Research Note

Anthelmintic efficiency of doramectin, fenbendazole, and nitroxynil, in combination or individually, in sheep worm control

Eficiência anti-helmíntica da doramectina, fenbendazole e nitroxinil, associados ou utilizados individualmente, no controle da verminose ovina

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Abstract

The anthelmintic efficiency of doramectin, fenbendazole, and nitroxynil, used individually or in combination, was determined by the Fecal Egg Count Reduction (FECR) test and cultivation of larvae of anthelminthic-treated sheep grouped as follows: G1 (doramectin), G2 (fenbendazole), G3 (nitroxynil), G4 (doramectin + fenbendazole), G5 (doramectin + nitroxynil), G6 (fenbendazole + nitroxynil), G7 (doramectin + nitroxynil + fenbendazole), G8 (untreated). In addition to individually used doramectin and fenbendazole, the helminths were also resistant to the combination of doramectin + fenbendazole; nitroxynil + fenbendazole; and doramectin + nitroxynil + fenbendazole, with their FECR rates ranging from 62-83%. The helminths showed possible nitroxynil-resistance, but had low resistance when the drug was administered in combination with doramectin. The evaluation of individual helminth species revealed that fenbendazole was fully effective against *Cooperia*; doramectin (G1), moderately effective against *Haemonchus* and insufficiently active against *Cooperia*; nitroxynil, effective against *Haemonchus* and insufficiently active against *Cooperia*. It was concluded from the results that herd nematodes are resistant to doramectin, fenbendazole, and nitroxynil, and that the combined use of the drugs not only fails to significantly improve the anthelmintic efficiency against *Haemonchus* and *Cooperia*, but is also cost-ineffective.

Keywords: Parasitic resistance, *Haemonchus*, *Cooperia*, small ruminants, anthelmintic association.

Resumo

Eficiências da doramectina, fenbendazole e nitroxinil, utilizados individualmente ou associadamente, foram determinadas através do Teste de Redução na Contagem de Ovos nas Fezes (RCOF) e cultivo de larvas. Os grupos experimentais foram os seguintes: G1 (ovinos tratados com doramectina), G2 (fenbendazole), G3 (nitroxinil), G4 (doramectina + fenbendazole), G5 (doramectina + nitroxinil), G6 (fenbendazole + nitroxinil), G7 (doramectina + fenbendazole + nitroxinil) e G8, não tratados. Os helmintos foram considerados resistentes a doramectina e ao fenbendazole isoladamente e às associações doramectina + fenbendazole, fenbendazole + nitroxinil, e doramectina + fenbendazole + nitroxinil, com taxas de RCOF variando de 62-83%. Helmintos foram considerados suspeitos de resistência ao nitroxinil e apresentaram baixa resistência, quando esta droga foi associada à doramectina. Dos tratamentos isolados, o fenbendazole demonstrou total eficácia (100%) contra *Cooperia*. Doramectina (G1) foi moderadamente efetiva contra *Haemonchus* e insuficientemente ativa contra *Cooperia*, e o nitroxinil efetivo contra *Haemonchus* (93,2%) e insuficientemente ativo contra *Cooperia* (0%). Concluiu-se neste estudo que os nematódeos do rebanho são resistentes à doramectina, fenbendazole e nitroxinil, e que, ainda que associadas, não devem ser utilizadas no rebanho por não melhorarem a eficiência anti-helmíntica nem a efetividade contra *Haemonchus* e *Cooperia* e por não apresentarem custo-benefício justificado.

Palavras-chave: Resistência parasitária, *Haemonchus*, *Cooperia*, pequenos ruminantes, associação anti-helmíntica.

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Introduction

According to the data from the Brazilian Institute of Geography and Statistics (IBGE), sheep farming in Paraná, Brazil, has been observed to be slowly increasing in recent years. In the penultimate livestock census, in 2009, the herd size was estimated to be 599,925 head of sheep, which corresponded to 12.5% of the total sheep population in southern Brazil (IBGE, 2013). The last census estimated a 6.7% increase in the number of sheep a small increment, considering the effective increase in the sheep population over the last four years in other states of southern Brazil, which was estimated to be 12.97% and 7.72% in Santa Catarina and Rio Grande do Sul, respectively.

The main obstacle to sheep production is the negative economic impact due to health problems that affect the sheep, among which, infections with gastrointestinal parasites stand out. Chemical method of control remains the most used among the endoparasite control methods. However, failure in using this method effectively has favored the emergence of resistant strains of parasites (SANGSTER, 2001).

Drugs with benzimidazoles and avermectins are commonly used for deworming sheep. However, due to reports of development of resistance in parasites to these anthelmintics, less common alternatives have been investigated, such as compounds with phenolic group substitutions and salinomycins, which are active against *Fasciola*, and are also used to treat haemoncose in sheep and cattle. Oxyclozanide is active mainly against adult worms, while nitroxynil, rafoxanide, and closantel are active against parasites in both adult and immature stages (BISHOP, 2005).

The resistance of helminths to anthelmintic drugs can be defined as an increase in the ability of certain strains of parasites to resist or survive doses of a drug that would normally destroy most individuals of the same species. The various mechanisms of resistance include modifying the action of the target molecule, increasing the number of targets receptors, increasing the rate of drug excretion, increasing the metabolism of the active ingredient, or simply, drug sequestration (KELLY & HALL 1979; TORRES-ACOSTA & HOSTE, 2008). A large variation in the degree of resistance of various nematode populations against anthelmintics have been widely reported the world over for several decades (JACKSON, 1993; MELO et al., 2003; COLES, 2005; BORGES et al., 2008; CONDI et al., 2009; SCZESNY-MORAES et al., 2010).

The aim of this study was to evaluate the anthelmintic efficacy of doramectin, fenbendazole, and nitroxynil, individually or in combination, in sheep that were naturally infected by gastrointestinal nematodes in a rural property in northern Paraná.

Materials and Methods

The study was conducted in a rural property in the municipality of Ribeirão Claro, north of Paraná State, between April 2012 and June 2012. Of the total 420 sheep of farm, 180 weaned Suffolk ewes were selected for this study. The number of eggs per gram of feces (EPG) was enumerated for all the 180 animals, and from

these, only those animals with EPG counts below 1000 were selected for the final study. Treatment groups were formed after randomization based on the EPG values, such that the mean EPG of the animals in each group was more than 150, which corresponds to the threshold for anthelmintic resistance and efficiency tests in sheep (COLES et al., 2006).

Seven treatment groups were formed, and treated with: injectable doramectin 1%-Dectomax®, Pfizer (a single dose of 0.2 mg/kg body weight); and/or injectable nitroxynil- Dovenix® Supra, Merial (a single dose of 10 mg/kg body weight); and/or oral fenbendazole- Panacur®, Intervet (a single dose of 5 mg/kg body weight); or were untreated. The groups were formed as follows: Group 1 (G1)- 10 sheep, mean EPG 315, treated with doramectin; Group 2 (G2)- 10 sheep, mean EPG 325, treated with fenbendazole; Group 3 (G3)- 10 sheep, mean EPG 305, treated with nitroxynil; Group 4 (G4)- 10 sheep, mean EPG 295, treated with doramectin + fenbendazole; Group 5 (G5)- 10 sheep, mean EPG 320, treated with doramectin + nitroxynil; Group 6 (G6)- 10 sheep, mean EPG 320, treated with fenbendazole + nitroxynil; Group 7 (G7)- 10 sheep, mean EPG 322, treated with doramectin + fenbendazole + nitroxynil; and Group 8 (G8)- 10 sheep, mean EPG 309, untreated. The sheep in G8 (untreated) received 10 mL of oral saline solution in place of the drugs on days that the animals from the other groups were treated.

The fecal samples from all of the animals were collected on day zero, prior to the treatment, and on days 7, 14, and 21 post-treatment, and evaluated by the technique developed by Gordon & Whitlock (1939). To calculate the fecal egg count reduction (FECR), the samples collected on day 7 were evaluated, and the corresponding anthelmintic efficiency was established according to the guidelines given by Coles et al. (1992). The larvae from the samples collected on day 7 were cultivated according to the technique developed by Roberts & O'Sullivan (1950), in order to verify the efficacy of the anthelmintics against each of the nematode genera identified in the samples. The FECR values were calculated using the following formula: FECR= 1 - (mean EPG treated group on day 7/mean EPG control group on day 7) x 100. The larvae (L3) were identified according to the criteria put forth by Keith (1953).

Based on the WAAVP recommendations (*World Association for the Advancement of Veterinary Parasitology*) helminths are considered to be resistant to a particular drug when their FECR values seven days post-treatment are less than 95%, and the lower limit of the confidence interval at 95% is less than 90%. In the absence of these results, the helminths are categorized as having suspected resistance (COLES et al., 1992). In accordance with these recommendations, we categorized the resistance as proposed by Vizard & Wallace (1987): the presence of resistance when the FECR was less than 95%, and the lower limit of the confidence interval at 95% was less 90%; low resistance when the FECR exceeded 95%, and the lower limit of confidence interval at 95% was less than 90%; and sensitive when the FECR exceeded 95%, and the lower limit of confidence interval at 95% was greater than 90%.

The efficacy of the treatment on different days of evaluation and that for the nematode genera that were identified were calculated using the formula below:

$$\text{Efficacy of X} = \frac{\left(\begin{array}{c}\text{Mean of LPG of X from GC } - \\ \text{Mean of LPG of X from GT}\end{array}\right) \times 100}{\text{Mean of LPG of X from GC}} \quad (1)$$

where:

LPG: larvae per gram of feces, calculated by multiplying the value of EPG with the percentage of each sex of the larvae found in the culture

X: genus of larvae identified

GC: control group

GT: treated group

The efficacies were categorized as: highly effective when Efficacy of X > 98%; effective when 90-98%; moderately effective 80-89%; and insufficiently active < 80% (Ordination 79/1996 MERCOSUL, GMC, 1996).

The cost of each of the treatments was evaluated by the analysis of variance and the comparison of the means by the Tukey's test. The values of EPG were log-transformed (log x + 1), and the means compared by paired and unpaired t-tests. Statistical analyses were performed using the statistical program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

Results and Discussion

The EPG of feces of the animals of all of the groups was observed to have decreased seven days after treatment. The mean EPG values of only those animals treated with a combination of doramectin and fenbendazole (G4) remained significantly lower (P = 0.002) than those of animals given treatment combinations throughout the experimental period. As expected, there was no significant decrease in the EPG values in the control animals (Table 1).

The comparison of the EPG values between the groups revealed that the animals treated with nitroxynil (G3), a combination of doramectin and fenbendazole (G4), and a combination of doramectin and nitroxynil (G5), had EPG values significantly lower than those of the control group (P = 0.22, 0.0456, and 0.0203, respectively), respectively, seven days after treatment. On day 14, the only groups that showed no significant decrease in the EPG values in comparison with those of the control group were those that were individually treated with doramectin (G1) and nitroxynil (G3). Until day 14 of the experiment, none of the treatment groups showed any significant differences in the EPG values between themselves. The EPG values of all of the treated groups were lower than those of the control group, on day 21. However, when comparing the treated groups with each other, it was found that the group of animals treated with doramectin in combination with fenbendazole and nitroxynil (G7) showed an FEC value significantly lower (P < 0.0001) than that of the group of animals treated with the same combination, but without doramectin (G6), thus, demonstrating an additive effect of doramectin. This effect might be attributed to two causes: a synergistic action by summation, since the three drugs have

different modes of action (NIES & SPIELBERG, 1996), and a slow rate of absorption (2.71 days) and more persistent plasma concentrations of the drug (MARRINER & BOGAN, 1981; ATTA & ABO-SHIHADA, 2000). This hypothesis is strengthened by comparing the FECR rates of the animals of G3 (nitroxynil) and G5 (doramectin + nitroxynil) (93% and 95%, respectively), which implies that the increase in the FECR rate of group G5 by 2.15% might have been due to doramectin treatment (Table 2).

Despite the significant decrease observed in the mean EPG values evaluated on several days in virtually all the treatment groups, the helminths were categorized as being resistant to treatment with doramectin (G1) and fenbendazole (G2) individually, and to treatment with combinations involving doramectin + fenbendazole (G4), fenbendazole + nitroxynil (G6), and doramectin + fenbendazole + nitroxynil (G7), as could be evaluated based on their FECR rates (83%, 80%, 83%, 83%, and 62%, respectively). Nitroxynil resistance was suspected in helminths from the animals of G3, which showed an FECR rate of 93%, with a 91.2% lower limit

Table 1. Mean (Log x+1) count of helminth eggs per gram of feces (EPG) in the animals of groups G1- Doramectin, G2- Fenbendazole, G3- Nitroxynil, G4- Doramectin + Fenbendazole, G5- Doramectin + Nitroxynil, G6- Fenbendazole + Nitroxynil, G7 Doramectin + Fenbendazole + Nitroxynil, and G8- Control, on the day of treatment (zero day) and days 7, 14, and 21 post-treatment.

Groups	Experimental days			
	zero	7	14	21
G1	(a) 1.97 (a)	(a,b) 0.61 (b)	(a,b) 1.63 (a,b)	(a,c) 1.59 (a,b)
G2	(a) 2.16 (a)	(a,b) 0.64 (b)	(a) 0.93 (b,c)	(a,c) 1.46 (a,c)
G3	(a) 2.40 (a)	(a) 0.22 (b)	(a,b) 1.86 (a)	(a,c) 1.66 (a)
G4	(a) 1.91 (a)	(a) 0.44 (b)	(a) 0.85 (b)	(a,c) 0.83 (b)
G5	(a) 1.91 (a)	(a) 0.34 (b)	(a) 1.22 (a)	(a,c) 1.02 (a,b)
G6	(a) 1.94 (a)	(a,b) 0.76 (b)	(a) 1.22 (a,b)	(a***) 1.61 (a)
G7	(a) 1.79 (a)	(a,b) 0.69 (b)	(a) 0.87 (a,b)	(c***) 0.53 (a,b)
G8	(a) 2.05 (a,b)	(b) 1.46 (a)	(b) 2.23 (b)	(b) 2.59 (a,b)

Different letters right of EPG averages represent significant difference (p <0.05) between days in the same group (lines), calculated by "*pared t*" test. Different letters left of EPG averages represent significant difference (p <0.05) between groups in the same day (column), calculated by "*unpared t*" test. ***most significant difference (p<0.0001).

Table 2. Fecal Egg Count Reduction (FECR) on day 7 in the sheep belonging to groups G1 -Doramectin, G2- Fenbendazole, G3- Nitroxynil, G4- Doramectin + Fenbendazole, G5-Doramectin + Nitroxynil, G6- Fenbendazole + Nitroxynil, and G7- Doramectin + Fenbendazole + Nitroxynil, and the lower limit of the confidence interval at 95%.

Groups	FECR (%)	LL-CI (95%)
G1 - Doramectina	83	74.97
G2 - Fenbendazole	80	60.72
G3 - Nitroxynil	93	91.20
G4 - Doramectina + Fenbendazole	83	77.32
G5 - Doramectina + Nitroxynil	95	88.98
G6 - Fenbendazole + Nitroxynil	83	62.41
G7 - Doramectina + Fenbendazole + Nitroxynil	62	55.95

LL-CI (95%) – lower limit of the confidence interval at 95%.

of the confidence interval at 95%; the helminths also showed low resistance when nitroxynil was used in combination with doramectin (G5).

Groups G5 and G3 had the best FECR rates. The animals of both the groups were administered nitroxynil, with only those in G5 being administered doramectin additionally. However, a group very similar to G3 and G5 in terms of the treatment, the G7 (doramectin + nitroxynil + fenbendazole), had the worst FECR rates (62%) among all the groups in the study. This was because of one particular animal in G7, which presented EPG values much higher than those of the rest of the animals in the other two groups (G3 and G5). If this animal were removed from the group, the reevaluated FECR rate would increase to 94%, which is comparable to those of G3 and G5 (93% and 95%, respectively), suggesting that fenbendazole neither contributed to the improvement of the efficacy of the treatment, nor was it responsible for any antagonism (Table 2).

Cezar et al. (2010) proposed that random combinations of drugs are not advisable as they might increase the treatment costs without adding to the efficacy of their anthelminthic activity, and might also speed up the problem of development of cross-parasitic resistance.

The cultivation of larvae from the feces of the animals of all the groups revealed a higher occurrence of the larvae of the *Haemonchus* and *Cooperia* genera, with the former being found at a higher frequency. The efficacy of the treatments against these genera varied from 62%, with the combination of doramectin + fenbendazole + nitroxynil (G7) to 93.2% with nitroxynil (G3) for the *Haemonchus* genus. Regarding the efficacy of the treatment against particular genera, only fenbendazole proved fully effective (100%) against *Cooperia*, and predictably, all other combinations containing this drug (G4, G6, and G7) were also effective against the parasite, demonstrating that, despite several years of exposure to this drug in the examined farm, *Cooperia* had not yet established a resistance to it.

The results indicate that fenbendazole was moderately effective against *Haemonchus* and highly effective against *Cooperia*. Doramectin (G1) was found to be moderately effective against *Haemonchus* and insufficiently active against *Cooperia*, and nitroxynil (G3) was effective against *Haemonchus* and insufficiently active against *Cooperia*. It was not possible to establish the efficacy of the combination of doramectin + nitroxynil (G5) against each of the nematodes genera because the cultivation of the larvae from the feces of the animals of this group yielded no growth (Table 3).

The anthelmintic properties of fenbendazole were first presented in 1975, who reported that the drug eliminated 100% of the *Haemonchus* contortus larvae in 3-10 days at doses of 3.5 mg/kg of body weight (KIRSCH & DÜWEL, 1975). Santiago et al. (1978) also reported high levels of efficacy of fenbendazole.

The results of this study corroborate those of studies by Chartier et al. (1998) and Palcy et al. (2010) in France, Bartley et al. (2004, 2005) in Scotland, and Farias et al. (1997), Melo et al. (2003), and Cruz et al. (2010) in Brazil, who identified the inefficacy of fenbendazole in most of the surveyed sheep farms.

In Chile, similar studies demonstrated, that in sheep treated with fenbendazole, the FECR rates 7-days post-treatment were 41%, and the genera that had highest resistance to fenbendazole were *Trichostrongylus* and *Teladorsagia* (TORO et al., 2014).

Table 3. The number of larvae per gram (LPG) of feces of the animals of groups G1- Doramectin, G2- Fenbendazole, G3- Nitroxynil, G4- Doramectin + Fenbendazole, G5-Doramectin + Nitroxynil, G6- Fenbendazole + Nitroxynil, G7- Doramectin + Fenbendazole + Nitroxynil, and G8- Control, on days 0 and 7, and the efficiency (%) of the treatments.

	Day-zero (LPG)		Day-7 (LPG and Efficiency)			
	Haemonchus	*Cooperia*	*Haemonchus*	EF(%) H.	*Cooperia*	EF(%) C.
G1	277	38	33	84.1	2	0
G2	273	52	40	80.5	0	100.0
G3	244	61	14	93.2	1	0
G4	266	29	35	82.9	0	100.0
G5	282	38	ng	-	ng	-
G6	266	54	35	82.9	0	100.0
G7	322	0	78	62.0	0	100.0
G8	275	34	205	-	0	-

LPG: larvae per gram of feces. EF(%)H.: Efficiency against *Haemonchus* genus. EF(%)C.: Efficiency against *Cooperia* genus. ng: no growth in culture.

The parasitic resistance against doramectin, and its low efficacy against the organisms of the *Haemonchus* and *Cooperia* genera found in this study corroborate the findings of the studies by Cruz et al. (2010) in Rio de Janeiro and Borgsteede et al. (2007) in the Netherlands. The latter reported only 15% efficacy of the drug, as indicated by its FECR values. However, the results of a previous study by Echevarria et al. (2000) in Rio Grande do Sul contradict these results.

The results observed in our experiment are consistent with those of several other recently published studies. Cruz et al. (2010) showed nitroxynil efficacies ranging from 76% to 100%, Vila Nova et al. (2014) too identified resistance in nematodes to nitroxynil treatment, with overall FECR rates of 66%, despite those of the larvae of *Haemonchus* being high (91%). In our study, despite the insufficient reduction in the total nematode count (less than 95%), the reduction in the larvae of the *Haemonchus* genera was noted to be high (93.2%).

With regard to the efficacy noted with the drugs administered individually or in combination, the results of this study corroborate those presented by Sczesny-Moraes et al. (2010), but contradict those reported by Soccol et al. (2004), Buzzulini et al. (2007), Cezar et al. (2010), and Holsback et al. (2013), all of which found higher efficacies with the administration of drug combinations compared to those obtained with individual drugs.

It is concluded from the results of this study that herd nematodes are resistant to doramectin, fendendazole, and nitroxynil, and that the efficacy levels of these drugs are unsatisfactory despite their administration in combination. The drug combinations of doramectin, fendendazole, and nitroxynil improve neither the anthelmintic efficiency, nor the efficacy against *Haemonchus* and *Cooperia*. In addition, the use of drug combinations is more expensive, and the resultant efficacy does not justify the high costs of treatment. Despite the cessation of the use of nitroxynil and fendendazole for several years, it can be concluded from our results that the parasitic resistance acquired by helminths persists for a long period after their use has been stopped.

References

Atta AH, Abo-Shihada MN. Comparative pharmacokinetics of doramectin and ivermectin in sheep. *J Vet Pharmacol Ther* 2000; 23(1): 49-52. http://dx.doi.org/10.1046/j.1365-2885.2000.00238.x. PMid:10747243.

Bartley DJ, Jackson E, Jackson F, Sargison N. Characterisation of two triple resistant field isolates of *Teladorsagia* from Scottish lowland sheep farm. *Vet Parasitol* 2004; 123(3-4): 189-199. http://dx.doi.org/10.1016/j.vetpar.2004.06.018. PMid:15325045.

Bartley DJ, Jackson E, Sargison N, Jackson F. Further characterisation of a triple resistant field isolate of *Teladorsagia* from a Scottish lowland sheep farm. *Vet Parasitol* 2005; 134(3-4): 261-266. http://dx.doi.org/10.1016/j.vetpar.2005.07.017. PMid:16168563.

Bishop Y. *The Veterinary Formulary*. 6th ed. London: Pharmaceutical Press; 2005.

Borges FA, Silva HC, Buzzulini C, Soares VE, Santos E, Oliveira G, et al. Endectocide activity of a new long-action formulation containing 2.25% ivermectin + 1.25% abamectin in cattle. *Vet Parasitol* 2008; 155(3-4): 299-307. http://dx.doi.org/10.1016/j.vetpar.2008.04.019. PMid:18562119.

Borgsteede FHM, Dercksen DD, Huijbers R. Doramectin and albendazole resistance in sheep in The Netherlands. *Vet Parasitol* 2007; 144(1-2): 180-183. http://dx.doi.org/10.1016/j.vetpar.2006.09.031. PMid:17113232.

Buzzulini C, Sobrinho AGS, Costa AJ, Santos TR, Borges FA, Soares VE. Eficácia anti-helmíntica comparativa da associação albendazole, levamisole e ivermectina à moxidectina em ovinos. *Pesq Agropec Bras* 2007; 42(6): 891-895. http://dx.doi.org/10.1590/S0100-204X2007000600017.

Cezar AS, Toscan G, Camillo G, Sangioni LA, Ribas HO, Vogel FSF. Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil. *Vet Parasitol* 2010; 173(1-2): 157-160. http://dx.doi.org/10.1016/j.vetpar.2010.06.013. PMid:20619543.

Chartier C, Pors I, Hubert J, Rocheteau D, Benoit C, Bernard N. Prevalence of anthelmintic resistant nematodes in sheep and goats in Western France. *Small Rumin Res* 1998; 29(1): 33-41. http://dx.doi.org/10.1016/S0921-4488(97)00116-8.

Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, et al. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 1992; 44(1-2): 35-44. http://dx.doi.org/10.1016/0304-4017(92)90141-U. PMid:1441190.

Coles GC, Jackson F, Pomroy WE, Prichard RK, Son-Himmelstjerna GV, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 2006; 136(3-4): 167-185. http://dx.doi.org/10.1016/j.vetpar.2005.11.019. PMid:16427201.

Coles GC. Anthelmintic resistance: looking to the future: a UK perspective. *Res Vet Sci* 2005; 78(2): 99-108. http://dx.doi.org/10.1016/j.rvsc.2004.09.001. PMid:15563915.

Condi GK, Soutello RGV, Amarante AFT. Moxidectin-resistant nematodes in cattle in Brazil. *Vet Parasitol* 2009; 161(3-4): 213-217. http://dx.doi.org/10.1016/j.vetpar.2009.01.031. PMid:19251366.

Cruz DG, Rocha LO, Arruda SS, Palieraqui JGB, Cordeiro RC, Santos-Junior E, et al. Anthelmintic efficacy and management practices in sheep farms from the state of Rio de Janeiro, Brazil. *Vet Parasitol* 2010; 170(3-4): 340-343. http://dx.doi.org/10.1016/j.vetpar.2010.02.030. PMid:20356679.

Echevarria F, Pinheiro AC, Alves-Branco FPJ, Caproni L Jr, Umehara O, Gonçalves LCB. Efficacy of doramectin against naturally acquired nematode infections in sheep. *Rev Bras Parasitol Vet* 2000; 9(1): 65-69.

Gordon HMCL, Whitlock HV. A new technique for counting nematode eggs in sheep faeces. *J Counc Sci Ind Aust* 1939; 12(1): 50-52.

Farias MT, Brodin EL, Forbes AB, Newcomb K. A survey on resistance to anthelmintics in sheep stud farms of Southern Brazil. *Vet Parasitol* 1997; 72(2): 209-214. http://dx.doi.org/10.1016/S0304-4017(97)01111-4. PMid:9404847.

Grupo Mercado Comum – GMC. *Regulamento Técnico para registros de produtos antiparasitários de uso veterinário: Mercosul resolução nº76: decisão nº4/91, resolução nº11/93*. GMC; 1996.

Holsback L, Marquez ES, Meneghel PP. Resistência parasitária de helmintos gastrointestinais e avaliação dos parâmetros hematológicos de ovinos no Norte do Paraná. *Rev Bras Med Vet* 2013; 35(1): 76-84.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Sistema IBGE de recuperação automática – SIDRA* [online]. Rio de Janeiro: IBGE; 2013 [cited 2015 Nov 9]. Available from: http://www.sidra.ibge.gov.br/bda/pecua/default.asp?t=2&z=t&o=12&u1=1&u3=1&u4=1&u5=1&u6=1&u7=1&u2=32.

Jackson F. Anthelmintic resistance: the state of play. *Br Vet J* 1993; 149(2): 123-138. http://dx.doi.org/10.1016/S0007-1935(05)80083-1. PMid:8485639.

Keith RK. The differentiation of the infective larvae of some common nematode parasites of cattle. *Aust J Zool* 1953; 1(2): 223-235. http://dx.doi.org/10.1071/ZO9530223.

Kelly JD, Hall AC. Resistance of animal helminths to anthelmintics. *Adv Pharmacol Chemother* 1979; 16: 89-128. http://dx.doi.org/10.1016/S1054-3589(08)60243-4. PMid:382801.

Kirsch R, Düwel D. Laboratory investigation in sheep with a new anthelmintic. *Vet Rec* 1975; 97(2): 28-31. http://dx.doi.org/10.1136/vr.97.2.28. PMid:1146192.

Marriner SE, Bogan JA. Pharmacokinetics of fenbendazole in sheep. *Am J Vet Res* 1981; 42(7): 1146-1148. PMid:7271033.

Melo ACFL, Reis IF, Bevilaqua CML, Vieira LS, Echevarria FAM, Melo LM. Nematódeos resistentes a anti-helmínticos em rebanhos de ovinos e caprinos do Estado do Ceará, Brasil. *Cienc Rural* 2003; 33(2): 339-344. http://dx.doi.org/10.1590/S0103-84782003000200024.

Nies AS, Spielberg SE. Principles of therapeutics. In: Goodman G. *The pharmacological basis of therapeutics*. 9th ed. New York: Mc Graw-Hill; 1996. p. 43-62.

Palcy C, Silvestre A, Sauve C, Cortet J, Cabaret J. Benzimidazole resistance in *Trichostrongylus axei* in sheep: Long-term monitoring of affected sheep and genotypic evaluation of the parasite. *Vet J* 2010; 183(1): 68-74. http://dx.doi.org/10.1016/j.tvjl.2008.09.012. PMid:18980850.

Roberts FHS, O'Sullivan JP. Methods for egg counts and larval cultures for strongyles infesting the gastrointestinal tract of cattle. *Aust J Agric Res* 1950; 1(1): 99-102. http://dx.doi.org/10.1071/AR9500099.

Sangster NC. Managing parasiticide resistance. *Vet Parasitol* 2001; 98(1-3): 89-109. http://dx.doi.org/10.1016/S0304-4017(01)00425-3. PMid:11516581.

Santiago MAM, Costa VC, Benevenga SF. Atividade anti-helmíntica do fenbendazole nos estágios imaturos de *Haemonchus contortus* de ovinos. *Rev Centro Cienc Rurais* 1978; 8(2): 123-125.

Sczesny-Moraes EA, Bianchin I, da Silva KF, Catto JB, Honer MR, Paiva F. Resistência anti-helmíntica de nematóides gastrintestinais em ovinos, Mato Grosso do Sul. *Pesq Vet Bras* 2010; 30(3): 229-236. http://dx.doi.org/10.1590/S0100-736X2010000300007.

Soccol VT, De Souza FP, Sotomaior C, Castro EA, Milczewski V, Mocelin G, et al. Resistance of gastrointestinal nematodes to anthelmintics in sheep (*Ovis aries*). *Braz Arch Biol Technol* 2004; 47(1): 41-47. http://dx.doi.org/10.1590/S1516-89132004000100006.

Toro A, Rubilar L, Palma C, Pérez R. Resistencia antihelmíntica en nematodos gastrointestinales de ovinos tratados con ivermectina y fenbendazole. *Arch Med Vet* 2014; 46(2): 247-252. http://dx.doi.org/10.4067/S0301-732X2014000200010.

Torres-Acosta JFJ, Hoste H. Alternative or improved methods to limit gastro-intestinal parasitism in grazing sheep and goats. *Small Rumin Res* 2008; 77(2-3): 159-173. http://dx.doi.org/10.1016/j.smallrumres.2008.03.009.

Vila Nova LE, Costa ME, Melo PGCF, Cunha LFC Fo, Barca FA Jr, Silva LC, et al. Resistência de nematoides aos anti-helmínticos nitroxynil 34% e ivermectina 1% em rebanho ovino no município de São João do Ivaí, Paraná. *Rev Bras Hig San Anim* 2014; 8(1): 160-171.

Vizard AL, Wallace RJ. A simplified faecal egg count reduction test. *Aust Vet J* 1987; 64(4): 109-111. http://dx.doi.org/10.1111/j.1751-0813.1987.tb09641.x. PMid:3619790.