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Reflection paper on anthelmintic resistance

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Reflection paper on anthelmintic resistance

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1. Introduction

Helminth infestations are common in most animals but, particularly in adult healthy animals, the immune system keeps the burden of helminths at such levels that clinical symptoms do not occur. However, if this balance is not obtained, which could be the case for young or diseased animals, or when infestation pressure is very high, helminth infestation may impact severely on the health status of the animals. This may in turn affect performance (e.g. sports horses) and production (e.g. reduced milk and weight gain in sheep) and could also lead to increased mortality. It is, therefore, important to ensure the availability of effective anthelmintics to treat animals. However, resistance to anthelmintics in veterinary medicines is an increasing problem worldwide, especially in ruminants and horses.

The scope of this reflection paper is to describe the current resistance situation in Europe for different helminths and anthelmintic classes and to reflect current knowledge on known resistance mechanisms. For companion animals, there is currently limited knowledge about the occurrence of anthelminthic resistance. For this reason the reflection paper focuses on food producing animals and horses. Monitoring systems and methods for detecting resistance are described, as well as the currently applied management strategies to delay resistance development.

This paper also provides some recommendations on measurements that might delay resistance development.

2. Definition of resistance

The definition of resistance varies in different publications. The following definition is given in the Guideline on anthelmintic combination products targeting nematode infections of ruminants and horses published by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Geary *et al.*, 2012): "*the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage"*. Resistance is inherited and selected for during treatment, as resistant helminths escape the effect of treatment and pass resistance to the next generation. The resistance genes that occur through mutation are initially rare in the population but, as selection continues, their relative proportion in the population increases and consequently the proportion of resistant parasites increases too. Cross resistance describes resistance between chemical classes (Dargatz *et al.,*2000).

3. Current resistance situation (overview)

Although there is a lack of systematic monitoring data within the EU, the occurrence of resistance in many helminth species against various classes of anthelmintics is evident through scattered reports concerning almost all domestic species. Several scientific reports indicate, to different extents, an increase in helminth resistance to the older classes of anthelmintics (benzimidazoles, tetrahydropyrimidines, imidazothiazoles and macrocyclic lactones) in the EU (Borgsteede *et al.,* 2007, Sargison *et al.,* 2001, Sargison *et al.,* 2005). Most recently, Geurden *et al.* (2015) reported on lower than expected efficacy for ivermectin and moxidectin (based on the reduction in egg excretion after treatment) on European cattle farms (Germany, UK, Italy, and France), with confirmed anthelmintic resistance on 12.5% of the farms tested. Isolated reports on cases of helminth resistance to the newest classes of anthelmintics have also been published (e.g. resistance of *Haemonchus contortus* to monepantel, an amino-acetonitrile derivative, Van den Brom *et al.,* 2015).

Benzimidazoles are the oldest class of authorised anthelmintics; thiabendazole was introduced in the 1960s. The first report of decreased efficacy of thiabendazole against *Haemonchus contortus* strains dates from 1964, just 3 years after its introduction to the market (Van den Bossche *et al.,* 1982).

Similarly, resistance has developed rapidly to other anthelmintic classes, particularly those used in sheep and horses, after their introduction to the market. For example, resistance to imidazothiazole-tetrahydropyrimidine and avermectin-milbemycin classes developed within 3-9 years after introduction to the market in sheep (Kaplan, 2004). Today, resistance to anthelmintics is a major constraint in the sheep industry in Australia but resistance is also evident in Europe. Papadopoulos *et al*. (2012) reported the widespread incidence of multidrug-resistant (benzimidazoles, imidazothiazoles and macrocyclic lactones) populations of *Haemonchus contortus*, *Teladorsagia* spp. and *Trichostrongylus* spp. in sheep throughout Europe.

A major concern which is emerging in the EU is the decreased efficacy of triclabendazole against liver flukes (*Fasciola hepatica*) in sheep and cattle (Moll, 2000, monitoring the Netherlands). Furthermore, resistant populations of *Cooperia* spp. to ivermectin are reported in cattle (El-Abdellati *et al.,* 2010). In horses, resistance to benzimidazoles, pyrantel and macrocyclic lactones, has been reported for Cyathostominae and *Parascaris equorum* (Geurden *et al.,* 2014, Matthews, 2014, Nielsen *et al.,* 2014). In addition, *Oesophagostomum* spp. in pigs have been reported to be resistant to pyrantel (Roepstorff *et al.,* 1987), levamisole and benzimidazoles (Gerwert *et al.,* 2002, Bjoern *et al.,* 1990, Várady *et al.,* 1996), and resistance to pyrantel in ascarids and hookworm has been observed in dogs and cats (Kopp *et al.,* 2008a, Riggio *et al.,* 2013).

4. Mechanisms of resistance

Due to advances in molecular technology, mechanisms of resistance in worms are becoming increasingly understood. As described by James *et al.* (2007) and Westenholm *et al.* (2004), resistance in worms can be the result of a variety of mechanisms and can be roughly categorised as genetic changes in the drug target, changes in the drug transport (e.g. ATP-binding Cassette (ABC) transporters), or changes in the metabolism of the drug within the parasite.

The relationship between the aforementioned changes and occurrence of resistance varies between helminth species. Whereas benzimidazole resistance in nematodes can be due to a mutation in the gene coding for the target site, the same mutation does not seem to cause resistance to triclabendazole in the trematode *Fasciola hepatica* (Wilkinson *et al.,* 2012). In addition, within a single worm species, different mutations can lead to resistance to the same anthelmintic. For instance, benzimidazole resistance in *Haemonchus contortus* can be caused by the phenylalanine to tyrosine mutation at amino acid position 200 of the isotype 1β-tubulin gene (Kwa *et al.,* 1994). However, the frequency of this resistance point mutation (single nucleotide polymorphism, SNP) varies considerably and it can be low in benzimidazole (BZ)-resistant populations (James *et al.,* 2007, Ghisi *et al.,* 2007) which carry other mutations (e.g. codon 167). Although genetic selection contributes to resistance, changes in drug transport mechanisms or in the metabolism of the drug within a worm species also account for different resistance mechanisms to the same anthelmintic (Blackhall *et al.,* 2008, Vokral *et al.,* 2013). The P-glycoprotein, a cell membrane transport protein able to transport many different drugs (including ivermectin, benzimidazoles and imidazothiazole derivatives), may lead to multi-drug resistance by increasing the active transport of drugs (James *et al.,* 2007, Kerboeuf *et al.,* 2003, Xu *et al.,* 1995). Therefore, it can be concluded that more research is needed in order to understand the mechanisms and to develop suitable assays for detection of resistance.

5. Monitoring resistance

Investigation of resistance in helminths is a demanding task, since mechanisms of resistance are complex and suitable methods of detecting and evaluating resistance are limited.

Prevalence of resistance in helminth species to different classes of anthelmintics in different target species is not systematically documented throughout Europe, and the development of resistance across Europe is therefore difficult to estimate.

It would be also of interest to evaluate the occurrence of cross-resistance and the subjacent / underlying mechanisms of resistance. Such information could be made available to the user in an appropriate way to inform about the selection of treatment.

5.1. Monitoring systems

There are only a few locally organised monitoring programmes running in the EU. It would be of significant value if surveillance programmes were established on a larger scale to allow systematic monitoring of resistance development within the EU. In the absence of such information, the continuous collection and analysis of publications within the area could aid in the monitoring of resistance development within the Community.

5.2. Pharmacovigilance system

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

However, the system has 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 and consequently the current pharmacovigilance data is of limited value to detect and monitor resistance.

5.3. Methods of detecting resistance

There are various *in vivo* and *in vitro* methods available to assess the efficacy of anthelmintics. Furthermore, specific laboratory methods can be applied to confirm a suspicion of resistance in the field, e.g. as described in the WAAVP study recommendations and guidelines (Wood *et al.,* 1995, Coles *et al.,* 1992). In this context it should be considered that identification of parasites (e.g. worm eggs) cannot always be performed in the field at species level, but sometimes only at genus or family level.

5.3.1. Nematodes

5.3.1.1. Faecal egg count reduction test

Reduced efficacy, that may reflect the development of resistance, can be detected by using the Faecal Egg Count Reduction Test (FECRT). This test estimates the anthelmintic efficacy by comparing the number of worm eggs in faeces of infected animals before and after treatment. This test can be used for all anthelmintic classes, which is a great advantage compared to other tests. However, the sensitivity of the FECRT may be low. For example, according to a study in sheep, it detects only BZ-resistance of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* when the proportion of resistant worms is greater than 25% (Martin *et al.,* 1989). In addition, the egg output of some

helminth species varies depending on the density of the adult worm population. This is the case for *Ancylostoma caninum* in dogs (Kopp *et al.,* 2008b) or *Oesophagostomum dentatum* in pigs (Christensen *et al.,* 1997). In cattle, there is also no clear correlation between egg output and worm number in cattle (Coles *et al.,* 2006, Graef *et al.,* 2013). This illustrates that FECRT has limitations as a tool for detecting resistance.

In general, FECRT can be used in horses, ruminants and pigs (Coles *et al.,* 1992) to detect nematodes which shed their eggs in the faeces. When evaluating the treatment effect the interval between treatment and second sampling should be shorter than the pre-patent period of the specific worm. Thus, the genus and (where possible) the species should be determined before testing. The correct sampling interval also depends on the type of anthelmintic, e.g. for persistent anthelmintics like macrocyclic lactones the interval between treatment and faeces sampling is recommended to be 14–17 days whereas for levamisole an interval of 3–7 days is advised (Coles *et al.,* 2006).

The appropriate study design for efficacy evaluation by use of FECRT and the thresholds for interpretation of efficacy depend on which anthelmintics and target species are to be evaluated. This could concern different minimum group sizes, minimum pre-treatment faecal egg counts, presence/absence of a control group, and time between anthelmintic administration and repeat of egg counting. According to the WAAVP guideline on anthelmintic resistance (Coles *et al.,* 1992)[1] a faecal egg count reduction of less than 90% (arithmetic mean) indicates resistance in pigs, provided that a minimum pre-treatment individual egg count was confirmed.

In horses, a reduction in FEC of less than 90% is suggested to indicate resistance but there is some disagreement regarding this threshold. In some reports a mean of 95% is regarded to be an appropriate cut-off level (Craven *et al.,* 1999, Larsen *et al.,* 2011) whereas in other reports different cut-off values for different classes of anthelmintics are proposed; e.g. 90% for pyrantel and 95% for benzimidazoles and macrocyclic lactones (Dargatz *et al.,* 2000). Further research is needed to conclude on the thresholds that indicate resistance when the FECRT is used in horses (Coles et al., 2006).

For small ruminants, according to the WAAVP guideline, resistance is confirmed when the percentage of reduction in egg count (arithmetic mean) is less than 95% and when the 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is only suspected. In cattle, according to El-Abdelatti *et al.* (2010), resistance in *Cooperia oncophora* can be suspected at a mean faecal egg count reduction of <95% and resistance is confirmed when the upper 95% confidence interval of the mean FECR was <95%.

As previously mentioned, the usefulness of the FECRT as a tool to identify resistance is limited by its lack of sensitivity. Another disadvantage is that it is not species-specific; eggs of different nematode species cannot be differentiated within the test. Moreover, the interpretation of the test depends upon various factors including the detection limit of the method, the number of animals per group, the host species, and the level of egg excretion by the helminths (Levecke *et al.,* 2012).

5.3.1.2. Egg reappearance period test

Egg reappearance period (ERP) is defined as the time interval between the last anthelmintic treatment and the resumption of significant helminth egg shedding (Coles *et al.,* 2006). To evaluate potential occurrence of resistance, the ERP after dosing should be compared with the historical ERP of the veterinary medicinal product. The ERP is a more sensitive method for detecting a reduction in efficacy than the FECRT for some helminth species (AAEP Guidelines, 2013, Nielsen *et al.,* 2014).

[1] The WAAVP anthelmintic resistance guideline is currently under revision. The date of publication of the revision is currently unknown

5.3.1.3. Molecular assays

Molecular techniques, such as polymerase chain reaction (PCR) or pyrosequencing, can reveal mutations in helminth genes responsible for resistance to a certain anthelmintic class. Currently, only resistance to benzimidazoles can be detected by PCR (Kwa *et al.,* 1994).

These methods are useful when resistance is caused by a single gene mutation (i.e. SNP), or by a small number of such mutations. Whether an observed mutation corresponds to detectable resistance to a certain anthelmintic would have to be substantiated by controlled laboratory studies, field studies or documented in literature.

5.3.1.4. Other methods

Other methods potentially useful for the detection of resistance are the egg hatch assay (EHA) (Robles-Pérez *et al.,* 2014) and the microagar larval development assay (LDA). These methods have been developed for detection of resistance to benzimidazoles or levamisole in horses, pigs or small ruminants. Coles *et al.* (2006) have described these assays and how to interpret the results. Yet another novel method for detection of drug resistant helminths is based on a digitalised evaluation of worm motility. Maintained motility of nematodes after administration of an anthelmintic that should lead to paralysis of the parasite could indicate a lack of efficacy. This method has been described for larval *Haemonchus contortus, Strongyloides ratti*, adult hookworms and blood flukes (Smout *et al.,* 2010).

5.3.2. Trematodes and cestodes

At present, there are no validated tests available for evaluation of resistance in trematodes and cestodes.

Coles and Stafford (2001) proposed a “dose and slaughter” trial to further substantiate suspected resistance of trematodes in the field; after artificial infestation followed by treatment with a flukicide (e.g. triclabendazole), the animals are killed and the number of flukes in the liver are counted. However, at the moment there is no agreed view on how to determine the occurrence of resistance on the basis of these counts.

The usefulness of the FECRT to reflect resistance has not been evaluated for tapeworms or flukes. An egg hatch assay (EHA), recently developed for the detection of resistance against albendazole in *Fasciola hepatica*, needs to be validated (Robles-Perez *et al.,* 2013). Fairweather *et al.* (2012) have developed an EHA test for the detection of triclabendazole (TCBZ) resistance in *Fasciola hepatica* which could be useful.

The coproantigen reduction test (ELISA test on faecal samples) might be a useful alternative to investigate resistance in flukes, also in the pre-patent stage, but further evaluation of interpretation criteria are needed (Flanagan *et al.,* 2011a, Flanagan *et al.,* 2011b, Gordon *et al.,* 2012, Novobilsky *et al.,* 2012).

PCR could potentially be used to confirm resistance suspected based on these tests (Robles-Perez *et al.,* 2013), but current literature on this topic is very scarce.

6. Management strategies to delay the development of resistance

Different management strategies are used with the purpose of preventing infestation and/or keeping infestation pressure low. This includes pasture management, refugia, and quarantine for animals which

are newly introduced into a flock or herd. The overall aim is to reduce the need for anthelmintics and consequently to delay the development of resistance. In addition, when anthelmintic treatment is applied, certain treatment practices are recommended with the purpose of reducing the risk for resistance development. Some methods are well established and often reflected in the product information of authorised VMPs, whereas others may require further investigation, as detailed below.

6.1. Correct use of anthelmintics

The prudent use recommendations currently established have the overall aim to target treatment in the best possible way so as to reduce unnecessary exposure and thus limit the risk for resistance.

Recommendations for prudent use of anthelmintics are generally based on an in-depth understanding of the helminth epidemiology (Sargison, 2011). It is stressed that deworming is based on the confirmation of worm burden and that treatment with a relevant product is applied at the right time in relation to the life cycle of the parasite so as to obtain sufficient effect without unnecessary exposure (Besier, 2012, Sargison, 2011).

It has been demonstrated that underdosing and/or a too frequent use of anthelmintics belonging to the same class will increase the risk for selection of resistance (Sargison, 2011). Furthermore, from a theoretical point of view it could be assumed that long-acting drugs (e.g. long acting boluses) and pour-on formulations pose a particular risk. An extended exposure period and sub-therapeutic tail concentrations could promote the selection of resistant strains and may also have implications for the refugia situation. It is recommended by experts that long-acting anthelmintics are applied only in situations when the grazing season is considerably longer than the duration of the effect (i.e. that these formulations are applied at the start of the grazing season [Rathbone and McDowell, 2012]). Pour-on formulations may be associated with substantial variation in drug exposure, i.e. there may be the risk of suboptimal treatment due to grooming behaviour, dirty coat or weather conditions.

To avoid unnecessary exposure it is prudent to limit the use of broad-spectrum (combined) products (e.g. with nematocidal and flukicidal activity) only when all substances included in the product are necessary to effectively treat the animal.

The limited number of anthelmintics authorised for minor species (e.g. fish, goats, alpacas and donkeys) leads to significant off-label use. Dosing strategies may then have insufficient scientific support which could cause, for example, unintentional under dosing.

Although there is a lack of scientific evidence, rotation of anthelmintic classes is often recommended to delay the development of resistance. Rotation of drugs was originally suggested based on the hypothesis that reversion to susceptibility might occur if resistant worms were less fit than susceptible worms, and counter selection was applied via treatment with a drug from a distinct chemical class. However, evidence that resistant worms are any less fit or that true reversion occurs in the field is scant (Fleming *et al.,* 2006).

Routine deworming which is still often practised, leads to unnecessary treatment and, thus, an increase of the selection pressure. This is of particular concern when used in farm animals in situations when environmental refugia (i.e. susceptible helminth population) is low. One example of routine use of anthelmintics on farms is the “dose -and- move” practice, which may provide a survival advantage for resistant parasites.

It is generally agreed that the maintenance of refugia through the implementation of appropriate treatment and pasture management routines is important to decrease the selection pressure and reduce the risk for resistance development (Graef *et al.,* 2013).

6.2. Refugia

Resistance spread is promoted if parasites carrying mutations that bring about reduced susceptibility to anthelmintics are provided with a survival advantage in the population. The refugia concept aims to keep the proportion of resistant worms within the population at a low level and it is thus advocated as a tool to slow the progress of anthelmintic resistance (Van Wyk, 2001). Parasites *in refugia* are those that have not been exposed to an anthelmintic, including those present as free-living stages in the environment, those in untreated individuals, and those in any lifecycle stages in the host that are not affected by the anthelmintic treatment (Fleming *et al.,* 2006, Van Wyk, 2001). Appropriate treatment strategies and pasture management need to be implemented to maintain *refugia* (Graef *et al.,* 2013).

Sargison has published an extensive overview of management measures to create refugia in sheep helminths (Sargison, 2011). The selective deworming of those animals that are predicted to be most infested by nematodes and/or to contribute most towards pasture contamination is implemented to slow the development of anthelmintic resistance but maintain a parasite population in refugia (Besier, 2012, Sargison, 2011). In horses, the usefulness of this practice needs to be further scientifically evaluated, however, the underlying principle remains the same (Nielsen *et al.,* 2014). According to Van Wyk (2001), the subpopulation of encysted equine cyathostomin larvae may be considered as a refugia population as they escape the effect of anthelmintic treatment and reduce the selection for resistance through excretion of susceptible worm eggs onto pasture.

The value of maintaining a population of parasites *in refugia* to slow down the development of anthelmintic resistance has been demonstrated in a bioeconomic model for sheep. In this model, besides the number of flock treatments, the proportion of the worm population in *refugia* had a significant influence on the rate of development of anthelmintic resistance (Pech *et al.,* 2009). The success of refugia (dilution) strategies relies on maintaining a sufficiently large susceptible population of worms. To be successful, the early implementation of helminth control strategies according to the refugia concept is regarded necessary, i.e. acting when resistant allele frequency in the parasite population is still low. It is also likely that implementation of control strategies according to this concept will be beneficial in regions or on individual farms where resistance is not currently a major concern (see also section 7.3).

6.3. Use of multiactive anthelmintic products

It is currently under discussion whether combination products that contain two or more active substances targeting the same helminth but through different mode of actions (so called multiactive anthelmintic products) could be advantageous with respect to delaying the emergence of resistance. Modelling studies and some field data have indicated that such products may delay the development of resistance to new active substances (Learmount *et al.,* 2012, Leathwick, 2012, Leathwick *et al.,* 2012), or delay development of anthelmintic resistance to existing anthelmintic classes (Leathwick and Hosking, 2009, Leathwick *et al.,* 2015). However, the use of multiactive anthelmintics might select for multiple resistance to different anthelmintic classes (Wrigley *et al.,* 2006, Leathwick and Besier, 2014, Geary *et al.,* 2012). Whether multi-actives offer a benefit with regard to resistance development that would outweigh any risk for promoting multiple resistance needs to be further substantiated. This is important to determine before formulating any recommendation on the use of such products.

6.4. Other options

Other measures to control helminth infestation in animals are different pasture management routines, *e.g.* removal of faeces from pasture to reduce the level of infective larvae, reducing stocking densities, preventing high degree of infestation or improving drainage of pastures to decrease the risk of liver

fluke infestations (Sargison, 2011). To be effective, such measures would have to be tailored according to the specific epidemiology situation on the individual farm. Knubben-Schweizer and Torgerson (2015) recommend that the farm epidemiological picture is determined by means of a detailed diagnosis of the affected pasture and the group of animals before implementing appropriate measures against *Fasciola hepatica* in dairy cattle. Appropriate quarantine protocols are also recommended as a useful measure to prevent introduction of resistant helminths.

In addition to this, other biological control methods are currently under development, e.g. vaccines (Heckendorn *et al.,* 2006, Waller *et al.,* 2006, Hertzberg and Sager, 2006, Nisbet *et al.,* 2016) and the selection for livestock that is genetically less susceptible to helminth infestation. The latter approach has been tested in sheep (Stear *et al.,* 2007).

7. Discussion

7.1. Resistance mechanisms and assessment of resistance

The development of anthelmintic resistance is a highly complex process influenced by the host, the parasite, the environment and the anthelmintic product. At present, the resistance mechanisms for a number of anthelmintic substances/classes are not yet fully understood. In addition, there is a lack of standardised/validated tests to confirm resistance in helminths. More research is necessary in order to understand the resistance mechanisms and to develop validated methods that are easy to use in practice. The commonly used FECRT is labour intensive. It has been shown to provide reliable results only if more than approximately 25% of the nematode population is resistant (Coles, 2001, Martin *et al.,* 1989). Moreover, for some target helminths and target species there is currently lack of consensus within the scientific community with regard to which cut-off levels should be employed to confirm the occurrence of resistance. Although FECRT can be very useful in the field situation, it is not sufficiently specific to confirm resistance; other methods have to be used to confirm any suspected finding.

For liver flukes, the coproantigen reduction test (ELISA test in faecal samples) might be a useful alternative to necropsy or to the FECRT, which is not reliable for this helminth. Resistance detection tests in helminths based on molecular techniques (PCR) can be very specific but are not useful in the field.

7.2. Monitoring of resistance

Currently, apart from smaller local initiatives, there are no systematic surveillance programmes running in any EU country.

A systematic EU monitoring system would be useful. However, the current lack of practical, affordable and sufficiently validated methods for determination of resistance, as outlined above in this document, puts restrictions on the possibility to implement such programmes. *In-vivo* tests such as worm counts and faecal egg count reduction tests (FECRT) are in principle possible to use, but unsuitable for monitoring purposes since either necropsy would be needed (worm counts) or they are time and cost intensive (FECRT). *In-vitro* assays [e.g. egg-hatch inhibition test, larval-development inhibition, allele specific molecular tools (PCR, Pyrosequencing)], which would be suitable for monitoring, are only available and validated for some worm species and/or active substances , or are not (yet) available and fully validated for routine diagnostic (e.g. larval-migration inhibition assays).

7.3. Treatment strategies

Current knowledge indicates that the implementation of certain treatment and management strategies can delay resistance development. Management practices related to the correct handling of animals on pasture and keeping stocking density at appropriate levels, as well as treating with anthelmintics only on the basis of a confirmed worm burden (targeted selected treatment) and administering the correct dose, are all well-known factors related to a delay in resistance development. Also, the use of the most appropriate anthelmintic substance for the target parasite and treating at an appropriate time point has similar positive effects. It is further agreed that combination products which aim at broadening the spectrum of activity should only be used when there is a confirmed need for all substances included in the product. Combination products which include two or more substances targeting the same helminth through different mechanisms (so called multiactive combinations) are proposed to delay the occurrence of resistance against the included substances. However, there are concerns that such practice could lead to development of simultaneous resistance to several anthelmintic classes. The assumption that the use of multiactive combination products delays resistance development is currently not sufficiently substantiated by data. The lack of information makes any conclusion on the benefits and risks associated with these combinations impossible. The benefits of these products need to be better quantified and practical guidance should be provided for prescribers, addressing the situations and timing when such multi-actives should be prescribed.

The implementation of the refugia strategy is proposed as a means to delay resistance development. Although published support for this concept is currently only available for sheep, experts in the field consider that the principle of refugia could be applicable for other species. Similarly, scientific evidence for important qualitative risk factors for the emergence of resistance (e.g. frequency of treatment) is currently only available in publications concerning sheep. Again, such evidence is considered supportive with regard to developing recommendations for the prudent use of anthelmintics in other species.

The concept of rotation of different anthelmintics with different mode of action on an annual basis remains under discussion and some parasitologists do not see real advantages in alternation either fast or slow (van Wyk, 2001, Fleming *et al.,* 2006).

Other features and management strategies aimed at reducing helminth burden and thereby delaying the emergence of resistance are good nutrition, acquired immunity and quarantine protocols to prevent introduction of resistant helminths to a flock or herd. Alternative methods, such as biological control methods, vaccines and selection of genetically less susceptible livestock are under development.

Detailed recommendations regarding management practices are not provided in this document. They would have to be tailored to each individual situation, taking into consideration epidemiology, environment, farm demographics, housing conditions, resistance situation, available VMPs, etc.

7.4. Assessment of anthelmintic product applications

Information on anthelmintic resistance needs to be provided in all marketing authorisation applications, in particular those where efficacy is claimed against helminth strains known to be resistant to other substances. However, guidance on how to determine anthelmintic resistance and how to reflect it in the SPC and product information is currently limited. As anthelmintic resistance is becoming more common, it is important to present any available data that could shed light on the resistance status of the claimed helminth species to the active substance(s) included in the product. Information on potential cross resistance could also be of value.

More guidance is needed on how to generate meaningful data (from laboratory and field trials) supporting treatment efficacy against helminth strains with documented resistance to other anthelmintics. Additionally, further guidance is required on how to address specific aspects of anthelmintic resistance/susceptibility in the product literature, apart from the technical advice already given in the Guideline on the summary of product characteristics for anthelmintics (EMEA/CVMP/EWP/170208/2005). This guideline was developed to recommend inclusion of standard warnings regarding the possible development of resistance in the SPC of anthelmintic products authorised for treatment of ruminants (sheep, goats and cattle) and horses. However, other animal species (pigs, companion animals, etc.) are not within the scope of that guideline. When efficacy is claimed against a helminth that has documented resistance to another substance it is particularly important that efficacy is sufficiently supported for this resistant strain (i.e. that there is no cross-resistance). Different biochemical changes or genetic mutations can lead to resistance to an active substance or to a class of anthelmintics. Moreover, a helminth can have separate (concomitant) resistance mechanisms to different classes of active substances. It can, therefore, never be assumed that a certain active substance will always remain effective against a helminth known to be resistant to another anthelmintic substance. The absence of cross resistance between anthelmintics in worm strains might be conveyed in the SPC for an authorised product, but given that the situation may change over time it would not be appropriate to include such information in the indication.

8. Conclusions

Scattered information from different sources in Europe makes it clear that anthelmintic resistance is present all across the region, mostly in small ruminants. Currently, there are no EU-wide programmes that systematically monitor the occurrence of resistance in helminths of relevant animal species. A few local monitoring programmes for specific target species are running, but trends regarding the development of anthelmintic resistance in Europe are difficult to follow on the basis of such local programmes. Knowledge regarding the extent of resistance to different active substances in different helminth species in various geographical areas is useful for decision-making regarding the implementation of activities aimed at controlling further spread. Therefore, a systematic monitoring programme in Europe would be of great value.

Demonstration of anthelmintic resistance is, however, difficult. For many helminth species, there is currently a lack of standardised/validated test systems for confirming resistance. More research is necessary in order to understand the mechanisms and to develop validated methods that are affordable and easy to use. The establishment of EU reference laboratories with the tasks of maintaining a reference strain library and evaluating and validating tools for monitoring anthelmintic resistance could be very useful.

There is some scarcity of scientific data regarding important risk factors for resistance development of helminths in the different target species. Nevertheless, there is a common understanding among experts that measures to reduce the need of anthelmintics and promoting an appropriate use of these drugs are important to delay resistance development. Examples of prudent use advice are: to base treatment on confirmation of worm burden or solid epidemiological information, to employ targeted selective treatment approaches at farm level and to avoid routine and frequent use, to dose correctly and particularly avoid under-dosing, to use combination products only when all substances are necessary for effective treatment, to manage pastures properly and to maintain an appropriate level of *refugia,* in particular by keeping a part of the herd untreated. Furthermore, although scientific support is currently lacking, it is often recommended to rotate between different anthelmintic classes over time. Particular care is necessary when pour-on formulations and prolonged release formulations are used with regard to the timing of administration and the management of animals after administration

of the product, i.e. to ensure sufficient drug exposure in all treated animals and to maintain a sufficient *refugia* population.

A lack of narrow-spectrum products might result in unnecessary use of active substances, e.g. inappropriate use of fixed combination products. In addition, there is a current shortage of authorised anthelmintic products for minor species, which may unintentionally lead to inappropriate off-label use.

9. Recommendations

There are several issues related to the use of anthelmintics 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 anthelmintics so as to reduce inappropriate use and consequently delay resistance development.

CVMP recommendations

- Treatment should be based on the confirmation of worm infestation pressure, using appropriate diagnostic measures e.g. faecal egg count. If this is not possible, treatment should be based on local (regional) epidemiological information regarding level of helminth infestation, e.g. contamination mapping or estimation of contamination.
- Promote targeted selective treatment at farm level which ideally should include a post-treatment check-up.
- 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.
- Harmonise prudent use warnings for similar products, as appropriate; and phrase such warnings in an unambiguous way to avoid misinterpretations. The review of the guideline on the summary of product characteristics for anthelmintics (EMEA/CVMP/EWP/170208/2005) is thus recommended and the scope should be extended to non-food species.
- Provide guidance on the resistance data that should be included in marketing authorisation applications for anthelmintic products (published literature and / or field data addressing the concerned regions in Europe) and on how to characterise and confirm suspected resistance in a helminth strain (e.g. addressing methods of detection, types of studies, number of strains to be considered).
- Promote increased availability of anthelmintics for minor species, especially goats, alpacas and donkeys. Encourage marketing authorisation applications for products with ‘MUMS’ indications
- Restrict use of combination products to situations where all active substances are necessary at the time of administration through appropriate statements in the product information.
- 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.

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 anthelmintics 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 National Competent Authorities (NCAs) to establish systematic monitoring systems at national level or EU-wide.
- Encourage the NCAs to control advertising for anthelmintic products.

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 anthelmintic resistance.

- Continue research on resistance mechanisms. Develop suitable and practical tests for detection of resistance in different parasite species. The threshold for confirming resistance in different helminth 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.
- Investigate resistance of helminths in companion and aquatic animals.
- Continue research on management strategies that could reduce the need of anthelmintics.
- Continue research on biological alternatives that could reduce the need for anthelmintics. .
- Educate and enhance awareness of anthelmintic resistance amongst veterinarians and other persons qualified to prescribe veterinary medicinal products in accordance with applicable national law, as well as animal owners.
- Scrutinise current deworming practices and husbandry procedures.
- Develop markers that trace early stages of resistance development in a helminth population
- Further explore through appropriate scientific evaluation the benefits and risks in relation to resistance development associated with the use of multiactive anthelmintics. More research / data are needed on the impact of all combination products on resistance development.
- Establish an EU reference laboratory for anthelmintic resistance; i.e. for the establishment and maintenance of reference strains, for the evaluation/validation of monitoring tools and for training in resistance detection/monitoring.
- Develop more narrow-spectrum anthelmintics with (reasonably) short withdrawal periods.

10. Glossary

Cross resistance: resistance against two drugs belonging to different anthelmintic drug classes.

Multiactive anthelmintic products: products containing two or more substances with activity against the same target helminths but with a different mode of action.

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