Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

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

H.W. Ploeger[a,*], R.R. Everts[b,c]

[a] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands
[b] Dutch Sheep and Goat Breeders Association (NSFO), P.O.Box 160, 5300 AD Zaltbommel, the Netherlands
[c] Veterinary Practice (Diergeneeskundig Centrum), Zuid-Oost Drenthe, Looweg 84, 7741 EE, Coevorden, the Netherlands

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).

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.

Table 1
Efficacy measured by FECR of six anthelmintic products tested in ewes following lambing in 2015.

Anthelmintic	No. flocks tested	No. flocks showing 90–95% efficacy	Flocks showing < 90% efficacy		Percentage Flocks showing < 90% FECR (*95% CI* [b])	
			No.	Median % efficacy (*range*)		
oxfendazole	30	3	22	63.9 (*0–88.0*)	73.3	(*55.6–85.8*)
levamisole	18	1	0		0	(*0–17.6*)
ivermectin	23	0	18	38.1 (*0–84.9*)	78.3	(*58.1–90.3*)
moxidectin	32	2	15	69.9 (*0–89.7*)	46.9	(*30.9–63.6*)
monepantel	26	0	2	65.3 (*46.0–84.5*)	7.7	(*2.1–24.1*)
closantel [a]	15	3	9	67.3 (*0–80.1*)	60.0	(*nc* [c])

[a] Closantel is only effective against blood-sucking gastrointestinal nematodes.

[b] 95% CI calculated using the program Epitools (Sergeant, 2017).

[c] nc = not calculated, because in many egg counts following treatment other species than *H. contortus* were represented as well.

Table 2
Presence of larvae from *Haemonchus contortus* (Hc) or the combination of *Teladorsagia circumcincta* and *Trichostrongylus* species (TT) in larval cultures after treatment in flocks showing a FECR of less than 90%.

Anthelmintic	Hc		TT	
	in % of flocks	min-max% in culture	in % of flocks	min-max% in culture
oxfendazole	90.5	6–100	76.2	1–100
levamisole	–	–	–	–
ivermectin	100	2–100	61.1	2–98
moxidectin	100	72–100	41.7	1–28
monepantel	100	33–97	0	–
closantel	44.4	2–61	100	28–100

were fully susceptible. In 4 of the 9 flocks (44.4%) showing a FECR < 90% against closantel, *H. contortus* larvae were found in the culture after treatment, suggesting closantel resistance in *Haemonchus*. Considering the culture results before treatment closantel efficacies amounted to 44.7, 82.6, 85.8 and 93.3% against *Haemonchus*.

Excluding closantel, in 30 flocks at least two products were tested. Multi-drug resistance was observed in 16 (53.3%) of these flocks, which involved products from different anthelmintic classes in 15 of these 16 flocks (Table S1). In 8 flocks AR was found against 2 products and in 7 flocks against 3 products. One flock showed resistance against 4 products, oxfendazole, ivermectin, moxidectin and monepantel. Of 29 flocks where both oxfendazole and a ML were tested, 15 (51.7%) showed AR against products from both anthelmintic classes. The two flocks showing monepantel resistance also showed resistance to both moxidectin and ivermectin. Of 22 flocks in which both ivermectin and moxidectin were tested, 4 (18.2%) showed no ML resistance, 9 (40.9%) showed resistance to ivermectin only, and the other 9 (40.9%) showed resistance to both MLs.

4. Discussion

Coles et al. (2006) gave recommendations how to perform FECR tests to evaluate AR in sheep. Our study design deviated in three aspects from those recommendations. One, fecal egg counts were performed mostly on composite samples rather than individual samples. Two, 7 animals per treatment group were used instead of at least 10. And three, the post-treatment sample was collected after 10–14 days for all products tested. Rinaldi et al. (2014) showed that FECR tests on composite fecal samples is possible and generally leads to the same conclusion as FECR tests based on individual samples, which was recently confirmed by others (Kenyon et al., 2016; George et al., 2017). They also showed

that there was little difference between composite samples based on 5, 10 or 20 animals as well as that the used egg counting technique, mini-FLOTAC or McMaster, gave largely similar results (Rinaldi et al., 2014; Kenyon et al., 2016). Only if many low or zero egg counts in a flock are present, a lesser accuracy with the McMaster technique using a sensitivity of 50 EPG might result (Kenyon et al., 2016). The present survey was conducted on ewes shortly after lambing. Due to the spring-rise in EPG in such animals, many low or zero egg counts were not expected, while high egg counts (up to thousands of EPG) usually do not present clinical problems in adult ewes. Nonetheless, one farm showed in all treatment groups mean EPGs below the threshold of 150 as recommended by Coles et al. (2006) for FECR tests. In the other 33 flocks sometimes a group had a pre-treatment EPG of less than 150, but in all these cases mean EPGs were close to the recommended threshold as well as that other groups in the same flocks did have EPGs above the threshold. In the expectation that an effective treatment should result in a zero egg count, finding eggs after treatment at least suggests presence of AR. Using triplicate egg counts on composite samples also ensured a fair accuracy of low mean egg counts. Therefore, no flock or treatment group was left out despite sometimes a low initial mean EPG. Using composite samples also influences one of the criteria used for AR, i.e. a lower 95% confidence limit of less than 90% based on counts from individual samples (Coles et al., 1992). However, 95% confidence limits for three replicate counts on a composite sample may result in smaller intervals, which may require a different interpretation. Therefore, without using 95% confidence limits we choose to be conservative in assessing AR by setting the threshold at less than 90% FECR.

The third deviation from the recommendations by Coles et al. (2006) involved the compromise we made on the interval of 10–14 days between pre- and post-treatment sampling. For levamisole a period of 3–7 days is recommended because this product may be less effective against inhibited larval stages. For the MLs a period of 14–17 days and for benzimidazoles a period of 8–10 days is recommended because these products may have a temporary sterilizing effect on the worms. However, Coles et al. (2006) also indicated that these intervals "are based on best guesses". It is reasonable to presume that highly effective products should result in a FECR of at least 95% and mostly resulting in zero egg counts after treatment before the prepatent period of the gastrointestinal nematodes involved ends. An interval of 10–14 days also does not deviate much from the recommended periods for benzimidazoles and MLs. Only in case of levamisole our chosen interval was much longer than recommended. But we considered this a minor issue because we did not expect much levamisole resistance yet, nor that we expected many positive egg counts because of the longer interval after treatment based on empirical experience in the field. Moreover, Borgsteede et al. (1997) also used a 14-day post-treatment interval, and more recently Playford et al. (2014) also reported FECR results based on 10–14 day intervals after treatment with a various anthelmintics, including levamisole. Therefore, the decision to visit the farms only once for the post-treatment sampling is considered to have had little effect on the results.

Cabaret (2014) discussed the difficulties in following the formal guidelines for conducting FECR tests and concluded that the deviations as used in the present study, do allow detection of AR, albeit that the estimated efficacies may be somewhat less accurate. A final point of discussion in our study pertains to the fact that we used lactating ewes whose physiology may differ from that of non-lactating animals, possibly affecting the efficacy of anthelmintic treatment. However, we never encountered differences between lactating and non-lactating sheep suggestive of reduced anthelmintic efficacy due to lactation. Cringoli et al. (2009) showed that both ivermectin and moxidectin were highly effective in lactating ewes, clearly indicating that FECR studies can be done in such animals. Overall, we consider the followed study design to be sufficiently appropriate to have produced accurate data on the level of anthelmintic resistance in the studied flocks.

To what extent our results give a true reflection of the situation in

the Netherlands may be more difficult to answer. The farms participating in this study were slightly larger with a mean of 85 and a median of 59 ewes compared to those in the study of Ploeger et al. (2016) (74 and 31, respectively). In the current study we specifically asked for farms with at least 21 and preferably 49 ewes present, which did not allow inclusion of very small flocks kept for hobby. Therefore, results of the current survey may not reflect the level of anthelmintic resistance present in small hobby flocks, even though anthelmintic treatments are applied in many of these flocks as well (Ploeger et al., 2016). Another bias may have resulted from the method of enrolling farms, with farmers volunteering to participate. It is difficult to judge the direction of bias resulting from this. Participants may had more experience than average with failing anthelmintics and were, therefore, more inclined to participate. On the other hand, some farmers also may had little experience with failing products but due to recent attention for AR were just interested to learn about their current situation. We unfortunately did not ask about the reason for volunteering. Whatever the reason for participating, it seems unlikely that our study only involved farms with extreme levels of AR. Results generally concur with results obtained over the last decade from several other smaller research projects (unpublished data). Therefore, we feel confident that results reflect the situation on Dutch farms keeping sheep for commercial purposes.

Anthelmintics were tested from all major groups as registered in the Netherlands. Of flocks tested, 73.3% showed resistance to oxfendazole. Benzimidazole resistance was already widespread in the mid 1990's as Borgsteede et al (1997) found oxfendazole resistance in 84% of sheep flocks by means of a FECR test. Over the last decades, most sheep farmers used oxfendazole (benzimidazoles) mainly to treat against nematodirosis or against tapeworm infection, which has been the general recommendation in the Netherlands so the MLs could be specifically used for treating against *H. contortus* and *Teladorsagia/Trichostrongylus* spp. The current resistance percentage of 73.3% is lower than the 84% found two decades ago. One can speculate whether this difference is real or just coincidence. Leignel et al. (2010) suggested that reversion of benzimidazole resistance may not be expected, and this appears to be generally accepted. However, benzimidazoles still are used on most sheep farms, even though it is mostly against nematodirosis or tapeworms. This may have kept AR levels high over the last decades. Interestingly, the median efficacy of oxfendazole in the current study was 63.9%, which implies that on many farms oxfendazole will reduce GI nematode infections substantially. Borgsteede et al. (1997) observed a mean FECR of 73%. Overall, it does appear that benzimidazole resistance, while apparently not reversed, also did not increase since 1997.

Ivermectin was introduced to the Dutch market in the 1980s and moxidectin in the 1990s. Until 2007 both anthelmintics showed satisfactory efficacies. Ivermectin resistance emerged in 2007 and was reported in 2010 (Borgsteede et al., 2010), and moxidectin resistance in 2012 (Van den Brom et al., 2013). Both products were the two most used anthelmintics for decades on sheep farms and kept their good efficacy for many years, especially ivermectin. However, ivermectin resistance now is even more widespread than benzimidazole resistance with 78.3% of flocks showing a reduced FECR. Moreover, the median efficacy for ivermectin was found to be 38.1%, which is much less than observed for oxfendazole. Moxidectin resistance occurs less frequent than ivermectin resistance, but even so, 46.9% of flocks already showed reduced FECR following moxidectin treatment. After the present study, emergence of moxidectin resistance was noted on several more farms, indicating that prevalence of moxidectin resistance is rapidly further increasing.

Monepantel was introduced just recently in 2011 in the Netherlands. Van den Brom et al. (2015) reported the first case of monepantel resistance in *H. contortus*, which was an exceptional finding so soon after monepantel became available. Authors noted that on the farm where resistance was found, monepantel was used as the sole anthelmintic 13 times in about 2–3 years. They also found that the

FECR was 0%, indicating monepantel had no efficacy at all. In the present study 2 sheep flocks (7.7%) showed monepantel resistance with efficacies of 46 and 84.5%. This suggests that monepantel resistance is not an all-or-nothing phenomenon and does develop gradually, albeit far more rapidly than for other anthelmintics. With *H. contortus* being the predominant gastrointestinal nematode species in sheep in the Netherlands, it may be recommended to either only use monepantel if everything else fails and even then as little as possible, or to use monepantel alternatingly with other still (mostly) effective products.

Of the other two products tested, closantel resistance in *H. contortus* was found in 44.4% of farms with a reduced FECR. No resistance was observed against levamisole. The latter product is a very old one, and has long been used rarely as the benzimidazoles and later the MLs were the preferred anthelmintics. The few eggs found after treatment in three flocks may have been due to resumed development of some inhibited larvae shortly after the treatment as we sampled 10–14 days post-treatment. Borgsteede et al. (1997) found similar results with just a few flocks showing positive egg counts 14 days after treatment. They concluded there was no clear indication of levamisole resistance. Because of the rapidly increasing resistance against the MLs, levamisole is increasingly used over the last couple of years (unpublished observations). Therefore, resistance to levamisole can be expected to emerge as well. By comparison, Playford et al. (2014) reported AR against levamisole in 73%–96% of FECR tests in Australia, where levamisole apparently is one of the commonly used anthelmintics. In the majority of these cases it involved *Teladorsagia* and *Trichostrongylus*, but in 30% of the cases it also involved *Haemonchus*. Indeed, reduced levamisole efficacy was observed on a Dutch farm in 2016 (unpublished data), one year after this study. The observed resistance to closantel is probably due to its use against liver fluke since the emergence of triclabendazole resistance in liver fluke in the late 1990s in the Netherlands (Moll et al., 2000). Until recently, closantel has not been used specifically against *Haemonchus*.

Results also suggest that multi-drug resistance is widespread on Dutch sheep farms. Of flocks in which at least two products (excluding closantel) were tested, 53.3% showed multi-drug resistance mostly involving drugs from different anthelmintic classes. Comparing resistance against both MLs showed that moxidectin could still be effective if there was ivermectin resistance. Moxidectin resistance in combination with a high ivermectin efficacy was not found.

In conclusion, AR appears to be present against every anthelmintic available in the Netherlands. Levels of AR are difficult to compare with levels found elsewhere as great variation is observed between countries and regions (Rose et al., 2015). Ivermectin resistance occurs most frequently, even more than benzimidazole resistance. Ivermectin is also the product with by far the lowest efficacy on average. These days, sheep farmers will have great difficulty controlling gastrointestinal nematode infections if solely relying on anthelmintics. Results show that there is a great urgency to minimize dependency on anthelmintics and stop using these preventively as much as possible and without prior checking for worm eggs in feces. It is also strongly recommended that sheep farmers check the efficacy of anthelmintic products every time a product is used, as this is still not widely practiced (Ploeger et al., 2016). Further, it is recommended to annually check anthelmintic efficacy before the grazing season starts in a small cohort of ewes randomly selected from the entire flock shortly after lambing.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

online version, at doi:https://doi.org/10.1016/j.vetpar.2018.09.007.

References

Boersema, J.H., Borgsteede, F.H.M., Eysker, M., Hendrikx, W.M.L., Jansen, J., Smith-Buys, C.M.C., 1987. Prevalence of benzimidazole resistance of nematodes in sheep in the Netherlands. Res. Vet. Sci. 43, 18–21.

Borgsteede, F.H.M., Pekelder, J.J., Dercksen, D.P., Sol, J., Vellema, P., Gaasenbeek, C.P.H., Van der Linden, J.N., 1997. A survey of anthelmintic resistance in nematodes of sheep in the Netherlands. Vet. Q. 19, 167–172.

Borgsteede, F.H.M., Dercksen, D.D., Huijbers, R., 2007. Doramectin and albendazole resistance in sheep in the Netherlands. Vet. Parasitol. 144, 180–183.

Borgsteede, F., Verkaik, J., Moll, L., Dercksen, D., Vellema, P., Bavinck, G., 2010. How widespread is resistance to ivermectin among gastro-intestinal nematodes in sheep in the Netherlands? Tijdschr. Diergeneeskd. 135, 782–785.

Cabaret, J., 2014. Reliable phenotypic evaluations of anthelmintic resistance in herbivores: how and when should they be done? In: Quick, W. (Ed.), Anthelmintics. Clinical Pharmacology, Uses in Veterinary Medicine and Efficacy. Nova science publ., Nova, New York, pp. 1–26.

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 (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

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.

Cringoli, G., Veneziano, V., Mezzino, L., Morgoglione, M., Pennacchio, S., Rinaldi, L., Salamina, V., 2009. The effect of moxidectin 0.1% vs ivermectin 0.08% on milk production in sheep naturally infected by gastrointestinal nematodes. Vet. Res. 5, 41.

George, M.M., Paras, K.L., Howell, S.B., Kaplan, R.M., 2017. Utilization of composite fecal samples for detection of anthelmintic resistance in gastrointestinal nematodes of cattle. Vet. Parasitol. 240, 24–29.

Kenyon, F., Rinaldi, L., McBean, D., Pepe, P., Bosco, A., Melville, L., Devin, L., Mitchell, G., Ianniello, D., Charlier, J., Vercruysse, J., Cringoli, G., Levecke, B., 2016. Pooling sheep faecal samples for the assessment of anthelmintic drug efficacy using McMaster and Mini-FLOTAC in gastrointestinal strongyle and *Nematodirus* infection. Vet. Parasitol. 225, 53–60.

Leignel, V., Silvestre, A., Humbert, J.F., Cabaret, J., 2010. Alternation of anthelmintic treatments: a molecular evaluation for benzimidazole resistance in nematodes. Vet. Parasitol. 172, 80–88.

MAFF (Ministry of Agriculture, Fisheries and Food agricultural development and advisory service), 1977. Manual of Veterinary Parasitological Laboratory Techniques. Technical Bulletin No.18. Her Majesty's Stationery Office, London.

Moll, L., Gaasenbeek, C.P.H., Vellema, P., Borgsteede, F.H.M., 2000. Resistance of Fasciola hepatica against triclabendazole in cattle and sheep in the Netherlands. Vet. Parasitol. 91, 153–158.

Playford, M.C., Smith, A.N., Love, S., Besier, R.B., Kluver, P., Bailey, J.N., 2014. Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009–2012). Aust. Vet. J. 92, 464–471.

Ploeger, H.W., Antonis, A., Verkaik, J.C., Vellema, P., Bokma-Bakker, M.H., 2016. Perceptions and actions of Dutch sheep farmers concerning worm infections. Vet. Parasitol. 229, 150–158.

Rinaldi, L., Levecke, B., Bosco, A., Ianniello, D., Pepe, P., Charlier, J., Cringoli, G., Vecruysse, J., 2014. Comparison of individual and pooled faecal samples in sheep for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC. Vet. Parasitol. 205, 216–223.

Rose, H., Rinaldi, L., Bosco, A., Mavrot, F., De Waal, T., Skuce, P., Charlier, J., Torgerson, P.R., Hertzberg, H., Hendrickx, G., Vercruysse, J., Morgan, E.R., 2015. Wide spread anthelmintic resistance in European farmed ruminants: a systematic review. Vet. Rec. 176, 546.

Sergeant, E.S.G., 2017. Epitools Epidemiological Calculators. Available at:. Ausvet Pty Ltd.. http://epitools.ausvet.com.au.

Van den Brom, R., Moll, L., Borgsteede, F.H.M., Doorn, D.C.K., van Lievaart-Peterson, K., Dercksen, D.P., Vellema, P., 2013. Multiple anthelmintic resistance of *Haemonchus contortus*, including a case of moxidectin resistance, in a Dutch sheep flock. Vet. Rec. 173, 552–553.

Van den Brom, R., Moll, L., Kappert, C., Vellema, P., 2015. *Haemonchus contortus* resistance to monepantel in sheep. Vet. Parasitol. 209, 278–280.