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Reflection paper on anthelmintic resistance

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Table of contents

1. Introduction

Helminth infections are common in most animals. Usually, a balance exists between helminths and the immune system of the animal and thus helminth infection will not lead to illness. However, in animals that do not have sufficient immunity against worms e.g. young or diseased individuals, or when exposure to eggs/larva is massive, helminth infections may have severe impact on the health status. This might subsequently impact on performance (e.g. racing horses) and production (e.g. reduced milk and weight gain in sheep).

Benzimidazoles were the first class of modern anthelmintics. Thiabendazole was introduced in the 1960's. The first report of decreased efficacy of thiabendazole to *Haemonchus contortus* strains dates from 1964, just 3 years after its introduction on the market (1). Other anthelmintic classes used in horses or small ruminants also demonstrated a rapid onset of resistance within a few years of their introduction on the market, i.e. 3 to 9 years in sheep, (2). Nowadays development of resistance against anthelmintics is considered a major threat for the sheep industry in Australia, and in Europe several reports have been published indicating increasing resistance developing against several classes of anthelmintics (3, 4, 5).

Currently, the few EU monitoring programs running are not funded or co-ordinated by government. Therefore, the prevalence of resistance to anthelmintics in different species is currently not systematically documented in Europe.

In a situation where anthelmintic resistance is becoming more common it is of interest to know if a certain anthelmintic is effective in worm populations that are resistant against other anthelmintic (classes), and, increasingly, applicants are seeking claims which state that certain anthelmintic products are effective in helminths that are resistant to other anthelmintic (classes).

However, it is under discussion how such information should be worded and where it could appear in the summary of product characteristics (SPC).

2. Definition of resistance

In line with the World Association for the Advancement of Veterinary Parasitology (WAAVP) Guideline on anthelmintic combination products targeting nematode infections of ruminants and horses (17), anthelmintic resistance can be defined as the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage. It is inherited and selected for because the survivors of drug treatments pass genes for resistance on to their offspring. These resistance genes are initially rare in the population or arise as rare mutations, but as selection continues, their proportion in the population increases as does the proportion of resistant parasites.

3. Mechanisms of resistance

Due to modern molecular technology, mechanisms of resistance in worms are becoming further understood. As reviewed by James et al. (9), Prichard (16) and Wolstenholme et al. (10), resistance in worms can be the result of a variety of mechanisms and can be roughly categorised as genetic changes in the drug target, in the drug transport (ABC transporters), or in the drug metabolism.

The cause of resistance in worms is often complex. Whereas nematode resistance to benzimidazoles 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 *Fasciola hepatica* (11). Even within a worm species different mutations can lead to resistance against the same anthelmintic. For instance, benzimidazole resistance

in *Haemonchus contortus* can commonly be caused by the phenylalanine to tyrosine mutation at amino acid position 200 of the isotype 1β-tubulin gene (12); however, the frequency of this major resistance point mutation varies considerably and it can be low even in resistant populations (9, 13). Therefore, besides this point mutation, benzimidazole (BZ)-resistant populations can carry different mutations that confer BZ-resistance. Furthermore, differences in drug transport or drug metabolism within a worm species also account for different resistance mechanisms against the same anthelmintic (14, 15). On the other hand, as P-glycoprotein is able to transport many different drugs (including ivermectin, benzimidazoles and imidazothiazole derivatives), changes in this protein might confer cross-resistance to many other drugs (9).

4. Methods of detecting resistance.

Depending on the type of helminth different methods of detecting resistance can be used, in line with the WAAVP Guideline on anthelmintic resistance (6).

4.1. Nematodes

4.1.1. General, faecal egg count reduction test

Reduced efficacy, including that which is due to resistance, can be detected by using the Faecal Egg Count Reduction Test (FECRT) which should be carried out in naturally infected animals, before and after treatment. The test is an estimation of anthelmintic efficacy by comparing faecal egg counts. Counts from a group of untreated animals provide a negative control during treatment. This test can be used with all anthelmintic classes, which is a great advantage as compared to other tests. However, it is not reliable if the proportion of resistant worms is less than 25% (7). FECRT can be used in horses, ruminants and pigs (6) for nematodes which shed their eggs via in the faeces. The interval between treatment and second sampling should be shorter than the pre-patent period of the worm, thus the genus and (where possible) the species should be determined. Furthermore, and as described by Coles (6, 18), the correct sampling interval depends on the type of anthelmintic.

The WAAVP guideline on anthelmintic resistance (6)[1] interprets a FECRT < 90% (arithmetic mean) as indicative of resistance in pigs, horses and cattle, provided that a minimum pre-treatment individual egg count is present as described in this guideline. In small ruminants the WAAVP guideline determines resistance when the percentage reduction in egg count (arithmetic mean) is less than 95% and the 95% confidence level is less than 90%; if only one of the two criteria is met, resistance is suspected. However, new insights reveal that these thresholds are not always applicable to all nematode species or to all substances/substance classes.

Resistance (indicated through FECRT in the field) must be confirmed in a laboratory study with induced infections using recently isolated European helminth strains suspected of being resistant, and confirmed by necropsy (19). All stages (adult and larval) mentioned in the SPC must be studied.

4.1.2. Molecular assays

Molecular techniques, such as polymerase chain reaction (PCR) or pyrosequencing, can expose mutations responsible for resistance against a certain anthelmintic class in helminth genes. Currently, in helminths, only resistance against benzimidazoles can be detected by PCR.

These methods are useful when resistance is caused by a single gene mutation, or by a small number of such mutations. For interpretation to be possible, the relevance of the mutation in the development

[1] The WAAVP anthelmintic resistance guideline is currently under revision. The revision is expected to be published by the end of 2014 (personal communication)

of resistance against a certain anthelmintic class should be substantiated by studies or described in literature.

4.1.3. Other methods

Other methods for detection of resistance are the egg hatch test and the microagar larval development test, which have been developed for detection of resistance against benzimidazoles or levamisole in horses or small ruminants. Coles et al. (18) have described the execution of these tests and interpretation of the results.

4.2. Trematodes and cestodes

Suspected resistance of trematodes in the field might be further substantiated with a “dose and slaughter” trial, as described by Coles et al. (8): after artificial infection followed by treatment with a flukicide (e.g. triclabendazole), the animals are killed and the number of flukes in the liver are counted. There is, however, currently no agreed view on how to determine the occurrence of resistance on basis of these counts. FECRT has not been standardised for tapeworms or flukes. PCR could potentially be used to confirm resistance in these worms but currently literature on this topic is very scarce.

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

5. Discussion

Currently there is a significant level of uncertainty regarding the clinical relevance of different types of resistance identified in the laboratory. Furthermore, the correlation between the outcome of available laboratory methods to identify resistance, and clinical signs of resistance among treated animals is unclear. Finally and most importantly, it can never be established that a certain substance will always be effective against a helminth which is resistant to another anthelmintic substance (i.e. a helminth can acquire separate resistance against both substances or cross-resistance may occur).

In order to establish the existence of helminths which are resistant to an anthelmintic substance, applicants should provide data (from published literature or own field data), to determine prevalence of resistance in an area or geographical region(s) in Europe where such problems have been identified. Suspected resistance, based on FECRT or helminth counts, should be confirmed by appropriate laboratory studies using these resistant field isolates. Molecular techniques such as PCR analysis of mutations may also be useful for confirmation of resistance.

Because of the wide variation in resistance mechanisms even within the same worm species, and considering the lack of knowledge on this subject, it is currently not possible to give guidance on how many worm strains with different genetic mechanisms of resistance should be investigated in laboratory and field studies.

Therefore, information on efficacy against worms which are accepted as being resistant to one antiparasitic substance or class cannot be presented in section 4.2 of the SPC of a competitor product which contains a different anthelmintic substance. However, provided that sufficient data have been presented to conclude on the occurrence of resistance in Europe, the number of studies, a summary of the outcome from these studies and details of the origin of the resistant challenge strains can be stated in the SPC section 5.1. Information such as this is only acceptable when the helminth in question is a target parasite listed in section 4.2 of the SPC.

If studies are only carried out with resistant strains derived outside Europe this should be clearly specified in section 5.1 of the SPC. The absence of cross resistance between anthelmintics in these worm strains could also be mentioned in section 5.1 of the SPC.

6. Management strategies / refugia

Discussion of management strategies/refugia is not recommended in the SPC because the refugia principle has not been proven in many species (only in sheep), and management strategies are not consistently applicable, as depending on the class of antiparasitic, the resistance situation might change over time.

7. Conclusion

A claim in section 4.2 of an SPC that a product is effective in helminths that are resistant against another anthelmintic substance/class is not acceptable because the potential occurrence of strains which are resistant to both substances can never be refuted.

Any reference to the efficacy of a certain product against helminths where resistance has been demonstrated against other antiparasitic substances should only be made in section 5.1 of an SPC and the number of studies, the outcome of these studies and details of the origin of the resistant challenge strains should be described.

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