Review Article

Anthelmintic resistance in parasites of small ruminants: sheep versus goats

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Summary

Anthelmintic resistance among parasites of sheep and goats has been known to occur for at least four decades. Both species of host have similar genera of nematodes, but the nematodes in goat herds usually develop anthelmintic resistance more rapidly. *In vitro* tests show higher ED_{50} values in goats than in flocks of sheep. Sheep and goats differ in many ways; for example, goats have a higher metabolic rate and require higher dose rates for drugs. The immune system of goats is also different. Additionally, these animals are reared under different management systems, i.e. sheep graze pastures and goats browse bushes, and lambing/kidding periods are different. Most anthelmintics used in goats have not been licensed for this animal species, and correct dose rates have rarely been experimentally determined. Possible explanations for such differences are discussed.

Keywords: anthelmintic resistance; sheep; goats

Introduction

Gastrointestinal nematode (GIN) infections remain one of the most prevalent and important issue affecting small ruminants worldwide. They are responsible for both direct and indirect major losses, causing decreased productivity, costs of control measures and deaths (Sykes, 1994; Torres-Acosta & Hoste, 2008). Up to now the control of GIN was largely based on the repeated use of chemical anthelmintic drugs. However, it is well known that by nowadays their efficacy has been reduced in many cases due to the development of anthelmintic resistance. Therefore, complementary or alternative solutions to the conventional chemical treatments have been implied offering novel approached to the sustainable control of GIN in sheep and goats. This is also supported by an enhanced public concern for more sustainable systems of production, less reliant on the use of chemicals. However, before adapting any of these approaches careful consideration is needed to apply it correctly since sheep and goats may demand different application (Papadopoulos, 2008; Torres-Acosta & Hoste, 2008).

Anthelmintic resistance in nematodes of sheep and goats

At present, the agents of several infectious diseases regularly exposed to therapeutic doses of drugs tend to gradually develop resistance against the drugs. Anthelmintic resistance has been defined as a genetic change in the ability of parasites to survive treatments with recommended doses of anthelmintic. The term 'resistance' describes the condition of nematode populations that, despite being previously sensitive to anthelmintics, inherit the ability to survive and evade the toxic effects of drugs after repeated administration.

Anthelmintic resistance has increased to become an important economic problem in several animal industries. Gastrointestinal nematodes are associated with production losses and even mortality. The modern broad-spectrum anthelmintics are currently widely used in prophylaxis and treatment of helminth infections in farm animals. As seen with antibiotics, an overuse of anthelmintics may result in the development of resistance in targeted organisms. The problem of resistance to chemotherapeutic drugs has gradually grown from its rather sporadic occurrence in the early 1960s to the current status where anthelmintic resistance threatens the sustainability of many intensive systems of production. At present, the problem of anthelmintic resistance occurs in several genera and classes of helminths with all three groups of commercially available anthelmintics - the benzimidazoles, imidazothiazoles and macrocyclic lactones.

Resistance to anthelmintics has particularly become a major problem in small ruminants infected with gastro-

intestinal nematodes of the family Trichostrongylidae. The nematode *Haemonchus contortus*, which parasitizes the abomasum of small ruminants, was the first parasite ever to develop resistance. Resistance to phenothiazine was reported in the USA in 1957 (Drudge *et al.*, 1964) within two decades of the drug's introduction onto the market. Resistance has developed mainly in *H. contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Ostertagia* spp. and *Cooperia* spp., affecting Australia, New Zealand, South Africa, many European countries, several Asian countries and both American continents. The problem of anthelmintic resistance is evidently more serious than has been documented by current data, as every country that participated in an occurrence survey reported resistant populations of gastrointestinal nematodes (Coles, 2004). Many areas with intensive farming of small ruminants, however, have yet to be surveyed. Since the early 1980s, resistance has been detected among the gastrointestinal nematode parasites of sheep and goats throughout the world, and large-scale surveys have shown that the situation is critical in many Latin American countries, South Africa, Australia and New Zealand. The severity of the situation is highlighted by a comparison of the resistance surveys conducted on sheep farms in Australia in 1991 and 2006. The earlier survey reported resistance to benzimidazole anthelmintics on 85 % of the farms, and 65 % of the farms harboured resistance to another group of anthelmintics - imidazothiazoles. On the other hand, resistance to macrocyclic lactones was not detected (Overend *et al.*, 1994). The survey conducted 15 years later recorded the same or moderately increased levels of resistance to benzimidazoles (90 %) and imidazothiazoles (80 %), but resistance to macrocyclic lactones had increased to 70 % (Besier, 2007). Surprisingly, the spread of benzimidazole resistance in several European countries (Greece, Italy, Spain, Sweden and Slovakia) is rather low (approx. 10 % of the farms), while countries such as Great Britain, the Netherlands and Switzerland report more than 80 – 90 % of the farms with resistance to benzimidazoles.

Cross resistance or multidrug resistance presents another peculiar problem often encountered. These terms refer to resistance to two or more anthelmintics from unrelated chemical groups with different modes of action. One of the worst scenarios was seen in South Africa, where a survey conducted on resistance of parasites of sheep revealed multiresistant parasites on 40 % of the farms, a case of cross resistance to all types of the broad-spectrum anthelmintics (Van Wyk *et al.*, 1999). A similar situation is also present in Australia, New Zealand and several countries of South America. This rapid evolution of multiple anthelmintic resistance leads to total anthelmintic failure in some areas (Chandrawathani *et al.*, 2004; Thomaz-Soccol *et al.*, 2004). Such outcomes have eliminated any possibility of therapeutic treatment. An ineffective therapy leads to an increased intensity of infection in the herd, thus directly affecting parameters of production such as reduced weight gain and slaughter weight of the animals (Sutherland *et al.*, 2010).

Goat farming is less common than sheep farming, as are studies on anthelmintic resistance in nematodes of goats. Less research on parasites of goats, however, does not necessarily mean less frequent resistance. The first case of benzimidazole-resistant *T. colubriformis* in goats was reported in 1970. Since then, dozens of cases have been documented throughout the world. The prevalence is particularly high in Australia and South America, but reports of elevated prevalence in Europe are increasing (Bauer, 1988; Bauer, 2001; Hertzberg & Bauer, 2000; Schnyder *et al.*, 2005; Artho *et al.*, 2007; Cringoli *et al.*, 2007). Populations of parasites tend to quickly lose sensitivity to administered drugs (Chartier & Hoste, 1994; Chartier *et al.*, 2001), particularly in large flocks with industrial schemes of production, high stocking rates, and frequent treatment. Resistance is believed to be more frequent in parasites of goats than in those of sheep. Cases of the occurrence of multiresistant populations to all types of broad-spectrum anthelmintics in goats have also been reported. It is worth noted that usually *in vitro* tests show higher benzimidazole-ED_{50} values in goats than in sheep flocks (Papadopoulos *et al.*, 2001; Gallidis *et al.*, 2009).

Methods for detection of anthelminthic resistance

The emergence and rapid spread of anthelminthic resistance worldwide have forced the development of several in vitro and in vivo techniques for the detection of resistance. Generally, in vivo techniques are rather time- and money-consuming and are often characterized by low reproducibility of results (accuracy and interpretation) that may be caused by differences in drug pharmacodynamics in treated animals. At present, the most accurate technique used for assessing anthelminthic efficacy is an in vivo control test, where the percentage reduction in adult nematodes is calculated after helminthological dissection. Considering the fact that the minimum number of animals needed to estimate the efficacy of an anthelminthic is seven per group, with data compared to those of a control group (an additional seven animals), then a total of 14 animals are needed to estimate a drug's efficacy, which is not negligible in terms of costs.

The most widely used in vivo technique is the Faecal Egg Count Reduction (FECR) test, recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). The test is based on a comparison of the number of eggs per gram (EPG) of faeces on the day of application to the number of eggs 10 to 14 days later. The tested population of nematodes is considered resistant if the reduction in EPG is less than 95 %. The technique itself is described in detail by Coles *et al.* (2006).

In vitro tests can be divided into two groups based on their effects: pharmacological and biochemical. In the former, physiological functions of the parasites are directly affected (e.g. production of parasite eggs or larvae), while in the latter, biochemical processes are affected (e.g. drug binding to larval tubulin or eserine to receptors). The

scientific literature offers several reviewes on using methods for the detection of anthelminthic resistance (Presidente, 1985; Johansen, 1989; Taylor & Hunt, 1989; Hazelby et al., 1994; Várady & Čorba, 1999; Taylor et al., 2002; Coles et al., 2006).

The most widely used in vitro technique is the Egg Hatch Test (EHT), which is a common term for several techniques used for the detection of benzimidazole resistance. They are based on the ovicidal properties of benzimidazoles and the ability of eggs of resistant populations to embryonate and hatch in a higher concentration of benzimidazole than can eggs from sensitive populations. The original test was described by Le Jambre (1976). Since then, several authors have added small modifications to the original technique. Currently, the most commonly used modified version recommended by the WAAVP is that according to Coles et al. (1992, 2006). The EHT is the most widely used in vitro method for the detection of benzimidazole resistance in field diagnostics. Kemp and Smith (1982), Cawthorne and Cheong (1984), Boersema et al. (1987), Hong et al. (1992), Praslička et al. (1994), Várady et al. (1994), Bartley et al. (2003) and Várady et al. (2006) used this test to detect resistance to benzimidazoles in a field survey of resistance to benzimidazole drugs in sheep. This test has also been used to detect resistance in parasites of goats. (Maingi et al., 1996; Requego-Férnandez et al., 1997; Dorny et al., 1994; Chartier et al., 2001).

The Larval Development Test (LDT) is another in vitro test, based on larval ability to survive and develop in environments of various concentrations of anthelminthics. Coles et al. (1988) was the first to describe the test where eggs recovered from faeces are incubated for seven days in an aquatic solution of the drug together with culture medium for developing larvae. Lyophilized E. coli (W strain) or Earle's balanced salt solution mixed with yeast extract (Taylor, 1990) was used as a medium. After incubation, the percentage of L1, L2 and L3 larvae is calculated for each drug concentration and for drug-free controls. The test can reliably detect resistance to benzimidazoles and levamizoles. Hubert and Kerboeuf (1992), Lacey et al. (1991) and Coles et al. (2006) described a Micro Larval Development Assay (MLDA) that relies on the same principle, but with an LD_{50} (concentration inhibiting development of 50 % of eggs into L3 infective larvae) being determined after the test. In addition to detecting benzimidazole and levamisole resistance, the test can detect ivermectin resistance in H. contortus. The test has the great advantage of the simultaneous detection of efficacy/inefficacy of the two broad-spectrum anthelminthics.

The Larval Paralysis test was the first test designed for the detection of levamisole and morantel-tartate resistance (Martin & Le Jambre, 1979) by determining the percentage of paralysed L3 larvae exposed in vitro to serial dilutions of anthelmintic. Gill et al. (1991) studied the effect of ivermectin on paralysis of H. contortus larvae. L3 invasive larvae resistant to ivermectin were less sensitive to this drug, resulting in reduced motility and paralysis of the larvae.

Another test relying on larval motility is the Migration-Inhibition Assay (Wagland et al., 1992; Rothwell & Sangster, 1993). This test is based on the ability of larvae to freely migrate through selected mesh sizes of nylon sieves and the reduced ability of larvae to migrate after preincubation with, and in the presence of, anthelminthics. Using this method, Rothwell and Sangster (1993) detected resistance to three broad-spectrum anthelminthics in H. contortus. Kotze et al., (2006) used the same principle for larvae of H. contortus migrating through sieves with a mesh size of 20 μm or agarose gels as a method for detecting populations resistant to ivermectin. The differences in larval motility are a basis for another test in which L3 infective larval motility is measured with a micromotility meter (Bennet & Pax, 1987).

Comparison of sensitive and resistant populations of H. contortus, O. circumcincta and T. colubriformis highlights the fact that invasive larvae of benzimidazole-resistant populations possess significantly greater amounts of acetylcholinesterase than larvae of susceptible populations of nematodes (Sutherland & Lee, 1989, 1990, 1993). Sutherland et al. (1988) described four biochemical assays for the detection of benzimidazole resistance based on comparisons of non-specific esterases and acetylcholinesterases in resistant and susceptible populations. The methods are accurate, sensitive and undemanding.

The majority of these tests, however, are unsuitable for widespread use in field screening surveys because they lack, to some degree, reliability, reproducibility, sensitivity and ease of interpretation. Only the EHT and LDT performed well enough to merit widespread use. The results of the EHT are usually interpreted using ED_{50} or ED_{99} values (the concentration of a drug producing 50 % or 99 % inhibition of hatching in the test, respectively). If the ED_{50} is used as a cut-off value, benzimidazole resistance is only detected when $\geq$ 25 % of resistant individuals are present in the population (Martin et al., 1989). The use of ED_{99} values can significantly increase the sensitivity of the test and identifies resistance when only a small proportion of the worm population is resistant (Várady et al., 2007). The principal question concerning delineation dose methods is when such tests are able to provide early detection during the development of resistance, especially if the alleles for resistance are rare in the parasite population. The delineation dose of 0.1 μg.ml^{-1} of thiabendazole obtained by the EHT provides a good estimate of genotypic resistance (Čudeková et al., 2010). The LDT was also able to clearly indicate the presence of low levels (4 %) of benzimidazole-resistant larvae amongst a susceptible background population (Várady et al., 2007). Additionally, knowledge of molecular basics of benzimidazole resistance has allowed the development of a number of PCR-based tests which provide high levels of specificity and sensitivity (Silvestre & Humbert, 2000; Álvarez-Sánchez et al., 2005; Walsh et al., 2007; von Samson-Himmelstjerna et al., 2009).

The situation with tests used to detect resistance to macrocyclic lactones is slightly more complicated, with smaller

differences between susceptible and resistant populations. The majority of these tests compare levels of paralysis of worms from resistant and susceptible populations. Macrocyclic lactones induce paralysis of the pharynx (Geary *et al*., 1993) and somatic muscles in the nematodes (Gill *et al*., 1991). The initial problem with the tests was their inability to produce a complete ivermectin dose-response, which resulted in failure to detect ivermectin-resistant isolates (Coles *et al*., 1988; Taylor, 1990). Several authors (Rohrer *et al*., 1994; Várady *et al*., 1996; Amarante *et al*., 1997) noted that use of an avermectin analogue (ivermectin aglycone, eprinemectin) increased the ability of the test to differentiate between ivermectin-resistant and -susceptible isolates (Kotze *et al*., 2002). Previous results suggest that the LDT has been the most sensitive test for detecting ML-resistant nematodes of sheep and goats.

Anthelmintic treatments

In most cases information for goats is accumulated from sheep data, which is not always the safe way (Hoste *et al*., 2010). Goats and sheep are infected mostly with the same nematode species, although there is some evidence that different caprine and ovine strains exist, at least for *Teladorsagia circumcincta* (Gasnier & Cabaret, 1996). However, several results have shown that the strategy to reduce worm infection largely differ between the two hosts, according to behavioural, immunological and physiological characteristics (Torres-Acosta & Hoste, 2008).

Another important issue in the GIN control strategy is the fact that within a flock, nematodes not only are unequally distributed amongst individuals, both for sheep and goats, but, on the contrary, generally a small number of animals are heavily infected whereas most individuals of the flock present a moderate worm burden (Sreter *et al*., 1994; Hoste *et al*., 2001; Torres-Acosta & Hoste, 2008). On this basis, targeted selective treatments based on parasitological and performance criteria, aiming to preserve worms *in refugia* and to administer anthelmintics solely to animals in need, were tested successfully in dairy sheep and goats (Gallidis *et al*., 2009). Several results examining directly or indirectly the effects of nematodes on dairy goats support that cases with high milk production, either due to peak of lactation or high producing individuals within the herd, correspond to higher susceptibility to parasitism (Hoste *et al*., 2005). Goats with the highest level of milk production, within a herd of experimentally infected animals, were found to be more infected with parasites and more susceptible to their negative effects, particularly at the peak of lactation (Hoste & Chartier, 1993). Hoste *et al*. (2005) concluded that goats represent a valuable model for basic research aiming at exploring the mechanisms of the host resilience and the complex interactions between parasitism and the feeding behaviour.

The feeding behaviour of these animal species differs significantly and may represent a major issue in their infection with nematode parasites. Sheep prefer to graze, while goats browse usually woody plants. In this way, goats avoid the 3rd stage infective nematode larvae, minimising the larval intake, which usually remain on the grass consumed by sheep, particularly when sheep and goats feed together. Additionally, in experiments conducted in goat breeds with different feeding behaviour, bred in the same conditions, was found that during the whole experimental period of 5 months nematode egg excretion was repeatedly higher in the goats of Angora breed, which can be considered as grazers, than the Saanen ones, which exhibit feeding activities close to browsers (Hoste *et al*., 2001). In grazing conditions, goats are constantly significantly more heavily infected than sheep carrying heavier worm burdens and expelling higher faecal egg counts (Le Jambre & Royal, 1976; Pomroy *et al*., 1986). However, in rangeland environment has been reported the opposite (Vercruysse, 1983; Hoste *et al*., 2001). On the other hand, Kanyari (1993) reported that sheep were found to be more heavily infected than goats not only with helminths, but also with coccidian, giving the possible explanation that this occurred due to the different feeding habits of these two animal species.

It is of great interest the fact that goats may offer a good model to study the potential of self-medication, because of their ability to feed on a much wider and diverse range of plants than sheep. For this reason, goats can be used to exploit the possible interactions/relationships against nematodes due to the development of immunity, feeding avoidance of infective larvae and alleviating worm challenges by self medication. (Hoste *et al*., 2010).

It has been demonstrated that goats are less efficient than sheep in the acquisition and expression of immune responses against gastrointestinal nematode parasites (Pomroy *et al*., 1986; Hoste *et al*., 2008). Furthermore, the development of a fully expressed immune response in goats appears delayed (Pomroy *et al*., 1986). Dorny *et al*. (1995) found that strongyle infections were acquired at an earlier age in sheep than in goats, with the mean faecal egg counts decreasing from the age of 8 months onwards in sheep while in goats this occurred from 12 – 18 months onwards. Goats despite previous exposure to nematodes in the field are less capable of restricting larval populations when compared with ewes. Differences are observed in mucosal mast cell (MMC) and globule leukocyte (GL) numbers as well as granule-associated mast cell proteinase (MCP) concentrations in goat intestinal tissues compared to sheep tissues (Huntely *et al*., 1995). Macaldowie *et al*. (2003), in experiments with worm-naïve animals enabling the study of comparative primary responses to infection, also confirmed the evident differences between caprine and ovine immunoregulatory mechanisms and the relative inability of goat yearlings and kids to reduce mean total gastrointestinal nematode burdens when compared with equivalently challenged lambs.

The more rapid metabolism of anthelmintics in goats compared with sheep results in lower short lived plasma levels of the active drug in other words a much lower bioavailability (Swan & Gross, 1985). The reduced efficacy of anthelmintics, such as albendazole, oxfendazole and levami-

sole, in goats compared with sheep may be caused by a difference in disposition. Hepatic metabolic activity, in particular oxidation and hydroxylation, proceeds at a faster rate in goats than in sheep (McKenna & Watson, 1987). The plasma concentrations of anthelmintics which are metabolized and secreted in bile by the liver, are greatly influenced by any change in the rate of hepatic metabolism. Hennessy *et al.* (1993) suggested that albendazole may be sequestered to a greater extent in the liver of goats than of sheep which results in lower concentrations of its active metabolites in plasma and abomasal fluid. It is suggested by the same researchers that this behaviour might be compensated for by administering albendazole to goats at a proportionally higher dose rate, which comes in good agreement with McKenna (1984) that it is necessary to have higher dose rates specific for effective treatment of goats. On the other hand, using the same dose rates in both animal species against gastrointestinal nematodes may result to a faster development of anthelmintic resistant strains in goats due to the reduced efficacy, which are transferred later to sheep (Gillham & Obendorf, 1985; Charles *et al.*, 1989). The results of experiments using ivermectin against gastrointestinal nematodes showed that a dose rate of at least 1.5 times that recommended for sheep should be implemented for goats (Mwamachi *et al.*, 1995) or even 2 times higher (Hennessy, 1994).

Conclusions

In the frame of the wide presence or the fast development of anthelmintic resistance, the effective control of nematodes in sheep and goats relies on the combination of effective anthelmintics with alternative strategies including immunomanipulation and management of the grazing environment. Detailed knowledge based on research is needed before applying to goats measures taken from our experience in sheep. This is vital in order not only to avoid past errors, but to think differently for goats than sheep due to several behavioural, immunological and physiological differences between these animal species. Furthermore, goats offer an excellent model to study the interactions/relationships against nematodes due to the development of immunity, feeding avoidance of infective larvae and alleviating worm challenges by self medication.

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