



Prevalence of Gastrointestinal Nematodes and Larval Stages in Horses from Romita, Guanajuato, Mexico



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Abstract

Parasites in horses are a serious problem worldwide, they can cause serious health problems, even death. The aim of the present study was to determine the prevalence of gastrointestinal parasites and larval stages in horses from the municipality of Romita, Guanajuato, Mexico. Twenty-eight horses were sampled in the Mexican Bajío Region. The Sex, Age, Zootechnical Function, Community of origin and previous deworming were recorded. Coproparasitoscopic examination was performed by flotation, the McMaster Technique, and the Larval Culture Technique. The horses were 78.5% female and 21.4% male, and both were positive for Strongyles spp. with a prevalence of 42.8%, for the Place the highest prevalence was for La Gavia with 21.5%, for the Zootechnical Function the highest prevalence was obtained by Work with 28.5%, for Age the highest prevalence was for > 5 years 32.1%. Finally for deworming the highest was for Never of 17.8%. The threshold of counts greater than 200 eggs per gram of feces was exceeded by 35.7%, showing equines with parasitosis of relevance to their health. The larvae obtained in the larval culture were from the older horses and the egg count per gram of feces.

Keywords: parasites, horses, animal health, Mexican Bajío Region, Strongylus.

Abbreviations: Eggs Per Gram of Feces (EPGF); gastrointestinal nematodes (GIN); Deworming (DWM)

Introduction

Gastrointestinal parasitism is a common animal health problem affecting equine populations. The main health problems caused by gastrointestinal parasites include poor performance, anemia, diarrhea, weight loss, poor growth, acute abdominal syndrome, malnutrition, and circulatory system disorders, leading to death of animals [1]. Parasites that infect equines can cause several degrees of damage, depending on the species, the number of parasites, and the immune status of the host [2]. The agricultural production farms that have horses in the municipality of Romita, Guanajuato are backyard type system, which they are used as traction and transport animals, these are in precarious conditions as far as preventive medicine is concerned, since the owners are unaware of the problems that parasitosis can cause in them, the study of the prevalence of parasites in equines is essential to

understand the species of parasites and their population dynamics [3]. Anthelmintic resistance and environmental concerns have forced a reconsideration of the use of best control practices, so monitoring parasite populations is of utmost importance, to increase the efficiency of treatments, while affecting the environment as little as possible [4]. The lack of susceptibility of parasites to anthelmintics is a problem since it is constantly generated by the parasite when, faced with a therapeutic dose of an anthelmintic, a greater number of individuals survive than those that survive in a population susceptible to parasites. Thus, this resistance is hereditary, which is aggravated by use without the correct orientation and knowledge about the parasites against which it will be medicated [5]. There is not enough literature on the prevalence of parasites in equines in the Mexican Bajío Region where there are mainly many animals in backyard type system

which are constantly affected by parasites, moreover, lacking studies difficult to carry out treatments properly [6]. Therefore, the aim of this study was to determine the prevalence of gastrointestinal parasites and larval stages in Romita, Guanajuato, Mexico horses.

Materials and Methods

Location: The municipality of Romita is located between the parallels 20°57' and 20°41' of northern latitude and the meridians 101°27' and 101°45' west longitude, with an altitude ranging from 1700 to 220 meters above sea level, with average rainfall is recorded from 600 to 800 mm, with a temperature range of 16 to 20°C and a semi-warm subhumid climate with summer rains [7]. Equines from the communities of Santa Rosalía, San Francisco, San Vicente and La Gavia were sampled.

Sampling: A convenience sampling was carried out, in which horses older than 6 months were sampled regardless of the conditions in which they were found, and data were registered and collected on the characteristics of the horse: Sex, Age, Zootechnical Function (activity), Place or community and Previous deworming were recorded. Feces for coprological examinations were taken directly from the rectum, first subjection and equines management, once the samples were taken, they were placed in plastic bags and placed in a polystyrene cooler to keep them between 4 to 7°C for transport to the laboratory.

Coproparasitoscopic method by flotation: Glucose solution was used for the review of the samples, for each sample 20 mL of solution was used and mixed with 3 g of feces, It was homogenized and filtered, placed in a 12 mL Falcon tube, centrifuged at 1500 revolutions per minute for 10 minutes in a centrifuge Sorvall ST40R, Later, with the help of a transfer pipette, the supernatant was taken and observed under the microscope 4, 10 and 40X, They were subsequently counted in Eggs Per Gram of Feces (EPGF) by means of the McMaster Technique [4,8]. The eggs found were identified morphologically, corroborating with the measurement under the microscope [9,10].

Larval Culture Technique: 15 grams of fecal matter positive for gastrointestinal nematodes (GIN) was mixed and 15 grams of sterile sawdust, distilled water was added until the humidity was 80 to 85%. The samples were placed in the incubator under aerobic conditions at 25±1°C during 7 to 12 days to develop infective larva (L3). Subsequently the contents were placed in a Baermann funnel [11] and the sample (liquid) was obtained in a Petri dish to observe with the stereoscopic microscope and identify the infective larvae [10].

Statistical analysis

The data were analyzed using descriptive statistics, which were expressed in percentages to determine the general prevalence, and evaluated: the sampled population, prevalence and egg count

per gram of feces. The methods used to analyze the data were non-parametric since the response variable hadn't a normal distribution. With the results of the McMaster Technique, the Kruskal-Wallis's test was performed, using the binary dependent variable EPGF, which was recorded as: 1) negative and 2) positive. As independent variables were evaluated: Sex with two levels: 1) male and 2) female; the Place (sampling community) with 4 levels: 1) Santa Rosalía, 2) San Francisco, 3) San Vicente and 4) La Gavia; the Zootechnical Function (activity) with three levels: 1) recreation, 2) work and 3) sport; Age in years with two levels: 1) < 5 and 2) >5; and the Use of Deworming with two levels: 1) ≥ 1 in the last year and 2) Never dewormed. Each factor was evaluated independently, to evaluate the association between eggs per gram of feces with each independent variable, Additionally, the Chi-square test ($P < 0.05$) of independence was performed. Statistical software Statgraphics Centurión, v18 was used for data analysis [12].

Results

Prevalence of positive cases by Factors

With cooperating owners were worked, collected and analyzed 28 samples from which positive samples were obtained for gastrointestinal parasites in equines, the nematodes found in all samples were of the genus *Strongylus* spp. with an overall prevalence of 42.8% (12) as shown in the Table 1. The horses sampled according to their characteristics were grouped as follows: for Sex, a higher percentage of Females was obtained with 78.5% (22) and the 21.5% (6) of Males.

The overall prevalence was 42.8% (12) and was presented as follows: for Sex the largest was for Females with a 35.7% (10), and the lowest was for males with 7.1% (2); For the Place the highest prevalence was for La Gavia with a 21.5% (6) while the minor was for San Francisco with a 3.6% (1) as for Sta. Rosalía and San Vicente the prevalence was 7.1% (2) and 10.8% (3) respectively; For the Zootechnical Function, the highest prevalence was obtained by level of the Work with a 28.5% (8), Recreation obtained a prevalence of 14.2% (4) and the lowest prevalence was for Sport with a 0%; For Age the prevalence for < 5 years was 10.8% (3) and to > 5 years of 32.1% (9); Finally for deworming ≥ 1 was 25.0% (7) and Never 17.8% (5).

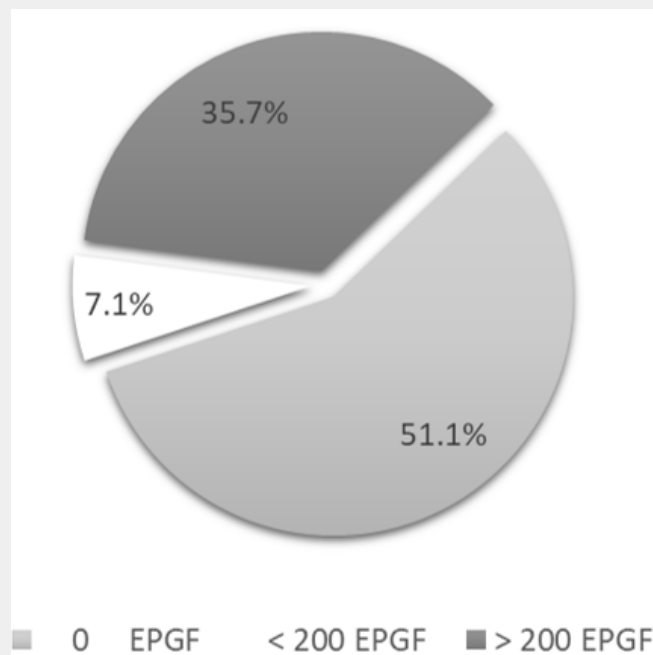
Distribution of positive cases based on parasitosis relevant to equine health

Regarding the EPGF count, the distribution was based on the threshold number of 200 EPGF the following was observed: the 51.1% of the samples resulted in 0 EPGF being negative for parasitosis, 7.1% were grouped in the range of <200 HPGH and the 35.7% resulted with parasitic load counts of >200 EPGF, with parasitosis of relevance to their health (Figure 1).

Table 1. Prevalence of parasites in equines, classified by zootechnical function (activity), age, sex, place, deworming and test results performed, in the municipality of Romita, Guanajuato, Mexico.

Factor	Level	n (%)	Positive (%)	EPGF [SD]	Chi-square	Kruskal-Wallis
Sex					NS	NS
	Female	22 (78.5)	10 (35.7)	592.5 [653.7]	0.6754	0.681153
	Male	6 (21.5)	2 (7.1)	1443.0 [1282.6]		
Place					NS	NS
	Santa Rosalía	7 (25.0)	2 (7.1)	1788.0 [794.7]	0.8337	0.841412
	San Francisco	3 (10.8)	1 (3.57)	792.0 [0.0]		
	San Vicente	7 (25.0)	3 (10.7)	1030.3 [970.3]		
	La Gavia	11 (39.2)	6 (21.4)	225.3 [163.4]		
Activity					**	**
	Recreation	12 (42.8)	4 (14.2)	970.7 [801.1]	0.0021	0.00264
	Work	9 (32.2)	8 (28.5)	616 [795.3]		
	Sport	7 (25.0)	0 (0.0)	0.0 [0.0]		
Age					NS	NS
(Years)	< 5	8 (28.5)	3 (10.7)	263.6 [103.3]	0.824	0.827253
	> 5	20 (71.5)	9 (32.1)	891.1 [850.1]		
DWM					**	**
	≥ 1	22 (78.5)	7 (25.0)	734.2 [779.8]	0.0054	0.00671
	Never	6 (21.5)	5 (17.8)	939.8 [878.2]		
Total		28 (100)	12 (42.8)	734.2 [779.8]		

DWM: deworming; EPGF: eggs per gram of feces; NS: not significant; Probability level (significance): *P<0.05; **P<0.01; [SD]: Standard deviation.

**Figure 1:** Distribution of the number of eggs per gram of feces (EPGF) based on the threshold of 200 EPGF in the samples of horses from the communities of the municipality of Romita, Guanajuato, Mexico.

Larval cultures

As for the larval stages of *Strongylus* spp. of equines in the laboratory, the positive samples were from equines from Sta.

Rosalía and San Vicente, from equines older than 15 years, with a zootechnical function of work and recreation (Table 2).

Table 2. Development from egg to larva *Strongylus* spp. in larval cultures of coproparasitoscopic samples from horses in Romita, Guanajuato, Mexico.

M	Lugar	Sexo	FZ	Edad	DWM	EPGF	LC
1	Sta. Rosalía	Male	Work	25	Never	2350	Positive
2	Sta. Rosalía	Female	Work	23	Never	1226	Positivo
3	San Vicente	Female	Recreation	15	>1	2146	Positivo
4	San Vicente	Female	Recreation	3	Never	383	Negativo
5	San Vicente	Female	Recreation	12	>1	562	Negativo
6	San Francisco	Female	Recreation	13	>1	791	Negativo

M: Sample number, DWM: Deworming; FZ: Zootechnical Function; EPGF: Eggs per gram of feces; LC: Larval culture.

Discussion

Based on the aim of this study, gastrointestinal parasites were found in more than 40% of the horses in Romita, Guanajuato, and the factors zootechnical function and last deworming were related to the prevalence of parasites, compared to the factors Sex, Age and Place, they did not show differences in the levels raised in the statistical study, however, the highest EPGF counts occur in male equines, older than 5 years and in the community of Santa Rosalía, in larval culture, positive cases were males and females, dedicated to work and recreation, over 15 years of age and with counts greater than 2000 EPGF. A high percentage of equines exceed the 200 EPGF threshold. Chaparro-Gutiérrez et al. [6] who conducted a study in Antioquia, Colombia with 664 horses, which 360 animals tested positive *Strongylus* spp., This was the family with the highest prevalence compared to the other species of parasites found in the study, which gives a prevalence of *Strongylus* spp. of 54% value very similar to that found in the present study, although there was a significant difference in the number of animals sampled, While in terms of sex no significant difference was found, since females had a prevalence of 56.3% and males 55.9%, which does not agree with what was found in this study, in which a significant difference was observed in the sex factor, This may be due to the fact that the majority of the equines dedicated to the zootechnical work function in the study are females, and have conditions of little or no preventive medicine due to lack of knowledge of the cause and low resources of the owners. Prada y Romero [13] carried out work in the savannas of Casanare, Colombia, where they collected 80 samples from 4 different municipalities from June 2006 to April 2007, with the aim of determining the populations of parasites that affect equines in that region, these authors reported a prevalence of 86.2% for small *Strongylus*, 6.2% for *Strongylus vulgaris*, 3.6% for *Strongylus edentatus* and 2.8% for *Strongylus equinus*, 1.1% for *Strongyliodes westeri* with an overall average of 984 EPGF, by municipality the average was 1569 Paz de Ariporo, 1319 Aguazul, 858 El Yopal and 190 for Maní, an average of 100 larvae were obtained per coproculture, which were observed 15

days after the samples were prepared, Their results were similar to those of the present study, with prevalence, EPGF counts and development of infective L3 larvae in cultures, which could surely be due to the conditions of the area with a tropical climate that favor the development of parasites. Bedoya et al. [14], conducted a study with 200 equines (horses, mules and donkeys) from different areas of municipalities in Colombia, using the McMaster test, in which 90% prevalence was determined but only 3% was *Strongylus* spp., a result that was below what was found in the present study, even though similar animal species were used, The results were probably influenced by environmental conditions at the study site and by the difference in endemic parasites in these areas.

The interest of the study of gastrointestinal parasites can have different objectives depending on the problems that arise at the field level in equines, Laugier et al. [15] worked with a total of 455 foals aged 3 to 9 months, from 11 Thoroughbred horse farms and four farms French Trotter horse in Normandy, France, collecting samples from July to October 2010, with the aim of estimating the prevalence of *Parascaris equorum* in foals, using the McMaster technique with saturated sodium chloride solution, with a detection limit of 50 HPGH, in addition to investigating the efficacy of ivermectin, using the Fecal Egg Count Reduction test (FECR) on three farms with suspected resistant populations. Most foals included in the study (80%) were Thoroughbreds, their average age was 5.5 months, the mares and their foals spent most of their time in pastures. From three of the farms with cases of parasitosis diagnosed postmortem, 36 foals naturally infected with *P. equorum* (≥ 100 EPGF) were selected to test the efficacy of ivermectin. The authors reported that for *P. equorum* the overall prevalence was 30.5% (388 EPGF), with variations from 6.9 to 76.2% depending on the location (farm). Regarding the prevalence with respect to age, the highest was in foals aged 3 to 4 months (38.1% and 596.6 EPGF) and lowest in those aged 8 to 9 months (15.3% and 186.5 EPGF). 6.9% of infected foals showed EPGF values of ≤ 200 . EPGF ranged from 30 to 39.6% (Farm 6) and from 64 to 68.9% (Farm

15). In the three farms, the overall efficacy of ivermectin against *P. equorum* was 52 to 54.2%, demonstrating that there were ivermectin-resistant populations of *P. equorum* in France. Also, in this trial the authors report that prior to ivermectin treatment, 14 foals (38.9%) had positive *Strongylus* spp. egg counts ranging from 50 to 3300 (average 574), which were susceptible to treatment. The present study differs in terms of population, because the equines were of different ages, this mainly because the focus was very directed to the problem in the case of foals in France, and most likely from this same thing, the differences between the EPGF ranges were derived. It is important to emphasize that both works highlight the importance of studying parasite populations, and based on this, horse breeders and veterinarians should consider the efficacy of anthelmintics and promote sustainable non-chemotherapeutic methods to control parasitic infections in animals with desirable characteristics to possess parasitosis, for example, age.

Morales et al. [16], conducted a study with thoroughbred horses, sampling horses that were in quarantine at the La Rinconada hippodrome, Caracas, Venezuela. The authors used the flotation and McMaster techniques, obtaining a prevalence of 60% of intestinal parasites, which were results much higher than those found in this study, where for the Activities performed factor, sport horses presented a null prevalence, this could be due to the environmental and management differences between both studies. Martin et al. [17] conducted a study with 142 foals aged 3 to 12 months, on farms in Sweden, using a similar technique to that used in the present study, the authors were able to find that the foals that tested positive had counts between 150 and 12,900 EPGF of *Parascaris* spp., these counts were similar to those found in the present study, since most of the positive samples when analyzed with the age factor, were within the range of <5 years, which was similar to the present study, because the EPGF counts were greater than 200 EPGF with an average of 263.6 EPGF, although the parasites found in the studies were of different species, this could be due to the differences between the regions in which the studies were conducted and the differences in their weather conditions.

Buzatu et al. [18] conducted a study in Romania with 195 horses, finding positive cases for *Strongylus* spp., 70% of the horses exceeded the value of 250 EPGF, which was very similar to that found in the present study, despite the differences between regions, however, two of the three farms analyzed by the authors, carried out up to 3 anthelmintic treatments per year and still had high levels of EPGF, which does not agree with what was found in the present study, since in the Last Deworming factor, the level of <6 months presented a prevalence of 0, which could be due to the generation of resistance to anthelmintics due to their inadequate use, Thereby so the authors recommend continuing to work in order to design better control programs, with a lower risk of selection of resistance to anthelmintics.

Sheferaw and Alemu [19] in Damot Gale, Ethiopia, studied gastrointestinal parasites in 500 equines, 283 donkeys and 217 horses, a prevalence of 100% for *Strongylus* was reported in both species, and 10.2% in donkeys and 5.5% in horses for *Parascaris*, they also report the presence of *Strongyloides*, *Fasciola*, *Gastrodiscus* and *Oxyuris*, significant variations were observed in the age and purpose factors of the animal, as well as in the parasitic load with an average of 689.2 EPGF (721.6 in donkeys and 641.5 in horses), being the sex factor the only one that did not present significance, which agrees with what was found in the present study, since both the factors and the average of EPGF were very similar despite the difference in the animals sampled and the prevalence found, being the age level, the only one in which the significance was different from that found in the present study, since the authors reported that those horses with a younger age were those that presented a higher parasitic load with an average of 963.1 EPGF, in addition to a higher prevalence of gastrointestinal parasites in general, which may be due to the different tests used, since the authors, in addition to the flotation technique, also used the sedimentation technique, this added to the geographical and management differences of both areas.

Bracken et al. [20] worked in Denmark (Zealand, Funen and Jutland) with fecal samples from 331 horses in 18 different farms collected from September to October 2009, with the aim of comparing the efficacy of traditional larval culture and individual and group polymerase chain reaction (PCR) testing. Selective deworming was used for 2 years in horses, the population per farm varied from 6 to 56 (average 18.4) and with ages from 2 months to 31 years (average 9.1), mainly of the Icelandic breed and its crosses, the zootechnical function was mainly recreational riding and a stud farm was included. Both tests were performed on all horses, PCR was performed on 66 groups of feces (3 to 5 horses/group). The authors report the presence of *Strongylus vulgaris*, with a prevalence of 12.1% (40) with individual PCR and 4.5% (15) with larval culture, with a range of 0 to 5440 EPGF. In total, 73 horses (22.1%) had negative egg counts, of which ten were positive for *S. vulgaris* by PCR, while two were positive by larval culture. No association was found between the occurrence of *S. vulgaris* and age, sex, farm or interactions between these using logistic regression. At farm level, eight farms were positive by larval culture, while 13 (72%) and 11 (61%) farms were positive by individual and combined PCR, respectively. Overall, horses that were positive by either method were between two months and 21 years old with a mean age of 8.2 years. Their results were like those of the present study, because they found the same parasite, and the Larval Culture test did not detect cases that other tests did. This could mainly be due to the difficulty of adapting the biological cycle of the gastrointestinal nematode to laboratory conditions. It should be noted that both studies contribute to the monitoring of parasite populations and emphasize the importance and applications that this has on control programs and the use of medications.

Based on the results obtained in this study, it is recommended to continue with the studies of parasites in horses from Romita, Guanajuato, in order to continue monitoring parasite populations, and based on the results, to make recommendations for better management in parasite control, since it was observed that none of the horses that were dewormed every 6 months presented parasitosis, so the interval between dewormings can be increased, in addition to strengthening control measures in those horses with the zootechnical function of work, as mentioned Valdéz-Cruz et al., [21] and those that were older than 10 years, since they were the ones that presented a higher prevalence regardless of sex. In addition, it is considered convenient to rotate the kind of dewormer in each deworming that is carried out, to avoid generating resistance to the chemical products used, as well as the use of alternatives such as biological control, strengthening of the immune system, use of disinfectants in facilities to cut the biological cycle in the phases outside the host, and a constant review of the health status of the animal, in order to timely detect any sign that it presents related to parasitosis. Obtaining infective L3 larvae from the cultures carried out in this study is very important since it greatly supports future research on the standardization of the biological cycle of parasites (nematodes) in the laboratory, this is a valuable tool in the detection tests for resistance of parasites to anthelmintics (McArthur et al., 2015).

Conclusion

The results of the present study allowed us to conclude that the equines sampled from the municipality of Romita, Guanajuato had a prevalence of 42.8% of parasites of the genus *Strongylus* spp. The community with the highest prevalence was La Gavia, although the highest EPGF count was obtained in the community of Sta. Rosalia. The equines with the highest prevalence were females and had the Zootechnical Working Function, however, those with the highest EPGF were those of Recreation, 35.7% had a parasitic load that could affect their health. The larvae obtained in the larval culture were from the oldest equines with the highest egg count per gram of feces.

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Conflict of Interest

The authors declare that they have no conflict of interest in relation to the study carried out.

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