LABORATORY STUDIES ON THE POSSIBILITY OF CULEX QUINQUEFASCIATUS TO HARBOUR HEPATOZOOON SP. INFECTING CERASTES CERASTES VIPER IN EGYPT

By
NAGWA A. RASHDAN, FATMA H. GALAL AND ALAA F. GAD-ALLAH
Department of Entomology, Faculty of Science, Cairo University, Giza 12211, Egypt

Abstract
A successful experimental infection of Culex quinquefasciatus with Hepatozoon sp. infecting Cerastes cerastes cerastes viper was carried out under laboratory conditions of 24±3°C and 60-70% R.H. The period monitored for complete sporogonic cycle was 21 days. The effect of high parasiticim blood meal was nonsignificant (P>0.05) on preoviposition period and hatchability. Meanwhile a highly significant reduction was observed in oviposition rate, number of deposited eggs, number of hatched larvae and longevity (P<0.01). On the contrary moderate infection with Hepatozoon revealed a great significant increase in fecundity (P<0.01) and a nonsignificant decrease in longevity (P>0.05).

Key words: Experimental, Culex quinquefasciatus, Hepatozoon sp, Cerastes Cerastes Cerastes

Introduction
Mosquitoes were incubrated since ancient times in the transmission of vertebrate diseases. Culex quinquefasciatus is a vector of many pathogens of humans, and both domestic and wild animals. This species include WNV, SLEV and Western equine encephalitis virus (WEEV), the main vector of SLEV in the southern U.S. (CDC, 2012), Reticuloendotheliosis virus (Ho-Ider et al., 1999), filarial worm Wuchereria bancrofti (Agrawal and Sashindran, 2006) and the protozoa Hepatozoon which is responsible for hepatozoonosis disease (Rashdan, 2007).

This study investigated the possibility of C. quinquefasciatus to harbor Hepatozoon sp. infecting the Egyptian viper Cerastes cerastes cerastes and to detect the influence of infection on some biological aspects of the mosquito host.

Materials and Methods
Culex quinquefasciatus was initially collected from Borg El Arab area, Alexandria Governorate and colonized in the laboratory of Entomology Department, Cairo University. Rearing technique was carried out according to Adham et al., (2003). Wild caught vipers Cerastes cerastes cerastes were obtained from Aswan Governorate. Each viper was housed in a specific mesh screened wooden cage under laboratory conditions of 24±3°C and 60-70% R.H., and was provided with constant access of water and maintained on a diet of mice each week. Parasite detection inside the vipers was carried out according to Bashtar et al. (1984) and parasitaemia percent was calculated (Galal, 2010). Mosquito infection and sporogony detection were according to Rashdan and El Sebaii (2006).

For studying the reproductive capacity, longevity and mortality assessment of Culex quinquefasciatus females infected with Hepatozoon sp. from the viper Cerastes cerastes cerastes three groups, 50 females each, of 3-4-day old were starved for 12 hrs prior to feeding. One mosquito group (G1) was allowed to feed on non-infected vipers. The second group (G2) was allowed to feed on naturally infected viper with moderate parasitaemia (3-10%). The third group (G3) was offered naturally infected viper with high parasitaemia (11-20%). Engorged females from each group were kept separately and examined daily for preoviposition period, number of deposited eggs, percent hatchability, oviposition rate together with longevity. The experiment was repeated three times.

Results
The results are shown in table (1) and figure (1)
Table 1: Effect of Hepatozoon sp. infection on reproductive capacity and longevity of Culex quinquefasciatus females

<table>
<thead>
<tr>
<th>Experiment al females</th>
<th>Preoviposition period (days)</th>
<th>Oviposition rate (%)</th>
<th>No. of deposited eggs</th>
<th>No. of hatched larvae</th>
<th>Hatchability percentage</th>
<th>Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min. + S.E</td>
<td>Max.</td>
<td>Min.</td>
<td>Max.</td>
<td>Min. + S.E</td>
<td>Min. + S.E</td>
</tr>
<tr>
<td>G1</td>
<td>3.00</td>
<td>22.00</td>
<td>90.91</td>
<td>100.00</td>
<td>47.00</td>
<td>284.00</td>
</tr>
<tr>
<td></td>
<td>12.06±1.55</td>
<td>94.41±4.81</td>
<td>123.29± 10.00</td>
<td>86.76± 5.72</td>
<td>76.30±4.21</td>
<td>28.22±1.74</td>
</tr>
<tr>
<td>G2</td>
<td>5.00</td>
<td>24.00</td>
<td>40.00</td>
<td>90.00</td>
<td>77.00</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td>13.18±1.95</td>
<td>60.00±12.25**</td>
<td>173.75±12.67**</td>
<td>142.08±13.25**</td>
<td>80.99±2.86</td>
<td>24.30±2.02**</td>
</tr>
<tr>
<td>G3</td>
<td>4.00</td>
<td>9.00</td>
<td>20.00</td>
<td>50.00</td>
<td>57.00</td>
<td>142.00</td>
</tr>
<tr>
<td></td>
<td>6.33±1.45</td>
<td>40.00±7.07**</td>
<td>90.20± 9.48**</td>
<td>65.60 ± 11.32**</td>
<td>69.51±9.54</td>
<td>9.00±1.93**</td>
</tr>
</tbody>
</table>

G1: females fed on non-infected vipers; G2: females fed on infected vipers with moderate parasitaemia; G3: females fed on infected vipers with high parasitaemia, P<0.05* = significant, P<0.01** = highly significant, same letter means not significant.

Discussion

In the present study, smears from both infected groups of *C. quinquefasciatus* females showed the developmental stages appearance of the Hepatozoon parasite. Female reared under 24±3°C and 60-70% RH showed complete gamogony and sporogony (Fig. 1). Zygote formation started by day 4 post infection. This result agreed with Bashatar et al. (1987) for *H. gracilis* and Fayed et al. (1995) for *H. malpiloni* but, disagreed with that obtained by Bashatar et al. (1984) for *A. aegypti*. Infected females gave rise to mature sporozoites by day 21 post infection. This result more or less agreed with the sporogonic period reported by Rashdan et al. (2006) for *H. sp. harbored by Uromastyx microlepis*. Meanwhile different periods of sporogonic cycle was observed by Abdel Ghaffar et al. (1994) for *H. ghaffari; Smith et al. (1994)* for *H. sipedon; Fayed et al. (1995)* for *H. malpiloni; Dessier et al. (1995)* for *H. catesbianae; Kim et al. (1998)* for *H. clamatae* and Ebreaheem et al. (2006) for *H. matrurensis*.

In the present study, *C. quinquefasciatus* females fed on the vipers *C. cerastes cerastes* with high parasitaemia of Hepatozoon reduced the preoviposition period and hatchability percent non-significantly (P>0.05). The reduction was great and highly significant (P<0.01) in oviposition rate, number of deposited eggs and number of hatched larvae. This result agreed with Hogg and Hurd (1995) for *Anopheles stephensi* infected with *Plasmodium yoelli nigeriensis* at high oocyst burdens and by Galal (2010) in case of *C. (C.) pipiens* fed on highly infected skinks with *H. gracilis*.

Using a viper with moderate Hepatozoon infection showed a non-significant increase in the preoviposition period (P>0.05). This result agreed with that recorded by Ebreaheem et al. (2006) in case of *C. pipiens* infected with *H. maturhensis* and with Rashdan et al. (2006) in case of *C. quinquefasciatus* and *C. pipiens* infected with Hepatozoon sp. Meanwhile, a highly significant reduction in oviposition rate together with a highly significant increase in number of deposited eggs and number of hatched larvae was obtained (P<0.01). This finding agreed with Ferguson et al. (2003) who reported the increase of fecundity of infected mosquitoes with malaria. On the contrary, reduction in fecundity was reported by Hogg and Hurd (1997) for *Anopheles gambiae* infected with malaria; Adham et al. (2003) for *C. pipiens* infected with *H. gracilis*, Ebreaheem et al. (2006) for *C. pipiens* infected with *H. maturhensis* and Rashdan et al. (2006) for *C. pipiens* and *C. quinquefasciatus* infected with Hepatozoon sp.

The hatchability percent was non-significantly affected by the presence of Hepatozoon sp. within female mosquitoes. This result agreed with that reported by Adham et al. (2003) for *C. pipiens* infected with *H. gracilis*, Ebreaheem et al. (2006) for *C. pipiens* infected with *H. maturhensis*, Rash-

Longevity severely reduced when females were fed on infected vespers with high parasitemia to 9±1.93 days in average (P<0.01). This result agreed with Galal (2010) in case of C. (C.) pipientis infected with H. gracilis. By using moderate parasitaemia vespers there was a non-significant decrease in longevity of C. quinquefasciatus females (P>0.05). This data agreed with Adham et al. (2003) in case of C. pipientis females infected with H. gracilis, Ebraheem et al. (2006) in case of C. (C.) pipientis females infected with H. maturhensis, Rashdan et al. (2006) in case of C. pipientis and C. quinquefasciatus infected with Hepatozoon sp. But, Galal (2010) reported a significant increase in longevity of C. (C.) pipientis infected with H. gracilis.

**Conclusion**

Members of Hepatozoon possess particularly complex life cycles which vary considerably among species. Appearance of complete sporogonic developmental stages of Hepatozoon infecting Ceratitis ceratitis ceratitis together with the increase of the biological parameters of female C. quinquefasciatus infected with moderate parasitaemia showing the adaptation of the mosquito towards Hepatozoon infection that confirm the ability of C. quinquefasciatus to be a good vector of Hepatozoon sp.

**References**


CDC, 2012: Saint Louis Encephalitis, USA.


Smith, TG, Desser, SS, Martin, DS 1994: development of *Hepatozoon sipedon* sp. nov. (Apicomplexa: Adeleina: Hepatozoidae) in its natural host, the Northern water snake (*Nerodia sipedon sipedon*), in the culicine vectors *Culex pipiens* and *Cx. territans* & in an intermediate host, the Northern leopard frog (*Rana pipiens*). Parasitol. Res. 80, 7:559-68

Fig.1: Sporogonic cycle of *Hepatozoon* sp. inside mosquito host. a: gametocytes inside viper RBCs. b: free gametocytes, c:micro-and macrogametes, d:mononucleated zygote, e:binucleated zygote, f:tetranucleated zygote, g: oocyst, h:sporoblast, i: sporocysts with mature sporozoites