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Methodology article

SEASONALITY AND DYNAMICS OF GASTROINTESTINAL HELMINTHS IN GRAZING HORSES

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The prevalence of helminths in horses may vary, depending on climatic factors and host susceptibility. The objective of this study was to evaluate the seasonal dynamics of helminth infection in grazing horses, from October 2015 to September 2016. In total, 104 crossbred horses of different age groups, males and females in a single herd, participated in this study. Individual fecal samples were collected every 28 days for counting of eggs per gram of feces (EPG) and fecal cultures. The horses were evaluated individually for weight, body condition score (BCS) and clinical status. Climatic information relating to the farm was also collected. The categories most affected by helminths were foals and senior horses, with EPG averages of 1271.9 and 1186.5, respectively. The EPG averages for all animal categories together, in the four seasons were: 1042.1 spring; 1508.9 summer; 817.8 autumn; and 571.1 winter. Through the body condition evaluation, it was seen that senior animals presented the lowest BCS in all seasons. It was concluded that the season in which these animals were most affected by helminth infections was the summer and that the most susceptible categories were foals and senior horses.

Keywords: equines, nematodes, prevalence, susceptibility

INTRODUCTION

Horses are animals of great economic importance that can be used in sports, leisure and work. In Brazil alone, the total population amounts to approximately 5,600,000 animals, and this is the fourth largest breeding stock in the world [1]. The horse agribusiness in the country generates U$ 1.8 billion and 640,000 jobs [2]. However, the majority of Brazilian horse rearing is still carried out under an extensive regime, where by the animals are kept in the pastures all the time, which favors constant infection by parasites that are present in the pastures [3].

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Horses are one of the animal species that are most susceptible to a range of gastrointestinal parasites and they may harbor several species concurrently [4]. The nematodes that most affect horses in tropical climate conditions are the small strongyles (Cyathostomins) [5]. Thus, parasitic infections are important causes of morbidity and mortality in this species [6].

Several studies on horse breeding farms worldwide have demonstrated that helminth populations are present under a wide range of different geographic and climatic conditions [6]. The environmental conditions of a given region have a great impact on the life stages of the parasites present in pastures, including temperature, rain, humidity and sunlight [5]. This is because pastures are the place where the eggs develop and hatch, the larvae develop and the hosts become infected. Helminths have seasonal characteristics, with periodic differences in many different regions of the world. The most favorable conditions for helminth development in temperate countries occur in spring and autumn, while in subtropical countries these occur in winter [7]. However, in regions with a tropical climate, less is known about the status of gastrointestinal helminths in horses. Information on the biology and epidemiology of cyathostomins could contribute towards controlling these helminths [8-10].

In a survey in Rio de Janeiro (Brazil), Martins et al. [11] found that the majority of horse owners did not know what the time of greatest occurrence of helminths was. Similarly, [12] observed the times of administration of anthelmintics in several horse herds in the state of São Paulo (Brazil) and found that there was great variability among the periods and frequencies chosen for deworming the horses. In general, it has been observed that antiparasitic treatments are administered without any strategic basis. Most of these treatments are carried out in months in which the application does not necessarily correspond to the period of greatest elimination of eggs in the feces. Use of drugs in this manner, without application of appropriate criteria, may lead to unsatisfactory or ineffective results, even favoring appearance of helminth resistance to the active agents used.

Worldwide, the emergence of antiparasitic resistance has become a serious threat to the ability to control nematode infections [13]. Therefore, it is important to find strategic control methods that can contain the intensity and severity of infection with cyathostomins. According to authors, [14,15] knowledge of the biology of helminth infection remains incomplete and information about the seasonal nature of transmission and the influence of environmental factors from different study regions is required for successful control. This lack of knowledge about the basic biology of helminths makes it difficult to control these parasites, and particularly, highly prevalent species [16].

Therefore, the aim of the present study was to evaluate the seasonal dynamics of helminth infection in horses that were kept on pasture and the influence of different climatic characteristics of the seasons on the level of helminthiasis; and to identify the seasons and categories of horses with higher incidence of helminths.

MATERIAL AND METHODS

Ethical animal research

The study was approved by the Ethics Committee on Animal Use (CEUA) of Universidade Estadual Paulista (Unesp) (n. 15/2014).

Experimental design, area of study and animals

This study was carried out between October 2015 and September 2016, on a farm located in the western part of the state of São Paulo (Brazil), in the municipality of Castilho (latitude 20° 52' 09.0" south; longitude 51° 29' 22.9" west). This region has a tropical climate that is characterized by high temperatures, with a range that does not exceed 10ºC. The summers are hot and humid and the winters tend to have lower temperatures and lower rainfall [12].

One hundred and twenty days before the start of the experiment, all the horses received an anthelmintic based on moxidectin. This drug had previously been tested by authors [12], who ascertained that it was effective on this horse herd. After this dosing, the horses did not receive any further anthelmintics.

A total of 104 animals were used, males and females, including 10 lactating foals, 21 juvenile fillies up to 03 years of age, 19 empty mares, 10 pregnant mares, 3 intact males, 33 castrated males and 8 elderly animals, all from the same herd. It should be noted that these numbers of animals per category are at the beginning of the experiment. There were changes during the study according to the numbers of foal's births and the advancing age of horses of all categories.

Coproparasitological analysis

Individual fecal samples were collected directly from the rectum of each animal studied every 28 days, over a 12-month period, for coproparasitological analysis. The feces were stored in plastic bags that were then packed into isothermal boxes and sent to the Parasitology Laboratory of the College of Animal Science, UNESP, in Dracena, SP. Individual Fecal Egg Count (FEC) was performed using a McMaster's chamber, in accordance with the technique recommended by [17], as modified.

Fecal cultures were used to identify larvae through about 60 grams of feces from each category of animals were weighed and the stool culture was performed, according to the technique proposed by Madeira de Carvalho et al. [18], in order to identify the main genres, present in the different categories. Differentiation of pairs of strongylid species cells was used as the basis for identifying intestinal cells, such as the complete larvae, larval sheath (presence or absence of sheath), layer perilarval and the appearance of the tail of the sheath.

Hematological analyses

Individual blood samples of 1 ml were collected from all horses, every 84 days, i.e., in 4 periods. These samples were collected directly from the jugular vein and were placed into Eppendorf tubes containing one drop of EDTA (potassium ethylenediaminetetraacetic acid). The tubes were then transported in a refrigerator (mini-fridge) at 4ºC to the Parasitology Laboratory of the College of Animal Science, UNESP, in Dracena, SP. Blood counts were then obtained using the BC-5000 vet hematological analyzer, to evaluate the globular volume (VG) and quantify the eosinophils. The total plasma protein (PPT) was estimated using a manual ocular.

Weighing of animals and evaluation of body score

All the horses were weighed every 28 days using a digital electronic scale. From the results, it was possible to evaluate any weight changes and developmental retardation caused by helminthic infection or by changes in climatic conditions. The weight and body condition score were evaluated and classified as described by Henneke et al. [19], using a scale from 1 to 9 (1 = excessively lean animal; and 9 = excessively obese animal), based on observation of the animal's appearance and palpation of its adipose tissue cover.

Clinical examination

A monthly clinical evaluation of the animals was performed, to monitor the characteristic symptoms of parasitic spoliation. Every time the animals were thus managed, their behavior, consistency of feces and degree of apathy were also carefully evaluated. The stool consistency was assessed on a scale from 1 to 5: extremely dry stools (1), dry stools (2), normal stools (3), pasty stools (4) and diarrheic stools (5) [20]. The degree of apathy was assessed through the physical examination and the horses were classified as presenting no apathy, moderate apathy or severe apathy [21].

Climatic analysis

Daily climatic records were collected by the Agroclimatological Station of Castilho, SP (21º 05' 12.4" S; 51º 35' 17.2" W), which is located eight kilometers from the experiment site. Data regarding average air temperature, rainfall and relative humidity were collected throughout the experimental period [22]. Subsequently, the climatic effects were interpreted in terms of helminth seasonality.

Data Analyses

The data analyses were performed using the Minitab 17 statistical analysis system. Descriptive statistics of the observed variables and their sequential variation over time were evaluated and presented, in order to assess the population dynamics of

helminths in grazing horses. Variance analysis techniques (ANOVA) and Tukey's test were applied at a 5% significance level.

Consent was declared for the client-owned animals included in this study.

RESULTS

The categories of animals most affected by the cyathostomins were foals and seniors, presenting general EPG averages of 1271.9 and 1186.5. The EPG averages of the other categories were: 1042.8 for older foals, 1056.3 mares, 1017.2 castrated males and 1035 stallions.

Regarding the 10 lactating foals, these animals presented a mean EPG count at two months of age of 350 and at three months of 1133.3, with a visible gradual increase until reaching a peak at sixth months of 1606.3. From that point on, the EPG count decreased until the time of weaning, when the mean was 558.3 (Figure 1). At 9 months of age, the mean EPG curve of the foals was in line with the mean EPG curve of the animals in the other categories that had been evaluated until the time of weaning of the foals (Figure 1). After weaning, the curve for the foals rose abruptly and no longer followed the EPG curve of the other categories of animals.

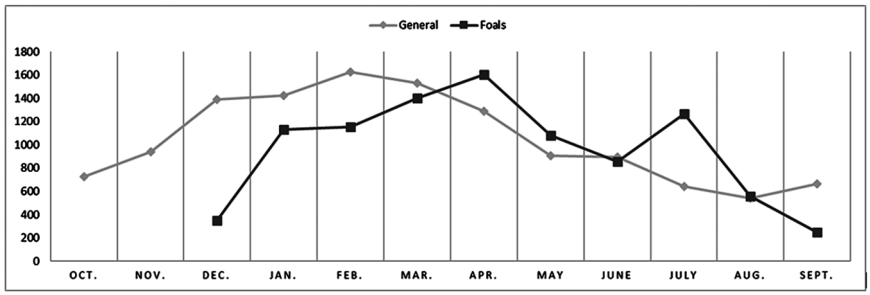


Figure 1. Mean egg count per gram of feces (EPG) of small strongyles for the entire experimental period of the animals studied in general and from the second month of life of the foals up to 10 months of age, reared on pasture.

When the mean EPG counts of the males (1081.3) and females (1071.7) were compared, no significant differences were observed.

The levels of parasitic infection of the animals were high at all collection times, and the lowest mean was found in August, with an EPG count of 540 (median 250). The highest mean or degree of infection was found in February with an EPG count of 1625.5 (median 1275) (Figure 1), which can be explained by climatic factors.

It could be seen that the EPG counts in the animals began to increase in the spring, with a peak in the summer (Table 1). The mean EPGs for all categories together, found in the spring, summer, autumn and winter were, respectively, 1042.1, 1508.9, 817.8 and 571.1, and all of the means were significantly different ($p < 0.05$) from each other.

Table 1. Mean of eggs per gram of feces (EPG), temperature (°C), relative humidity (%), and sum of precipitation (mm).

Season / Parameters	EPG	Temperature	Relative Humidity	Precipitation
Spring	1042.14b	26.65a	70.99ab	464.60a
Summer	1508.91a	26.91a	75.58a	544.40a
Autumn	817.81c	22.72b	65.82b	85.00b
Winter	571.13d	21.69b	54.18c	146.00b

Equal letters do not differ between lines.

The climatic variables of the region influenced the population dynamics of small strongyles (Cyathostomins) in these horses (Figure 2). The mean temperatures found during this study were 26.65°C in the spring, which did not differ from 26.91°C in the summer, but these were significantly different from the mean temperatures found in the autumn, of 22.72°C, and in the winter, of 21.69°C (Table 1).

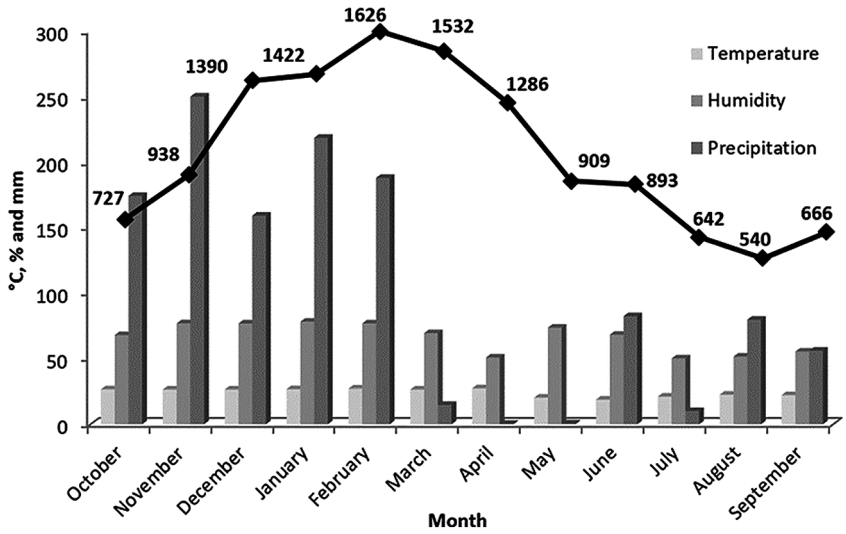


Figure 1. Mean egg count per gram of feces (EPG) of small strongyles for the entire experimental period of the animals studied in general and from the second month of life of the foals up to 10 months of age, reared on pasture.

The highest average for relative air humidity was found in the summer, and this was 75.58%. This was not significantly different from the average found in the spring, which was 70.99% (Table 1). The latter did not differ from the autumn average of 65.82%. In winter, the lowest relative humidity, of 54.18%, was observed, and this was significantly different from the means of the other seasons. The total rainfall was 464.60 mm in the spring, 544.40 mm in the summer, 85.0 mm in the autumn and 146.0 mm in the winter. And the season with the highest rainfall (summer) also presented the highest EPG count.

Regarding the clinical examination, only 8 horses in the spring and 7 in the summer (the periods of greatest challenge) presented pasty feces (score 4), with averages EPG counts of 760 and 1575, respectively. The rest of the horses presented feces scores of 3 (normal). In the autumn and winter (less challenging periods), no alterations in the consistency of feces were observed.

In the evaluation of apathy, only three horses, in the senior category, were observed to present moderate or severe apathy, because they presented high EPG values.

Overall, the horses also maintained good body condition during the study period, and the mean body condition scores (BCS) observed in each season were: 4.25 in spring, 4.41 in summer, 4.58 in autumn and 4.55 in winter. In the evaluation according to category in each season (Table 2), it could be seen that the senior horses had the lowest BCS in all seasons: spring, summer, autumn and winter, with scores of 3.83, 3.68, 3.56 and 3.14, respectively.

Table 2. Mean Body Condition Score of horses studied in the four seasons of the year

Category / Season	Spring	Summer	Autumn	Winter
Foals	4.05b	4.78a	4.95a	5.00a
Old Foals	4.24b	4.33ab	4.46a	4.51a
Mares	4.46bc	4.38c	4.78a	4.66ab
Castrated Males	4.19b	4.37ab	4.49a	4.53a
Stallions	4.19b	4.37ab	4.49a	4.53a
Senior Horses	3.83a	3.68a	3.56a	3.14a

Equal letters do not differ between lines.

The mean weights (kg) found for the foals according to season (spring, summer, autumn and winter) (Table 3) were 181.63, 162.44, 200.23 and 230.67. The older foals presented mean weights of 306.16, 327.85, 323.64 and 321.80, respectively, thus demonstrating an increase in mean weight in this category. Only the mean weight in spring differed significantly from the means of the other seasons.

Table 3. Mean weight (Kg) of horses reared on pasture in the four seasons of the year

Category/ Season	Spring	Summer	Autumn	Winter
Foals	181.63b	162.44b	200.23a	230.67a
Old Foals	306.16b	327.85a	323.64a	321.80a
Mares	369.14a	383.76a	395.03a	401.62a
Castrated Males	372.37a	385.94a	396.33a	393.72a
Stallions	445.89a	425.67a	444.89a	448.13a
Senior Horses	401.75a	384.44a	367.44a	362.67a

Equal letters do not differ between lines.

The results from the fecal cultures showed that 100% of the gastrointestinal larvae found were in the family of small strongyles (Cyathostomins). *Parascaris equorum* eggs were found only in the feces of the young foals (< 6 months of age).

No alterations were found in the hematological examinations of the animals, when the means of the blood parameters (leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, hemoglobin, globular volume and total plasmatic protein) of the four seasons were analyzed and of six categories. At the individual level, some values were observed below or above the reference values [23], presented. Despite the high level of parasitic infection, eosinophilia was not observed in the animals studied here, even in those most infected.

DISCUSSION

The foals and senior horse categories presented high EPG counts that were significantly different from the means of the other categories studied. These results corroborate the findings from a study on Dutch horses, which showed that the animals most affected by helminths were foals and animals over 23 years of age, and animals with more frequent or even permanent access to pasture [24]. Another author also observed that foals did not have immunity to gastrointestinal parasites and were therefore more susceptible to their pathogenic action [25].

In relation to cyathostomins infection in foals, it was observed that the OPG count increased from the third month of age, with a peak in the sixth month. According to Madeira de Carvalho et al. [18], foals are infected very early, since they begin to ingest grass in the first week of life, with a gradual increase in the elimination of eggs from two months of age (corresponding to the minimum pre-patent period of cyathostomins). Infection is controlled by the host itself, through reducing the number of migrating larvae and, consequently, the number of adults in the intestine [26,27].

The results from the fecal cultures showed that 100% of the gastrointestinal larvae found were in the family of small strongyles (Cyathostomins). *Parascaris equorum* eggs were found only in the feces of the young foals (< 6 months of age).

The level of infection of the animals was high at all collection times, with the lowest average found in August and the highest in February (Figure 2). This high parasitism in the population studied here is concordant with management situations involving long-lasting grazing, in which only one annual, non-selective deworming is performed [28,29].

The climatic variables of the region had a significant influence on the population dynamics of the helminths in these horses. In tropical countries the temperature and humidity are the most important factors, and these factors are responsible for the development of eggs and larvae in the environment [30].

Temperature is the climatic variable that most influences the development of the eggs of different species of Strongylidae to the point of larval hatching [31]. The velocity of larval development is directly proportional to temperature, and the L3 stage can be reached in a few days under high temperatures, or in a few weeks when the temperature is lower [32,33]. The main peaks of EPG counts occur during hotter periods [34]. It has been reported that temperatures between 25 and 33°C are optimal for the development of eggs and larvae [35,36], with complete development to the infectious form within a period of 3-4 days. This explains the lower number of eggs found in the winter, given that the average temperature of this season is below the optimum temperature. In addition to influencing larval development, temperature also influences migratory behavior and survival, together with precipitation, which is the main route for larval dispersal [37].

According to authors [38], the minimum humidity for larval development is be 30%. In the present study, all the seasons presented humidity above this limit, thus providing adequate conditions for larval development throughout the experimental period.

In the current study, the season that presented the highest precipitation (summer) also had the highest EPG counts (Table 1). These results agree with those found by Couto et al.[38], who observed that in the rainy season, in tropical climates (high temperature), horses presented higher EPG counts, which may be related to greater egg elimination by female horses when weather conditions are more favorable.

Migration of the L3 larval stage from the fecal mass to the grass occurs during the rainy season. During this period, the grass is more palatable for horses and they therefore increasing their food intake and, consequently, their L3 intake. According to a study by Bezerra et al. [39], rainfall is an essential element in the behavior of larvae, since it provides the necessary moisture for their migration to the pasture, thereby enabling active ingestion by horses. It is important to note that although 100% of the horses were parasitized and almost all the horses presented massive levels of infection, the majority of these animals did not show any clinical signs associated with helminthiasis. In the autumn and winter, which was the period of lesser challenge, no animals with altered feces consistency or presenting apathy were observed. These observations are in agreement with those of Larsen et al.[40] in Denmark, who also found that there was no association between diarrhea and high loads of gastrointestinal parasites in horses. In other studies, absence of clinical signs of helminths in horses was observed with EPG counts above 1000 [18,41-44].

In evaluating the body condition score, it was observed that the horses maintained a good condition score throughout the study. In the evaluation according to category in each season (Table 3), it was found that the senior horses presented lower BCS in all seasons. This score observed in senior horses was probably due to musculoskeletal problems that led to muscle atrophy due to inactivity of muscles [45]. The category that presented the highest BCS in the spring was that of the mares, as was also seen in the summer, autumn, and winter.

The results from the fecal cultures showed that all the gastrointestinal larvae found were of the small strongyles family. This can be explained by the deworming that was performed 120 days before the start of the experiment. It is known that some parasites have a very long-life cycle, and that it may take 12 months for the adult forms to appear [46]. The pre-patent period of cyathostomins is two to three months [47]. Others authors also found, in all groups of horses studied, that cyathostomins were abundant in the fecal cultures (proportions of 95 to 100%) [48].

Regarding the hematological evaluation, no alterations were found in the samples. Despite the high level of parasitic infection, eosinophilia was not found in the horses studied here, as also observed in the study by Lhamas et al. [23]. This partly contradicts the concept that eosinophilia should accompany situations of endoparasitism among horses. The explanation for this may be that eosinophilia occurs particularly as a consequence of larval-phase parasite migration after pure solid experimental infections, especially by *Strongylus vulgaris*, and that in cases of natural infections, cyathostomins account for the great majority of parasites present in the intestinal tract, compared with large strongyles [49].

It was concluded that the population dynamics of the helminths of these horses were influenced directly by the climatic characteristics, and that the seasons with higher temperature, humidity and precipitation were also the periods when the horses had higher degrees of helminth infection. The categories most susceptible to helminth infection were foals and senior horses. Thus, one possible treatment strategy would be to concentrate anthelmintic administration in the spring and summer, regardless of body condition, with greater attention given to the most susceptible categories.

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Authors' contributions

IL participated in research planning and field data collection, laboratory and data analysis, and writing the manuscript. JA field data collection, laboratory data analysis, and preparation of tables and figures. TA participated in the corrections of the manuscript, and the preparation of tables and figures. MO supported laboratory analysis and participated in data analysis and manuscript writing. IA participated in laboratory analysis and data tabulation. GM participated in statistical analysis and manuscript writing. HJ field data collection and laboratory tests. RV conceived of the study, participated in its design and coordination, and helped draft the manuscript. GJ data tabulation. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SEZONSKO POJAVLJIVANJE I DINAMIKA GASTROINTESTINALNIH HELMINATA KOD KONJA NA PAŠI

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Prevalencija parazitoza izazvanih helmintima kod konja može značajno da varira u zavisnosti od klimatskih faktora i prijemčivosti domaćina. Cilj studije je bio da se obavi evaluacija sezonske dinamike helmintoza konja na ispaši u periodu od oktobra 2015. do septembra 2016. Ukupno je ispitano 104 konja, različitih starosnih kategorija, pastuva i kobila u jednom krdu. Individualni uzorci fecesa su sakupljani svakih 28 dana u cilju određivanja broja jaja parazita po gramu uzorka kao i pravljenja kulture fecesa. Konji su individualno ispitivani u odnosu na telesnu masu, skor telesne kondicije (BCS) i klinički status. Isto tako, sakupljeni su i podaci u vezi klimatskih prilika na farmi. Najviše ugrožena kategorija, u odnosu na invaziju helmintima bili su ždrebad i stariji konji kod kojih je prosečan broj jaja po gramu fecesa bio 1271.9 odnosno 1186.5. Prosečan broj jaja po gramu fecesa zbirno za sve kategorije ispitivanih konja, za četiri sezone bio je 1042.1 tokom proleća, 1508.9 tokom leta, 817.8 u jesen i zimi 571.1. U odnosu na evaluaciju telesne kondicije, uočeno je da su odrasli stariji konji imali najniži skor, tokom svih ispitivanih sezona. Na osnovu rezultata, zaključeno je da je najveći pritisak invazije helmintima kod ispitivane grupe konja bio tokom leta. Najugroženije kategorije su bili ždrebad i odrasli konji.