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Short communication

Evaluation of the action of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) isolate HP88 on the biology of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

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ABSTRACT

The objective of this work was to evaluate the effect of different concentrations of the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* strain HP88 on the biological parameters of the non-parasite phase of engorged females of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Six groups were formed, each containing 20 engorged females, which were exposed to the following concentrations of infective juveniles of this nematode: 0, 75, 150, 300, 600 and 1200 EPNs/female, respectively. The following biological parameters were observed: female weight before oviposition, egg mass weight, pre-oviposition period, oviposition period, survival period, hatching percentage, egg production index (%EPI), nutritional index (%NI) and efficacy of treatment. There were no statistically significant differences in the female weight before the oviposition and pre-oviposition period ($p > 0.05$) between the groups. The nematode action caused significant alterations ($p < 0.01$) in the egg mass weight, oviposition period, survival period, hatching percentage, %EPI and %NI between the treated groups and the control group. Treatment efficacy was higher than 90% in all groups, reaching 99% at a dosage of 1200 EPNs/female. The present study demonstrates that under laboratory conditions, *H. bacteriophora* HP88 has a deleterious effect on the majority of the parameters of the non-parasitic phase of engorged *R.* (*B.*) *microplus* females, making this species a potential biological control agent of cattle ticks.

Among the various species of ticks existing in Neotropical regions, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae) (=*Boophilus microplus*), commonly known as the cattle tick, causes the highest economic losses to breeders (Martins et al., 2006). This tick can cause direct damage due to blood spoliation and its consequences, and indirect damages through transmission of pathogenic agents and expenses for medicines and specialized labor (Furlong et al., 2007). The resulting economic losses amount to some two billion dollars a year in Brazil (Grisi et al., 2002).

The control of *R.* (*B.*) *microplus* is mainly carried out during its parasite phase, through the use of chemical products. However, the indiscriminate and incorrect use of acaricides has been causing serious problems of resistance, besides leaving residues in meat, milk and the environment in general (Furlong et al., 2007; Labruna, 2008). Therefore, it is necessary to find new alternatives that can be used in programs for integrated management of this pest, to minimize

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the environmental impacts and reduce production costs (Kaaya et al., 2000; Samish, 2000).

A promising alternative is biological control through the use of entomopathogenic nematodes (EPNs) (Samish and Glazer, 2001; Reis-Menini et al., 2008). Studies conducted as part of the research program of the Embrapa Dairy Cattle Research Center (Embrapa Gado de Leite) in Brazil have demonstrated that different species of entomopathogenic nematodes are efficient against cattle tick under laboratory conditions (Vasconcelos et al., 2004; Freitas-Ribeiro et al., 2005; Silva, 2007; Reis-Menini et al., 2008). The nematode *Heterorhabditis bacteriophora* Poinar 1975 (Rhabditida: Heterorhabditidae), strain HP88, has been shown to have high potential to control other tick species (Samish and Glazer, 1992; Hill, 1998; Kaaya et al., 2000). Therefore, we designed the present study to evaluate the influence of different concentrations of infective juveniles (IJs) of *H. bacteriophora* HP88 on the biological parameters of the non-parasite phase of engorged females of *R.* (*B.*) *microplus*.

The study was carried out in the Parasitology Laboratory of the Embrapa Dairy Cattle Research Center, located in Juiz de Fora, Minas Gerais, Brazil. We chose a strain of *R.* (*B.*) *microplus* originating from the municipality of Campina Verde, Minas Gerais. The nematodes of the species *Heterorhabditis bacteriophora* used in this study were donated by Dr. Cláudia Dolinski of North Fluminense State University (UENF). This nematode was bred of according to Lindegren et al. (1993) and Kaya and Stock (1997).

The experiment was based on the method used by Silva (2007). The engorged females were divided into six treatment groups of 20 ticks. Each group was divided into four subgroups with five females, identified with nontoxic paint for individual monitoring (each female = an experimental unit), and distributed in Petri dishes (6 cm in diameter) containing 15 g of sterilized sand as substrate.

Each subgroup was sprayed with 4 ml of a aqueous solution containing the nematodes, at concentrations of 375; 750; 1500; 3000 and 6000 IJs per dish, so that the concentration of EPNs per female in each treatment was 75; 150; 300; 600 and 1200. The control consisted of 4 ml of distilled water free of nematodes. The groups were kept in a climate-controlled chamber at $27 \pm 1\,^{\circ}C$ and $RH > 80 \pm 10\%$ for a period of 72 h.

The females were observed daily to check for mortality and egg-laying until the last tick died. The egg masses were placed individually in labeled 5-ml adapted syringes, and kept in the climate-controlled chamber under the same temperature and relative humidity conditions mentioned before. After the exposure period, with the use of adhesive tape the females from each treatment were affixed in dorsal decubitus position in 12-cm Petri dishes and placed in the climate-controlled chamber at $27 \pm 1\,^{\circ}C$ and $RH > 80 \pm 10\%$, for continued biological monitoring of the non-parasite phase. The following biological parameters were evaluated: female weight before oviposition (mg), egg mass weight (mg), pre-oviposition, oviposition and survival periods (days), hatching percentage, egg production index (%EPI), nutritional index (%NI) (Bennett, 1974) and the efficacy of treatments, obtained through offspring inhibition by the formula proposed by Drummond et al. (1973).

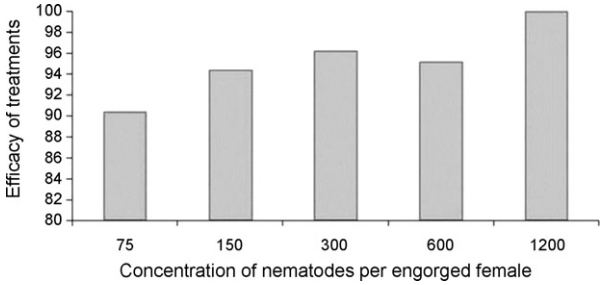

Fig. 1. Efficacy of different concentrations of *Heterorhabditis bacteriophora* HP88 against engorged females of *Rhipicephalus* (*Boophilus*) *microplus*, under laboratory conditions ($27 \pm 1\,^{\circ}C$ and $RH > 80 \pm 10\%$). Embrapa Gado de Leite Parasitology Laboratory, Juiz de Fora, MG, Brazil.

The statistical analysis was performed using the software Biostat version 5.0. The percentage values were transformed into $\sqrt{}$arcsen x. The median values of each treatment were analyzed by ANOVA and the Tukey test ($p < 0.05$). In the case of nonparametric distributions, the values were compared through the nonparametric tests of Kruskal–Wallis and Student–Newman–Keuls ($p < 0.05$). The Spearman test was used to calculate the correlation between the nematode concentration and egg mass weight and survival period of the engorged females. A linear regression was employed to establish an equation describing the relation between the nematode concentration and efficacy of treatments, and this equation was used to calculate the 50% and 90% offspring inhibition concentrations. To calculate the regression, we placed the nematode concentration on the *X*-axis and the concentration × efficiency transformation on the *Y*-axis, according to Ikemoto and Takai (2000).

The results of the present work are presented in Tables 1 and 2, and Fig. 1. The weights before oviposition of the females in the different groups did not mutually vary significantly ($p > 0.05$) (Table 1), suggesting that the changes in the other biological parameters probably were related to the action of the nematodes.

The values obtained for egg mass weight, egg production index (%EPI) and nutritional index (%NI) of all the treated groups differed significantly ($p < 0.01$) from the respective figures for the control group (Table 1). The pre-oviposition period was not affected by the action of *H. bacteriophora* HP88 at the different concentrations ($p > 0.05$) (Table 2). The oviposition and survival periods of the treated groups varied, respectively, between 1.00 and 2.60 and 2.59 and 5.12 days (Table 2). In both cases these values were lower than for the control group (13.88 and 16.87 days), evidencing highly significant differences ($p < 0.01$). The hatching percentage of the treatments varied from 55.00% and 66.73%, and in all cases was significantly different ($p < 0.01$) than the percentage of the control group (92.60%) (Table 2).

The action of *H. bacteriophora* HP88 induced significant alterations in most of the analyzed parameters, presenting deleterious effect in the oviposition process of the cattle ticks, often killing them before they started oviposition or interfering in the process of converting blood into eggs. Vasconcelos et al. (2004) employed different concen-

Table 1
Mean female weight before oviposition, egg mass weight, egg production index (%EPI) and nutritional index (%NI) of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* treated with different concentrations of infective juveniles of *Heterorhabditis bacteriophora* HP88, under laboratory conditions (27 ± 1 °C and RH > 80 ± 10%). Embrapa Gado de Leite Parasitology Laboratory, Juiz de Fora, MG, Brazil.

Concentration of nematodes	Female weight before oviposition (mg)	Egg mass weight (mg)	Egg production index, EPI (%)	Nutritional index, NI (%)
0	223.15^{a} ± 30.18 (16)	111.43^{a} ± 22.44 (16)	50.04^{a} ± 8.40 (16)	69.42^{a} ± 10.38 (16)
75	228.82^{a} ± 25.14 (17)	15.41^{b} ± 24.55 (17)	7.01^{b} ± 10.94 (17)	29.15^{b} ± 26.44 (17)
150	228.45^{a} ± 34.73 (17)	9.40^{b} ± 14.99 (17)	3.92^{b} ± 5.56 (17)	23.79^{b} ± 26.37 (17)
300	225.29^{a} ± 29.75 (17)	6.54^{b} ± 7.60 (17)	2.84^{b} ± 3.18 (17)	18.30^{b} ± 17.26 (17)
600	226.12^{a} ± 29.83 (17)	8.73^{b} ± 20.79 (17)	3.25^{b} ± 7.67 (17)	21.42^{b} ± 30.03 (17)
1200	226.69^{a} ± 29.48 (17)	0.16^{c} ± 0.33 (17)	0.07^{c} ± 0.16 (17)	2.37^{c} ± 4.73 (17)

(*n*): Sample size. Means followed by equal letters in the same column do not differ statistically at 5% significance.

trations of *H. bacteriophora* CCA and *Steinernema glaseri* (Steiner, 1929) (Rhabditida: Steinernematidae) SANTA ROSA against this cattle tick, and found that only concentrations of 1000 and 5000 EPNs per female of the second nematode caused a significant alteration in most of the parameters. The results of the present study are similar to those of Freitas-Ribeiro et al. (2005) and Silva (2007), both of which reported that females of the same tick were affected in all treatments with different dosages of two strains (SANTA ROSA and ALL) of *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae) and *Heterorhabditis indica* (Poinar et al., 1992) (Rhabditida: Heterorhabditidae) strain LPP1, respectively.

We observed a negative correlation between the nematode concentration and survival period ($r = -0.6669$) ($p < 0.01$) and the egg mass weight ($r = -0.7466$) ($p < 0.01$) of the engorged females. Vasconcelos et al. (2004) and Freitas-Ribeiro et al. (2005), utilizing concentrations of *S. glaseri* SANTA ROSA and *S. carpocapsae*, strain ALL and SANTA ROSA, respectively, also observed that increasing nematode concentrations progressively reduced the survival period and egg mass weight of this tick.

The efficacy in all the treatments was greater than 90% and the treatment with 1200 EPNs/female was 99.92% efficient (Fig. 1). The efficacy in the treatment with the lowest nematode concentration (75 EPNs/female) exceeds the values obtained by Vasconcelos et al. (2004), who observed efficiency above 80% for *H. bacteriophora* CCA at a concentration of 300 EPNs/female and greater than 90% for *S. glaseri* only at concentrations of 1000 and 5000 EPNs/female. The efficacy found in the present work was similar to that found by Silva (2007), using the nematode *H. indica* LPP1, where the efficiency was above 90% starting at a concentration of 75 EPNs/female.

To calculate the polynomial regression, we used only the values obtained at the concentrations of 75, 150 and 300 EPNs/female, the most linear part of the curve, as recommended by Bean (1961), to obtain values closer to reality. The polynomial regression returned the following equation: $D = -571.50005 + 98.054286 \times \mathrm{DE}$ (R2 = 1.0), and from this equation we obtained the 50% and 90% offspring inhibition concentrations which were 11.89 and 71.00 EPNs/female, respectively. These results show that low concentrations of infective juveniles of *H. bacteriophora* HP88, under laboratory conditions, are sufficient to keep engorged females from producing offspring.

At the highest concentration (1200 EPNs/female), we observed rupture of the cuticle of some engorged females because of the nematodes' action. This was also reported by Hill (1998) and Vasconcelos et al. (2004), utilizing *H. bacteriophora*, isolates HP88 and CCA, respectively. According to those authors, the rupture is due to the penetration of the infective juveniles, since nematodes of the *Heterorhabditis* Poinar, 1976 genus have a keratinous tooth in their front part that permits them to penetrate the host's tegument (Kaya and Gaugler, 1993). In the present study this result can be related to the high concentration of invaders, since we only observed rupture of the cuticle in the treatment with 1200 EPNs/female.

The present study demonstrates that under laboratory conditions, *H. bacteriophora* HP88 had high patogenicity against *R.* (*B.*) *microplus*, with efficacy rates above those found with the use of other nematodes, such as *H. bacteriophora* CCA, *S. glaseri* SANTA ROSA and *S. carpocapsae* SANTA ROSA and ALL strains (Vasconcelos et al., 2004; Freitas-Ribeiro et al., 2005). Therefore, new studies should be conducted to investigate and validate the methods for possible use of this nematode in field conditions.

Table 2
Mean pre-oviposition, oviposition and survival periods of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* treated with different concentrations of infective juveniles of *Heterorhabditis bacteriophora* HP88, under laboratory conditions (27 ± 1 °C and RH > 80 ± 10%) and hatching percentage of larvae obtained of the eggs these females. Embrapa Gado de Leite Parasitology Laboratory, Juiz de Fora, MG, Brazil.

Concentration of nematodes	Pre-oviposition period (days)	Oviposition period (days)	Survival period (days)	Hatching percentage, %EC
0	1.38^{a} ± 0.51 (16)	13.88^{a} ± 2.31 (16)	16.87^{a} ± 1.85 (16)	92.60^{a} ± 6.95 (16)
75	1.79^{a} ± 0.58 (15)	2.60^{b} ± 1.68 (15)	5.12^{b} ± 1.65 (17)	66.73^{b} ± 31.61 (15)
150	1.79^{a} ± 0.43 (14)	1.93^{b} ± 1.07 (14)	3.88^{bc} ± 0.78 (17)	63.43^{b} ± 32.65 (14)
300	1.67^{a} ± 0.49 (13)	1.46^{b} ± 0.88 (13)	3.75^{bc} ± 1.24 (17)	61.85^{b} ± 37.68 (13)
600	1.73^{a} ± 0.47 (11)	2.00^{b} ± 1.61 (11)	3.63^{cd} ± 1.41 (17)	58.50^{b} ± 32.06 (8)
1200	1.80^{a} ± 0.45 (5)	1.00^{b} ± 0.00 (5)	2.59^{d} ± 0.62 (17)	55.00* ± 49.50 (2)

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

References

Bean, J.L., 1961. Predicting emergence of second-instar spruce budworm larvae from hibernation under field conditions in Minnesota. Ann. Entomol. Soc. Am. 54, 175–177.

Bennett, G.F., 1974. Oviposition of *Boophilus microplus* (Canestrini, 1887) (Acarina: Ixodidae). I. Influence of tick size on egg production. Acarologia 16, 52–61.

Drummond, R.O., Ernest, S.E., Trevino, J.L., Gradney, W.J., Graham, O.H., 1973. *Boophilus anulatus* and *Boophilus microplus*: laboratory tests of insecticides. J. Econ. Entomol. 66, 30–133.

Freitas-Ribeiro, G.M., Furlong, J., Vasconcelos, V.O., Dolinski, C., Ribeiro, A.L., 2005. Analysis of biological parameters of Boophilus microplus Canestrini, 1887 exposed to entomopathogenic nematodes Steinernema carpocapsae SANTA ROSA and ALL STRAINS (Steinernema: Rhabditidae). Braz. Arch. Biol. Technol. 48, 911–919.

Furlong, J., Martins, J.R.S., Prata, M.C.A., 2007. O carrapato dos bovinos e a resistência: temos o que comemorar? Controle estratégico do carrapato dos bovinos. A Hora Vet. 27, 53–56.

Grisi, L., Massard, C.L., Moya-Borja, G.E., Pereira, J.B., 2002. Impacto econômico das principais ectoparasitoses em bovinos no Brasil. A Hora Vet. 21, 8–10.

Hill, D.E., 1998. Entomopathogenic nematodes as control agents of developmental stages of the black legged tick, Ixodes scapularis. J. Parasitol. 84, 1124–1127.

Ikemoto, T., Takai, K., 2000. A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environ. Entomol. 29, 671–682.

Kaaya, G.P., Samish, M., Glazer, I., 2000. Laboratory evaluation of pathogenicity of entomogenous nematodes to African ticks species. Ann. N. Y. Acad. Sci. 916, 306–308.

Kaya, H.K., Gaugler, R., 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38, 181–206.

Kaya, H.K., Stock, P., 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic, CA, pp. 281–324.

Labruna, M.B., 2008. In: Pereira, M.C., Labruna, M.B., Szabo, M.P.J., Klafke, G.M. (Eds.), Combate contra Rhipicephalus (Boophilus) microplus. *Rhipicephalus* (*Boophilus*) *microplus*: Biologia, Controle e Resistência. MEDVET, pp. 15–56.

Lindegren, J.E., Valero, K.A., Mackey, B.E., 1993. Simple in vivo production and storage methods for *Steinernema carpocapsae* infective juveniles. J. Nematol. 5, 93–197.

Martins, J.R.S., Furlong, J., Leite, R.C., 2006. Controle de carrapatos. In: Barrosbattesti, D.M.B., Arzua, M., Brechara, G.H. (Eds.), Carrapatos de importância médico-veterinária da Região Neotropical. Um guia ilustrado para a identificação de espécies. Instituto Butantan, São Paulo, pp. 145–153.

Reis-Menini, C.M.R., Prata, M.C.A., Furolng, J., Silva, E.R., 2008. Compatibility between the entomopathogenic nematode *Steinernema glaseri* (Rhabditida: Steinernematidae) and an acaricide in the control of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Parasitol. Res. 2, 1391–1396.

Samish, M., Glazer, I., 1992. Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) to female ticks of *Boophilus annulatus* (Acari: Ixodidae). J. Med. Entomol. 29, 614–618.

Samish, M., 2000. Biocontrol of ticks. Annals of New York Academy of Sciences 916, 172–178.

Samish, M., Glazer, I., 2001. Enthomopatogenic nematodos for the biocontrol of ticks. Trends Parasitol. 17, 368–371.

Silva, E., 2007. Ação do nematóide entomopatogênico *Heterorhabditis indica* LPP1 (Poniar, Kranukar & David, 1992) (Rhabditida: Heterorhabditidae) sobre a biologia reprodutiva de fêmeas ingurgitadas de *Boophilus microplus* (Canestrnii, 1887) (Acari: Ixodidae). Dissertação PhD Thesis, Universidade Federal de Juiz de Fora, Minas Gerais, Brasil, 45p.

Vasconcelos, V.O., Furlong, J., Freitas, G.M., Dolinski, C., Aguillera, M.M., Rodrigues, R.C.D., Prata, M.C.A., 2004. *Steinernema glaseri* Santa Rosa strain (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* CCA strain (Rabditida: Heterorhabditidae) as biological control agents of *Boophilus microplus* (Acari: Ixodidae). Parasitol. Res. 94, 201–206.