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First report of pathogenicity of entomopathogenic nematodes of the genus *Heterorhabditis* on partially engorged females of *Dermacentor nitens* (Acari: Ixodidae)

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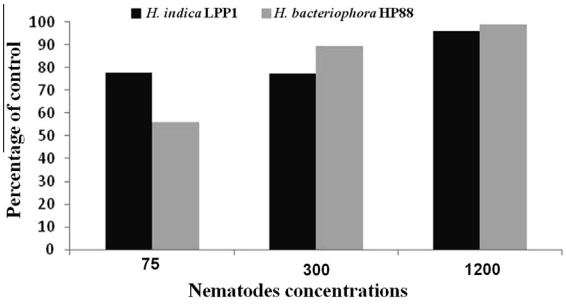
HIGHLIGHTS

- First record of nematodes of genus *Heterorhabditis* infecting *D. nitens*.
- *H. bacteriophora* HP88 and *H. indica* LPP1 showed virulence to *D. nitens*.
- *H. bacteriophora* HP88 and *H. indica* LPP1 in the highest concentration resulted in efficacy above 95%.
- *H. bacteriophora* HP88 and *H. indica* LPP1 are promising agents for biological control for *D. nitens*.

GRAPHICAL ABSTRACT

Percentage of control of partially engorged females of *Dermacentor nitens* treated with differents concentrations of *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis indica* LPP1 under laboratory conditions (27 ± 1 °C and RH 80 ± 10%).



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ABSTRACT

The aim of this study was to evaluate the effect of different concentrations of the entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis indica* LPP1 on the reproductive biology of partially engorged females of *Dermacentor nitens*. Four groups were formed, with each group containing 10 females and exposed to concentrations of 0, 75, 300, and 1200 nematodes for each female. This procedure was performed separately for each nematode. The following biological parameters were evaluated: egg mass weight, egg production index, hatching percentage, and percentage of control. *H. bacteriophora* HP88 at the two highest concentrations (300 and 1200 EPNs/female) caused a reduction ($p < 0.05$) on the egg mass and egg production index. Was noted a significant reduction ($p < 0.05$) in the percentage of hatched in all the treated groups. For *H. indica* LPP1, all treatments resulted in decreased ($p < 0.05$) values for all the parameters. The percentages of controls obtained at concentrations of 75, 300, and 1200 EPNs/female were 56.3, 89.3, and 98.8 and 77.5, 77.1, and 95.9 for *H. bacteriophora* HP88 and *H. indica* LPP1, respectively. Therefore, it is concluded that these nematodes showed pathogenicity toward partially engorged females of *D. nitens*, thereby negatively affecting the reproductive biology of this tick.

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1. Introduction

Dermacentor nitens (Neumann, 1897), also known as the tropical horse tick, is found mainly inside the ear of equines. However, in cases of large infestations it can also settle in other parts of the animal's body (Borges and Leite, 1993). The parasitism by these ticks causes animal stress, blood loss, predisposition to myiasis and secondary bacterial infections and expenses in equipment and treatment of animals (Guimarães et al., 2001; Guglielmone et al., 2006). Moreover, this tick is the vector of *Babesia caballi* (Nuttall and Strickland, 1910), an etiologic agent of equine piroplasmosis (Labruna and Machado, 2006).

Entomopathogenic nematodes (EPNs) are important agents in controlling agricultural pests (Grewal et al., 2001; Hazir et al., 2003). Recently, the use of these nematodes has also been considered for the control of parasites of medical and veterinary importance (Glazer et al., 2005), particularly in the search for a new alternative for controlling ticks (Kaaya et al., 2000; Vasconcelos et al., 2004; Samish et al., 2008; Monteiro et al., 2012). Studies have shown that several species of ixodids and two argasids are susceptible to infection by EPNs, of which the virulence varies with the strain of the nematode and stage and species of the tick that is tested (Hill, 1998; Kaaya et al., 2000; Samish et al., 2008).

Among the strains tested against cattle tick, *Rhipicephalus microplus* (Canestrini, 1888), *Heterorhabditis bacteriophora* (Poinar, 1975) strain HP88 and *Heterorhabditis indica* (Poinar, Karanukar and David, 1992) strain LPP1 were identified as the most virulent (Monteiro et al., 2010a; Silva et al., 2012). Regarding *D. nitens*, however, only one study was conducted and the results showed that these ixodids were susceptible to infection by two strains of *Steinernema carpocapsae* (Weiser, 1955) (Freitas-Ribeiro et al., 2009); however, no data is available on the action of the *Heterorhabditis* genus against this species. Thus, the objective of this study was to evaluate the efficacy of different concentrations of *H. bacteriophora* HPP8 and *H. indica* LPP1 on the reproductive biology of partially engorged females of *D. nitens*.

2. Materials and methods

The study was conducted in the Laboratório de Parasitologia of Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil. Partially engorged females used in the experiment with *H. bacteriophora* HP88 were obtained from naturally infested horses in the city of Seropédica, RJ, Brazil, while the females used in the experiments with *H. indica* LPP1 were obtained in the same circumstances from the municipality of Cabo Frio, RJ, Brazil. The nematodes used were provided by the Laboratório de Nematologia of the Universidade Federal Norte Fluminense Darcy Ribeiro and are kept in the EPN bank of the Laboratório de Parasitologia of Embrapa Gado de Leite, through *in vivo* multiplication in *Galleria mellonella* (Linnaeus, 1758) according to the methodologies proposed by Lindegren et al. (1993) and Kaya and Stock (1997).

The experimental procedure for both experiments was based on the methodology used by Monteiro et al. (2010a). Partially engorged females were divided into four groups containing 10 ticks with uniform weights ($p > 0.05$). Each group was divided into two subgroups with five females properly identified using non-toxic paint and were distributed in Petri dishes (6 cm) containing 15 g of sterile sand. Each tick was considered a repetition (each female = one experimental unit).

The groups were pipetted using 3 ml of nematode suspension at concentrations of 375, 1500, and 6000 EPNs/plate; thus, the concentration of female EPNs in each treatment was approximately 75, 300, and 1200. The control group was pipetted with 3 ml of distilled water, free of nematodes. The groups were kept in a climate-controlled chamber at 27 ± 1 °C and relative humidity (RH) more than 80 ± 10% for 48 h.

After exposure, the females that were still alive in each treatment group were fixed (with adhesive tape) in dorsal decubitus position on 12-cm Petri dishes using tape and placed in a climate-controlled chamber under the same temperature and humidity conditions mentioned previously. The collection and weighing of eggs were performed every day, and the egg masses collected were weighed and placed individually in identified syringes (10 ml) with the distal portion cut and sealed using cotton, and kept in a climate-controlled chamber (27 ± 1 °C and RH > 80 ± 10%) to evaluate the hatching percentage of larvae.

The egg production index (EPI%) was calculated according to Bennett (1974).

$$\text{EPI} = (\text{egg mass/female weight before oviposition}) \times 100$$

To evaluate the efficacy of the treatments, the estimated reproduction (ER) was calculated.ER = (egg mass weight/female weight) × hatching% × 20,000 (Drummond et al., 1973).

Next, the values obtained were used to calculate the percentage of control:%C = (ER control group − ER-treated group) × 100/ER control group (Drummond et al., 1973).

The statistical analysis was performed using the software Biostat version 5.0. The percentage values were transformed into $\sqrt{}$ arcsen x. The means values were compared using analysis of variance (ANOVA) followed by Tukey test. In the case of non-normal distribution, the Kruskal–Wallis test followed by Student–Newman–Keuls test were used. Spearman's test was used to verify the possible correlations between the concentration of nematodes and the egg mass weight, EPI and hatching percentage.

3. Results

The average egg mass weight and EPI of females treated with *H. bacteriophora* HP88 at a concentration of 75 EPNs/female were, respectively, 27 mg and 23.5% and were statistically similar values ($p > 0.05$) to those observed for the control (38.1 mg and 35.8%). However, at concentrations of 300 and 1200 EPNs/female, the values were 8.0 and 4.6 mg and 7.8% and 3.9%, respectively, indicating the action of nematodes at concentrations starting from 300 EPNs/female results in reduced in the amount of eggs ($p < 0.05$) (Table 1).

The hatching percentage of larvae of control group was 92%, while the hatching percentage of the treated groups ranged from 57% to 9%, with a significant reduction in all concentrations (Table 1). Further, negative correlations were observed between the concentrations of nematodes of *H. bacteriophora* HP88 and the egg mass weight ($r = -0.6924$; $p < 0.01$), EPI ($r = -0.7068$; $p < 0.01$), and hatching percentage of larvae ($r = -0.5953$; $p < 0.01$).

In the experiment where the females were treated with *H. indica* LPP1, the egg mass weights and EPI were, respectively, 39.9, 14.2, 14.4, and 3.2 mg and 34.5, 11.6, 9.8, and 2.8% at concentrations of 0, 75, 300, and 1200 EPNs/female. These results showed that the nematodes caused significant deleterious ($p < 0.05$) effects on the egg laying process at all concentrations tested (Table 1).

This fact was also observed in relation to the hatching percentage of larvae, in which the values obtained for the treated groups (55.6%, 56.7%, and 47.5%) also showed significant differences ($p < 0.05$) than that for the control (89.0%) (Table 1). Results of the Spearman test showed a negative correlation between the concentrations of *H. indica* LPP1 and the egg mass weight ($r = -0.6620$; $p < 0.01$) and EPI ($r = -0.6825$; $p < 0.01$). However, the same was not observed for the hatching percentage ($r = -0.2971$; $p < 0.05$).

For *H. bacteriophora* HP88 the control percentages were 56.3, 89.3 and 98.8% and those for *H. indica* LPP1 were 71.1, 75.5, and 95.9% at concentrations of 75,300 and 1200 EPNs/female, respectively.

Table 1
Mean, egg mass weight (mg), egg production index (%EPI), hatching percentage of larvae (%) and percentage of control of *Dermacentor nitens* treated with different concentrations of *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis indica* LPP1 under laboratory conditions (27 ± 1 °C and RH 80 ± 10%).

Nematode	Concentration of EPNs per female	Egg mass weight (mg)	Egg production index (%)	Hatching percentage of larvae (%)	Percentage of control (%)
H. bacteriophora HP88	0	38.1[a] ± 14.0 (10)	35.8[a] ± 7.3 (10)	92.0[a] ± 6.8 (10)	
	75	27.0[a] ± 24.8 (10)	23.5[a] ± 16.7 (10)	57.0[b] ± 41.6 (10)	56.3
	300	8.0[b] ± 14.3 (10)	7.8[b] ± 13.3 (10)	47.4[b] ± 46.6 (05)	89.3
	1200	4.6[b] ± 7.5 (10)	3.9[b] ± 6.6 (10)	9.0[b] ± 17.3 (04)	98.8
H. indica LPP1	0	39.9[a] ± 14.7 (10)	34.5[a] ± 14.6 (10)	89.0[a] ± 13.8 (10)	
	75	14.2[b] ± 18.7 (10)	11.6[b] ± 14.6 (10)	55.6[b] ± 35.9 (08)	77.1
	300	14.4[b] ± 22.2 (10)	9.8[b] ± 13.6 (10)	56.7[b] ± 47.3 (06)	77.5
	1200	3.2[b] ± 10.1 (10)	2.8[b] ± 8.7 (10)	47.5[*] ± 627.2 (02)	95.9

(n): Sample size. Means followed by equal letters in the same column for same nematode do not differ statistically at 5% significance.
[*] Statistical test not performed due to the small sample size.

4. Discussion

Entomopathogenic nematodes are used successfully in various locations around the world to control different insect pests (Grewal et al., 2001; Dolinski et al., 2006), and in the past two decades, research has sought to enable the use of EPNs to control ticks (Samish et al., 2008). Most studies have been directed toward the control of *R. microplus* (Vasconcelos et al., 2004; Reis-Menine et al., 2008; Molina-Ochoa et al., 2009; Monteiro et al., 2010b; Carvalho et al., 2010; Monteiro et al., 2012) and *Rhipicephalus annulatus* (Say, 1921) (Samish and Glazer, 1991; Samish and Glazer, 1992; Samish et al., 2000; Glazer et al., 2001; Alekseev et al., 2006). To the best of our knowledge, this is the second study on the pathogenicity of EPNs on *D. nitens* and the first to investigate the efficacy of nematodes of the genus *Heterorhabditis*, which have been identified as the most virulent types on ticks (Samish et al., 2008).

The two strains tested led to a reduction in the number of eggs produced by partially engorged *D. nitens* females, and at the highest concentrations (300 and 1200 EPNs/female), many females died before starting ovipositing. On the positive side, we highlight the fact that EPNs rapidly kill the females, and in many cases completely preventing egg laying, which under field conditions would result in the reduction of large numbers of larvae in the next generation. Reduction in egg mass was also observed when *H. bacteriophora* HP88 (Monteiro et al., 2010a) and *H. indica* LPP1 (Silva et al., 2012) tested against engorged females of *R. microplus*. Similar results were reported by Freitas-Ribeiro et al. (2009) in tests using two strains (ALL and Santa Rosa) of *S. carpocapsae* on engorged females of *D. nitens*.

Further, our study results showed lower values for the EPI of females in the treated groups. Similar results were reported by Freitas-Ribeiro et al. (2009) by using ALL and Santa Rosa strains of *S. carpocapsae* on *D. nitens* females. The reported results indicated that a low nutrient intake rate by partially engorged females was used for egg conversion (Freitas-Ribeiro et al., 2009). This process was also observed when the nematodes *H. bacteriophora* HP88 (Monteiro et al., 2010a) and *H. indica* LPP1 (Silva et al., 2012) were tested against engorged females of *R. microplus*.

H. bacteriophora HP88 and *H. indica* LPP1 also caused reduction in viability of eggs produced by partially engorged females, because the figures for the hatching percentage of the treated groups were lower than the controls. Similar results were reported by Monteiro et al. (2010a,b) and Silva et al. (2012) by using *H. bacteriophora* HP88 and *H. indica* LPP1, respectively, where the hatching percentages of the treated groups were lower than the control. Interferences in the processes of oocytes formation, fertilization, water absorption by the cuticle, and lubrication of eggs by Gene's organ could explain this reduction (Monteiro et al., 2010a; Silva et al., 2012). For confirmation, further studies are needed on histology of organs and structures related to the process of oviposition and evaluation of the profile of lipids and proteins in eggs from treated females.

Increasing concentrations resulted in decreased values for all parameters evaluated, except for the hatching percentage in the experiment with infective juveniles of *H. indica* LPP1. The same was observed for the egg mass weight and EPI in the study by Monteiro et al. (2010a) and Silva et al. (2012). The increase in infective juveniles penetrating into the hemocoel can contribute to the establishment of the infection process, allowing EPNs to overcome the host's immune barriers (Dowds and Peters, 2002).

Contrary to that observed in tests performed using *R. microplus*, wherein the concentration of 75 EPNs/female of *H. bacteriophora* HP88 and *H. indica* LPP1 resulted in the percentage of control exceeding 90% (Monteiro et al. 2010a,b; Silva et al., 2012), in the present study, only the highest concentration (1200 EPNs/female) resulted in a percentage of control exceeding 90%. From these results, we can infer that *D. nitens* females have greater resistance to infection by infective juveniles of *H. bacteriophora* HP88 and *H. indica* LPP1. In addition, the results and data shown in the studies by Freitas-Ribeiro et al. (2005, 2009), both testing two strains of *S. carpocapsae*, All and Santa Rosa, indicate that *R. microplus* females are more susceptible to this nematode than engorged *D. nitens* females.

Comparing the performances of the two nematodes used in this study, we conclude that *H. bacteriophora* HP88 and *H. indica* LPP1 showed similar results, with superior performance of the second nematode at a low concentration (75 EPNs/female), with a reversal observed in the next concentration. At the highest concentration, the results were very close with a slight advantage for *H. bacteriophora* HP88.

In this study, tests were performed on partially engorged *D. nitens* females; however, we consider it reasonable to assume that similar results would be found in the case of tests on the pathogenicity of *H. bacteriophora* HP88 and *H.indica* LPP1 on engorged female of *D. nitens*. Freitas-Ribeiro et al. (2005) reported that the nematode strains of *S. carpocasae*, ALL and Santa Rosa showed similar performance on engorged and semi-engorged *R. microplus* females.

Thus, we conclude that partially engorged females of *D. nitens* are susceptible to infection by *H. bacteriophora* HP88 and *H. indica* LPP1 *in vitro* assays. The application this EPNs as new alternative for the control of ticks deserves be investigated, because after the process of engorgement, female *D. nitens* seek in soil, sites with low incidence of direct solar radiation and high humidity for make the posture, conditions are also favorable for survival of EPNs.

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