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Multiple anthelmintic resistance in a goat herd

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Abstract

Anthelmintic resistance was monitored over a 30 month period within a goat herd in eastern Virginia, USA. Resistance to ivermectin, levamisole and benzimidazole drugs was detected in *Haemonchus contortus* using the fecal egg count reduction test (FECRT). When levamisole use was discontinued for 1 year, susceptibility to levamisole appeared to return. Although a single treatment with fenbendazole was able to reduce fecal egg counts by only 50%, two doses administered in a 12 h interval increased efficacy to 92%, however, confidence intervals indicated that resistance was still present. When fecal egg counts were determined the following year after several treatment using this protocol, the efficacy of fenbendazole had fallen again to 57% reduction in fecal egg counts. The predominant genus present in cultured composite fecal samples was *Haemonchus*. *Trichostrongylus*, *Cooperia* and *Teladorsagia* were also present in smaller numbers.

Keywords: Resistance; *Haemonchus*; Goat; Anthelmintics; Levamisole; Ivermectin; Fenbendazole

1. Introduction

Anthelmintic resistance in trichostrongyloid parasites of small ruminants has been described throughout the world and has become an important factor in limiting efficient production in many countries (Conder and Campbell, 1995). In the US, results of a survey of sheep flocks in North Carolina indicated at least 90% efficacy in only three of 13 sheep flocks treated with benzimidazoles (Uhlinger et al., 1992). Ivermectin resistance has also been described in lambs in the southeast (Miller and Barras, 1994). In goats in the US,

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benzimidazole resistance has been described in Texas and Pennsylvania (Craig and Miller, 1990; Uhlinger et al., 1988). Ivermectin resistance was first reported in the US in an Angora goat in Texas and subsequent work with the same herd showed resistance to levamisole, ivermectin and fenbendazole (Craig and Miller, 1990; Miller and Craig, 1996). One factor which may have contributed to the high worldwide prevalence of anthelmintic resistance in small ruminant trichostrongyles is the common use of the sheep dosage of these products in both sheep and goats (Conder and Campbell, 1995). Coles (1997) has recommended that goats require higher dosages than sheep to achieve similar efficacy against trichostrongyles.

Several strategies have been proposed to maximize the efficacy of benzimidazole products in the face of developing anthelmintic resistance. Studies have recently been conducted to show that benzimidazole efficacy can be improved by withholding food before treatment (Hennessy, 1997) and by administering two doses of benzimidazole drug at 12 h intervals (Sangster et al., 1991). However, the length of time over which this strategy will sustain drug efficacy is not well documented. Also, once anthelmintic resistance develops in a flock or herd, the length of time required for reversion to susceptibility is not well established, although benzimidazole resistance appears to persist for many years after use of this drug group is discontinued (Herd et al., 1984; McKenna, 1990).

In the south-central and southeastern US the most important helminth of small ruminants is *H. contortus*. Multiple anthelmintic treatments are commonly required throughout the grazing season to prevent serious morbidity and mortality. The purpose of this study was to examine the extent of anthelmintic resistance in a goat herd in the southeastern US, to determine if the extent of resistance changed over a period of approximately 30 months and to attempt to prolong efficacy of benzimidazole products by using multiple drug doses.

2. Materials and methods

2.1. Goats

The study was conducted with male and female goats of various ages and breeds belonging to the herd maintained by Virginia State University in eastern Virginia, USA. This herd was established in 1992 as part of a program to develop a mixed breed goat for meat production in southeastern US. The herd consists of approximately 200 goats including progeny of male and female goats purchased from several southern states including Texas, Mississippi, Georgia and Tennessee. Goats are maintained on pasture with supplemental feeding as needed and have required repeated anthelmintic treatments during the year to prevent severe losses from haemonchosis. At the time the study was begun, several goats had recently died from severe *H. contortus* infection despite treatment with levamisole. From the time of establishment of the herd to the beginning of the present study in 1995, ivermectin was the anthelmintic of choice, although several treatments with levamisole had been given during the 8 months preceding the study. Throughout the period of the study, goats were dewormed at approximately monthly intervals during the grazing season (April–November). The following anthelmintics were used in the herd before and during the study: ivermectin (10/92–12/94, 10/95–1/96, 1/97–6/98), levamisole (1/95–9/95, 7/97–6/98) and fenbendazole (2/96–12/96).

A total of 286 goats (159 females and 127 males) were used in the FECRTs between fall, 1995 and summer, 1998. All levamisole tests were done with the male herd. Males were also used for the 1995 fenbendazole test and the ivermectin test in February, 1996. Non-lactating females were used for the remainder of the FECRT. As much as possible, the same animals were used in sequential tests. Movement of goats through pastures on the farm was common and it is unlikely that parasite susceptibility to anthelmintics varied on the different pastures of the farm. During the period of the study a small number of goats was added to the herd.

2.2. Anthelmintics

Three anthelmintics were tested for resistance over a period of approximately 30 months. Drug dosages followed the guidelines of Coles (1997). Levamisole drench (11.8 mg kg^{-1}) was tested in goats in 1995–98. Resistance to ivermectin was tested in early 1996 using the formulation for subcutaneous injection (0.3 mg kg^{-1}). When resistance was detected, the dosage was increased (0.4 mg kg^{-1}). Ivermectin was tested again in June 1996, 1997, 1998. The product was administered by injection in 1996 and 1998. In 1997, ivermectin was given either by subcutaneous injection or by oral administration of the drench formulation. Fenbendazole was first tested in late 1995 in a single dose of 10 mg kg^{-1}. When resistance was detected, it was tested again in June 1996 using two doses of 10 mg kg^{-1} separated by a 12 h interval. In 1997, this test was repeated and, in addition, food was withheld for 6–8 h prior to anthelmintic treatment. At that time a second group of goats was given two treatments at 5 mg kg^{-1} to examine whether splitting the appropriate dose and withholding food would be enough to demonstrate efficacy of the product. In 1998, goats received two treatments of 10 mg kg^{-1} after food was withheld for approximately 12 h.

2.3. Fecal egg count reduction test

Goats used for testing each anthelmintic were randomly divided into treatment and control groups. Goats in the treatment groups received anthelmintic based on individual body weight. In 1995, levamisole was administered to 23 goats with 20 goats acting as controls. In 1996, 25 goats received levamisole and another 25 goats were controls and in 1997, there were 18 levamisole treated goats and 16 untreated goats. Twelve goats received levamisole in the 1998 test, with 16 goats in the untreated control group.

In the tests of fenbendazole efficacy, similar numbers of goats were used. In November 1995, 18 goats received fenbendazole and 12 untreated goats served as controls. In June 1996, 20 treated and 20 untreated goats were tested and in May 1997, 16 goats each were treated in the low and high dose groups with 20 controls. In 1998, 18 goats were untreated and 19 received fenbendazole.

Ivermectin was given to 29 goats in February 1996 and to 12 goats in June 1996. Thirty and 14 goats, respectively, served as controls in these two trials. In 1997, ivermectin was tested in 34 goats, 15 received the drug orally and 19 by subcutaneous injection. Fourteen goats were untreated. In the final trial in 1998, 18 goats were untreated and 18 received ivermectin.

Feces were collected from goats on the day of treatment and 7–10 days following treatment, a second rectal fecal sample was collected from each goat. In 1997, the levamisole posttreatment fecal sample was collected after 5 days in view of evidence suggesting that a longer interval may produce an underestimation of levamisole efficacy (Grimshaw et al., 1996). Individual fecal egg counts were determined by a modified McMaster's technique (Whitlock, 1948) with a sensitivity of 25 eggs per gram. Fecal egg count reductions were determined following the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP), (Coles et al., 1992), using arithmetic mean egg counts and the formula: $100\,(1 - T2/C2)$, where T2 is the mean egg count at 10–14 days post-treatment and C2 is the mean control fecal egg count at 10–14 days post-treatment. Confidence intervals were also determined by the method of WAAVP (Coles et al., 1992). Resistance is considered to be present if the egg count reduction following treatment is less than 95% and the lower 95% confidence interval is less than 90% (Coles et al., 1992). Fecal egg count reductions were also determined by the method of Dash et al. (1988), using both pre-treatment and post-treatment fecal egg counts. The reductions calculated by this method were similar and are not included in this paper.

In 1996 and 1997, portions of individual fecal samples were also pooled within treatment and control groups for coproculture and identification of third stage larvae (Ministry of Agriculture, 1986). Results for each sample are expressed as the proportion of L3 of each species in each culture multiplied by the mean fecal egg count of the group from which the samples came.

3. Results

3.1. Levamisole

Levamisole did not significantly reduce fecal egg counts when it was first tested (lower confidence interval < 0) in 1995 (Table 1) and use of the anthelmintic was discontinued at that time. The 0.75% reduction in fecal egg counts was followed 9 months later, in June 1996, by a 74% reduction and in May 1997, levamisole treatment produced a 97% egg count reduction. Confidence intervals for the egg count reductions for the tests in 1995–97 did not overlap. However, following use of levamisole in 1997, the fecal egg count reduction in 1998 was only 73%. The reduction in numbers of *Cooperia* spp. and *H. contortus* larvae in post-treatment 1996 larval cultures appeared to parallel the overall decline in fecal egg counts. In the 1997, post-treatment larval cultures, numbers of *H. contortus* showed a decrease consistent with the 97% reduction in FECRT (Fig. 1). *Trichostrongylus* spp. and *Teladorsagia circumcincta* larvae were also found in cultures.

3.2. Fenbendazole

Although benzimidazole drugs had not been used in the herd, no significant reduction in fecal egg counts resulted from a single treatment with fenbendazole at a dosage of $10\,\mathrm{mg\,kg^{-1}}$ in 1995 (lower confidence interval < 0, Table 1). Efficacy was higher (92%)

Table 1
Mean post-treatment fecal egg counts (FEC), egg count reductions and 95% confidence intervals (CI) in levamisole (L), fenbendazole (FBZ) and ivermectin (I) FECRT conducted in Virginia goats

Anthelmintic	Test date	Dosage and ROA [a]	*n* [b]	Control FEC, range, s.e.[c]	Treatment FEC, range, s.e.	FEC reduction	95% CI
Levamisole	11/95	11.8 mg kg^{-1} PO [d]	25 C [e], 25 L	1114 50–3500, 223	1105 50–4000, 255	0.75%	−83%, 43%
Levamisole	6/96	11.8 mg kg^{-1} PO	25 C, 25 L	1408 0–4500, 231	361 0–2125, 943	74%	52%, 86%
Levamisole	5/97	11.8 mg kg^{-1} PO	16 C, 18 L	728 0–2950, 207	22 0–75, 24	97%	93%, 99%
Levamisole	6/98	11.8 mg kg^{-1} PO	16 C, 12 L	1794 50–5300, 458	477 0–800, 153	73%	38%, 89%
Fenbendazole	11/95	10 mg kg^{-1} PO	13 C, 18 FBZ	562 50–3100, 230	283 0–850, 72	50%	−36%, 81%
Fenbendazole	6/96	2 × 10 mg kg^{-1} PO	20 C, 19 FBZ	234 0–700, 45	18 0–200, 11	92%	72%, 98%
Fenbendazole	5/97	2 × 5 mg kg^{-1} PO	20 C, 16 FBZ	1020 50–5450, 281	606 0–2500, 165	41%	−31%, 73%
Fenbendazole	5/97	2 × 10 mg kg^{-1} PO	20 C, 16 FBZ	1020 50–5450, 281	440 50–1450, 100	57%	10%, 79%
Fenbendazole	6/98	2 × 10 mg kg^{-1} PO	18 C, 19 FBZ	1075 0–7675, 422	388 25–1700, 103	76%	6%, 86%
Ivermectin	2/96	0.3 mg kg^{-1} Inj[f]	36 C, 35 I	354 0–1875, 70	160 0–600, 31	54%	20%, 74%
Ivermectin	6/96	0.4 mg kg^{-1} Inj	14 C, 12 I	830 0–2700, 179	202 0–1425, 117	76%	14%, 93%
Ivermectin	6/97	0.4 mg kg^{-1} PO	14 C, 15 I	421 25–2500, 172	125 0–325, 30	70%	22%, 89%
Ivermectin	6/97	0.4 mg kg^{-1} Inj	14 C, 19 I	421 25–2500, 172	82 0–450, 27	90%	35%, 92%
Ivermectin	6/98	0.4 mg kg^{-1} Inj	18 C, 18 I	1075 0–7675, 422	253 0–875, 65	76%	38%, 91%

[a] Route of administration.
[b] Number of goats.
[c] Standard error.
[d] By mouth.
[e] Control.
[f] Subcutaneous injection.

in 1996 with two treatments separated by a 12 h interval. However, the lower confidence interval of the 1996 test of 72% indicated that fenbendazole treatment was not fully effective. After using fenbendazole for the remainder of 1996, no significant reduction in fecal egg counts were observed when next tested in 1997. In 1998, treatment with fenbendazole resulted in an egg count reduction of 76%. Parasite genera present in 1996 included *Trichostrongylus*, *Haemonchus* and *Cooperia* (Fig. 2). Numbers of all three genera were reduced in post-treatment samples. In 1997, *H. contortus*, *T. circumcincta* and *Cooperia* spp. were found and numbers were not reduced by treatment.

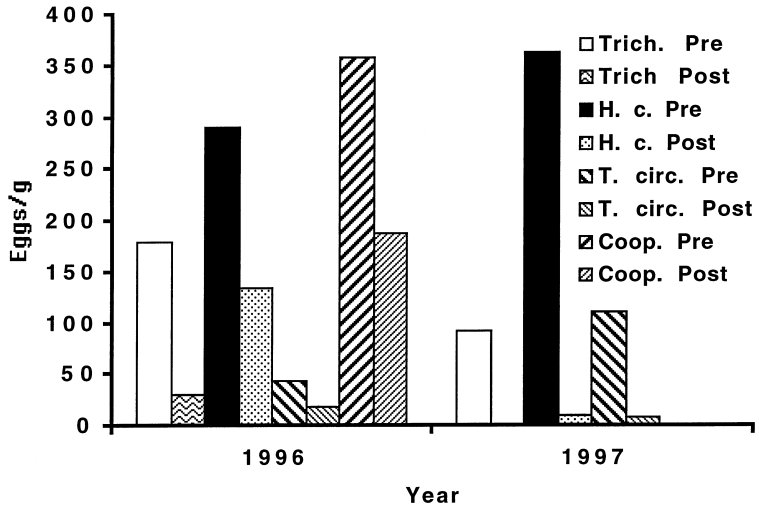

Fig. 1. Species distribution of goat parasites determined by larval culture and identification before and after treatment with levamisole (Trich — *Trichostrongylus*, H. c. — *H. contortus*, T. circ. — *T. circumcincta*, Coop — *Cooperia* spp., pre — pre-treatment, post — post-treatment).

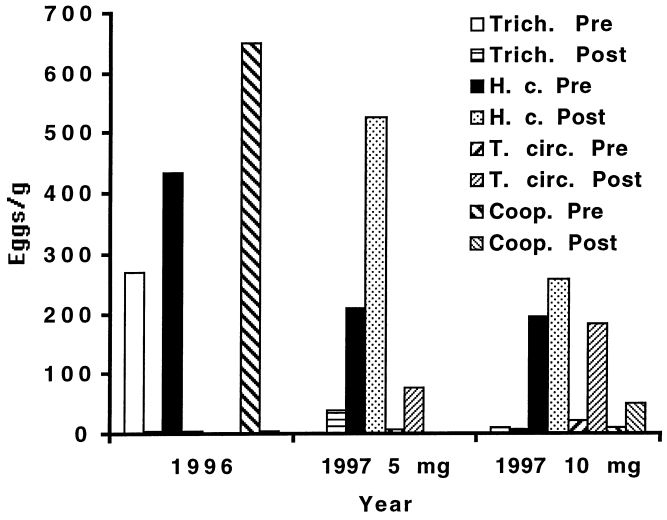

Fig. 2. Species distribution of goat parasites determined by larval culture and identification before and after treatment with fenbendazole (Trich — *Trichostrongylus*, H. c. — *H. contortus*, T. circ. — *T. circumcincta*, Coop — *Cooperia* spp., pre — pre-treatment, post — post-treatment).

3.3. Ivermectin

The FECRT performed early in 1996 indicated a 54% reduction in fecal egg counts (Table 1). The test was repeated in June using an increased dosage with only a small increase in the egg count reduction. The following year treatment was repeated with both

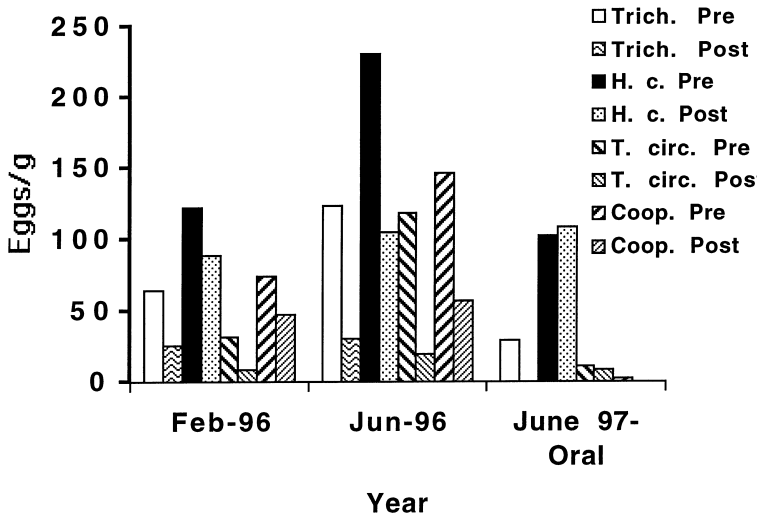

Fig. 3. Species distribution of goat parasites determined by larval culture and identification before and after treatment with ivermectin (Trich — *Trichostrongylus*, H. c. — *H. contortus*, T. circ. — *T. circumcincta*, Coop — *Cooperia* spp., pre — pre-treatment, post — post-treatment).

injectable and oral formulations. Oral treatment produced a 70% reduction in egg counts. Subcutaneous injection of ivermectin resulted in a 90% reduction. In 1998, subcutaneous injection of ivermectin produced a 76% reduction in egg counts. In both 1996 and 1997, *Haemonchus* was the most common genus found in larval cultures (Fig. 3). Treatment with ivermectin did not substantially reduce numbers of *H. contortus* larvae in tests performed in 1996 or 1997. In the 1996 tests, the level of *Cooperia* spp. in the post-treatment sample also did not fall below 90% of pre-treatment levels.

4. Discussion

Anthelmintic resistance has been documented in small ruminants in the US in only a few publications, principally from the southeast and Texas (Uhlinger et al., 1988; Craig and Miller, 1990; Uhlinger et al., 1992; Miller and Barras, 1994; Miller and Craig, 1996). However, it is likely that resistance to anthelmintics is now well established in the south-eastern and south-central US, especially in the common species, *H. contortus*. Results of this study demonstrated the presence of multiple resistance in the mid-Atlantic region of the southeast. Resistance was present to all major groups of anthelmintics available for treating GI nematodes of grazing animals, even though only ivermectin had been used extensively in the herd. It seems likely that the assembly of goats from several locations in the southeast, when the herd was established, probably resulted in the introduction and combination of several anthelmintic resistant parasite strains.

When levamisole was first tested for resistance in 1995, it had been used for less than a year in this herd. However, the FECRT indicated that the drug had no effect on fecal egg

counts. Use was immediately discontinued and 7 months later it appeared that levamisole had begun to regain some of its activity, although FECRT results still indicated that resistance was present. In 1997, levamisole treatment resulted in a 96% reduction in fecal egg counts with a lower 95% CI of 90%. Fecal egg counts have been shown to increase more rapidly after treatment with levamisole than with other anthelmintics (Grimshaw et al.). In the first two levamisole trials the second sample was collected 9–10 days after treatment. In 1997 and 1998, the post-treatment samples were collected 5 days after goats were treated. The longer interval between samples in the first two tests may have underestimated the level of drug efficacy. However, in 1995, both FECRT results and clinical observations suggested a reduction in the efficacy of levamisole. For the same reason, in the second test, susceptibility may have been greater than estimated by the FECRT. In 1997, the interval between samples was only 5 days and susceptibility of the parasites appeared to have been restored. Following the results of the 1997 test, levamisole use was reinstated. When an FECRT was repeated in 1998, the results indicated that levamisole had again become ineffective.

Resistance to benzimidazole anthelmintics was expected in view of some survey work (Uhlinger et al., 1992) available and anecdotal evidence. In Australia, producers are advised to administer 2–3 benzimidazole treatments separated by 12 h intervals when resistance is suspected (Taylor, 1994). In this study, the two dose protocol seemed to improve efficacy of fenbendazole, but the wide confidence interval indicated that resistant parasites were present. Because both ivermectin and levamisole resistance were present in 1996, fenbendazole continued to be used in the two dose protocol for the remainder of the year. When fenbendazole was retested the following year, fecal egg counts were unaffected by treatment. These results emphasize the importance of continued monitoring of animals when anthelmintic use is altered in an attempt to overcome resistance or slow its development. Extension personnel and veterinarians who suggest alternative treatment regimes to producers must also emphasize that they represent only temporary solutions in the face of existing resistant parasites. Since 1998, the manager of the herd used in this study has controlled the development of clinical parasitism with a combination of careful pasture management and simultaneous administration of fenbendazole and levamisole when goats are dewormed. However, recent testing suggests that fecal egg counts are no longer being reduced by treatment.

Egg count reductions with ivermectin were first tested in early 1996. Reduced drug efficacy was evident at this time which was not unexpected since ivermectin had been used in the herd almost continuously at 1.5 times the sheep dosage since the herd was established in 1992. Even when ivermectin was tested again in 1996 at an even higher dosage, the egg count reduction of 77% still fell well below the 95% level. Although results from 1997 demonstrated a higher egg count reduction for goats injected with ivermectin compared to oral administration, the lower 95% confidence intervals were 35% and 22%, respectively, for the two routes of administration, and did not indicate that the orally administered drug was effective. Confidence intervals of all ivermectin tests do not suggest any change in ivermectin efficacy over the course of the study.

There are few studies documenting the length of time required for reversion to susceptibility of drug resistant parasite populations. Field observations with benzimidazoles suggest that reversion may require a period of many years after drug use is discontinued (Herd et al., 1984; McKenna, 1990). Ivermectin resistance was still present on a New Zealand farm

after 4 years of restricted use of the drug (Watson et al., 1993), although the efficacy did not decrease further after the initial FECRT.

Results of this study suggested that susceptibility to levamisole might have returned after 1 year of discontinued use. The confidence intervals in the first three tests do not overlap and may indicate significant differences in efficacy of levamisole in the first three tests. If resistance does not persist long in a population of trichostrongyloid nematodes after discontinuing treatment, use of levamisole in an annual or biannual rotation of anthelmintics may help maintain its efficacy. An alternative explanation for the changes in levamisole efficacy is the introduction of susceptible parasite strains which replaced the resistant parasites. There was very limited introduction of new goats into the herd during the course of this study and it seems unlikely that such rapid change could have occurred in the parasite population since winter in the mid-Atlantic region decreases the rate of development of free living stages for several months. However, to evaluate changes in levamisole efficacy over any period of time with accuracy, total worm burden data should be collected following treatment of goats.

The parasite of greatest concern in this study was *H. contortus* since it causes the most severe clinical problems in this region and elsewhere in the world. *Haemonchus contortus* predominated in larval cultures of feces during the study. The technique of fecal culture may not accurately represent species distribution of eggs in feces due to differential survival of the parasites during the culture period (Dobson et al., 1992). However, clinical observation and results of total worm burdens from other animals in this herd supported the dominance of *H. contortus* in the goats. The data indicated *H. contortus* was resistant to all anthelmintics tested. It cannot be determined from the data whether these results represent the presence of a single population of worms with multiple drug resistance or several populations, each resistant to one or more anthelmintics. However, given the length of time over which resistance has been detected, it seems likely that normal reproductive cycles would have produced worms with multiple drug resistance. Other species of worms were present in variable proportions and, in most cases, were present in such low numbers that it was difficult to conclude with confidence that resistance was present.

5. Conclusion

These results provided further documentation of anthelmintic resistance in small ruminants in the USA. Veterinarians and extension personnel must increase their efforts to educate producers in techniques to reduce selection pressure for resistance, if the USA is to avoid the serious resistance problems which have developed in other countries.

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References

Coles, G.C., 1997. The control of parasites in goats. Goat Vet. Soc. J. 17, 28–32.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Conder, G.A., Campbell, R.K., 1995. Chemotherapy of nematode infections of veterinary importance with special reference to drug resistance. Adv. Parasitol. 35, 2–84.

Craig, T.M., Miller, D.K., 1990. Resistance by *Haemonchus contortus*to ivermectin in Angora goats. Vet. Rec. 126, 580.

Dash, K.M., Hall, E., Barger, I.A., 1988. The role of arithmetic and geometric mean worm counts in faecal egg count reduction tests and in monitoring strategic drenching programs in sheep. Aus. Vet. J. 65, 66–68.

Dobson, R.J., Barnes, E.H., Birclijin, S.D., Gill, J.H., 1992. The survival of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in faecal culture as a source of bias in apportioning egg counts to worm species. Int. J. Parasitol. 22, 1005–1008.

Grimshaw, W.T.R., Hong, C., Hunt, K.R., 1996. Potential for misinterpretation of the faecal egg count reduction test for levamisole resistance in gastrointestinal nematodes of sheep. Vet. Parasitol. 62, 267–273.

Hennessy, D.R., 1997. Physiology, pharmacology and parasitology. Int. J. Parasitol. 27, 145–152.

Herd, R.P., Streitel, R.H., McClure, K.E., Parker, C.F., 1984. Control of hypobiotic and benzimidazole-resistant nematodes of sheep. J. Am. Vet. Med. Assoc. 184, 726–730.

McKenna, P.B., 1990. The use of benzimidazole-levamisole mixtures for the control and prevention of anthelmintic resistance in sheep nematodes: an assessment of their likely effects. NZ Vet. J. 38, 45–49.

Miller, J.E., Barras, S.R., 1994. Ivermectin resistant *Haemonchus contortus* in Louisiana lambs. Vet. Parasitol. 55, 343–346.

Miller, D.K., Craig, T.M., 1996. Use of anthelmintic combinations against multiple resistant *Haemonchus contortus* in Angora goats. Small Rum. Res. 19, 281–283.

Ministry of Agriculture, Fisheries and Food, 1986. Manual of veterinary parasitological laboratory techniques. Her Majestyís Stationery Office, London, 159 pp.

Sangster, N.C., Rickard, J.M., Hennessey, D.R., Steel, J.W., Collins, G.H., 1991. Disposition of oxfendazole in goats and efficacy compared with sheep. Res. Vet. Sci. 51, 258–263.

Taylor, R., 1994. Making smarter use of drenches. Rural Res. 163, 26–28.

Uhlinger, C., Fetrow, J., Johnstone, C., 1988. Field evaluation of benzimidazole and nonbenzimidazole drugs in a herd of dairy goats. J. Vet. Int. Med. 2, 113–116.

Uhlinger, C., Fleming, S., Moncol, D., 1992. Survey for drug-resistant gastrointestinal nematodes in 13 commercial sheep flocks. J. Am. Vet. Med. Assoc. 210, 77–80.

Watson, T.G., Hosking, B.C., McKee, P.F., 1993. Stability of ivermectin resistance in a field strain of *Ostertagia* spp.. Proc. NZ Soc. Anim. Prod. 53, 239–241.

Whitlock, H.V., 1948. Some modifications of the McMaster helminth egg-counting technique apparatus. J Coun. Sci. Ind. Res. 21, 177–180.