Effect of infestation of *Pyxinia firma* on the Total Haemocyte Counts (THC) and Larval Growth of the *Dermestes vulpinus* (Dermestidae : Coleoptera)

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Abbreviation: THC – total haemocyte count

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A B S T R A C T

Pyxinia firma has not previously been recorded in the mid gut of the larvae of scavenger beetle *Dermestes vulpinus*. Infected larvae of the scavenger beetles *were* investigated in order to determine the effects of their infection by the eugregarine, *Pyxinia firma*. Infected larvae were found to have higher total haemocyte counts and greater weight gain than uninfected ones. Infected larvae that were starved, however, lost weight much faster than uninfected larvae. The impact of infection on the physiology of the insect is also discussed and it is observed that the variation in the pattern of the total haemocyte counts between infected and uninfected larvae is indicative of the effect of the infection on the immune system of *D. vulpinus*.

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1. Introduction

Eugregarines are known entomophilic parasites of the invertebrates [1, 2 and 3]. Except for two species which are reported to be in commensal association unless under nutritional stress [4-6] relatively few of these have had species their host-parasite relationships described. Among the family dermestidae, only *Attagenus megatoma* has had its relationship with its enzootic parasite *Pyxinia frenzeli* fully reported [7-8].

This study presents an original record of the species, *P. firma* in *Dermestes vulpinus* and attempts to evaluate the association between the two species. *D. vulpinus* , in its gut *P. firma* was encountered where it remains attached to the epithelial layer. During the survey of gut contents of the larvae in Saudi Arabia an identified and apparently parasitic infection was observed and advice of Natural History Museum was sought. A colony of *Pyxinia firma* and infected *D. vulpinus* separately was maintained in the laboratory for 12 months to study the host parasite relationship and transmission is carried through cannibalistic means.

2. Materials and Methods

Infected and uninfected, adults and larval stages of *D. vulpinus* were collected from the skin and carcasses of sacrificed sheep and goats on the outskirts of the Al-Kharj district, 100km south of Riyadh. A colony was maintained in the laboratory at 28±1˚C and 70% relative humidity. During the experiment, individuals were examined regularly through dissections for signs of and gut contents were smeared and stained with Giemsa stain each day to identify the occurrence of

sporonts stage of the *Pyxinia firma* in the gut and their haemolyph was collected through piercing the abdomen of the 4^{th} instar larvae by a micro pipette and Neubaur counting chamber was used to calculate their total haemocyte counts The location of the parasites was determined by the histological and surgical study of the whole body. Control groups were obtained by adding 8% w/w sorbic acid into the diets of a selection of the colony.

A statistical analysis was undertaken of the THC among the various larval and adult stages of multiple generations within the colony , with the counts calculated through the Neubauer chamber being analysed using the SPSS software package version 12.0 to perform a least significant difference (LSD) test and to obtain significant values where ($P< 0.05$). In addition the degree of weight loss was recorded for larvae between the 4^{th} and 5^{th} instar in the five replicates of 120 infected and uninfected each . This wight loss data were calculated with standard deviations

3. Results and Discussion

The average total counts (THC) between the fourth and fifth instars of treated larvae of the D. vulpinus ranged between 4500 to 5000mm^{-3}, with a mean of 4750±90. An approximately two fold higher total haemocyte counts (THC) was recorded in infected (untreated) larvae between the fourth and fifth instar than in the treated laravae at a similar developmental stage with a range between 9000 and 11000mm^{-3}. The first progeny of treated larvae exhibited a higher THC than that in the treated larvae, with the second progeny exhibiting higher THC still(although still lower than untreated larvae) (Fig. 1 & 3).

These differences in the THC levels are reflective of the organism's active role on the immune system of the beetle. Starvation of infected larvae had the effect of reducing their weight three times faster than starved eugregarine free larvae after only three weeks from the commencement of a starvation diet (Fig.2). This rate of weight loss slowed down , however in the next two generations of treated specimens.(Figs.2 & 4). Strikingly, when one considers these dramatic differences in weight between different categories of starved specimens, no significant difference was observed in the degree of weight gain between infected and uninfected groups of fed specimens.

Histological examination, meanwhile, revealed on lesions in the epithelial layer of the mid gut.

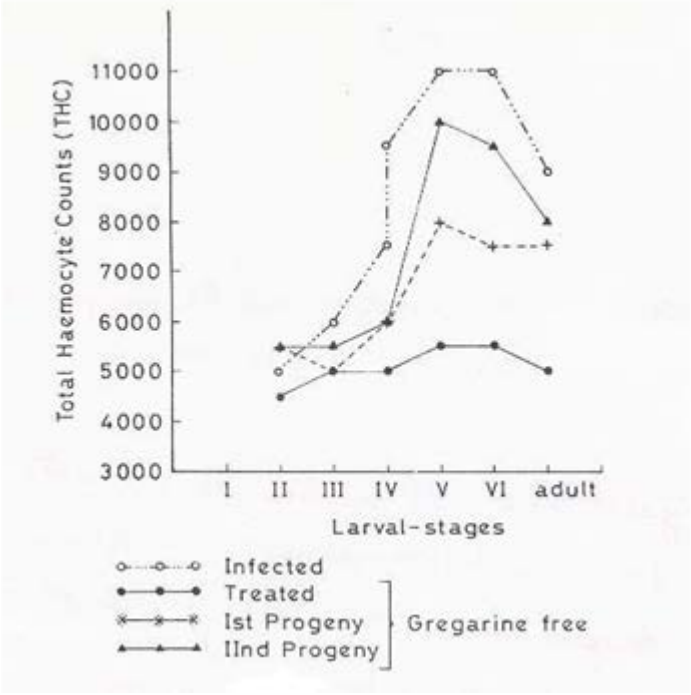
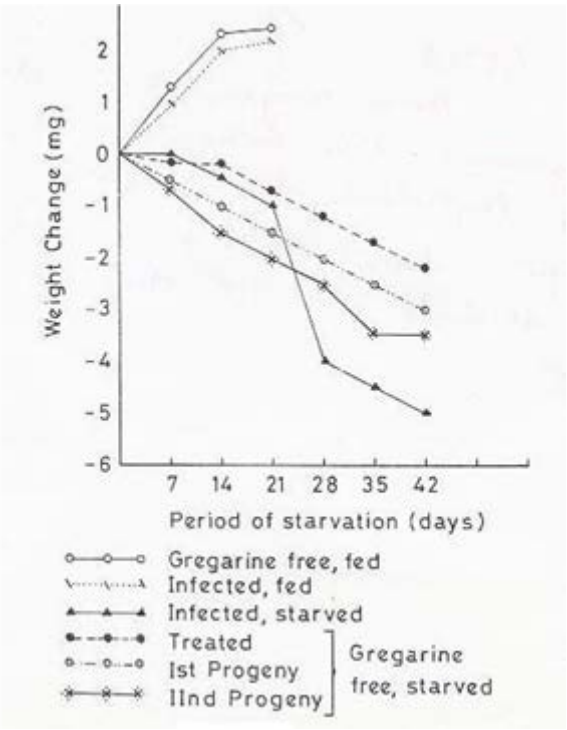


Figure 1 : Graphs showing the pattern of variation in the THC at different larval and adult stages of infected and gregarine free *D. vulpinus*



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106 Figure 2: Graphs showing the weight gain in fed larvae and the weight loss in starved fourth instar
107 larvae of *D. vulpinus*
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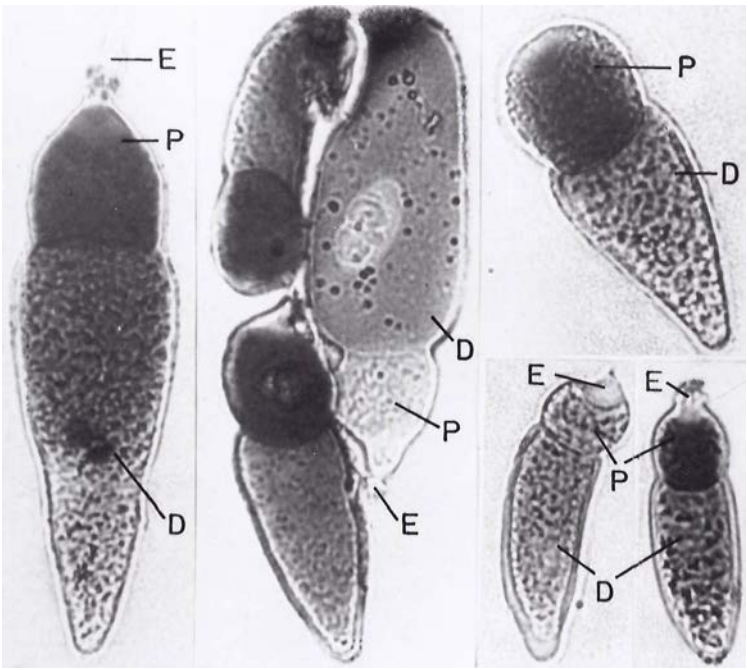
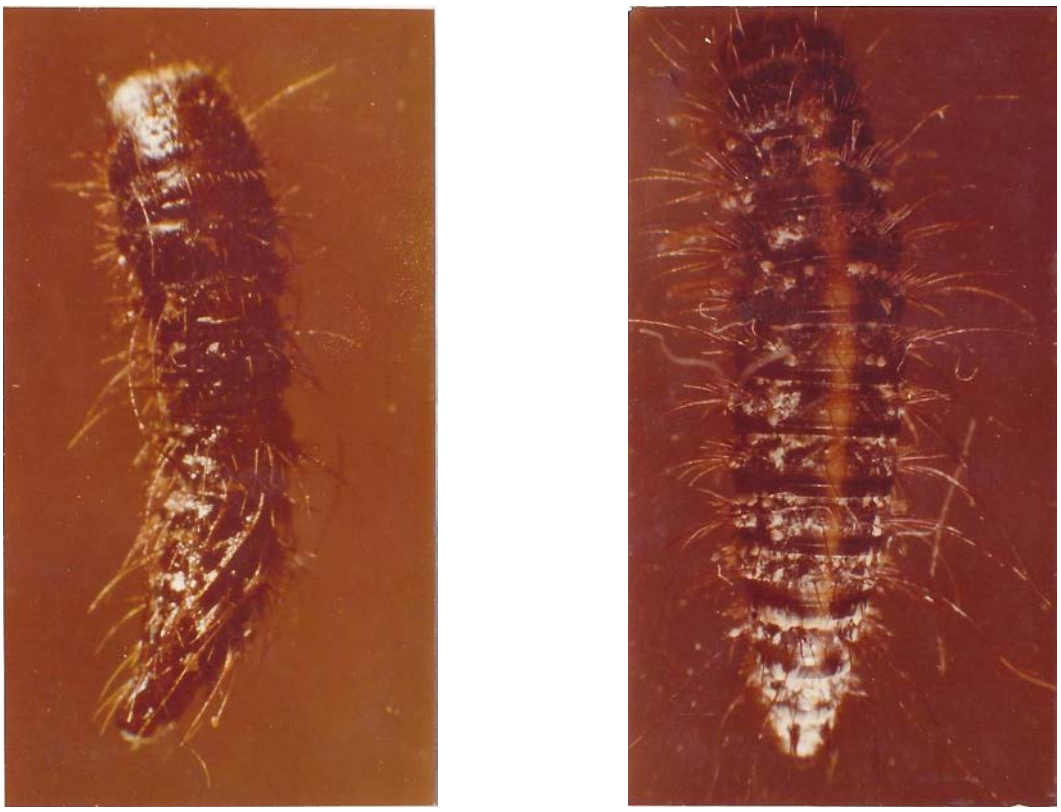


Fig 3: Photomicrographs showing different forms of *P. firma;* E -,epimerite ; P-,protomerite ; D-deutomerite.



(a) (b)

Figure 4: Photographs of 4th instar larvae of *D.vulpinus* (a) Starved and infected (b) fourth (b) Starved and infection- free

P. firma [8] is known to be an inhabitant of *Dermestes frischli,* while *D. vulpinus* is known as a host of *Pyxinia crystalligera* . Identification of the species in this instance was provided by "The Natural History Museum, London". Our results suggest that the impact of infection is manifested most obviously when larvae of *D. vulpinus* were starved . Starved larvae that were infected exhibited a much faster rate of weight loss than those that were gregarine free. These observations are compatible with those of [7] in respect to the effect of *P. frenzli* on the carpet beetle, *Attagenus megatoma* .

Our results also indicate a higher THC in infected larvae than in uninfected larvae which conforms with the findings of Weiser [9] in respect to the effect of infection by *Serucesthis* on scarabid beetle larvae..

The elevation in the THC and the rate of weight loss in infected compared to gregarine free larvae becomes less significant if larvae are treated with sorbic acid . The toxic effect of the sorbic acid, however, appears to subside with successive generations since both the THC and the rate of weight loss begin to rise again the following progeny. This tends to to support the argument of Trehan and Payni [10] that poison causes a reduction in the total haemocyte counts in infected insects. The THC in *Locusta migratoria* [11] and in *P. americana* [12-13] for example increased at each mout during the nymphal period to justify their role in the immune system [14-15] Qamar and Jamal [16]). In fact, insects and other arthropods present different physico-chemical methods to combat and, check the challenges posed by their biological enemies including viruses, bacteria, protozoans, fungi and cestods [17-18] .

Since no injuries were caused to the site of inhabitance and on account of the fact that normally fed larvae showed little signs of infection other than elevated THC compared to uninfected larvae , our results would tend to suggest that *P. firma* in *D. vulpinus* should be considered to be more a commensal than a parasite.

4. Conclusion

Pyxinia firma is a new record in the midgut of the scavenger beetle, *Dermestes vulpinus*. It shows a negative effect on the growth of the larvae of the beetle however only when these are starved. Infected specimens exhibit a significant increase in the total haemocyte counts and

these counts also rise in the progeny of treated larvae , presumably as an indication of immunological response of haemocytes to the Pyxinia infection as reported earlier also [19]

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