## HIGH ANTHELMINTIC RESISTANCE IN SMALL RUMINANTS FROM THE

## SEMIARID OF PARAÍBA STATE, BRAZIL

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# (ELEVADA RESISTÊNCIA ANTI-HELMÍNTICA EM PEQUENOS RUMINANTES DO

## SEMIÁRIDO DA PARAÍBA, BRASIL)

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## **SUMMARY**

This study aimed to evaluate the effect of Ivermectine 0.08% and Hydrochloride of 8 9 Levamisole 5% in controlling sheep and goat gastrointestinal helminthiasis from Agreste region of Paraíba State, Northeastern Brazil. The experiment was developed from July 2011 10 to February 2012. Were used 28 farms, with animals of both sexes and ages between three to 11 12 48 monthes. In each farm were chosen 18 animals without anthelmintic treatments at least 13 three months. The animals were divided into three groups: group 1, treated with Ivermectine 0.08%, orally, in a single dose of 2.5 mL/ 10 kg l. w.; group 2, treated with Hydrochloride of 14 15 Levamisole 5%, orally, in a single dose 1.0 ml/ 10 kg l. w. and group 3, which received no anthelmintic treatment, serving as a control group. Fecal samples were collected on days zero 16 17 and ten days after treatments for fecal analysis. The treatment with Hydrochloride of Levamisole reduced 86.7% and 93% the parasite load of goats and sheep, respectively. 18 19 Although, the treatment with Ivermectine reduced only 30.9% in goats and 24.6% in sheep. 20 The most prevalent helminth gender was *Haemonchus* sp. The gastrointestinal nematodes of 21 goats and sheep from Agreste of Paraíba State are highly resistant to Ivermectine. Hydrochloride of Levamisole is still effective in sheep, but already shows resistance traces in 22 23 goats.

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**KEY WORDS:** Goats. ivermectine. semi-arid. sheep.

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#### **RESUMO**

- 28 Este estudo objetivou avaliar o efeito da Ivermectina 0,08% e do Cloridrato de Levamisole
- 29 5% no controle das helmintoses gastrintestinais de ovinos e caprinos da mesorregião do
- 30 Agreste do Estado da Paraíba, Nordeste do Brasil. O experimento foi desenvolvido no período

de julho de 2011 a fevereiro de 2012. Foram utilizadas 8 propriedades, com animais de ambos os sexos e idades entre três e 48 meses. Em cada propriedade foram escolhidos 18 animais sem tratamento anti-helmíntico a pelo menos três meses. Os animais foram divididos em três grupos: grupo 1, tratado com Ivermectina 0,08%, via oral, em dose única de 2,5 mL/ 10 kg p. v.; grupo 2, tratado com Cloridrato de Levamisole 5%, via oral, em dose única de 1,0 mL / 10 kg p. v. e grupo 3, que não recebeu tratamento anti-helmíntico, servindo como grupo controle. Amostras fecais foram coletadas nos dias zero e dez dias após os tratamentos para realização das análises fecais. O tratamento com Cloridrato de Levamisole reduziu 86,7% e 93% a carga parasitária de caprinos e ovinos, respectivamente. Entretanto, o tratamento com Ivermectina reduziu apenas 30,9% em caprinos e 24,6% em ovinos, O helminto mais prevalente nas coproculturas foi o *Haemonchus* sp. Os nematódeos gastrintestinais de caprinos e ovinos do Agreste da Paraíba encontram-se altamente resistentes à Ivermectina. O Cloridrato de Levamisole ainda é efetivo em ovinos, mas já apresenta traços de resistência em caprinos.

## PALAVRAS-CHAVE: Caprinos. ivermectina. ovinos. semiárido.

## INTRODUCTION

The goat and sheep production occupies a prominent place in the brazilian Northeastern, where farmers use their products in food and commerce, resulting in stability and development. In the Agreste region of Paraíba State, the small ruminant production is a viable activity, generating a major source of animal protein for human consumption. However, there are some limiting factors in the productivity of the herds, including the gastrointestinal helminthiasis, responsible for the decrease in food intake and nutrient absorption, growth retardation, decrease in the meat and milk production and mortality (LIMA et al. 2010a).

The main form of parasite control is done by the use of chemicals with broad spectrum of activity, most often administered empirically (CEZAR et al. 2010). Among the most widely used anthelmintics, the Ivermectine stands out, belonging to the macrocyclic lactones group, which act by opening chloride channels targeted by glutamate causing parasite

neuromuscular paralysis. Another compound widely used is the Hydrochloride of Levamisole, belonging to the imidazothiazoles group, which has action on acetylcholine receptors, causing muscle contractions and worm paralysis (COLES et al. 2006).

The irrational use of anthelmintics has contributed to the resistance to the most available drugs of the small ruminant gastrointestinal helminthes. Several studies reports the resistance of these helminthes to Ivermectine and Hydrochloride of Levamisole in Brazil (CEZAR et al. 2010; MORAES et al. 2010; LIMA et al. 2010b), however no studies of anthelmintic resistance in the Agreste region of Paraíba State were conducted.

Due to lack of studies evaluating the efficacy of anthelmintics in this region, this study aimed to evaluate the effect of Ivermectine 0.08% and Hydrochloride of Levamisole 5% in the control of sheep and goats gastrointestinal helminthiasis.

## MATERIAL AND METHODS

The experiment was conducted on farms of small ruminant production system in the Gado Bravo city, Agreste of Paraíba State, during July 2011 to February 2012. The region has a semi-arid climate, with a rainy season from April to July, which 90% of rainfall occurs and a dry season. The annual temperature average is 23,5°C (minimum of 18°C and maximum 29°C), with little variation over the year (VILELA et al. 2008). This region includes the transition zone between the moist coast and the semiarid backwoods, presenting vegetation of the Caatinga biome.

Were used 28 herds (504 animals), 15 producing goats and 13 sheep (270 and 234 animals, respectively), of both sexes, between three to 48 months-old and without defined breed. In each herd18 animals were chosen, they should be without anthelmintic treatment for at least three months and presenting OPG $\geq$ 500. Subsequently, animals were individually identified and randomly assigned into three groups: group 1, treated with Ivermectine 0,08%,

orally, in a single dose of 2,5 mL/ 10 kg l. w.; group 2, treated with Hydrochloride of Levamisole 5%, orally, in a single dose of 1,0 mL/ 10 kg b. w. and group 3, received no anthelmintic treatment, serving as a control group.

Fecal samples were individually collected on day zero and ten days after treatments and sent to the Laboratory of Parasitic Diseases of Domestic Animals of the Universidade Federal de Campina Grande (UFCG), Patos - PB, for the fecal analyzes realization. Were performed the counting of Eggs Per Gram of feces (EPG), according to Whitlock & Gordon (1939) and larval culture, according to Roberts & O'Sullivan (1950).

The Fecal Egg Count Reduction test (FECR) was performed according to Coles et al. (1992). Subsequently, the data were subjected to one-way variance analysis, and followed by Tukey test at 5% probability. The EPG values were analyzed using logarithmic transformation log (x + 1), however, are present as arithmetic averages of untransformed values. The analyses were performed using the BioEstat 5.0 Software. The efficacy of the drugs was based on Technical Regulation Ordinance N°. 48/1997 of the Minitério da Agricultura, Pecuária e Abastecimento (MAPA) for chemicals endowed with antiparasitic activity using the following criteria: is highly effective when it reduces more than 98%; 90-98% effective, moderately effective 80-89% and insufficiently active<80% (BRASIL, 1997).

## RESULTS

Was observed a statistical difference (p<0,05) between the goat and sheep anthelmintic treatments (Table 1).

Table 1. Values of EPG e FECR of goat and sheep submitted to anthelmintic treatments in the Agreste region of Paraíba State, Brazil.

Groups	Goats			Sheep		
Groups	0	10	FECR	0	10	FECR
Ivermectine 0,08%	5376 <sup>Aa</sup>	4081 Ab	30,9%	1800 <sup>Aa</sup>	1255 <sup>Ba</sup>	24,6%
Hyd. of Levamisole 5%	5516 <sup>Aa</sup>	783 <sup>Bc</sup>	86,7%	1521 <sup>Aa</sup>	116 <sup>Bb</sup>	93,0%
Control	5798 <sup>Aa</sup>	5914 <sup>Aa</sup>	-	1682 <sup>Aa</sup>	1665 <sup>Aa</sup>	-

Values followed by the same letters maiuscle in lines and minuscle in columns did not statistically differ (p>0.05) – Tukey's test.

Was observed that the Ivermectine 0,08% EPG did not reduced satisfactorily, especially in sheep, where this was not statistically different (p>0,05) than the control group. Hydrochloride of Levamisole 5% was the best treatment, differing significantly (p<0,05) from the other groups in EPG values post-treatment in both species.

According to Brasil (1997), Ivermectine was insufficiently active in goats (30,9%) and sheep (24,6%). Hydrochloride of Levamisole already appeared moderately effective in goats (86,7%) and effective in sheep (93%).

The helminths percentages recovered from fecal cultures are described in Table 2.

Table 2. Percentages of gastrointestinal helminths recovered from fecal cultures of goat and sheep submitted to anthelmintic treatments in the Agreste region of Paraíba State, Brazil.

	_	Control		Ivermectine 0,08%		Hyd. of Levamisole 5%	
		0	10	0	10	0	10
Goats	Н	70	68	78	56	38	42
	T	25	31	20	34	60	51
	S	0	1	2	4	0	2
	O	5	0	0	6	2	5
Sheep	Н	40	49	35	53	45	63
	T	55	38	58	34	40	37
	S	2	11	3	10	11	0
	O	3	2	4	3	4	0

H: Haemonchus sp.; T: Trichostrongylus sp.; S: Strongyloides sp.; O: Oesophagostomum sp.

The most prevalent helminth gender in fecal cultures was *Haemonchus* sp., followed by *Trichostrongylus* spp., *Strongyloides* sp., and *Oesophagostomum* sp.

### **DISCUSSION**

It was observed that the Ivermectine 0,08% do not satisfactorily reduced the animals worm burden, with reductions of only 30,9% in goats and 24,6% in sheep. High anthelmintic resistance was also observed by Lima et al. (2010a), when evaluating the efficacy of Ivermectine 0.02% in goat herds from Cariri region of Paraíba State, obtaining reduction of 50,1%. Pereira et al. (2008), evaluated this anthelmintic in goats and sheep from Rio Grande do Norte State, Brazil, showed a reduction of only 14,2% and 20 7% respectively. However, George et al. (2011) evaluated the Ivermectine in sheep from Trinidad and Tobago getting 95-97% of FECR, showing up effective. Lima et al. (2010b) in Pernambuco State, Brasil, observed that Ivermectine showed an efficacy of 67,33% in goats, indicating resistance, although in sheep presented 100% of efficacy considering highly effective.

Several studies have confirmed the resistance of gastrointestinal helminthes of small ruminants to Ivermectine worldwide (LIFSCHITZ et al. 2010; BARTLEY et al. 2012; LEATHWICK et al. 2012).

The Hydrochloride of Levamisole 5% showed reductions of 86,7% and 93% for sheep and goats, respectively, similar to which was observed by Rodrigues et al. (2007), when tested this anthelmintic in goats from the Sertão region of Paraíba, with 93.3% of efficiency. Duarte et al. (2012), testing this anthelmintic in Northern of Minas Gerais State, Southeastern Brazil, achieved efficacy ranging from 90% to 100%. However, Leathwick et al. (2012) observed anthelmintic resistance to this anthelmintic (efficacy<70%) in New Zealand sheep and George et al. (2011) observed efficacies between 53% to 81% in sheep from Trinidad and Tobago.

The resistance traces to Hydrochloride of Levamisole 5% observed in some farms may be due to its low efficacy against immature stages of nematodes in general (MELO et al. 2003).

The most prevalent helminth gerder was *Haemonchus* sp., corroborating with Vilela et al. (2012). Probably, this worm acquires faster resistance due to its high biotic potential, a high genetic variability as well as hosting the allele that causes decreased susceptibility to a drug (BLACKHALL et al. 1998). Besides *Haemonchus* sp. were found *Trichostrongylus* sp. and *Strongyloides* sp. and, to a lesser extent, *Oesophagostomum* sp. Similar percentages of these helminths were also observed in other studies conducted in Northeastern Brazil (LIMA et al. 2010a; COSTA et al. 2011).

Several factors contribute to the development of anthelmintic resistance and consequently inefficiency of antiparasitic drugs. The indiscriminate use of these drugs most often occurs by the producers lack of knowledge, poor management practices and the ease in acquiring these drugs. According to Vilela et al. (2012), the high resistance to anthelmintics observed in studies conducted in semi-arids regions of Northeastern Brazil may be occurs due

to the fact that it is a common practice the deworming of all the small ruminant herds four tosix times per year.

Must be widespread the idea that the anthelmintic control based only on massive herd deworming is a wrong practice. The control must be integrated, where alternative forms of control such as the use of anthelmintic plants, the Famacha<sup>©</sup> method and/ or in the near future, nematophagous fungi, along with synthetic anthelmintics and also associated with good practices of herd management are the solution to minimize the effects of gastrointestinal helminthiasis.

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### CONCLUSION

The goats and sheep gastrointestinal nematodes of the Agreste region of Paraíba State are highly resistant to Ivermectine 0,08%. Resistance traces to Hydrochloride of Levamisole 5% were observed in goats.

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## REFERENCES

BLACKHALL, W.J.; POULIOT J. F.; PRICHARD, R. K.; BEECH, R. N. *Haemonchus contortus*: selection at a glutamate-gated chloride channel gene in ivermectine and moxidectin selected strains. **Exp. Parasitol.** 90:42-48, 1998.

191

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). **Regulamento Técnico para Licenciamento e/ou Renovação de Licença de Produtos Antiparasitários de**194 **Uso Veterinário.** Portaria nº 48, de 12 de maio de1997. Diário Oficial da União de
195 16/05/1997, Seção 1, Página 10165.

196

197 CEZAR, A. S.; TOSCAN, G.; CAMILLO, G.; SANGIONI, L. A.; RIBAS, H. O.; VOGEL, F. S. F. Multiple resistance of gastrointestinal nematodes to nine different drugs in sheep flock in southern Brazil. **Vet. Parasitol.** 173:157-160, 2010.

200

COLES, G. C.; JACKSON, F.; PONROY, W. E.; PRICHARD, R. K., SAMSONHIMMELSTJERNA, G. V.; SILVESTRE, A.; TAYLOR, M. A.; VERCRUYSSE, J. The detection of anthelmintic resistance in nematodes of veterinary importance. **Vet. Parasitol.** 136:167-185, 2006.

205206

- 209 COLES, G. C.; BAUER, C.; BORGSTEEDE, F. H.; GEERTS, S.; KLEI, T. R.; TAYLOR,
- 210 M. A.; WALLER, P. J. World Association for the Advancement of Veterinary Parasitology
- 211 (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary
- 212 importance. **Vet. Parasitol.** 44:35-44. 1992.

213

- 214 COSTA, K. M. F. M.; AHID, S. M. M.; VIEIRA, L. S.; VALE A. M.; BLANCO, B. S.
- 215 Efeitos do tratamento com closantel e ivermectina na carga parasitária, no perfil hematológico
- e bioquímico sérico e no grau Famacha de ovinos infectados com nematódeos. Pesq. Vet.
- 217 **Bras.** 31:1075-1082, 2011.

218

- DUARTE, E. R.; SILVA, R. B.; VASCONCELOS, V. O.; NOGUEIRA, F. A.; OLIVEIRA,
- 220 N. J. F. Diagnóstico do controle e perfil de sensibilidade de nematódeos de ovinos ao
- albendazol e ao levamisol no norte de Minas Gerais. Pesq. Vet. Bras. 32:147-152, 2012.

222

- GORDON, H. M. & WHITLOCK, H.V. A new technique for counting nematode eggs in
- sheep faeces. **J. Coun. Sci. Ind. Res.** 12:50-52, 1939.
- 225 GEORGE, N.; PERSAD, K.; SAGAM, R.; OFFIAH, V. N.; ADESIYUN, A. A.,
- 226 HAREWOOD, W.; LAMBIE, N.; BASU, A. K. Efficacy of commonly used anthelmintics:
- 227 First report of multiple drug resistance in gastrointestinal nematodes of sheep in Trinidad.
- 228 **Vet. Parasitol.** 183:194-197, 2011.

229

- LEATHWICK, D. M.; WAGHORN, T. S.; MILLER, C. M.; CANDY, P. M.; OLIVER, A.
- M. B. Managing anthelmintic resistance Use of a combination anthelmintic and leaving
- some lambs untreated to slow the development of resistance to ivermectine. Vet. Parasitol.
- 233 187:285-294, 2012.

234

- LIMA, W. C.; ATHAYDE, A. C. R.; MEDEIROS, G. R.; LIMA, D. S. D.; BORBUREMA, J.
- B.; SANTOS, E. M.; VILELA, V. L. R.; AZEVEDO, S. S. Nematóides resistentes a alguns
- anti-helmínticos em rebanhos caprinos no Cariri Paraibano. **Pesq. Vet. Bras**. 30:1003-1009,
- 238 2010a.

239

- LIMA, M. M.; FARIAS, M. P. O.; ROMEIRO, E. T.; FERREIRA, D. R. A.; ALVES, L. C.;
- FAUSTINO, M. A. G. Eficácia da moxidectina, ivermectinea e albendazole contra helmintos
- 242 gastrintestinais em propriedades de criação caprina e ovina no estado de Pernambuco. Ciê.
- 243 **Anim. Bras.** 11:94-100, 2010.

244

- 245 LIFSCHITZ, A.; ENTROCASSO, C.; ALVAREZ, L.; LLOBERAS, M. Glycoprotein
- improves ivermectine activity against adult resistant nematodes in sheep. Vet. Parasitol.
- 247 172:291-298, 2010.

248

- MELO, A. C. F. L.; REIS, I. F.; BEVILAQUA, C. M. L.; VIEIRA, L. S.; ECHEVARRIA, F.
- 250 A. M.; MELO, L. M. Nematódeos resistentes a anti-helmíntico em rebanhos de ovinos e
- caprinos do estado do Ceará, Brasil. Ciê. Rur. 33:339-344, 2003.

252

- 253 MENEZ, C.; ALVINERIE, M.; JACKSON, F.; DEVIN, L.; LESPINE, A. Influence of
- 254 Pluronic 85 and ketoconazole on disposition and efficacy of ivermectine in sheep infected
- with a multiple resistant *Haemonchus contortus* isolate. **Vet. Parasitol.** 187:464-472, 2012.

- MORAES, E. A. S.; BIANCHIN, I.; SILVA, K. F.; CATTO, J. B.; HONER, M. R.; PAIVA,
- F. Resistência anti-helmíntica de nematóides gastrintestinais em ovinos, Mato Grosso do Sul.
- 259 **Pesq. Vet. Bras.** 30:229-236, 2010.

260

- PEREIRA, R. H. M. A.; AHID, S. M. M.; DIÓGENES, A. C., BEZERRA, S.; SOARES, H.
- S.; FONSECA, Z. A. A. S. Diagnóstico da resistência dos nematoides gastrintestinais a anti-
- helmínticos em rebanhos caprino e ovino do Rio Grande do Norte. Acta. Vet. Bras. 2:16-19,
- 264 2008.

265

- 266 RODRIGUES, A. B.; ATHAYDE, A. C. R.; RODRIGUES, O. G.; SILVA, W. W.; FARIA,
- 267 E. B. Sensibilidade dos nematóides gastrintestinais de caprinos a anti-helmínticos na
- mesorregião do sertão paraibano. **Pesq. Vet. Bras.** 27:162-166, 2007.

269

- VILELA, V. L. R.; FEITOSA, T. F.; LINHARES, E. F.; ATHAYDE, A. C. R.; MOLENTO,
- 271 M. B.; AZEVEDO S. S. FAMACHA© method as an auxiliary strategy in the control of
- 272 gastrointestinal helminthiasis of dairy goats under semiarid conditions of Northeastern Brazil.
- 273 **Vet. Parasitol.** 190:281-284, 2012.

274

- 275 VILELA, V. L. R.; SOLANO, G. B.; ARAÚJO, M. M.; SOUSA, R. V. R., SILVA, W. A.;
- FEITOSA, T. F.; ATHAYDE, A. C. R. Ensaios preliminares para validação do método
- FAMACHA© em condições de semi-árido paraibano. Rev. Bras. Parasitol. Vet. 17:154-157,
- 278 2008.

279280

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284285

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