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Chapter

Anthelmintic Resistance in Livestock

Morutse Mphahlele, Nthatisi Molefe, Ana Tsotetsi-Khambule and Thekisoe Oriel

Abstract

For decades anthelmintics have been used as the primary control measure for worm infections in livestock. However, there has been continuous development of anthelmintic resistance (AR) by the parasitic worms infecting livestock. This chapter reviews AR in livestock with a special focus on treatment and control, modes of action of different anthelmintic classes, risk factors leading to development of AR, conventional and molecular tools used to detect AR, FAMACHA© and holistic control strategy to control anthelmintic resistance.

Keywords: anthelmintic resistance, helminths, livestock, benzimidazoles, imidazothiazoles, macrocyclic lactones

1. Helminths infecting livestock

Livestock can be infected with a variety of helminths on pastures, through ingestion of the larvae of the parasites on the contaminated grass, the most common of which are gastrointestinal nematodes and flukes [1]. It goes without saying that helminths have constantly been problematic and without doubt a long-standing concern that threatens the livestock industry [2] given that these parasites have a negative impact on animal productivity and welfare, affecting among other things feed intake, growth rate and milk yield [3]. Parasitic worms include tapeworms, roundworms, lungworms, liver flukes, ring worms, hook worms and whip worms. Transmission of GIT parasites is fairly direct in most cases; the infective eggs or oocyst are passed with the faeces when the animal defecates, the next animal would be infected if they graze in the contaminated areas, and humans could be infected through ingestion of contaminated food and water and/or through close interactions of humans with the infected animals [4]. The annual cost associated with parasitic diseases has been estimated at 1 billion dollars in Australia [5], 7.11 billion dollars in Brazil [6], and believed to be tens of billions of dollars worldwide [5].

2. Treatment and control

2.1 Chemotherapy

Worm control in most farms is exclusively based on anthelmintic treatments rather than on management practices that embraces integrated strategies. The currently

available anthelmintics belong to different drug classes, i.e. macrocyclic lactones (MLs), benzimidazoles (BZs), tetrahydropyrimidines-imidazothiazoles, aminoacetonitrile derivatives (AADs) and spiroindoles. The compounds of these drug classes are potent against a broad range of nematode species, and, furthermore, MLs are effective against many arthropod parasites, whilst BZs also versus some flat worm species [7]. However, even with correct administration of treatment, figures point that the use of anthelmintics is still an expensive way of controlling parasitic diseases [5].

2.2 Modes of action of different anthelmintic classes

Each class of anthelmintics has a unique mode of action against parasites [8]. Imidazothiazoles (IM), such as levamisole, are acetylcholine agonists that act on the nervous system of the parasite [8]. These drugs cause muscle contraction and paralysis in the helminth, resulting in the eventual expulsion of the parasite from the body [9]. Macrocyclic lactones, on the other hand, act on glutamate-gated chloride channels (GluCl) causing paralysis of the parasite neuromusculature, including the pharynx, thereby preventing the worm from feeding [8]. The target of benzimidazoles is, however, the tubulin within the parasite intestinal cells, which forms into microtubules that are necessary for nutrient acquisition [10]. Benzimidazoles bind to the β-tubulin component preventing it from forming microtubules within the intestinal cells of the helminth. This impairs the uptake of nutrients and prevents the transportation of necessary digestive enzymes resulting in death due to starvation [9]. Additional effects of benzimidazoles on nematodes include depletion of energy reserves and the inhibition of waste excretion [11]. The only available aminoacetonitrile derivative on the market today is monepantel [11]. It acts as an agonist of the mptl-1 channel, a channel belonging to a class of nicotinic acetylcholine receptors in the process causing constant fluctuation in muscle ions leading to muscle depolarisation and irreversible nematode paralysis [11]. Benzimidazoles and macrocyclic lactones are effective against the adult and immature stages of the parasite, whilst the imidazothiazoles are effective against the adults and the later stages of immature larvae [8]. In short, these drugs enter the worm and interact with its target receptor in order to trigger a harmful physiological effect [12]. **Figure 1** shows a schematic

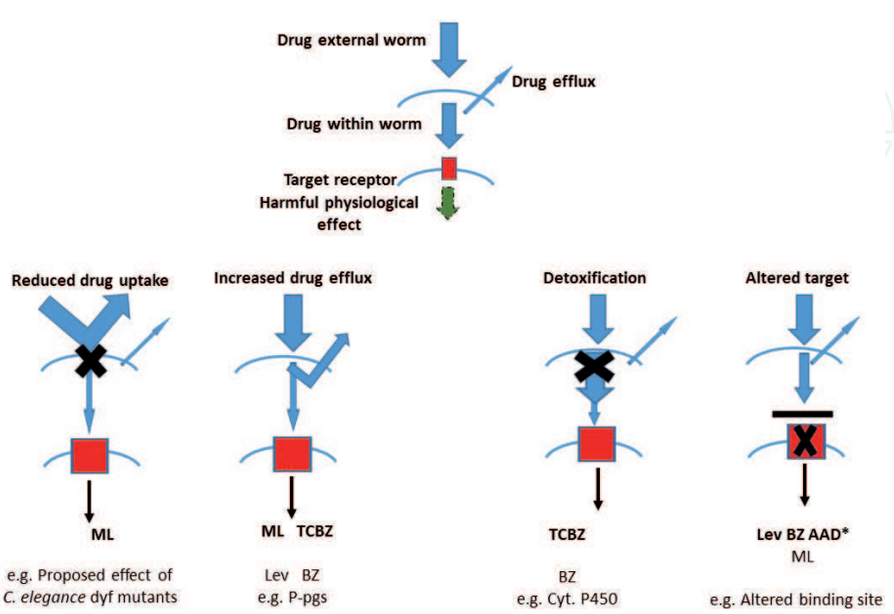

Figure 1.
Schematic representation of principally known anthelmintic resistance pathways and their relevance to each of the current anthelmintic drug classes [8].

representation of principally known anthelmintic resistance (AR) pathways and their relevance to each of the current anthelmintic drug classes.

The classes of broad-spectrum anthelmintics range from benzimidazoles, imidazothiazoles/tetrahydropyrimidines and macrocyclic lactones, but salicylanilides, phenolic substitutes and organophosphates are also popular [13]. Broad-spectrum anthelmintics are more commonly used in ruminants because they are capable of eliminating large numbers of parasites, besides being of easy administration and safe to the hosts [14].

3. Anthelmintic resistance

For decades anthelmintics have been used as the primary control measure for nematode parasites in sheep [15]. However, over the years there has been continuous and significant development of AR by the parasitic worms infecting livestock. 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 treatments pass genes for resistance onto their offspring. These resistant 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 [16].

Earlier work evaluated the knowledge that defined resistance in the year 1980, and from their study, they predicted the spread and future impact of resistance and also set goals for future research [17]. The earliest report of AR was in 1964 for *H. contortus* resistance to benzimidazole in treated sheep and was also the first for a modern drug in production animals [18]. Within 10 years of the first report of AR, resistance was found regularly in sheep parasites, followed by reports of resistance in horse and cattle nematodes [19]. Although anthelmintics have been efficient and work quickly, nematodes have developed resistance in a number of sheep-producing countries such as Australia [20], South Africa [21], New Zealand, [22], Switzerland [23] and Italy [24]. To this end the highest resistance has been observed with ivermectin (Ivomec®) and albendazole (Valbazen®) or fenbendazole (Safeguard® or Panacur®), and low to moderate resistance has been observed with levamisole (Levasole®, Tramisol®). Resistance to moxidectin (Cydectin®) is also prevalent and on the rise on many livestock farms [25]. In Africa, anthelmintic resistance has been reported in both the commercial and resource-poor farming sectors in at least 13 countries, and, among the commercial farms in South Africa, the situation is considered the worst in the world, with high levels of *Haemonchus contortus* resistance to all classes of anthelmintics [26].

Resistance to the two newer classes, the aminoacetonitrile derivatives and paraherquamide derivatives, is expected to follow [27]. There are anthelmintics still available, but multiple drug-resistant helminth strains have quickly developed, and producers and animal health professionals must now seek alternative methods of treatment and prevention [28]. Below are some prominent cases of anthelmintic resistance reported in the world (**Table 1**).

Sadly, anthelmintic resistance is now considered the status quo in most sheep-producing countries of the world [45], and repeated cross-sectional studies in Europe and South America have shown a worsening situation, with both multidrug and multispecies resistance which are increasingly more common [46, 47]. Although it is not widespread, resistance has already developed to two new active ingredients, monepantel and derquantel. This was despite spiroindole—derquantel—being marketed as a combination product to slow the development of resistance [48]. All of these highlight the urgent need to identify risk factors associated with AR development, to inform future recommendations on sustainable parasite control [49].

Country	Anthelmintic (class)	Nematode genera	Year AR reported	References
South Africa	Levamisole, morantel	*Trich*/*Tel* spp.	1990	[29]
South Africa	Benzimidazole, fenbendazole, rafoxinide, levamisole (BZ, SCL, IMID)	*Haemonchus* spp.	1992–1996	[30]
South Africa	Albendazole, closantel, ivermectin, levamisole (BZ, SCL, AVM, IMID)	*Haemonchus* spp., *Trich*/*Tel* spp. and *Oesophagostomum* spp.	2003 and 2013	[31, 32]
Zimbabwe	Fenbendazole, albendazole, oxfendazole, levamisole (BZ, IMID)	*Haemonchus* spp., *Cooperia* spp.	1997 and 2003	[33, 34]
Zimbabwe	Fenbendazole, levamisole, rafoxanide (BZ, IMID, SCL)	*Haemonchus* spp.	1997	[35]
Zambia	Ivermectin, albendazole (AVM, BZ)	*Haemonchus* spp.	2001	[36]
Kenya	Ivermectin, fenbendazole (AVM, BZ)	*Haemonchus* spp., *Trich*/*Tel* spp. and *Oesophagostomum* spp.	1995	[37]
Germany	Levamisole, ivermectin (IMID, AVM)	*Trich*/*Tel* spp.	2012	[38]
Norway	Albendazole (BZ)	*Trich*/*Tel* spp.	2012	[39]
Northern Ireland	Benzimidazole, moxidectin, avermectin, levamisole (BZ, MLB, AVM, IMID)	*Trich*/*Tel* spp., *Cooperia* spp.	2013	[40]
Switzerland	Avermectin (AVM)	*Haemonchus* spp., *Trich*/*Tel* spp.	2007	[41]
Brazil	Ivermectin (AVM)	*Haemonchus* spp.	2013	[42]
India	Fenbendazole, benzimidazole, thiabendazole, tetramisole (BZ, IMID)	*Haemonchus* spp., *Trich*/*Tel* spp.	2013, 2011	[43], [44]

BZ, benzimidazoles; ML, macrocyclic lactones (AVM, avermectines, or MLB, milbemycin); nicotinic agonists (IMID, imidazothiazoles, or TETR, tetrahydropyrimidines); AAD, aminoacetonitrile derivatives; SCL, salicylanilides; Tel, Teladorsagia; Trich, Trichostrongylus

Table 1.
Some cases of anthelmintic resistance.

4. Risk factors for development of AR

The control of gastrointestinal parasitism for small ruminants has long been under threat from the development of anthelmintic resistance by parasite populations [46]. However, in recent years it has become evident that this is also an emerging problem for cattle [50]. Resistance against drugs belonging to the same anthelmintic drug class is called side resistance, whereas cross and multidrug resistance refer to resistance against two or multiple drugs belonging to different anthelmintic drug classes [47]. Development of AR can be limited by ensuring that the parasites are exposed to an effective drug dose and to consider the timing and

frequency of anthelmintic drug treatments so that only a small proportion of the population is exposed to the anthelmintic [51]. The main factors for the selection for anthelmintic resistance are high-treatment frequency, [52] underdosing and the use of the same anthelmintic class over several years [48]. These factors, individually or in combination, together with the risk of underdosing and continued use of one class of anthelmintics, irrespective of efficacy status are frequently encountered factors enhancing development of anthelmintic resistance [53]. Underestimation of real weight has a potential to lead to underdosing, which can contribute to the development of AR [48]. The results of a South African study attributed AR observed in goats to underdosing caused by visual appraisal of an animal to estimate its weight as opposed to the actual weighing before dosing to determine the correct anthelmintic dosage [31]. In consideration of ensuring a correct dose, livestock farmers have to determine the weight as accurately as possible, preferably by individually weighing each animal [40]. Alternatively, the use of a heart girth measurement tape is also recommended as this would certainly provide small-scale farmers with a practical tool to be used in determining the live weight of their small stock [54]. The use of faecal egg count reduction tests (FECRT) and egg hatch assays in combination with morphological identification of third-stage larvae recovered from pre- and post-treatment cultures may provide a solid indication of the presence of anthelmintic resistance.

5. Anthelmintic resistance monitoring

5.1 Faecal egg count reduction test

The faecal egg count reduction test is the main method of detection of anthelmintic resistance in nematodes of veterinary importance [55]. In the FECRT, populations of gastrointestinal nematodes of sheep are considered susceptible when drug efficacy exceeds 95% (reduction in FECRT). Conversely, resistance is present when efficacy is <95%. The equivalent efficiency benchmark for resistance is 90% for other host species. However, reductions in efficacy require interpretation in the light of different situations [56], where, for instance, the 95% cutoff is more complex than it seems because some drugs have very high efficacy (99.9%) against some parasite species but lower (say, 95%) for others in the same host. FECRT is an in vivo method that involves the nematodes in the sheep as the experimental unit [57–59]. An advantage of FECRT is that it can be used with all groups of anthelmintics that are available today. The disadvantage is that the faecal egg count (FEC) levels do not always correspond to the number of adult worms inside the animals. However, FECs in young sheep correlate fairly well to the burden of adult worms, at least compared to the situation in adult sheep [58, 59]. Furthermore, the FECRT can only detect AR if there are over 25% of resistant nematodes in a population and also requires a large number of sheep and is therefore difficult to be used in small flocks [58, 59]. Whilst FECRT has been used for over 30 years, more recent work has revealed shortcomings in the diagnosis of resistance based on proportional reduction. The problem is that diagnosis overestimates resistance when it is emerging. A study by Lyndal-Murphy [60] reported on the use of statistical simulation studies to consider situations for sheep where resistance is defined as <95% efficacy. This study has shown that FECRT results too often diagnose resistance where it does not exist. FECRT has been used successfully to detect AR in many other countries including Zimbabwe [33], Zambia [36], Brazil [42], Kenya [37] and Switzerland [41].

5.2 Egg hatch test

Egg hatch test (EHT) is an in vitro test that can be used to measure AR [61]. EHT can only measure BZ resistance. In practice, fresh eggs are either diluted in increasing concentrations of thiabendazole (TBZ) or diluted in a predetermined concentration (discriminating dose) and incubated for 48 hours. The eggs hatched are then counted under an inverted microscope. Discriminating doses have been established in nematode species such as *H. contortus*. A discriminating dose is the dose required to prevent hatching of 99% of susceptible eggs. The EHT can detect resistance if there are at least 2–3% resistant eggs [58].

Egg hatch test and other in vitro tests generate dose-response lines [10]. This allows the calculation of parameters, such as the concentration that kills 95% of eggs (the EC95), a single parameter used to compare isolates. Resistant worms will have a higher EC95 because a higher drug concentration is required to kill them. Such assays are underutilised tools for measuring resistant phenotypes. However, they have been fundamental tools for studying the results of experimental genetic crosses [62].

5.3 Larval development assay

Two versions of larval development test are used. The first was described in detail by Hubert and Kerbouf in 1992 [63]. The counted number of eggs in a 0.5 mL of egg suspension is put into each well in a 96-microtitre plate. The contents of the wells are then mixed, and the plates placed in an incubator under humidified conditions at 27°C for 48 hours for incubation of the eggs. After 48 hours, thiabendazole is added to the plates containing the egg suspension. The plates are incubated for 5 days; after which they will be examined to determine the survival of the larvae at different concentrations. All the L_3-stage larvae in each well must be counted, and the percentage inhibition of larval development is calculated using the formula [64]:

$$E = \frac{(\text{Eggs} + \text{L1}) - \text{L1}}{\text{Eggs} + \text{L1}} \times 100 \quad (1)$$

In the second version, the micro-agar larval development test (MALDT) is performed as described by Coles et al. [57]. This test is also performed on 96-microtitre well plates. Stock solutions of thiabendazole/levamisole are prepared by predissolving the drugs in dimethyl sulfoxide (DMSO) with subsequent dilution in distilled water (1:4). Nematode eggs recovered from faecal samples are incubated for 7 days at 27°C in 96-well microtitre plates with the drug solution. The plates will normally have a culture medium (yeast extract with Earle's balanced salt solution and physiologic salt solution) in an aquatic solution of various concentrations of thiabendazole/levamisole and the determined proportion of nematode eggs in each well. After 7 days, the numbers of unhatched eggs and L_1–L_3 larvae in each well are counted under an inverted microscope. The rate of L_3 development in the discriminating dose (0.02 and 0.5 μg/ml for thiabendazole and levamisole, respectively) compared to the control is then used to determine if resistance is present; thus, the number of larvae developing from L_1 to L_3 stage in the discriminating dose of 0.02 μg/ml thiabendazole and 0.5 μg/ml levamisole is a clear indication of resistance.

5.4 Use of molecular techniques for AR monitoring

Nowadays, the traditional parasitological diagnostic techniques involving mainly microscopy have been complemented by a variety of new techniques and

tools, mostly molecular in nature. To date, traditional methods are still routinely used despite the fact that they can be labour and time intense to perform [25]. PCR-based procedures have been proven to have greater sensitivity and specificity than 'conventional' diagnostic approaches reliant on microscopy and/or immune detection [65]. Studies with other models of resistance to xenobiotics demonstrated that migration plays a fundamental role in such things as the dispersion of insecticide-resistant genes in mosquitoes [66] and of antibiotic resistance among some species of bacteria [67]. There have been studies on the origin of the BZ-resistant alleles in worm populations. For instance, using RFLP studies on the isotype 1-tubulin gene, it was established that there are various BZ-resistant alleles in different resistant populations of *H. contortus* [68]. Using the same approach on two BZ-resistant populations, it was also found that the BZ-resistant alleles were probably already present in two *H. contortus* populations before this class of drugs was even developed [69].

6. FAMACHA© and targeted selective treatment

With nematode resistance now present to all three of the broad-spectrum anthelmintic classes (benzimidazoles, levamisole and macrocyclic lactones) used on ruminants [52], control strategies aiming to sustain effective parasitic control are of key importance. Methodologies designed to maintain refugia which are the size of the unselected proportion of the nematode population can help to reduce the build-up of resistance by preserving susceptible nematode genotypes which helps to dilute the frequency of resistance alleles and maintain anthelmintic efficacy [70].

One strategy that aims to achieve this is targeted selective treatment (TST), which involves the treatment of selected individuals that require treatment as opposed to treatment of the entire group [71]. Individuals are generally identified as needing to receive treatment on the basis of their level of parasitism [3]. Although TST strategies have been developed and applied successfully in sheep, there are considerably fewer studies on cattle, with the first insights into the application of TST having occurred relatively recently [72]. As there are important differences in host-parasite interactions and parasite epidemiology between cattle and sheep, differences in the methodology and application of TST in cattle can be expected. Although TST strategies in sheep have been shown to be beneficial in reducing selection for anthelmintic resistance [72], it is difficult to know which of the various strategies would be most effective under various scenarios. At present there are no direct comparisons of TST strategies in cattle, in part due to difficulties arising from confounding variables [72].

Simulation modelling on the other hand may offer an effective alternative and be highly beneficial in assessing the feasibility of novel control strategies. In the FAMACHA© system, operators assess the severity of parasitism by using a conjunctival colour chart which correlates to anaemia to choose affected animals for selective treatment. FAMACHA© was developed by a South African veterinarian, and it stands for Faffa Malan Chart. The application of FAMACHA© has been a pivotal example of a practical approach in managing resistance, as targeted treatment provides many potential benefits. One benefit is that it helps in the removal of worms from the most severely infected and affected animals and so reduces production losses in the most impacted animals. These animals also shed more eggs than other animals, so targeted treatment of a small proportion of the flock reduces a large proportion of pasture contamination. Most importantly, it reduces selection by reducing chemical use and maintaining refugia.

7. Holistic control strategy to control anthelmintic resistance

General risk factors for the development of AR in livestock include overuse of anthelmintics, underdosing, frequent movement and transfer of animals from one area to another and poor pasture management. Techniques such as body condition scoring, faecal egg detection, larval detection and FAMACHA© are still relatively underutilised when in combination with the use of anthelmintics. We propose that countries must develop integrated holistic system that will be a combined effort between animal health professionals, extension officers, farmer unions and drug companies where education is one of the most important components for helminthosis control and prevention of AR development (**Figure 2**). Farmers, both at large-scale commercial and small-scale communal farming, need to be constantly conscientised on the proper use of anthelmintics, pasture management and purchase and transportation of livestock from one area to another.

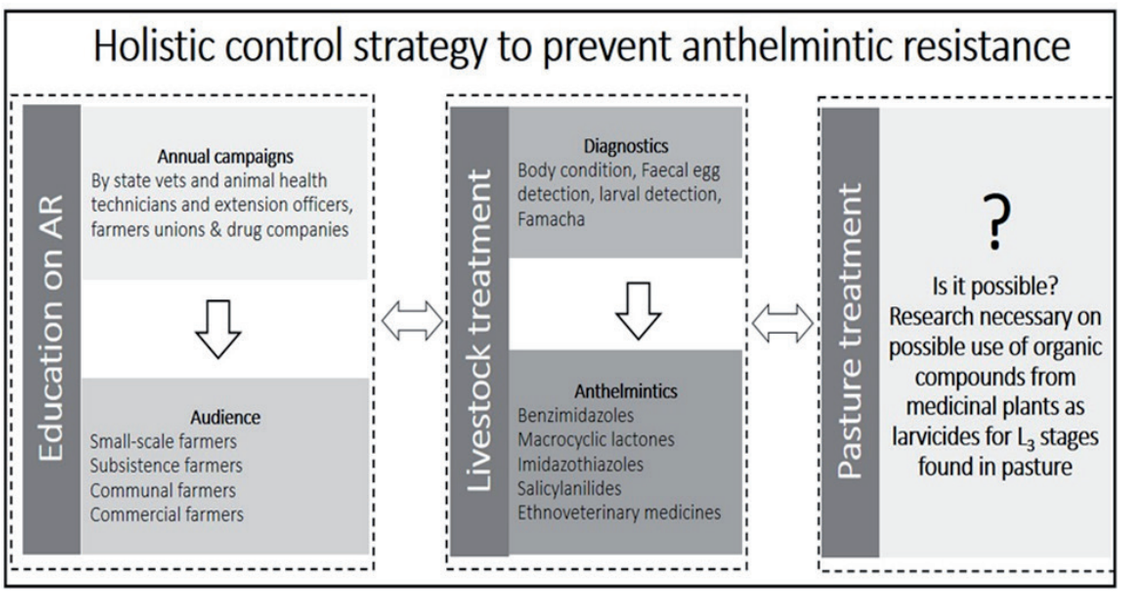

Figure 2.
A holistic AR prevention strategy which includes annual education campaigns to all types of farmers and application of different diagnostic techniques which then dictates necessary anthelmintic treatment.

Direct anthelmintic-like effects have been demonstrated in in vitro assays, which have shown that incubation in crude condensed tannin extracts reduced the development, viability, motility and migratory ability of parasite larvae [73]. Whilst there will be continuous development of synthetic compounds which will be used as anthelmintics, there is a need for increased scientific studies of conversion and adoption of natural compounds extracted from medicinal plants as a substantial number of them has been reported to contain anthelmintic activity [74]. Future research should also focus on possible treatment of pasture with organic compounds from medicinal plants in an attempt to control the larval stages of helminths.

8. Perspective (future control and prevention methods, necessary research)

Many of the approaches that are available for prevention of AR are still being researched and evaluated, and most of them are at present not suitable for the

communal grazing systems of many resource-poor farmers; therefore, further research must still be conducted to ensure adaptability to both commercial and resource-poor farming operations. Another challenge facing both the farmers and researchers alike could be that even though the AR monitoring techniques has been used for years, correlation between in vivo and in vitro tests for detecting BZ resistance is not always good [75]. This is probably because in vitro tests are more sensitive than in vivo tests [20], and those shortcomings concerning sensitivity and specificity could be subjugated by the use of molecular techniques than are not reliant on microscopy. In order to win the battle against the emergence of AR, correct use of anthelmintics and on-farm training about gastrointestinal helminths infecting livestock must be provided. Such training should be ongoing and provided by extension officers together with animal health technicians. Training initiatives should incorporate practical demonstrations and focus on aspects such as the importance of correct dosage, when to alternate anthelmintic classes and treatment frequency. Furthermore, a sustainable integrated parasite management must become the new paradigm, where anthelmintics are used much less frequently and in a more targeted and strategic manner following the principles of smart drenching and FAMACHA© together with a variety of nondrug-based practices. These strategies can be employed in combination with faecal egg counts.

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Conflict of interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this book chapter.

Declarations

The authors declare that all the literature and sources used in the writing of this book chapter have been properly cited in the text and in the reference section.

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References

[1] Van Dijk J, Sargison ND, Kenyon F, Skuce PJ. Climate change and infectious disease: Helminthological challenges to farmed ruminants in temperate areas. Animal. 2010;**4**:377-392

[2] Waller PJ. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology. 2006;**126**:277-289

[3] Charlier J, Van der Voort M, Kenyon F, Skuce PJ, Vercruysse J. Chasing helminths and their economic impact on farmed ruminants. Trends in Parasitology. 2014;**30**:361-367

[4] Sanchez VVV, Patiño AS, Segundo VJP, Sandoval JACS, Esquivel CVC, Sanchez TAC. Prevalence of gastro-intestinal parasites among captive primates in Panama. Journal of Animal and Veterinary Advances. 2009;**8**:2644-2649

[5] Roeber F, Jex AR, Gasser RB. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance: An Australian perspective. Parasites & Vectors. 2013;**6**:153

[6] Grisi L, Leite CR, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, et al. Reassessment of the potential economic impact of cattle parasites in Brazil. Brazilian Journal of Veterinary Parasitology. 2014;**23**(2):150-156. DOI: 10.1590/S1984-29612014042. PMid:25054492

[7] Traversa D, Von Samson-Himmelstjerna G. Anthelmintic resistance in sheep gastrointestinal strongyles in Europe. Small Ruminant Research. 2015;**135**:75-80

[8] Kohler P. The biochemical basis of anthelmintic action and resistance. International Journal for Parasitology. 2001;**31**:336-345

[9] Mansour TE. Chemotherapeutic Targets in Parasites: Contemporary Strategies. Cambridge: Cambridge University Press; 2002. pp. 156-188

[10] Sangster NC, Dobson RJ. Anthelmintic resistance. In: Lee DL, editor. The Biology of Nematodes. London: Taylor & Francis; 2002. pp. 531-567

[11] Vercruysse J, Claerebout E. Overview of anthelmintics. In: The Merck Veterinary Manual. 2014. Retrieved from: http://www.merckvetmanual.com/mvm/pharmacology/anthelmintics/overview_of_anthel mintics.html

[12] Kotze AC, Hunt PW, Skuce P, Von Samson Himmelstjerna G, Martin RJ, Sager H, et al. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. International Journal for Parasitology: Drugs and Drug Resistance. 2014;**4**:164-184

[13] Oliveira-Sequeira TCG, Amarante AFT. Parasitologia animal: Animais de produção. 1st ed. Rio de Janeiro: EPUB; 2001

[14] Monteiro SG. Parasitologia na medicina veterinária. 1st ed. São Paulo: ROCA; 2011

[15] Baloyi MA, Laing MD, Yobo KS. Use of mixed cultures of biocontrol agents to control sheep nematodes. Veterinary Parasitology. 2012;**184**:367-370

[16] Geary TG, Hosking BC, Skuce PJ, Von Samson-Himmelstjerna G, Maeder S, Holdsworth WP, et al. WAAVP

guideline on anthelmintic combination products targeting nematode infections of ruminants and horses. Veterinary Parasitology. 2012;**190**:306-316

[17] Prichard RK, Hall CA, Kelly JD, Martins ICA, Donald AD. The problem of anthelmintic resistance in nematodes. Australian Veterinary Journal. 1980;**56**(5):239-251. DOI: 10.1111/j.1751-0813.1980.tb15983.x. PMid:7002142

[18] Drudge JH, Szanto J, Wyant ZN, Elam G. Field studies on parasitic control in sheep: Comparison of thiabendazole, ruelene, and phenothiazine. American Journal of Veterinary Research. 1964;**25**:1512-1518

[19] Jackson R, Rhodes AP, Pomroy WE, Leathwick DM. Anthelmintic resistance and management of nematode parasites on beef cattle-rearing farms in the North Island of New Zealand. New Zealand Veterinary Journal. 2006;**54**:289-296

[20] Overend DJ, Phillips ML, Poultion AL, Foster C. Anthelmintic resistance in Australian sheep nematode populations. Australian Veterinary Journal. 1994;**71**:117-121

[21] Ahmed M. Gastrointestinal (nematode) infections in small ruminants: Epidemiology, anthelmintic efficacy and the effect of wattle tannins [MSc thesis]. University of KwaZulu-Natal: Pietermaritzburg, South Africa; 2010

[22] Leathwick DM, Pomroy WE, Heath AC. Anthelmintic resistance in New Zealand. New Zealand Veterinary Journal. 2001;**49**:227-235

[23] Schnyder M, Torgerson PR, Schönmann M, Kohler L, Hertzberg H. Multiple anthelmintic resistance in *Haemonchus contortus* isolated from South African Boer goats in Switzerland. Veterinary Parasitology. 2005;**128**:285-290

[24] Cringoli G, Veneziano V, Rinaldi L, Sauve C, Rubino R, Fedele V, et al. Resistance of trichostrongyles to benzimidazoles in Italy: A first report in a goat farm with multiple and repeated introductions. Parasitology Research. 2007;**101**:577-581

[25] Burke J. Management of Barber pole Worm in Sheep and Goats in the Southern U.S. Booneville AR. Dale Bumpers Small Farms Research Update, n. p. 2005

[26] Anon. The World Factbook. CIA Directorate of Intelligence. 2007. Available from: http://www.cia.gov/ciapublicatins/pubs.html (Accessed: March 2007)

[27] Kaminsky R, Ducray P, Jung M, Clover R, Rufener L, Bouvier J, et al. A new class of anthelmintics effective against drug-resistant nematodes. Nature. 2008;**452**(7184):176-180. DOI: 10.1038/nature06722

[28] Jackson F, Coop RL. The development of anthelmintic resistance in sheep nematodes. Parasitology. 2001;**120**:95-107. DOI: 10.1017/S0031182099005740

[29] Van Wyk JA. Occurrence and dissemination of anthelmintic resistance in South Africa, and management of resistant worm strains. In: Boray JC, Martin PJ, Roush RT (eds). Resistance of parasites to antiparasitic drugs. Round Table Conference held at the VIIth International Congress of Parasitology, Paris; August 1990; MSD Agvet, Division of Merck, Rahway: New Jersey; 1990. pp. 103-113

[30] Van Wyk JA, Stenson MO, Van der Merwe JS, Vorster RJ, Viljoen PG. Anthelmintic resistance in South Africa: Surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort Journal of Veterinary Research. 1999;**66**:273-284

[31] Bakunzi FR. Anthelmintic resistance of nematodes in communally grazed goats in a semi-arid area of South Africa. Journal of the South African Veterinary Association. 2003;**74**:82-83

[32] Tsotetsi AM, Njiro S, Katsande TC, Moyo G, Baloyi B, Mpofu J. Prevalence of gastrointestinal helminths and anthelmintic resistance on small-scale farms in Gauteng Province, South Africa. Tropical Animal Health and Production. 2013;**45**:751-761

[33] Mukaratirwa S, Charakupa R, Hove TA. Survey of anthelmintic resistance on ten sheep farms in Mashonaland East Province, Zimbabwe. Journal of the South African Veterinary Association. 1997;**68**(4):140-143

[34] Matika O, Van Wyk JB, Erasmus GJ, Baker RL. Genetic parameter estimates in Sabi sheep. Livestock Production Science. 2003;**79**:17-28

[35] Boersema JH, Pandey VS. Anthelmintic resistance of trichostrongylids in sheep in the Highveld of Zimbabwe. Veterinary Parasitology. 1997;**68**(4):383-388. DOI: 10.1016/S0304-4017(96)01089-8

[36] Gabriel S, Phiri IK, Dorny P, Vercruysse J. A survey on anthelmintic resistance in nematode parasites of sheep in Lusaka, Zambia. Onderstepoort Journal of Veterinary Research. 2001;**68**:271-274

[37] Mwamachi DM, Audho JO, Thorpe W, Baker RL. Evidence for multiple anthelmintic resistance in sheep and goats reared under the same management in coastal Kenya. Veterinary Parasitology. 1995;**60**:303-313

[38] Voigt K, Scheuerle M, Hamel D. Triple anthelmintic resistance in *Trichostrongylus* spp. in a German sheep flock. Small Ruminant Research. 2012;**106**:30-32

[39] Domke AVM, Chartier C, Gjerde B, Hoglund J, Leine N, Vatn S, et al. Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. Parasitology Research. 2012;**111**:185-193

[40] McMahon C, Edgar HW, Barley JP, Hanna REB, Brennan GP, Fairweather I. Control of *Nematidirus* spp. infection by sheep flock owners in Northern Ireland. Irish Veterinary Journal. 2017;**70**:31. DOI: 10.1186/s13620-0109-6

[41] Artho R, Schnyder M, Kohler C, Torgerson PR, Hertzberg H. Avermectin resistance in gastrointestinal nematodes of Boer goat and Dorper sheep in Switzerland. Veterinary Parasitology. 2007;**144**:68-73

[42] Fortes FS, Kloster FS, Schafer AS, Bier D, Buzatti A, Yoshitani UY, et al. Evaluation of resistance in a selected field strain of *Haemonchus contortus* to ivermectin and moxidectin using the larval migration on agar test. Pesquisa Veterinaria Brasileira. 2013;**33**:183-187

[43] Rialch A, Vatsya S, Kumar RR. Detection of benzimidazole resistance in gastrointestinal nematodes of sheep and goats of sub-Himalayan region of northern India using different tests. Veterinary Parasitology. 2013;**198**:312-318

[44] Swarnkar CP, Singh D. Role of quarantine in management of anthelmintic resistance in strongyle worms of sheep. Indian Journal of Small Ruminants. 2012;**18**(1):95-99

[45] Kaplan RM, Vidyashankar AN. An inconvenient truth: Global worming and anthelmintic resistance. Veterinary Parasitology. 2012;**186**:70-78

[46] Papadopoulos E, Gallidis E, Ptochos S. Anthelmintic resistance in sheep in Europe: A selected review. Veterinary Parasitology. 2012;**189**:85-88

[47] Torres-Acosta JFJ, Mendoza-De-Gives P, Aguillar-Caballero AJ, Cuellarordaz JA. Anthelmintic resistance in sheep farms: Update of the situation in the American continent. Veterinary Parasitology. 2012;**189**:89-96

[48] Sangster NC, Cowling A, Woodgate RG. Ten events that defined anthelmintic resistance research. Trends in Parasitology. 2018;**34**:553-563. DOI: 10.1016/j.pt.2018.05.001

[49] Morgan ER. Detail and the devil of on-farm parasite control under climate change. Animal Health Research Reviews. 2013;**14**:138-142

[50] Sutherland IA, Leathwick DM. Anthelmintic resistance in nematode parasites of cattle: A global issue? Trends in Parasitology. 2011;**27**:176-181

[51] Sargison ND. Keys to solving problems in small ruminants: Anthelmintic resistance as a threat to sustainable nematode control. Small Ruminant Research. 2016;**142**:11-15

[52] Vijayasarathi MK, Sreekumar C, Venkataramanan R, Raman M. Influence of sustained deworming pressure on the anthelmintic resistance status in strongyles of sheep under field conditions. Tropical Animal Health and Production. 2016;**48**:1455-1462

[53] Čerňanská D, Várady M, Čudeková P, Čorba J. Worm control practices on sheep farms in the Slovak Republic. Veterinary Parasitology. 2008;**154**:270-276. DOI: 10.1016/j.vetpar.2008.03.026

[54] De Villiers JF, Gcumisa ST, Gumede SA, Thusi SP, Dugmore TJ, Cole M, et al. Estimation of live body weight from the heart girth measurement in KwaZulu–Natal goats. Applied Animal Husbandry and Rural Development. 2009;**1**:1-8

[55] Calvete C, Uriarte J. Improving the detection of anthelmintic resistance: Evaluation of faecal egg count reduction test procedures suitable for farm routines. Veterinary Parasitology. 2013;**196**:438-452

[56] Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, et al. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology. 1992;**44**(1-2):35-44. DOI: 10.1016/0304-4017(92)90141-U

[57] Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology. 2006;**136**:167-185

[58] Vatta AF, Lindberg ALE. Managing anthelmintic resistance in small ruminant livestock of resource-poor farmers in South Africa. Journal of the South African Veterinary Medical Association. 2006;**77**:2-8

[59] Coles GC. Sustainable use of anthelmintics in grazing animals. Veterinary Record. 2002;**151**:165-169

[60] Lyndal-Murphy M, Swain AJ, Pepper PM. Methods to determine resistance to anthelmintics when continuing larval development occurs. Veterinary Parasitology. 2014;**199**(3-4):191-200. DOI: 10.1016/j.vetpar.2013.11.002

[61] Le Jambre LF. Egg hatch as an *in vitro* assay of thiabendazole resistance in nematodes. Veterinary Parasitology. 1976;**2**:385-391

[62] Elard L, Cabaret J, Humbert JF. PCR diagnosis of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, Teladorsagia

circumcincta. Veterinary Parasitology. 1999;**80**:231-237

[63] Hubert J, Kerbouf DA. Microlarval development essay for the detection of anthelmintic resistance in sheep nematode. Veterinary Record. 1992;**130**:442-446

[64] Cala AC, Chagas ACS, Oliveira MCS, Matos AP, Borges LMF, Sousa LAD, et al. *In vitro* anthelmintic effect of *Melia azedarach* L. and *Trichilia claussenii* C. against sheep gastrointestinal nematodes. Experimental Parasitology. 2012;**130**:98-102

[65] Garcia LS, Arrowood M, Kokoskin E, Paltridge GP, Pillai DR, Procop GW, et al. Laboratory diagnosis of parasites from the gastrointestinal tract. Clinical Microbiology Reviews. 2018;**31**: e00025-17. DOI: 10.1128/CMR.00025-17

[66] Raymond M, Callaghan A, Fort P, Pasteur N. Worldwide migration of amplified insecticide resistance genes in mosquitoes. Nature. 1991;**350**:151-153

[67] O'Brien TF. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. Clinical Infectious Diseases. 1997;**24**:2-8

[68] Kwa MS, Veenstra JG, Roos MH. Molecular characterization of β-tubulin genes present in benzimidazole-resistant populations of *Haemonchus contortus*. Molecular and Biochemical Parasitology. 1993b;**60**:133-144

[69] Blouin MS, Yowell CA, Courtney CH, Dame JB. Host movement and the genetic structure of populations of parasitic nematodes. Genetics. 1995;**141**:1007-1014

[70] van Wyk JA. Refugia e overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort Journal of Veterinary Research. 2001;**68**:55-67

[71] van Wyk JA, Hoste H, Kaplan RM, Besier RB. Targeted selective treatment for worm management-how do we sell rational programs to farmers. Veterinary Parasitology. 2006;**139**:336-346

[72] O'Shaughnessy J, Earley B, Mee JF, Doherty ML, Crosson P, Barrett D, et al. Nematode control in suckler beef cattle over their first two grazing seasons using a targeted selective treatment approach. Irish Veterinary Journal. 2015a;**68**:13

[73] Molefe NI, Tsotetsi AM, Ashafa AOT, Thekisoe OMM. *In vitro* anthelmintic activity of *Artemisia afra* and *Mentha longifolia* against parasitic gastro-intestinal nematodes of livestock. Bangladesh Journal of Pharmacology. 2012;**7**:157-163

[74] Waller PJ. International approaches to the concept of integrated control of nematode parasites of livestock. International Journal for Parasitology. 1999;**29**(1):155-164. DOI: 10.1016/S0020-7519(98)00178-7

[75] Dolinka M, Ivanisinova O, Königová A, Várady M. Anthelmintic resistance in sheep gastrointestinal nematodes in Slovakia detected by in-vitro methods. BMC Veterinary Research. 2014;**10**:233