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Parasite prevalence among equidae in the backland of the State of Pernambuco, Brazil

Frequência parasitológica em equídeos do sertão Pernambucano, Brasil

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Abstract

The present study aimed to assess the prevalence of gastrointestinal and lung parasites in equidae reared in the backland of the state of Pernambuco, Brazil and also evaluate the influence of equidae species, rearing system, age group, and anthelmintic administration schedule the infection level. 185 fecal samples were randomly collected in cities in the backland of Pernambuco. After collection, the material was submitted to a qualitative coproparasitological analyses and number of eggs and larvae of parasites. The horses were found to have lower EPG count than the donkeys. Regarding the rearing system, equidae reared in pasture had higher EPG counts than those confined in pens. Moreover, foals under one year old have lower EPG count than those between one and two years old. No difference was found in EPG count among de equidae that received anthelmintic at different frequencies, while the horses and donkeys had a higher amount of Cyathostominae than of other nematodes researched. It is concluded that, in the backland of Pernambuco, most equidae had light to moderate parasite loads, with a predominance of the Cyathostominae e Strongylinae subfamilies, with no horses, donkeys, or mules infected by *Dictyocaulus arnfieldi*. In addition, the donkeys had higher parasite loads than the horses and the extensive grazing system presents a greater risk of infections by nematodes.

Key words: Caatinga. Donkeys. Equines. Mules. Nematodes.

Resumo

O presente estudo teve como objetivo verificar a frequência de parasitos gastrointestinais e pulmonar em equídeos criados no sertão pernambucano e também avaliar a influência das espécies equídeas, sistema de criação, faixa etária e frequência de vermifugação no grau de infecção. Foram coletadas 185 amostras fecais de forma aleatória em municípios do sertão pernambucano. Após coleta, o material foi submetido à análises coproparasitológicas qualitativas e números de ovos e larvas dos parasitos. Observou-se que os equinos apresentaram menor contagem de OPG que os asininos. Em relação aos sistemas de criação, equídeos mantidos a pasto apresentaram maior contagem de OPG que os animais criados confinados em baias. Além disso, potros com menos de um ano de idade apresentaram menor contagem de OPG que os animais na faixa etária entre um e dois anos e meio. Não houve diferença na contagem de OPG dos equídeos submetidos às diferentes frequências de vermifugação, e nos equinos e asininos a quantidade de larvas de parasito da subfamília Cyathostominae foi superior aos demais nematóides pesquisados. Concluiu-se que no sertão pernambucano a maioria dos equídeos apresenta cargas parasitárias leves a moderadas, com prevalência das subfamílias Cyathostominae e Strongylinae, não havendo nesta região infecção de equinos, asininos e muare por *Dictyocaulus arnfieldi*. Além disso, os asininos apresentam maiores cargas parasitárias que os equinos e o sistema extensivo, à pasto, apresenta maiores riscos de infecção por nematóides.

Palavras-chave: Asininos. Caatinga. Equinos. Muare. Nematóides

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Introduction

The backland of the state of Pernambuco, Brazil, is characterized by tropical semi-arid climate with negative water balance due to the mean annual rainfall below 800 mm, mean annual sunlight incidence of 2,800 h, mean temperatures between 23 °C and 27 °C, evaporation of 2,000 mm per year, and 50% mean relative air humidity (MENDES et al., 2014). The local vegetation is hyperxerophyllous caatinga with patches of deciduous forest and is periodically affected by droughts that cause partial or total losses in agriculture and cattle farming (ASSIS et al., 2014).

According to Morales et al. (2012), equidae are infected by a broad range of internal parasites, with the ones in the gastrointestinal tract being the most prevalent. According to Dias et al. (2014), equidae rearing systems in Brazil favor a high incidence of parasite infections at as early as the first weeks of life. Given the negative correlation between high parasite load and performance of horses, donkeys, and mules in their working roles, adopting appropriate sanitary practices is important to control worms in the herd (MOLENTO, 2005).

The main clinical symptoms shown by the helminth-infected equidae are weakness, rough hair, slow growth, intestinal cramps, and diarrhea (TEIXEIRA et al., 2014). The parasitic helminth fauna in horses, donkeys, and mules is broad and important for animal sanity (FERREIRA et al., 2014). Among such fauna, the small strongyles (*Cyathostomum* spp., *Cylicostephanus* spp.), large strongyles (*Strongylus vulgaris*, *S. equinus*, *S. edentatus*), *Parascaris equorum*, *Oxyuris equi*, *Strongyloides westeri*, *Trichostrongylus axei*, *Habronema* spp., *Dictyocaulus arnfieldi*, and *Anoplocephala* spp. (MOLENTO, 2005) stand out.

Nematodes infect equidae of all age groups, but are more aggressive in young animals due to their late immunity development (ALMEIDA et al., 2008). In adults, inappropriate nutrition associated to excessive physical strain compromises

the immune system, which exposes the animal to infections (FERRARO et al., 2008). In the extensive grazing rearing system, equidae become infected mainly by consuming grass contaminated with larvae, more commonly in the rainy season since the higher moisture in the pastures favors infection (LANGROVÁ et al., 2003). The animals reared in confinement in small pens or stalls, in turn, may have high parasite loads deriving from the high population density (CARVALHO et al., 2007).

With this in mind, the present study aimed to assess the prevalence of gastrointestinal and lung parasites in equidae reared in the backland of the state of Pernambuco, Brazil, and also evaluate the impact of equidae species, rearing system, age group, and anthelmintic administration schedule on the infection level.

Material and Methods

The study was carried out with samples of equidae feces randomly collected in nine cities of the backland of Pernambuco: Afogados da Ingazeira, Belém do São Francisco, Custódia, Floresta, Flores, Inajá, São José do Belmonte, Serra Talhada e Triunfo. The samples were directly collected from the rectal ampulla or from the surface of the fresh fecal bolus from a total of 185 equidae of different ages, being 152 horses, 20 donkeys, and 13 mules. During collection, a questionnaire was applied to the animals' owners inquiring about the rearing system, deworming schedule, and animal age. After collection, the samples were identified and stored in an isothermic box with recycled ice for analyses at the Biology Laboratory of the Federal Rural University of Pernambuco, Academic Unit of Serra Talhada.

In order to assess the effect of the equidae species on the prevalence of gastrointestinal parasites, a completely randomized design was used with three treatments: horses, donkeys, and mules. In order to evaluate the effect of the equidae rearing system on parasite prevalence, a completely randomized

experimental design was used with two treatments: animals reared in the extensive grazing system or confined in pens. The effect of the animals' age groups on parasite prevalence was assessed using a completely randomized design with five treatments: animals below one year old, between one and two and a half years old, between three and five years old, between six and ten years old, and above ten years old. The effect of the deworming schedule on the presence of gastrointestinal parasites was evaluated using a completely randomized design with seven treatments: equidae dewormed monthly, every other month, every three months, every four months, every six months, annually, and non-dewormed.

The eggs per gram of feces (EPG) were counted according to Gordon and Whitlock (1939) modified technique, weighing 2g of feces and transferring to plastic cups, homogenizing in 58 ml of saturated sugar solution. Afterwards, two areas of McMaster's camera were fulfilled with the alicot of this fecal suspension. The Reading was done after one to two minutes on optical. The counting was done in the two cells of the McMaster's camera, the number of found eggs was multiplied by 100, to achieve the number of eggs per grams of feces. While coproculture was performed according to Roberts and O'Sullivan (1950). The presence of the lung parasite *Dictyocaulus arnfieldi* was verified using the Baermann technique described by Willcox and Coura (1989).

The EPG and coproculture results did not show normal distribution and homoscedasticity among the treatments after analysis by non-parametric statistical tests. Thus, in order to compare the effect of rearing systems on EPG count, Mann-Whitney test was used, while Kruskal-Wallis test was employed to compare the effects of equidae species, age groups, and deworming schedules on EPG count.

The relations among the coproculture results and the studied equidae species and age groups underwent a frequency dispersion study. Information on equidae species and the role they perform were submitted to Pearson's correlation. All statistical analyses were processed using the softwares Excel (version 2010) and Infostat (version 3.10).

Results and Discussion

The EPG count of gastrointestinal parasites of the Strongylidae family showed that 50.6% of the samples collected from horses were positive, with an average count of 327.6 ± 578.9 ; 85% of the samples from donkeys were positive, with an average of 700.0 ± 778.7 ; and 69.2% of the samples from mules were positive, with an average of 561.5 ± 672.7 .

A difference ($p=0.0035$) was found among the EPG counts of horses, donkeys, and mules (Table 1), with the horses showing a lower count than donkeys. This result may be associated to the owner's profile and consequently to the different activities in which horses and donkeys are employed given the highly significant correlation ($p<0.001$) found between the equidae species and their roles, with a coefficient of 43.87%. In the backland of Pernambuco, 100% of the donkeys are used for traction, where due to the low purchase power and little access to technical assistance, the animal's owners, hardly used deworming as a health practice routine. The equines, on the other hand, are mainly used for sports (64.5%), which matches the reports of Leite et al. (2014) that the horses in the region are bred mainly for vaquejada (a bull-catching contest typical of the Brazilian Northeast). In this case, high spending power by the vaquejada horse's owners, combined with a higher technical assistance, favors the adoption of better health practices to the flock, including the periodic deworming practice.

Table 1. Eggs and oocysts per gram of feces from horses, donkeys, and mules reared in the countryside of Pernambuco.

	Horses (n=152)	Donkeys (n=20)	Mules (n=13)	p
Strongylidae	327.6 ^b	700.0 ^a	561.5 ^{ab}	0.0035
<i>Parascaris equorum</i>	-	-	-	-
<i>Anoplocephala</i> spp.	-	-	-	-
<i>Eimeria</i> spp.	-	-	-	-

Different letters in the rows differ among the equidae species according to Kruskal-Wallis test ($p < 0.05$).

Ferreira et al. (2014) also found a higher parasite load in donkeys compared to horses in the state of Maranhão, Brazil. According to those authors, even with the high parasite loads, the donkeys did not show any clinical sign of nematodiosis, which suggests they have greater resistance against nematodes than horses.

Getachew et al. (2010) assessed the gastrointestinal helminth population in traction donkeys in Ethiopia and found that 55% of the animals had EPG count above 1,000. According to those authors, high multi-parasite loads are common among traction donkeys with no evident compromise to their physical activities. According to Mendoza Hortúa et al. (2014), traction equidae in developing countries are owned by people with low income whose subsistence depends on daily work with these animals. The equidae are submitted to long workloads and inappropriate nutritional and sanitary management, which makes them more exposed and susceptible to different infectious agents, including nematodes.

Regarding the prevalent gastrointestinal parasites species in the backland of Pernambuco, the EPG count found nematode eggs of the family Strongylidae, matching the reports of Barbosa et al. (2001) in the state of São Paulo, Ferraro et al. (2008) in the state of Paraná, Schuster et al. (2011) in the state of Rio Grande do Sul, and Dias et al. (2014) in the state of Espírito Santo.

Parascaris equorum eggs were found in only three animals and, even then, at low counts. This result is in accordance with Almeida et al. (2009),

who also reported low incidence of *Parascaris equorum* in equidae in the state of Rio Grande do Sul. Those authors associated the low incidence of this parasite with the joint rearing of horses and sheep. The low prevalence of *Parascaris equorum* in equidae in the backland of Pernambuco may also be related to the joint rearing with other domestic species since, according to Leite et al. (2014), most equidae in the region are kept in pastures along with cows, sheep, and/or goats. Besides that, low infestation by *Parascaris equorum* can be related to the animal's age, once adult equidae are more resistant to these parasites and in this sample, most of the animals were adults.

Out of the 185 samples analyzed, *Eimeria* spp. oocytes were found only in the feces of one five-month-old foal and all samples were negative for *Anoplocephala* spp.

The 133 equidae reared in the extensive grazing system had higher counts of Strongylidae eggs (412.03) than the 52 animals confined in pens (313.46) ($p = 0.0309$). The opposite was expected since, according to Cintra (2010), the longer the confinement period and population density, the higher the risks of infectious disease transmission and infestation by external and internal parasites, among which gastrointestinal nematodes.

Moreover, the edaphoclimatic characteristics of the caatinga biome are unfavorable since temperature and humidity directly impact the development of the infecting larvae (L_3) of nematodes in pastures, which would require a moisture film on the vegetation for the L_3 to migrate (STROMBERG,

1997). According to Langrová et al. (2003), only the humid grass would represent a risk of infection to the equidae. Supporting this hypothesis, Couto et al. (2009) observed the highest EPG counts in horse feces precisely in the hot and rainy season and related this result to the higher spawning rates by the females since the more favorable climate conditions facilitate L_3 larvae migration from the fecal mass to the grass. Those authors also pointed out that, in that season, the grass is more palatable to the horses, which increases the intake of food and, consequently, of L_3 . Therefore, in the backland of Pernambuco, the low air relative humidity and rainfall could decrease spawning and the migration of infecting larvae.

According to Lima et al. (2006), the horses used for rural work and traction in Brazil have low maintenance cost and only receive some treatment against endoparasites and/or concentrated supplementation during the dry season at an average individual cost of 82.27 BRL (approximately 27 USD in April 2015) per year. The opposite is true for horses used in sports, which have a mean monthly cost with pharmaceuticals alone of 44 BRL (approximately 14.5 USD) per animal. In the present study, 94.2% of the animals confined in pens are horses and mainly reared to compete in vaquejadas (LEITE et al., 2014). The adoption of better sanitary practices, confirmed by the higher monthly cost with pharmaceuticals (LIMA et al.,

2006) could justify the lower EPG count in those animals.

The equidae under one year old had lower EPG count than those between one and two and a half years old (Table 2). A different result was reported by Barbosa et al. (2001), who, when evaluating 14 horses naturally infected by nematodes in the state of São Paulo, found that, out of 537,128 nematodes recovered from the horses' necropsies, 72.64% were found in young animals and the other 27.36% were found in adult animals. According to Almeida et al. (2008), larger gastrointestinal parasite infections are found in young animals, mainly between 2 and 6 months old, due to their still immature immunity. Low parasite burden in equidae under one year old can be related to a high frequency of deworming practice applied to them, because usually the young equidae are submitted to higher deworming programs than adults.

No difference ($p=0.2538$) was found in EPG count among the equidae submitted to different deworming schedules (Table 3). Except for those that were not dewormed, the animals submitted to other schedules had light ($0 \leq \text{EPG} \leq 300$) to moderate ($300 \leq \text{EPG} \leq 500$) (FERREIRA et al., 2014) parasite loads. Only the non-dewormed equidae had high parasite loads ($\text{EPG} \geq 500$) (FERREIRA et al., 2014). Nevertheless, only one non-dewormed animal had diarrhea at the moment of collection.

Table 2. Eggs and oocysts per gram of feces of equidae in different age groups reared in the countryside of Pernambuco.

Parasites	Age (years)					P
	< 1.0 (n=11)	1.0 a 2.5 (n=19)	3.0 a 5.0 (n=40)	6.0 a 10.0 (n=76)	> 10.0 (n=35)	
Strongylidae	72.7 ^b	615.8 ^a	370.0 ^{ab}	371.1 ^{ab}	402.9 ^{ab}	0.0423
<i>Parascaris equorum</i>	-	-	-	-	-	-
<i>Anoplocephala</i> spp.	-	-	-	-	-	-
<i>Eimeria</i> spp.	-	-	-	-	-	-

Different letters in the rows differ among the age groups according to Kruskal-Wallis test ($p < 0.05$).

Table 3. Eggs and oocysts per gram of feces of equidae subjected to different deworming schedules in the countryside of Pernambuco.

Deworming schedule	Strongylidae	<i>Parascaris equorum</i>	<i>Anoplocephala</i> spp.	<i>Eimeria</i> spp.
Monthly (n=16)	275.0	-	-	-
Every other month (n=10)	420.0	-	-	-
Every 3 months (n=14)	442.9	-	-	-
Every 4 months (n=7)	371.4	-	-	-
Every 6 months (n=27)	348.1	-	-	-
Once a year (n=20)	390.0	-	-	-
No deworming (n=54)	513.0	-	-	-
p	0.2538	-	-	-

According to Couto et al. (2009), nematode control in horses has been done for decades with anthelmintics, often indiscriminately and with no appropriate control strategy, which leads to parasite resistance against most anthelmintics available. Furthermore, little effort is made to research alternative treatments to control these agents in horses other than pharmaceuticals (DIAS et al., 2014). Hence, the similarity in EPG count among the animals at different deworming schedules in the present study, besides the close results between the deworming carried out every other month or once a year (420.0 and 390.0, respectively), suggest that longer deworming intervals can be adopted in the backland of Pernambuco.

A difference ($p=0.0009$) was found among the equidae only in the presence of *Strongylus*

edentatus larvae, found only in the mules (Table 4). The other gastrointestinal parasite species were found in similar amounts in horses, donkeys, and mules. A comparison of the proportion of gastrointestinal parasites in each equidae species showed that the amount of Cyathostominae larvae in horses and donkeys was larger than of the other nematodes investigated. Umur and Açıçi (2009), when comparing the parasite prevalence in horses, donkeys, and mules in the central region of the Black Sea, in Turkey, found a higher prevalence of Cyathostominae larvae not only in horses and donkeys, but also in mules. According to Rodrigues (2012), the most important nematodes used to be those of the genus *Strongylus* due to the migration through different organs, but the advent of anthelmintic resistance turned Cyathostominae into the greatest problem to equidae farming.

Table 4. Relative frequency (%) of nematode larvae in the feces of horses, donkeys, and mules in the countryside of Pernambuco.

Parasites	Horses (n=76)	Donkeys (n=20)	Mules (n=7)	P
Cyathostominae	67.5 ^A	75.0 ^A	71.4 ^A	0.8055
<i>Strongylus vulgaris</i>	9.10 ^B	15.0 ^B	14.3 ^A	0.7064
<i>Strongylus equinus</i>	1.30 ^B	5.00 ^B	-	0.5220
<i>Strongylus edentatus</i>	-	-	16.7 ^A	0.0009
<i>Trichostrongylus</i> spp.	5.48 ^B	25.0 ^B	16.7 ^A	0.0952

Different letters in the rows differ among the parasite species according to Kruskal-Wallis test ($p<0.001$)
 p =Degree of significance in the comparison among the equidae species according to the Chi-squared test.

No difference ($p=0.0931$) was found in the prevalence of nematode larvae among the age groups of the equidae (Table 5). Only Cyathostominae larvae were found in all the age groups investigated, and that was also the only subfamily found in the feces of foals under one year old. In the other age groups, other nematodes were found, although Cyathostominae was always the most prevalent larvae. The samples from foals

between one and two and a half years old had larvae of subfamily Cyathostominae and *Trichostrongylus* ssp. In the equidae between three and five years old, Cyathostominae. *Strongylus vulgaris*, *Strongylus equinus*, and *Trichostrongylus* ssp. larvae were found. In the adult animals between six and ten years old, larvae of all nematodes investigated were found, while those above ten years old had no *Strongylus equinus* and *Strongylus edentatus* larvae.

Table 5. Relative frequency (%) of the nematode larvae in the feces of equidae of different age groups in the countryside of Pernambuco.

Parasites	Age (years)					P
	< 1.0 (n=7)	1.0 a 2.5 (n=13)	3.0 a 5.0 (n=20)	6.0 a 10 (n=42)	> 10 (n=20)	
Cyathostominae	50.0	84.6 ^A	80.0 ^A	69.0 ^A	45.0 ^A	0.0931
<i>Strongylus vulgaris</i>	-	-	25.0 ^B	11.9 ^B	5.00 ^B	0.1176
<i>Strongylus equinus</i>	-	-	5.00 ^B	2.38 ^B	-	0.7726
<i>Strongylus edentatus</i>	-	-	-	2.38 ^B	-	0.8198
<i>Trichostrongylus</i> ssp	-	7.69 ^B	10.0 ^B	9.52 ^B	10.0 ^B	0.9394

Different letters in the rows differ among the parasite species according to Kruskal-Wallis test ($p<0.001$)

p=Degree of significance in the comparison among the equidae species according to the Chi-squared test.

In the feces samples of the equidae submitted to the Baermann technique, no larvae of the lung parasite *Dictyocaulus arnfieldi* was found. According to Nascimento (2008), this species has high incidences in regions with hot and humid climate. It is likely that the hot and dry climate in the backland of Pernambuco prevents the larvae of this parasite from staying in the pastures.

In the backland of Pernambuco, most equidae have light to moderate parasite loads, with a predominance of the subfamily Strongylinea and Cyathostominae, with no horses, donkeys, or mules infected by *Dictyocaulus arnfieldi*. In addition, the donkeys had higher parasite loads than the horses and the extensive system in pasture presents a greater risk for infections by nematodes.

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