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PREVALENCE OF INTESTINAL HELMINTHS AND TREATMENT OF OSTENSIVE POLICING HORSES FROM THE MILITARY BRIGADE OF RIO GRANDE DO SUL, BRAZIL

PREVALENCIA DE HELMINTOS INTESTINALES Y TRATAMIENTO DE CABALLOS POLICÍAS OSTENSIVOS DE LA BRIGADA MILITAR DE RIO GRANDE DO SUL, BRASIL

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ABSTRACT

This study describes the prevalence of intestinal helminths in 98 adult working military horses of the Brazilian Sport Horse and Crioulo breeds from the Military Brigade of Rio Grande do Sul, Brazil, before and after treatment with a formulation containing ivermectin and praziquantel. Fecal samples were collected and sent to the laboratory of the Faculty of Veterinary Medicine at the Federal University of Rio Grande do Sul. Parasitological diagnosis was obtained using the eggs per gram of feces (EPG) count method and coproculture for identification of infective larvae. The prevalence of parasitized horses was 40.8% (40/98), with 62.5% (25/40) males and 37.5% (15/40) females. Positive samples showed eggs from the Strongylidae family, Baird, 1853, and, in 20%, eggs of *Parascaris equorum* Goeze, 1782. After anthelmintic treatment and repeated fecal examinations, 13.3% (13/98) of the tests showed Strongylidae eggs, and 86.7% (85/98) were negative. Infective larvae of *Gyalocephalus capitatus* Looss, 1900, *Cylicocyclus radiatus* (Looss, 1900) Chaves, 1930, and *Trichostrongylus axei* Looss, 1905 were recovered from coproculture. The recorded efficacy was 67.5%. Parasitological examinations should be performed periodically to determine parasitic load and select the therapeutic protocol.

Keywords: Helminth – Horse – Intestinal nematodes – Fecal egg counts – Strongylidae – Treatment

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RESUMEN

Este estudio describe la prevalencia de helmintos intestinales en 98 caballos militares de trabajo adultos de las razas Caballo Deportivo Brasileño y Criollo de la Brigada Militar de Rio Grande do Sul, Brasil, antes y después del tratamiento con una formulación que contiene ivermectina y praziquantel. Se recolectaron muestras fecales y se enviaron al laboratorio de la Facultad de Medicina Veterinaria de la Universidad Federal de Rio Grande do Sul. El diagnóstico parasitológico se obtuvo utilizando el método de recuento de huevos por gramo de heces (HPG) y coprocultivo para la identificación de larvas infectivas. La prevalencia de caballos parasitados fue de 40,8% (40/98), con 62,5% (25/40) machos y 37,5% (15/40) hembras. Las muestras positivas mostraron huevos de la familia Strongylidae Baird, 1853 y, en 20%, huevos de *Parascaris equorum* (Goeze, 1782). Tras el tratamiento antihelmíntico y repetidos análisis fecales, el 13,3% (13/98) de las pruebas mostró huevos de Strongylidae, y el 86,7% (85/98) fueron negativos. Se recuperaron larvas infectivas de *Gyalocephalus capitatus* Looss, 1900, *Cylicocyclus radiatus* Looss, 1900, Chaves, 1930, y *Trichostrongylus axei* Looss, 1905 mediante coprocultivo. La eficacia registrada fue del 67,5%. Se deben realizar exámenes parasitológicos periódicamente para determinar la carga parasitaria y seleccionar el protocolo terapéutico.

Palabras clave: Caballo – Helminto – Nematodos intestinales – Recuento de huevos fecales – Tratamiento

INTRODUCTION

Gastrointestinal parasitosis in horses have a cosmopolitan distribution and cause a wide spectrum of health effects, ranging from asymptomatic infections to sudden death. There are 29 genus and 83 species of nematodes; the majority includes 19 genus and 64 species, and belong to the family Strongylidae. The genus *Strongylus* Müller, 1780 includes three species: *S. equinus* Müller, 1780, *S. edentatus* Looss, 1900, and *S. vulgaris* Looss, 1900, which exhibit higher pathogenicity, yet their prevalence is low due to the widespread availability of anthelmintics (Kaplan & Nielsen, 2010; Marama *et a*l., 2025). Ascarids and small strongyles (cyathostomins) are the most common parasites worldwide (Saeed *et al.*, 2019), with frequent infections involving 15 to 25 species and global prevalence rates of up to 100% across all age groups (Bellaw & Nielsen, 2020). Emerging cyathostomin larvae from the intestinal wall can lead to larval cyathostominosis syndrome, resulting in diarrhea, weight loss, lethargy, colic, edema, hypoproteinemia, and mortality in up to 50% of hospitalized cases. Although the relative importance of each species remains unknown, the development of species-specific DNA methods provides a means to establish the pathogenicity of different species (Ghafar *et al.*, 2023).

In Brazil, prevalence rates are significant, with regional variations ranging from 60% to 100% (Molento *et al.*, 2024), and critical records are present in the states of Rio Grande do Sul (Piccoli *et al.*, 2015; Lignon *et al.*, 2020; Menetrier *et al.*, 2020; Marques *et al.*, 2025), Paraná (Godéski & Pedrassani, 2018), Minas Gerais (Barbosa *et al.*, 2018), and Amazonas (Ferreira *et al.*, 2024). Recent studies in Australia (Abbas *et al.*, 2021; Abbas *et al.*, 2023; Saeed *et al.*, 2019), Germany (Boelow *et al.*, 2023), Denmark (Beasley *et al.*, 2020), the United States (Nielsen *et al.*, 2024; Nielsen *et al.*, 2025), Mexico (Romero *et al.*, 2020), Romania (Bulgaru *et al.*, 2021), Nigeria (Alaba *et al.*, 2022; Ogbein *et al.*, 2022), Ethiopia (Marama *et al.*, 2025), and Peru (Almeyda & Porras, 2019) have reported prevalence rates oscillating from 46% to 93%.

Anthelmintic drugs are widely used to treat nematodes in equines; however, resistance to nearly all of them is attributed to continuous and indiscriminate use, representing a challenge for their control (Nielsen *et al.*, 2018; Abbas *et al.*, 2021). Alternative programs, including treatment strategies based on egg shedding levels and epidemiological factors, have been proposed to address anthelmintic resistance (Kaplan & Nielsen, 2010; Saeed *et al.*, 2019). Usually, 10% to 30% of horses contribute 80% of the eggs deposited on pastures, known as high egg excretors and their treatment can help reduce overall anthelmintic use, allowing most horses to avoid frequent treatments and keep parasite refugia (nematode populations not exposed to anthelmintics) (Nielsen *et al.*, 2024).

Although several diagnostic tests are used for fecal egg counts, there is no consensus on their methodologies, and the modified McMaster technique (Gordon & Whitlock method or fecal egg count) remains the most widely method used in equine parasitology (Ghafar *et al.*, 2021). This study aimed to collect fecal samples to measure helminth parasitic loads before and after broad-spectrum anthelmintic treatment, dosed quarterly according to the military unit's protocol, in working military horses from the Military Brigade in Porto Alegre, Rio Grande do Sul, Brazil.

MATERIAL AND METHODS

A total of 98 horses, 60 males and 38 females, all adults, of the Brazilian Sport Horse and Crioulo breeds, belonging to the 4th RPMon – Regimento Bento Gonçalves of the Military Brigade in the state of Rio Grande do Sul, Brazil were subjected to parasitological examinations. The stool samples were collected from the rectal ampulla of 20 animals per week by military handlers and sent to the Helminthology Laboratory at the Faculty of Veterinary Medicine, Federal University of Rio Grande do Sul. Coprological diagnosis was made using the modified Gordon and Whitlock method (eggs per gram of feces - EPG); in the laboratory, the samples were kept refrigerated at 4°C and analyzed within 24 hours (Hoffmann, 1987). The five EPG ranges were: (1.) Zero; (2.) 100-400; (3.) 500–800; (4.) 900-1000; (5.) above 1100, according to Nielsen *et al.* (2019).

Coprocultures were performed on 14 fecal samples, corresponding to EPG above 500. The identification of infective larvae from coprocultures, conducted before and after treatment, enabled the identification of L_3 larvae with morphological characteristics of the Subfamily Cyathostominae, based on observation of the number and shape of intestinal cells, total larva length, presence or absence of sheath, and tail sheath aspect (Madeira de Carvalho *et al.*, 2004; Madeira de Carvalho *et al.*, 2008). To evaluate the product's efficacy, the fecal egg count reduction test (FECRT) was performed, using the formula by Barbosa *et al.* (2018): Efficacy (%) = (EPG (pre-treatment) – EPG (post-treatment) / EPG (pre-treatment) × 100.

Ethic aspects: For this study formal consent is not required.

RESULTS AND DISCUSSION

In the pre-treatment coprological analysis, the overall prevalence of parasitized horses by the EPG method was 40.8% (40/98), with 62.5% (25/40) and 37.5% (15/40) for males and females, respectively. The positive samples showed eggs from the Strongylidae family, and in 20%, eggs of *P. equorum* Goeze, 1782. After anthelmintic treatment and repetition of fecal exams, 13.3% (13/98) of the exams showed Strongylidae eggs, and 86.7% (85/95) exhibited negative results. Infective larvae of *Gyalocephalus capitatus* Looss, 1900, *Cylicocyclus radiatus* Looss, 1900 Chaves, 1930, and *Trichostrongylus axei* Looss, 1905 were recovered from the coproculture, in similar proportions (30-35%). After deworming, no *P. equorum* eggs were detected in the coprological exams.

The anthelmintic treatment performed on all animals was the formulation with 1.55% ivermectin and 7.75% praziquantel, according to the manufacturer's recommendation. Two weeks after the application of the anthelmintic, new collections and fecal exams were performed, resulting in 13.3% (13/98) of horses positive and 86.7% (85/95) of animals negative. The results are shown in Table 1.

Table 1. Parasitological examination results (PG) of military working horses from the 4th RPMon – Bento Gonçalves Regiment of the Military Brigade (RS), Brazil, before and after treatment with an ivermectin- and praziquantel-based anthelmintic.

EPG values	Before treatment (%)	After treatment (%)
Zero	58 (59.18)	85 (86.73)
100-400	26 (26.53)	9 (9.18)
500-800	7 (7.14)	3 (3.06)
900-1000	2 (2.04)	0 (0)
≥ 1100	5 (5.10)	1 (1.02)

The average efficacy recorded was 67.5%. The *in vivo* test has been used for many years, mainly based on procedures described in the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) published by Coles *et al.* (1992), whose test is based on fecal egg counts (EPG) before and after treatment in the same animals. Anthelmintic efficacy is estimated by comparing egg counts in feces before and after treatment, with the time defined according to the tested group. The evaluation of this equine population was conducted two weeks post-treatment. The reduction test, according to WAAVP recommendations (Coles *et al.*, 1992), is considered the method of choice for monitoring anthelmintic efficacy due to its easy execution and interpretation, being performed with a sequence of fecal egg count exams. Regarding the calculation of EPG counts, it is a phenotypic test whose count directly depends on the host effect and is considered indirect, as it reflects the egg-laying by females, which depends on the host's immune response (Fortes & Molento, 2013). Furthermore, the use of arithmetic means of egg counts in the feces of the same animals before and after anthelmintic administration, instead of randomly, can provide more reliable results (Dobson *et al.*, 2009). The test evaluation can be impaired due to variations in the correlation between fecal egg counts and adult parasite burden among different parasite species. Anthelmintic resistance is widely common in small strongyles, and recent studies have documented the increasing incidence of resistance to the macrocyclic lactone class of drugs (Barbosa *et al.*, 2018; Abbas *et al.*, 2021).

Several European countries have implemented legal restrictions on the use of anthelmintics, such as prescription-only use, aiming to reduce the intensity of anthelmintic treatment and decrease the selection pressure for resistance; however, the long-term results of this approach have not been fully evaluated (Nielsen *et al.*, 2018; Nielsen, 2022). The sanitary management of working military horses is rigorous, with quarterly deworming supervised by career military veterinarians. The acquisition of medications and supplies follows the official supply protocol, ensuring predictability for both the herd and the police personnel. Therefore, routine health evaluations are essential for logistical planning of new products and materials.

According to Aromaa *et al.* (2018), different types of hygienic management affect each parasite differently: pasture management is critical for strongylids, while hygiene of stalls/facilities is more relevant for *P. equorum*, highlighting the need for customized control strategies. However, Almeyda & Porras (2019) investigated gastrointestinal parasite rates in 384 Thoroughbred horses in the province of Chincha, Peru, and found a prevalence of *Strongylus* spp. of 74.22%, identifying that animal age, humidity, and rainy seasons (typical climatic conditions in the region) were factors associated with this prevalence. Romero *et al.* (2020) investigated horses from different regions in central Mexico with a prevalence of 47.24%, with *Strongylus* spp. being the most prevalent (23.85%), followed by *Trichostrongylus* spp. (21.56%), and concluded that breed and place of origin showed a significant association with helminth infection.

Alaba *et al.* (2022) investigated the prevalence, severity, and predisposing factors (age, sex, and breed) of gastrointestinal parasites in 56 polo horses in Ibadan, Nigeria, and demonstrated a prevalence of 92.9%, dominated by *Strongylus* spp. (89.3%), showing no association between EPG prevalence and the age, sex, and breed of the horses. However, there was a higher occurrence and more severe infections in adult horses, females, and exotic breeds. Meanwhile, Ogbein *et al.* (2022) tested fecal samples from 108 horses in Jos (Nigeria), consisting of 25 males and 83 females, over a six-month period covering three months in the dry season and three months in the rainy season, with an overall prevalence of 82.41%, of which 51.85% showed single infections and 30.56% mixed infections. The most relevant gastrointestinal parasites in this study were *Strongylus* spp., *Strongyloides westeri* Ihle, 1917, *Trichonema* spp. K. Möbius, 1888, *P. equorum*, and *Triodontophorus* spp. Looss 1900. The fecal egg count (EPG) prevalence was zero (3.70%), low (46.30%), medium (24.07%), and high (9.26%), and had a higher expression in young animals, during the rainy season, and in animals with poor body condition scores (84.62%). For these authors, under the rearing conditions of the horses, different factors such as inadequate nutrition, incorrect management practices, health status, and other physiological factors can affect the horses' immune system, making them more susceptible to gastrointestinal parasitic infections. In the context of the Military Brigade, the health of the horses is the foundation of operational efficiency. Unlike leisure horses, policing horses face a workload that demands perfect physical and metabolic condition, where any parasitosis can compromise the performance and safety of the man-horse duo. The facilities for the horses and the vehicles that transport them to the working locations have to be qualified to preserve health and avoid stress during displacements.

In Germany, Boelow *et al.* (2023) evaluated the prevalence and potential severity of nematodiasis through fecal exams of 1,067 horses on German farms, with 46.5% for strongylids. Marama *et al.* (2025) described the strong dependence on anthelmintics for parasitic control in horses in Ethiopia, with growing concern about drug resistance, and therefore conducted a study to estimate helminth prevalence, assess risk factors, and evaluate anthelmintic efficacy. Standard parasitological methods were applied to 382 samples to detect parasite eggs or larvae, allocated into three treatment groups: fenbendazole, ivermectin, and an untreated control group. Fecal egg counts were performed on the day of treatment and 14 days later. The overall prevalence of gastrointestinal helminths was 72%, and the identified parasites included *Strongylus* species (63.87%), *S. westeri* (4.71%), *O. equi* (4.45%), *P. equorum* (5.5%), and most animals presented mild (51.0%) or moderate (38.2%) infections, while 10.7% had severe infections. The anthelmintic efficacy study demonstrated resistance to fenbendazole in horses, while parasites in donkeys remained susceptible to both fenbendazole and ivermectin, reinforcing the importance of gastrointestinal parasite control in equines and the judicious use of anthelmintics to control resistance and maintain effective parasitic control.

Piccoli *et al.* (2015) evaluated the occurrence of intestinal helminths in working horses (N=131) used in carts for recyclable waste collection and compared them with leisure horses (N=145) raised in Porto Alegre, Rio Grande do Sul. Positivity was 73% (202/276), with 64.8% (94/145) and 82.4% (108/131) for leisure and working horses, respectively, with a higher frequency of eggs from the Strongylidae family and low occurrence of *P. equorum*, *S. westeri*, and *Anoplocephala* spp. Menetrier *et al.* (2020) recorded 94% (64/68) gastrointestinal infection in horses treated at the university hospital in Porto Alegre, Rio Grande do Sul, with predominance of Strongyloidea eggs (78%), *P. equorum* (7.81%), and *O. equi* (9.4%). The study by Godéski & Pedrassani (2018) reinforces an important pattern in equine parasitology: ubiquity versus intensity. Even with 100% prevalence (N=35), the average EPG of 102 is low, suggesting a balance between the horses' immune system and herd management. There was 100% predominance of nematodes from the order Strongylida and 71.4% by *P. equorum* in animals up to 2 years; for horses aged 3 to 15 years, 96.4% presented parasitism by nematodes from the order Strongylida. The hatched larvae from the coprocultures of the eggs identified small strongyles. A study conducted by Lignon *et al.* (2020) in draft horses in the city of Pelotas, southern Rio Grande do Sul, recorded a rate of 90.2% (74/82) for some helminth, presenting an average count of 739.2 EPG, with higher prevalence of infections by parasites from the Strongylidae family (74.3%). In northern Brazil, Ferreira *et al.* (2024) studied horses raised on farms in the state of Pará. The result, through EPG, revealed 66.67% (20/30) eggs of parasites from the Strongylidae family.

Routine diagnostic methods can be evaluated for parameters such as sensitivity, specificity, accuracy, and precision. There are numerous variables that can affect accuracy in egg counting, including biological ones such as: uneven distribution of parasite eggs in a fecal sample; composition of parasite species within the animal, and the number of internal parasites present, which can affect egg production (Denwood *et al.*, 2023; Kaplan & Vidyashankar, 2012). Modern anthelmintic treatment programs in equines in North America use surveillance-based methods, in which only horses that contribute the highest number of parasite eggs to the pasture, and consequently increase infection pressure, are treated with an anthelmintic drug (Nielsen *et al.*, 2019; Rendle *et al.*, 2019). The American Association of Equine Practitioners currently recommends two fecal egg count methods to determine the number of strongylid parasite eggs: a modified McMaster technique and a modified Wisconsin technique. Both techniques require specialized equipment, including a flotation solution with density, a microscope, and a specialized counting slide or microscope slides (Nielsen *et al.*, 2019); however, these routine methods are widely used, and their cost is well-supported by breeders.

The control of endoparasites is one of the main challenges of equine health management and requires constant vigilance, based on diagnosis and monitoring of therapy and resistance to anthelmintics. The reviewed studies demonstrate that the issue is global, with reports on different continents involving several drug classes, which highlights the extent and severity of the phenomenon. Comparative analyses of international guidelines show that there is no absolute consensus on surveillance and control protocols, with regional differences prevailing related to the importance attributed to certain species and the availability of diagnostic resources, which reinforces the complexity of the scenario. In addition to scientific effort and technological innovations, addressing anthelmintic resistance in equines also depends on consistent public policies and extension programs that guide producers and veterinarians (Bulgaru *et al.*, 2021).

The development of new drugs is extremely expensive and is expected to be more expensive than older drugs. Therefore, it seems clear that the "global deworming" approach that has consolidated over the last 40 to 50 years needs to change, and animal producers need to develop a new vision for parasite control and production sustainability (Bellaw & Nielsen, 2020; Ghafar *et al.*, 2023). Furthermore, parasitologists must improve the study design and data analysis methods used for diagnosing anthelmintic resistance, especially for the fecal egg count reduction test. One critical point is the lack of a standard for comparing data between different studies (Ghafar *et al.*, 2021). The view of Kaplan & Vidyashankar (2012) highlights that we cannot just "wait for a new drug," as resistance tends to evolve faster than pharmaceutical innovation. It is necessary to adopt molecular methods that are more sensitive and cost-effective to become routine and early in identifying important parasites.

MacDonald *et al.* (2023) reviewed data on the Efficacy Reduction Period (ERP) in cyathostomins against the three main classes of anthelmintics: macrocyclic lactones, tetrahydropyrimidines, and benzimidazoles in 54 studies published over three decades (1972-2022). Until early 2022, there was no consensual definition for ERP, with eight definitions identified in the literature, which makes it difficult to compare the studies. It is not clear whether the reduction of ERP of these anthelmintics to such levels is due to the development of anthelmintic resistance (AR) or biological factors related to horses, cyathostomin species, and/or the environment. The ERP for other anthelmintics (fenbendazole and pyrantel) was often not reported due to already established resistance against these drugs. Nielsen *et al.* (2025) reported increasing incidence of resistance to the macrocyclic lactone class of drugs in cyathostomin parasites. Several European countries have implemented legal restrictions on the use of anthelmintics, allowing only under medical prescription, to reduce treatment intensity and decrease selective pressure for drug resistance, however, the long-term results of this approach have not been comprehensively evaluated. In Danish horses parasitized by *S. vulgaris*, it was selected 299 horses from 30 herds and four collaborating veterinary clinics. All horses with fecal egg counts were treated with ivermectin. The efficacy of ivermectin and the effective remission period were determined following current guidelines. Coproculture and PCR were used for the detection of *S. vulgaris*. Fecal egg count reduction tests based on egg counts by the McMaster method suggested inconclusive efficacy of ivermectin in two equine operations and total efficacy in all other populations, while the automated system suggested resistance to ivermectin in six operations and inconclusive results in another 8. The ERP of ivermectin was determined at least 8 weeks with both methods in all cases. The prevalence of *S. vulgaris* was 2.7% and 5.7% with coproculture and PCR, respectively, and all samples were negative with both methods at 8 and 24 weeks after treatment. Overall, ivermectin efficacy was high, although some results suggest that a reduction in efficacy may be occurring, justifying deeper monitoring. ERP estimates exceeded 8 weeks, suggesting no reduction. The two egg counting techniques showed general agreement, but the automated system detected more positive results at low egg count levels, leading to lower efficacy estimates in some populations.

Advances in equine parasite diagnosis involve molecular techniques that facilitate a deeper understanding of the epidemiology of different parasite species in a given horse population. Next-generation sequencing of target DNA regions has revolutionized the identification of gastrointestinal nematodes in production animals, potentially providing the relative abundance of prevalent species (Ghafar *et al.*, 2023). The use of ITS-2 region sequencing to characterize the nemabiome represents the most modern in parasitology; while EPG reveals how much infection exists, Nielsen (2022)'s work with ITS-2 amplicon sequencing identifies nematode species that the common microscope cannot differentiate. However, these advances in molecular biology are well-suited to unravel the helminthic fauna, but are unfeasible in laboratory conditions due to the high cost of equipment and necessary infrastructure, in addition to the high cost of parasitological exams for equine breeders. The breeder needs to know the parasitic burden of their herd and which treatment to perform, having difficulty affording high-cost exams. This precision veterinary medicine is still not accessible in most countries.

The most prevalent parasites were strongylids. The prevalence of negative animals reached 86.73% of the herd. There was no detection of *P. equorum* post-treatment. Infective larvae of *Gyalocephalus capitatus*, *Cylicocyclus radiatus*, and *Trichostrongylus axei* were recovered from the coproculture.

Author contributions: CRediT (Contributor Roles Taxonomy)

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