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Research paper

Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands

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ABSTRACT

In a survey involving 34 sheep flocks spread over the Netherlands anthelmintic resistance (AR), based on a fecal egg count reduction (FECR) test, was determined for six different products. The study was conducted in ewes shortly after lambing during spring 2015. A FECR of less than 90%, indicating presence of AR against one or more nematode genera producing strongylid eggs, was found in 22 of 30 (73.3%) flocks against oxfendazole, 18 of 23 (78.3%) flocks against ivermectin, 15 of 32 (46.9%) flocks against moxidectin, and 2 of 26 (7.7%) flocks against monepantel. No AR was observed against levamisole. If oxfendazole resistance was observed, *Haemonchus contortus* was involved in 90.5% of the cases. If resistance against ivermectin, moxidectin or monepantel was observed, it invariably involved *H. contortus*. In the majority of cases resistance was also observed for *Teladorsagia circumcincta* and/or *Trichostrongylus* spp, between which no distinction was made in this study. Based on FECR 9 of 15 (60.0%) flocks showed resistance against closantel, which was mainly due to closantel not being effective against most other nematode species than *H. contortus*. However, in 44.4% of flocks showing reduced FECR it did involve *H. contortus* as well.

Multi-drug resistance (excluding closantel) was found in 16 flocks, of which 8 showed resistance against 2 products, 7 against 3 products and 1 flock showed resistance against 4 products. If resistance against 3 or 4 products was present, there invariably was resistance against both ivermectin and moxidectin. Overall, of the 22 flocks in which both macrocyclic lactones (ML) were tested, 4 (18.2%) showed no resistance against both products, 9 (40.9%) showed resistance against ivermectin only, and 9 (40.9%) showed resistance against both MLs.

It is concluded that AR is widespread in sheep in the Netherlands and involves products from all major anthelmintic classes, with possibly the exception of levamisole. It appears that the macrocyclic lactones have lost much of their efficacy against sheep nematodes over the last decade.

1. Introduction

In the Netherlands, gastrointestinal (GI) nematode infections, particularly haemonchosis, belong to the most important diseases threatening sheep production (Ploeger et al., 2016). As elsewhere in the world, control of these infections has been and still is largely based on the use of anthelmintics, but anthelmintic resistance (AR) is of increasing concern as recently reviewed by Rose et al. (2015). In the Netherlands benzimidazole resistance became widespread in the 1980-1990's (Boersema et al., 1987; Borgsteede et al., 1997). Until 2007 no resistance was reported against anthelmintics other than the benzimidazoles. In 2007 and 2010 doramectin and ivermectin resistance became apparent on several farms (Borgsteede et al., 2007, 2010), followed by reports on AR against moxidectin and even monepantel (Van den Brom et al., 2013, 2015). The last survey on AR was carried out by Borgsteede et al. (1997). Here, results are presented of a survey to establish the current level of AR carried out in 2015 for six products representing every major anthelmintic class (oxfendazole representing the benzimidazoles; ivermectin and moxidectin representing the macrocyclic lactones (ML); levamisole representing imidazothiazoles; monepantel representing amino-acetonitrile derivatives; and closantel representing salicylanilides).

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2. Materials and methods

2.1. Farms and animals

Farms were invited to participate in the study through the Dutch Sheep and Goat Breeders Association (NSFO) by means of their web- and news-site, and by making the study public in a well-read sheep trade journal. Farmers were asked to have preferably 49, but at least 21, animals available for testing. Animals were adult ewes and entered the study approximately one to four weeks following lambing. Ewes were not treated by the owner prior to or around lambing. Suckling ewes were chosen for this study as they usually excrete worm eggs in sufficient numbers over a period of many weeks after lambing without showing clinical symptoms and therefore allowing a save fecal egg count reduction (FECR) test. This study was performed during spring and early summer of 2015. Data were available from 34 farms which were located in 10 of the 12 Dutch provinces (2–6 farms per province). The 34 farms owned 19–375 ewes (≥1 year) with a mean of 85 and a median of 59 ewes (see Supplementary data Table S1). One farm owned less than the minimum requested 21 ewes (19 ewes), but was kept in the study. In total, 14 of the 34 farms had less than 49 ewes available.

2.2. Treatment groups

The WAAVP guidelines indicate to test 15 animals per group for accurate evaluation of FECR following treatment (Coles et al., 1992), but this was later reduced to 10 animals per group if available (Coles et al., 2006). Given the average size of Dutch sheep farms (see Ploeger et al., 2016), it was anticipated that requiring 10–15 animals per group would lead to fewer farms where all six products could be tested. Therefore, it was decided to reduce the number of animals to seven per group, about half of those recommended by the original WAAVP guidelines (Coles et al., 1992). This choice was supported by results from Rinaldi et al. (2014), who showed that fecal egg counts made on pooled samples from 5, 10 or 20 sheep correlated strongly with each other and with the mean of the individually examined samples and gave similar results when examining anthelmintic drug efficacy. The six products tested were oxfendazole (Bovex®), levamisole (Endex®), ivermectin (Oramec®), moxidectin (Cydectin®), monepantel (Zolvix®), and closantel (Flukiver®). Products were administered as a drench, according to manufacturer's instructions and in a single product, except for levamisole which was administered using a combination product that included triclabendazol as this was the only registered levamisole product. In the Netherlands, closantel is only registered in a combination product with mebendazole. Therefore, closantel was obtained from the UK as a single product. Dosage for each product was based on the weight of the heaviest ewe in the flock. If no weighing scale was available, the weight of the heaviest ewe was estimated by visual inspection with 10 kg added. On all farms a control group of seven animals was included. Animals on a farm were randomly allocated to each treatment group. Ewes remained housed up to the post-treatment visit 10 to 14 days after treatment when again fecal samples were collected. Coles et al. (2006) recommended different post-treatment intervals depending on the product used, ranging from 3 to 7 days for levamisole to 14–17 days for macrocyclic lactones. However, abiding by these intervals would result in severe logistic difficulties and it was, therefore, decided to choose a convenience interval of 10–14 days for all products tested.

2.3. Sampling and laboratory analysis

Fecal samples were collected from the rectum from all individual animals in each group using a plastic bag on the day of treatment (day 0). After taking the sample, the bag was closed as airtight as possible and identified with the eartag number of the ewe. Samples were processed the same day or stored at 4 °C until the next day. Fecal egg counts on day 0 were done on composite samples, except from 9 farms of which samples were examined individually because these farms also participated in another study. After treatment (day 10–14), the same sheep were sampled again but all egg counts were done on composite samples per group. Composite samples were chosen to allow more farms to be included. Composite samples were prepared in the laboratory and consisted of equal amounts of feces (3 g per animal) from each individual sample per treatment group and were thoroughly mixed with a mortar and pestle. Three separate egg counts were made from each composite sample using a McMaster technique with a detection limit of 50 eggs per gram feces (EPG), to ensure an accurate egg count from each composite sample even though this partly eliminated the time and labour advantage of using composite samples.

On the day of treatment a composite feces sample from 14 randomly selected ewes, irrespective of treatment group, was cultured for 10–13 days at room temperature for larval identification. For the composite sample 10 g of feces was taken from each individual sample. Following the second farm visit a composite feces sample was made for each treatment group separately and cultured under similar conditions, provided strongyle eggs had been found. Culturing, larval collection and identification were as described by MAFF (1977). Hundred larvae, if present, were identified with for practical purposes no distinction made between *Teladorsagia circumcincta* and *Trichostrongylus* spp.

2.4. Statistical analysis

Percentage FECR (%FECR) was calculated as 100*(1-(T2/T1)*(C1/C2)), with C1 and C2 the mean arithmetic fecal egg count of the control group at day 0 and day 10–14, respectively, and likewise T1 and T2 the mean arithmetic fecal egg count of a treatment group. Means were calculated from the three replicate egg counts for composite samples, or in case of the 9 farms also participating in another study from the individual pre-treatment egg counts. In five flocks no control group was available on request by the farmer. In one other flock a C2 sample missed as well as a T1 sample for levamisole. In these cases, FECR was calculated as 100*(1-(T2/T1)) or 100*(1-(T2/C1)). No 95% confidence limits were calculated for the FECR as all FECRs involved triplicate egg counts from composite samples 10–14 days after treatment. To allow for the missing lower 95% confidence limit, AR was deemed present if FECR was < 90%. If FECR was between 90% and 95%, presence of AR was suspected.

3. Results

Table S1 in the supplementary data presents the results for all 34 flocks, showing flock size, involved sheep breeds, which products were tested in each flock and mean pre- and post-treatment egg counts for the treatment groups with resulting %FECR. The mean arithmetic EPGs for all treatment groups in the 34 flocks on day 0 ranged between 17 and 5767 EPG (Table S1). Of 172 treatment groups 14 showed a pre-treatment mean EPG lower than 150 as recommended by Coles et al. (2006), with specifically one flock having low EPGs pre-treatment in all 7 groups examined in this flock. All other cases of less than 150 EPG concerned groups in flocks also having groups with mean EPGs higher than 150.

Table 1 shows the observed efficacies for the six products tested based on FECR following treatment. Only levamisole showed a good efficacy on all farms tested, with just one flock with suspected AR. Ivermectin showed the lowest median efficacy compared to the other products. The apparent AR based on FECR for closantel is largely due to its lack of efficacy against GI nematodes other than *H. contortus*.

Table 2 shows the proportion of flocks with < 90% FECR for a product in which *H. contortus* or *T. circumcincta/Trichostrongylus* spp. larvae were present in cultures after treatment. If AR was present against the MLs or monepantel, it always involved *H. contortus*, whereas there still are some flocks in which *T. circumcincta/Trichostrongylus* spp.