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LABORATORY STUDIES ON THE POSSIBILITY OF *CULEX QUINQUEFASCIATUS* TO HARBOR *HEPATOZOON* SP. INFECTING *CERASTES 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 parasitimic 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 incriminated 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-lder *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 viper. 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

Experimental females	Preoviposition period (days)		Oviposition rate (%)		No. of deposited eggs		No. of hatched larvae		Hatchability percentage		Longevity (days)	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
	Mean ± S.E		Mean ± S.E		Mean ± S.E		Mean ± S.E		Mean ± S.E		Mean ± S.E	
G1	3.00	22.00	90.91	100.00	47.00	284.00	44.00	127.00	39.08	100.00	8.00	40.00
	12.06±1.55 A		94.41±4.81 A		123.29± 10.00 B		86.76± 5.72 B		76.30±4.21 A		28.22± 1.74 A	
G2	5.00	24.00	40.00	90.00	77.00	225.00	59.00	215.00	59.69	97.73	1.00	48.00
	13.18±1.95 A		60.00±12.25** B		173.75±12.67** A		142.08±13.25** A		80.99±2.86 A		24.50±2.02** A	
G3	4.00	9.00	20.00	50.00	57.00	142.00	4.00	116.00	6.78	95.77	1.00	16.00
	6.33±1.45 A		40.00±7.07** B		90.20 ± 9.48** B		65.60 ± 11.32** B		69.51±9.54 A		9. 00±1.93** B	

G1: females fed on non-infected viper, G2: females fed on infected viper with moderate parasitaemia, G3: females fed on infected viper 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. Females reared under 24±3ºC and 60-70% RH showed complete gamogony and sporogony (Fig. 1). Zygote formation started by day 4 post infection. This result agreed with Bashtar *et al.* (1987) for *H. gracilis* and Fayed *et al.* (1995) for *H. malpoloni* but, disagreed with that obtained by Bashtar *et al.* (1984) for *H. 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. malpoloni*; Desser *et al.* (1995) for *H. catesbiance*; Kim *et al.* (1998) for *H. clamatae* and Ebraheem *et al.* (2006) for *H. matruhensis.*

In the present study, *C. quinquefasciatus* females fed on the viper *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 yoelii 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 Ebraheem *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*, Ebraheem *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*, Ebraheem *et al*. (2006) for *C. pipiens* infected with *H. maturhensis*, Rash-

dan *et al*. (2006) for *C. quinquefasciatus* and *C. pipiens* infected with *Hepatozoon* sp. and Rashdan and El-Sebaii (2006) for *C. neavei* infected with *H. maturhensis.*

Longevity severely reduced when females were fed on infected viper with high parasitaemia to 9±1.93 days in average (P<0.01). This result agreed with Galal (2010) in case of *C. (C.) pipiens* infected with *H. gracilis*. By using moderate parasitaemic viper 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. pipiens* females infected with *H. gracilis*, Ebraheem *et al*. (2006) in case of *C. (C.) pipiens* females infected with *H. matruhensis*, Rashdan *et al.* (2006) in case of *C. pipiens* and *C. quinquefasciatus* infected with *Hepatozoon* sp. But, Galal (2010) reported a significant increase in longevity of *C. (C.) pipiens* 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 *Cerastis cerastis cerastis* 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.

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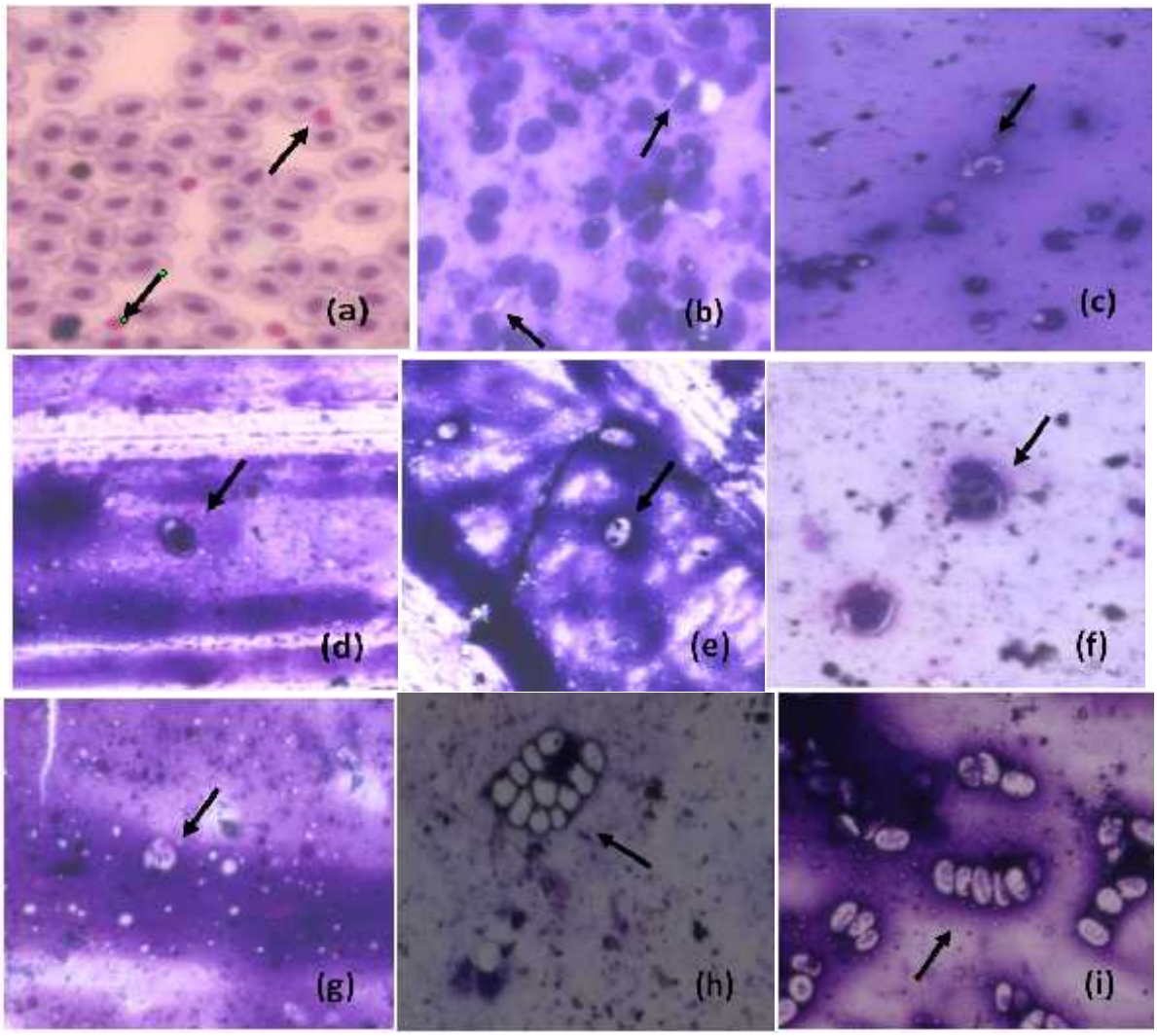

Fig.1: Sporogonic cycle of *Hepatozoon* sp. inside mosquito host. a: gametocytes inside viper RBCs, b: free gametocytes, c:micro-and macrogametes, d:mononucleated zygote, e:binucleated zygote, f:tetranucleated zygote, g: oocyst, h:sporoblast, i: sporocysts with mature sporozoites