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# Susceptibility of helminth species parasites of sheep and goats to different chemical compounds in Brazil

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ABSTRACT

A total of 160 sheep and 160 goats were necropsied to evaluate the degrees of susceptibility or resistance of different helminth species to 0.2 mg/kg ivermectin (subcutaneous route), 0.2 mg/kg moxidectin (subcutaneous route), 100 mg/kg trichlorfon (administered orally) and the combination of 5 mg/kg albendazole + 7.5 mg/kg levamizole + 0.2 mg/kg ivermectin (administered orally). To achieve this objective, eight experiments were performed, four with each animal species. In each experiment, naturally infected sheep or goats were divided into five groups with eight individuals each, as follows: T01, untreated control; T02, trichlorfon; T03, ivermectin; T04, moxidectin; and T05, albendazole + levamizole + ivermectin, based on average counts of eggs per gram of feces (EPG) before treatment (experimental dates −3, −2 and −1). Seven days post-treatment (DPT), all animals were euthanized and necropsied for the recovery of helminth burdens. Based on the obtained results, it is possible to conclude that the resistance of some helminth species parasitizing sheep and goats is different for the tested chemical groups. Ivermectin, at 0.2 mg/kg dosage, presented inferior anthelmintic efficacy against some of these parasites. Of these species, populations of *Haemonchus contortus*, followed by *Trichostrongylus colubriformis*, *Cooperia curticei* and *Oesophagostomum columbianum*, exhibited the greatest resistance to the aforementioned chemical compound, whereas *Trichostrongylus axei* displayed higher susceptibility to ivermectin. For moxidectin (0.2 mg/kg), 75% of all *H. contortus* populations were considered resistant to this drug, whereas all populations of *T. colubriformis, T. axei*, *C. curticei* and *O. columbianum* were susceptible. Trichlorfon and albendazole + levamizole + ivermectin were effective against the analyzed nematode populations, except against one strain of *H. contortus* and one strain of *T. colubriformis*. All three *Strongyloides papillosus* populations evaluated were susceptible to the tested formulations, except for moxidectin, as this compound presented low efficacy indices against all populations of this helminth species.



## 1. Introduction

The sheep and goat breeding performed in tropical countries primarily focuses on the production of the meat, milk and wool from these animals. However, gastrointestinal nematodes represent one of the main sanitary problems in small ruminants and are responsible for increased economic losses in the sheep and goat industries worldwide (Gazda et al., 2012).

Although new alternatives for controlling sheep and goats nematodiasis exist and are used in the field by producers with excellent results, the administration of chemical compounds with anthelmintic activity remains the most reliable methodology. Even on properties where alternative methods of control are employed, producers and owners are eventually forced to rely on chemical products during specific periods of the year for small ruminant production (Maciel et al., 2014). Helminth control in sheep and goats represents a major factor in productivity; however attempts to eliminate these endoparasites are usually performed

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inappropriately, which increases production costs and accelerates the development of parasite resistance, likely the main sanitary problem in animal breeding (Lopes et al., 2013). This is evidenced by several publications on the resistance of small ruminant endoparasites against active principles available in the market for their control (Buzzulini et al., 2007; Ahid et al., 2008; Lima et al., 2010; Holsback et al., 2013; Mahineu et al., 2014). In addition to these aspects, it is also essential to note the presence of residues from such chemicals in products of animal origin (Sindan, 2008).

Due to severe losses caused by helminth infections in sheep and goats, together with studies about the resistance of these parasites to chemical compounds, it is essential to constantly monitor the efficacy of the available active components against endoparasites that affect small ruminants of a given region. Based on this premise, the objective of the present study was to evaluate the degree of susceptibility or resistance of different helminth species parasitizing naturally infected sheep and goats, against anthelmintic formulations administered via different routes (0.2 mg/kg ivermectin, subcutaneous; 0.2 mg/kg moxidectin, subcutaneous; 100 mg/kg trichlorfon, oral; and 5 mg/kg albendazole + 7.5 mg/kg levamizole + 0.2 mg/kg ivermectin combination, oral).

## 2. Materials and methods

### 2.1. Animal selection and experimental design

This study was performed between January 2012 and February 2014. One hundred and sixty sheep and 160 goats, from eight different rural properties (four of each animal species) from the state of São Paulo, Southeast region of Brazil, located in the following cities: Jaboticabal, Viradouro, Pontal, Morro Agudo, Sertãozinho, Ribeirão Preto, Taquaritinga and São João da Boa Vista. All 320 animals were mixed breed, both males and females, with ages ranging from eight to 24 months, housed under extensive farming conditions without contact with any other animal species and naturally infected by helminths. Only animals that were not treated with any type of anthelmintics for a minimum period of 60 days before the beginning of the experiment and that presented eggs per gram of feces (EPG) counts (strongylid eggs) greater than 500 (Gordon & Whitlock, 1939) were selected for this study. None of the properties from which the experimental animals were obtained used alternative (non-chemical) methods of helminth control. All farms treated the sheep/goats against gastrointestinal parasites every 40 or 50 days or before this interval when submandibular edema was observed in the animals.

Seven days before the experimental treatments, the selected animals were transported to the Center of Researches in Animal Health, CPPAR/FCAV/UNESP, where they were housed during the entire study in suspended boxes with slatted floors, preventing helminthic reinfections. Animals (sheep and goats) were fed corn silage, commercial ration, mineral supplementation and *ad libitum* water.

At experimental day zero, the sheep and goats were allocated to treatment groups based on their origin herds and utilizing average counts of strongylid eggs per gram of feces (EPG), obtained on days −3, −2 and −1, as criteria for their distribution in pairs, forming eight blocks per herd. Four trials were conducted with sheep and another four with goats. For each experiment, independent of the species involved, the animals were divided in five groups of eight animals each, as follows: T01, saline solution (untreated control); T02, 100 mg/kg trichlorfon administered orally (Neguvon®—Bayer Animal Health, commercially available for sheep and goats, Lopes et al., 2014b); T03, subcutaneous 0.2 mg/kg ivermectin (Ivomec®, Merial Animal Health, commercially available for sheep); T04, subcutaneous 0.2 mg/kg moxidectin (Cydectin®, Zoetis, commercially available for sheep); and T05, 5.0 mg/kg albendazole + 7.5 mg/kg levamizole + 0.2 mg/kg ivermectin (Trimix®, Merial Animal Health, commercially available for sheep), administered orally.

### 2.2. Parasitological necropsies

In all experiments, animals (control and treated groups) were necropsied on the 7th day post-treatment (DPT). The digestive systems of each animal were separated by double ligature marks into different anatomical segments, including the abomasum, small and large intestine. The fresh organs were washed, and the wash was preserved in 10% formalin and heated to 80 °C. The fresh abomasums (not fixed) were individually subjected to digestion with a pepsin hydrochloric acid solution. Each abomasum was placed in a 1% pepsin solution. The volume (by weight) of these solutions was at least three times that of the mucosa. The mucosal material was digested with this solution in a water bath at 37–40 °C for no longer than 4–6 h (Wood et al., 1995). The time between the harvest of the helminthes and the euthanasia of animals was approximately 40–50 min.

Lungs and livers from all animals were also dissected and visually inspected to determine the number of helminths in these organs (Wood et al., 1995).

### 2.3. Helminth species identification

A 10% aliquot from the total contents of each segment was retained for examination and an estimation of parasite loads. Helminths were collected using a magnifying glass, and the generic and species identifications of 10% of the helminthes in the aliquot were performed using a stereoscopic microscope (magnification 100–400×) according to the taxonomic criteria described by Levine (1968), Ueno and Golçalves (1998) and Achi et al. (2003). One or two drops of lacto phenol were added to facilitate identification.

### 2.4. Efficacy

Based on arithmetic means of helminth quantification for each experimental group, the therapeutic efficacy percentages of different formulations were calculated for each helminth species diagnosed using the formula described by Wood et al. (1995):

$$\%\text{efficacy} = \frac{\text{Average number of helminths on the control group} - \text{Average number of helminths on the treated group}}{\text{Average number of helminths on the control group}} \times 100$$

### 2.5. Data analysis

For later calculations, the counts were first log transformed [ln (x + 1)], according to VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) guidelines (Vercruysse et al., 2000).Because of the relative helminth counts, statistical analysis was performed using a generalized linear mixed model with treatment-fixed and block random effects and a block-treatment interaction (SAS, 1996).

Differences between treatments were considered statistically significant at $P \leq 0.05$.

### 2.6. Criteria for the diagnosis of resistance, susceptible or inconclusive

According to Presidente (1985) and Vercruysse et al. (2001), a nematode population is considered resistant when the efficacy of a formulation is less than 90%. Additionally, VICH GL12 (2001)

emphasizes the importance of attaining a significant difference between treated and control groups at the 5% probability level. This same guideline reports that, generally, the minimum average number of nematodes recommended as adequate for comparisons is 100, but lower counts are to be expected for *Bunostomum* spp., *Oesophagostomum* spp., *Trichuris* spp. and *Dictyocaulus* spp. Recent studies indicate that arithmetic means should be used to diagnose a strain of helminths, which is resistant to a specific formulation (Dobson et al., 2009; Vercruysse et al., 2011).

When less than four animals in the control group were infected with specific helminthes, it was not possible to evaluate the degree of sensitivity or resistance for one helminth or complete a statistical analysis. Therefore, the results obtained from one species of helminth were considered inconclusive.

Therefore, the diagnosis of nematode species with resistance, susceptibility or inconclusive to different formulations was determined based on the aforementioned criteria, using the arithmetic means of helminth counts.

## 3. Results

### 3.1. Sheep and goats analyzed together

When data from all significant (with more than six animals from the control group infected) helminth populations recovered were analyzed together, independent of the species of hosts, it was possible to verify that 87.5% and 62.5% of all *Haemonchus contortus* and *Trichostrongylus colubriformis* strains were resistant to 0.2 mg/kg ivermectin. For *Trichostrongylus axei*, *Cooperia curticei*, *S. papillosus* and *Oesophagostomum columbianum*, 25%, 50%, 0.0% and 33.3% of all detected populations, respectively, were resistant to this particular formulation (Tables 1–4).

For moxidectin (0.2 mg/kg), 75% of all *H. contortus* populations presented resistance to this formulation. When *T. colubriformis*, *T. axei*, *C. curticei* and *O. columbianum* were analyzed, all populations were classified as susceptible to moxidectin. In contrast, the evaluation of sensibility or resistance of *Strongyloides papillosus* was possible in only three populations because more than six animals from the untreated control group were infected by this particular helminth species and all were considered resistant to moxidectin (Tables 1–4).

For the 100 mg/kg trichlorfon and 5 mg/kg albendazole + 7.5 mg/kg levamizole + 0.2 mg/kg ivermectin treatments, only one population of *H. contortus* and one population of *T. colubriformis* were considered resistant to these compounds. However, 100% of all *T. axei*, *C. curticei*, *S. papillosus* and *O. columbianum* populations were susceptible to trichlorfon and to the albendazole + levamizole + ivermectin combination (Tables 1–4).

Despite the increased efficacies observed for trichlorfon against previously discussed helminth species, two goats from different experiments showed clinical signs of intoxication with the active component approximately 40 min after being treated. These caprines presented ataxia, sialorrhea, shivering, constriction of pupils, loud dyspnea, involuntary urination and defecation, spastic paresis, tympanism and watery eyes. Immediately after the detection of these symptoms, approximately 45 min after administering trichlorfon and, consequently, five minutes after the intoxication signs were observed, 1% atropine sulfate was administered to these animals at a dose of 0.5 mg/kg, together with support fluid therapy. Nearly 20 min after, both animals were able to stand again. At 24 h after the administration of trichlorfon and subsequent treatment for intoxication, the two goats were standing and feeding normally; therefore, they were not excluded from the study.

*Cooperia punctata*, Ctenarytaina spatulata, *Trichostrongylus logispicuralis*, *Capillaria bovis* and *Trichuris ovis* were present in some of the sheep and goat samples at a very low frequency, which did not permit statistical evaluations of drug efficacy against these species, leading to their exclusion from further analysis (Tables 1–4).

Visual inspection of livers and lungs during the necropsies of the animals (sheep and goats) in all eight studies did not reveal the presence of any parasites (Tables 1–4).

## 4. Discussion

Studies using EPG counts have been performed in several different regions of southern Latin America (Brazil, Paraguay and Argentina), and several studies (Eddi et al., 1996; Echevarria et al., 1996; Maciel et al., 1996; Waller et al., 1996) report the inefficacy of albendazol, levamizole, albendazole + levamizole, ivermectin and closantel for the treatment of sheep populations. Melo et al. (1998), Ahid et al. (2007), Byaruhanga and Okwee-Acai, 2013 and Mahineu et al. (2014) report the inefficacy of ivermectin for the treatment of sheep and goats populations. In these cases, the genus *Haemonchus* was the most abundant, followed by *Trichostrongylus* and *Oesophagostomum*. In the present study, *H. contortus* presented the highest resistance to ivermectin, followed by *T. colubriformis*, *C. curticei*, *O. columbianum* and *T. axei*. According to Blackhall et al. (1998), *H. contortus* likely develops resistance at a faster pace due to its high biotic potential. Moreover, this nematode has increased genetic variability and possibly harbors the allele that leads to decreased susceptibility to a specific chemical compound (Gilleard, 2013). Increased sensitivity of *T. axei* to ivermectin was also reported by Lopes et al. (2013), Lopes et al. (2014a) and Felippelli et al. (2014).

Recent studies with moxidectin in ruminants indicate that the active principle is most promising for the control of nematodes in these animals (Houlsback et al., 2013; Lopes et al., 2014a; Mahineu et al., 2014 Lopes et al., 2014a; Mahineu et al., 2014) compared to the remaining avermectins, ivermectin, abamectin, doramectin and eprinomectin. Consistent with Shoop et al. (1993), moxidectin may be more potent in eliminating nematodes such as *Haemonchus* than ivermectin, but this difference is lower for others helminths such as *Cooperia*. The efficacy obtained in the present study, with ivermectin and moxidectin against *H. contortus* in sheep and goats, support the study of Shoop et al. (1993), and the superior efficacy of moxidectin compared to ivermectin against *H. contortus* and *T. colubriformis* was observed in both animal species (sheep and goats). Prichard et al. (2012) suggests that these molecules, although similar, are not identical with regard to their mechanisms of resistance and consequent effects on helminths. In contrast, six of all eight populations analyzed in the present study were resistant to this antiparasitic drug at a dose of 0.2 mg/kg. Results similar to those obtained with moxidectin against *H. contortus* were observed by Buzzulini et al. (2007) near the region where this study was conducted. Moreover, all *S. papillosus* populations were classified as resistant to the aforementioned active compound. It is highly probable that if parasitological necropsies were not performed in the present study, the resistance of *S. papillosus* to moxidectin would not have been detected.

Despite the results of this study, it is important to note that some formulations, such as ivermectin, moxidectin and the combination of albendazole + levamizole + ivermectin used in goats, can presents problems at the same dose rates as sheep because many are not licensed for use in goats and the drugs are metabolized much more rapidly in goats than in sheep (AFRC, 1998). Furthermore, it is know that the injectable formulations in small ruminants has lower concentration of active in target tissues, which can hamper the elimination of some species of helminths as *Haemonchus* and *Trichostrongylus* (Lloberasa et al., 2012; Lanusse et al., 2014).

**Table 1**
Mean counts of helminth species (complete sample) collected from sheep belonging to the control and treated groups; results of variance analysis of helminths and efficacy percentages (a).

Studies sheep

Experiment 1—Viradouro city

| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[c] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] | TRIC | IVER | MOX | Alb + Le + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 4565.5 (3–11,293) A | 37.0 (0–263) D | 2366.4 (0–4356) B | 928.0 (0–4567) C | 35.9 (0–153) D | 99.2 | 48.2 | 79.7 | 99.2 | SUS | RES | RES | SUS |
| *Trichostrongylus axei* | 194.0 (0–1239) A | 0.0 (0–0) B | 7.8 (0–41) B | 2.5 (0–20) B | 0.0 (0–0) B | 100.0 | 96.0 | 98.7 | 100.0 | SUS | SUS | SUS | SUS |
| *Trichostrongylus colubriformis* | 8172.8 (3464–18,023) A | 48.3 (0–200) B | 5535.7 (0–18,023) A | 50.3 (0–241) B | 47.9 (0–152) B | 99.4 | 32.3 | 99.4 | 99.4 | SUS | RES | SUS | SUS |
| *Cooperia curticei* | 1028.0 (30–3053) A | 0.0 (0–0) B | 1157.6 (30–4056) A | 0.1 (0–1) B | 1.3 (0–10) B | 100.0 | 0.0 | 99.9 | 99.8 | SUS | RES | SUS | SUS |
| *Strongyloides papillosus* | 127.0 (0–655) A | 0.0 (0–0) B | 0.0 (0–0) B | 45.0 (0–300) B | 0.0 (0–0) B | 100.0 | 100.0 | 64.5 | 100.0 | SUS | SUS | RES | SUS |
| *Capillaria bovis* | 0.8 (0–3) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Oesophagostomum columbianum* | 198.6 (44–440) A | 0.0 (0–0) B | 221.1 (12–632) A | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 0.0 | 100.0 | 100.0 | SUS | RES | SUS | SUS |
| *Trichuris ovis* | 0.9 (0–5) A | 0.0 (0–0) A | 0.1 (0–2) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 85.1 | 100.0 | 100.0 | INC | INC | INC | INC |

Experiment 2—Pontal city

| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[d] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 4598.2 (542–11,791) A | 328.1 (0–1119) C | 1854.7 (210–4350) B | 1093.2 (54–4325) B | 309.2 (1–1306) C | 92.8 | 59.6 | 76.2 | 93.2 | SUS | RES | RES | SUS |
| *Trichostrongylus axei* | 2.3 (0–14) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Trichostrongylus colubriformis* | 4426.9 (0–32,923) A | 0.0 (0–0) C | 850.6 (0–2502) B | 0.0 (0–0) C | 0.0 (0–0) C | 100.0 | 80.79 | 100.0 | 100.0 | SUS | RES | SUS | SUS |
| *Trichostrongylus logispicuralis* | 4.6 (05–46) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Cooperia curticei* | 513.8 (50–4223) A | 0.4 (0–5) B | 398.0 (0–3215) B | 0.3 (0–3215) B | 0.1 (0–1) B | 99.9 | 22.5 | 99.9 | 99.9 | SUS | RES | SUS | SUS |
| *Cooperia punctata* | 68.8 (0–277) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Cooperia spatulata* | 1.6 (0–16) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Strongyloides papillosus* | 14.0 (0–110) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.1 (0–1) A | 0.0 (0–0) A | 100.0 | 100.0 | 99.3 | 100.0 | INC | INC | INC | INC |

SUS = susceptible/RES = resistant/INC = inconclusive.

[a] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.

[b] Commercial formulation purchased in the local market.

[c] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean and the results of the data analysis.

[d] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean and the results of the data analysis.

**Table 2**
Mean counts of helminth species (complete sample) collected from sheep belonging to the control and treated groups; results of variance analysis of helminths and efficacy percentages (b).

| Studies sheep | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment 3—Ribeirão Preto city | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[c] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 5074.0 (726–18,756) A | 31.4 (0–102) C | 5818.6 (45–23,450)A | 803.8 (0–3210) B | 18.6 (0–78) C | 99.3 | 0.0 | 84.1 | 99.6 | SUS | RES | RES | SUS |
| *Trichostrongylus axei* | 132.3 (0–875) A | 6.9 (0–34) B | 7.5 (0–50) B | 0.6 (0–5) B | 0.1 (0–1) B | 94.8 | 99.5 | 99.5 | 99.9 | SUS | SUS | SUS | SUS |
| *Trichostrongylus colubriformis* | 9105.1 (7–23,450) A | 10.5 (0–23) C | 4415.5 (3–8379.0) B | 97.3 (0–423) C | 8.0 (0–21) C | 99.8 | 98.9 | 98.9 | 99.9 | SUS | SUS | SUS | SUS |
| *Cooperia curticei* | 1530.3 (0–7067) A | 29.5 (0–134) B | 1357.8 (0–8504) A | 24.1 (0–123) B | 16.9 (0–76)B | 98.0 | 98.4 | 98.4 | 98.9 | SUS | SUS | SUS | SUS |
| *Oesophagostomum columbianum* | 227.O (43–556) A | 0.1 (0–1) B | 203.3 (32–531) A | 1.5 (0–12) B | 0.1 (0–1) B | 99.9 | 99.3 | 99.3 | 99.9 | SUS | SUS | SUS | SUS |
| Experiment 4—São João da Boa Vista city | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5mg/kg + Lev7.5mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[d] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 2460.4 (249–15,348) A | 1030.3 (20–1699) B | 1734.4 (20–7868) B | 1364.5 (50–4379) B | 183.1 (0–1250) C | 58.1 | 29.5 | 44.5 | 92.5 | RES | RES | RES | SUS |
| *Trichostrongylus axei* | 3.2 (0–17) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Trichostrongylus colubriformis* | 1304.1 (40–6811) A | 114.0 (0–990) B | 112.0 (0–970) B | 98.0 (0–570) B | 209.0 (0–1720) B | 91.2 | 91.4 | 92.5 | 83.4 | SUS | SUS | SUS | RES |
| *Cooperia curticei* | 56.0 (0–210) A | 0.0 (0–0) B | 0.0 (0–0) B | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 100.0 | 100.0 | 100.0 | SUS | SUS | SUS | SUS |
| *Strongyloides papillosus* | 51.9 (0–312) A | 0.0 (0–0) B | 0.0 (0–0) B | 11.9 (4–32) B | 0.0 (0–0) B | 100.0 | 100.0 | 77.1 | 100.0 | SUS | SUS | RES | SUS |
| *Oesophagostomum columbianum* | 8.0 90–30) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |

SUS = susceptible/RES = resistant/INC = inconclusive.

[a] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.

[b] Commercial formulation purchased in the local market.

[c] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean and the results of the data analysis.

[d] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean and the results of the data analysis.

**Table 3**
Mean counts of helminth species (complete sample) collected from goats belonging to the control and treated groups; results of variance analysis of helminths and efficacy percentages (a).

| Studies Goat | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment 1—Pontal city | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[2] | Efficacy (%) | | | | Classification of strain[c] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 3206.8 (1015–6337) A | 102.1 (0–422)B | 312.5 (0–876) B | 65.6 (0–214) B | 203.4 (0–987) B | 96.8 | 90.2 | 97.9 | 93.6 | SUS | SUS | SUS | SUS |
| *Trichostrongylus axei* | 14.6 (0–90) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Trichostrongylus colubriformis* | 2336.6 (130–8103) A | 5.5 (0–32) B | 456.9 (0–1023) B | 6.6 (0–22) B | 7.6 (0–61) B | 99.7 | 80.4 | 99.7 | 99.6 | SUS | RES | SUS | SUS |
| *Capillaria bovis* | 0.25 (0–2) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.1 (0–1) A | 0.0 (0–0) A | 100.0 | 100.0 | 50,00 | 100.0 | INC | INC | INC | INC |
| *Oesophagostomum columbianum* | 9.4 (0–75) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 98.9 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Trichuris ovis* | 1.1 (0–7) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.1 (0–1) A | 0.0 (0–0) A | 100.0 | 100.0 | 88.8 | 100.0 | INC | INC | INC | INC |
| Experiment 2—Morro Agudo city | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[d] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 1542.8 (10–3060.0) A | 0.2 (0–1) C | 887.7 (0–2818) B | 93.2 (1–411) C | 20.9 (0–90) C | 99.9 | 42.4 | 93.9 | 98.6 | SUS | RES | SUS | SUS |
| *Trichostrongylus axei* | 147.6 (2–268) A | 0.0 (0–0) B | 23.0 (0–23) B | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 84.23 | 100.0 | 100.0 | SUS | RES | SUS | SUS |
| *Trichostrongylus colubriformis* | 6668.6 (360–15,069) A | 2.5 (0–20) C | 2333.2 (321–8743) B | 3.0 (0–23) C | 19.0 (0–100) C | 99.9 | 65.0 | 99.9 | 99.7 | SUS | RES | SUS | SUS |
| *Cooperia curticei* | 54.3 (32–240) A | 0.0 (0–0) B | 16.6 (0–78) B | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 69.4 | 100.0 | 100.0 | SUS | RES | SUS | SUS |
| *Strongyloides papillosus* | 79.1 (1–342) A | 0.0 (0–0) B | 0.0 (0–0) B | 10.0 (0–321) A | 0.0 (0–0) B | 100.0 | 100.0 | 1.3 | 100.0 | SUS | SUS | RES | SUS |
| *Oesophagostomum columbianum* | 23.7 (0–100) A | 0.0 (0–0) A | 15.1 (0–98) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 36.3 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Trichuris ovis* | 5.0 (0–10) A | 0.0 (0–0) A | 5.5 (0–14) A | 1.4 (0–10) A | 0.0 (0–0) A | 100.0 | 100.0 | 72.5 | 100.0 | INC | INC | INC | INC |

SUS = susceptible/RES = resistant/INC = inconclusive.

[a] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.

[b] Commercial formulation purchased in the local market.

[c] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean and the results of the data analysis.

[d] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean and the results of the data analysis.

**Table 4**
Mean counts of helminth species (complete sample) collected from goats belonging to the control and treated groups; results of variance analysis of helminths and efficacy percentages (b).

| Studies goat | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment 3—Sertaozinho city | | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2 mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[c] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 3778.7 (934–9843) A | 8.4 (0–23) C | 3335.8 (2–845.0) A | 633.8 (0–4563) B | 7.8 (0–17) C | 99.8 | 11.7 | 83.2 | 99.8 | SUS | RES | RES | SUS |
| *Trichostrongylus axei* | 5.6 (0–23) A | 6.8 (0–25) A | 1.7 (0–10) A | 0.1 (0–1) A | 4.4 (0–15) A | 0.0 | 69.5 | 97.8 | 22.2 | INC | INC | INC | INC |
| *Trichostrongylus colubriformis* | 2578.3 (210–9342) A | 3.2 90–14) B | 1769.0 (0–10,453) A | 134.6 (0–901) B | 2.2 (0–12) B | 99.8 | 31.4 | 94.8 | 99.9 | SUS | RES | SUS | SUS |
| *Capillaria bovis* | 0.7 (0–3) A | 0.0 (0–0) A | 0.6 90–3) A | 0.4 (0–2) A | 0.1 (0–1) A | 100.0 | 23.8 | 50.0 | 83.3 | INC | INC | INC | INC |
| *Oesophagostomum columbianum* | 14.2 (0–98) A | 0.4 (0–2) A | 23.8 (0–112) A | 0.4 (0-2) A | 0.2 (0-1) A | 97.3 | 0.0 | 97.3 | 98.6 | INC | INC | INC | INC |
| *Trichuris ovis* | 2.0 (0–13) A | 0.1 (0–1) A | 0.0 (0–0) A | 0.2 (0–1) A | 0.0 (0–0) A | 93.7 | 100.0 | 87.5 | 100.0 | INC | INC | INC | INC |
| Experiment 4—Taquaritinga city | | | | | | | | | | | | | | |
| Helminth species | T01: control (saline solution) | T02: trichlorfon 100 mg/kg[b] | T03: ivermectin 0.2 mg/kg[b] | T04: moxidectin 0.2mg/kg[b] | T05: Alb 5 mg/kg + Lev7.5 mg/kg + Iver 0.2 mg/kg[b] | Efficacy (%) | | | | Classification of strain[d] | | | |
| | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | Mean count[a] (range) | TRIC | IVER | MOX | Alb + Lev + Iver | TRIC | IVER | MOX | Alb + Lev + Iver |
| *Haemonchus contortus* | 588.2 (262–1429) A | 15.6 (0–91) C | 252.2 (0–902) B | 157.4 (0–570) C | 18.6 (0–105) C | 97.3 | 57.1 | 73.2 | 96.8 | SUS | RES | RES | SUS |
| *Trichostrongylus axei* | 3790.6 (38–16,491) A | 0.0 (0–0) B | 1.6 (0–12) B | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 99.9 | 100.0 | 100.0 | SUS | SUS | SUS | SUS |
| *Trichostrongylus colubriformis* | 11675.1 (1310–26,382) A | 21.7 (0–80) C | 583.9 (20–3214) B | 52.3 (0–310) C | 14.2 (0–100) C | 99.8 | 95.0 | 99.5 | 99.8 | SUS | SUS | SUS | SUS |
| *Cooperia curticei* | 93.0 (10–320) A | 0.0 (0–0) B | 8.0 (0–34) B | 0.0 (0–0) B | 0.0 (0–0) B | 100.0 | 91.4 | 100.0 | 100.0 | SUS | SUS | SUS | SUS |
| *Strongyloides papillosus* | 2.0 (0–2) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |
| *Oesophagostomum columbianum* | 130.0 (10–430) A | 0.0 (0–0) B | 35.9 (0–321) B | 1.0 (0–1) B | 0.0 (0–0) B | 100.0 | 72.4 | 99.2 | 100.0 | SUS | RES | SUS | SUS |
| *Trichuris ovis* | 6.0 (0–30) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 0.0 (0–0) A | 100.0 | 100.0 | 100.0 | 100.0 | INC | INC | INC | INC |

SUS = susceptible/RES = resistant/INC = inconclusive.

[a] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.

[b] Commercial formulation purchased in the local market.

[c] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean and the results of the data analysis.

[d] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean and the results of the data analysis.

For the groups treated orally with 100 mg/kg trichlorfon, only one *H. contortus* population was diagnosed as resistant to this compound, whereas the remaining nematode populations evaluated, *T. colubriformis*, *T. axei*, *C. curticei*, *S. papillosus* and *O. columbianum*, were sensitive to this dosage. Falbo et al. (2009) described the elevated efficacy of this same drug against *Haemonchus* in the state of Paraná, South Brazil, whereas Sczesny-Moraes et al. (2010) reported *Haemonchus* resistance to trichlorfon in the state of Mato Grosso do Sul in the central-western region of the country. In a study conducted by Lopes et al. (2009) in cattle, the researchers observed increased anthelmintic efficacy of 48.5 mg/kg trichlorfon against nematode populations resistant to 200 and 630 μg/kg ivermectin, including *H. placei* and *C. punctata*.

Regarding intoxication of the caprines treated with 100 mg/kg trichlorfon in the present study, Lopes et al. (2014b) also observed clinical cases of intoxication by such active principles administered at therapeutic dosages, in which eight animals, from a group of 20 that received 100 mg/kg trichlorfon, presented intoxication signs and had to be medicated with atropine sulfate. These authors emphasize that this animal species may present an elevated sensitivity to the recommended trichlorfon dosage described for goats on the labels (100 mg/kg), even with all recommendations for the active component.

For the albendazole + levamizole + ivermectin combination, only one population of *T. colubriformis* was considered resistant to this medication, whereas the remaining nematode populations evaluated, *T. colubriformis*, *T. axei*, *C. curticei*, *S. papillosus* and *O. columbianum*, were diagnosed as susceptible to this compound. Although albendazole, levamizole and ivermectin were not tested separately, studies conducted worldwide with benzimidazoles reinforce the inefficacy of this antiparasitic class against helminths parasitizing sheep and goats (Cunha Filho et al., 1998; Melo et al., 1998; Sczesny-Moraes et al., 2010; Duarte et al., 2012; Mahineu et al., 2014). However, there are several reports of helminthic resistance against levamizole, the active principle in ruminants (Souza and Thomas-Soccol, 1997; Souza and Thomas-Soccol, 1997), and susceptible populations remain, depending on the region (Lima et al., 2010; Duarte et al., 2012). The increased efficacy of the albendazole + levamizole + ivermectin combination was also reported by Buzzulini et al. (2007) and Holsback et al. (2013) for sheep from the states of São Paulo (Southeast region of Brazil) and Paraná (South region of Brazil), respectively. In contrast, using EPG counts, sheep nematode populations resistant to this combination of chemicals were previously detected in the state of Mato Grosso do Sul, Center-West region of Brazil (Sczesny-Moraes et al., 2010).

Based on the results obtained in the present study, it is possible to conclude that the resistance of some helminth species parasitizing sheep and goats occurs for different evaluated chemical groups, of which ivermectin at a dosage of 0.2 mg/kg had the poorest anthelmintic activity against some of these species. Populations of *H. contortus*, followed by *T. colubriformis*, *C. curticei* and *O. columbianum*, presented greatest resistance to the aforementioned chemical, whereas *T. axei* demonstrated superior sensitivity to ivermectin. For moxidectin (0.2 mg/kg), 75% of all *H. contortus* populations were diagnosed as resistant to this compound, whereas for *T. colubriformis*, *T. axei*, *C. curticei* and *O. columbianum*, all analyzed populations were susceptible to moxidectin. Trichlorfon and the albendazole + levamizole + ivermectin combination demonstrated efficacy against the different evaluated nematode populations, with the exception of one *H. contortus* and one *T. colubriformis* strain, respectively. All three populations of *S. papillosus* observed in the present study were considered sensitive to the tested formulations, with the exception of moxidectin, as this drug presented low efficacy against all populations of this helminth species.

## Conflicts of interest

There were no conflicts of interest that may have biased the work reported in this paper.

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