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Research paper

Development of *Haemonchus contortus* resistance in sheep under suppressive or targeted selective treatment with monepantel

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ABSTRACT

This study examined the development of resistance to anthelmintics in *Haemonchus contortus* in lambs under suppressive or selective treatment regimens that included monepantel. Twenty Ile de France and 20 Santa Ines lambs were allocated to two anthelmintic treatment regimens, based on body weight and nematode faecal egg counts (FEC): targeted selective treatment (TST) or suppressive treatment, both with monepantel. Lambs of the TST group were treated individually when they presented with a packed cell volume (PCV) ≤20%. On 7 October 2016, the lambs were allocated to clean pastures, where they grazed in separated paddocks by group until late February 2017. The experimental area was contaminated with nematodes that were introduced with the experimental Ile de France and Santa Ines lambs, naturally infected with gastrointestinal nematodes. To maintain the grazing lambs in the suppressive treatment group and their pasture as free of worms as possible, these lambs were treated with anthelmintics before being allocated to their paddock and then were periodically treated with monepantel. However, the use of a suppressive treatment regimen that included monepantel over a period of 3 months resulted in the emergence of a population of resistant *H. contortus*. In the TST group, there was a rapid and progressive reduction in the efficacy of monepantel, which at the end of the experiment was only 76%. The Ile de France lambs were all treated one or more times during the experiment, whereas only two Santa Ines lambs in the TST required treatment. In conclusion, a population of *H. contortus* resistant to monepantel emerged quickly during the rainy season, even when sheep were submitted to selective treatment.

1. Introduction

Parasitism by gastrointestinal nematodes (GIN) is an important cause of economic losses in the sheep industry. Prophylaxis against GIN infections is based on anthelmintic treatment. However, lack of professional guidance and ease of access to anthelmintics have led farmers to frequently and indiscriminately use these drugs on their flocks. Therefore, parasites with resistance to most important chemical compounds (benzimidazole, macrocyclic lactone, imidazothiazoles, tetrahydropyrimidines, and salicylanilides) have emerged, resulting in a global problem that jeopardizes the control of nematode infections in ruminants (Kaplan et al., 2004; Gilleard, 2006; Amarante, 2014; Bartley et al., 2015).

A new class of anthelmintic, derived from amino-acetonitrile (AAD) and called monepantel (Kaminsky et al., 2008), was introduced into the market in 2009 and is considered an important alternative treatment when there is multiple resistance to other classes of antiparasitics used to treat sheep (Ciuffa et al., 2013). In 2013, two nematode species (*Teladorsagia circumcincta* and *Trichostrongylus colubriformis*) resistant to monepantel were first reported in New Zealand (Scott et al., 2013). Since then, resistance in *Haemonchus contortus* has been reported in Uruguay (Mederos et al., 2014), the Netherlands (Van den Brom et al., 2015), Australia (Sales and Love, 2016; Lamb et al., 2017), and Brazil (Martins et al., 2017).

The mechanisms that underlie the emergence of resistance are complex and may include mutations in the genomes of parasites (Rufener et al., 2013). In *Caenorhabditis elegans* resistant to monepantel, a spontaneous mutation in the *acr-23* allele was identified, whereas in *H. contortus*, a mutation was found to occur in *Hco-mptl-1* and *Hco-des-2H* (Rufener et al., 2009; Bagnall et al., 2017).

Frequent treatment is considered one of the main risk factors for the development of anthelmintic resistance, especially when the anthelmintic is administered to all of the sheep in a flock. Resistant parasites, which survive treatment, are selected and reproduce, resulting in a

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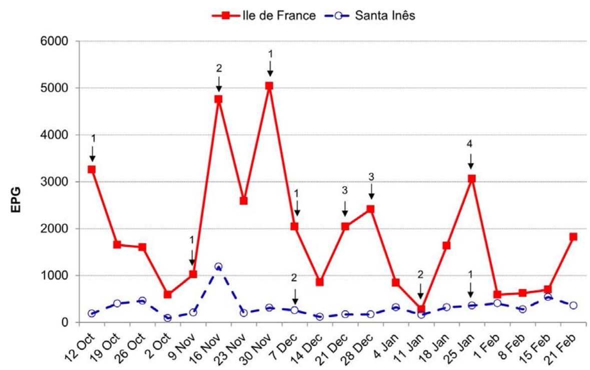

Fig. 1. Average of eggs of strongyles per gram of faeces (EPG) of lambs under targeted selective treatment with monepantel. Arrows indicate the number of animals treated on each occasion owing to PCV ≤ 20%.

progressive increase in the frequency of genes in the population that confer resistance (Besier, 2012; Falzon et al., 2014). To increase the size of the worm population in refugia, targeted selective treatment (TST) has emerged as an alternative treatment regimen that can be used to delay the onset of anthelmintic resistance (Besier, 2012).

From October 2016 to February 2017 (the rainy season), in an experiment designed to determine the productive performance of lambs, a decrease in the efficacy of monepantel was observed. Thus, in the present study, we aimed to describe the development of *H. contortus* resistance in lambs naturally infected by GIN and submitted to suppressive or selective treatment with monepantel.

2. Material and methods

2.1. Animals and pasture

All the procedures involving animals in this study are in accordance to international ethical standards and were approved by the Animal Use Ethics Committee of the FMVZ/Unesp (47/2016).

Twenty Ile de France and 20 Santa Ines 3-month-old uncastrated male lambs were purchased from farms located in São Manuel and Vitoriana, respectively, in São Paulo state, Brazil. The lambs were raised indoors at their farms of origin. The Santa Ines and Ile de France lambs arrived in the experimental area of the University on September 29 and 30, 2016, respectively, where they were housed until the beginning of the experiment. On the day of arrival, blood and faecal samples were collected for haematological and parasitological examinations. The Ile de France and Santa Ines lambs presented averages of 435 (range of 0–1100) eggs per gram (EPG) and 1553 (0–7300) EPG, respectively. Based on faecal culture, Santa Ines and Ile de France lambs were found to harbour *Haemonchus* spp. (63% and 86%, respectively) and *Trichostrongylus* spp. (37% and 14%, respectively) infections. All lambs received a clostridiosis vaccine (Poli-Star®, Vallée), with a booster dose administered 2 months later, and toltrazuril (14 mg/kg, Farmacox®, Farmabase Animal Health) to prevent coccidiosis.

The experiment was in a 2 × 2 factorial, with two anthelmintic treatment regimens (TST or suppressive) and two sheep breeds (Santa Ines and Ile de France). The animals of each breed were allocated to two groups balanced as far as possible for body weight and nematode faecal egg counts (FEC). The Ile de France showed average of 300 EPG (range 0–1100 EPG) and the Santa Ines average of 950 (0–7300 EPG) in the Group under suppressive treatment, while in the TST group, the Ile de France presented average of 300 (0–1100 EPG) and the Santa Ines 600 (0–6700 EPG).

At the beginning of the experiment, one Santa Ines and one Ile de France lamb died. For this reason, the experimental groups had the following number of animals:

- Group of lambs with low exposure to GIN that received suppressive treatment: 10 Ile de France and 9 Santa Ines lambs maintained in the same paddock.
- Group of high exposure to GIN that received TST: 9 Ile de France and 10 Santa Ines lambs maintained in the same paddock.

On 7 October 2016, the lambs were allocated in groups to separate paddocks in a pasture of *Cynodon* spp. and *Urochloa decumbens* that was maintained without ruminants from 1 March 2016 until the beginning of the present experiment. At the study site, nematode infective larvae were recovered for a maximum of 3–4 months after pasture contamination (Carneiro and Amarante, 2008). Because the pasture

Table 1
Faecal egg count reduction (FECR) in lambs under target selective treatment with monepantel (high-exposure group). Date of the treatment, number of eggs of strongyles per gram of faeces (EPG) on the day of the treatment (pre-treatment) and 14 days post-treatment.

Month	Lamb identification	Date	EPG		FECR (%)
			Pre-treatment	14 days post-treatment	
October	Ile de France – 33	12-Oct	15400	0	100%
November	Ile de France – 33	09-Nov	2700	400	98% (100; 91)
	Ile de France – 34	16-Nov	6700	0	
	Ile de France – 38	16-Nov	6200	100	
	Ile de France – 40	30-Nov	15500	0	
December	Santa Ines – 22	07-Dec	1100	0	83% (98; 0)
	Santa Ines – 29	07-Dec	200	0	
	Ile de France – 33	07-Dec	4200	0	
	Ile de France – 35	07-Dec	7600	0	
	Ile de France – 31	21-Dec	1800	0	
	Ile de France – 38	21-Dec	400	4800	
	Ile de France – 34	28-Dec	10100	300	
	Ile de France – 36	28-Dec	4500	100	
January	Ile de France – 31	11-Jan	100	0	76% (95; 0)
	Ile de France – 39	11-Jan	300	3800	
	Ile de France – 33	25-Jan	6300	600	
	Ile de France – 34	25-Jan	4400	400	
	Ile de France – 35	25-Jan	8000	400	
	Ile de France – 40	25-Jan	1900	100	
	Santa Ines – 22	25-Jan	1500	0	

FECR (%) in each month = 100 × (1–post-treatment mean EPG/pre-treatment mean EPG). Upper and lower confidence interval limits (95%) are in parentheses.

Table 2
Third stage larvae (%) in faecal cultures of lambs under target selective treatment with monepantel.

Data	Breed	*Haemonchus*	*Trichostrongylus*	*Cooperia*	*Oesophagostomum*
05-Oct	Ile de France	94	6	0	0
	Santa Inês	55	44	0	1
12-Oct	Ile de France	91	6	0	3
	Santa Ines	63	34	1	2
19-Oct	Ile de France	92	8	0	0
	Santa Ines	50	43	0	7
26-Oct	Ile de France	95	1	0	4
	Santa Ines	74	26	0	0
02-Nov	Ile de France	83	6	11	0
	Santa Ines	37	58	4	1
09-Nov	Ile de France	78	22	0	0
	Santa Ines	46	52	2	0
16-Nov	Ile de France	95	0	5	0
	Santa Ines	67	30	3	0
23-Nov	Ile de France	60	40	0	0
	Santa Ines	38	62	0	0
30-Nov	Ile de France	98	0	2	0
	Santa Ines	43	55	2	0
07-Dec	Ile de France	94	6	0	0
	Santa Ines	54	46	0	0
14-Dec	Ile de France	88	7	5	0
	Santa Ines	59	39	2	0
21-Dec	Ile de France	98	1	1	0
	Santa Ines	41	54	5	0
28-Dec	Ile de France	96	4	0	0
	Santa Ines	42	50	8	0
04-Jan	Ile de France	61	28	11	0
	Santa Ines	40	46	14	0
11-Jan	Ile de France	92	2	6	0
	Santa Ines	56	39	5	0
18-Jan	Ile de France	83	9	8	0
	Santa Ines	56	36	8	0
25-Jan	Ile de France	75	20	5	0
	Santa Ines	35	52	13	0
01-Feb	Ile de France	86	14	0	0
	Santa Ines	62	31	7	0
08-Feb	Ile de France	97	0	3	0
	Santa Ines	51	46	3	0
15-Feb	Ile de France	84	7	9	0
	Santa Ines	69	29	2	0
21-Feb	Ile de France	82	15	3	0
	Santa Ines	31	54	15	0

remained without animals for a period of 7 months, the grazing area used was considered "clean". Therefore, gastrointestinal nematodes in the experimental area were introduced by the naturally infected experimental Ile de France and Santa Ines lambs. In the paddocks, the lambs had free access to tap water and mineral salt (Presencefós, Presence Animal Nutrition) and received a daily dietary supplement (Supplementa Ovinos, Presence Animal Nutrition). It is important to emphasize that the groups always remained in the separate paddocks.

2.2. Suppressive anthelmintic treatment in the group with low exposure to GIN

Just after lambs were assigned into groups (7 days before being allocated to the paddocks), they were treated with the following combination of anthelmintics: albendazole (10 mg/kg, Endazole® 10% CO, Hipra), levamisole (9.4 mg/kg, Ripercol® L 150 F, Zoetis), and monepantel (2.5 mg/kg, Zolvix®, Novartis). Following this treatment, no nematode eggs were detected in a series of faecal examinations. Then, to keep the grazing lambs and their pastures as free of worms as possible, the lambs were treated periodically with monepantel (details in Fig. 2). In addition to periodic treatments with monepantel, lambs in the suppressive group received moxidectin (0.4 mg/kg, Cydectin® Zoetis) on November 16, because some of them were shedding *Strongyloides papillosus* eggs (mean of 42 and range of 0–200 EPG). On January 14, lambs also received a combination treatment of monepantel, levamisole (9.4 mg/kg), and albendazole (10 mg/kg), because they were shedding strongyle eggs (379, 0–1000 5300EPG) and *S. papillosus* (874, 0–5300 EPG).

2.3. TST in the group with high exposure to GIN

The lambs in the TST group (high exposure) were not treated on arrival at the experimental facilities. Instead, they were introduced into the clean pasture harbouring natural infections of parasites acquired at

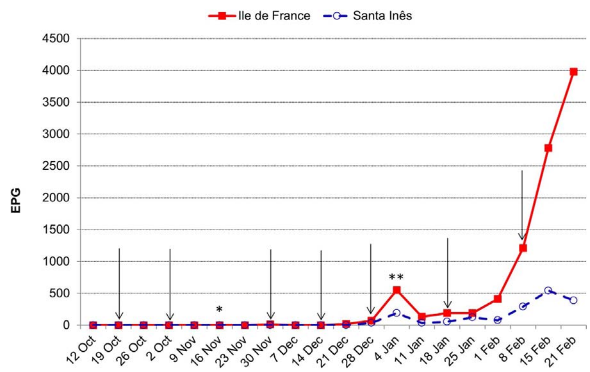

Fig. 2. Average number of strongyles eggs per gram of faeces (EPG) from lambs under suppressive anthelmintic treatment. The black arrows indicate the dates of treatment with monepantel, the orange arrow indicates the date of treatment with moxidectin, and the blue arrow indicates the date of treatment with a combination of monepantel, levamisole, and albendazole. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

their farm of origin. These lambs underwent selective treatment with monepantel when they showed a packed cell volume (PCV) ≤20%.

2.4. Faecal examination, haematological tests, and body weight measurement

Blood samples were collected weekly from each lamb to determine the PCV by micro-haematocrit centrifugation. Faecal egg counts were determined using a modified McMaster technique, in which each worm egg counted represented 100 EPG. A composite faecal culture to obtain infective larvae of gastrointestinal nematodes was performed separately for each group and breed (Ueno and Gonçalves, 1998). These larvae were identified according to descriptions by Ueno and Gonçalves (1998). At the same time as blood and faecal sampling, body weights were recorded to determine the precise amount of anthelmintic to be administered to each animal.

2.5. Faecal egg count reduction test (FECRT)

A FECRT was performed to evaluate the efficacy of the anthelmintic treatments (Coles et al., 1992). RESO 2.0 software (Wursthorn and Martin, 1990) was used to calculate anthelmintic efficacy. Resistance was considered present when the percentage reduction in egg count was less than 95% 14 days after anthelmintic treatment.

3. Results

At the arrival in the experimental facilities (September 30), the Ile de France lambs presented 435 EPG on average. However, one week later, the TST group displayed 3110 EPG on average. This increase in the EPG values indicates that the animals were harbouring immature worms that reached patency at the beginning of the trial. For example, the animal 33 presented 1100 EPG at the arrival and 15,400 EPG two weeks later (October 12).

Of the two Santa Ines lambs under TST that presented PCV < 20% and therefore required anthelmintic treatment, one of them was treated twice. On the day of treatment, the EPG counts of these animals were relatively low, between 200 and 1500 EPG (Fig. 1; Table 1). After treatment, no eggs were found in these Santa Ines lambs, indicating high treatment efficacy (Table 1). In contrast, all nine Ile de France lambs required anthelmintic treatment at some point in the experiment: three animals required one treatment; three animals needed two treatments; two animals needed three treatments; and one animal required four treatments (Table 1). The efficacy of monepantel varied from animal to animal, but there was a clear tendency toward a reduction in efficacy as the experiment progressed (Table 1). At the end of the experiment, based on seven animals treated in January, a monepantel efficacy of 76% was recorded (Table 1). The predominant genera of strongyles in the faecal cultures were *Haemonchus* spp. and *Trichostrongylus* spp. (Table 2). On some occasions, individual post-treatment faecal cultures were performed: on December 7 and on January 1, a culture of faeces from lamb 33 showed 100% *Haemonchus* spp. larvae; on January 12, culture of faeces from animal 23 showed 81% *Haemonchus* spp. and 19% *Cooperia* spp. larvae; and, on the same day, culture of faeces from animal 31 showed 93% *Haemonchus* spp. and 7% *Cooperia* spp.

No eggs were detected in the faeces of lambs in the group under suppressive treatment up to December 14 (Fig. 2). However, larvae were detected in faecal cultures on November 16 (Table 3), indicating that mild infections, not yet detected by egg counting, had already been established in animals. On December 21, two animals presented detectable eggs in their faeces (100 EPG) for the first time. Combined treatment with monepantel, levamisole, and albendazole on January 4 showed high efficacy against *S. papillosus* infection, with an FECR of 99% (mean of 874 EPG before treatment and 5 EPG after treatment). However, this regimen showed less efficacy against *H. contortus* infection, with animals shedding an average of 126 EPG (range of 0–500) two weeks after treatment on January 18 (Fig. 2). In February, monepantel treatment did not reduce EPG counts in lambs in the suppressive group; EPG counts increased progressively until the end of the experiment (Fig. 2). In faecal cultures, larvae of *Haemonchus* spp. and *Cooperia* spp. were detected; however, only *Haemonchus* larvae were identified in the animals in the suppressive treatment group from December 28 to the end of the trial (Table 3).

4. Discussion

The use of a suppressive treatment regimen with monepantel over a period of 3 months resulted in the rapid emergence of a resistant *H. contortus* population in the present study. When the population of parasites in refugia is small, hosts are reinfected with the progeny of worms that survived the anthelmintic treatment, which, even with a small number of survivors, produce offspring that are the only individuals available for reinfection (Bartley et al., 2015). The rapid emergence of resistance in animals under suppressive treatment observed in this study was not surprising. At the end of the experiment, the selected population consisted only of parasites that survived the treatment, i.e., resistant worms.

In the TST group, there was a rapid and progressive reduction in efficacy, which, at the end of the experiment, was only 76%. This rapid emergence of resistant parasites in the paddocks where there was a nematode population in refugia was unexpected, although in sheep under TST, Cintra et al. (2016) and Mederos et al. (2014) observed the appearance of monepantel-resistant *T. colubriformis* and *H. contortus*, respectively. Selective treatment of animals has been suggested as an important alternative strategy to delay the onset of resistance to anthelmintics. By reducing the number of animals treated, there should be reduced selection pressure and an increase in the proportion of nematodes in refugia. This population consists of parasites at stages within the host and those of free-living stages in the pastures that are not exposed to anthelmintic treatment and can pass on susceptible alleles to the next generation of parasites (Besier, 2012). To a certain extent, this was demonstrated in the present study, because in the group under TST, a considerable percentage of the parasite population, approximately 76% based on FECRT, was still susceptible. If the experiment lasted longer, it is possible that a resistant population would come to predominate. Also noteworthy was the fact that, although lambs submitted to each treatment regimen were held in separate paddocks throughout the experiment, the appearance of resistance to monepantel occurred at similar times, with a difference of approximately 1 month between the groups.

In conclusion, the emergence of a population of *H. contortus*

Table 3
Third stage larvae (%) in faecal cultures of lambs under suppressive treatment with monepantel (low-exposure group).

Data	Breed	*Haemonchus*	*Trichostrongylus*	*Cooperia*	*Oesophagostomum*
05-Oct	Ile de France	89	11	0	0
	Santa Ines	67	33	0	0
12-Oct	Ile de France	*	*	*	*
	Santa Ines	*	*	*	*
19-Oct	Ile de France	*	*	*	*
	Santa Ines	*	*	*	*
26-Oct	Ile de France	*	*	*	*
	Santa Ines	*	*	*	*
02-Nov	Ile de France	*	*	*	*
	Santa Ines	*	*	*	*
09-Nov	Ile de France	*	*	*	*
	Santa Ines	*	*	*	*
16-Nov	Ile de France	89	0	11	0
	Santa Ines	80	0	20	0
23-Nov	Ile de France	100	0	0	0
	Santa Ines	*	*	*	*
30-Nov	Ile de France	97	0	3	0
	Santa Ines	100	0	0	0
07-Dec	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
14-Dec	Ile de France	100	0	0	0
	Santa Ines	*	*	*	*
21-Dec	Ile de France	99	1	0	0
	Santa Ines	100	0	0	0
28-Dec	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
04-Jan	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
11-Jan	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
18-Jan	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
25-Jan	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
01-Feb	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
08-Feb	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
15-Feb	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0
21-Feb	Ile de France	100	0	0	0
	Santa Ines	100	0	0	0

* No larvae were recovered from the faecal culture.

resistant to monepantel was shown to occur quickly during the hot and wet season, even when sheep are selectively treated. With a decrease in the efficacy of anthelmintics, prophylaxis of GIN infections in sheep has become more challenging. It is of utmost importance that the efficacy of new compounds such as ADD is preserved through the use of sustainable control measures that minimize the use of anthelmintics.

Conflicts of interest

The authors have no conflicts of interest.

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References

Amarante, A.F.T., 2014. Sustainable worm control practices in South America. Small Rum. Res. 118, 56–62.

Bagnall, N.H., Ruffell, A., Raza, A., Elliott, T.P., Lamb, J., Hunt, P.W., Kotze, A.C., 2017. Mutations in the *Hco-mptl-1* gene in a field-derived monepantel-resistant isolate of *Haemonchus contortus*. Int. J. Parasitol. Drugs Drug Resist. 7 (2), 236–240.

Bartley, D.J., Devin, L., Nath, M., Morrison, A.A., 2015. Selection and characterisation of monepantel resistance in *Teladorsagia circumcincta* isolates. Int. J. Parasitol. Drugs Drug Resist. 5, 69–76.

Besier, R.B., 2012. Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. Vet. Parasitol. 186, 2–9.

Carneiro, R.D., Amarante, A.F.T., 2008. Seasonal effect of three pasture plants species on the free-living stages of *Haemonchus contortus*. Arq. Bras. Med. Vet. Zootec. 60 (4), 864–872.
Cintra, M.C.R., Teixeira, V.N., Nascimento, L.V., Sotomaior, C.S., 2016. Lack of efficacy of monepantel against *Trichostrongylus colubriformis* in sheep in Brazil. Vet. Parasitol. 216, 4–6.
Ciuffa, A.Z., Brasão, S.C., Urzedo, M.C., Ramos, G.B., Gomes, D.O., Rosalinski-Moraes, F., 2013. Monepantel efficacy to control gastrointestinal Strongylid parasites in sheep. Ars Vet. 29 (4), 52.
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.
Falzon, L.C., O'Neillb, T.J., Menziesc, P.I., Peregrine, A.S., Jones-Bitton, A., vanLeeuwen, J., Mederos, A., 2014. A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. Prev. Vet. Med. 117, 388–402.
Gilleard, J.S., 2006. Understanding anthelmintic resistance: the need for genomics and genetics. Int. J. Parasitol. 36, 1227–1239.
Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Mäser, P., 2008. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452, 176–180.
Kaplan, R.M., Burke, J.M., Terrill, T.H., 2004. Validation of the Famacha© eye color chart for detecting clinical anemia in sheep and goats on farms in the Southern United States. Vet. Parasitol. 123, 105–120.
Lamb, J., Elliott, T., Chambers, M., Chick, B., 2017. Broad spectrum anthelmintic resistance of *Haemonchus contortus* in northern NSW of Australia. Vet. Parasitol. 241, 48–51.
Martins, A.C., Bergamasco, P.L.F., Felippelli, G., Tebaldi, J.H., Duarte, M.M.F., Testi, A.J.P., Lapera, I.M., Hoppe, E.G.L., 2017. Haemonchus contortus *Haemonchus contortus* resistance to monepantel in sheep: fecal egg count reduction tests and randomized controlled trials. Semin: Ciên. Agrar. 38 (1), 231–238.
Mederos, A.E., Ramos, Z., Banchero, G.E., 2014. First report of monepantel *Haemonchus contortus* resistance on sheep farms in Uruguay. Parasit. Vectors 7, 598.
Rufener, L., Maser, P., Roditi, I., Kaminsky, R., 2009. Haemonchus contortus *Haemonchus contortus* acetylcholine receptors of the DEG-3 subfamily and their role in sensitivity to monepantel. PLoS Pathog. 5, e1000380.
Rufener, L., Bedoni, N., Baur, R., Rey, S., Glauser, D.A., Bouvier, J., Beech, R., Sigel, E., Puoti, A., 2013. acr-23 *acr-23* encodes a monepantel-sensitive channel in *Caenorhabditis elegans*. PLoS Pathog. 9 (8), e1003524.
Sales, N., Love, S., 2016. Resistance of *Haemonchus* sp. to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW Australia. Vet. Parasitol. 228, 193–196.
Scott, I., Pomroy, B., Paul, K., Greg, S., Barbara, A., Moss, A., 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Vet. Parasitol. 198, 166–171.
Ueno, H., Gonçalves, P.C., 1998. Manual Para Diagnóstico Das Helmintoses De Ruminantes, 4th ed. Japan International Cooperation Agency, Tokyo, Japan.
Van den Brom, R., Moll, L., Kappert, C., Vellema, P., 2015. Haemonchus contortus *Haemonchus contortus* resistance to monepantel in sheep. Vet. Parasitol. 209 (3–4), 278–280.
Wursthorn, L., Martin, P., 1990. Reso© FECRT analysis program. Version 2.0. CSIRO. Animal Health Research Laboratory, Parkville, Australia.