Identification of ivermectin and doramectin-resistant *Cooperia punctata* (LINSTOW, 1907) in a dairy herd in the State of Rio de Janeiro, Brazil

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Abstract

Anthelmintic resistance is a potential problem to nematodes control in cattle and may cause economic loss in the dairy and beef cattle industries. The objetive of this study was to determinate the efficacy of ivermectin, doramectin and abamectin in naturally and experimentally infected calves for *Cooperia punctata* in a brazilian dairy herd. Faecal egg count reduction tests were carried out employing naturally infected calves that were treated with injectable solutions of ivermectin, doramectin and abamectin. Faecal samples were collected on the day of the treatment, day 0, and at 7 and 14 days after treatment and larvae culture were made in the positive samples. A control test was carried out using 18 artificially infected calves, alocatted in three groups with six animals each: Group I - control, no treatment; Group II – ivermectin, injectable solution, 200 μg/kg; Group III – doramectin, injectable solution, 200 μg/kg. Faecal samples were collected on day of the treatment, day 0, and at 3, 7, 9 and 14 days after treatment. On days 14, 15 and 16, two animals of each group were slaughtered and their lung and gastrointestinal parasite burdens determined. The results of faecal egg count reduction tests using naturally infected calves showed a reduction percentage at 14 day after treatment from –4.45 to 11.49% for ivermectin; 32.31 to 60.40% for doramectin and 85,05% for abamectin. The only parasite identified in the larvae culture was *Cooperia spp.* Control test showed a faecal egg count reduction percentage at 14 day after treatment of 51.47% and 96.08% for ivermectin and doramectin, respectively. Reduction of adult worm counts in this control test was of 53.91% and 82.43% for ivermectin and doramectin, respectively. Only *C. punctata* was recovered in the necropsies. Thus, this *C. punctata* strain was considered resistant to ivermectin and doramectin treatments and suggest a possible resistance to abamectin treatment by faecal egg count reduction tests.

Key words:
Resistant.
Ivermectin.
Doramectin.
Cooperia.
Bovine.

Introduction

Ivermectin, doramectin and abamectin are closely related avermectins; macrocyclic lactones produced as fermentation products by the bacterium *Streptomyces avermetilis*.[1] The avermectins are widely used as an anthelmintic for cattle in Brazil and around the world because of its high efficacy against a broad spectrum of nematode parasites and certain ectoparasites. Several studies have reported the efficacy of ivermectin [2,3,4,5], doramectin [6,7,8,9,10] and abamectin [11,12,13,14] against the most important cattle gastrointestinal nematodes, the efficacy against adult *Cooperia punctata* of 95% is demonstrated.

Unless an anthelmintic treatment is always one hundred per cent effective, the possibility exists that treatment will select for anthelmintic resistance. Resistance is present when is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population

of the same species and is heritable.[15] The development of resistance to various chemical groups of anthelmintics is widespread in nematode parasites of sheep, goats and horses. Anthelmintic resistance is also recognized as a potential problem of resistance to nematodes in cattle[16] and has been reported in naturally infected cattle.[17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33]

The objetive of this study was to determinate the efficacy of ivermectin, doramectin and abamectin in naturally and experimentally infected calves for *Cooperia punctata* in a brazilin dairy herd.

Material and Methods

Faecal egg count reduction tests in naturally infected calves.

The apparently resistant parasites were discovered during a test of eficacy of different anthelmintics against naturally infected cattle. The faecal egg count reduction tests (FECRT) in naturally infected calves were conducted in the Experimental Station from Itaguaí, Seropédica, R.J., Brazil, from January to June, 1998. The climate of the area is subtropical with predominantly rainfall from November to April.

The number of animals for each test ranged from 8 to 14 calves. Animals were selected based on the presence of infection by using egg counts. Across all studies, test animals ranged from 1 to 10 months of age and 52 to 194 kg in body weight at the beginning of the study. Both female and male and cross-bred calves were used in the tests. Calves were individually identified by a numbered ear tag and kept together in the pastures. The animals were naturally infected.

Faecal samples were harvested directly from the rectum of the calves on the day of the treatment, Day 0, and on days 7 and 14 after treatment. All animals received injectable formulations and were treated by subcutaneous injection in the lateral midline of the neck. On day 0, the calves were individually identified, weighed and allocated to a treatment group based on faecal egg count before treatment, as follows: Trial I - 14 animals treated with ivermectin, 200μg/kg bodyweight (Ivomec, Merial); Trial II - 8 animals treated with ivermectin, 200μg/kg bodyweight (Ivomec, Merial); Trial III - 8 animals treated with ivermectin, 200μg/kg bodyweight (Ivomec, Merial); Trial IV - 9 animals treated with doramectin, 200μg/kg bodyweight (Dectomax, Pfizer); Trial V - 8 animals treated with doramectin, 200μg/kg bodyweight (Dectomax, Pfizer); Trial VI - 7 animals treated with abamectin, 200μg/kg bodyweight (Duotin, Merial).

Control test in experimentally infected calves

The controled test in experimentally infected calves was performed in the Universidade Federal Rural Rio de Janeiro, Brazil, from September 21 to November 24, 1998. Initially, 24 weaned calves were used, male and female, 2-8 month old, cross-breed from Holstein x Zebu. They were purchased from several local farms and brought to the research site, then they were housed in pen with concrete floors. The floor was cleaned every day. Fresh water and trace mineral salt were supplied *ad libitum*. Animals were also offered a supplemental grain ration at the rate of 1kg/head/day and forage.

When the animals arrived in September, 1998, they were weighed, individually identified by a numbered ear tag and treated with Fenbendazol given orally at the dose rate of 5mg/kg body weight and Levamisole Hydrochloride injectable solution at the dose rate of 7.5mg/kg body weight. No other anthelmintic treatments were given and faecal samples were colleted at regular intervals to monitor the worm burden. One week later, each animal was infected with approximately 85,000 infective larvae of *Cooperia spp*, given orally in single dose. The larvaes used in this test were from

faecal cultures of donor calves used in previous tests and they were harvested from faecal cultures no more than 9 days before oral inoculation to calves. On day 40 after inoculation the patency of the *Cooperia spp* infection was confirmed by faecal analysis, the calves were ranked in decrescent order on the basis of the average e.p.g. counts. The first three animals on the list were randomly allocated to either a control group or one of two treated groups. The procedure was repeated with the second three animals and thus successively, until all animals were allocated to the three treatments with six calves each.

All animals which received injectable formulations were treated by subcutaneous injection in the lateral midline of the neck. On day 0, day of the treatment, the calves were weighed and treated, as follows: Group I - control, no treatment; Group II – ivermectin, 200 ìg/kg (Ivomec, Merial); Group III – doramectin, 200 µg/kg (Dectomax, Pfizer). Faecal samples were collected directly from the rectum of the calves on day of the treatment, day 0 and at 3, 7, 9 and 14 days after treatment. On days 14, 15 and 16, two animals of each group were slaughtered and their lung and gastrointestinal parasite burdens determined.

Parasitological techniques

Faecal egg counts were estimated for all calves, using a modified McMaster method with 4g of faeces, in which each egg counted represents 50 eggs per gram of faeces.[34] Three exams were made for each faecal sample and the result of each animal was the arithmetic mean from these three exams. Larval cultures were carried out on individual pre- and post-treatment faecal samples as described by Roberts and O'Sullivan[35]. The infective and adults stages of the parasites were identified as described by Keith [36] and Pinto [37], respectively.

FECRT, larval cultures, necropsies of cattle, collection of worms from specific gastrointestinal compartments, aliquoting of samples, identification of species and the methodology for the detection of anthelmintic resistance in nematodes, was carried out as recommended by the W. A. A. V. P..[38,39] The equations used to calculate the reduction percentages were: before and after treatment evaluation in treated hosts (without control group): FECRT = 100(1-[T2/T1]) where T2 is the post treatment and T1 is the pretreatment arithmetic mean of the epg; after treatment evaluation in the control and treated hosts: Adult Worm Counts and FECRT = 100(1-[T2/C2]) where T2-treated and C2-control are the arithmetic mean of adult worm counts and FECRT. Resistance is present if the percentage reduction in egg counts is less than 95% and the 95% confidence level is less than 90%. If only one of the two criteria is met resistance is suspected.

Statistical analysis

Statistical analysis was performed using JMP v.5.0.1.2 (SAS Institute Inc., USA). All data were examined using descriptive statistics prior to testing for associations between the outcomes of interest, using Tukey-Kramer Test for categorical variables. Proportions are displayed wherever possible as point estimates with 95% confidence intervals.

Results and Discussion

FECRT in naturally infected calves on the day of the treatment, Day 0, and at 7 and 14 days after treatment are given in table 1. Individual pre- and post-treatment larval culture from each calf within each trial were carried out and only *Cooperia spp*. were identified. Statistical analyses were performed in all treatment groups of naturally infected calves and demonstrated a association between the results and resistance and/or possible resistance.

Table 1 - Arithmetic mean faecal trichostrongyle egg counts and standard deviations (S.D.) before and after treatment with ivermectin, doramectin and abamectin

Treatment Trials	n	Faecal egg counts				
		Day0	Day7		Day14	
		MFEC (+S.D.)	MFEC (+S.D.)	FECRT%	MFEC (+S.D.)	FECRT%
I. Ivermectin	14	802.38 (879.75)	483.33(504.38)	39.76	838.10 (1,020.04)	-4.45
II. Ivermecin	8	870.83 (1,005.89)	2,277.08 (2,176.86)	-161.48	800.00 (1,210.60)	8.13
III. Ivermectin	8	362.50(216.35)	268.75(344.05)	-25.86	320.83 (373.93)	11.49
IV.Doramectin	9	738.89 (1,147.16)	257.41(323.62)	65.16	292.59 (359.89)	60.40
V. Doramectin	8	406.25(432.41)	283.33(337.83)	30.26	275.00 (343.77)	32.31
VI. Abamectin	7	669.05(899.15)	9.52(25.20)	98.58	100.00 (145.30)	85.05

a FECRT%: reduction percentage of faecal egg counts.

The results demonstrated that the reduction percentages in the Trials I, II and III, calves treated with ivermectin, 200μg/kg, at 7 and 14 days after treatments were 39.76% and –4.45%; -161.48% and 8.13%; -25.86% and 11.49%, respectively. The data of the present study are lower than the data published to ivermectin-resistant *Cooperia spp* in calves that found a reduction between 13-92%. [18,19,20,21,22,23,25,26,27,29,30,31,32,33]

The reduction rates of Trials IV and V, calves treated with doramectin, 200μg/kg, at 7 and 14 days after treatments were 65.16% and 60.40%; 30.26% and 32.31%, respectively. These results were similar to Rangel et al. [32] and Vermunt, West and Pomroy [25] that found, respectively, a reduction of 50.6% and 27% on day 14 after treatment with doramectin in calves that were naturally infected with *Cooperia spp*.

In Trial VI, calves treated with abamectin, 200μg/kg, showed reduction of 98.58% and 85.05% at 7 and 14 days after treatments, respectively. Loveridge et al. [28] carried out a study using abamectin pour on in dairy cattle and found an efficacy of 79% for *C. oncophora* and 99% for *C. punctata* by FECRT.

Results of the FECRT and adult worm counts of the control test that used artificially infected cattle with *Cooperia spp.* larvae are given in table 2. Infected calves were in good body condition, and showed no clinical signs of parasitic gastroenteritis. Statistical analyses were carried out between groups at the beginning of the control test showed a similar distribution the e. p. g. counts.

The percentage reduction by FECRT on days 3, 7, 9 and 14 after treatment in the Group II, treated with injectable ivermectin, 200μg/kg, was 50%, 84.35%, 76.62% and 51.47% and in the group III, treated with injectable doramectin, 200μg/kg, was 75.69%, 99.32%, 96.10% and 96.80%, respectively. These results suggest an ineffective action of ivermectin and a lack of efficiency of doramectin in the control of the infection.

However, the reduction percentage in adult worm counts in the necropsy at day 14-16 after treatment in calves from Group II, treated with injectable ivermectin, was 53.91% and in the Group III, treated with injectable doramectin, was 82.43%. All parasites recovered in the necropsies were *Cooperia punctata.* It was found association between parasite burden and resistance because there was no statistical difference of adult parasites number recovered in the control group and both treated groups. Bisset, Brunsdon and Forbes [17], Coles, Stafford and Mackay [26], Coles, Watson and Anziano [27] and Mejía et al. [22] carried out control tests using calves treated with injectable and pour on ivermectins and only found a inefficacy in the reduction of parasite burden for *C. oncophora.*

Based on the criteria promulgated by the W. A. A. V. P. [38,39], the results of the present study demonstrate that strains of *Cooperia punctata* of dairy calves in State Rio de Janeiro exhibit resistance to ivermectin and doramectin. The results of the FECRT using abamectin suggest a possible resistance. This study is the first that identified ivermectin and doramectin-resistant *Cooperia punctata* in dairy herd.

Table 2 - Mean faecal egg counts, adult worm counts and efficacy (%) of anthelmintic treatments in experimentally infected calves

Group / Animal Number	Mean faecal egg counts					Number of *Cooperia punctata*
	Day 0	Day 3	Day 7	Day 9	Day 14	
Control						
6	583.30	716.70	1,033.30	483.30	1,433.30	2,080.00
41	50.00	0.00	0.00	0.00	350.00	7,680.00
49	650.00	900.00	616.70	366.70	683.30	800.00
129	66.70	216.70	33.30	100.00	66.70	9,660.00
135	116.70	150.00	133.30	216.67	83.30	1,460.00
674	416.70	416.70	633.30	116.70	783.30	1,320.00
Arithmetic Mean	313.90	400.02	408.32	213.90	566.65	3,833.33
Ivermectin SC						
43	433.30	450.00	16.70	50.00	0.00	2,780.00
69	650.00	216.70	83.30	50.00	0.00	3,440.00
112	33.30	50.00	33.30	100.00	50.00	1,120.00
138	83.30	33.30	50.00	16.70	50.00	220.00
164	150.00	200.00	66.70	50.00	733.30	1,340.00
675	250.00	250.00	133.30	33.30	816.70	1,700.00
Arithmetic Mean	266.65	200.00	63.88	50.00	275.00	1,766.67
FECR%[a]	-	50.00	84.35	76.62	51.47	53.91
Doramectin SC						
26	433.30	200.00	0.00	16.70	16.70	1,100.00
29	100.00	66.70	16.70	16.70	33.30	180.00
36	600.00	66.70	0.00	0.00	0.00	0.00
39	266.70	150.00	0.00	16.70	16.70	2,300.00
45	16.70	0.00	0.00	0.00	0.00	0.00
73	50.00	100.00	0.00	0.00	66.70	460.00
Arithmetic Mean	244.45	97.23	2.78	8.35	22.23	673.33
FECR%[a]	-	75.69	99.32	96.10	96.08	82.43

a. Percentage reduction in arithmetic mean.

Identificação de *Cooperia punctata* (LINSTOW, 1907) resistente a ivermectina e doramectina em um rebanho leiteiro no Estado do Rio de Janeiro, Brasil

Resumo

Resistência antihelmíntica é um problema potencial para o controle de nematodas em bovinos e pode causar perdas econômicas na indústria de bovinos leiteiros e de cortes. O objetivo deste estudo foi determinar a eficácia da ivermectina, doramectna e abamectina em bezerros infectados naturalmente e experimentalmente para *Cooperia punctata* em um rebanho bovino leiteiro. Testes de redução da contagem de ovos fecais foram conduzidos em bezerros infectados naturalmente que foram tratados com soluções injetáveis de ivermectina, doramectina e abamectina. Amostras fecais foram colhidas no dia do tratamento, dia 0, e nos dias 7 e 14 após o tratamento e cultura de larvas foram feitas nas amostras positivas. Um teste controlado foi realizado usando 18 bezerros infectados artificialmente,

Palavras-chave: Resistência. Ivermectin. Doramectina *Cooperia*. Bovino.

alocados em três grupos com seis animais cada: Grupo I - controle, sem tratamento; Grupo II – ivermectina, solução injetável, 200μg/kg; Grupo III – doramectina, solução injetável, 200μg/kg. Amostras fecais foram colhidas no dia do tratamento, dia 0, e nos dias 3, 7, 9 e 14 após o tratamento. Nos dias 14, 15 e 16, dois animais de cada grupo foram eutanaziados e cargas de parasitos pulmonares e gastrintestinais foram determinados. Os resultados dos testes de redução da contagem de ovos fecais em bezerros infectados naturalmente mostraram redução no dia 14 após o tratamento de – 4,45 a 11,49% para ivermectina; 32.31 a 60.40% para doramectina e 85,05% para abamectina. O único parasito identificado na cultura de larvas foi *Cooperia spp.* O Teste Controlado mostrou uma redução da contagem de ovos fecais no dia 14 após o tratamento de 51.47% e 96.08% para ivermectina e doramectina, respectivamente. Redução da contagem de adultos neste teste controlado foi de 53.91% e 82.43% para ivermectina e doramectina, respectivamente. Somente *C. punctata* foi recuperado nas necropsias. Assim, esta cepa de *C. punctata* foi considerado resistente ao tratamento com ivermectina e doramectina e sugere uma possível resistência para abamectina pelo teste de redução da contagem de ovos fecais.

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