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Short communication

Viability of *Haemonchus placei* parasitism in experimentally infected young goats

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ABSTRACT

The present study aimed to evaluate the viability of *Haemonchus placei* parasitism in experimentally infected goats. For that, 14 75 days old kids male Saanen kids were placed in one of the four experimental groups: GI – infected with 5000 *H. placei* L3 (n = 4); GII – infected with 5000 *H. contortus* L3 (n = 4); GIII – infected with 2500 *H. contortus* L3 + 2500 *H. placei* L3 (n = 4), and GIV – control, inoculated with distilled water (n = 2). Each kid received, orally, the infective dose in a single inoculum. Based on daily fecal egg counts, the average pre-patent period was determined as 24 days for *H. contortus*, and 31 days for *H. placei*. Regarding the *Haemonchus* spp. recovered at necropsy, the experimental groups GI, GII, and GIII had, respectively, an average of 25.5, 619.5, and 724.75 (120 *H. placei*, and 604.75 *H. contortus*) adult specimens, and no immature forms. Under the conditions of this study, the viability of goat infection by *H. placei* was confirmed, although, with low susceptibility. Nevertheless, the parasitism of this helminth species was more intense when associated with *H. contortus*. This fact indicates that in common grazing between cattle and young goats, when the latter end up ingesting both *Haemonhcus* species, especially in a mixed infection, *H. placei* may also parasitize them.

1. Introduction

Regarding goat gastrointestinal nematodes (GINs), the *Haemonchus* genus play the most important role, for its high pathogenicity, and prevalence. Due to its hematophagous activity, this parasite is responsible for causing varying degrees of anemia, which could lead to animal's death, when the parasitic load is high (Bichuette et al., 2015). It is known that *H. placei* has high specificity for cattle, whereas *H. contortus* is highly adapted to small ruminants (Amarante et al., 1997). However, mixed infections by these species under natural conditions have been described in cattle, sheep, and goats (Amarante et al., 1997; Akkari et al., 2012). In experimental conditions, it was possible to demonstrate the susceptibility of lambs to *H. placei* (Santos et al., 2014a,b), and of calves to *H. contortus* (Favero et al., 2016). The present study aimed to evaluate the susceptibility, and parasitological aspects of experimental primo-infection of young goats by *H. placei*, compared to *H. contortus*.

2. Material and methods

2.1. Study location and animals

The experiment was carried out at the Animal Health Research Center (CPPAR/ FCAV/UNESP). Fourteen male, newborn, Saanen goats, were used. The animals were fed bovine milk, by means of bottles, until age of 60 days old (weaning). Concentrate corn meal (60%), soybean meal (40%), and Tifton hay (*Cynodon* sp.) were supplied as early as the first week of life, to encourage solid food consumption. After artificial infection, feed was restricted to 150 g of daily concentrate, Tyfton hay (*Cynodon* sp.), and water, supplied *ad libitum*.

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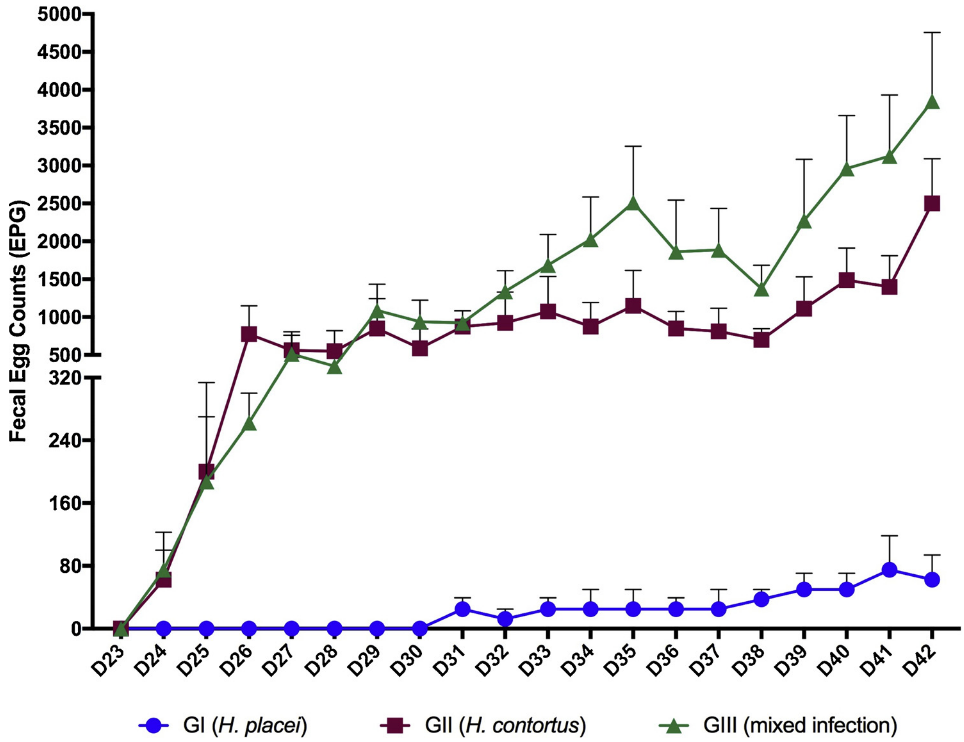

Fig. 1. Graphic representation of the arithmetic means (± standard errors) of the FEC for *Haemonchus* sp. of the experimentally infected kids, throughout the experimental period.

2.2. Distribution of the experimental groups

At an age of 75 days old (day zero), 15 days after weaning, the animals were weighed and distributed in four experimental groups: GI - infected with *H. placei* (n = 4); GII - infected with *H. contortus* (n = 4); GIII - infected with *H. placei* and *H. contortus* (n = 4) and GIV - control group (n = 2). The kids were individually allocated in suspended metal cages, thus not having contact with the soil or between the other kids, protected under a cemented and ventilated shed. The feces were removed daily and the urine washed.

2.3. Artificial infections with H. placei and H. contortus

To obtain the *H. placei* larvae, two cattle raised isolated of others animals as sheep and goats, from the same local of one commercial slaughterhouses of Jaboticabal, were used. After the slaughter, the abomasums were removed, and opened through the greater curvature (Amarante et al., 1997), and recovery of 150 *H. placei* adult specimens. The female ones had their oviducts dissected in physiological solution at 37 °C. Was possible to recovered 47,145 eggs that were then added to vermiculite moistened with distilled water, being placed in a BOD incubator for seven days. The infective third stage larvae (L_3) were extracted from the cultures according to Robert and O'Sullivan (1950), before inoculation.

The *H. contortus* L_3 were recovered from a mono-infected donor sheep's fecal cultures, and were kindly provided by Dr. Ana Carolina de Souza Chagas, of the Laboratory of Veterinary Parasitology of the EMBRAPA Livestock, São Carlos, SP, Brazil.

On day zero of the study, the animals were artificially infected, following the recommendations of the World Association for the Advancement of Veterinary Parasitology (Wood et al., 1995). The kids from GI were infected with 5000 *H. placei* L_3, the GII were infected with 5000 *H. contortus* L_3, and the GIII received a mixed infection with 2500 *H. placei* L_3 + 2500 *H. contortus* L_3. The kids from GIV received only distilled water.

2.4. Coproparasitological examinations

Weekly, between days D-28, and D-7, and daily, between days D-5, and D-1, fecal samples were collected, directly from the rectums, for fecal egg counts – FEC (GORDON and WHITLOCK, 1939), in order to confirm the absence of a natural GIN infection. After the artificial infection, daily FEC were performed, between days D + 7, and D + 42, in order to determine the pre-patent period (PPP) for *Haemonchus* species evaluated.

2.5. Euthanasia, parasitological necropsy and identification of helminths

On day D42 after the artificial infection, all goats were euthanized. The abomasums were digested in 3% hydrochloric acid (Wood et al., 1995) to recover possible immature forms present in the mucosa.

All the parasite specimens were collected from the gastric contents of each animal, and were stored in 70% alcohol, separated by sex (male and female parasites). The other organs were also inspected for the diagnosis of possible helminths present.

For the diagnosis of the *Haemonchus* species, the females were cut at their esophagus-intestine junctions, for counting of the furrows (grooves), according to Lichtenfels et al. (1994); Jacquiet et al. (1997); Santos et al. (2014a,b). For specific identification of the male specimens, the same cuts were performed (esophagus-intestinal junction), and the hooks and dorsal radius of the copulatory bursa were morphologically evaluated The diagnosis was too confirmed by PCR with both primer pairs (HpBotuF/R and HcBotuF1/R2), in acordante with the methodology described by Amarante et. al (2017).

2.6. Statistical analysis

The data were analyzed according to a completely randomized design, in a split-plot in time scheme, considering the treatments (experimental infections) as the main plot, and observation dates as subplots. The groups were compared by repeated measures ANOVA, and the means were compared by the Tukey test, at a 5% significance level

Table 1
Pre-patent period (days) for *Haemonchus placei* e *Haemonchus contortus* in kids experimentally infected with 5000 L_3.

Group	Kid	Pre-patent period	FEC[a]
GI: *H.placei*	248	38	50
	243	–[b]	–
	431	31	50
	114	31	50
	Mean	33	50
GII: *H.contortus*	231	25	50
	212	29	50
	213	24	100
	214	24	150
	Mean	25	87.5
GIII: *H. placei* + *H. contortus*	249	26	200
	415	24	200
	313	25	300
	268	24	100
	Mean	25	200

[a] FEC identified on the first day with presence of eggs in faeces.
[b] Animal did not shed Haemonchus sp. eggs throughout the experimental period.

($p \le 0.05$). The FEC were transformed to log10 (X + 1) prior to the analyzes. The analyzes were carried out with the statistical package SAS (2001).

3. Results

3.1. Pre-patent period

The first strongylid eggs were identified on the D24 post-inoculation (Fig. 1), in two goats belonging to the GII (*H. contortus*), and two of the GIII (*H. placei* + *H. contortus*). In the GI (*H. placei*), the FEC was positive from the D31. The goats belonging to the control group did not present any parasite eggs in the feces during the whole study period. Based on the results, the average PPP for the young goats experimentally infected was 31 to 38 days for *H. placei*, and 24 to 29 days for *H. contortus*. For the mixed infection, the observed PPP was 24 to 26 days (Table 1).

3.2. Helminths recovered at necropsy

The control group remained uninfected, with no GIN found at necropsy date (Table 2).

In the mixed infection group (GIII), a percentage of 83.44% was found for *H. contortus*, and 16.56% for *H. placei*. The total number of *H. contortus* recovered for this group was significantly higher ($p < 0.001$) than that of *H. placei*, with averages of 604.8 ± 44.07, and 120 ± 18.46, respectively. The number of adult *H. placei* specimens recovered was higher ($p < 0.01$) in GIII (120 ± 18.46) compared to GI (25.5 ± 5315). Regarding the number of *H. contortus* specimens recovered, the GII and GIII groups did not differ significantly ($p > 0.05$) Table 2.

The establishment rates for *H. placei* in the mono-infected animals (GI) were very low, ranging from 0.26% to 0.74% (mean of 0.405 ± 0.169%), significantly lower ($p \le 0.01$) than those obtained for the mixed infection group (GIII), ranging from 2.8% to 6.32% (mean 4.8 ± 0.738%). However, the establishment rates of this *Haemonchus* species in both GI and GIII groups were significantly lower ($p < 0.01$) than those of *H. contortus*, which ranged from 4.08% to 17.12% (mean of 12.39 ± 2.876%) in GII (mono-infection) and from 19.24% to 27.56% (mean of 24.19 ± 1.763%) in mixed infection (GIII). Nevertheless, higher establishment rates ($p < 0.05$) for *H. contortus* were found in GIII, compared to than in GII.

4. Discussion

Natural infection of goats by *H. placei*, species not adapted to these host, have been diagnosed in some studies carried out with animals from slaughterhouses by Achi et al. (2003) and Akkari et al. (2012). These studies, under natural conditions, highlight the susceptibility of goats to *H. placei* infection, and evident participation of mixed infections with *H. contortus.*

In the present study, the susceptibility of young goats to *H. placei* was confirmed experimentally, as well as the comparison of the kinetics of the infection by this species in comparison to *H. contortus*. The mean PPP observed for *H. contortus* and *H. placei* was 25 and 33 days, respectively, being in agreement with the literature regarding *Haemonchus* sp. infections in both cattle and small ruminants (Riggs, 2001; Santos et al., 2014a,b; Favero et al., 2016; Reiniger et al., 2017). Based on these works, it was possible to verify that *H. placei*, when parasitizing cattle, its most common hosts, presented an earlier PPP than those observed for goats in the present study (average of 33 days). Considering the results described in the studies cited above, regardless of the animal species involved, the PPP for *H. contortus* has always occurred earlier than for *H. placei*. No studies were found on the kinetics of *H. placei* infection in the caprine species.

According to the present results, it is possible to verify that *H. placei*, when inoculated alone in young goats (GI), presented a low establishment rate, which caused low FEC in the animals, and, consequently, few parasite specimens were recovered at necropsy. However, when this species was associated with *H. contortus* (mixed infection, GIII), it was possible to observe a higher *H. placei* infection rate. In these situations, some kind of synergism seems to occur between these two *Haemonchus* species, with a superior establishment rate for both *H. placei* and *H. contortus* in this group. This fact indicates that in common grazing between cattle and young goats, when the latter end up ingesting both *Haemonchus* species, especially in mixed infection, *H. placei* can also parasitize them. Experimental mixed infection by *H. contortus* and *H. placei* was also evaluated by Santos et al. (2018), but in sheep. The authors recovered similar amounts of both parasites on the D42 post-infection, which differed from the present study in which the number of *H. contortus* recovered was significantly higher than *H. placei*. These authors, however, did not evaluate mono-infected animals for comparative purposes regarding the possible synergy between the

Table 2
Means, standard deviations and comparison of data analysis obtained from the amount of *Haemonchus* collected in the necropsied experimental goats.

		Experimental groups/ Mean = [Σlog(x + 1)/n][a] and standard deviation			
Helminths		GI: *H. placei*	GII: *H. contortus*	GIII: *H. placei* + *H. contortus*	GIV: Controle
Haemonchus placei	Female	1.11 ± 0.24B	0.00 ± 0.00C	1.72 ± 0.15 A	0.00 ± 0.00C
	Male	1.11 ± 0.13B	0.00 ± 0.00C	1.81 ± 0.16 A	0.00 ± 0.00C
Haemonchus contortus	Female	0.00 ± 0.00B	2.42 ± 0.37A	2.50 ± 0.11 A	0.00 ± 0.00B
	Male	0.00 ± 0.00B	2.44 ± 0.22A	2.45 ± 0.06 A	0.00 ± 0.00B
Means and standard deviations resulting from the body lengths of the females for GI, GII and GII					
GI: *H. placei*		GII: *H. contortus*		GIII: *H. placei* / *H. contortus*	
19.60 ± 1.06		19.95 ± 1.35		20.00 ± 0.91 / 20.4 ± 1.35	

[a] Values followed by the same letter in the line do not differ from one another by the *t*-test ($P > 0.05$).

Haemonchus species.

However, it is also possible that, with the aging of these animals and time of exposure to the parasite, the amount of *H. placei*, a species less adapted to goats, ends up decreasing significantly in their abomasums. Santos et al., 2014a,b compared the aspects of artificial primo-infections by *Haemonchus* sp. in sheep, and a smaller patent period (PP) was found for *H. placei* (288–364 days) compared to *H. contortus* (302–538 days), evidencing an early parasite expulsion in the first group. The authors evaluated the role of continuous exposure to both parasites, through serial infections, in the acquisition of immunity to future infections by these parasites. It was observed that, for the re-infections, the animals serially infected with *H. placei* presented significantly lower FEC than the group serially infected with *H. contortus*, which did not occur in the primo-infection, when both groups presented high FEC, with no significant differences. The authors also observed that the number of adult *H. placei* recovered after slaughter was significantly lower in the serially infected group, which did not occur for *H. contortus*, with a similar number of adults recovered among the primo-infected or serially infected groups. In the present study, however, it was not possible to determine the PPP of goats for both *Haemonchus* species, nor the role of continuous exposure to these parasites in the acquisition of immunity to future infections, with a lack of such studies on this subject for goats.

5. Conclusion

Based on the present results, taking into account the experimental design adopted, it was possible to conclude that *H. placei* when inoculated alone in goats, is not well adapted to this host, in view of the low establishment rates and absent clinical signs of haemonchosis. However, when associated with *H. contortus*, the percentage of *H. placei* individuals which completed its life cycle was approximately ten-fold higher. This fact indicates that, in common grazing between cattle and young goats, the latter may become infected by *H. placei*, most notably in mixed infection with *H. contortus*.

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