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Review

RESISTANCE TO ANTHELMINTICS

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ABSTRACT

In pasture-raised animals, parasitic infections result in significant financial losses. On the other hand, their prevention and limitation require significant financial resources. Climate change, the existence of causative agents resistant to anthelmintic used for prevention and treatment raise questions requiring focused research under current animal husbandry practices, and premise the frequent presence of gastrointestinal parasites in pasture-raised domestic animals in Europe. The most common nematodes found in sheep in Europe that exhibit anthelmintic resistance are those belonging to the genera *Teladorsagia*, *Haemonchus*, and *Trichostrongylus*. Species from the genus *Cooperia* and the genus *Nematodirus* are reported to exhibit resistance less frequently. There is variation in the distribution from west to east and from north to south.

Keywords: anthelmintic resistance, strongyloids, anthelminthics, sheep and goats.

Definition of anthelmintic resistance

The emergence of anthelmintic resistance depends on the host, the management method, the parasite species, the climatic conditions, the kind of the used anthelmintic and its application (1). The earliest published information on anthelmintic resistance was to a phenothiazine (2), followed by that to thiabendazole (3), established 3 years after its introduction to the market. The efficacy of imidazothiazole-tetrahydropyrimidine and avermectin-milbemycin against gastrointestinal strongyloids in sheep declined for three to nine years after their introduction (4).

There are several definitions of anthelmintic resistance. *The ability of parasites to withstand effective doses that would normally kill them, evade the effects of treatment, and pass this trait on to their progeny is known as anthelmintic resistance*, according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) (5). Prichard et al. (6) defined anthelmintic resistance as a *heritable decreased sensitivity of the parasite population to the action of anthelmintic medicines*, which is one of the most often quoted definitions.

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Anthelmintic resistance types

Nipane et al. (7) distinguish between three forms of anthelmintic resistance: multiple, indirect, and cross resistance. According to Singh et al. (8), *cross-resistance* refers to a strain's capacity to withstand exposure to therapeutic dosages of medications that have a distinct or unrelated chemical mechanism of action. The expression of tolerance brought about by the selection of parasites resistant to a different substance with a comparable mechanism of action is known as *indirect resistance*. Strains that are resistant to levamisole, for instance, have also been demonstrated to be resistant to morantel (9), moxidectin (10), and ivermectin. Benzimidazole compounds have also been shown to exhibit indirect resistance (11). Helminths that exhibit *combined resistance* are resistant to two or more anthelmintics, either as a consequence of indirect resistance or separate selection from each group. Green et al. (12) reported the observation of *H. contortus* strains that are resistant to levamisole, benzimidazole, and naphthalphos (trichlorofen).

Despite the use of numerous methods to control helminth infestations, treatment failure has been linked to the rise in parasite populations that are resistant to anthelmintics (13). This poses a threat to the continuation of sustainable and effective animal husbandry. The fact that

populations resistant to many classes of anthelmintics are becoming more common (14) and that the disease is spreading to new areas (15) exacerbates the problem of anthelmintic resistance.

Mechanisms for building anthelmintic resistance

Genetic alterations that impact the drug's transport or metabolism inside the parasite are the processes responsible for the development of anthelmintic resistance (16). They are mixed at random to form various parasite groups and anthelmintic classes. For instance, a mutation in the gene encoding the target of action is frequently the cause of resistance to benzimidazole in nematodes, but it does not result in resistance to triclabendazole in *F. hepatica* (17).

Point mutations occur and provide a particular genetic mechanism for the development of helminth resistance to benzimidazole and other drugs (18).

According to Sangster (19), there are two possible mechanisms for the emergence of resistance to levamisole: either a decrease in the number of nicotine-acetylcholine receptors, which is consistent with data from Richmond and Jorgenson (20), or a decrease in the receptors' affinity for the levamisole molecule. Fleming et al. (21) claim that these alterations are the result of mutation.

Methods for proving anthelmintic resistance

There are two categories of techniques for demonstrating anthelmintic resistance: *in vitro* and *in vivo*.

The controlled efficacy test (CET) and the fecal egg count reduction test (FECRT) are two examples of "*in vivo*" techniques.

In field studies of anthelmintic resistance, FECRT is a frequently employed diagnostic technique (22). It is useful for examining the efficacy of both narrow- and broad-spectrum anthelmintics in animal infestations with single and mixed nematodes (23). Comparing the amount of eggs released per gram of feces (EPG) before and after treatment is the main objective of the methodology (24). One of the method's drawbacks is that there is a weak correlation between the number of eggs released and the actual number of adult parasites in the host, which can lead to an inaccurate assessment of anthelmintic effectiveness due to the biological peculiarities of the causative agents (25), particularly in *Nematodirus* species infestations (26), *T. circumcincta* infestations (27), and *T. colubriformis* infestations, but not in *H. contortus* infestations. Some nematode species produce eggs differently, which causes a discrepancy in the percentage ratio between them and compromises the accuracy of the results (28). When resistant nematodes make up less than 25% of the host's total population, FECRT is unable to identify them (29).

The CET, is limited to use in experimental settings but is more accurate in demonstrating anthelmintic resistance (30). It serves as the benchmark for assessment when creating novel approaches or changing established ones. In order to determine the success of the anthelmintic treatment based on the quantity of live helminths, an experimental infestation must be replicated.

Larval development assays (LDA), egg hatch assays (EHA), tubulin binding assays, larval motility tests, larval feeding inhibition assays, and egg hatch paralysis assays (EHPA) are examples of "*in vitro*" techniques.

An egg hatch test (EHA) was developed by Le Jambre (31) to identify nematode resistance to benzimidazoles in horses (32), pigs (33), and ruminants (34). It was essential to determine the concentration at which 50% of the eggs ceased developing by incubating unembryozed eggs in increasing concentrations of thiabendazole (22, 35).

Coles et al. (36) made modifications to the larval development assay (LDA), which was first proposed by Ibarra and Jenkins (37). According to Babbar et al. (38), it can be used to demonstrate resistance to broad-spectrum anthelmintics such levamisoles, benzimidazoles, and macrocyclic lactones. It stems from their larvicidal and/or ovicidal behaviors. Initially, *E. coli* was employed as a growth medium (36). Later, Hubert and Kerboeuf (39) added bacteria, yeast, and a balanced salt solution. If at least 10% of the parasite population is resistant to the relevant anthelmintic, the approach establishes the presence of resistance (40).

According to Johansen (41), the assay for tubulin binding is extremely specific and highly sensitive. It is predicated on benzimidazoles tagged with tritium having the capacity to attach to tubulin within parasite cells. Populations of resistant nematodes show less inclination towards labeled benzimidazole (42).

When ezerine, macrocyclic lactones, levamisole, and morantel are present, larval motility assays can be used to determine anthelmintic resistance to thiabendazole. When pure anthelmintic substances were added to L3 for an incubation period, the larvae's changes in motility were measured using an electronic detector (43), migration through a microsieve (Larval migration inhibition assay - (44), or direct observation under a microscope for a predetermined amount of time Larval paralysis assay; (45).

Developed by Geary et al. (46) a technique for measuring larval feeding inhibition was modified for use with larvae after it was first created to identify adult gastrointestinal strongylid resistance to macrocyclic lactones (47). It is predicated on the fact that macrocyclic lactones can prevent L1 feeding when fluorescein isothiocyanate (FITC) attached to *E. coli* is present. Larvae of resistant gastrointestinal strongyles that have taken up *E. coli*-FITC appear as green-glowing "sticks" under fluorescence microscopy.

The Egg Hatch Paralysis Assay (EHPA) was used by Dobson et al. (48) to show anthelmintic resistance to levamisole. This assay involves incubating gastroenteric strongylid eggs, completing L1 development, and monitoring the period just before hatching in the presence of levamisole. Because it is challenging to synchronize larval hatching, EHPA is regarded as being unreliable in mixed infestations.

The goal of molecular techniques for anthelmintic resistance determination is to demonstrate alterations in the nucleotide sequence of genes that, in the case of a mutation in the P-tubulin gene at position 200, encode resistance, most frequently to benzimidazoles (49). More research is needed to fully understand the genetic mechanism behind the development of resistance to macrocyclic lactones and levamisole.

Research on anthelmintic resistance prevalence in Europe

The literature contains reports of anthelmintic resistance in a number of European countries **(Table 1).** In the USA, reports of resistance to all classes of given anthelmintics have been made (50).

Over the past ten years, benzimidazole or levamisole have been the most often reported anthelmintic resistance cases in most European countries. A growing number of cases have also been reported to be resistant to macrocyclic lactones, particularly ivermectin (24). There are further reports of moxidectin (51), whose efficacy of 44% was not deemed sufficiently advantageous in Switzerland and southern Germany, and doramectin (52), for which 15% efficacy was observed in the Netherlands. There have also been reports of triclabendazole resistance (53).

Every successful control programme must include an evaluation of the effectiveness of on-farm anthelmintic treatments, according to Fleming et al. (54). Partially resistant helminths are more likely to survive in situations when the medicine is administered ineffectively or at the wrong dosage, which prevents the best possible absorption. Consequently, the administration method should be selected to maximize the chance that the treatment would eradicate partially resistant helminths. According to Sangster et al. (55), the esophageal groove closes when the medication solution enters the oral cavity as opposed to the throat and esophagus. This process avoids the rumen. Benzimidazole resistance is inherited as an incomplete dominant trait, meaning that heterozygous specimens have an advantage over entirely resistant ones when it comes to surviving deworming (40). Worms may be sensitive to the drug but tolerant of greater dosages if they only possess a portion of the resistance genes for levamisole, moxidectin, and ivermectin. One of the multigene properties of these medications is resistance.

According to Anderson et al. (56), there are a growing number of reports of established anthelmintic resistance in South Africa, Australia, New Zealand, Malaysia, Spain, France, Denmark, the United Kingdom, Brazil, and the USA. Resistance to every class of anthelmintics has been reported in the United States (50). Due to the intricate mechanisms involved and the scarcity of techniques for both detection and evaluation, monitoring for resistance in helminths is challenging (57). The prevalence of helminth resistance to various anthelmintics in various animal target groups has not been systematised in Europe. The presence of cross- and multi-resistance adds to the problem's complexity.

Table 1. *Published data on detected anthelmintic resistance in ruminant helminths in Europe.*

Country	Anthelmintic	A type of helminth	Source
Great Britain	Benzimidazoles	*Teladorsagia* spp.	Taylor et al. (2009) (76)
	Imidazothiazoles		
	Moxidectin		
	Multidrug resistant		
Germany	Levamisole Ivermectin	*Teladorsagia* spp. *Trichostrongylus* spp.	Voigt et al. (2012) (77)
Greece	Benzimidazole	*Teladorsagia* spp.	Geurden et al. (2014) (75)
		Haemonchus contortus	Gallidis et al. (2011) (78)
Spain	Benzimidazole Macrocyclic lactones	*Teladorsagia* spp. *Trichostrongylus* spp.	Diez-Banos et al. (2008) (79)
Italy	Ivermectin	*Trichostrongylus* spp.	Traversa et al. (2007) (81)
Norway	Albendazole	*Trichostrongylus* spp. *Teladorsagia* spp.	Domke et al. (2012) (80)
Northern Ireland	Benzimidazole Moxidectin Avermectin Levamisole	*Trichostrongylus* spp. *Teladorsagia* spp. *Cooperia* spp.	McMahon et al. (2017) (84)
Slovakia	Albendazole	*Ostertagia* spp. *Trichostrongylus* spp. *Chabertia* spp. *Oesophagostomum* spp.	Cernanska et al. (2006) (87)
	Ivermectin	*Ostertagia* spp. *Trichostrongylus* spp. *Chabertia* spp. *Cooperia* spp. *Haemonchus* spp. *Nematodirus* spp.	
France	Benzimidazole	*Trichostrongylus axei*	Palcy et al. (2010) (82)
The Netherlands	Benzimidazoles Ivermectin	*Haemonchus contortus*	Borgsteede et al. (2010) (86)
Switzerland	Avermectin	*Haemonchus* spp. *Trichostrongylus* spp. *Teladorsagia* spp.	Artho et al. (2007) (85)
Sweden	Benzimidazoles	*Haemonchus contortus*	Hoglund et al. (2009) (83)

The earliest class of anthelmintics is benamidazoles. In sheep across Europe, multiresistant populations of *H. contortus*, *Teladorsagia* spp., and *Trichostrongylus* spp. have been found to be highly prevalent, according to Papadopoulos et al. (58). Benzimidazoles, imidazothiazoles, macrocyclic lactones, and tetrahydropyrimidines are among the older groups of anthelmintics that have been linked to increased helminth resistance in EU member states (52). Germany, England, Italy, and France have shown lower-than-expected efficacies of ivermectin and moxidectin, with 12.5% of farms polled confirming anthelmintic resistance (59). There have been isolated reports of helminth resistance to the newest classes of anthelmintics, such as *H. contortus*'s resistance to monepantel (60). It was discovered that triclabendazole was less effective in the Netherlands against *Fasciola hepatica* in sheep and cattle (61), and that ivermectin was more effective in Belgium against *Cooperia* spp. in cattle (62). Resistance to benzimidazoles, pyrantel, and macrocyclic lactones has also been reported in horses from *Parascaris equorum* and members of the superfamily Cyathostominae (63); in pigs from *Oesophagostomum* spp. to pyrantel (64); in dogs and cats from roundworms and hookworms to pyrantel (65).

Studies on the presence and spread of anthelmintic resistance in Bulgaria

In Bulgaria, targeted, organized surveillance for helminth manifestations of anthelmintic resistance in productive animals is uncommon. Zhelyazkov et al. (66) carried out the earliest investigations on anthelmintic resistance in our

country. A study including sheep from the Sofia, Blagoevgrad, Shumen, and Troyan regions revealed that individual flocks exhibited resistance to levamisole, and in *Ostertagia ostertagi*, the reduction in egg count was less than 80%.

Prelezov et al. (67) did not detect anthelmintic resistance. As a result of the study, the authors concluded that benzimidazoles are highly effective against gastrointestinal strongyloides. Iliev et al. (68) suggest that benzimidazole efficacy may be diminished by intensive and sometimes incorrect application. As a result, two in vitro tests were carried out to identify the benzimidazole resistance of gastrointestinal strongyloids in sheep: the egg hatch test and the larval development test. The data collected demonstrated that the gastrointestinal strongyloids in sheep from two of the thirteen flocks under examination were resistant to benzimidazole.

The Larval Development Assay has demonstrated for the first time in Bulgaria that gastrointestinal strongyloids belonging to the genera *Haemonchus* and *Teladorsagia* are resistant to levamisole in sheep from South-East Bulgaria (69).

CONCLUSION

Possible measures to prevent anthelmintic resistance are:

- to be avoid long-term treatment with the one and same anthelmintic (70);
- anthelmintics should not be applicate in subtherapeutic doses (28);
- to be maintain ‘refugium’ nematode population (71);
- do not raise sheep and goats together (72);
- to be select appropriate breeding technology and type of sheep for each farm (73);
- it is necessary to take into account local climatic conditions (74).

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