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INTENSITY OF INFECTION WITH STRONGYLIDS NEMATODES IN HORSES FROM SOUTHEAST MEXICO

INTENSIDAD DE INFECCIÓN POR NEMATODOS ESTRONGÍLIDOS EN CABALLOS DEL SURESTE DE MÉXICO

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RESUMEN

Se realizó un estudio coprológico para determinar la intensidad de infección por nematodos gastrointestinales (NGI) en caballos y por otra parte se analizó la influencia de las condiciones climáticas en la reinfección de los animales. Para determinar la intensidad de infección, se recolectaron muestras de heces de caballos de Tabasco y Chiapas, México. En el segundo trabajo se utilizaron 20 caballos que se desparasitaron al inicio de cada época del año con fenbendazol (7,5 mg kg^{-1} de peso vivo, PV). Las muestras fueron procesadas con la técnica de McMaster para estimar el número de huevos por gramo de heces (HPG) y se realizaron coprocultivos para identificar los principales géneros y especies de estrongílidos. Se estudiaron varios factores intrínsecos (edad y género), origen de los animales (estado y localidad) y la época del año sobre el nivel de infección. Las principales especies identificadas fueron: *Strongylus equinus, S. edentatus, S. vulgaris, Trichostongylus* spp., y larvas clasificadas como Cyathostominae. El 86% de los equinos resultaron positivos a estrongílidos. La intensidad de la infección alcanzó 1182 ± 131 HPG en promedio. Los conteos fecales de huevos (CFH) fueron mayores en caballos adultos (1093 ± 143 HPG) que en caballos jóvenes y viejos (829 y 936 HPG, respectivamente). Valores superiores a 1000 HPG se observaron dos meses después de la aplicación del antihelmíntico en las tres épocas del año (frío (nortes), seco y lluvioso). Se concluyó que existe una alta reinfección de estrongílidos en caballos, especialmente por Cyathostominae durante todo el año en condiciones cálido-húmedas en el sureste de México.

Palabras clave: antihelmíntico, Cyathostominae, equinos, nematodos, *Strongylus*.

ABSTRACT

A coprological study was conducted to determine the intensity of infection with gastrointestinal nematodes (GIN) in horses, and the influence of climatic conditions on the reinfection of the animals. To determine intensity of infection, samples of faeces were collected in horses from Tabasco and Chiapas, Mexico. In the second study, 20 horses were treated with fenbendazole (7.5 mg kg^{-1} body weight, BW) at the beginning of each season. The samples were processed by the McMaster te-

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chnique to estimate the number of eggs per gram of faeces (EPG), and coprocultures were performed to identify the main genres and species of strongylids. Several intrinsic factors (age, gender), the origin of animals (state and locality) and season over the level of infection of animals were studied. The main species identified were: *Strongylus equinus, S. edentatus, S. vulgaris, Trichostongylus* spp., and also larvae were classified as Cyathostominae. Results showed that 86% of the horses tested positive for strongylids. Intensity of infection reached 1182 ± 131 EPG on average. The FEC were higher in adult horses (1093 ± 143 EPG) than in young and old horses (829 and 936 EPG, respectively). Values above 1000 EPG were observed two months after the application of anthelmintic in the three seasons (cold (Norths), dry and rainy). It was concluded that reinfection by strongylids is high in horses, especially by Cyathostominae, throughout the year in warm humid climates of the southeast of Mexico.

Key words: anthelmintic, Cyathostominae, equids, nematodes, *Strongylus*.

INTRODUCTION

According to FAO (FAOSTAT, 2014), the equine population in Mexico was estimated in 6.35 million heads in 2011, ranking third after the U.S.A. and China. Despite this large population of horses, most of them do not receive medical care, such as regular vaccinations, trim hooves and anthelmintic treatment, which contributes to the fitness condition of the animals and their lifespan (de Aluja et al., 2000). The most common parasitic diseases in horses are caused by gastrointestinal nematodes. The damage caused by these parasites results in the following clinical signs: cramping, weight loss and anorexia, oedema, shaggy hair, pale mucous membranes, chronic diarrhoea and occasionally death when there is high burden of either *Strongylus* spp (Morales et al., 2010) or cyathostomins (Stratford et al., 2011).

The most important gastrointestinal nematodes belong to the family Strongylidae: large strongyles (Strongylinae) and small strongyles (Cyathostominae). Large strongyles are common parasites of the large intestine, the main species are: *Strongylus vulgaris*, *S. equinus* and *S. edentatus* (Morales et al., 2010). Small strongyles, known as cyathostomins, are represented by over 52 species (Lichtefels et al., 2002; Traversa et al., 2007; Kornaś et al., 2009). Cyathostomins are the most frequently detected strongylids in horses, and the animals usually present between five to 10 different species (Stratford et al., 2011). Other gastrointestinal nematodes are: *Parascaris* (Ascaridida), *Oxyuris* (Oxyurida) and *Trichostrongylus* (Trichostrongyloidea) (Holland et al., 2001; Güiris et al., 2010; Bedoya et al., 2011; Worku and Afera, 2012).

Abundance of gastrointestinal nematodes in horses has been found on the west center of Mexico (Nayarit), where the climate is temperate with little rainfall (Peña, 2004). Presence of *Strongylus* spp, *Trichonema* and *Cyathostomum* has been reported in this region. However, parasitological studies in horses are scarce for south-eastern Mexico. A study on the diversity of species was conducted in Chiapas (Güiris et al., 2010) and a recent study to evaluate the effect of parasites in haematological values and body condition of horses was carried out in Veracruz (Valdéz-Cruz et al., 2013). However, little information is available in the country if we consider the importance that equines have for work, transport and entertainment activities, especially in humid tropical areas, where animals are constantly exposed to infection with gastrointestinal parasites (Güiris et al., 2010). The problem is not simple because the effect of parasites on horses' health is still unknown for this region. Therefore, the aim of this study was to determine FEC in horses that are not frequently treated, as it is the case of many horses in Chiapas. This epidemiological study provides some basis to reduce the FEC of the animals but it is also important to consider that anthelmintic resistance has been observed in equines in many countries worldwide (von Witzendorff et al., 2003; Stratford et al., 2011).

In addition, prevalence of strongylids in the different months of the year has not been recorded in other places of Mexico at similar latitude. This information is especially relevant for the humid tropics, where the environmental conditions promote the growth of gastrointestinal parasites. This study reports the level of reinfection of strongylids in horses and its intensity along the different seasons in Tabasco and Chiapas, Mexico.

MATERIALS AND METHODS

Location

Two studies were performed. Firstly, an observational study was carried out during 10 months in Salto de Agua (17°34′ N, 92°29′ W, and 85 m.a.s.l.), Chiapas, Mexico and then a second study on the intensity of infection was conducted in horses of five localities in Chiapas and two localities in Tabasco (Fig. 1) for seven months (December 2012-June 2013).

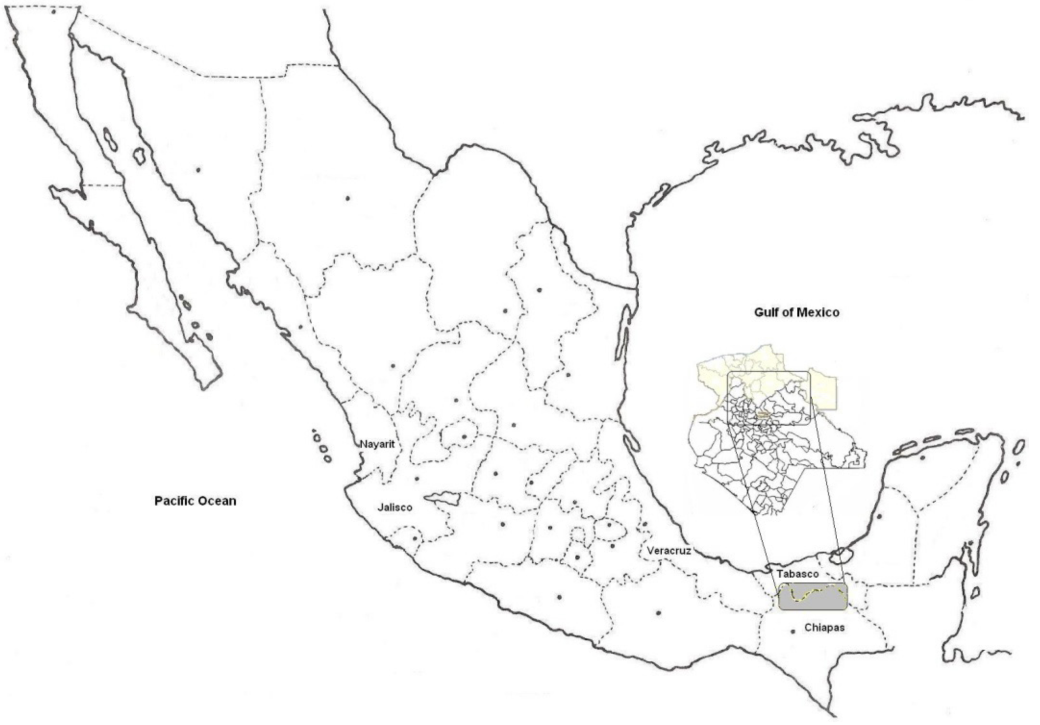

Fig. 1. Location of the study area in Tabasco and Chiapas in south-eastern Mexico.
Fig. 1. Localización del área de estudio en Tabasco y Chiapas en el sureste Mexicano.

The climate of this region is equatorial, hot and humid with rainfall throughout the year (Kottek et al., 2006). The average annual temperature is 26.6°C and the annual rainfall is 3289 mm (CONAGUA, 2014).

The climatic conditions in the study region are very similar in terms of temperature, but with slight differences in rainfall compared to Salto de Agua (Fig. 2). The local seasons were defined based on humidity, temperature and other climatologic characteristics of the region (Larios and Hernández, 1992): dry season (February-

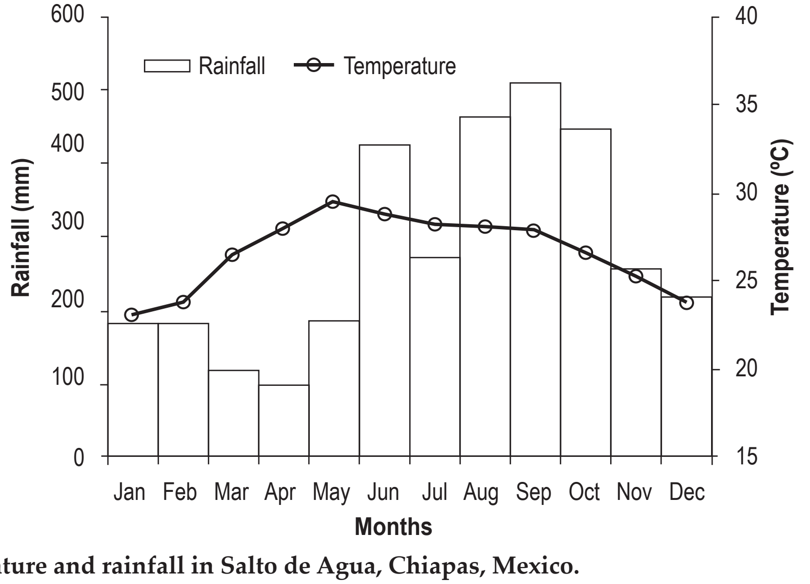

Fig. 2. Temperature and rainfall in Salto de Agua, Chiapas, Mexico.
Fig. 2. Temperatura y precipitación en Salto de Agua, Chiapas, México.

May), rainy season (June-September) and cold season (“Norths”; October-January).

Parasitological methods

The faecal egg counts were determined using a McMaster technique (Thienpont et al., 1986), with a lower detection of 50 eggs per gram of faeces. Coprocultures were also done to identify the main strongylids genera affecting the horses. Fifty grams of faeces of each animal were placed in plastic containers and every third day 5 mL of water were added. Faecal samples were cultured 10 days at room temperature to allow the development of infective third stage larvae (L3), which was collected using the Baermann procedure. Genera were identified according to their morphology and size (Thienpont et al., 1986). The species of *Strongylus* were identified according to the number of intestinal cells. In addition, larvae were categorized as Cyathostominae when they had eight intestinal cells (Bowman, 2011).

Intensity of nematode infection
Sample size determination

Sample size was determined according to the total equine population in each state (Tabasco N = 28095, and Chiapas N = 72095; INEGI, 2009) with an absolute precision of 5%, and 95% confidence level (CL). The variable EPG was transformed to log (EPG + 1), based on the following formula (Steel and Torrie, 1980):

$$n = \frac{NZ^2_{\alpha/2}s^2_{n'}}{Nd^2 + Z^2_{\alpha/2}s^2_{n'}}$$

where, n = required sample size; N = population size; d = desired absolute precision (α* previous sample average); Z = Z value (95% CL);s^2 = variance of one sample for the log (EPG + 1).

The sample sizes estimated for Chiapas and Tabasco were 52 and 35 horses, respectively. Nevertheless, 149 horses were sampled in Chiapas and 39 horses in Tabasco because owners in some communities were interested in the diagnosis of parasites in their working horses. The horses included in this study did not receive anthelmintic treatment for at least the 2 months prior to the study, according to information provided by the owners. In general, horses in Chiapas are not usually treated with anthelmintics, while in the localities of Tenosique and Centro (Tabasco) animals are dewormed every three months.

Statistical analysis

The EPG were transformed to log (EPG + 1) to correct the variance and approximate the results to the normal distribution (Gauly and Erhardt, 2001). The variance analysis was evaluated by using PROC GLM of SAS (SAS, 2003). Results were considered according to various intrinsic factors (age, gender), the origin of animals (state and locality), age (young < 3 years, adult 3-10 years, old > 10 years), season, using the following model:

$$Y_{ijklmno} = \mu + \varrho_i + \zeta_j + \tau_{k(j)} + \psi_l + \omega_m + \eta_{n(i)} + \psi^*\omega_{ml} + \psi^*\tau_{k(j)l} + \omega^*\tau_{k(j)m} + \psi^*\eta_{n(i)l} + \omega^*\eta_{n(i)m} + \varepsilon_{ijklmno}$$

where $Y_{ijklmno}$ = EPG, μ = overall mean, ϱ_i = fixed effect of season (rainy, cold and dry seasons), ζ_j = effect of the j-esim state (Tabasco, Chiapas), $\tau_{k(j)}$ = effect of the k-esim locality (state), Ψ_l = effect of the l-esim sex, ω_m = effect of the m-esim age, $\eta_{n(i)}$ = effect of the n-esim month (season). Interactions terms: $\psi^*\omega_{ml}$, $\psi^*\tau_{k(j)l}$, $\omega^*\tau_{k(j)m}$, $\psi^*\eta_{n(i)l}$, $\omega^*\eta_{n(i)m}$ and $\varepsilon_{ijklmno}$ = residual error.

Efficacy of anthelmintics and reinfection

Faecal samples from the rectum of 20 horses were taken from December 2012 to September 2013. The horses were treated by oral route with fenbendazole at the commercial dose (7.5 mg kg^{-1} BW). The percentage of efficacy was calculated in each one of three local seasons, according to Vidyashankar et al. (2012).

Efficacy = (FEC pre-treatment – post-treatment)/ FEC pre-treatment

The horses were sampled before the treatment and 15 days after (Dobson et al., 2012), and later on a monthly basis. The samples were collected directly from the rectum. Individual faecal samples from each horse were labelled and transported to the parasitology laboratory of the Unidad Regional Universitaria Sursureste (URUSSE), Universidad Autónoma Chapingo (UACh), in Tabasco, Mexico.

A model using the GLM procedure (SAS, 2003) was used to study the influence of climatic conditions on reinfection, considering the effect of period (each anthelmintic treatment represents the beginning of a period in cold, dry and rainy season) and months within each period (December to September) as fixed effects. The mean separation was conducted by using Duncan test.

$$Y_{ijk} = \mu + \varrho_i + \eta_{j(i)} + \varepsilon_{ijk}$$

Y_{ijk} = EPG, μ = overall mean, ϱ_i = fixed effect of season (period I, II and II), $\eta_{j(i)}$ = effect of the n-esim month nested in period. ε_{ijk} = residual error.

RESULTS

Intensity of nematode infection

The analysis of the gastrointestinal eggs observed in the faeces show that the horses were parasitized only by strongylids, except in one horse that was parasitized by *Oxyuris*. No eggs from other gastrointestinal nematode and cestoda were observed in the faeces. By means of the coprocultures, cyathostomins (91.5%) were obtained and identified as the main nematode group, while large strongylids represented 8.2% with the species: *S. equines* (1.0%), *S. edentatus* (1.9%) and *S. vulgaris* (5.3%). Additionally, *Trichostongylus* spp (0.3%) were found in a few samples.

The state, locality, age of animals and the interaction of the month and season were significant (P < 0.01) among the factors analyzed. No differences were obtained in the FEC of strongylids based on gender and interactions with the other factors (P > 0.05).

Faecal egg count (FEC) was higher in Chiapas compared to the value obtained in Tabasco (P < 0.01). Tenosique was the locality which showed the lowest FEC (Table 1). In addition, significant differences were observed in terms of age of animals (P < 0.01). The greatest egg excretion numbers were observed in adult horses (1093 ± 143 EPG), whereas no differences were observed in old and young horses (829 and 936 EPG, respectively).

In general, the prevalence for strongylids in horses from Chiapas and Tabasco (Table 1) was greater than 85%. In 54.1% of the horses, only one species was found, two species in 40.3%, three species in 4.2% and four species in 1.4%.

The lowest FEC occurred in February and June, while the highest FEC values were recorded in December and May (Fig. 3).

Anthelmintic efficacy and reinfection

Before the anthelmintic treatment (December), 77.6% of larvae obtained by coprocultures were cyathostomins. *S. equinus* and *S. vulgaris* were also identified in 1.2% and 21.2% of animals, respectively. After the first treatment, only cyathostomins larvae were observed during all the experimental period.

As shown in Fig. 4, the effect of time was significant (P < 0.01) on FEC in horses from Salto de Agua (Chiapas), while the reappearance of egg excretion was observed throughout the year. FEC increased above 1000 EPG after three months in the cold-dry season (December to March), two and half months at the onset of rainy season, and two months in the rainy season (July-August). Anthelmintic efficacy was 96.4% (1621 EPG pre-treatment and 58 EPG post-treatment) in the first application (December), 93.7% (2179 EPG pre-treatment and 138 EPG post-treatment) in the second application (March), and 87.4% (1181 EPG pre-treatment and 149 EPG post-treatment) in the third application (June).

DISCUSSION

Intensity of nematode infection

This study determined that the infection by strongylids (small and large) is highly frequent.

Table 1. Faecal egg count (FEC) and prevalence of strongylids of horses from different localities of two states of south-eastern Mexico (n = 188).

Tabla 1. Conteos fecales de huevos y prevalencia de estrongílidos de caballos de diferentes localidades de dos estados del sureste Mexicano (n = 188).

State/locality	n	EPG		Prevalence	
		Mean	Std. error	Positive	(%)
Chiapas	149	1182 a	131	145	97.3
Cenobio Aguilar	17	921 ab	221	16	94.1
Juárez	23	765 ab	113	23	100
Palenque	13	1131 a	165	13	100
Reforma	7	1171 ab	343	7	100
Salto de Agua	89	1198 ab	116	86	96.6
Tabasco	39	428 b	87	36	92.3
Centro	18	517 bc	158	18	100
Tenosique	21	352 c	91	18	85.7

n: number of horses. Different letters in each column indicate differences (P < 0.01).

Faecal samples were obtained in different months. EPG: eggs per gram of faeces.

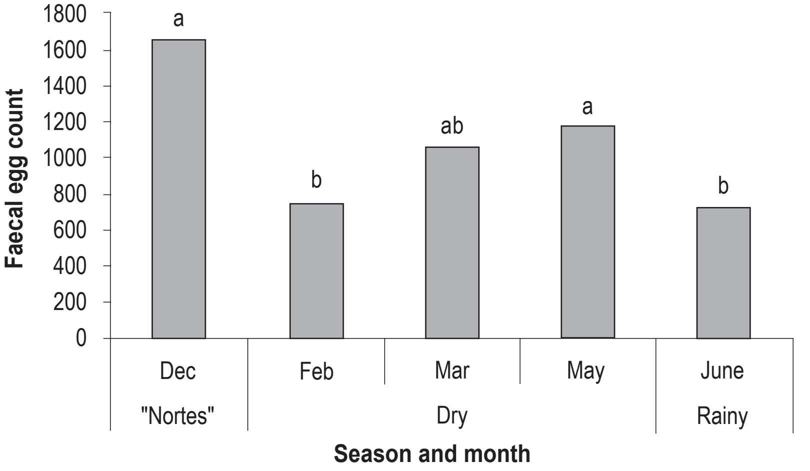

Fig. 3. Faecal egg counts of gastrointestinal nematodes by season and month in horses from Tabasco and Chiapas.

Fig. 3. Conteo fecal de huevos de nematodos gastrointestinales por estación y mes in horses de Tabasco y Chiapas.

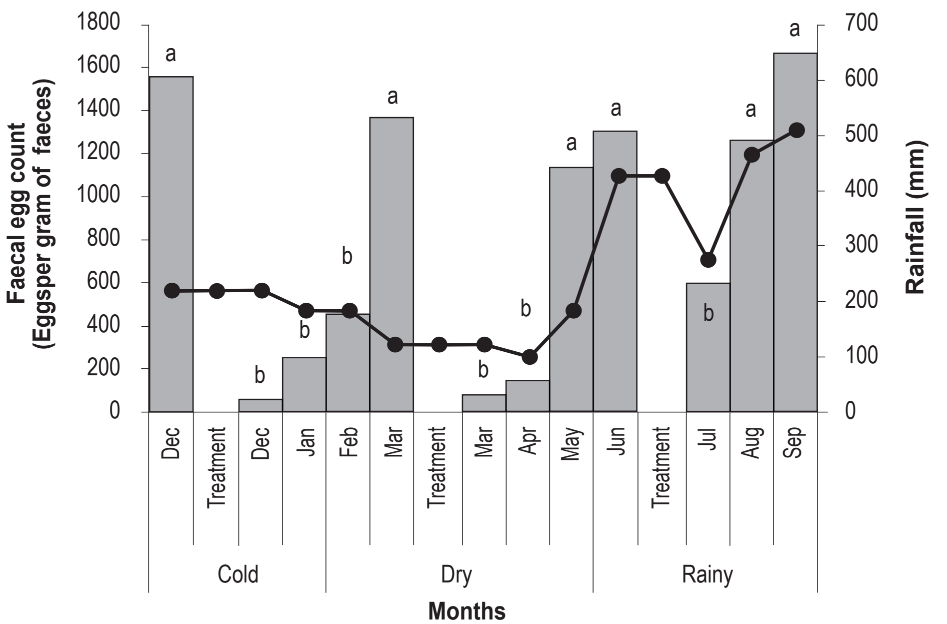

Fig. 4. Faecal egg count of strongylids in horses from Salto de Agua, Chiapas, Mexico, after the treatment with fenbendazol (n = 20).

The columns represent the faecal counts and the lines are the amount of rainfall. Different letters on columns indicate statistical differences (P < 0.01).

Fig. 4. Conteos fecales de huevos de estrongilidos en caballos de Salto de Agua, Chiapas, México, después del tratamiento con febendazol (n = 20).

Las columnas representan los conteos fecales y las líneas son la cantidad de precipitación. Letras diferentes en la columna indican diferencias estadísticas (P < 0.01).

The most common strongylids were cyathostomins, followed by large strongylids: *S. equinus*, *S. vulgaris* and *S. edentatus*, which have also been reported as the main parasites in several studies (Holland et al., 2001; Chapman et al., 2003; Valdéz-Cruz et al., 2013). In addition, *Trichostrongylus* spp. was found in Chiapas, which agrees with data reported in Veracruz, Mexico (Valdéz-Cruz et al., 2013). Nevertheless, the results of another study reported that the prevalence of horses passing *Trichostrongylus* by faeces was 90%, 7% *Trichonema,* and 3% *Strongylus* (Bedoya et al., 2011).

A high prevalence of strongylids in all localities of Chiapas (above 90%) and Tabasco (above 86%) was observed, similar to that reported for Veracruz, Mexico (Valdéz-Cruz et al., 2013). In a study conducted in Colombia, only 8% of the horses were free of parasites, whereas 92% of equines had some genera of nematodes, which negatively affected their productive performance and health (Bedoya et al., 2011). In Cameroon, 100% of the analyzed horses (N = 894) were parasitized (Lem et al., 2012). On the contrary, a lower infection has been reported in several studies. For instance, prevalence reached 61% for strongyles, 8% for *Parascaris equorum* and 1% for *Oxyuris equi* in a study conducted in Venezuela, while no parasite eggs were observed in 30% of horses (Morales et al., 2012). In Ethiopia, the prevalence of intestinal nematodes was 52.1%, and most of the equines were passing eggs of nematodes as strongyles, *Ascaris* and *Oxyuris* (Worku and Afera, 2012). In contrast to these studies, no *Ascaris* eggs were detected in Tabasco and Chiapas. By using the flotation technique, a lot of cyathostomins, some large strongylids and a few *Trichostrongylus* were detected. *Oxyuris* eggs were detected only in one sampled horse in Tenosique ,Tabasco. The high prevalence obtained in humid tropical climate is due to the favorable conditions for the development of main species of gastrointestinal nematodes in horses. The effect of climate has been reported in Brazil (Navarro-dos Santos et al., 2012), where high survival of cyatostominae larvae was observed in *Brachiaria* grass during one year.

In the present study, the existence of infection by two or three species of parasites was in agreement with other authors (Bedoya et al., 2011). Similarly, poly-parasitic infections (92.3%) were more common in Ethiopia and Cameroon (Lem et al., 2012; Worku and Afera, 2012). In addition, other study on racehorses reported that the infection was by a single species (Morales et al., 2012).

Higher levels of FEC of strongylids in horses from Chiapas could be explained by the fact that owners do not treat their horses with anthelmintics, whereas horses receive the treatment every three months in Tabasco. The results showed that the average value of FEC (954 ± 858 EPG) was similar to that obtained in Veracruz, where most of the working equines evaluated had gastrointestinal infections ranging from mild to moderate (500 to 1000 EPG) (Valdéz-Cruz et al., 2013). In Venezuela, the FEC of strongyles ranged between 600 and1900 EPG (Morales et al., 2012). The mean of FEC reported in Bac Can (Vietnam) was higher (2053 EPG), and the maximum was 11300 EPG. In Bac Giang, (Vietnam) the mean FEC was 732 EPG, and the maximum was 2950 EPG (Holland et al., 2001). In the present work, the maximum value of FEC was obtained in three horses in Chiapas (4250, 7350 and 17550 EPG, respectively), and only one horse in Tabasco (3000 EPG).

To get more knowledge of the factors that influence infections by strongylids in horses, the coprological data were examined considering age and gender. The results indicate that age influences the FEC of strongylids, being adult horses more infected than young and old ones. These findings agree with those reported by some researchers (Worku and Afera, 2012), but disagree with others who have reported no effect of age and gender on the infection (Bedoya et al., 2011). Similarities in the prevalence and FEC of strongylids observed when considering gender are in disagreement with other study (Morales et al., 2012), in which mares were more infected (62%) than males (38%). Similar results by gender were reported in Spain (Francisco et al., 2009).

Anthelmintic efficacy and reinfection

Faecal egg counts of gastrointestinal nematodes were very high in Chiapas during all sampling events and, therefore, many horses required treatment according to the thresholds indicated in the literature (1000 EPG; Quiróz, 2003). However, no studies were found on interval dose programs and anthelmintic efficacy in horses in Mexico. This topic is important in warm climates because there is a high risk of infection during all year due to the prevalent environmental conditions of temperature and humidity. Therefore, one objective was to determine the time in which the level of reinfection reaches the threshold to implement a new treatment. Fenbendazole was highly effective in the first application; the fact that anthelmintic treatments of horses are not performed frequently in this region can account for this situation. However, the effectiveness was markedly reduced when the following treatment was conducted. The results indicated 96.4% of effectiveness in the first treatment, whereas it reached only 87.4% in the third treatment. No faecal egg count reduction test (FECRT) was performed to determine the anthelmintic resistance because producers only had a few working horses and were using different management techniques. Thus, only anthelmintic effectiveness was measured.

The reduced effectiveness in the second and third treatment and the presence only of small strongyles (cyathostomins) is worrisome because anthelmintic resistance has been described in these nematodes in several cases (Lake et al., 2009; Stratford et al., 2011; Matthews et al., 2012). For this reason, it is important consider some alternatives of sustainable parasite control programs based on systematic surveillance of parasite levels (Andersen et al., 2013). The loss of efficacy is one of the main causes of development of the AR in horses worldwide (Vidyashankar et al., 2012).

Due to the frequent reports of AR in horses (Nielsen 2012), specific procedures to reduce the infection have been studied. As a result for these studies, a method has been proposed based on treatment intensity (dose) programs (von Samson-Himmelstjerna, 2012). Coprological surveys constitute a useful tool to provide recommendations for the owners in terms of measures to control strongylids, especially if we consider that anthelmintics showed a high efficacy in the present study. Because of the increasing levels of anthelmintic resistance, it is also important to note that recommendations should be made with caution (Lake et al., 2009; Matthews et al., 2012).

CONCLUSIONS

The results of this study showed a high infection by strongylids, especially cyathostomins, in working equids in Tabasco and northern Chiapas, Mexico. The infection of horses with infective third-stage larvae of strongylids, particularly cyathostomins, occurred throughout the year in humid climatic conditions of south-eastern Mexico. The results also indicate that locality, age of animals and interaction of the month and season affect faecal egg counts. Further research is required in order to determine suitable measures, including frequency of the chemical treatment, for the control of nematodes that affect horses.

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