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THE EFFECT OF HEPATOZOON SP.INFECTION ON SOME BIOLOGICAL PARAMETERS OF THE MOSQUITO CULEX PIPIENS COMPLEX

By
FATMA K. ADHAM[1], FATMA H. GALAL[1] and ALI SOBHY MOHAMED SALAMA[2]
Departments of Entomology, Faculties of Science, Cairo University[1], Giza, and Tanta University[2], Tanta, Egypt (*Correspondence: adhamfk@gmail.com)

Abstract

Hepatozoon is a very diverse genus with more than 300 species currently assigned to it, based largely on morphological characters, host specify and life cycle patterns. Peripheral blood smears of naturally infected vipers *Cerastes cerastes cerastes* (hornless) showed intracytoplassmic banana-shaped gametocytes in the erythrocytes. Upon initial examination in 12% out of 100 collected vipers harbored *Hepatozoon* sp. Effect of *Hepatozoon* sp. infection on some biological parameters of *Culex pipiens* complex showed no significant difference between longevity of non-infected and infected ones, and between mortality rate, and pre-oviposition period of both. Fertility female mosquitoes were significantly affected. Infected females produced significantly fewer eggs/female as compared to those deposited by non-infected ones, without significant difference incubation periods of eggs deposition. The mean oviposition rate of fully engorged infected *Cx. pipiens* complex female was highly significant less than that of non-infected female ones. Statistical analysis revealed a highly significant difference ($P<0.0001$) between the larval mortality from infected and non-infected female ones.

Keywords: *Culex pipiens* complex, Biology, *Hepatozoon* sp.

Introduction

Blood parasites of the genus *Hepatozoon* Miller 1908 (Apicomplexan) are transmitted between vertebrates and arthropod hosts, creating dynamic relationships subject to influence both the host and parasite. They were described from all groups of terrestrial vertebrates among which many reptiles, mammals, amphibians as well as a wide range of hematophagous arthropods (Telford, 2009). Sexual reproduction and sporogonic development occur within the hemocoel of the invertebrate host, which consumed by the vertebrate host. Smith (1996) reported that *Hepatozoon* spp. life cycle is characterized by sporogonic development in hematophagous arthropods that results in the oocysts, each contained sporozite-filled sporocyst. He added that both merogonic and gametogonic developed in internal organs of an infected vertebrate host.

The viper *Cersastes cerastes cerastes* was present in the Eastern Desert and Sinai, except in the Northern dune fields (Schnurrenburger, 1959), as well as along the Nile Valley margins, particularly along the shores of Lake Nasser (Gasperetti, 1988). One of the most characteristic feature is the presence of supraorbital 'horns', which may be predominantly, entirely hornless, or mixed, but with eyes on head both sides of significant sexual dimorphism as being larger in males (Sterer, 1992).

This paper aimed to evaluate *Cerastes cerastes cerastes* (hornless) natural infection with *Hepatozoon* sp. in Aswan Governorate.

Materials and Methods

Collections of vipers: One hundred viper; *Cerastes cerastes cerastes* (hornless) were collected from Aswan Governorate. On laboratory examination only 12/100 were infected with the protozoan blood parasite. Each infected viper was housed in a separate mesh screened wooden cage (35x35x35cm) under laboratory conditions of temperature of 24 + /-3^{o}C & RH 70% They were weekly allowed water & diet of a clean laboratory lizard.

Detection of *Hepatozoon*s by thin blood smears: Blood samples were obtained from the vipers by clipping the last 2mm of tail's tip with a sterilized razor blade. Blood was collected in heparinized micro-hematocrit capillary tube and the wound was sprayed with Bactine (R) antiseptic spray before vipers were returned to their cages. Blood smears were air-dried and fixed with acetone

free absolute methanol for 3min, stained in 10% Giemsa (pH 7.3) for half an hour and microscopically examined for gamonts of *Hepatozoon* sp. using bright field at 250x (Adham *et al,* 2003).

Culex pipiens rearing: *Cx. pipiens* complex larvae were collected from Abu-Rawash water bodies at Giza Governorate. They were reared and maintained under laboratory conditions of 27+/-3^{o}C and RH 60-70%. No attempt to control the light conditions in the laboratory which received supplementary illumination during daytime from overhead fluorescent lamps. Emerging adults were released into a mesh screened wooden cage (35x35x35cm) with the front side holding a hole fitted with a piece of cloth sleeve to facilitate food supply, to remove the deposited eggs and to clean the cage. Each cage was provided with a small petri-dish containing a cotton pad soaked with 10% sucrose solution as a food source for both males and females. Females were provided with blood meals from a domesticated pigeon **(***Columba livia domestica***)** lied over on the cage top, as well as human volunteer. Small beakers (250ml) three-quarter filled with dechlorinated water were placed inside the cage holding the adults for oviposition. The eggs were directly transferred to white enamel pans (20cm in diameter) with fresh dechlorinated water for hatching. Batches of about 200newly hatched larvae were reared in large white enamel pans (40cm in diameter) contained 250ml dechlorinated water. Daily renewal of the water was necessary in the first and second larval instars. Dechlorinated water was added to compensate the water lost via evaporation. Overcrowding of larvae was avoided to prevent undersized emerging adults.

Larval feed on TetraMin® Tropical Flakes (Baby fish food), active life formula helps nutritionally support fish's immune system for optimal health and long life. Additives: vitamins A376001u/kg, vitamin D32000kg, Vitamin E125mg/Kg, L-ascorbyl-2-polyphosphate 265mg/Kg, Lecitin-contained EEC permitted colorants and preservatives. A very small amount of was given to newly hatched larvae, and the amount was increased as they grown until pupation. Pupae were daily collected by a handle sieve and transferred to a mesh screened wooden cage for adult emerging, mating and oviposition as given before.

Statistical analysis: Data between means were determined by using F-test statistical program. Changes were considered non-significant and highly significant when P-value was >0.05 & <0.0001 respectively.

Results

Preliminary study on survival of engorged females *Cx. pipiens* complex on highly infected *C. c. cerastes* (hornless), and allowed to feed on vipar with high parasitemia (28%).

Effect of *Hepatozoon* sp. on some biological parameters of females *Cx. pipiens* complex**:** Two groups of 100 individuals each of 3-4 day-old females were starved for 12hrs. The first group was allowed to feed blood meal from non-infected viper (control), but the second one was fed on a blood meal from the infected one (10% parasites). The fully engorged females from each group were transferred separately to oviposition vials containing 1.5 ml dechlorinated water. Cotton imbibed with 10% sugar solution was put on the top of the vial glass. Continuous access of sugar was not provided in order to avoid any inhibition of oviposition promoted by prolonged sugar feeding. Dead female mosquitoes were counted and discarded and survival ones was recorded. Preoviposition period, incubation period and fecundity for each female were recorded. Hatchability, fertility and oviposition rate were determined. Fertility was measured by number of hatched larvae from each egg raft, and oviposition rate was measured by total number of females deposited egg rafts divided by total number of fully engorged females. Each experiment was repeated 3 times under same laboratory conditions.

Life cycle: a- Longevity revealed that the mean longevity of the non- infected females

was (18.50+10.53days), from the first while that of the infected ones (15.50+ 8.80days), without significant difference (P =0.3) between the longevity of non-infected and infected females. b- Mortality rate showed that the means daily mortality rate of infected and non- infected females were (3.33+/-3.02 & 2.77+/- 2.17) respectively, without significant difference (P=0.38).

c- Accumulative mortality rate showed that the accumulative mortality rate of infected females started from the first day post feeding on blood of infected viper was 20% of the colony size by day 9th post feeding and continue gradually to 60% by day 21st post feeding. Starting from day 20th post feeding the curve was moving at higher rate than in case of non-infected females till all females diminished by the day 30 post feeding.

In non- infected viper the accumulative mortality rate of non-infected females started from the 2nd day post feeding on clean blood meal from non-infected vipers then increased to reach almost 20% by the day 10th post feeding then continue smoothly till all normal female mosquitoes diminished by day 37 post-feeding. Accumulative death rates of both infected and non-infected female mosquitoes were similar.

Reproductive capacity: a- Pre-oviposition period was not significant (P=0.25) in pre-oviposition period of female*s* fed on infected (9.57±3.30days) vipers compared to non-infected ones (7.66± 2.16 days). b- Fecundity showed a highly significant (P<0.0001) decrease in eggs deposited by infected female*s* complex (52.05±23.67eggs/female), as compared to eggs deposited by non-infected ones (143.62± 26.142ggs /female).

Incubation period showed that of eggs deposited period by non-infected and infected females were (43.47±15.19& 39.18±13.82 hrs) respectively, without significant difference (P=0.06) between non-infected and infected females.

Fertility (hatchability) showed that fertility was affected significantly (P<0.0001) by *Hepatozoon* sp. in females (48.97±19.32 & 82.40±6.23) respectively.

Oviposition rate showed that the mean oviposition rate of fully engorged infected females was (28.55±2.34) less than that of non-infected ones (63.08±3.73), with highly significantly different (P=0.0002).

Effect of *Hepatozoon* sp. on mortality rate among newly hatched larvae from infected and non-infected female of first gonotrophic cycle were (36.15±44.11& 3.44±5.37) respectively, with significance (P< 0.0001).

Details were given in tables (1 & 2) and figures (1 & 2).

Table 1: Effect of *Hepatozoon sp*. infection on longevity and mortality rates of female *Culex pipiens* complex

Culex pipiens complex	Mortality/day (dead females/total females (%) Mean+/- SD (min-max)	Longevity in days Mean+/- SD (min-max)
Non-infected female	2.77+/-2.17^{A} (0-7.95)	18.50+/-10.53^{A} (1-36)
Infected	3.33+/-3.02^{A} (0-12.2)	15.50+/-8.80^{A} (0-30)

Table 2: Effect of *Hepatozoon sp.* on reproductive capacity of *Cx. pipiens* complex

Culex (*Cx*) *pipiens* complex	Preoviposition period (days) (min-max)	Fecundity (egg/female) (min-max)	Total oviposition rate (%) (min-max)	Incubation (hour) (min-max)	Hatchability (%) (min-max)	Mortality of new larval generation (%) (min-max)
Non infected female	7.66+/_2.16^{A} (5-11)	143.62+/_26.17^{A} (29-175)	63.08+/_3.73^{A} (63.33-67.33)	43.47+/_15.19^{A} (24-72)	82.40+/_6.23^{A} (51.72-93.60)	3.44+/_5.37^{A} (0-18.500)
Infected	9.57+/_ 3.30^{A} (6-16)	52.05+/_23.67^{B} (12-110)	28.55+/_2.34^{B} (26.100-30.770)	39.18+/_13.82^{A} (21-72)	48.97+/_19.32^{B} (0-80.41)	36.15+/_44.11^{B} (0-100)

Discussion

In the present study, 12/100 vipers (125) were naturally infected with *Hepatozoon* species. The highest parasites rate was during October (33.33%) of the collected vipers, dropped to zero during March and April. Bashtar *et al* (1991) reported *Hepatozoon seurati* from \viper *C. c.* (horned). Also, Smith *et al.* (1994) reported *H. sipedon* from water snake *Nerodia sipedon sipedon*.

In the present study, Cx. pipiens female fed on infected viper with low infection rate (10%), but harbored the parasite and maintained their life cycle normally till all dimin-

ished by the 30th day post-infection. The curve of accumulative mortality rate showed that infected females started from the 1st day post-infection to reach 20% by the 9th day and continued gradually to 60% by the 21th day. But on the 20th day post-infection the curve jumped at higher rate till diminished by the 30th day. This agreed with Brown *et al* (2006) who found that *Hepatozoon* spp. infection was considered relatively benign in its vector. Also, Ebrahemm *et al* (2006) reported that *Hepatozoon* spp. infection did not affect the vector longevity. The present results suggested that *Cx. pipiens* complex were vector of *Hepatozoon* sp.

Bashtar *et al.* (1984a, b) described a new species; *H. aegypti* from snake *Spalerosophis diadema*, and followed its complete life cycle in *Cx. p. molestus*, using optical and electron microscopy. Also, Bashtar *et al.* (1991) described a new species, *H. melhorni* from the viper *Echis carinatus* and followed the sporogony in *Cx. pipiens'* hemocoel.

The present results proved role *Cx. pipiens* complex as a vector of *Hepatozoon* sp.

In the present study, mosquitoes showed high mortality (28%) after feeding on infected vipers. Ball *et al* (1967) and Wosniak and Telford (1991) found a relationship between high parasitemia and mosquito mortality. Also, Ferguson *et al* (2012) reported mosquito death after feeding on frogs with high levels of *H. sipedon* and *H. clamatae*. Thus, parasitemia was risky factor to determine the mosquito effective capacity.

In the present study, pre-oviposition period of female mosquitoes was not affected by *Hepatozoon* sp. in blood meal. But, Adham *et al* (2003) reported a significant increase in the pre-oviposition period of *Cx. pipiens* infected with *H. gracilis*.

In the present study significant change was found in the incubation period between infected and non-infected females. In the 1st gonotrophic cycle infected females produced significantly fewer eggs (28.55±2.34) than the non-infected ones (63.08±3.73). Also, a complete fecundity reduction (100%) was seen in some infected females after infected blood meal. Also, the new hatched larvae from infected females showed a high mortality rates than those from non-infected ones. Thus, *Hepatozoon* sp. has a large impact on the fitness and survival of new generation resulting from the infected mother mosquito. This agreed with Madsen *et al* (2005) they found that *Hepatozoon* in pythons effected on growth rate and juvenile survival, with significant risky impact on the reproductive capacity of *Cx. pipiens* complex. This agreed with Webb and Hurd (1999) or low concentration or infrequent provision of sugar which effectively lower total lipid resources of mosquitoes and decrease fecundity (Briegel *et al*, 2002), or a protozoan and insect interaction (Adham *et al*, 2003). The parasite might produce factors to reduce fecundity or induce modulators secretion in hosts that affect oogenesis (Hurd, 2009). After fertilization, and oocysts grown rapidly divided during sporogony with multiple fissions, they required substantial quantities of more nutrients for protein synthesis. The shortness of available nutrients reduced fecundity. This agreed with Sanders *et al*. (2003) who found that after blood meal digestion, nutrients destined for the ovaries co-mprise a pool of resources that targeted for use by an invading parasite for its own metabolic demands. For a parasite to reduce the hosts' fecundity target two areas of oocyte development; the fat body where vitellogenin (Vg) produced and the ovaries where Vg uptake occurred (Ahmed *et al*, 2001). Vitellogenin production and Vg uptake by the ovaries (Hogg *et al*, 1997) reduced or inhibited ovarian protein content (Renshaw and Hurd, 1994; Jahan and Hurd,1998) followed by follicle resorption (Hopwood *et al,* 2001)) and a reduction in the number of eggs produced (Warr *et al,* 2004). Moreover, the fat body may be localized in particular regions and if large numbers of *Hepatozoon* spp. occupied cells responsible for Vg, fecundity reduction could occur (Arresse and Soulages, 2010). Eggs deposited during infection

may be deficient in protein and thus reduced the viability (Ahmed *et al,* 1999). Also, parasite may actively produce factors that reduced fecundity (Hurd, 2009) or induce the modulators secretion of hosts that affect oogenesis (Adamo, 2002). Modulators released by parasites or hosts can also trigger an immune response led to fecundity reduction via energy depletion (Hurd, 2001) or host compensatory response to infection (Hurd, 2009).

Conclusion

Hepatozoon species affected its arthropod host and most of its food resources and is directing it to achieve personal benefit to ensure its success in order to continue its life cycle and be able to be transmitted to the new developmental phase within the vertebrate host. Level of parasitemia in the blood of infected viper is an essential factor to determines the ability of mosquitoes to be effective vectors.

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Explanation of figures

Fig. 1: Effect of *Hepatozoon* sp. on mortality rate & longevity of female *Cx. pipiens* complex

Fig. 2: Effect of *Hepatozoon* sp. on accumulative mortality rate & longevity of female *Cx. pipiens* complex.

Fig. 3: Gamonts of *Hepatozoon* sp.