PARASITIC RESISTANCE OF GASTROINTESTINAL NEMATODES IN GOATS FROM THE SEMIARID REGION OF PERNAMBUCO, NORTHEASTERN BRAZIL

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ABSTRACT

The aim of this study was to observe the situation of parasite resistance in goat farms located in Petrolina municipality, São Francisco Valley region, Northeastern Brazil. Four-hundred and twenty goats of both sexes, older than seven months, and with the highest parasite load were selected from each of the 10 farms and were divided into six homogeneous groups of seven animals. Each group was treated with an anthelmintic drug: Group I–Ivermectin 0.08%; Group II–Albendazole 10%; Group III–Levamisole 5%; Group IV–Moxidectin 1%; Group V–Closantel 10%; and Group VI–Control (no treatment). The samples were collected on days D0 (before treatment), D7, D14 and D21 post-treatment. The Fecal Egg Count Reduction Test (FECRT) was applied to evaluate anthelmintic resistance. The groups treated with Moxidectin showed inefficacy in D7 and low efficacy in D14, with an average reduction of 79.8% and 88.69%, but high efficacy in D21 (93.43%). Closantel showed low efficacy with average of 84.72% only in D14. Levamisole, Albendazole and Ivermectin showed drug inefficacy, with averages lower than 80%. All the tested drugs except moxidectin (D21) showed less than 90% antiparasitic efficacy in the goat herds, indicating the presence of parasites with multidrug resistance.

Key words: Efficacy, Anthelmintic, Helminthiasis, FECRT.

RESISTÊNCIA PARASITÁRIA DE NEMATÓDEOS GASTRINTESTINAIS DE CAPRINOS DA REGIÃO SEMIÁRIDA DE PERNAMBUCO, NORDESTE DO BRASIL

RESUMO

O objetivo deste estudo foi observar a situação de resistência parasitária em propriedades rurais caprinas localizadas no município de Petrolina, região do Vale do São Francisco, Nordeste do Brasil. Um total de 420 caprinos de ambos os sexos e idades acima de sete meses foram selecionados em 10 diferentes propriedades. Cada estabelecimento foi constituído por seis grupos homogêneos de sete animais, conforme a administração dos medicamentos: Grupo I-Ivermectina 0,08%; Grupo II-Albendazole 10%; Grupo III-Levamisole 5%; Grupo IV-

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Moxidectina 1%; Grupo V-Closantel 10%; e Grupo VI-Controle (sem tratamento). As amostras foram coletadas nos dias D0 (prévio ao tratamento), D7, D14 e D21 pós-tratamento. Para avaliar a resistência parasitária, aplicou-se o teste de redução na contagem de ovos por grama de fezes (RCOF). Para identificação das larvas foi realizada a técnica de coprocultura. Os grupos tratados com Moxidectina apresentaram ineficácia no D7 e baixa eficácia no D14, com redução média de 79,8% e 88,69%, mas alta eficácia no D21 (93,43%). Closantel mostrou baixa eficácia com média de 84,72% apenas no D14. Levamisol, Albendazol e Ivermectina mostraram-se ineficazes, com médias inferiores a 80%. Todos os medicamentos testados, com exceção da Moxidectina (D21) apresentaram resultados inferiores a 90%, indicando a presença de parasitos multirresistentes nos rebanhos estudados.

Palavras–chave: Eficácia, Anti-helmínticos, Helmintoses, RCOF.

RESISTENCIA PARASITARIA DE NEMATODOS GASTROINTESTINALES EN CABRAS CREADAS EN UNA REGIÓN SEMIÁRIDA DE PERNAMBUCO, NORESTE DE BRASIL

RESUMEN

El objetivo de este estudio fue observar la situación de la resistencia parasitaria en granjas rurales de cabras ubicadas en el municipio de Petrolina, en la región del Valle de São Francisco, noreste de Brasil. Se seleccionaron un total de 420 cabras de ambos sexos y edades de más de siete meses de 10 granjas diferentes. Cada establecimiento estuvo conformado por seis grupos homogéneos de siete animales, según la administración de medicación: Grupo I-Ivermectina 0.08%; Grupo II-Albendazol al 10%; Grupo III-Levamisol 5%; Grupo IV-Moxidectina al 1%; Grupo V-Closantel 10%; y Grupo VI-Control (sin tratamiento). Las muestras se recogieron los días D0 (antes del tratamiento), D7, D14 y D21 después del tratamiento. Para evaluar la resistencia parasitaria se aplicó la prueba de reducción del recuento de huevos por gramo de heces (RCOF). Para identificar las larvas se realizó la técnica de cocultivo. Los grupos tratados con Moxidectina mostraron ineficaz en D7 y baja eficácia en D14, con una reducción media del 79,8% y 88,69%, pero alta eficacia en D21 (93,43%). El closantel mostró una eficacia baja 82,0% solamente en D14. Se demostró que el levamisol, el albendazol y la ivermectina eran ineficaces, con un promedio de menos del 80%. Todos los fármacos probados, a excepción de la moxidectina (D21), presentaron resultados inferiores al 90%, lo que indica la presencia de parásitos multirresistentes en los rebaños estudiados.

Palabras clave: eficiencia, antihelmínticos, helmintos, RCOF.

INTRODUCTION

Gastrointestinal nematode (GIN) infection is the main health problem in small ruminant breeding systems from Brazil (1). It can impair animal growth causing weight loss, decreased food intake, low productivity and low fertility (2). It is considered one of the main obstacles to achieving good zootechnical performance indexes (3).

GINs are usually controlled by administering anthelmintics to the animals (4), but the indiscriminate use of these drugs contributes to the emergence of parasite resistance to the anthelmintics (5). Moreover, few producers apply strategic treatments that involve the rotation of drugs with different active ingredients (6). Anthelmintic resistance is a biological phenomenon, in which a particular drug, when used under the same conditions as when it was

effective, cannot maintain the same efficacy against parasites after a certain time (7). It is considered that the occurrence of anthelmintic resistance in farms is now a rule and no longer an exception in Latin American countries (8).

Goat farming, an activity present in almost all countries, is often cited as one of the most suitable activities for the Brazilian semiarid region. Pernambuco is spread over an area of 98,148,323 km² (9). Its population in 2010 was 8,796,448 people (4.6% of the national population) spread over 185 municipalities. About 80.2% of the population lives in urban areas and 19.8% in rural areas. Among some of the factors favorable to goat farming in the northeast are the suitability of livestock to the semi-arid agro-ecosystems, the low need for initial capital, the capacity to accumulate income on a small scale, the high potential for generating productive occupations, the easy sociocultural appropriation, and the offer of products with great appeal in new markets (10). Despite the high incidence of parasite resistance in Brazil´s northeastern flocks, few studies have focused on goats. This information is essential to maximize control over and delay the development of anthelmintic-resistant nematodes in small ruminant herds in rural areas of the semi-arid regions. Therefore, this research aimed to evaluate the resistance of GINs in goat farms to anthelmintic drugs (ivermectin, albendazole, levamisole, moxidectin and closantel) in the municipality of Petrolina, which is situated in the San Francisco valley in the semi-arid region of the state of Pernambuco, Brazil.

MATERIAL AND METHODS

Study area

The study was carried out on farms in the municipality of Petrolina (9º23'55" S 40º30'03" W), where it has 118,725 head of goats, is located in the region of the lower middle São Francisco River in the Caatinga biome, with a semi-arid tropical climate. In Petrolina the relative humidity of the air varies in average from 66% to 71.5% in the rainy season. The wettest month is April, which corresponds to the end of the rainy season, and the driest is October, corresponding to the end of the dry season. Regarding the air temperature, present average variations from 24.2ºC to 28.2ºC (11,12).

Sampling procedures and experiment

Ten farms with herds of no fewer than 70 goats were selected for convenience from August to December 2015. The animals in the study met the following inclusion criteria: (1) positive diagnosis for gastrointestinal helminths with a minimum number of 200 eggs per gram of feces (EPG), and (2) absence of treatment with any type of anthelmintic drug for a minimum period of 90 days prior to the study. The study involved 42 animals from each farm, making a total of 420 goats older than seven months, regardless of their reproductive condition. All the animals were identified by means of ear and neck tags. Before starting the deworming process to all the entire heard, each animal was weighed and clinically examined to obtain information about sex, age, mucosal color, body condition score, and physiological category.

The 42 animals from each farm were divided into six homogeneous groups of seven animals considering sex, age, breed and EPG; and each group was treated with a different anthelmintic drug, as follows: Group I – 0.2 mg/kg of ivermectin (IVM), applied orally (Ivomec® drench for sheep); Group II – 4.5 mg/kg of albendazole (ALB), applied orally (Aldazol®, Vallée); Group III – 5 mg/kg of levamisole (LVS), applied orally (Ripercol®, Fort

Dodge); Group IV – 0.2 mg/Kg of moxidectin (MXD) 1% injectable, applied subcutaneously (Cydectin/Fort Dodge); Group V – 10 mg/Kg of closantel (CLO), applied orally (Diantel®/Irfa), and Group VI – no treatment, served as the control group. The dosages applied were those recommended for sheep by the drug manufacturers, since the package inserts do not mention treatment for goats.

Fecal samples were collected directly from the rectal ampulla of each goat naturally infected by gastrointestinal nematodes. The samples were placed in sterile plastic bags, labeled, and stored in a refrigerator until they were processed. Sampling was performed the day of treatment, D0 (prior to treatment), and on D7, D14, and D21 (post treatment). EPG counts were performed as proposed by Gordon e Whitlock (13), while larvae were quantified by the coproculture method described by Roberts e O'Sullivan (14) and identified based on the characteristics described by Keith (15).

Analysis of eggs per gram of feces

The arithmetic mean of the EPG count of each treated group (EPGt) was calculated and compared with the mean EPG count of the control group (EPGc). A fecal egg count reduction test (FECRT) was performed using the formula described by Coles et al. (16): FECRT = [1-(EPGt/ EPGc)] x 100, where FECRT = test to reduce egg count per gram of feces; EPGt = mean number of eggs per gram of feces from the group of treated animals; EPGc = mean number of eggs per gram of feces from the control group. The effectiveness of the aforementioned anthelmintics was classified as follows: a) FECRT above 90%: effective drug; b) FECRT of 80% to 90%: low efficacy drug; c) FECRT below 80%: ineffective drug (17,18).

Statistical analysis

The groups were compared based on an analysis of variance of repeated measurements using the GLM (Generalized Linear Model) procedure, with multiple comparisons by the Tukey test (19) at a 5% significance level. The analysis was performed using the statistical package SPSS version 20 for Windows.

RESULTS

All the treated groups showed a reduction in EPG count compared control group (Table 1). Between days 7 and 21, group I-IVM showed a percentage of post-treatment effectiveness between 22.23% and 33.13%, indicating that the medication was ineffective. In group II-ALB it was 53.66% and 35.29%, proving ineffective. The group III-LVS, it ranged from 70.75% and 70.56%, indicating ineffectiveness. The group IV-MXD it was 79,81% to 93,43%, showing efficacy in the day 21. In group V-CLO it was 75,42% to 73,94%, indicating also ineffectiveness (Table 2).

Even showing a reduction in EPG count, Group III-LVS, Group IV-MXD and Group V-CLO showed significantly better efficacy compared to Group I-IVM and Group II-ALB, which demonstrated the greatest ineffectiveness among all medicaments used on day 21. In addition, Group IV-MXD was the only one that demonstrated efficacy on day 21. There was no significant influence of the moments (D7, D14 and D21) on the group means (Table 2).

The nematodes identified in the coprocultures were *Haemonchus contortus* (67%), followed by *Oesophagostomum* spp. (26%) and *Trichostrongylus* spp. (7%).

Table 1. Rate of reduction of the number of eggs per gram (EPG) of feces of gastrointestinal nematodes in goats in the municipality of Petrolina, Pernambuco state, Brazil.

Group	D0		D7		D14		D21	
	Mean	IC 95%	Mean	IC 95%	Mean	IC 95%	Mean	IC 95%
GI	862.90^{a,A,}	728.03; 997.77	416.13^{b,A}	330.12; 502.14	441.94^{b,A,D}	346.07; 537.80	424.19^{b,A}	346.93; 501.46
GII	722.73^{a,A,B}	592.01; 853.45	262.12^{b,A,B}	178.76; 345.49	301.52^{b,A,B}	208.60; 394.43	372.73^{b,A}	297.84; 447.62
GIII	616.42^{a,A,B}	486.68; 746.16	119.40^{b,B,C}	36.66; 202.14	162.69^{b,B,C}	70.47; 254.91	117.91^{b,B}	43.58; 192.24
GIV	597.06^{a,B}	568.28; 725.84	63.24^{b,C}	-18.89; 145.36	41.18^{b,C}	-50.36; 132.72	30.88^{b,B}	-42.90; 104.66
GV	543.94^{a,B}	413.22; 674.66	103.03^{b,B,C}	19.67; 186.40	71.21^{b,C}	-21.71; 164.13	89.39^{b,B}	14.50; 164.28
GVI	507.69^{a,B}	375.97; 639.41	586.15^{a,D}	502.15; 670.16	606.15^{a,D}	512.53; 699.78	520.00^{a,A,C}	444.54; 595.46

*GI: Ivermectin; GII: Albendazole; GIII: Levamisole; GIV: Moxidectin; GV: Closantel; GVI: Control. Different superimposed lowercase letters on the same line indicate statistical difference between the means ($P < 0.05$); different overlapping capital letters in the same column indicate statistical difference between the means ($P < 0.05$)

Table 2. Average anthelmintic drug efficacy rates in goats in the municipality of Petrolina, Pernambuco state, Brazil.

Group	D7		D14		D21	
	Mean (E%)	IC 95%	Mean (E%)	IC 95%	Mean (E%)	IC 95%
GI	22.23^{a,A}	-4.64; 49.05	27.58^{a,A}	-2.45; 57.61	33.13^{a,A}	16.28; 49.97
GII	53.66^{a,A,B}	27.65; 79.68	48.30^{a,A,B}	19.20; 77.41	35.29^{a,A}	18.96; 51.62
GIII	70.75^{a,A,B}	44.93; 96.57	48.92^{a,B}	20.03; 77.80	70.56^{a,B}	54.36; 86.77
GIV	79.81^{a,B,C}	54.18; 105.44	88.69^{a,B,C}	60.02; 117.37	93.43^{a,B}	77.34; 109.52
GV	75.42^{a,B,C}	49.60; 101.25	84.72^{a,A,B}	55.84; 113.61	73.94^{a,B}	57.73; 90.14
GVI	-31.73^{a,D}	-57.75; -5.71	-33.28^{a,D}	-62.38; -4.17	-28.18^{a,C}	-44.51; -11.85

*GI: Ivermectin; GII: Albendazole; GIII: Levamisole; GIV: Moxidectin; GV: Closantel; GVI: Control; E%: Efficacy. Different superimposed lowercase letters on the same line indicate statistical difference between the means ($P < 0.05$); different overlapping capital letters in the same column indicate statistical difference between the means ($P < 0.05$)

DISCUSSION

In the present study, the only medication that was effective was MXD, followed by CLO, which had low efficacy. IVM, ALB and LMS obtained ineffective results in helminth control. MXD on the 14th day after treatment showed low efficacy, which is in agreement with Borges et al. (20) who performed the anthelmintic activity in goats from the Caatinga and Mata Atlântica biomes, raised in pastures in the semi-arid, which were treated with albendazole, ivermectin, levamisole, moxidectin and closantel and found efficacy percentages ranging from 0 to 97%, and that the majority of herds were less than 95% effective, with the exception of one herd where the effectiveness for moxidectin was 97%.

Moxidectin obtained satisfactory results, which corroborated with Papadopoulos et al. (21) who under similar experimental conditions registered an oral moxidectin efficacy of 100% in goats at 14 days post-treatment and 93.5% at 56 days. According to Ahid et al. (22) evaluated the anthelmintic resistance in goats in the forest zone of the State of Alagoas of the

drugs moxidectin, ivermectin and albendazole and found a percentage of post-treatment efficacy ranging between 71.2% and 98.74%. In addition, the same five drugs in the present study were tested in sheep, in which almost all showed some level of resistance, however, Moxidectin was the one that obtained the best result (23).

Lima et al. (24) evaluated the efficacy of anthelmintic products based on moxidectin, ivermectin and albendazole on goat and sheep farms in Pernambuco and found ineffectiveness with an index below 95%. Moxidectin was effective in goats and sheep, ivermectin in goats with 67.33% and sheep with 100%, while albendazole with a percentage between 30% and 70.50%, proving to be ineffective. Traversa et al. (25) determined the effect of febantel, levamisole, ivermectin and moxidectin in the control of infections in 50 sheep in Italy and resistance was found to levamisole with 89%, to ivermectin with 93%, while moxidectin was 100% effective.

Melo et al. (4) estimated the occurrence of resistance to oxfendazole, levamisole and ivermectin in breeding sheep and goats, in the Jaguaribe region, and identified the prevalence of nematodes resistant to oxfendazole, levamisole and ivermectin in sheep was 88%, 41 % and 59%, and in goats of 87.5%, 75% and 37.5%, respectively.

CLO, which showed low efficacy on the day 14 of evaluation and inefficacy in the others days, was similar to those found by Borges et al. (19), who tested CLO in the Caatinga biome and found efficacy rates of 0%, 63% and 85% on days 10, 11 and 12 after treatment, respectively. Melo et al. (26) treated goats and sheep with CLO and reported 85% drug efficacy in goats and 83% in sheep. In the research conducted by Sczesny-Moraes et al. (27) in sheep herds in the state of Mato Grosso do Sul, CLO showed 97.4% efficacy in only one herd, but was ineffective in the other herds, with an overall reduction of 49.8% in egg count.

The results found with the drug IVM are consistent with the results found in the dry season of Paraíba state (28), Rio Grande do Norte state (29) and Pernambuco state (24). However, these authors reported that the drug was 100% effective in sheep, all located in northeastern Brazil. On the other hand, still in the Northeast of Brazil, were found in the state of Alagoas, efficacy with the use of IVM 7 days after treatment and low efficacy at 14 and 21 days after treatment (22). In Porto Alegre, in the south of the country, different results were also obtained, where efficacy was observed in goats in the region (30). Authors who observed these higher efficiencies in sheep herds may be justified by having performed the treatments in regions with different characteristics and used a variety of deworming protocols for different periods of time.

Some factors may have been responsible for the ineffectiveness of ALB on farms in the present study. This is one of the most widely used pharmaceutical ingredients in northeastern Brazil as it is easy to handle, relatively inexpensive and is contained in several brands available on the market. In addition, most drugs on the market are directed to sheep, and the use of goat benzimidazoles at the indicated dose for sheep is less effective, as the biotransformation of benzimidazoles in goats is different from sheep (31). The results obtained in the present study corroborate those found in the Cariri region of Paraiba state during the dry season (28), Paraíba state (32) and Rio Grande do Norte state (29). However, AHID et al. (22) reported egg count reduction rates of 97.89%, 71.2% and 80% in goat herds in the state of Alagoas.

The 5% LMS hydrochloride resistance traits observed in some farms may be due to the low efficacy against immature stages of nematodes in general (4), i.e., the larvae residing in the gastrointestinal tract may have matured and started producing eggs (33). The results obtained in the present study corroborate the findings in the state of Ceará (4). However, efficacy was observed in the backwoods region of Paraíba state (31) and in the northern part of the state of Minas Gerais, located in southeastern Brazil (34). In the state of Rio Grande do Sul, it was observed a low efficiency of 85.5% in lambs, being suggested to use with caution

in the studied herd, that is, to use appropriate management methods to aid in a good effectiveness of the drug (35).

Although some drugs in the study were ineffective, it was observed that all had a reduction in egg counts per gram of feces when compared to the control group. In a study conducted in the United States, it was also observed that there was a decrease in egg counts when compared to the control group, but all drugs tested (moxidectin, levamisole, albendazole and ivermectin) were ineffective (33).

IVM and MXD showed statistical difference when compared between groups, even belonging to the same class of anthelmintics, macrocyclic lactones. However, MXD increased power makes it more effective against IVM resistant parasites in goats (36). Resistance to MXD seems to develop more slowly than resistance to avermectins (37,38).

The purpose of the coprocultures performed in the present study was to identify the most prevalent parasites in the goat herd, but a comparison of antiparasitic efficacy was not made. The most prevalent species was *Haemonchus contortus*, which is in agreement with results of experiments involving goat herds in the state of Ceará and other states in northeastern Brazil (39), followed by *Oesophagostomum* spp. Charles (40) considered *O. columbianum* one of the most prevalent helminth parasites in goats in Petrolina, Pernambuco state, causing pathogenic effects among different breeds and ages. In the present study, *Trichostrongylus* spp. was the third most frequently identified genus (7%). These data differ from those reported by Coelho et al. (41), who identified, in descending order of frequency, *Haemonchus* sp., *Trichostrongylus* spp. and *Oesophagostomum* spp. in goats in the municipality of Mossoró, state of Rio Grande do Norte.

According to Vilela et al. (39), the high anthelmintic resistance observed in studies conducted in the semi-arid northeastern Brazil is due to the common practice of massive deworming of the entire small ruminant herd four to six times a year. It should be considered that the rate of development of resistance to anthelmintic may vary according to geographic area, number of treatments, and repeated use of the same chemical group and time of treatment (20,42). In addition, Melo et al. (43) highlighted the administration of anthelmintic drugs in the dry period can accelerate the development of resistance in the semiarid region of northeastern Brazil, as this period is characterized by a small or no refuge population. Thus, the results of this study indicate that the development of anthelmintic resistance and, consequently, the ineffectiveness of antiparasitic drugs can be attributed to several factors, including the indiscriminate use of anthelmintic, leading to increased cattle production costs, declining production rates and, even worse, the lack of an effective antiparasitic drug to control GINs. In addition, this study contributed to assist in the biosecurity of goat breeding farms, making it possible to implement effective parasite control measures. The use of sustainable control measures can be considered, such as the use of pasture rotation system, plant-based products, as well as certain fungi and earthworms that can reduce parasitism (44).

CONCLUSION

In conclusion, commercially available anthelmintic drugs were ineffective, since parasites were found to be highly resistant to IVM, ALB, LMS and CLO. All the drugs tested here, except for MXD (D21), showed less than 90% antiparasitic efficacy in the goat herds, suggesting the presence of multiresistant parasites.

DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

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