (2004). It has been suggested that this assay when combined with the discriminating concentration concept may be used as an inexpensive and rapid resistance diagnostic technique (Eiden *et al.*, 2015). A discriminating concentration is a single concentration of an insecticide that will kill a large portion of the susceptible genotype while the resistant genotypes remain alive. The LPT is not suitable for acarine growth regulators.

These types of tests are not suitable for testing resistance of IGRs which act by disrupting the moulting process and/or inhibiting the hatching of eggs. For testing IGR resistance in temporary pests like flies, the fly eggs are usually incubated in rearing media with increasing concentrations of the IGR (Jandowsky *et al.,* 2010). For ectoparasites that remain on the host permanently, specific test conditions might be required, e.g. the use of wool or skin scrapings of the host are considered essential for egg hatching in lice (Levot and Sales, 2008; James *et al.,* 2008).

5.2. Topical application to adults or larvae

Adults: An often used method is the topical application at a chosen location on the body surface of the arthropod. Using different dilutions, small droplets of the chemical under investigation are applied by micro-syringe to the arthropods that are immobilised, for example by carbon dioxide or cooling. As before (see 5.1), at the end of the test, the mortality of the arthropods is evaluated (Pessoa *et al.,* 2015).

Another type of topical application is the immersion test. During this test the arthropods are submerged in different dilutions of the chemical under investigation (Castro-Janer *et al.,* 2009).

Larvae: For larvae an analogous test is the Larval Immersion Test (LIT) (Shaw, 1966). This test is not so widely used and has not been promoted by the FAO.

5.3. Feeding tests with treated rearing media

The basic principle is that the tested chemical, at different concentrations, is added to the culture rearing media for the larval stages of the ectoparasite. The larvicidal efficacy can be tested with such bioassays (Kelly *et al.,* 1987, Rust *et al.*, 2014).

A bioassay to determine resistance in sea lice has been described by Sevatdal *et al*. (2005). Pre-adult II sea lice are put in boxes and placed in seawater. The sea lice are then exposed to different doses of

ectoparasiticides for 30 to 60 minutes. Twenty four hours after exposure survival rates of sea lice can be evaluated.

5.4. Biochemical and molecular assays

These tests have the potential to investigate resistance mechanisms in an individual ectoparasite and thus confirm resistance. However, these tests are currently only used for research purposes. Several biochemical and immunological assays are described by the WHO (1998) to test elevation or alteration of ectoparasite enzymes involved in higher tolerance to ectoparasiticides. For example, the biochemical microtitre plate tests allow for the same ectoparasite to be used for all assays to test enzyme activity, e.g., for detecting altered acetylcholinesterase, elevated esterase, glutathione-S-transferase. The enzyme activities are quantified visually or with a spectrophotometer. It should be stressed that biochemical assays do not exist for all known resistance mechanisms and can, therefore, not completely substitute the standard susceptibility tests.

6. Resistance monitoring programmes

There are currently no systematic monitoring programmes for resistance in ectoparasites in Europe, except monitoring programmes for resistance occurrence in salmon lice in Norway and a monitoring programme currently starting for stable flies (*S. calcitrans* and *M. domestica*) in Germany.

Various projects monitor the environment and the health status of honey bee colonies including distribution of Varroa mite infestation at a national level in EU member states (e.g. Italy with BeeNet, i.e. an Italian beekeeping monitoring network, the German bee monitoring project, Spain etc). However, they do not specifically study levels of resistance and there is no EU-wide monitoring project that homogeneously collects data on Varroa resistance according to a standardized study protocol. In France, the field efficacy of products authorised against *V. destructor* in bees is monitored annually on a voluntary basis supervised by FNOSAD (Federation Nationale des Organisations Sanitaires Apicole Departementales) in order to detect any lack of expected efficacy. This is carried out using *in vivo* efficacy tests. The international honey bee research association COLOSS (prevention of honey bee COlony LOSSes) has a Varroa control task force; however, it does not primarily focus on resistance monitoring (http://coloss.org/taskforces/varroacontrol).

Pharmacovigilance system

Lack of expected efficacy should be reported within the EU pharmacovigilance system. These reports could be supportive in providing evidence of potential development of resistance to a specific active substance.

However, the system has its limitations as resistance is difficult to recognise in the field, and lack of expected efficacy is generally underreported. Thus, the true incidence of lack of efficacy is likely to be underestimated. Consequently, the current pharmacovigilance system is of limited value to detect and monitor resistance.

7. Management strategies to delay the development of resistance

According to the WHO (2014) the occurrence of resistance is of focal nature and requires local decisions. From a general perspective, however, the following measures for reducing the development of resistance are addressed in the related literature:

7.1. Monitoring

Regular resistance monitoring before choosing an appropriate ectoparasiticide for application has been recommended in the public literature (FAO, 2004; Abbas *et al.,* 2014; Karakus *et al.,* 2017). Monitoring requires a recognized laboratory responsible for resistance testing, a defined standard methodology including a susceptible reference strain and, if necessary, also a known resistant strain (FAO, 2004).

7.2. Use of ectoparasiticides

7.2.1. Reduction of number of treatments

There is general consensus that the reduction of the selection pressure for resistance in the field may delay the emergence of resistance (FAO, 2004; Thullner *et al.,* 2007), and it has been recommended reducing the use of ectoparasiticides (e.g. timing the treatments according to epidemiology) or avoiding the treatment of uninfested animals (FAO, 2004; Heath and Levot, 2015).

This was supported by a case control study performed in Australian dairy farms (Queensland), where regional differences were noted in the prevalence of acaricide resistance to the cattle tick *R. (Boophilus) microplus*. Certain regions and the frequency of acaricide application were consistently associated with resistance; it could e.g. be observed that the risk of resistance to synthetic pyrethroids and to amitraz increased when more than 5 applications of acaricide were made in the previous year (Jonsson *et al.,* 2000).

7.2.2. Method of administration of a VMP

The method of administration has also been taken into account (Jonsson *et al.,* 2000; FAO, 2004). For tick eradication programmes, topical application via plunge dips or spray races were considered superior with regard to efficacy compared to administration with an hand-held spray apparatus, since the latter method might provide insufficient distribution and/or wetting of the animals with the possibility of ticks being exposed to sublethal concentrations. This was supposed to be a possible factor that mediates the development of resistance (Jonsson *et al.,* 2000; WHO, 2014).

7.2.3. Rotation of different classes of ectoparasitics

Furthermore, the use of rotation or alternation of different groups of insecticides/acaricides, which have no cross-resistance has been discussed (Kunz and Kemp, 1994; Cloyd, 2010; Abbas *et al.,* 2014; WHO report, 2014). This approach assumes that within an ectoparasite population the frequency of resistant individuals to each chemical used before will decline during the application of the alternate substances (Kunz and Kemp, 1994). In this respect the means of maintaining refugia of susceptible ectoparasites to dilute resistance alleles has been considered (Kunz and Kemp, 1994; FAO, 2004; WHO report, 2014) although this appears to be difficult to apply in practice (Heath and Levot, 2015). The use of either strategy is considered controversial as it has not been adequately demonstrated that these strategies actually mitigate resistance (Cloyd, 2010).

With regard to acaricides there is evidence from a study performed under laboratory conditions with defined *R. microplus* tick strains that rotation of pyrethroid acaricides (deltamethrin) with organophosphate acaricides (coumaphos) could delay the development of pyrethroid resistance. However, field trials are considered necessary to confirm such strategy (Thullner *et al.,* 2007).

7.2.4. "Multi-active products"

A further strategy still under discussion to delay resistance is the use of products containing two or more ectoparasiticidal substances with different modes of action against the same parasite (multi-active products). This approach is based on the assumption that an individual parasite is unlikely to carry resistant alleles for two or more acaricides or insecticides with different modes of action (Kunz and Kemp, 1994; Abbas *et al.,* 2014; WHO report, 2014). This strategy requires that the active substances in a multi-active product are compatible, of equal persistence (to prevent that sublethal concentrations of one component would select for resistant heterozygotes) and that they are used at recommended concentrations. However, the potential risk of the development of multiresistance cannot be fully excluded and further thorough clarification on this strategy appears necessary before firm conclusions on its usefulness can be drawn.

7.3. Synergists

Piperonyl butoxide (PBO)

PBO is widely used as a synergist to certain ectoparasiticides (e.g. pyrethroids, carbamates) for the control of arthropods. PBO has no intrinsic killing properties against arthropods and is practically non-toxic to birds and mammals (NPIC, 2017). PBO inhibits numerous enzymes in the arthropods that can break down the active substance before they can operate. Specifically, PBO inhibits the detoxification of ectoparasiticides by binding to the Cyt P450 dependent mixed function oxidases (MFOs), which are responsible for the degradation of active substances (Weber, 2005). Therefore, by adding PBO to a product, resistance based on increased activity of insect's MFOs might be overcome to some extent; thereby preserving the toxicity of carbamates and synthetic pyrethroids.

7.4. Environmental control measures

To delay the development of resistance, additional measures which may reduce the infestation pressure and thereby the frequency of ectoparasiticide application have been addressed in the relevant literature: Pasture management (e.g. pasture alternation and/or rotation, in combination with ectoparasiticides) and/or housing management (e.g. good ventilation, thorough manure removal, optimum animal density, low stress) (Jonsson *et al.,* 2000; Abbas *et al.,* 2014). Treatment of the surroundings to reduce or eliminate reinfestations is also a common strategy to reduce the infestation pressure, e.g. as practiced in the case of fleas. Management measures may include mosquito traps, horsefly traps and fly traps (lights, sticky strips) (Heath and Levot, 2015). Moreover, quarantine of bought-in livestock may be considered as a strategy to prevent possible infestation and the need for treatment of the whole flock at a later time (FAO, 2004). This has been recommended for one host ticks, lice and mites e.g. *Ampblyomma* in Africa and South America, prevention of transmission of biting louse *Bovicola (B.) ovis* or chorioptes mites Sarcoptes (non flying obligatory ectoparasites).

7.5. Alternative management strategies

Alternative methods for controlling ectoparasiticide infestations are, for example, the use of natural enemies (e.g. predator mites) and vaccination:

7.5.1. Natural enemies

The black dump fly *Hydrotaea aenescens* (formerly *Ophyra*) has been used successfully for controlling house fly populations on swine and poultry farms in Europe and the United States (Betke *et al.,* 1989, Ruszler, 1989; Turner and Carter, 1990; Jespersen, 1994; Hogsette and Jacobs, 1999).

Leclercq *et al.* (2014) studied the efficacy of cleaner fish (wrasse, Labridae), which feed on the skin of other fish, as a biological control against sea lice. The authors concluded that farmed Ballan wrasse (*Labrus bergylta*) are highly effective controls against sea lice.

In poultry production, the release of predator mites such as *Androlaelaps casalis* that consume the poultry red mite *D. gallinae* is used. However, although commercially available, the use of predator mites under field conditions needs further research (Sparagano *et al.,* 2014).

7.5.2. Vaccination

For few arthropod species the development of vaccines is considered a possible alternative approach for the control of ectoparasites. For many years research efforts focused on the development of a vaccine against the single host tropical cattle tick *R. microplus*, which has considerable negative impact on livestock production (de la Fuente *et al.,* 2007; Vargas *et al.,* 2010; Guerrero *et al.,* 2012 b; McNair, 2015, Schetters *et al.,* 2016). Presently, only one vaccine containing the gut antigen Bm86 of *R. microplus* is commercially available (Guerrero *et al.,* 2012 b). However, efficacy of this vaccine is said to be variable because of strain-to-strain variation, and acceptance is not widespread (Guerrero *et al.,* 2012 b). Cattle tick vaccine research is ongoing in order to develop improved vaccines.

Similar approaches for other veterinary infestations are also considered useful (e.g. sheep scab, sea lice) (McNair, 2015). However, the selection of suitable antigens as vaccine candidates is generally a major constraint (Smith *et al.,* 2001; Smith and Pettit, 2004), and so far no vaccine against ectoparasites is available in the EU.

8. Discussion

Worldwide expanding resistance against ectoparasitic substances contained in veterinary medicinal products and biocides is a major concern for animal welfare, for livestock production and partly also for human safety. Differences in the life cycle and prevalence of a parasite as well as in husbandry and environmental conditions, have resulted in region-specific reports about arthropod's resistance. For ectoparasite species with worldwide occurrence (e.g. fleas, mosquitoes, flies, mites) the resistance situation appears to have been more evenly investigated.

8.1. Resistance mechanisms

The development of resistance to antiparasitics is known to be influenced by the host, the parasite, the frequency of use of antiparasiticidal products and the environment/husbandry system. Presently, in ectoparasites two major resistance mechanisms have been identified, which in general are i) point mutations and ii) enzyme-based detoxification mechanisms. For several ectoparasite species resistance mechanisms against some of the relevant substance classes have been determined. However, clinically relevant resistance has also been observed for which the underlying resistance mechanism is presently not exactly known, e.g. for amitraz or macrocyclic lactone compounds in ticks. The possibility that resistance against a substance or substance class is based on more than one mechanism needs to be considered. To summarize, more information in this area including the inheritance of resistance genes

is needed not least for the benefit of establishing resistance management programs. Therefore, continuation of research in the highly complex process of resistance development is needed.

8.2. Detection of resistance

Usually, resistance will be suspected through lack of efficacy during clinical use. However, lack of efficacy could also occur due to inadequate application of a product, e.g. underdosing, inappropriate dosing frequency or timing of treatment or poor application techniques. Such inappropriate practices could, however, also lead to the selection of resistant ectoparasite species. Dryden and Rust (1994) suspected that the reason for lack of efficacy of ectoparasiticides in fleas is most likely not linked to resistance development but rather to treatment deficiencies related to the absence of environmental control, i.e. poor penetration of insecticides into carpets with subsequent re-emergence of adult fleas.

Based on experiences in Australia, frequent use of ectoparasiticides of the same class over an extended period of time is considered a risk factor for the development of ectoparasiticide resistance (Levot *et al*., 1995; Wilson *et al*., 1997; Jonsson *et al*., 2000). Furthermore, it may be a problem when the same products are used to control different species of parasites and where the epidemiology of the different species infestations also differ, e.g. the use of macrocyclic lactones as anthelmintics may also select for resistance in ectoparasites (FAO, 2004). Therefore, the knowledge of preceding treatment practices may help to find the reason for an observed lack of efficacy of an ectoparasiticide.

A complicating factor is that it is generally difficult to confirm that lack of efficacy observed in the field is due to resistance against the veterinary medicinal product. Currently, most of the methods available to verify suspected resistance require time-consuming laboratory conditions. In addition, special expertise is necessary to propagate an ectoparasite population in the laboratory before resistance testing. Thus, there is a need for the development of resistance detecting methods that can be routinely performed under field conditions, and which can provide results in a timely manner with regard to the resistance/susceptibility status of an ectoparasite population.

8.3. Monitoring of resistance

In Europe, published information on resistance in ectoparasites is rather sporadic, focusing predominantly on mites, sealice, lice and flies and to a lesser extent on ticks, fleas and mosquitoes.

Structured resistance surveillance programs are only available in very few EU Member States, and only for specific parasites. Apart from this, there is a huge absence of information of the resistance situation and possible trends over time in most ectoparasite species in European countries in relation to currently used ectoparasiticides. Thus, there is a need for systematic monitoring throughout the European region. Experience from countries outside Europe (e.g. Australia, New Zealand) show that such information is a prerequisite to manage ectoparasite resistance development. (Jonsson *et al*., 2000; FAO, 2004; Abbas *et al*., 2014; Karakus *et al*., 2017).

8.4. Strategies to delay resistance development

To reduce the risk of resistance development and to achieve the expected treatment benefits a sufficiently confirmed diagnosis as well as a correct application of the veterinary medicinal product are inherent measures.

In order to ensure that a suitable ectoparasiticide is selected regular regional monitoring for the development of resistance against different chemical classes would be useful. The present difficulties, however, connected to the establishing of a monitoring program have already been addressed above.

Based on the general notion that increased exposure will increase the risk for resistance development, it can be assumed that reducing unnecessary routine preventive use of chemical controls is essential. This would fit into the concept of targeted selective treatment such as the refugia concept, which has been shown to be beneficial for delaying resistance development against anthelmintics. According to the refugia concept, a certain proportion of the parasite population is left untreated to reduce the selection pressure for genes that confer resistance. At the moment the available information is insufficient to draw final conclusions on the usefulness of this concept for ectoparasites; however, the refugia concept could be considered applicable in delaying resistance against ectoparasites, e.g. in ticks, lice, mites (Kunz and Kemp, 1995; FAO, 2004; Cloyd, 2010; Abbas *et al*., 2014; McNair, 2015). Further clinical investigation is deemed necessary though (FAO, 2004; Cloyd 2010).

Apart from targeted selective treatment there are further strategies proposed in the literature, which could delay resistance development: e.g. rotation in the use of ectoparasiticide classes or use of multi-active product combinations with different mode of action targeted against the same parasite species (Abbas *et a*l., 2014; Heath and Levot, 2015). These strategies are believed to be most successful if implemented before resistance develops to any of the active substances included. However, it is described that in practice such strategies are often implemented when resistance has already developed against one or more active substances (Heath and Levot, 2015), which is likely to make them less effective. Moreover, there is always the potential risk of selection of multiresistant parasite species. Thus, at present, there is insufficient information to conclude on the effectiveness of rotation or the use of multi-active products with regard to delaying resistance. This emphazises the need for further scientific evaluation.

Furthermore, there are treatment independent options to reduce the need for treatment and, thus, selection for resistance. These are environmental control measures like appropriate pasture and/or housing management, the use of specific insect traps or the use of natural enemies of particular ectoparasites. The latter strategy, however, is often negatively influenced by concomitant use of ectoparasiticides. Nevertheless, it is generally agreed that non-chemical strategies could contribute to a reduced use of ectoparasiticides and, thus, to the delay of development of resistance. Therefore, it would be beneficial to put further emphasis on the research and the integration of such sustainable strategies into management programs and on the education of responsible persons.

8.5. Assessment of product applications for ectoparasiticides

For marketing authorisation applications information on the potential emergence of resistant arthropod species of clinical relevance is required. Applicants are also requested to provide data on the resistance mechanism as far as known. Furthermore, it would be useful if scientifically supported risk mitigation measures aimed at reducing the risk for resistance development could be presented. It is acknowledged, however, that the possibility to provide information on resistance is restricted due to the current lack of both surveillance and useful methods for detecting resistance. Even though there is some information on resistance mechanisms available in the literature the data base is currently limited, particularly with regard to information on the mode of heredity. Nevertheless, applicants are encouraged to provide all available data in the field of resistance development for the active substance in the product to be authorised. Unlike anthelmintic products, only a few SPCs of ectoparasiticide products currently contain precautions aimed at mitigating the risks for resistance development. It could be useful to develop guidance information regarding scientifically supported risk mitigation measures to be included in the SPC of ectoparasiticides. Considering that information on the resistance situation in the EU is scarce, there is limited information to include in the product literature. A sufficient number of different pack sizes should be made available for the market to allow treatment of different numbers of animals without causing left-overs that could be used inappropriately.

9. Conclusion

There is limited knowledge on ectoparasiticide resistance in Europe, as documentation on the subject is scattered and incidental. For most ectoparasite species in European countries there is no comprehensive data base, which provides an overview regarding the resistance situation against commonly used ectoparasiticides and possible trends over time. However, such information is considered essential for managing resistance development. In order to establish a sound basis for action regarding resistance management it would not only be important to initiate systematic monitoring programmes but also to continue the research regarding resistance mechanisms.

Resistance to ectoparasiticides is difficult to determine in the field, and it can be assumed by lack of efficacy of an insecticidal/acaricidal product. However, other causes may also result in insufficient or lacking efficacy, e.g. inadequate application of a product. In addition, reinfestations from the surroundings or from untreated animals could simulate lack of efficacy. Therefore, suspected resistance should require verification by laboratory tests. Since most of the available tests are arduous and time-consuming, further research regarding the development of quick tests for diagnostic purposes, which can be routinely used, should be encouraged.

Even though there is a lack of knowledge on the current situation of ectoparasiticide resistance in Europe, it might be prudent to include warnings in the SPC of ectoparasiticidal products to prevent inappropriate use. It should, however, be stressed that the knowledge on the prudent use of ectoparasiticides is by no means complete.

10. Recommendations

There are several issues related to the use of ectoparasiticides with the purpose of reducing the risk for resistance development that do not fall within the mandate of the CVMP/EWP. For many issues, action requiring professional expertise and input from other parties is needed to improve understanding, monitoring, management practices, and the prudent use of ectoparasiticides so as to reduce inappropriate use and consequently delay resistance development.

10.1. CVMP recommendations

- Use of an ectoparasitic product for therapeutic/treatment purposes should be based on the confirmation of ectoparasitic infestation, using appropriate diagnostic methods, if necessary, e.g. skin scraping investigations in the laboratory to verify mange mites.
- Improve pharmacovigilance reporting. Veterinarians and other qualified individuals, as well as farmers and animals keepers, should be encouraged to identify and report any lack of expected efficacy.
- Develop and harmonise prudent use warnings for similar products, as appropriate.
- Provide guidance on the resistance data that should be included in marketing authorisation applications for ectoparasiticides (e.g. published literature addressing the concerned regions in Europe) in line with the requirements of the new veterinary legislation.
- Promote increased availability of ectoparastiticides for minor species to reduce off-label use. Develop specific guidance for minor species in line with the requirements of the new veterinary legislation.

- Restrict the use of fixed combination products extending the parasite spectrum to situations where all active substances are necessary at the time of administration through appropriate statements in the product literature.
- A sufficient number of different pack sizes should be made available for the market to allow treatment of different numbers of animals without causing left-overs that could be used inappropriately.

10.2. Responsibility of Member States

- Decisions on prescription status are not within the responsibility of the CVMP for nationally authorised products. Nevertheless, the prescription-only status is recommended for ectoparasiticides for food-producing animals to avoid inappropriate use.
- A sufficient number of different pack sizes should be made available for the market to allow treatment of different numbers of animals without causing left-overs that could be used inappropriately.
- Encourage Member States to establish systematic monitoring systems at national level or EU-wide to detect resistance in ectoparasites, in particular parasites for which there is a particular concern.
- Encourage the NCAs to control advertising for ectoparasitic products which are available as non-POM to be sure that it is coherent with the summary of product characteristics and not include information which could be misleading or lead to overconsumption of the product.

10.3. Research and education

The following topics fall outside the mandate of the CVMP and national regulatory agencies. However, they are of importance for understanding and monitoring the development of ectoparasitic resistance.

- Continue research on resistance mechanisms. Develop suitable and practical tests for detection of resistance in different ectoparasite species, and markers that trace early stages of resistance development in an ectoparasitic population. A threshold to confirm resistance in different ectoparasite species needs to be established for each target animal species. Support development of better monitoring tools, e.g. user-friendly software/apps that could be routinely used (by farmers).
- Continuous validation of tests, e.g. by carrying out inter-laboratory ring tests.
- Continue research on management strategies that could reduce the need of ectoparasiticides.
- Continue research on biological alternatives that could reduce the need for ectoparasiticides.
- Educate and enhance awareness of ectoparasitic resistance amongst veterinarians and other persons qualified to prescribe veterinary medicinal products in accordance with applicable national law, as well as animal owners.
- Further explore through appropriate scientific evaluation the benefits and risks in relation to resistance development associated with the use of multi-active ectoparasiticides. More research / data are needed on the impact of all combination products on resistance development.

Definitions

ABC transporters: ATP binding cassette (ABC) transporter proteins which are expressed in all organisms and which are essential to several physiological processes (e.g. translocation of substances, cellular defense).

Cross-resistance: Decreased susceptibility to more than one active substance within different chemical classes.

Discriminating dose/concentration: The dose which kills 100% of susceptible test ectoparasites within a given population. Any individuals from field collected isolates which survive at this dose are by definition resistant.

Multiactive product: Products containing two or more substances with activity against the same target parasite but with a different mode of action.

Refugia: The theoretical basis behind the refugia concept is to leave a certain proportion of the parasite population untreated to reduce the selection pressure for genes that confer resistance.

Resistance factor (RF): The ratio of the LC 50 of a field population relative to the LC 50 of a susceptible reference strain. In addition to LC 50 also other LC values (e.g. LC 90, LC 95 or LC 99.9) are used.

Resistance ratio (RR): see RF

Side-resistance: Decreased susceptibility to more than one active substance within the same chemical class.

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