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Changes in Haemolymph Proteins Pattern of *Hyalomma dromedarii* Ticks Infected with Entomopathogenic Nematodes

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Abstract: The current study was designed to follow up the changes in engorged female protein haemolymph (HL) of *Hyalomma dromedarii* infected with entomopathogenic nematodes (EPNs). *Steinernema sp*.SII, *S.carpocapsae* DD136, *Heterorhabditis sp*.TWF and *H.bacteriophora* Hp88 were used to infect the engorged females. HL samples obtained after four intervals time (24, 48, 96 and 178 hr).Quantitative and qualitative changes were detected in SDS-PAGE electrophoresis protein separation. Parasitism caused HL protein lyses as shown by changes in number of fractions. *H.bacteriophora* Hp88 was the most effective species in engorged female. In conclusion this study revealed that the main etiology of tick mortality is related to marked changes in HL protein of *H. Dromedarii* following infection with entomopathogenic nematodes.

Key words: Entomopathogenic nematodes · Hyaloma dromedarii · Protein · Biocontrol

INTRODUCTION

Tick-related diseases are a serious problem throughout the world. The principal measures used to control ticks and tick-borne pathogens are applications of acaricidal or chemotherapeutical agents which are costly and required trained personnel [1, 2]. The effectiveness of an acaricide used is being decreased by the development of resistant strains [3, 4]. Therefore, search of alternative solution for ticks control became necessary. entomopathogenic nematodes (EPNs) of two families, Steinernematidae and Heterorhabditidae are used as microbial insecticides and are produced commercially by various companies around the world [5]. The term of "entomopathogenic nematode" refers to the nematode's ability to quickly kill hosts (1-4 days depending on nematode and host species) that is facilitated by their mutalistic association with bacteria in the genus *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae. The infective Juvinile (IJs) carry cells of their bacterial symbiontn in their intestines. After location in a suitable host, the IJs invade it through natural openings (mouth, spiracles, anus) or thin areas of the host's cuticle [6] and penetrate into the host haemocoel. The IJs release their symbiotic bacteria that probagate, kill the host by septicemia and metabolize its tissues. The nematodes start developing and feed on the bacteria and metabolized host tissues. Although, engorged females of *Boophilus annulatus* and *Hyalomma dromedarii* ticks were highly susceptible to infection by nematodes of the families steinernematidae and Heterorhabditidae but, the IJs couldn't developed inside its haemolymph [7-9].

Insect immune response consists of interactive cellular and humoral actions. The haemocytic mechanisms comprising phagosytosis, nodule formation and cellular encapsulation. The humoral response including melanotic encapsulation and the production of antimicrobial peptides, either induced (e.g. cecropins) or constitutive (e.g. lysozyme). The immediate response against nematodes is encapsulation and against bacteria is phagosytosis, or nodulation in the case of a large load. Also, injury and microbe infection induce the production of antibacterial peptides. However, the details of the response vary with the species of insect and pathogen and their physiological states [10, 11].

The present study aimed to 1- detect the changes in ticks HL proteins following entomopathogenic nematodes infection using Sodium dedcylsulphate Polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Collection of Ticks: Engorged females of *Hyalomma dromedarii* (Acari: Ixodidae) ticks collected from sandy soil of camel's resting house, Borkash, Giza, Egypt were used.

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Maintaining of Nematodes: Four different species of entomopathogenic nematodes were used in this study, *Steinernema carpocapsae* DD136 (Sc); *Stienernema sp*. SII (un identified native isolate); *Heterorhabditis bacteriophora* Hp88 and *H.bacteriophora* TWF (un identified native isolate). The nematodes were maintained on the last instar larvae of *Galleria mellonell* L. according to the method of Dutky *et al*. [12].

Experimental Design: The engorged female ticks of *H. dromedarii* were frankly exposed to 2000 infective juveniles (IJs) nematodes /female. Ticks were cooled for 30-60 min at 4°C, 19 samples of HL were collected during intervals of 24, 48, 96 and 178 hr. In case of Hp88, HL could not be collected at interval 178 hr due to tick mortality. Control groups have been treated with tape water during different intervals. The protein concentration of the all prepared HL was measured as described by Lowry *et al*. [13].

Polyacrylamide Gel Electrophoresis (SDS-PAGE): Haemolymph of infected (24, 72, 96 and 178hr PI) and non-infected *H. dromedarii* were electrophorsed by SDS-PAGE using a dis continuous gel system of Laemmli [14]. After staking, separating and simultaneously pouring gel, the comb was inserted by a slop way. The haemolymph protein (40ug/weel were treated with the reducing buffer 12% SDS containing 0.7 M 2-mercoptoethanol, 5% glycerol and 0.001% bromophenol bleu) in the ration of 1:2. The treated protein were immersed in a boiling water bath for 2 min to ensure protein denaturation. After polymerization of the gel (about 2 hr) and removing of the comb, unstained protein molecular weight marker (Bio-Rad Labs, Richmond, CA) and the treated protein were loaded in the wells. A voltage of 100 v. was applied until the bromophenol blue had reached the bottom of the gel. The gel was then stained with 0.025% commassie blue (0.25 g L^{-1}) at room temperature, over night. To visualize the protein bands, the gel was washed several times with destaining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) until the back ground become completely clear. Finally, the gel was photographed.

Statistic analysis, was made using a software package Costat, a product of Cohort Software Inc. Berkeley, California.

RESULTS

Soluble HL proteins of infected engorged females were assayed and illustrated in Figs. 1,2 and Tables 1- 4. The entomopathogenic nematode species (TWF,SII,

Table 1: Effect of four species of entomopathogenic nematodes (TWF, SII, Sc, Hp88) on ticks haemolymph proteins kDa after 24 hrs

Marker (kDa)	Control	TWF	SII	Sc	Hp88
220	218.55*	218.55*	218.55*	220	217.11
97	102.79	102.79	101.34	101.34	101.34
	87.642*	87.057*	87.642*	86.472	87.057
66	67.755*	64.062	67.755*	63.738	65.031
	62.769*	61.154	62.446*	60.185	59.215
	55.662*	55.662*	55.662*	55.015*	55.662*
		50.492	51.138	50.492	50.169
45		44.016	45.323	45	46.938
	39.836*	39.098*	39.836*	41.557	39.59*
30	29.317*	29.886*	29.317*	31.23	30.984
20.1					
		19.424	19.734		19.565
	18.777				
143					

*P<0.05

Table 2: Effect of four species of entomopathogenic nematodes (TWF, SII, Sc, Hp88) on ticks haemolymph proteins kDa after 48 hrs

Marker (kDa)	Control	TWF	SII	Sc	Hp88
220	221.45*	221.45*	220	220	212.76
97	101.34*	101.34*	101.34*	101.34*	101.34*
	85.887	87.642	89.396	87.057	88.811
		82.962			
66	70.679	73.604	72.434		
		67.755	63.415	63.415	64.708
	62.769	61.8			60.508
	56.631*		58.246	56.631*	
		49.523		49.846	50.492
45	45.646				
	41.803				
		33.689	37.131	37.869	36.639
30	30.492		28.634	28.976	28.976
	20.214*	20.783*	19.959	20.1*	19.875

*P<0.05

Table 3: Effect of four species of entomopathogenic nematodes (TWF, SII, Sc, Hp88) on ticks haemolymph proteins kDa after 96 hrs

Marker (kDa)	Control	TWF	SII	Sc	Hp88
220	226.74	225.05	225.05	223.37	221.68
97	105.42	103.74	112.16	103.74	102.05
	88.654*	89.846	91.038	88.654*	88.058*
	83.885		84.481	82.692	
	68.385	73.154	69.577		
66	60.96*			64.04	
		60.4*	59.28	59.56	58.16
			48.64		
45	46.96*	47.8		46.96*	47.52
30	33.243*	30.811	33.649*	32.838	30
			28.945	28.539	
	23.021*	23.508*	23.184*	22.616	23.67*
20.1					
14.3		14.402			
					13.817

*P<0.05

Table 4: Effect of four species of entomopathogenic nematodes (TWF, SII, Sc, Hp88) on ticks haemolymph proteins kDa after 178 hrs

Marker (kDa)	Control	TWF	SII	Sc
220	220*	220*	218.32	218.32
97	103.74	105.42	102.05	98.685
	86.865*	86.865*	88.058	88.654
	77.327	68.981	75.538	
66	64.88			
	54.8	59.28	57.04	58.44
			50.32	
45	43.176	46.4		
		36.486	37.703	37.5
30	31.622		30.608	30.608
		24.401	24.563	25.05
20.1				
14.3		14.427		14.122

*P<0.05

Sc and Hp88) and tape water were applied during different interval of times (24, 48, 96 and 178 hrs) for the infected and control groups, respectively. At 24 hr post infection (pi) the protein patterns showed quantitative and qualitative differences. The visual analysis of polypeptide bands showed that TWF consists of 11 bands (ranged between 19.424 - 218.55 kDa). SII separated into 11 bands (19.734 - 218.55 kDa). Sc separated into 10 protein bands (31.23 - 220kDa) and Hp88 separated 11 protein bands (217.11 to 19.565 kDa). Protein band of 55 kDa was detected as dominant band in infected HL with TWF, SII, Sc, Hp88 and control too. Protein band 101.34 kDa detected in all infected HL with the three nematode species except TWF (Table 1). This specific common band 101.34 kDa appeared in HL of TWF, N_2, Sc, Hb and the control in 48hr Pi (Table2). Also, The 55.662 kDa band was

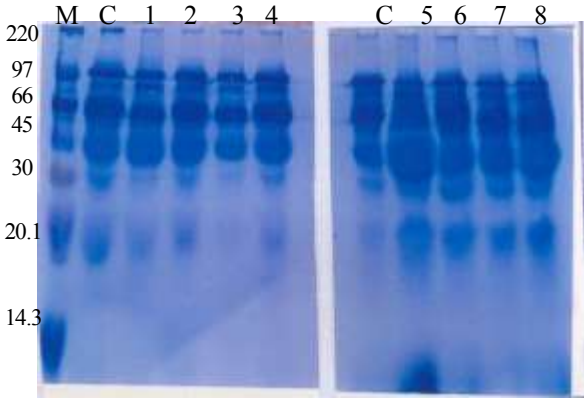

Fig. 1: 10% SDS/PAGE electrophoretic profile for protein of engorged female of *H. dromedarii* hemolymph infected with four species of entomopathogenic nematodes. Lan 1, represents TWF = Heterorhabditis sp., Lan 2, SII = Steinernema sp., Lan 3, Sc = S.carpocapsae DD163, Lanes 4, Hp88= H.bacteriophora.After 24hr.Lan 5,6,7,8 represent TWF, SII, Sc, Hp88 after 48hr respectively. Lane M, represents molecular weight marker Lane C, represents control.

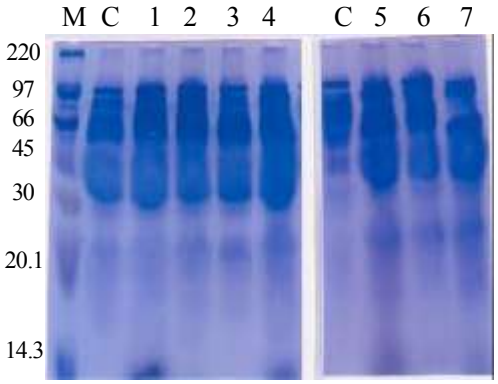

Fig. 2: Lanes 1, 2, 3, 4 represent TWF, SII, Sc, Hp88 after 96hr respectively. Lanes 5,6,7 represent TWF,SII, Sc. after 178hr. 10% SDS/PAGE electrophoretic profile for protein of female *H. dromedarii* hemolymph infected with various entomopathogenic nematodes. Lane M, represents molecular weight marker. Lane C, represents control.

detected in all infected and control HL as common band. In addition the last band 18.777kDa was disappeared from all infected HL in comparing of the control (Table1).

The analysis of 48hr Pi protein patterns showed missing of the two bands 45.646 and 41.803 kDa in HL infected with the four nematode species (Table 2, Fig. 1). After 96 hr commassie blue staining showed the increasing in last two bands of ticks HL infected of nematode belong to heterorhabditis species TWF and Hb88 if compare with control. The specific low molocular weight band ranged from 22.616 - 23.508 kDa was detected in all treated and nontreated HL (Table 3, Fig. 2). With the advance of infection time, there was increased in separated HL bands in TWF and Sc nematode species 178hr Pi. in comparing with control. In this interval of time, two protein bands of low molecular weight were appeared in TWF and SII. but, in Sc just one band of molocular weight 24.563kDa was detected (Table 4, Fig. 2). Because of the rapid death of infected ticks with Hb88, haemolymph after 178hr couldn't be extracted.

In general, it could be noticed that high molecular weight (85.887 – 220 kDa) revealed no changes in infected as compared to non-infected HL. On the other hand, the most observed changes were seen in low molecular weight (13.817 – 61.8 kDa) protein. These changes were obviously observed in last two time intervals 96 and 178hr pi.

DISCUSSION

Parasitism starts with nematodes entry by natural opening (mouth, anus, spircles) or directly through the integument (mainly *Heterorhabditis* nematodes) of the insects. *Steinernema spp*. induce a toxicogenesis [15, 16] and produce an immune depressive factor active against antimicrobial peptides from the insects [17]. Infective juveniles of both genera release their bacterial symbionts in the host body cavity and develop into fourth-stage juviniles and adults. The insect died mainly due to a septicaemia [18]. In agricultural pests nematodes reproduce in the insect cadaver and feed on the symbiont biomass and insect tissues was metabolized by the bacteria. But in ticks, nematodes could invade and kill it without any further developmental stage inside the ticks haemolymph [19]. So, this study focused on the changes in haemolymph protein during the different intervals of times following infection of ticks with entomopathogenic nematodes. This chosen four intervals of time were depending on previously studies which revealed that *H. dromedarii* and *B.annulatus* ingorged females are susceptible to entomopathogenic nematode infection and died after 72hr to 4days [19]. Moreover, it was reported that *Heterorhabditis spp* were more virulence on engorged female of *H. dromedarii* than *Steinernematidis spp*. [20, 21]. This finding coincide with the current, whereas, they mentioned that engorged females infected with *H.bacteriophora* Hp88 died after 96hr. SDS-PAGES of Ticks haemolymph showing remarkable increase in protein patterns of infected females if compared with no-infected control. In the first 24hr post infection, whereas, it reached to 11 bands in *Heterorhabditis sp* TWF, *H.bacteriophora* Hp88 and *Stteinernema sp*.SII and 10 bands in *S.carpocapsae* DD136. This results were confirmed with previous study [20, 22], which revealed that the SDS-PAGES of mid-gut and salivary gland of infected *H.dromedarii* and *B. annulatus* showed increased in protein bands. It reached 25 and 21 bands in infected salivary gland and mid-gut versus 19 and 13 bands in non-infected ones respectively. Ticks haemolymph proteine paterns post infection with all nematode species showed the presence of additional bands. This may be attributed to the proteases secreted by the primary cells of both *Photorhabdus* and *Xenorhabdus* while the secondary cells generally produce lower levels of protease activity. Wee *et al*. [23], showed that secondary cells of *P.luminescens* produce a protease inhibitor which helps to explain why protease activity is detected in primary, but not in secondary cells. It's generally believed that protease activity is involved in the breakdown of insect proteins thereby providing nutrition for bacterial and nematode growth [24].

This study showed marked quantitative and qualitative differences in ticks haemolymph electrophoresis by SDS/PAGE resulting in entompathogenic nematodes infection in different intervals time. therefore, it may be speculated that the rate of nematode penetration and perhaps the rate of development and virulence of the symbiotic bacteria within ticks, played a major role in the nematode tick interaction as Similarly previously shown for ticks & insects [25, 26]. The relation between the penetration site of the various nematodes and their efficiency in killing engorged female of *Ixodes scapularis* had been demonstrated by Glazer [27]. Moreover, The appearance or disappearance of protein bands in 96hr and 178hr post infection may be explained as, the activity of *Photorhabdus* and *Xenorhabdus* are released into the host body cavity whereas they disable the immune system and the disappearance of haemolymph sample and occur hydrolyzes of the host protein. This results

were similar to that of Schmidt and Platzer [28]. who stated that, the losses of soluble protein from the host haemolymph during parasitism may be explained by the parasite secretion of proteolytic enzymes into the haemocoel of the insect and hydrolyze the host proteins [29]. Gillespie *et al*., observed that, there was reduction in total protein content of the hemolymph of *Schistocerca gregaria* during the course of infection with the entomopathogenic fungus, *Mertarhizium var acridium.*

It could be concluded that the main cause of ticks mortality is the protein lyses resulting of symbiotic bacterial metabolites. Although the nematodes doesn't developed inside the engorged females but it can be used as bacterial injection tool to ticks haemolymph. Sprayed entomopathogenic nematodes on infected animals with *B.annulatus* gave excellent results as biocontrol agent.

REFERENCES

1. Kumar, R. and R. Kumar, 1995. Artificial immunization of rabbits with *Hyalomma dromedarii* tick derived midgut antigen. Experimental Applied of Acarology, 19: 319-324.
2. Marc-Jan, G., V. Javier, A.H. Miguel and J. Frans, 2000. Characterization of a henmaed *Theileria annulata* vaccines from Spain and the Sudan. Annual New York Academy Socity, 916: 521-532.
3. Willadsen, P., J. Bird, G.S. Cobon and J. Hungerford, 1995. Commercialization of recombinant vaccine against *Boophilus micropuls*. Parasitology, 110: S43-S50.
4. Wikel, S.K., 1996. Tick modulation of host cytokines. Experimental Parasitology, 84: 304-309.
5. Bedding, R., 1993. Biological control of *Sirex noctilio* using the nematode *Deladenus siricidicola*. In East Melbourne, Ausrailia: Nematodes and the Biological Control of Insect: Eds., Bedding, R., R. Akhurst and H. Kaya, CSRIO, pp: 11-20.
6. Peters, A. and R.U. Ehlers, 1994. Susceptibility of Leather-jackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematodes *Steinernema feltiae*. Journal of Invertebrate Pathology, 63: 163-171.
7. Samish, M. and I. Glazer, 2001. Entomopathogenic nematodes for the boicontrol of ticks. Trends Parasitology, 17: 368-71.
8. El-Sadawy, H.A. and S. Abdel-Shafy, 2007. Laboratory and field studies on entomopathogenic nematodes as biocontrol agent for the attle tick *Boophilius annulatus* (Ixodoidea: Ixodidae). Acarologia, XLVII, 1-2: 25-31.
9. El-Sadawy, H.A., A.A. Zayed and A.El-Shazly, 2008. Characterization of Midgut and Salivary Gland Proteins of *Hyalomma dromedarii* Females Controlled by EntomopathogenicNematodes. Pakistan Journal of Biological Sciences, 11: 508-516.
10. Dunphy, G.B. and G.S. Thurston, 1990. Insect immunity. In: Gaugler, R. and H.K. Kaya, eds.,Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, Florida, pp: 301-323.
11. Duphny, G.B. and R.S. Bourchier, 1992. Responses of nonimmune larvae of the gypsy moth, *Lymantria dispar*, to bacteria and influence of tannic acid. Journal of Invertebrate Pathology, 65: 25-34.
12. Dutky, S.R., J.V. Thompson and G.E. Cantweel, 1964. A technique for the mass propagation of the DD-136 nematode. Journal of Insect Pathology, 5: 417-422.
13. Lowery, O.H., M.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemestry, 193: 265-275.
14. Laemm Li, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriopahge T_4. Nature, London, 227: 680-685.
15. Boemare, N.E., C. Laumond and J. Luciani, 1982. Mise en évidence d'une toxicogénèse provoquée par le nématode entomophage *Neoaplictanan carpocapsae* Wieser chez l'insecte *Galleria mellonella* L. Comptes Rendus des séance de l'Académie des Sciences, Paris, Sér. III. 295: 543-546.
16. Boemare, N.E., E. Bonifassi, C. Laumond and J. Luciani, 1983. Etude experiméntale de l'action pathogene du nématode *Neoaplictanan carpocapsae* Wieser, Recheches gnotoxéniques chez l'insecte *Galleria mellonella* L. Agronomie, 3: 407-415.
17. Götz, P., A. Boman and H.G. Boman, 1981. Interactions between insect immunity and an insect-pathogenic nematode with symbiotic bacteria. Proceeding of the Royal Socity of London Series B, 212: 333-350.
18. Forst, S., B. Dowds, N.E. Boemare and E. Stackebrandt, 1997. *Xenorhabdus spp.* and *Photorhabdus spp.*: bugs that kill bugs. Annual Review of Microbiology, 51: 47-72.
19. Hassanain, M.A., A.A. Derbala, N.A. Abdel-Barry, M.A. El-sherif and H.A. EL-Sadawy, 1997. Biological control of ticks (Argasidae) by Entomopathogenhic nematodes. Egyptian Journal of Biolcal pest control, 7: 41-46.

20. El-Sadawy, H.A., A.A. Zayed and A. El-Shazly, 2008. Characterization of Midgut and Salivary Gland Proteins of *Hyalomma dromedarii* Females Controlled by Entomopathogenic Nematodes. Pakistan Journal of Biological Sciences, 11(4): 508-516.
21. Sadawy, H.A. and S.M. Habeeb, 1998. Testing some entomopathogenic nematodes for the biocontrol of *Hyalomma dromedarii* koch (Acarina: Ixodidae). Journal Union Arab Biology Cairo, 10: 1-11.
22. Abdel Wahab, K.S., E.G.E. Helal and K.M. Kammah, 1999. *Boophilus annulatus* (Acari: Ixodidae) Salivary gland and gut antigens. Arab Journal of Biotechnology, 2: 135-142.
23. Wee, K.E., C.R. Yonan and F.N. Change, 2000. A new broad-spectrum protease inhibitor from the entomopathogenic bacterium *Photorhabdus luminescens.*Microbiology, 146: 3141-3147.
24. Schmidt, T.M., B. Bleakley and K.H. Nealson, 1988. Characterization of an extracellular protease from the insect pathogen *Xenorhabdus luminescens*. Applied and Environmental Microbiology, 54: 2793-2797.
25. Glazer, I., E. Alekseev and M. Samish, 1991. Factors affecting the virulence of entomopathogenic nematodes to engorged female *Boophilus annulatus* ticks. Journal of Parasitology, 87: 808-812.
26. Glazer, I., E. Alekseev and M. Samish, 2001. Factors affecting the virulence of entomopathogenic nematodes to engorged female *Boophilus annulatus* ticks. Journal of Parasitology, 87: 808-12.
27. Glazer, I., 1992. Invasion rate as a measure of infectivity of *steinernamatide* and *Heterorhabditid*. Nematodes to insects. Journal of Invertbrate Pathology, 59: 90-94.
28. Schmidt, S.P. and E.G. Platzer, 1980. Hemolymph composition of mosquito larvae infected with amermithid nematode. Journal of Nematolgy, 10: 299-304.
29. Gillespie, J.P., C. Burnettand and A.K. Charnley, 2000. The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* var *acridum*. Journal of Physiolgy, 46: 429-437.