



## Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection<sup>☆</sup>

M.A. Taylor<sup>\*</sup>, J. Learmount, E. Lunn, C. Morgan, B.H. Craig

Food and Environment Research Agency (FERA), Sand Hutton, York YO41 1LZ, UK

### ARTICLE INFO

#### Article history:

Available online 13 October 2009

#### Keywords:

Sheep  
Anthelmintic resistance  
FECRT  
LDT

### ABSTRACT

Intensification of animal production systems has led to an increased reliance on effective anthelmintics to control parasitic worms. However, the excessive and continued use of these “wormers” can lead to high selection pressures and have resulted in increased reports of emerging nematode populations exhibiting resistance to all of the main anthelmintic classes. Faecal egg count reduction tests (FECRT) were conducted on six farms in England and Wales according to standardised guidelines produced by the World Association for the Advancement of Parasitology. Selected farms were identified from a network of 40 study farms participating in a study investigating implementation of Sustained Control of Parasites in Sheep (SCOPS) principles. Resistance to the larval development test (LDT) to either benzimidazole- or imidazothiazole-group anthelmintics had been previously detected on these farms. In this study, resistance was indicated as present by LDT and/or FECRT to one or more groups of anthelmintics on a number of the study farms. Comparisons were made between results obtained by the two tests on the six farms. Benzimidazole resistance was identified by FECRT on five farms; imidazothiazole resistance on four farms and macrocyclic lactones resistance on five farms. The LDT identified the presence of benzimidazole resistance on all six farms, and imidazothiazole resistance on five farms. Generally, there was good agreement between the two tests in identifying both benzimidazole and imidazothiazole resistance. On one farm, the LDT identified the presence of benzimidazole-resistant nematodes, not detected by FECRT and on two farms the presence of imidazothiazole-resistant nematodes not detected by FECRT. On two farms “triple” resistance (i.e. resistance to all three groups of anthelmintics) was identified by FECRT and on one farm moxidectin resistance was suspected based on an early return to egg laying at 28 days post-treatment. Resistance was present in one or more genera, but most commonly in *Teladorsagia* on all six farms.

© 2009 Elsevier B.V. All rights reserved.

### 1. Introduction

Resistance to all the main anthelmintic classes has been exhibited by nematode populations in most sheep-rearing

countries over the last twenty years. Gastro-intestinal nematodes can cause considerable disease and loss of production in livestock (Kloosterman et al., 1992) and the farming industry in the United Kingdom could not exist in its current form without an effective arsenal of anthelmintic drugs (Stear et al., 2000; Roger, 2008). The emergence of resistance to all three main anthelmintic groups (Wolstenholme et al., 2004; Papadopoulos, 2008) and recent accounts of multiple resistance in single nematode strains (Bartley et al., 2004; Sargison et al., 2007) is of major concern. However, as often only one species, mainly

<sup>☆</sup> This paper is part of the special issue entitled: Keynote lectures of the 7th International Sheep Veterinary Congress 2009, Guest Edited by Snorre Stuen, Martha J. Ulvund and George C. Fthenakis.

<sup>\*</sup> Corresponding author.

E-mail address: [mike.taylor@fera.gsi.gov.uk](mailto:mike.taylor@fera.gsi.gov.uk) (M.A. Taylor).

*Teladorsagia circumcincta*, is involved, acceptable control may still be achievable by appropriate parasite and resistance monitoring and careful management.

The presence of anthelmintic resistance can be measured in a number of ways and has been reviewed by Taylor et al. (2002). Under field conditions, anthelmintics may continue to give clinical responses in parasitised sheep despite the apparent presence of resistant genotypes. Consequently, sheep farmers may remain unaware of any sub-optimal production performances and that the severity of resistance will increase rapidly if the anthelmintic remains in use. By detecting resistance at an early stage, farmers can employ appropriate control recommendations to prolong the activity of the anthelmintic group(s) concerned, so that both wormer and animal performance can be maintained for longer.

## 2. Materials and methods

A network of over 40 farms was selected and matched for an ongoing study aimed at monitoring the update of new SCOPS worm control recommendations (Abbott et al., 2007) and based around participating practice-based veterinarians and their clients.

### 2.1. Parasitological techniques

Faecal samples were routinely collected from ewes and lambs in the above farms on at least four occasions throughout 2007 and 2008. Sampling dates were designed wherever possible, to coincide with the traditional sheep handling times of lambing (visit 1: March to April), "marking" (visit 2: May to June), weaning of lambs (visit 3: July to August) and "drafting" (Visit 4: September to October). Fresh faecal samples were collected from 20 ewes and/or lambs depending on the visit date and submitted to the laboratory. On receipt, individual samples were weighed, consistency scored and bulked in batches of 10 as described by Morgan et al. (2005). Faecal egg counts were performed in duplicate on each of the batched samples by using a modified McMaster Method, to an accuracy of 50 eggs per gram (epg) of faeces (MAFF, 1986). Where the mean bulked FEC was >200 epg, then remaining faeces were cultured (MAFF, 1986) and 3rd stage infective larvae were identified by using larval identification keys (Taylor et al., 2007).

### 2.2. Larval development tests (LDT)

Larval development tests were conducted at the Veterinary Laboratories Agency (VLA) and the Central Science Laboratory (now FERA) on samples submitted at visit 1 and visit 4 for each of the two grazing seasons (2007 and 2008). The method used was a modification to the method originally described by Taylor (1990). In the modified method, the culture medium comprised lyophilised *Escherichia coli* and heat-treated (autoclaved) sterile sheep faeces. In all other respects, (e.g., volumes and concentration ranges) it was as described originally. Only benzimidazole and imidazothiazole groups of anthelmintics were screened by LDT, as the test is not applicable for macrocyclic lactones (Taylor et al., 2002).

### 2.3. Faecal egg count reduction tests (FECRT)

Farms selected for FECRT were identified among those that had been routinely visited by veterinarians engaged on the research project. As such, full farm histories were available and each of these farms had either reported or confirmed, the presence of resistance to one or more anthelmintics groups by LDT. FECRTs were conducted according to standardised guidelines produced by WAAVP (Coles et al., 2006). FECRTs were only conducted on farms where a pre-treatment FEC was >200 epg; three treatment groups and one untreated control group (each with 20 sheep) were included in the test. FECs were conducted on all groups before treatment (D0), on the control and the imidazothiazole-treated groups 7 days post-treatment (D7) and on the control, the benzimidazole-treated and the macrocyclic lactone-treated groups 14 days post-treatment (D14). For all farms screened by FECRT, the test products contained fenbendazole (a benzimidazole), levamisole (an imidazothiazole) and ivermectin (a macrocyclic lactone) and were administered to animals in the form of oral drench at the recommended dose rates. All lambs were weighed prior to treatment and dose rates given were calculated according to the heaviest lamb in each group.

### 2.4. Data management and analysis

The criterion used in assessing the sensitivity of the LDT was the presence or absence of live larvae at the discriminating doses of 0.1 µg per ml of thiabendazole (for testing benzimidazole resistance), or 1 µg per ml of levamisole (for testing imidazothiazole resistance).

FECRT results were calculated on arithmetic means using the formula:  $FECR\% = 1 - [(Treatment_2/Treatment_1) \times (Control_1/Control_2)] \times 100$ , as described in Coles et al. (2006). Resistance was considered present if the percentage reduction in arithmetic mean FEC of a test group compared to that of the untreated controls, was <95%.

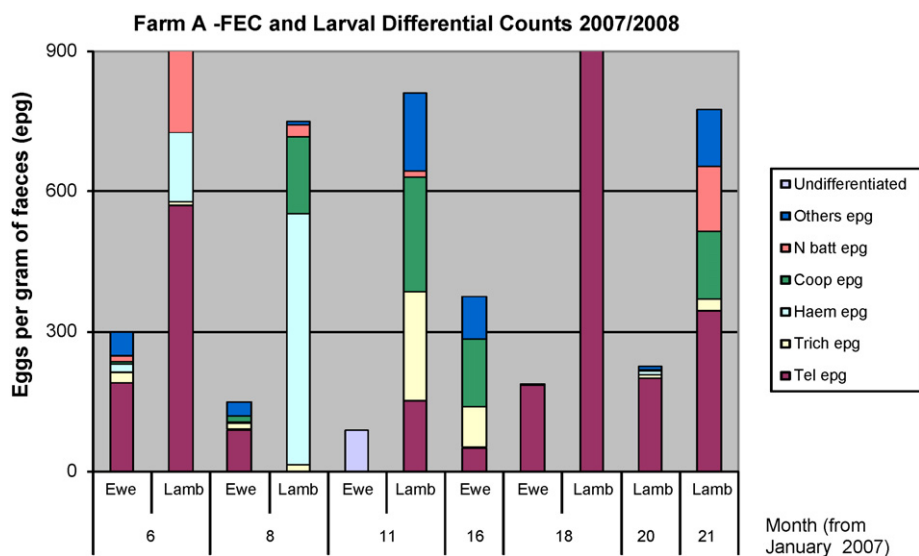


Fig. 1. Seasonal variation in faecal egg counts and parasite genera based on larval differentiation in ewes and lambs (farm A) [count of months below x axis starts from January 2007: 6 = June 2007 to 21 = September 2008].

**Table 1**

Comparison of results for faecal egg reduction test and larval development test for benzimidazole (BZ), imidazothiazole (IDZ) and macrocyclic lactone (ML) classes of anthelmintics.

Farm	Test employed	BZ	IDZ	ML	Main parasitic genus involved	Comments
A	FECRT	6	80	84	<i>Teladorsagia</i>	Tel: triple resistance, Tric: IDZ, Coop: BZ,
	LDT	>0.5	3	–	<i>Teladorsagia</i>	Tel, Trich: BZ, IDZ, Coop: LV
B	FECRT	0	0	0	<i>Teladorsagia</i>	Tel: triple resistance, Trich: IDZ, Coop: IDZ,
	LDT	0.5	>5	–	<i>Teladorsagia</i>	Tel: IDZ, Trich: BZ, Coop: BZ
C	FECRT	0	99	100	<i>Teladorsagia</i>	Haem, Trich, Coop: BZ
	LDT	>0.5	5	–	<i>Teladorsagia</i>	Tel: BZ, Trich: BZ, IDZ, Coop: BZ
D1	FECRT	21	14	70	<i>Teladorsagia</i>	Tel: triple resistance
	LDT	>0.3	>1	–		
D2	FECRT	60	100	36	<i>Teladorsagia</i>	Tel: BZ, ML
	LDT	0.4	3	–		Tel: BZ, IDZ, Trich: BZ, IDZ
E	FECRT	93	96	93	<i>Teladorsagia</i>	Tel: BZ, IDZ
	LDT	3	–	3	<i>Teladorsagia</i>	Tel: BZ, IDZ, Trich: IDZ
F	FECRT	100	90	100	<i>Teladorsagia</i>	Tel: BZ, IDZ, Trich: BZ, Coop: BZ
	LDT	<1	–	<1	<i>Teladorsagia</i>	Tel: BZ

In faecal egg reduction test (FERCT) results are expressed as percentage reduction of egg shedding <95% indicates resistance; in larval development test (LDT) results are expressed as minimum inhibitory concentrations >0.3 g/ml indicates resistance.

### 3. Results and discussion

For all 40 farms visited, *Teladorsagia* was the predominant genus and LDT results suggested that all farms except one (97.5%), had populations containing alleles conferring resistance to benzimidazole-groups of anthelmintics. A much lower frequency of resistance alleles to imidazothiazoles was found, with a lower prevalence (40%) of resistance on farms. The general prevalence of *Trichostrongylus* spp. was lower on all farms (85%) and consequently, fewer LDT assessments could be made. Populations resistant to benzimidazoles were identified on 16 farms (44%) and populations resistant to imidazothiazoles on 18 farms (50%), but the detectable presence of resistant genotypes was not always consistent and varied between seasons and the time of year that the LDT was conducted. Although *Haemonchus* was present on 55% of the farms as determined by larval differentiation, infection levels were generally low compared to other genera present and as a consequence few LDT test results were available for this genus. Only 11 of the study farms had *Haemonchus* present in any of the samples tested by LDT and LDT results were generally inconclusive and difficult to interpret for this genus.

Parasitic gastroenteritis is a complex multi-species disease, with the causative nematodes having both free living, as well as host-dependent life stages (Coop and Jackson, 2000). Suppressive treatments are carried out only on the host-dependent stages and free-living stages are said to be in refugia (Gaba et al., 2006). This, coupled with the fact that animals are frequently moved between pastures and that ewes and lambs are often treated differently due to differences in immune states (Stear et al., 2000), means that resistance alleles in the worm population will often be in a state of flux. Additionally, differences in lifecycle parameters (O'Connor et al., 2006) may result in differences in prevalence for the different genera across the grazing season Boag and Thomas (1977), such that appearance of different genera (Fig. 1) and observed resistance levels may vary on the same farm, depending on the time of year that testing was performed.

Multiple anthelmintic resistance was identified on 14 of 40 farms by using the LDT; on six farms, visited in 2008, further FECRT data was obtained and compared with LDT results (Table 1). On all six farms, resistance was present in one or more genera but most commonly in *Teladorsagia*. On two farms “triple” resistant *Teladorsagia* was identified by means of FECRT and on one farm (Farm D2), moxidectin resistance was suspected based on an early return to egg shedding at 28 days post-treatment. In fact, that farm D2 presents an interesting case in that triple resistance had been identified by FECRT in 2007 (Table 1). Sheep were subsequently quarantined and moved from another farm, Farm D1, under the same ownership. The quarantine procedure involved withholding feed overnight, sequential treatment with moxidectin, levamisole and albendazole, before holding on concrete for 48 h (Abbott et al., 2007) followed by turn out onto pasture previously grazed by cattle only. Despite the precautions taken, the new lambs showed a steady increase in FEC and the FECRT in September 2008 showed both benzimidazole and macrocyclic lactone resistance (Table 1) and an early return to patent infection following moxidectin treatment (D28). LDT confirmed resistance to benzimidazole for *Teladorsagia* and *Trichostrongylus*, but also detected resistance to imidazothiazoles in these species. The implication that moxidectin failed to completely remove this triple resistant worm population is of particular concern, as it is often the drug strategically used in sustainable integrated parasite control programmes (Wilson and Sargison, 2007). Since few UK farmers can avoid introducing sheep to their farms to maintain their flocks, this report and recent more serious cases of flock closure (Sargison et al., 2005; Blake and Coles, 2007) due to triple resistance are of particular concern.

### Acknowledgements

The authors are grateful to all farmers, veterinarians and advisors involved on the project. The authors also gratefully acknowledge Rowan Wood at the VLA, Ruth Grant and Valerie Boughtflower at FERA for laboratory assistance. Funding was provided by both Defra and EBLEX.

## References

- Abbott, K.A., Taylor, M., Stubbings, L.A., 2007. Anthelmintic resistance—new guidelines. In: Sustainable Worm Control Strategies for Sheep. SCOPS.
- Bartley, D.J., Jackson, F., Jackson, E., Sargison, N.D., 2004. Characterisation of two triple resistant field isolates of *Teladorsagia* from Scottish lowland sheep farms. *Vet. Parasitol.* 23, 189–199.
- Blake, N., Coles, G., 2007. Flock cull due to anthelmintic-resistant nematodes. *Vet. Rec.* 161, 36.
- Boag, B., Thomas, R.J., 1977. Epidemiological studies on gastrointestinal nematode parasites of sheep: the seasonal number of generations and succession of species. *Res. Vet. Sci.* 22, 62–67.
- Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* 136, 167–185.
- Coop, R.L., Jackson, F., 2000. Gastrointestinal helminthosis. In: Martin, W.B., Aitken, I.D. (Eds.), *Diseases of Sheep*. Blackwell Science, Oxford.
- Gaba, S., Cabaret, J., Ginot, V., Silvestre, A., 2006. The early drug selection of nematodes to anthelmintics: stochastic transmission and population in refuge. *Parasitology* 133, 345–356.
- Kloosterman, A., Parmentier, H.K., Ploeger, H.W., 1992. Breeding cattle and sheep for resistance to gastrointestinal nematodes. *Parasitol. Today* 8, 330–335.
- M.A.F.F., 1986. *Manual of Veterinary Parasitological Laboratory Techniques*. HMSO, London.
- Morgan, E.R., Cavill, L., Curry, G.E., Wood, R.M., Mitchell, E.S., 2005. Effects of aggregation and sample size on composite faecal egg counts in sheep. *Vet. Parasitol.* 131, 79–87.
- O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Vet. Parasitol.* 142, 1–15.
- Papadopoulos, E., 2008. Anthelmintic resistance in sheep nematodes. *Small Rumin. Res.* 76, 99–103.
- Roger, P.A., 2008. The impact of disease and disease prevention on sheep welfare. *Small Rumin. Res.* 76, 104–111.
- Sargison, N.D., Jackson, F., Bartley, D.J., Moir, A.C.P., 2005. Failure of moxidectin to control benzimidazole-, levamisole-, and ivermectin-resistant *Teladorsagia circumcincta* in a sheep flock. *Vet. Rec.* 156, 105–109.
- Sargison, N.D., Jackson, F., Bartley, D.J., Wilson, D.J., Stenhouse, L.J., Penny, C.D., 2007. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. *Vet. Parasitol.* 145, 65–76.
- Stear, M.J., Mitchell, S., Strain, S., Bishop, S.C., McKellar, Q.A., 2000. The influence of age on the variation among sheep in susceptibility to natural nematode infection. *Vet. Parasitol.* 89, 31–36.
- Taylor, M.A., 1990. A larval development test for the detection of anthelmintic resistance in nematodes of sheep. *Res. Vet. Sci.* 49, 198–202.
- Taylor, M.A., Hunt, K.R., Goodyear, K.L., 2002. Anthelmintic resistance detection methods. *Vet. Parasitol.* 103, 183–194.
- Taylor, M.A., Coop, R.L., Wall, R.L., 2007. *Veterinary Parasitology*, 3rd ed. Blackwell, Oxford.
- Wilson, D., Sargison, N., 2007. Anthelmintic resistance in *Teladorsagia circumcincta* in sheep in the UK. *Vet. Rec.*, 535–536.
- Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C., 2004. Drug resistance in veterinary helminths. *Trends Parasitol.* 20, 469–476.