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Multiple anthelmintic resistance of *Haemonchus contortus*, including a case of moxidectin resistance, in a Dutch sheep flock

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Benzimidazole resistance in sheep was first described in 1964 (Drudge and others 1964). Nowadays, multiple anthelmintic resistance is of major concern for the sheep and goat industry (Sargison 2012). In Europe, resistance to moxidectin (MOX) has been described in Germany in *Haemonchus contortus* (Scheuerle and others 2009), in the UK in *Teladorsagia circumcincta* (Wilson and Sargison 2007, Sargison and others 2010), and recently to long-acting injectable MOX in northwestern Spain (Martinez-Valladares and others 2013), and is not as common as resistance to ivermectin (IVM) and doramectin (DRM) (Maingi and others 1997, Ambrosini 2000, Sargison and others 2001, Cernanska and others 2006, Borgsteede and others 2007).

On a Dutch sheep farm, MOX resistance was suspected, and a study was conducted aiming (1) to investigate this suspected case, (2) to simultaneously test the efficacy of DRM, monepantel (MPL), fenbendazole (FBZ) and levamisole/triclabendazole (LEV/TCBZ) and (3) to identify the genus and species of nematodes before and after treatment by larval culture.

On a farm with 700 breeding ewes, around 150 ewe lambs were purchased annually as replacements. On arrival, these lambs were treated with MOX (Cydectin 0.1 per cent Oral Solution for Sheep, Zoetis BV), and subsequently placed onto low-contaminated pastures

with a pre-existing population of the farm's endemic worm population. In preceding years, all breeding ewes were treated with MOX (Cydectin 0.1 per cent Oral Solution for Sheep, Zoetis BV) directly after lambing, and with DRM (Dectomax Solution for Injection, Zoetis BV) six weeks prior to breeding. Lambs were treated in spring first with oxfendazole (Bovex Oral Suspension, Chanelle), and subsequently every four to five weeks alternately with DRM and MOX. In August 2012, lambs were in poor condition and, after treatment with MOX, high strongyle-type egg counts were found in pooled faecal samples.

During late summer, 6 groups of 10 lambs were randomly selected out of a flock of 100 crossbred Texel lambs, and were weighted, marked and individually identified. On day 0 and day 10, individual faecal samples were collected. Lambs from group 1 remained as untreated controls. Group 2–6 were treated with MOX (Cydectin 0.1 per cent Oral Solution for Sheep, 0.2 mg/kg bodyweight, Zoetis BV), DRM (Dectomax Solution for Injection, 0.2 mg/kg bodyweight, Zoetis BV), MPL (Zolvix, 2.5 per cent Oral Solution for Sheep, 2.5 mg/kg bodyweight, Novartis Animal Health), FBZ (Panacur 2.5 per cent Oral Suspension for Sheep, 5 mg/kg bodyweight, MSD Animal Health), LEV/TCBZ (Endex 8.5 per cent Oral Suspension for Sheep, 7.5 mg LEV/kg bodyweight, Novartis Animal Health), respectively, at the manufacturer's recommended dose rate.

A faecal egg count reduction test (FECRT) was carried out according to the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles and others 1992). Faecal egg counts (FEC) were performed using a modified McMaster method with a sensitivity of 17 eggs per gram (EPG). The percentage reduction was calculated according to the following formula: $\text{Efficacy} = 100 \times (1 - \text{arithmetic mean EPG of the treated group} / \text{arithmetic mean EPG of the control group})$. A composite larval culture was made of each group, and cultures were incubated for two weeks at 20°C and thereafter processed according to the method of Roberts (Roberts and O'Sullivan 1950). For each group, a maximum of 100 larvae were identified according to the tables of Eckert (Eckert 1960).

FEC reductions were calculated using the Reso FECRT analysis program V.4 (<http://sydney.edu.au/vetscience/sheepwormcontrol/>) for Excel. Analyses for resistance were based on WAAVP recommendations (Coles and others 1992).

FEC results are shown in Table 1. At day 0, faecal samples of 59 out of 60 lambs were positive for strongyle-type eggs. At day 10, one DRM-treated lamb was presented with severe pneumonia and no faeces could be collected. At day 10, no strongyle-type eggs were seen in samples from the MPL and LEV/TCBZ groups. In all other samples, strongyle-type eggs could be detected. Calculated efficacies indicated that resistance was present to MOX, DRM and FBZ, while the worm population was fully susceptible to MPL and LEV/TCBZ.

Results of coprocultures are also given in Table 1. At day 0, all larvae (n=100) in the control group were identified as *H. contortus*. In the MOX group, DRM group, MPL group, FBZ group and the LEV/TCBZ group, the percentage *H. contortus* was 97, 95, 94, 97 and 98 per cent, respectively. All remaining larvae were identified as *Teladorsagia trichostrongylus* species.

At day 10 again, all larvae in the control group (n=100) were identified as *H. contortus*. In the MOX group, DRM group and FBZ group, the percentage *H. contortus* was 99, 100, and 98 per cent, respectively. In the LEV/TCBZ group, five larvae were detected, and one was identified as *H. contortus*. All remaining larvae were identified as *Teladorsagia trichostrongylus* species. No larvae were detected in the MPL group.

Multiple anthelmintic resistance is a worldwide threat to the small ruminant industry. Although MOX resistance has been described before in Europe (Wilson and Sargison 2007, Scheuerle and others 2009, Sargison and others 2010, Martinez-Valladares and others 2013), this study confirmed a case of *H. contortus* resistance to milbemycins and avermectins, MOX and DRM, in a sheep flock in Europe. Also, resistance to FBZ was found in this flock. Outside Europe,

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TABLE 1: Results of FEC (mean EPG, range (minimum–maximum) and number of positive samples), larval identification at day 0 and day 10 after treatment, and results of FECRT (reduction percentage and 95% CI) within each group

Group	Day 0	Day 10	Reduction% (R/S) (95% CI)
Control (n=10/10)			
Mean EPG St (minimum–maximum)(n pos)	1352 (17–5350) (10)	2680 (250–5900) (10)	NA
Larval id (n)	100% Hc (100)	100% Hc (100)	NA
MOX (10/10)			
Mean EPG St (minimum–maximum) (n pos)	1148 (17–2250) (10)	630 (133–1350)(10)	76.5 (R) (57.1 to 87.1)
Larval id (n)	97% Hc, 3% T/T (100)	99% Hc, 1% T/T (100)	NA
DRM (10/9)			
Mean EPG St (minimum–maximum) (n pos)	795 (100–2850) (10)	1613 (233–5900) (10)	39.8 (R) (0 to 75.6)
Larval id (n)	95% Hc, 5% T/T (100)	100% Hc (100)	NA
MPL (10/10)			
Mean EPG St (minimum–maximum) (n pos)	2105 (0–8350) (9)	0 (0) (0)	100 (S)
Larval id (n)	94% Hc, 6% T/T (100)	NA (0)	NA
FBZ (10/10)			
Mean EPG St (minimum–maximum) (n pos)	1748 (83–3600) (10)	1137 (83–3350) (10)	57.6 (R) (14.3 to 79.0)
Larval id (n)	97% Hc, 3% T/T (100)	98% Hc, 2% T/T (100)	NA
LEV/TCBZ (10/10)			
Mean EPG St (minimum–maximum) (n pos)	833 (183–2250) (10)	0 (0) (0)	100 (S)
Larval id (n)	98% Hc, 2% T/T (100)	20% Hc, 80% T/T (5)	NA

For all groups, the number of lambs sampled at day 0 and day 10 is given

DRM, Doramectine; FBZ, Fenbendazole; FEC, Faecal egg counts; FECRT, Faecal egg count reduction test; Hc, *Haemonchus contortus*; id, Identification; LEV/TCBZ, Levamisole/triclabendazole; MOX, Moxidectine; MPL, Monepantel; NA, Not applicable; R, Resistant; S, Susceptible; St, Strongyle-type; T/T, *Teladorsagia/Trichostrongylus* species

resistance to MOX is much more common (Watson and others 1996, Wooster and others 2001, Ranjan and others 2002, Chandrawathani and others 2003, Hughes and others 2004, Almeida and others 2010). In The Netherlands, resistance of *H. contortus* to LEV and MPL has not been found.

On this farm, MOX treatments during quarantine and around lambing, and repeated treatments of lambs with DRM and MOX before turning the animals on low contaminated pastures possibly offered resistant alleles a survival advantage, and established a heavily preselected population. Limited use of anthelmintics and correct anthelmintic dose rates, combined with targeted grazing management, are important features of modern parasite control, trying to prevent selection for resistance especially when the proportion of susceptible nematodes exposed to the anthelmintic compared with that on pasture is high at the time of treatment. These control measures are even more important taking into account that long-term reversion to susceptibility is unusual or, in all probability, does not occur within flocks (Sargison 2012).

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Competing interests At the time of this study, D P D was a paid employee of Zoetis BV.

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