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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)