

Resistance of bovine gastrointestinal nematodes to four classes of anthelmintics in the semiarid region of Paraíba state, Brazil

Resistência de nematódeos gastrintestinais de bovinos à quatro classes de anti-helmínticos no semiárido do Estado da Paraíba, Brasil

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How to cite: Melo LRB, Sousa LC, Oliveira CSM, Alvares FBV, Ferreira LC, Bezerra RA, et al. Resistance of bovine gastrointestinal nematodes to four classes of anthelmintics in the semiarid region of Paraíba state, Brazil. *Braz J Vet Parasitol* 2021; 30(3): e010921. <https://doi.org/10.1590/S1984-29612021077>

Abstract

The effectiveness of four anthelmintic classes on cattle gastrointestinal nematodes in the semi-arid region of Paraíba State, Brazil, was evaluated. Twenty farms were used, testing 40 animals in each one, totaling 800 animals. Cattle were divided into four groups composed with ten animals: I, treated with albendazole sulfoxide 15%; II, treated with ivermectin 1%; III, treated with closantel 25%; IV, treated with levamisole hydrochloride 7.5%. All treatments were administered subcutaneously. For the Fecal Egg Count Reduction Test (FECRT), individual fecal samples were collected on days 0 and 14, and sent for analysis of egg count per gram of feces (EPG) and larval cultures. It was observed that multiresistance was present in 95% (19/20) of the farms. Resistance to ivermectin and albendazole was observed in 95% (19/20), to closantel in 75% (15/20) and to levamisole in 20% (4/20). The most used management system was semi-intensive (75%; 15/20) and the ivermectin was the most reported drug for controlling helminths (65%; 13/20). *Haemonchus* spp. was the most prevalent helminth genus. It was concluded that the anthelmintic resistance of bovine gastrointestinal nematodes is high in the semi-arid of Paraíba State, Brazil, with multiresistance observed mainly to ivermectin, albendazole and closantel.

Keywords: Antiparasitics, helminthiasis, multiresistance, ruminants.

Resumo

Avaliou-se a eficácia de quatro classes de anti-helmínticos sobre nematódeos gastrintestinais de bovinos na região semiárida da Paraíba, Brasil. Foram utilizadas 20 fazendas, sendo testados 40 animais em cada uma, totalizando 800 animais. Os bovinos foram distribuídos em quatro grupos compostos por dez animais: I, tratado com sulfóxido de albendazol 15%; II, tratado com ivermectina 1%; III, tratado com closantel 25%; IV, tratado com cloridrato de levamisole 7,5%. Para o Teste de Redução da Contagem de Ovos Fecais (TRCOF), amostras fecais individuais foram coletadas nos dias 0 e 14 e enviadas para análises de contagem de ovos por grama de fezes (OPG) e coproculturas. Observou-se que a multirresistência estava presente em 95% (19/20) das fazendas. Foi observada resistência à ivermectina e ao albendazol, em 95% das fazendas (19/20); ao closantel, em 75% (15/20) e, ao levamisole, em 20% (4/20). O sistema de manejo mais utilizado foi o semi-intensivo (75%; 15/20) e a ivermectina foi o fármaco mais relatado para controle de verminose (65%; 13/20). O gênero de helminto mais prevalente foi *Haemonchus* spp. (76,7%). Conclui-se que é alta a resistência anti-helmíntica por nematódeos gastrintestinais de bovinos no Semiárido da Paraíba, Brasil, com multirresistência observada principalmente à ivermectina, ao albendazol e ao closantel.

Palavras-chave: Anti-parasitários, helmintoses, multirresistência, ruminantes.

Received June 11, 2021. Accepted August 19, 2021.

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Introduction

Brazil stands out among the milk and beef-producing countries, with the fastest-growing agribusiness in the world. This country finished 2020 with a record final balance of US\$ 87.7 billion, which contributed positively and decisively to the total trade balance (Kreter et al., 2021). In this country, the profitability of livestock activities can be significantly reduced by the effects of parasites, which affect the welfare and productivity of cattle. Infections with gastrointestinal nematodes have an annual economic impact of 7.11 billion dollars (Grisi et al., 2014).

The main genera of nematodes that parasitize cattle are *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., belonging to the *Trichostrongylidae* family; *Oesophagostomum* sp., belonging to the *Strongylidae* family; *Strongyloides* sp., to the *Strongyloididae* family; and *Trichuris* spp., to the *Trichuridae* family. In cattle, the parasitic infection occurs under ideal temperature and humidity conditions, and transmission occurs during the ingestion of pasture contaminated with infective larvae, which, in the animal's gastrointestinal tract, become adults, reaching reproductive maturity, reproducing and eliminating their eggs in the environment through feces (Bowman, 2010; Neves, 2014). This cycle is completed around 28 to 35 days. However, the infecting larvae can remain in the environment for a few months (Taylor et al., 2013). Usually, cattle nematode infections are mixed, in which more than one species parasitizes the same animal (Fávero et al., 2020). The absence of adequate anthelmintic control can lead to significant losses, which gives rise to decreased food intake, gastrointestinal disorders and impaired animal development, leading to death in extreme cases (Cezar et al., 2008; Almeida et al., 2020).

To avoid losses relating to helminth infections, the main means of control is through use of chemical compounds with a broad spectrum of activity, mainly comprising macrocyclic lactones, benzimidazoles, imidazothiazoles and salicylanilides (Taylor et al., 2013; Silva et al., 2017; Ramos et al., 2020). Most of the time, these are administered without applying any technical criteria for drug selection, in an empirical and indiscriminate manner. This has additional implications for the effectiveness of anthelmintic treatments, since it causes the emergence and dissemination of parasitic resistance (Neves et al., 2014; Geurden et al., 2015. Ramos et al., 2018; 2020).

The resistance of bovine gastrointestinal nematodes to most drugs available on the market has become an emerging problem worldwide (O'Shaughnessy et al., 2014; Rose et al., 2015; Berk et al., 2016; Lobayan et al., 2017; Baiak et al., 2019). In several countries, there are high rates of resistance, such as in New Zealand (Leathwick & Luo, 2017), Australia (Bullen et al., 2016), Germany, Belgium and Sweden (Demeler et al., 2009), Sudan (Mohammedsalih et al., 2021), United States (Gasbarre, 2014) and Argentina (Cristel et al., 2017). In Brazil, there have been reports of resistance to benzimidazoles (Ramos et al., 2020; Fávero et al., 2020), macrocyclic lactones (Neves et al., 2014; Borges et al., 2015; Ramos et al., 2020), imidazothiazoles (Neves et al., 2014; Ramos et al., 2020) and salicylanilides (Silva et al., 2017; Ramos et al., 2020).

In the semiarid region of Brazil, information about the effectiveness of anthelmintics is scarce. Therefore, the objective of the present study was to obtain more information on anthelmintic resistance in cattle in the semiarid region of the state of Paraíba, northeastern Brazil. The efficacy of injectable formulations of albendazole sulfoxide, ivermectin, closantel and levamisole hydrochloride was evaluated in naturally infected cattle in 20 farms from 20 different municipalities across the state of Paraíba.

Material and Methods

Ethical approval and location of the study

This study was approved by our institution's research ethics committee, under registration number 23000.000663.2019-81.

It was carried out in the semi-arid region of the state of Paraíba, northeastern Brazil. This area forms part of the Caatinga biome. Its rains are irregular and are concentrated in the months from January to May, with average annual precipitation between 250 and 800 mm. The average maximum temperature is 32 °C and the average minimum temperature is 20 °C. There are high rates of evaporation and the relative humidity of the air is around 70% (IBGE, 2019).

Fecal samples were collected between January and December 2020, on 20 cattle farms that had herds of more than 40 animals, with a history of problems with nematode control or indiscriminate anthelmintic use, and on which the cattle had not been dewormed for at least 90 days. Each farm was located in a different municipality, as shown in Figure 1.

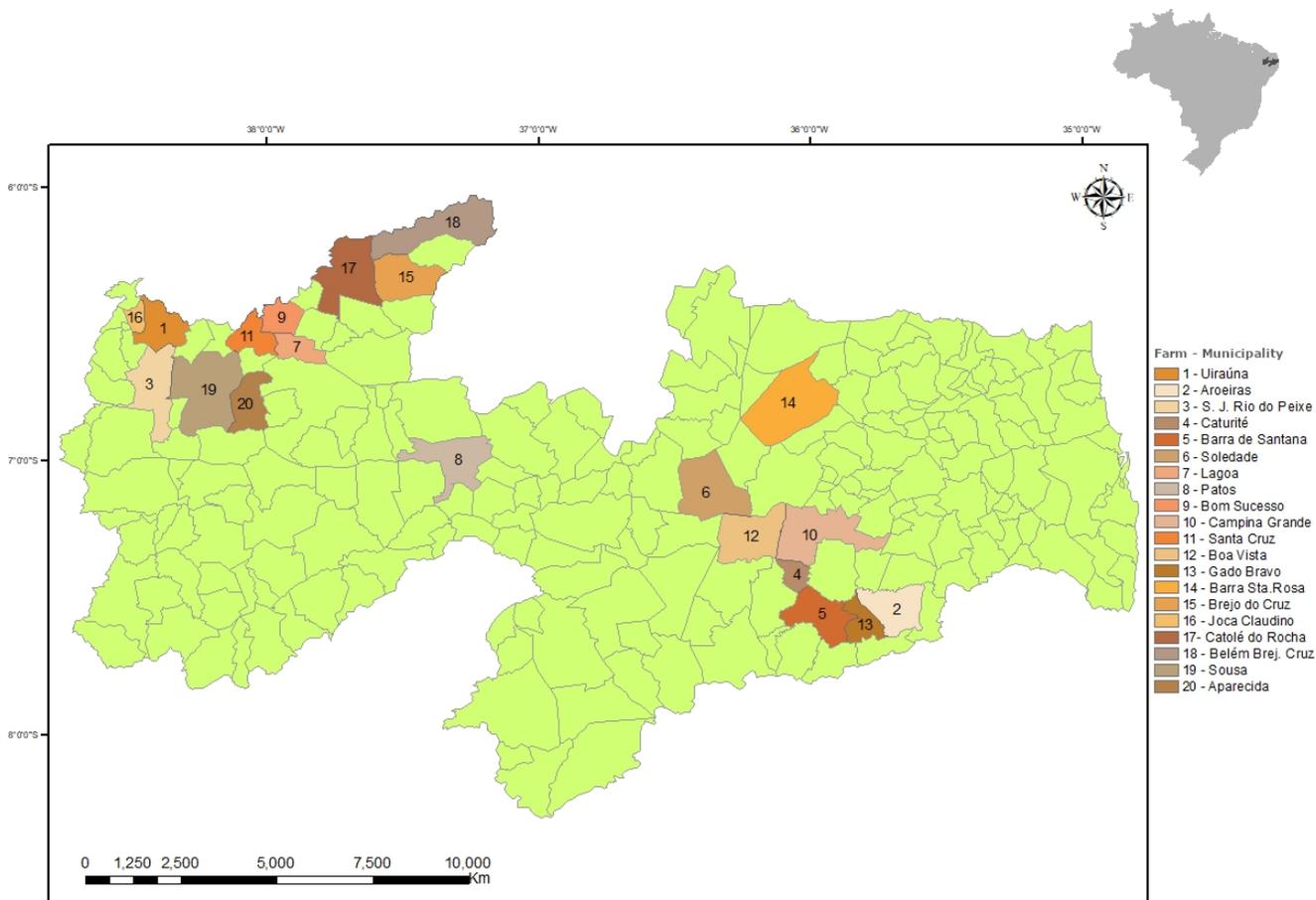


Figure 1. Geographical distribution of the municipalities in which farms were visited for undertaking anthelmintic resistance tests on bovine gastrointestinal nematodes, in the semiarid region of Paraíba state, Brazil.

Diagnosis of anthelmintic resistance

On each farm, 40 naturally infected animals were used, of both sexes, aged between four months and eight years, regardless of breed. These 40 animals had an egg count per gram of feces (EPG) ≥ 150 . Subsequently, the animals were identified individually through ear tags and were randomly distributed into four groups composed of ten animals each: group I, treated with albendazole sulfoxide 15% (3.4 mg / kg) (Agebendazol®, Agener União); group II, treated with ivermectin 1% (0.2 mg / kg) (Ivomec®, Boehringer Ingelheim); group III, treated with closantel 25% (5 mg / kg) (Taitec®, Calbos); group IV, treated with levamisole hydrochloride 7.5% (3.75 mg / kg) (Ripercol®, Zoetis). Prior to the treatments, all animals underwent weight estimation by means of a weighing tape (Fita Torácia para Pesar Gado®, MultitecAgro). All treatments were performed as a single dose, administered subcutaneously after previous antiseptics of the application site, in accordance with the manufacturers' recommendations.

Fecal samples were collected individually and directly from the rectal ampoule, on the same day, before the anthelmintic treatment (day 0) and 14 days after (day 14) (FAO, 2004). Subsequently, these were placed in plastic bags, labeled, kept refrigerated in an isothermal box and promptly sent to the Veterinary Parasitology Laboratory (VPL) of the Instituto Federal da Paraíba (IFPB), Sousa, Paraíba, for analysis.

EPG counts were performed by a McMaster modified technique (Gordon & Whitlock, 1939), with a sensitivity of 50 EPG. Larval cultures (Roberts & O'Sullivan, 1950) were carried out per group and per collection, from a pool of samples. However, when the mean EPG was zero in a given group on day 14, larval cultures were not performed.

Were used eggCounts package version 2.3, which is a "R" package developed to analyze faecal egg count reduction using Bayesian hierarchical models (Wang & Paul, 2018). The percentage efficacy was calculated, along with the lower (L95) and upper (U95) 95% confidence limits. The models are tailored for a variety of practical situations, including individual treatment efficacy, zero inflation, small sample size and potential outliers (Wang et al., 2018).

The anthelmintic resistance status was interpreted using the method described by Lyndal-Murphy et al. (2014), and based on the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines on anthelmintic resistance (Coles et al., 1992), considering the EPG reduction percentage and the upper and lower 95% confidence limits.:

- Efficacious (E): percentage reduction and upper 95% confidence limit above 95% and lower 95% confidence limit above 90%.
- Confirmed anthelmintic resistance (R): percentage reduction and upper 95% confidence limit below 95% and lower 95% confidence limit below 90%.
- Suspicion of resistance (S): neither of the above criteria fulfilled.

Epidemiological questionnaire

A structured epidemiological questionnaire was used at the farms visited, to collect information about the rearing system (extensive, intensive or semi-intensive), number of animals, type of herd exploitation, average daily milk production, deworming strategy, drugs used, frequency of drug use, routes of administration, use of endectocides and movements of animals (purchase and/or sale).

Results

The arithmetic mean (AM), minimum and maximum faecal egg counts before and after treatment, the percentage efficacy calculated based on AM, along with the lower (L95) and upper (U95) 95% confidence limits per anthelmintic and per farm are shown in Tables 1-4. Multidrug resistance was observed in 95% (19/20) of the farms evaluated in this study. Among these farms, 5% (1/20) showed resistance to one drug, 20% (4/20) to two drugs, 60% (12/20) to three drugs and 15% (3/20) to all four of the drugs evaluated (Tables 1-4). Anthelmintic resistance to ivermectin and albendazole was detected in 95% (19/20) of the herds, closantel in 75% (15/20) and levamisole in 20% (4/20) (Table 5).

In larval cultures, presence of five genera of gastrointestinal helminths was observed. These were predominantly *Haemonchus* spp. (76.7%), which was most prevalent in all larval cultures, followed by *Trichostrongylus* spp. (13.2%), *Oesophagostomum* sp. (8.5%), *Cooperia* spp. (1.1%) and *Strongyloides* sp. (0.5%) (Table 6).

It was observed that the management system most used was semi-intensive (75%; 15/20), followed by extensive (20%; 4/20) and intensive (5%; 1/20). Dairy farms were the most frequent type among the farms studied (40%; 8/20), followed by mixed farms (35%; 7/20) and beef farms (25%; 5/20). The number of cattle per farm ranged from 45 to 600 animals and the average daily milk production ranged from zero (beef herds) to 1700 liters.

Among the active ingredients most reported by the farmers for controlling worms, ivermectin was cited as the only drug used in 65% (13/20) of the farms, followed by doramectin (20%; 4/20), which are both in the group of macrocyclic lactones. In addition, ivermectin, doramectin and moxidectin were administered alone or in association also for controlling *Rhipicephalus microplus* and/or *Haematobia irritans* on 95% (19/20) of the farms. Levamisole was mentioned in 15% (3/20) of the farms, used separately on one farm, in association with ivermectin on another and in association with ivermectin and doramectin on the third. There was no report of previous use of albendazole or closantel.

Anthelmintics were administered by means of injection on 100% of the farms. This took place in the entire herd at least once a year, mostly during the rainy season (January to May), on 75% (15/20) of the farms. On the remaining 25% (5/20) of the farms, this was only done when verminosis was suspected and, in these cases, administration was individual.

Discussion

In the semiarid region of northeastern Brazil, the present study was the first to test the anthelmintic efficacy of four distinct pharmacological groups against bovine gastrointestinal nematodes. Multiresistance was found in 19/20 herds tested. The phenomenon of multidrug resistance was also observed by Ramos et al. (2018, 2020) in

Table 1. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Ivermectin 1% (IVM) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	IVM									
	Pre			Post			Efficacy			Status
	AM	Min	Max	AM	Min	Max	%	L95	U95	
1	240	150	850	120	0	400	50.9	39.6	88.7	R
2	160	150	200	120	0	150	24.2	0	44.4	R
3	650	150	2500	200	0	500	68.1	32.1	82.0	R
4	155	150	200	155	0	450	0	0	0.19	R
5	155	150	200	155	0	250	0	0	0.9	R
6	233.3	150	500	133.3	0	550	42.7	12.6	75.9	R
7	165	150	200	15	0	50	91.2	83.3	98.6	S
8	175	150	400	120	0	200	31.8	0	87.5	R
9	160	150	250	160	0	250	0	0	0.7	R
10	170	150	350	170	0	600	0	0	0.23	R
11	155	150	200	155	0	200	0	0	0.15	R
12	230	150	900	85	0	300	62.8	48.8	87.1	R
13	200	150	600	160	0	550	21.9	0	37.2	R
14	395	150	1650	130	0	900	66.2	23.2	78.2	R
15	410	150	2450	90	0	400	76.1	44.1	91.6	R
16	160	150	200	160	0	250	0	0	0.7	R
17	255	150	1000	100	0	250	63.2	31.2	78.8	R
18	170	150	350	150	0	650	13.7	0	32.2	R
19	205	150	550	140	0	450	30.9	0	89.2	R
20	465	150	3450	50	0	250	86.2	52.2	92.1	R

the state of Rio Grande do Sul in 100% (10/10) of the farms studied and by Soutello et al. (2007) in 20% (5/25) of the farms assessed in the northwest of the state of São Paulo.

The results regarding ivermectin demonstrated that anthelmintic resistance was present on 95% (19/20) of the farms, with 36.5% of efficacious mean for FECR. In São Paulo, Soutello et al. (2007) and Neves et al. (2014) detected anthelmintic resistance in 92% (23/25) and 100% (10/10), respectively, of cattle herds evaluated, after treatment with this drug. Ramos et al. (2020), in Rio Grande do Sul, also detected resistance on all the farms examined (7/7). In Argentina, resistance to ivermectin was seen on 93.5% (58/62) (Lobayan et al., 2017) and 100% (4/4) (Cristel et al., 2017) of the cattle farms evaluated.

In line with the lower efficacy rates of ivermectin, it was observed that macrocyclic lactones, especially avermectins, were the drugs most used for anthelmintic treatments on the farms studied, similarly to what had been observed in other studies (Pereira, 2011; Suarez & Cristel, 2014). Soutello et al. (2007) and Ramos et al. (2018, 2020) stated that the higher rate of resistance to ivermectin, compared with other drugs, was probably related to its frequent use, and to its easy availability and ease of acquisition by farmers. Use of this drug has also been reported for controlling ticks (*R. microplus*) and horn flies (*H. irritans*), which indiscriminately boosts its use in cattle

Table 2. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Albendazole Sulfoxide 15% (ALB) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	ALB									
	Pre			Post			Efficacy			Status
	AM	Min	Max	AM	Min	Max	%	L95	U95	
1	340	150	1950	145	0	650	57.8	42.3	78.7	R
2	155	150	250	100	0	150	36.1	0	73.2	R
3	355	150	1150	225	50	500	35.7	0	78.9	R
4	160	150	300	160	0	450	0	0	0.17	R
5	160	150	450	40	0	350	77.2	66.1	94.4	R
6	300	150	500	160	0	600	42.1	0	83.1	R
7	150	150	250	75	0	200	50.3	37.2	78.9	R
8	155	150	250	90	0	150	42.1	0	77.2	R
9	250	150	500	30	0	100	88.3	71.2	92.0	R
10	185	150	500	185	0	1050	0	0	0.3	R
11	200	150	350	65	0	100	67.9	61.2	91.1	R
12	315	150	1100	65	0	200	78.7	70.4	89.0	R
13	285	150	650	210	0	600	29.1	0	69.3	R
14	245	150	900	160	0	550	22.6	0	70.1	R
15	265	150	1300	10	0	100	97.7	87.2	100.0	S
16	195	150	300	75	0	100	62.3	50.7	88.2	R
17	420	150	2900	45	0	150	88.3	79.2	93.2	R
18	165	150	250	165	0	400	0	0	0.21	R
19	205	150	400	65	0	150	71.2	36.4	94.1	R
20	855	150	4900	135	0	450	81.9	54.3	89.8	R

herds, thus generating greater pressure for selection and dissemination of resistant alleles in parasite populations (Graef et al., 2013; Neves et al., 2014; Vilela et al., 2020). These actions can promote faster development of drug resistance (Leathwick & Luo, 2017).

For albendazole, anthelmintic resistance was also observed on 95% (19/20) of the farms, with 51.5% of efficacious mean for FECR. Ramos et al. (2020) obtained similar values for the FECR (49.8%) and detected resistance to this drug on all the farms evaluated (7/7). On the other hand, Soutello et al. (2007) detected anthelmintic resistance on only 20% (5/25) of the farms examined, with an average FECR of 75.9%. In Sudan, Mohammedsalih et al. (2021) reported that a reduction (< 90%) in the effectiveness of albendazole on bovine gastrointestinal nematodes had occurred.

Resistance to closantel was observed on 75% (15/20) of the farms, with 55.8% of efficacious mean for FECR, which was similar to the results obtained by Ramos et al. (2020), of 55.2%, and Silva et al. (2017), of 45.45%. However, Bushra et al. (2019) and Maqbool et al. (2018) found reductions of 94.44% and 100%, respectively, in India. In the present study, despite the observed resistance, no use of this drug was reported in the herds. According to Neves (2014), the low use of closantel among cattle may be due to the fact that most products sold are for oral use, thus limiting the possibility of administering them to cattle.

Table 3. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Closantel 25% (CLO) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	CLO									
	Pre			Post			Efficacy			Status
	AM	Min	Max	AM	Min	Max	%	L95	U95	
1	210	150	750	70	0	250	67.2	28.3	83.2	R
2	150	150	150	100	0	200	31.7	0	44.2	R
3	170	150	250	25	0	100	86.9	64.7	97.3	S
4	180	150	250	40	0	50	77.6	41.3	94.3	R
5	345	150	1900	25	0	200	91.1	81.4	97.6	S
6	266,7	150	600	11,1	0	50	94.3	71.4	100.0	S
7	165	150	350	35	0	200	79.6	44.4	91.4	R
8	190	150	550	65	0	300	66.6	42.1	93.7	R
9	240	150	1050	295	0	1000	0	0	0.15	R
10	150	150	150	150	0	200	0	0	0.1	R
11	155	150	200	155	0	250	0	0	0.14	R
12	535	150	1550	295	0	600	47.2	0	83.3	R
13	220	150	600	195	0	350	12.3	0	44.1	R
14	490	150	1200	85	0	250	88.2	75.3	94.1	R
15	210	150	350	15	0	50	96.1	91.4	100.0	E
16	185	150	500	90	0	600	47.3	12.1	71.3	R
17	300	150	800	165	0	1150	49.1	84.6	91.3	R
18	245	150	600	170	0	300	32.2	0	66.2	R
19	375	150	1700	15	0	50	98.8	94.6	100.0	E
20	230	150	750	110	0	500	50.7	79.3	87.2	R

Resistance to levamisole was observed on 20% (4/20) of the farms. Similar results regarding resistance to this drug were reported by Ramos et al. (2020), of 28% (2/7), and Bullen et al. (2016), of 25% (5/20), in Australia. In the present study, levamisole demonstrated the highest efficacious mean (93.1%). Efficacious status was obtained on 55% (11/20) of the farms. The good efficacy of this drug in the herds tested may have resulted of its low selection pressure, as it was not used frequently by the farmers, such that it was only mentioned on 15% (3/20) of the farms.

The *Haemonchus* was the most prevalent parasite genus in all larval cultures, both before and after treatments. This has also been reported in several other studies evaluating bovine gastrointestinal nematodes (Borges et al., 2015; Lobayan et al., 2017; Silva et al., 2017; Ramos et al., 2020). This nematode probably acquires resistance faster due to its high biotic potential and great genetic variability. In addition, it harbors the allele that causes decreased susceptibility to a drug (Blackhall et al., 1998; Chaudhry et al., 2015). *Haemonchus placei* is the species that is considered most relevant in cattle (Borges et al., 2015; Santos et al., 2015). It causes gastric hemorrhagic lesions, due to its high capacity for hematophagy (Taylor et al., 2013).

Visual estimation of the animals' weight was the practice generally used by the farmers. According to Leathwick & Luo (2017), this can be reflected in inefficiency of the treatment, given that doses below or above those recommended

Table 4. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Levamisole Hydrochloride 7.5% (LEV) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	LEV									
	Pre			Post			Efficacy			
	AM	Min	Max	AM	Min	Max	%	L95	U95	Status
1	255	150	2150	0	0	0	100	100.0	100.0	E
2	175	150	350	10	0	50	93.1	87.2	100.0	S
3	485	150	1500	40	0	250	95.7	90.1	99.2	E
4	230	150	800	0	0	0	100	100.0	100.0	E
5	170	150	600	25	0	200	84.2	63.2	94.1	R
6	261	150	950	6	0	50	98.9	91.3	100.0	E
7	155	150	200	0	0	0	100	100.0	100.0	E
8	165	150	300	55	0	200	72.3	37.3	91.7	R
9	175	150	300	20	0	150	90.5	82.3	100.0	S
10	300	150	700	20	0	150	91.9	83.9	96.0	S
11	235	150	400	15	0	100	92.7	66.2	98.3	S
12	240	150	750	0	0	0	100	100.0	100.0	E
13	165	150	300	45	0	150	73.9	36.4	89.1	R
14	225	150	850	0	0	0	100	100.0	100.0	E
15	290	150	1500	0	0	0	100	100.0	100.0	E
16	180	150	350	0	0	0	100	100.0	100.0	E
17	160	150	250	35	0	350	77.6	36.3	93.2	R
18	320	150	1550	20	0	150	94.2	90.6	98.0	S
19	370	150	1400	0	0	0	100	100.0	100.0	E
20	978	150	3000	5	0	50	98.1	98.6	100.0	E

Table 5. The number of farms with efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S) for Ivermectin 1% (IVM), Albendazole Sulfoxide 15% (ALB), Closantel 25% (CLO) and Levamisole Hydrochloride 7.5% (LEV), and the percentage of efficacious mean (EM%) of the anthelmintics on cattle gastrointestinal nematodes in the semiarid region of Paraíba state, Brazil.

Treatment	N animals	N farms	EM (%)	R	S	E
IVM	200	20	36.5	19	1	0
ALB	200	20	51.5	19	1	0
CLO	200	20	55.8	15	3	2
LEV	200	20	93.1	4	5	11
Total				57	10	13

by the manufacturers might be estimated. In addition, most of the farmers (75%; 15/20) used anthelmintics at the beginning of the rainy season. However, if the animals are treated and transferred to clean pastures, or if they are treated during the dry season, resistance can develop quickly, even if the animals receive few annual treatments

Table 6. Percentage (%) of cattle gastrointestinal nematodes by genus and farm recovered from larval cultures before (pre) and after (post) anthelmintic treatments with Ivermectin 1% (IVM), Albendazole Sulfoxide 15% (ALB), Closantel 25% (CLO) and Levamisole Hydrochloride 7.5% (LEV) in the semi-arid region of Paraíba state, Brazil.

		Farms																			
		IVM																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pre	H	84	91	85	75	71	94	50	72	80	75	100	89	51	82	80	62	74	90	100	80
	T	10	6	9	16	14	4	40	13	18	25	0	3	49	0	4	23	4	5	0	19
	O	6	3	0	6	11	0	10	11	2	0	0	1	0	18	16	15	22	5	0	1
	C	0	0	6	3	3	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	0	0	0	7	0	0	0	0	0	0	0	0
Post	H	94	74	78	95	97	61	93	75	98	70	60	100	81	85	76	95	74	74	94	58
	T	6	7	12	3	2	34	7	8	2	30	30	0	1	5	18	2	6	21	4	42
	O	0	9	5	2	1	5	0	17	0	0	0	0	18	10	6	3	20	5	2	0
	C	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	10	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
		ALB																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pre	H	92	95	93	83	70	100	82	71	80	51	94	93	100	76	64	86	87	85	80	57
	T	2	5	2	9	13	0	8	14	19	49	4	2	0	0	30	7	3	15	20	33
	O	6	0	5	6	13	0	6	11	0	0	2	0	0	24	5	7	10	0	0	10
	C	0	0	0	2	3	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	1	0	0	5	0	0	1	0	0	0	0	0
Post	H	98	89	90	69	100	79	97	75	80	64	79	100	76	94	100	82	89	86	98	85
	T	2	7	5	25	0	7	2	25	20	30	13	0	21	4	0	0	11	5	1	15
	O	0	4	5	0	0	14	1	0	0	6	8	0	2	2	0	18	0	9	1	0
	C	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		CLO																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pre	H	75	70	71	100	85	65	94	70	69	71	70	51	66	75	81	84	79	82	95	86
	T	17	13	12	0	10	0	6	12	20	13	13	37	3	0	6	0	8	6	2	13
	O	6	13	14	0	5	11	0	14	11	12	14	12	31	25	13	16	13	12	3	1
	C	0	3	2	0	0	24	0	3	0	3	2	0	0	0	0	0	0	0	0	0
	S	2	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
Post	H	90	95	100	76	98	71	91	87	77	91	64	90	90	97	97	68	90	95	72	91
	T	8	3	0	9	2	0	9	0	21	4	13	3	0	3	1	27	5	2	22	9
	O	2	2	0	12	0	29	0	13	2	0	23	5	10	0	2	5	5	3	6	0
	C	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	5	0	2	0	0	0	0	0	0	0	0
		LEV																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pre	H	95	87	86	67	70	75	72	71	51	94	72	90	51	96	74	91	100	80	75	52
	T	4	13	9	18	14	0	6	14	16	3	13	0	32	0	5	7	0	19	25	39
	O	1	0	5	6	12	25	17	11	33	3	11	1	17	4	21	2	0	1	0	9
	C	0	0	0	9	3	0	5	3	0	0	3	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	0	0	1	9	0	0	0	0	0	0	0	0
Post	H	-	95	100	-	98	100	-	95	80	100	95	-	100	-	-	-	100	83	-	97
	T	-	5	0	-	2	0	-	3	8	0	5	-	0	-	-	-	0	0	-	3
	O	-	0	0	-	0	0	-	1	12	0	0	-	0	-	-	-	0	17	-	0
	C	-	0	0	-	0	0	-	0	0	0	0	-	0	-	-	-	0	0	-	0
	S	-	0	0	-	0	0	-	0	0	0	0	-	0	-	-	-	0	0	-	0

H: *Haemonchus* spp.; T: *Trichostrongylus* spp.; O: *Oesophagostomum* sp.; C: *Cooperia* spp.; S: *Strongyloides* sp. For those groups with high efficacy no coproculture was performed (-).

with anthelmintics (Martin et al., 1981; Papadopoulos et al., 2001). Therefore, management methodologies and strategies should be designed to keep parasites in *refugia* in herds, thereby prolonging the effectiveness of current anthelmintics and preserving susceptible nematode genotypes (Berk et al., 2016).

Movement of animals without previous knowledge of the clinical history was a common practice reported by all the farmers. Moreover, no investigations or parasitological examinations were conducted on newly acquired animals. Both of these practices contribute to dispersion of resistant nematode populations. Bullen et al., (2016) suggested that, without prior knowledge of the farm's anthelmintic resistance status, movement of dairy cattle constitutes a considerable risk with regard to introduction of anthelmintic resistance on unaffected farms.

In addition, through PCR, Ramos et al. (2020) demonstrated the presence of co-infections of species of *Haemonchus* spp. that affect different cattle and sheep that share pastures. This matter deserves further study and may explain the high rates of anthelmintic resistance found on the farms studied, especially in relation to the drugs albendazole and closantel. Although no previous use of these drugs was reported in the herds evaluated, they are widely used among small ruminants and anthelmintic resistance to them in the semi-arid region of Paraíba has already been reported (Lima et al., 2010; Silva et al., 2018).

To optimize the effectiveness of anthelmintics in populations of multidrug-resistant nematodes, Ramos et al. (2016) suggested that combinations of two drugs belonging to different chemical groups should be used. However, they stressed the importance of conducting anthelmintic efficacy tests in order to choose the chemical groups to be used, as well as introduction of control measures for gastrointestinal worms within the management of cattle. Acquisition of cattle without adequate sanitary management before or after transporting them needs to be avoided. This is especially important in relation to acquisition from farms on which grazing combined with goats and/or sheep is practiced: this can be considered to be a critical point regarding worm control. Chaudhry et al. (2015) reported the first genetic confirmation of hybridization between *H. contortus* and *H. placei* in the field, thus increasing the possibility of inter-species transmission of anthelmintic resistance mutations.

Conclusion

It was concluded that the anthelmintic resistance of bovine gastrointestinal nematodes in the semiarid region of Paraíba, northeastern Brazil, is high. Multidrug resistance was observed on almost all the farms evaluated, especially in relation to the drugs ivermectin, albendazole and closantel. Levamisole was considered to be the drug with the best anthelmintic efficacy. It can be suggested that the sanitary management of cattle herds in the semiarid region should be adapted so as to avoid mass deworming without applying technical criteria. Moreover, animals should only be transported after a parasitological diagnosis has been made, especially in the case of acquisition from farms with a history of problems regarding worm control and from farms use pastures intercropped between cattle and small ruminants.

Acknowledgements

The authors acknowledge finding from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and IFPB (Instituto Federal da Paraíba), for scholarship grants.

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