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Review article

Cattle nematodes resistant to anthelmintics: why so few cases?

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Abstract – The apparent lack of anthelmintic resistant nematodes in cattle is due to the management systems used with most cattle and the lack of surveys for resistance. With extensive beef grazing or with beef suckler herds a large percentage of worms are in refugia (not exposed to anthelmintic) and few anthelmintic treatments are used. With dairy replacement heifers resistance can become a problem unless only first year animals are treated and different fields are used for grazing the animals each year. Intensive beef production on grass is unsustainable and in New Zealand resistance is already becoming a serious problem. Individual farms require monitoring for resistance and steps taken to avoid the introduction of resistance. New validated tests and surveys for resistance are required as the extent of anthelmintic resistant bovine nematodes is not known.

anthelmintic / cattle / *Cooperia* / ivermectin / nematodes / refugia / resistance

Résumé – Les nématodes des bovins résistants aux anthelmintiques : pourquoi si peu de cas sont-ils recensés ? L'absence apparente de nématodes résistants aux anthelmintiques chez les bovins est liée aux systèmes d'élevage pratiqués pour la majorité des bovins et à la rareté des enquêtes sur la résistance. Chez les bovins menés en troupeaux extensifs ou chez les bovins allaitants, un pourcentage important d'helminthes est en zone de refuge sur les pâtures (non exposé aux anthelmintiques) et les traitements anthelmintiques sont peu nombreux. Dans les systèmes avec des génisses de remplacement, la résistance peut devenir un problème, à moins que seuls les animaux dans leur première année soient traités et que chaque année des parcelles différentes soient affectées à la pâture des animaux. La production intensive de bétail à viande à l'herbe n'est pas durable et en Nouvelle Zélande la résistance commence à devenir un problème sérieux. Les exploitations devraient avoir recours à une surveillance de la résistance et mettre en place des dispositifs pour éviter l'introduction de nématodes résistants. De nouveaux tests diagnostics et des enquêtes complémentaires sur la résistance sont nécessaires car l'étendue de la résistance des nématodes de bovins n'est pas connue.

anthelmintique / bovin / *Cooperia* / ivermectine / nematode / refuge / résistance

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1. INTRODUCTION

To understand why anthelmintic resistant nematodes are not as common in cattle as in sheep, it is important to recognise that management and worm control practices differ between sheep and cattle and between beef and dairy cattle. The potential for resistance to develop depends on the frequency of genes for resistance in unselected populations of worms, the genetics of resistance, the fitness of resistant worms, but most importantly the numbers of worms in refugia. In intensive beef units in New Zealand resistance is very common indicating that bovine nematodes have the potential to develop resistance. However, if correct strategies are used it should be possible to prevent anthelmintic resistant nematodes becoming a serious problem in cattle in most regions.

2. ANTHELMINTIC RESISTANCE

Resistance in parasitic nematodes has been defined by Prichard et al. [31]: 'resistance is present in a population when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable'. An essentially similar definition was used by Conder and Campbell [11]. A problem of resistance arises when naturally occurring individuals, that contain genes permitting survival of anthelmintic treatment, are given an opportunity to increase as a percentage of the total worm population. It is often assumed that before drug treatment commences resistant individuals will be rare. This is not true. Higher doses of oxamniquine, used to treat the parasitic trematode *Schistosoma mansoni,* are required in East Africa than in Brazil. In a study in Kenya, Coles et al. [8] found oxamniquine resistant *S. mansoni* in patients before significant use of drug had occurred in the area under investigation, thus explaining the need for higher levels of oxamniquine in East Africa. In their paper they referred to tolerance of *S. mansoni* in Kenya but later revised the definition of resistance in *Schistosoma* [7] so that surviving worms were resistant, not tolerant. Tolerant nematodes are species that never respond to a particular type of anthelmintic in any part of the world. Resistant worms are present in a population if there is a significantly lower response to treatment than in the most sensitive population of the same species. In New Zealand ivermectin failed to work properly as a pour-on against *Cooperia* species in cattle [3]. The authors concluded that it was very unlikely to be due to resistance as ivermectin had not been used before on this property. However, on the correct understanding of resistance, resistant worms were present before they encountered the anthelmintic as ivermectin and other macrocyclic lactones have been successfully used in other trials to control

Cooperia sp. in many parts of the world for several years. In the review by Coles in 1988 [5] on resistance in *Ostertagia* a number of trials were listed in which anthelmintics failed to give the expected response [5]. Assuming the drugs were correctly administered and that the pharmacokinetics of anthelmintics did not vary widely between different groups of cattle, resistance had already been found quite extensively in bovine nematodes. Therefore, with the exception of New Zealand, why is anthelmintic resistance apparently not a large problem in nematodes of cattle?

A number of factors may contribute. In *O. ostertagi* the biology of the adult worm may affect the development of resistance. Adult worms only survive for a relatively short period (25–50 days) in cattle [25], and therefore those that survive treatment have a relatively small advantage over susceptible worms. By contrast adult *Haemonchus contortus* in sheep can persist for many months giving a large advantage to resistant worms to produce the next generation. Even where there has been apparent reduced efficacy of anthelmintics [3] trials to investigate this have not been undertaken, presumably due to the relatively high cost of working with cattle. In addition in vitro tests for anthelmintic resistance in cattle have been inadequately researched and validated, thus discouraging their use. However, the major reason for anthelmintic resistance not being more common has to do with the population biology of bovine nematodes.

3. DIFFERENCES BETWEEN GRAZING SPECIES

An important difference between cattle, sheep and goats is in their development of immunity to nematodes and thus the frequency of treatment and the percentage of animals treated. Being browsers by preference, goats have evolved the lowest levels of immunity to nematodes and both young and adults that are made to graze grass require anthelmintic treatment to maintain their health. Adult sheep develop a higher level of immunity than goats as they are naturally grazers. Unless they face a heavy challenge of nematode larvae, particularly with *H. contortus,* treatment of adults may not be essential. However, it is widely practised. In contrast to sheep, providing that first year calves encounter a sufficient nematode challenge to develop a good level of immunity, older cattle do not require treatment for nematodes, although treatment may improve productivity somewhat (reviewed by Vercruysse and Claerebout [36]). The major difference between the three groups of ruminants is the percentage of animals and thus of nematodes that are exposed to anthelmintic. Less use of anthelmintics will be reflected in lowers levels of resistance and this in turn is shown by the percentage of papers on anthelmintics in sheep and cattle that mention resistance, 0.07% compared with 0.01% [4]. The difference in the degree of immunity developed by sheep and cattle could be due to mites (*Psoroptes ovis*) being a major killer of sheep but not cattle. A higher immune response is important in immunity to worms but it could result in a higher pathogenicity to the mites that cause an acute allergic dermatitis.

4. CATTLE MANAGEMENT

In temperate regions four distinct management systems are used for grazed cattle each of which have differing effects on the population dynamics of the nematodes.

(i) *Extensive ranching*. This frequently involves low rainfall regions and a low stocking density. Anthelmintic treatments are not usually required and few are given. Under these conditions anthelmintic resistance would be unlikely to develop.

(ii) *Beef suckler herds*. An important part of the regulation of nematode numbers is the dilution effect provided by the grazing together of immune adults and calves. The large majority of the herbage is eaten by the adults, which produce relatively few eggs, so the challenge faced by young calves should be quite low. Under these conditions few treatment are given to calves and none to adults and resistance would not be expected to become common. However, where macrocyclic lactones (ML) are used for tick control as, for example, in South America, nematodes are being exposed regularly to anthelmintics.

(iii) *Dairy replacement heifers*. Young calves are reared away from their mothers and once weaned usually graze outside for the summer months. The pasture used is frequently near the farm to permit regular inspection of the calves and the same field or fields may be used each year. Under these conditions nematodes that infect subsequent groups of calves come from treated animals and resistance will be likely to develop. If, however, different fields are used each year for calves, the over wintering nematodes that infect the next generation of calves will come from untreated adults and so resistance will be unlikely to develop.

(iv) *Intensive beef calf rearing*. Young calves are usually from dairy herds or males and females from dairy beef crosses that are grazed away from adults. To maximise growth anthelmintic treatments may be given quite frequently helping to ensure that pasture contamination comes from worms surviving treatment. This is the most likely cause for ML resistant *Cooperia* species that were found on 16/18 cattle farms in New Zealand [15].

5. NEMATODE POPULATION BIOLOGY

To be able to fully predict the rate at which anthelmintic resistance can develop in bovine nematodes it is essential to know:

(i) *The frequency of genes for resistance in an unselected population*. This information is unavailable for any population of nematodes. The changes involved in benzimidazole resistance have been investigated at the molecular level in ovine nematodes [13, 19, 30], but not in bovine nematodes. However the frequency of genes for resistance in unselected populations have not been determined. The molecular changes associated with resistance to levamisole and MLs are not known. Because of the widespread use of anthelmintics it will be difficult to find populations of cattle nematodes that have not been exposed to anthelmintics. Providing the necessary probes become available, tests on existing populations could give indications of the potential for benzimidazole resistance to develop rapidly under different management and climatic conditions.

The frequency of genes for resistance can be expected to vary between different species of bovine nematodes being highest in the dose limiting species. *Cooperia* is the dose limiting genus of bovine nematodes for the MLs. It was therefore to be expected that this would be the genus first showing ML resistance.

(ii) *The genetics of resistance*. No research has been undertaken on the genetics of anthelmintic resistance in bovine nematodes but it is likely to be the same as in ovine nematodes. Although there has been a report of benzimidazole resistance being dominant [20], the population of worms used had been highly selected and probably the genetics were of a high level resistance rather than normal benzimidazole resistance. Benzimidazole resistance is recessive as is resistance to levamisole [32], but resistance to the MLs will be dominant [21]. Where resistance is dominant it can be expected to increase the rate at which resistance spreads as heterozygotes (RS) as well as homozygotes (RR) will survive treatment.

(iii) *The fitness of resistant worms*. Fitness includes the ability to develop and survive on pasture, the ability to climb on herbage and be ingested, survival in the host including the rate of egg laying and persistence in the host both as inhibited larvae and as adults. Persistent adult worms will probably be less immunogenic or better able to avoid the immune response of the host. The only information on changes in fitness of resistant nematodes is on benzimidazole resistance in the ovine nematode *Teladorsagia* (*Ostertagia*) *circumcincta* where resistant worms appear to be as fit as susceptible nematodes [14]. In *H. contortus* benzimidazole resistant worms were reported to be fitter than susceptible ones [18]. However, to be confident that a fitness factor is associated with resistance, it must either be checked using genetic crosses or several susceptible and several resistant isolates have to be compared. The report by Kelly et al. [18] could just be natural variation between populations. To prove association between change in fitness and anthelmintic resistance could be very expensive in cattle. As benzimidazole resistance is associated with a change in one amino acid at position 200 in β-tubulin so that in resistant worms the sequence is now the same as in mammals [19], a change in biology associated with resistance may not occur. Initially in a population of benzimidazole resistant *H. contortus* the worms laid less eggs than the susceptible ones, but after further selection egg production increased [23]. Whether levamisole and ivermectin resistant nematodes will be as fit as susceptible ones is not known. Subjective impressions suggest that ML resistant *Cooperia* may be more pathogenic than susceptible isolates [10] and it appears to persist longer than a laboratory susceptible isolate in calves. This could mean that resistant worms produce more eggs over time than susceptible ones. Leignel and Cabaret [22] have reported that selection for resistance may increase the size of worms and thus the rate at which they lay eggs. More research is required to confirm these observations and into possible differences between susceptible and resistant nematodes of other species and its implications for the spread of resistance.

(iv) *The numbers of nematodes in refugia*. The most important issue in the development of resistance is the contribution that those individuals surviving therapy make to the next generation, which in turn depends on the numbers of worms escaping treatment (in refugia). Van Wyk [34] has argued strongly that this is the single most important issue in the development of anthelmintic resistance and its implication for management of grazing animals including cattle has been discussed by Coles [6]. Deliberate incomplete spraying of crops with insecticides is used to try to ensure that the next generation comes from insects that have not been exposed to insecticide. A similar approach is required in nematode control, but the large difference is that insects can fly but nematodes usually only move in animals.

Nematodes in refugia come from three sources, pasture, untreated animals and inhibited stages surviving treatment in the host. Pastures contaminated with nematode larvae form an excellent source of nematodes in refugia so that continuously grazed pastures are less likely to permit the development of resistance providing that there is not heavy reliance on anthelmintic treatments. Whether survival in the soil, as distinct from on herbage, is important will depend on soil type and is unknown. By killing larvae on pasture drought will tend to increase the risk of resistance developing [2, 26]. Treatment of animals and movement to clean grazing has been an important strategy of nematode control in cattle and was recommended by Michel [25]. By encouraging the infection of pasture with eggs from nematodes surviving treatment it will select for resistance. Prevention of build up of nematodes populations in the summer has been very popular with slow release or pulse release boluses or spaced anthelmintic treatments being given at turn out in the spring. These treatments should have selected for resistance, but do not appear to have done so. The reason lies in the management of cattle as already described, i.e. in the percentage of animals treated. Where only calves are treated contamination is coming primarily from untreated worms in second year and older animals. This is also true for dairy calves providing the same pasture is not used each year for the calves. This is, in the author's opinion, the major reason why anthelmintic resistance is not common in bovine nematodes. If there are stages not responding to therapy this may help delay the development of resistance and is probably very important in explaining why pyrantel and ML resistance has been so slow to appear in equine nematodes. In bovine nematodes it may help explain why levamisole resistance appears to have been slower to appear than ML and benzimidazole resistance, at least in New Zealand [24]. Levamisole is not very effective against immature bovine nematodes, especially *O. ostertagi*.

Since there is very little information on any of the crucial indicators of nematode biology, models to predict the occurrence of resistance are unlikely to be accurate. In addition there is not much information on the way farmers are using anthelmintics in cattle under different management conditions. Such information as is available suggests that farmers will be tending to over treat and thus select for resistance [33, 36].

6. SURVEYS FOR RESISTANCE

Anthelmintic resistance may be more common than is supposed. In a 1988 review of resistance in *Ostertagia* trials were listed where results had been poorer than expected [5]. Assuming the definition of resistance used in the present article then resistance was present in numerous herds of cattle to several anthelmintics. With the exception of trials of new formulations of the avermectins/milbemycins, there have been very few trials in cattle with oral anthelmintics since 1988. In a trial in Louisiana Williams [37] found low activity of levamisole pour-on against adult *O. ostertagi* suggesting the presence of resistant worms. A similar conclusion was reached by Williams and Brossard [38] using pour-on levamisole. Williams et al. [39] found lower than expected activity of albendazole, fenbendazole and oxfendazole against immature and adult *O. ostertagi* suggesting some resistance. There were no cases of anthelmintic resistance in bovine nematodes in the UK when a survey was begun in 1998, but that work found the first case of ML resistance reported in the northern hemisphere [33]. Interestingly in this case the poor condition of the cattle had been attributed to coccidiosis as the animals had been treated with anthelmintic. How many other cases of anthelmintic failure are being missed ? A second case was found following an enquiry from a veterinary surgeon [10]. Doubtless others would be found if a larger survey was undertaken. In New Zealand ML resistant *Cooperia* has been found on 16/18 (89%) farms [15]. Resistance to MLs is being found in *Cooperia* sp. in Brazil [12] and Argentina [1, 16] so resistance in cattle nematodes is becoming more common. The full extent of the problem remains unknown.

The detection of resistance is hampered by the lack of simple sensitive tests. Ideally these would be based on molecular probes but these are not available. Benzimidazole resistance can be detected with an egg hatch test but fresh or anaerobically stored eggs are required. The definition of resistance used by the World Association for the Advancement of Veterinary Parasitology methods [9], with resistance being present if the LD_{50} is above 0.1 ppm thiabendazole, is not very sensitive and a delineating dose is much better (unpublished results). Delineating doses require determining for cattle nematodes using PCR probes to confirm whether there are any resistant worms in 'fully susceptible' populations. The micro agar larval development test (MALDT) can be used in cattle [33] but the optimum conditions have not been determined and the delineating doses are not known. Temperatures may need to be below room temperature. In equine nematodes the MALDT with thiabendazole is much less sensitive than the egg hatch tests for detecting low level benzimidazole resistance (unpublished results) but the reasons are not known. Whether ivermectin is the optimal analogue for the detection of ML resistance is not known. In sheep ivermectin is not satisfactory for the detection of ML resistance in *H. contortus* and avermectin B_1 is required [17]. So currently the only proven way of detecting anthelmintic resistance in cattle nematodes is the faecal egg count reduction test which is both slow and expensive. With better tests and more surveys anthelmintic resistant nematodes will undoubtedly be found to be more common on cattle farms.

7. PREVENTION OF RESISTANCE

Under European conditions anthelmintic resistance should not develop to any extent in nematodes of extensively grazed beef herds and in beef suckler herds due to the numbers of worms in refugia and the few treatments given. Active steps to prevent the development of resistance need adopting on dairy replacements and intensive beef production units.

In dairy replacements it should be possible to avoid the development of resistance by:

(i) treating first year animals only unless serious problems of infection develop in older animals. Although treatment of lactating animals may increase milk production it should not be used;

(ii) allowing sufficient exposure of animals to nematode larvae in the first year to permit development of immunity. Work in The Netherlands suggests that reduced growth in year one is better than in year two [27, 28]. Tests based on titres to a specific *Cooperia* antigen are being developed in The Netherlands to determine whether calves have received sufficient exposure to nematode larvae in their first year of grazing [29];

(iii) ensuring that calves are not grazed on the same pasture each year. This means that first year calves are infected with larvae coming from untreated older animals;

(iv) changing the type of anthelmintic used each year. The necessary experiments to prove that annual rotation of anthelmintic types will prevent the development of resistance have not been undertaken, but changing types may be of benefit;

(v) avoiding the introduction of resistant nematodes. The resistance status of the farm from where animals are purchased should be known. Failing that, animals should be treated with more than one anthelmintic type and then held on concrete for 48 hours. Levamisole should be included to remove ML resistant *Cooperia*.

Intensive beef production on grass with regular use of anthelmintics is not sustainable in the medium to longer term as is being shown by the experience in New Zealand. In the short term it may be possible to use it providing that anthelmintic efficacy is monitored regularly and any anthelmintic to which resistance starts to develop is abandoned. Where resistance is already present to two types of anthelmintic it may be possible to follow the idea of Van Wyk and Van Schalwyk [35] and dilute out resistant worms with susceptible ones. This requires field evaluation before it is adopted.

8. CONCLUSION

Resistance can and will become a practical problem in cattle nematodes if the incorrect type of management is used. Just how widespread resistance has become is not known. New validated tests for resistance require development and application to study the extent of resistance in different management systems. It should be possible to delay or prevent the development of resistance in dairy replacement calves with correct management which ensures sufficient numbers of worms are in refugia, but intensive beef production on grass with frequent anthelmintic treatments is unsustainable.

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