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Susceptibility of helminth species from horses against different chemical compounds in Brazil

Gustavo Felippelli[a], Breno Cayeiro Cruz[a], Lucas Vinicius Costa Gomes[a], Welber Daniel Zanetti Lopes[a,b,*], Weslen Fabrício Pires Teixeira[a], Willian Giquelin Maciel[a], Carolina Buzzulini[a], Murilo Abud Bichuette[a], Gabriel Pimentel Campos[a], Vando Edésio Soares[a], Paula Luzia Formigoni Bergamasco[a], Gilson Pereira de Oliveira[a], Alvimar José da Costa[a]

[a] CPPAR - Animal Health Research Center, Faculdade de Ciências Agrárias e Veterinárias, UNESP, Campus de Jaboticabal, Via de acesso Prof. Paulo Donatto Castellane, s/n°. CEP: 14884-900, Jaboticabal, São Paulo, Brazil
[b] Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO 74605-050, Brazil

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ABSTRACT

By means of parasitological necropsies, the present study aimed to evaluate, in six experiments, the degree of susceptibility or resistance of different helminth species which naturally infect horses to ivermectin 0.2 mg/kg, abamectin 0.2 mg/kg, moxidectin 0.4 mg/kg, trichlorfon 35 mg/kg, ivermectin 0.2 mg/kg + praziquantel 2.5 mg/kg, abamectin 0.2 mg/kg + praziquantel 2.5 mg/kg and ivermectin 0.2 mg/kg + 6.6 mg/kg pyrantel. At experimental day zero, the horses were allocated to treatment groups based on average counts of strongylid eggs per gram of feces (EPG) obtained on days -3, -2 and -1. *Oxyuris* sp. infections were confirmed as positive or negative. All the animals in the six experiments were naturally infected by this helminth species. Each group (control or treated) consisted of six animals. All the assessed *Habronema muscae* populations analyzed were susceptible to ivermectin, abamectin and moxidectin. Of the six Trichostrongylus axei populations, four were susceptible to ivermectin, abamectin, moxidectin, trichlorfon and ivermectin + praziquantel, and two were resistant to abamectin + praziquantel and ivermectin + pyrantel. Both *Strongyloides westeri* populations analyzed were susceptible to ivermectin, abamectin, moxidectin and abamectin + praziquantel. For O. equi, resistance was found in four different populations treated with ivermectin, abamectin, moxidectin, trichlorfon and ivermectin + praziquantel. Only combinations of abamectin + praziquantel and ivermectin + pyrantel were effective against this parasite species. All the large strongyles diagnosed in the present study (*Strongyus edentatus*, *Strongyus vulgaris* and *Triodontophorus serratus*) were susceptible to all the chemicals tested, with the exception of trichlorfon. Of the Cyathostominae populations, one was diagnosed as resistant to ivermectin and another to trichlorfon. The remaining populations from this nematode group were considered to be sensitive to ivermectin, abamectin, moxidectin, ivermectin + praziquantel, abamectin + praziquantel and ivermectin + pyrantel. New studies should be performed in different regions to evaluate the efficacy of trichlorfon in others field populations of helminthes.

1. Introduction

According to Teixeira et al. (2014), parasitic infections in equines cause important economic losses both directly in individuals who develop clinical disease and indirectly by interfering with physical condition and performance. Horses are susceptible to more than 60 internal parasites and may harbor several species of worms at any given time (Stoltenow and Purdy, 2006). This vast parasitic fauna comprises several distinct families and genera, with a predominance of small strongyles (Teixeira et al., 2014).

Nematodes can cause diverse clinical signs ranging from minor abdominal discomfort to fulminating episodes of cramping and even death (Canaver et al., 2013). Due to their ease of administration and good cost/benefit ratio, chemical compounds are the main tools utilized by animal owners for equine endo- and ectoparasite control (Molento, 2005).

* Corresponding author. Fax: +55 1632092605.
E-mail address: wdzlopes@hotmail.com (W.D.Z. Lopes).

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Of the chemical groups available on the veterinary market, four are most commonly used: benzimidazoles, organophosphates, pyrimidines and macrocyclic lactones. Parasite control in racehorses is usually performed with a suppressive treatment several times throughout the year, which results in selecting for drug-resistant parasites (Kaplan, 2002). A lack of efficacy of chemical compounds for small strongyles or *Parascaris equorum* has been reported in many countries (Molento et al., 2008; Reinemeyer, 2008; Traversa et al., 2009; Lester et al., 2013; Geurden et al., 2014). In contrast, there are few studies on the efficacy of equine helminth control that have used parasitological necropsies. Therefore, the present study aimed to evaluate the degree of susceptibility or resistance of different helminth species which naturally infect horses to ivermectin 0.2 mg/kg, abamectin 0.2 mg/kg, moxidectin 0.4 mg/kg, trichlorfon 35 mg/kg, ivermectin 0.2 mg/kg + praziquantel 2.5 mg/kg, abamectin 0.2 mg/kg + praziquantel 2.5 mg/kg and ivermectin 0.2 mg/kg + 6.6 mg/kg pyrantel.

2. Material and methods

2.1. Animal selection and experimental design

During this study, all procedures using animals complied with the Ethical Principles in Animal Research adopted by the College of Animal Experimentation (COBEA) and were approved by the Ethical Committee for Research Institute for Animal Health, Formiga, under protocol number 003J2/2012.

Ninety-six horses belonging to six different rural properties in the states of São Paulo and Minas Gerais in the Southeast region of Brazil were selected for this study. All 96 animals were mixed breeds. They were both males and females over 24 months old, bred under extensive farming conditions without contact with any other animal species and naturally infected by helminths. Only animals that were not treated with anthelmintics for a minimum period of 90 days before the beginning of each experiment were selected for this study. Additionally, the animals had EPG counts (strongylid eggs) greater than 900 (Gordon and Whitlock, 1939) and were positive for *Oxyuris*.

Seven days before the experimental treatments, the selected animals were transported to the Center of Researches in Animal Health, (CPPAR). They were housed in boxes with concrete floors during the entire study to prevent helminth reinfection. The animals were fed with hay and a commercial ration with mineral and *ad libitum* water. At experimental day zero, the horses were allocated to treatment groups based on average counts of strongylid eggs per gram of feces (EPG) obtained on days -3, -2 and -1. *Oxyuris* sp. infections were confirmed as positive or negative (using eggs counts and a tape or Graham method, described by Costa, 2012). All the animals in the six experiments were naturally infected by this helminth species. The animals were allocated to treatment groups according to a randomized complete block design. The block formation was based on the counts (arithmetic means) of strongylid eggs per gram of feces (EPG), obtained of each animal on days -3, -2 and -1 (Brazil, 1997). In the experiments 1 and 2, the horses were divided into six blocks of four animals, and in the experiments of 3, 4, 5 and 6, the animals were divided into six blocks of two animals.. Within each block, the animals were randomly placed in one group, as described in Table 1. Each group (control or treated) was composed by six animals.

The products used were: ivermectin 0.2 mg/kg (Iver Pasta Equinos® – Ouro Fino Agronegócios), abamectin 0.2 mg/kg (Animax Gel® – Agener União), moxidectin 0.4 mg/kg (Equest® – Zoetis Animal Health), trichlorfon 35 mg/kg (Neguvon® – Bayer Animal Health), ivermectin 0.2 mg/kg + praziquantel 2.5 mg/kg (Iver gel Composto® – Ouro Fino Agronegócios), abamectin 0.2 mg/kg + praziquantel 2.5 mg/kg (Aba gel Composto® – Ouro Fino Agronegócios) and ivermectin 0.2 mg/kg + 6.6 mg/kg pyrantel (Mectimax Plus® – Agener União). All products were stored according to label specifications. In each experiment, the horses were weighed individually one day prior to treatment to calculate an exact dose for the animal. Moreover, weighing scales were tested. Treatments were performed individually in the horses.

The drug was withdrawn from a commercial plastic syringe and weighed on a precision scale (Digimed KN500 serial number 05F36) in sterile petri dishes. Subsequently, we calculated the exact dose of the product for each animal. The dose was administered directly into the animal's mouth with the aid of a forefinger using a pair of disposable gloves per animal. Only for trichlorfon was used a syringe to administering the product, because the active was dissolved in water. The products were deposited over each animal's tongue. The animals of the control group received saline solution.

2.2. Parasitological necropsies

In all experiments, animals in the control and treated groups were necropsied on the 14th day post-treatment (DPT). All the animals were subjected to 24 h of fasting and then sacrificed in accordance with ethical procedures described in Brasil, (2008). The method used for sacrificing experimental animals is described in the Note Guidelines on Euthanasia of the American Veterinary Medical Association (AVMA, 2013). Each animal was sedated (0.3 mg/kg – Rompum® – Bayer Animal Healthe) before administering with 4 mg/kg sodium thiopental (Thiopentax® – Cristália Ltda.). After complete unconsciousness was confirmed (absence of palpebral and masticatory reflexes), they were bled by means of transection of the great vessels of the neck. Death occurred through hypovolemia.

After death, the digestive tracts of the horses were removed. The anatomical segments (stomach, small intestine and large intestine) were isolated and separated using double ligatures. These segments were then opened using an enterotome, and all the mucosa were washed with running water to remove all contents and parasites. The total content extracted from each segment was subjected to a second washing under running water and was strained through a sieve (Tyler 48, 297.0 μm) to conserve helminth samples, which were then stored in plastic jars with the addition of heated 10% formaldehyde at 80 °C.

The stomach and small intestine were individually subjected to digestion with a pepsin hydrochloric acid solution. The volume by weight of these solutions was at least three times that of the mucosa. Mucosal material was digested by this solution in a water bath at 37–40 °C for no longer than 4–6 h (Duncan et al., 2002). The mucosa of the large intestine was carefully inspected for the presence of parasites, but this mucosa was not digested.

During necropsies, all lungs were dissected and visually inspected for presence of *Dictyocaulus* possibly present in the lungs (Herlich, 1956; VICH GL15, 2001; Duncan et al., 2002).

2.3. Helminth species identification

A 10% aliquot of each intestinal content solution was separated. Helminths were identified using optical microscopes (100–400 × amplification) in accordance with taxonomical criteria described by Lichtenfels et al. (2008) and Costa (2012).

2.4. Efficacy

Based on arithmetic means of helminth quantifications for each experimental group, a percent of therapeutic efficacy for each