

Infectivity of steinernematid and heterorhabditid nematodes for the human body louse *Pediculus humanus humanus* (Anoplura : Pediculidae) ⁽¹⁾

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Accepted for publication 16 March 1993.

Summary – The susceptibility of the human body louse *Pediculus humanus humanus* to the entomopathogenic rhabditids is described. Exposure of female lice to infective juveniles (IJs) of *Steinernema carpocapsae* “Pye” strain and *S. glaseri* in a Petri-dish assay resulted in high mortality levels (> 85 %) within 24 h. Among female lice exposed to IJs of the “HP88” strain of *Heterorhabditis bacteriophora* 45 % mortality was recorded after 42 h exposure. Lice exposure to the “Mexican” strain of *S. carpocapsae* for 42 h resulted in 65 % mortality. The mortality of the louse females was directly related to increased dosage of *S. glaseri* IJs. Complete lice mortality was achieved with this nematode at 400 IJs/dish. Exposure of the lice to 800 IJs of *H. bacteriophora* HP88/dish resulted in 27.5 % mortality. The highest number of nematodes was recovered from dead lice infected with *S. glaseri* (7.7 nematodes/louse). Abnormal development was observed among females of *S. glaseri*, which were shrunken, whereas males developed normally. The lowest number (0.2 nematodes/louse) was recorded in the lice infected with the “HP88” strain of *H. bacteriophora*.

Résumé – *Pouvoir infestant de nématodes Steinernematidae et Heterorhabditidae envers le pou de corps humain, Pediculus humanus humanus (Anoplure : Pediculidae)* – La sensibilité du pou de corps humain, *Pediculus humanus humanus*, aux Rhabditidae entomopathogènes est étudiée. Lors d'un essai en boîtes de Petri, l'exposition de poux femelles aux juvéniles infestants (JI) de *Steinernema carpocapsae* souche “Pye” et de *S. glaseri* conduit en 24 h à un taux de mortalité élevé (> 85 %). Après 42 h d'exposition au JI d'*Heterorhabditis bacteriophora* souche “HP88”, le taux de mortalité des poux femelles est de 45 %, et de 65 % après exposition pendant le même temps aux JI de *S. carpocapsae* souche “Mexican”. La mortalité des poux femelles est en relation directe avec le nombre croissant de JI de *S. glaseri* utilisés. La mortalité complète est obtenue avec 400 JI de ce dernier nématode par boîte de Petri. L'exposition de poux à 800 JI de *H. bacteriophora* par boîte de Petri conduit à une mortalité de 27,5 %. Le plus grand nombre de nématodes est observé dans les poux morts lors d'infestation avec *S. glaseri* (7,7 nématodes par pou). Il a été observé un développement anormal des femelles de *S. glaseri*, qui restent ratatinées, alors que les mâles se développent normalement. Le plus faible nombre (0,2 nématode par pou) est observé chez les poux infestés par *H. bacteriophora* souche “HP88”.

Key-words : Nematode, Steinernematidae, Heterhabditidae, *Pediculus h. humanus*.

Among the alternative pest control measures for replacement of chemical insecticides in agriculture, particular attention has focused in recent years on biological control using entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Rhabditida : Nematoda). The third stage infective juveniles (IJs) of these nematodes, which harbour bacteria of the genus *Xenorhabdus* (Akhurst & Boemare, 1990), are capable of infecting and killing a wide range of insects within 24-48 h (Georgis, 1990 a, b).

As nematodes inhabit the soil, most applications have involved the targeting of insects which dwell in the soil throughout, or for a part of their life cycle (Klein,

1990). However, entomopathogenic nematodes can also be applied to off-ground cryptic habitats such as tunnels made by tree borers (Kaya, 1990).

Unlike agricultural entomology there has been only limited use of entomopathogenic rhabditids against insects of medical importance. Laboratory assays indicated that maggots of the house fly (*Musca domestica*) are susceptible to steinernematid and heterorhabditid infection (Geden *et al.*, 1986; Mullens *et al.*, 1987). However, environmental conditions in the natural habitats of these human pests have prevented the use of nematodes for biological control (Geden *et al.*, 1986; Begley, 1990). Despite the susceptibility of mosquito larvae and black

(1) Contribution from the Agriculture Research Organization (ARO), Bet Dagan, Israel. No. 3517-E, 1992 series.

flies to entomopathogenic rhabditids, a number of factors cause reduced efficacy, including damage to nematodes during ingestion, host immune response, and spatial separation of host and nematodes (Begley, 1990).

Other than the malaria mosquito, no other insect has caused more deaths to mankind than the body louse *Pediculus humanus humanus* (Anoplura: Pediculidae). This ectoparasite, which thrives in conditions of overcrowding, filth and famine may have a devastating effect on humans due to its association with epidemic typhus (*Rickettsia prowazekii*), trench fever (*Rochalimea quintana*) and louse-borne relapsing fever (*Borrelia recurrentis*), diseases which most probably have changed the course of history (Taplin & Meinking, 1987).

The infectivity of entomopathogenic steinernematids and heterorhabditids for the body louse has not yet been reported. In the present study the susceptibility of *P. h. humanus* to nematode infection is described.

Materials and methods

The following nematode strains were used throughout this work: *Steinernema glaseri* Steiner (obtained from Dr. Ehlers, Kiel University, Germany), Strains "Mexican" and "Pye" of *S. carpocapsae* Weiser and the "HP88" strain of *Heterorhabditis bacteriophora* Poinar (all obtained from Biosys, Palo Alto, CA, USA). The nematodes were reared on the greater wax moth *Galleria mellonella* according to the method described by Poinar (1979). The IJs of the steinernematids and heterorhabditids were stored in water suspensions at 6 °C and 10 °C, respectively.

The louse *P. h. humanus* was reared in the laboratory according to methods described elsewhere (Mumcuoglu et al., 1990).

The effect of different concentrations of the entomopathogenic nematodes on lice mortality was determined by exposing female lice to IJs of the nematodes *S. glaseri* and *H. bacteriophora* "HP38" in 5-cm-diam. plastic Petri dishes padded with filter paper (Whatman, No. 1). The nematodes were placed in the Petri dishes in 0.5 ml distilled water at concentrations of 0, 100, 200, 400 or 800 IJs/dish and 20 lice were added to each dish. The plates were placed without covers in an incubator at 30 ± 1 °C and $87 \% \pm 3$ RH. Lice mortality was recorded after 24 h incubation. Each treatment was repeated in two dishes.

To evaluate the susceptibility of lice nymphs (N_{1-3}), male or female to nematode infection twenty lice from each developmental stage were separately exposed to IJs of *S. glaseri* (1500 nematodes/dish) for 24 h at 30 ± 1 °C and $87 \% \pm 3$ RH before lice mortality was determined. In a separate experiment different developmental stages of lice were exposed to 1000 IJs of *S. glaseri*/dish under the same experimental conditions. Lice were transferred into a nematode-free Petri dish padded with a filter paper following one hour exposure to the nema-

todes. Mortality was recorded after an additional 23 h of incubation. In each experiment the treatments consisted of two replicates. The experiments were repeated twice.

The infectivity of various nematode strains was compared by exposing 20 female lice in each dish to 400 IJs of the nematode strains listed above. Control treatment consisted of distilled water only. Lice mortality was recorded after 24 and 42 h of incubation at 30 ± 1 °C and $87 \% \pm 3$ RH. Each treatment was repeated twice. Four of the dead lice from each treatment were dissected under a stereoscopic microscope and the number of nematodes which were recovered from each louse was recorded. The experiment was repeated three times.

Lice mortality presented in percentage form were normalized using $\arcsine \sqrt{x}$ transformation (Little & Hills, 1978). The number of nematodes in the lice body were normalized using \sqrt{x} transformation (Box & Cox, 1964). Both transformation were used for the analysis of variance. Difference between means was evaluated by the F test ($P = 0.05$) in a general linear model (GLM) procedure.

Results

The mortality of the louse *P. h. humanus* females was directly related to increased dosage of *S. glaseri* IJs (Fig. 1). Total lice mortality was achieved with this nematode at 400 IJs/dish. Exposure of the lice to *H. bacteriophora* "HP88" resulted in poor mortality at all concentrations. Even at the highest concentration only 27.5 % mortality was recorded.

Exposure of different lice developmental stages to IJs of *S. glaseri* for 24 h resulted in a level of mortality in the range 75-99 % (Fig. 2A) with no significant differences between the stages ($P = 0.24$). One hour's exposure to the nematodes was sufficient to cause substantial mortality ranging from 41 % to 79 % among the various lice developmental stages (Fig. 2 B).

Among the various nematode strains tested against female lice high mortality levels ($> 85 \%$) were recorded with *S. carpocapsae* "Pye" strain and with *S. glaseri* (Fig. 3). This effect was achieved within 24 h. An additional 18 h exposure to these two nematode species resulted in only a slight increase in lice mortality. The "HP88" strain of *H. bacteriophora* was found to be the least infectious among the four nematode species tested. Mortality among louse females which were exposed to this nematode for 24 h did not differ significantly ($P > 0.05$) from the control. Exposure for 42 h to IJs of the "HP88" strain resulted in a significant (2.5 fold) increase in lice mortality as compared to the control. The effect of exposure time was also recorded with the "Mexican" strain of *S. carpocapsae* whereby lice mortality increased by 25 % between 24 and 42 h exposure. However, even after this period lice mortality was lower than 70 %, indicating poor infectivity as compared to the *S. carpocapsae* "Pye" strain and *S. glaseri* (Fig. 3).

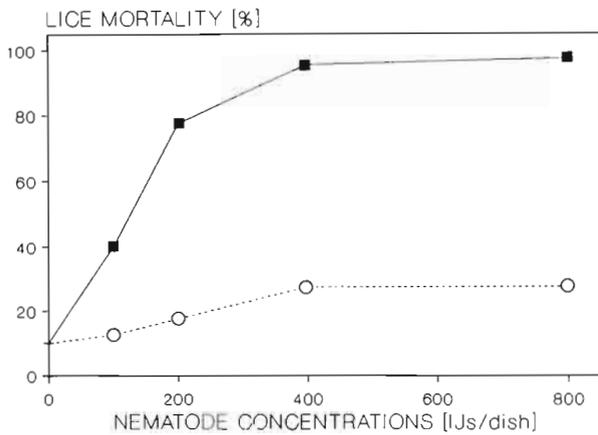


Fig. 1. Effect of different concentrations of the nematodes *Steinernema glaseri* (■) and *Heterorhabditis bacteriophora* "HP88" (○) on the mortality of *Pediculus humanus humanus* females.

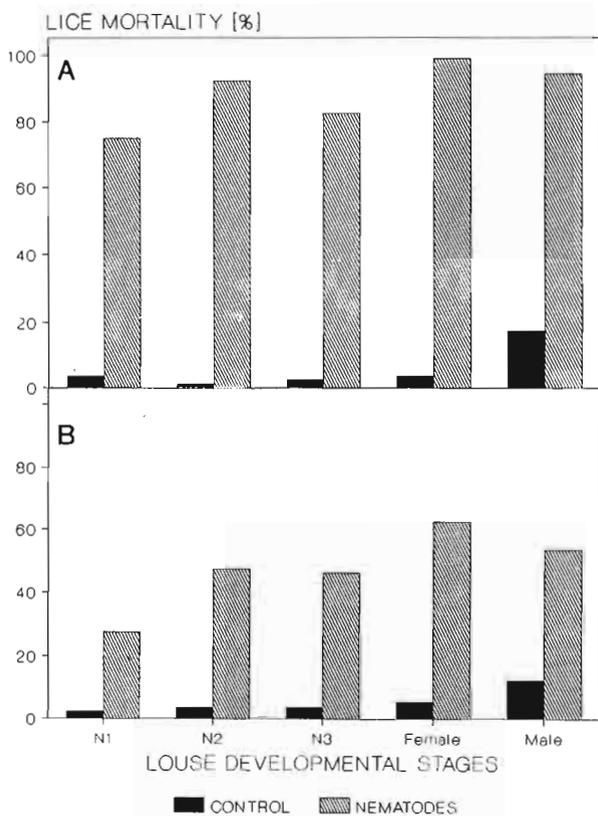


Fig. 2. Infectivity of the nematode *Steinernema glaseri* to different developmental stages of *Pediculus humanus humanus* (N = nymph). Lice mortality after 24 h exposure (A) and 1 h exposure (B).

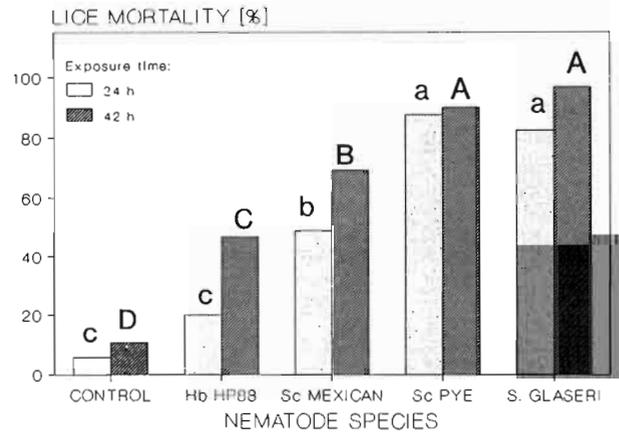


Fig. 3. Infectivity of different nematode species for female *Pediculus humanus humanus*. Means with different letters are significantly different within each exposure period ($P < 0.05$). (Hb = *Heterorhabditis bacteriophora*; Sc = *Steinernema carpocapsae*).

Dissection of dead louse females infected with the various nematode strains indicates that the highest number of nematodes per louse was recovered from dead lice infected with *S. glaseri* (Table 1). The number of nematodes found in the dead lice infected by *S. carpocapsae* "Pye" was significantly ($P < 0.05$) lower than that found with *S. glaseri*. The lowest number was recorded in lice infected with the "HP88" strain of *H. bacteriophora*.

Table 1. Number of nematodes recovered from dead female *Pediculus humanus humanus* following 48 h exposure to infective juveniles of *Steinernema carpocapsae*, *S. glaseri* and *Heterorhabditis bacteriophora*.

Nematode species	Strain	Average number * nematodes/ouse
<i>S. glaseri</i>		$7.7 \pm 0.007 a^{**}$
<i>S. carpocapsae</i>	"Pye"	$3.5 \pm 0.012 b$
	"Mexican"	$1.5 \pm 0.012 c$
<i>H. bacteriophora</i>	"HP88"	$0.2 \pm 0.008 d$

* Total of twelve dead lice were dissected for each nematode species.
 ** Means with different letters are significantly different ($P < 0.05$).

The nematodes were found at various developmental stages in the dead lice, from IJ to adults. Abnormal development was observed among females of *S. glaseri* which were shrunken, whereas males developed normally.

The dead, nematode-infected female louse, is transparent, and nematodes could be seen in all parts of the body, including the abdomen (Fig. 4 A) and the legs (Fig. 4 B).

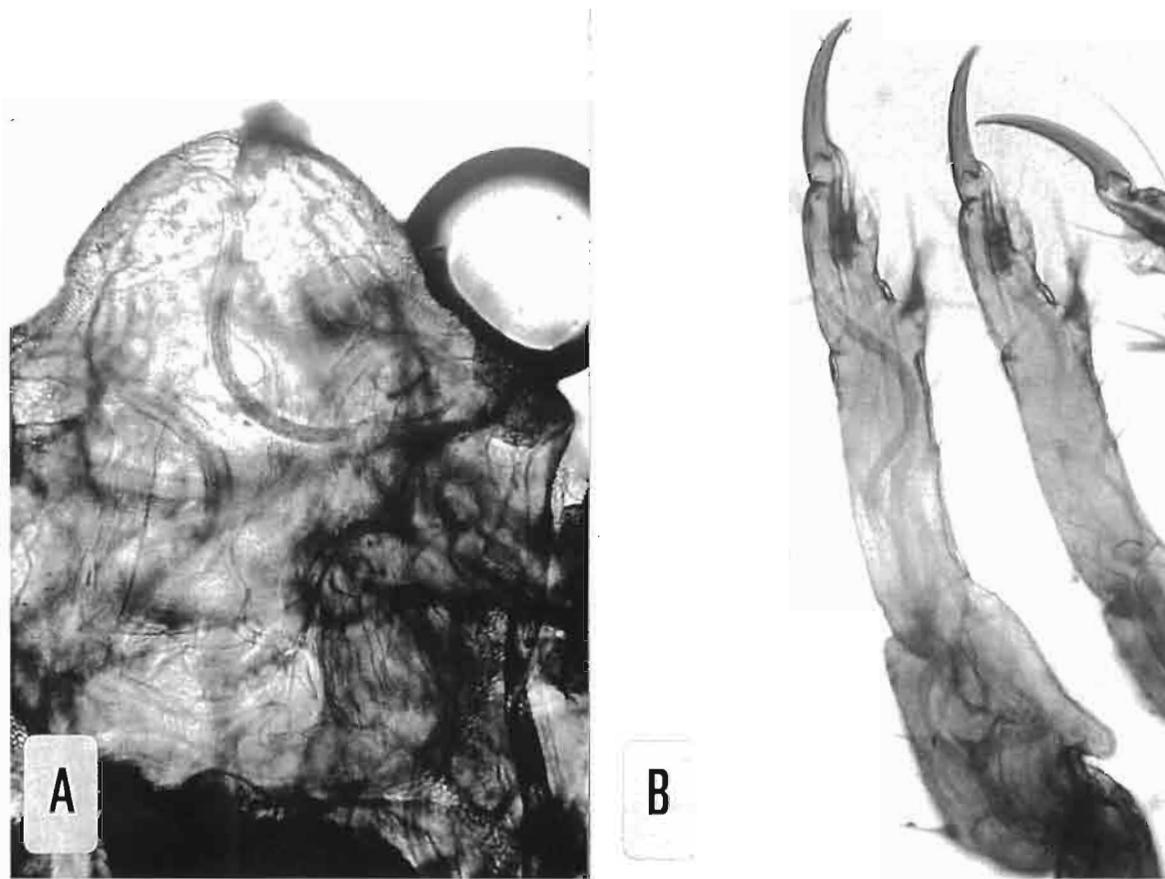


Fig. 4. Infective juveniles of the nematode *Steinernema glaseri* in the abdomen (A) and legs (B) of the louse *Pediculus humanus humanus* females.

Discussion

The data presented here are the first evidence for infectivity of entomopathogenic steinernematids and heterorhabditids for a member of the Pediculidae. The rapid lice mortality which was achieved within 24 h indicates that the louse *P. h. humanus* is highly susceptible to the nematode infection. Furthermore, the substantial mortality which was achieved by short exposure of the lice to the nematodes indicates that these pests are easily invaded by the parasite. The invasion rate was shown to be the most important factor in the infectivity process which affects the mortality rate among lepidopteran larvae, known to be highly susceptible to the nematode infection (Glazer *et al.*, 1991). The present findings emphasize this notion with respect to non-lepidopteran insects.

The penetration site of the nematodes to the lice body has not yet been defined. Commonly, entomopathogenic rhabditids invade the host through natural openings (Klein, 1990). However, the narrow blood sucking

mouth part (haustellum) of the louse and the small size of the trachea openings would prevent penetration of the nematode IJs, particularly those of *S. glaseri* which are relatively large. Some penetration has been observed through the anus but the possibility of invasion through the cuticle, as observed with other insects (Bedding & Molyneux, 1983), is not excluded.

Difference in susceptibility of various developmental stages have been recorded with many insect species (Kondo, 1987; Kaya, 1990; Glazer, 1992). These differences were commonly attributed to rapid motility of young larvae which did not allow the IJs to infect the target host. However, under the experimental conditions of the assays conducted here the comparison of various stages of *P. h. humanus* did not show significant differences with regard to susceptibility to *S. glaseri* infection. This phenomenon can be explained by the poor motility of all louse stages. Furthermore no significant difference was recorded between the level of mortality of starved (24 h from last feeding) and engorged louse females which were exposed to IJs of *S. glaseri*. Louse eggs which

were exposed to *S. glaseri* IJS for 10 days on moist black silk cloth were not infected by the nematodes.

Differences in infectivity of nematode strains has been documented with many insect hosts (Bedding & Molyneux, 1983; Forschler & Nordin, 1988; Griffin *et al.*, 1989; Glazer *et al.*, 1991). Significant differences in infectivity were also recorded among the various nematode strains tested in this study. This variation can be attributed to their ability to invade the host, to release the symbiotic bacteria as well as to the growth rate of the bacteria (Glazer *et al.*, 1991). The high number of nematodes recovered from the dead lice infected with *S. glaseri* indicates that this nematodes penetrates the host more efficiently than the others. However, even a lower number of nematodes, as recorded with *S. carpocapsae* "Pye" was sufficient to cause a similar mortality to that found with *S. glaseri*.

The low mortality caused by *S. carpocapsae* "Mexican" strain and *H. bacteriophora* "HP88" strain is due to the low number of nematodes recovered from the dead lice, indicating poor penetration ability. The low activity of the heterorhabditid strain could be partially influenced by the experiment temperature (30 °C) which is in the higher range for the IJs activity (Kaya, 1990). Although lice mortality with the "HP88" strain was significantly higher than that in the control at 42 h exposure, nematodes were recovered only from one out of four dead lice. The disappearance of the nematodes could be explained by their rapid degradation in the dead lice.

With the exception of the *H. bacteriophora* "HP88" strain, the louse *P. h. humanus* was found to be a suitable host for nematode development as adult stages were recovered from the dead lice 48 h post infection. The abnormal development of *S. glaseri* females is most likely due to their large size in comparison to the small insect host.

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