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Experimental infection of calves with *Haemonchus placei* and *Haemonchus contortus*: Assessment of parasitological parameters

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ABSTRACT

The present study evaluated the viability and possible effects of *Haemonchus contortus* infections in experimentally prime infected calves, comparing them to infections by *Haemonchus placei*. Ten male Holstein newborns were used. All calves were individually weighed for subsequent group formation, in which two animals were kept as a control group, inoculated with water (GI); four animals were inoculated with 10,000 third stage (L3) *Haemonchus contortus* larvae (GII); and the remaining four calves were inoculated with 10,000 third stage (L3) *H. placei* larvae (GIII). All experimental animals were necropsied on the 42nd day after inoculation. Based on results obtained by the present study, it can be concluded that bovine calves were susceptible to infections by both *Haemonchus* species (*placei* and *contortus*). *H. contortus* presented an inferior pre-patent period when compared to *H. placei*. No significant difference ($P > 0.05$) was observed between *Haemonchus* burdens recovered from both infected groups (GII and GIII). Moreover, *H. contortus* females maintained an egg production rate similar to *H. placei* females in young animals, which can contribute to pasture contamination by both *Haemonchus* species. This could possibly lead to negative reflexes on helminth control based on a mixed pasture with bovines and ovines, especially when it involves younglings.

1. Introduction

Gastrointestinal endoparasites are amongst the most important health concerns found in bovine herds, affecting the physiological development of young (growth phase) animals (Felippelli et al., 2014). Of all nematode species that affect ruminants, those belonging to the *Haemonchus* genus stand out, mainly due to hematophagy and lesions caused on abomasum mucosa. In order to prevent or minimize production losses caused by these gastrointestinal helminths, the main resources available are anthelmintic treatments (Lopes et al., 2014).

According to Fernandes et al. (2004), an alternative adopted to reduce pasture contamination with infecting larvae of these nematodes is the use of alternate or simultaneous/mixed grazing between different herbivore species (equines, caprines, ovines and bovines). Studies conducted in naturally infected animals created in a grazing system (Amarante et al., 1997) demonstrate the possibility of crossed infections between ovine's *Haemonchus contortus* and bovine's *Haemonchus placei*. However, as time passes, animals usually develop resistance and naturally eliminate helminth species that are not well adapted (Silva et al., 2015). Despite these works conducted by the aforementioned researchers, we are aware of no studies performed with the aim of confirming these hypotheses in young, experimentally prime infected animals. Based on this prerogative, the present study evaluated the viability and possible effects of an infection by *H. contortus* in young calves that were experimentally prime infected in comparison to infection by *H. placei*.

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2. Material and methods

2.1. Location and animals

This experiment was conducted at the Center for Research in Animal Health (Centro de Pesquisas em Sanidade Animal—CPPAR), FCAV/UNESP, Jaboticabal campus, São Paulo, Brazil.

Ten male Holstein newborns were used. All of them were separated from their mothers at the moment they were born. These animals received colostrum in the first hours of life and were then kept in suspended paddocks, with the objective of avoiding any possibility of helminth infections. The animals received substituted, granulated commercial rations and Tifton until they were 60 days old. After weaning (60 days old), animals were fed for another 30 days with 70% Tifton hay and 30% commercial rations. Water was provided *ad libitum*.

2.2. Experimental group formation and animal inoculation with H. contortus *and* H. placei *and L3 dosages*

Animals were randomized into groups when calves were 90 days old. Each animal was individually weighed, and groups were then formed.

Larvae (L_3) of *H. placei* and *H. contortus* were kindly donated by Prof. Dr. Alessandro F.T. Amarante (Departamento de Parasitologia do Instituto de Biociências da Universidade Estadual Paulista—UNESP, Botucatu campus, São Paulo). The identification of different *Haemonchus* species used in the present study was performed on both L_3 and adults, based on morphological criteria and PCR criteria previously established by Santos et al. (2014) and Silva et al. (2015).

The number of infecting larvae (inoculum) was defined based on international standards defined by the World Association for the Advancement of Veterinary Parasitology (Wood et al., 1995), which recommends a single infection with approximately 5000–10,000 infecting larvae (L_3). Bovines from groups GII and GIII received approximately 10,000 infecting larvae (L_3) of the respective *Haemonchus* species (Table 1) orally with the aid of a 10 mL syringe. The volume was divided in three consecutive dosages. Each animal belonging to group GI (control) received oral placebo, which consisted of 30 mL of water.

2.3. Fecal collection, parasitological necropsies and helminth species identification

In the five days that preceded inoculation, daily EPG counts (Gordon and Whitlock, 1939 and adapted by Lopes et al., 2014) were conducted to confirm the absence of helminth infections in the experimental bovines. Starting on the 6th post-inoculation day, EPG exams were performed daily, seeking to determine the pre-patent period—to detect the first eggs in fecal samples that originated from each of the studied nematode species.

Euthanasia of all ten experimental calves was performed on the 42nd post-inoculation day (AVMA, 2013). The digestive system of each calf was separated into different anatomical segments (abomasum, small and large intestine). Abomasums were individually subjected to digestion with a pepsin hydrochloric acid solution (Wood et al., 1995). The contents of each segment were fixed in 70.0% alcohol (Borges et al., 2011). Remaining organs were also inspected for the presence of possible helminth species (Wood et al., 1995).

From all parasites recovered, which were subsequently stored in 70% alcohol, 10% of the total amount of specimens of each sex was randomly selected from each animal for analysis.

These parasite samples were measured to obtain their total length. For species classification in females, tails were measured, vulva types were registered, and cuts on the esophagus-intestine junctions were performed in order to count the synlophes (grooves), based on methodology described by Santos et al. (2014) and Silva et al. (2015).

In order to differentiate adult males, cuts of the esophagus-intestine junctions were also performed and spicules and hooks (located in the copulatory bursa) were measured, since these usually present larger dimensions in *H. placei* (Santos et al., 2014; Silva et al., 2015).

2.4. Data analysis

The experimental design used in EPG counts was completely random in a split plot in time scheme, with treatments as main parcels and observation dates as secondary parcels. EPG values underwent a logarithmic transformation ($\log(x+1)$) in order to normalize responses homogenize variances.

When evaluating helminth burdens recovered in necropsy, values underwent logarithmic transformations ($\log(x+1)$), and a completely randomized design was used.

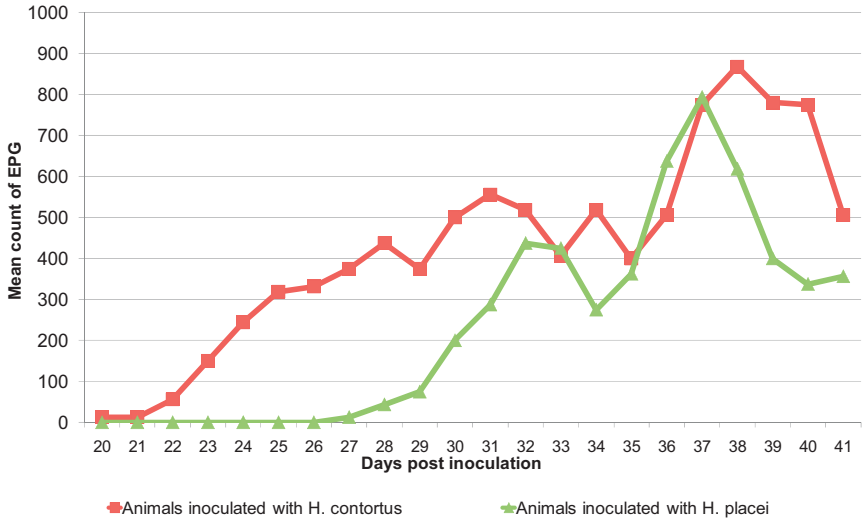

Fig. 1. Egg counts "strongyles" (*Haemonchus*) per gram of feces (EPG) in calves belonging to the two groups infected experimentally.

Table 1
Prepatent period (post-inoculation day), eggs per gram of feces (EPG) and quantifications of *Haemonchus contortus* and of *Haemonchus placei* collected from 10 necropsied cattle.

		Fecal exams		Number of – *Haemonchus*			
Animal	Group	Prepatent period (days)	EPG*	Male	Female	Total	Fixing rate (%)
3790	GI: Control	0	0	0	0	0	0
3674		0	0	0	0	0	0
Total		0	0	0	0	0	–
Mean		0	0	0	0	0	0
4670	GII: Infected with	27	162	348	414	762	7.62
294	*Haemonchus contortus*	20	852	446	636	1082	10.2
3337		20	706	296	470	766	7.66
4803		29	77	161	200	361	3.61
Total		–	–	1251	1720	2971	–
Mean		24	428.41A	312.75	430.0	742.75	7.43
2925	GIII: Infected with	28	209	234	229	463	4.63
2985	*Haemonchus placei*	27	457	129	119	248	2.48
4115		28	448	700	643	1343	13.43
4798		27	333	537	519	1092	10.92
Total		–	–	1636	1510	3146	-
Mean		28	350.83A	409.0	377.5	786.5	7.86

Means and standard deviations resulting from the recovery of adult specimens of *Haemonchus* ofcalves belonging to infected experimental groups

	Experimental groups	
Sex	GII: *Haemonchus contortus* Means[a] ± SD**	GIII: *Haemonchus placei* Means[a] ± SD**
Male	312.75 ± 118.75 Aa	409.00 ± 271.17 Aa
Female	430.00 ± 179.99 Aa	377.50 ± 244.53 Aa

*Mean of EPG counts from the date on which was recorded the presence of eggs for each animal.**Standard deviations.
[a] Means values followed by the same capital letter on the same line and lowercase in the colun, do not differ significantly at a 95% confidence interval.

The proposed treatment averages were evaluated by t tests ($p \leq 0.05$) (SAS, 2001).

3. Results

The first batch of nematode eggs in fecal samples was observed after the 20th post-inoculation day, first diagnosed in animals belonging to the group inoculated with *H. contortus*. Bovines infected with *H. placei* presented positive EPG counts after the 27th day post-inoculation (Table 1). The highest EPG counts occurred near the final observation dates for both groups (Table 1 and Fig. 1). Animals that were not inoculated (control group) did not present nematode eggs in fecal samples during the entire experimental period. No significant differences ($P > 0.05$) were observed when comparing mean EPG counts from both inoculated groups.

Immature forms of *Haemonchus* were not observed in any experimentally infected calf. Total *Haemonchus* burdens did not differ ($P > 0.05$) between both infected groups. Moreover, the number of males and females recovered for each *Haemonchus* species did not differ between themselves ($P > 0.05$) within the same experimental group, as seen in Table 1. This same Table (Table 1) also shows that the average establishment rates of different *Haemonchus* species were 7.43% and 7.86% for *H. contortus* and *H. placei*, respectively.

Mean EPG counts were superior in the group infected with *H. contortus*, though female specimens were more common in the group infected by *H. placei* (Table 2). The average female length found in both groups demonstrates that *H. placei* females were larger when compared to *H. contortus* females recovered from calves belonging to group II (Table 2).

4. Discussion

Coproparasitological results allow the inference that the prepatent periods for prime infected bovines were between 20 and 29 and 27 to 28 days for *H. contortus* and *H. placei*, respectively. In prime infected sheep, Santos et al. (2014) observed that *H. placei* infections were not only possible, but also presented a prolonged period of egg elimination in feces, resulting in a pre-patent period of 18–22 days for *H. contortus* and 26–32 days for *H. placei*. Using ovines as common hosts, Reiniger (2012) pre-patent periods of 22 and 26 days for *H. contortus* and *H. placei*, respectively.

Concerning parasitic burdens and percentage rates of nematodes, in a study with lambs prime infected and subsequently reinfected with *H. contortus* and *H. placei* (5000 L_3 of the respective species), Reiniger (2012) obtained mean recovery rates of 316 specimens in animals that only had contact with *H. contortus*, 397 specimens in lambs with previous contact with *H. contortus* that were later infected by *H. placei*, and 429 specimens in animals previously infected with *H. placei*, treated and then infected with a *H. contortus* inoculum.

A study with sheep slaughtered at abattoirs in Egypt presented higher nematode burdens of *H. contortus* when compared to *H. placei* (Khalafalla et al., 2011). Abomasums collected on the Ivory Coast during the dry season and analyzed for the number and species of *Haemonchus* sp. That parasitized 77 ovines, 75 caprines, 65 *Bos indicus* cattle and 58 *Bos taurus* cattle showed that *H. placei* parasitized more bovines than sheep, though the latter was also infected to a lesser degree (Achi et al., 2003). Meanwhile, *H. contortus* was observed only in abomasums from ovines and caprines.

Foreyt and Trainer (1970) recovered a superior average number of male adult *H. contortus* specimens using three animals per group, with inoculums of 25,000 and 100,000 L_3. In the present study, mean EPG counts were numerically superior in the group infected by *H. contortus*, though the female total was larger in the group infected with *H. placei*. This may demonstrate that *H. contortus* females are more prolific than *H. placei*, though future studies must be conducted in order to reinforce such hypotheses.

The inoculum used in the present study was the maximum recommended by Wood et al. (1995) in experimental *Haemonchus* spp. infections in ruminants. The authors reported percentage rates ranging between 15% and 20%. In this study, observed percentage rates were between 3.61–10.82 and 2.48–13.43 for *H. contortus* and

Table 2
EPG counts and total number of females of *Haemonchus* and the length mean of the parasites recovered from infected animals in each group.

Animal	Group	Variables		
		EPG*	Total of females	Leght mean of the parasites (mm)[a]
4670	GII: infected with	162	414	18.81
0294	*Haemonchus contortus*	852	636	21.97
3337		706	470	20.13
4803		77	200	18.30
Mean		428.41	4300	19.80
2925	GIII: infected with	209	229	22.00
2985	*Haemonchus placei*	457	119	23.83
4115		448	643	22.12
4798		333	519	22.38
Mean		350.83	3775	21.04

*Mean of EPG counts from the date on which was recorded the presence of eggs for each animal.
[a] Mean obtained from 5% of females collected from each calf.

H. placei, respectively. It is essential to reinforce that L_3 used for the inoculation of calves were active and had good motility, which did not interfere with the percentage rates of different *Haemonchus* species in experimental bovines.

The results obtained in the present study regarding the length of *H. contortus* and *H. placei* females were similar to normal values of the species obtained in sheep and cattle, respectively considered to be central hosts of such nematodes (Hoberg et al., 2004). Lichtenfels et al. (1994) obtained sizes varying between 14.8 mm to 27.2 mm for *H. contortus* and 12.5 mm to 25.5 mm for *H. placei*, demonstrating greater body length values for the first species. In the present study, *H. contortus* presented lower values when compared to the average length of parasites recovered in the group infected by *H. placei.*

Based on results found throughout the present study, it can be concluded that calves were susceptible to infection by both *Haemonchus* species (*placei* and *contortus*). *H. contortus* presented a pre-patent period that was relatively shorter than *H. placei.* There was no significant difference ($P > 0.05$) between the numbers of *Haemonchus* recovered in both infected groups (GII and GIII). Moreover, *H. contortus* females maintained an egg production rate equivalent to *H. placei* females in young animals, which can contribute to pasture contamination by both *Haemonchus* species and eventually lead to possible negative effects on helminth control by mixed grazing between sheep and cattle, especially in calves.

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