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COMPARATIVE SUSCEPTIBILITIES OF *CULEX PIPIENS* AND *CULEX QUINQUEFASCIATUS* TO THE INFECTION WITH *HEPATOZOON* SP . INFECTING Saudi lizard (*UROMASTYX MICROLEPIS.*)

NAGWA , A . RASHDAN

Department of Entomology , Faculty of Science , Cairo University , Egypt .

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SUMMARY

C . pipiens and *C.quinquefasciatus* females infected with *Hepatozoon sp.* present in the Saudi lizard *Uromastyx microlepis* were examined for sporogonic development . Mature zygotes formed synchronously at 8 hr then quickly subsided in *C . pipiens* . In *C.quinquefasciatus* zygote formation was more protracted and its density peaked at 12 hr . Losses in parasite abundance during the conversion of zygote tooocyst was nonsignificantly higher in *C.pipiens* than that in *C .quinquefasciatus* ($P > 0.05$) . Melanotic encapsulation of oocysts was not observed for both mosquito species . On the 20th day sporozoites were present in the salivary glands of *C. pipiens* , while *C.quinquefasciatus* showed sporozoite - negative glands.Nearly half of mosquitoes with mature oocysts gave rise to sporozoite infected ones. Parasite populations were not normally distributed in either mosquito species but were described by a negative binomial type of distribution .

INTRODUCTION

Culicine mosquitoes were recorded to be one of the natural vectors of *Hepatozoon* sp. The experimental transmission of *Hepatozoon* by mosquito bites was based mainly on offering the mosquito vector infected hosts and refeeding the vector on clean recipient ones. The present investigation was designed to follow up the formation and kinetics of the different developmental stages of the *Hepatozoon* sp . infecting the Saudi lizard *Uromastyx microlepis* inside *Culex pipiens* and *Culex quinquefasciatus* t o clarify some of the underlying mechanisms that perform support to *Hepato-*

zoon sporogony causing various degrees of vector infectivity .

MATERIALS AND METHODS

Mosquito host maintainance : *Culex pipiens* and *Culex quinquefasciatus* were initially collected from Al Rass Governorate , Al Quaseem Area , Kingdom of Saudi Arabia and colonized in the laboratory of biology in Al Rass Girls College . Mass production of adult mosquitoes was maintained according to Adham et . al . (2003) .

The reptile host : Naturally infected *Uromastyx microlepis* Saudi lizard with *Hepatozoon sp.* collected from Al Rass Governorate , were detected by examining thin smears of blood after clipping off the tail tip and the infected individuals were used .

Initiating sporogony : Gametocytemic lizard were placed on the screened tops of the rearing cages of mosquitoes . The invertebrate host infection was examined and determined according to Rashdan et al. (2006 b) . Mosquito species were allowed to feed from the same infected host to avoid any variability in gametocyte infectiousness (co-infection) and only engorged females were used .

Zygote kinetics : The two culicine species (about 40 females per species) were co-infected and 4 - 6 engorged females of each species were picked up by an aspirator at intervals of 4 , 8 , 12 , 16 , 20 and 24 hr after infection and processed for zygote densities examination according to Vaughan et al. (1991) . The number of formed zygotes was recorded in 4 separate co- infections using a hemocytometer under phase-contrast microscopy .

Oocyst production : Oocyst production was quantitated in 4 separate co-infections for each mosquito species . For each infection 6 to 10 females were dissected and zygotes were quantitated at 8 hr for *C.pipiens* and at 12 hr for *C . quinquefasciatus* . At 10 - 14 days post-infection , all remaining mosquitoes were dissected and examined for oocysts . The geometric means of zygote and oocyst densities were calculated . The decrease in parasite density from zygote to oocyst stage was expressed as mortality (k) according to Varely et al. (1973) . The percentage of infection was calculated .

Sporozoite production : Mosquito females from both species were co-infected. By day 10 postinfection a subsample (4 - 10 females) from each mosquito species was examined to determine the initial infection rates . On the 20th day after infection the remaining females were dissected

and examined for the oocysts and sporozoites . The percentage of oocyst infection revealed in sporozoite infections was calculated .

Parasite distribution : Zygotes and oocysts were quantified through 15 infectious feeds. For each mosquito species, frequency of distribution was constructed for both zygotes and oocysts .

Data were analysed by using one way ANOVA , t - test , chi - square test and Duncan multiple range test computer program , copyright © , 1989 (H . S .) Motulasky , version 1.0 , Dr. Leo P . Schouest , UC Riverside , and serial # 8901685 .

RESULTS

Zygote kinetics : Complete vector - phase development of *Heptozoon* was described by Rashdan el al. (2006 a). Fig. (1) illustrated the two general zygote kinetic patterns. In *C. pipiens* zygote density peaked at 8 hr after infected blood meal and subsided within 12 hr . Meanwhile in *C. quinquefasciatus* the density peaked later at 12 hr after infected blood meal with a broad peak over the course of 12 - 24 hr. For 4 co - infections , the zygote density was nonsignificantly lower inside the gut of *C. pipiens* (119 .2) than in the case of *C . quinquefasciatus* (150 . 8) (Duncan multiple range test , P > 0 . 05 , Table 1) . A nonsignificant increase in the zygote infection prevalence was recorded in case of *C.pipiens* (89 . 3 %) than in case of *C . quinquefasciatus* (80 . 6 %) (chi - square test P > 0 . 05 , Table 1) .

Oocyst production : In 4 co - infections the oocysts densities increased nonsignificantly between *C.pipiens* (3 . 2) and *C . quinquefasciatus* (2.9) (Duncan multiple range test , P > 0 . 05 , Table 1) . Also no significant difference was reported in the losses of the parasite abundance during the conversion to oocysts (K- values) between the two mosquito species (37- fold and 52 fold losses for *C. pipiens* & *C.quinquefasciatus* , respectively) (Duncan multiple range test , 0.05, Table 1) .*C . pipiens* showed a nonsignificant higher infection prevalence (42 %) than that observed for *C. quinquefasciatus* (29 . 1 %) (chi -square test , P > 0 . 05 , Table 1) . Melanotic encapsulation of the parasite was not observed in both *C.pipiens* and *C. quinquefasciatus* .

Sporozoite production : Oocyst infection prevalences at day 10 in *C.pipiens* (92 %) was greater than in *C.quinquefasciatus* (43 %) . This did not change appreciably by day 20 (Table 2) . Free sporozoites were present in the salivary

glands of *C. pipiens* females by day 20 post infection , but only half of the oocyst - infected mosquitoes had concurrent gland infections (52 % , Table 2) . *C . quinquefasciatus* glands were sporozoite - negative while mature free sporozoites were abundant in the hemolymph . Many oocysts contained mature sporozoites were seen . Nearly half of the oocyst infected females showed sporozoite prevalence (46 % ,Table 2) .

Parasite distribution : Parasite populations were not normally distributed in both species , but were adequately described by a negative binomial type of distribution (chi - square goodness of fit , $P < 0.05$) . Skewness moments (g) and overall infection prevalences (%) for zygotes were g = 1 . 14 , 89 % and g = 1 . 39 , 75 % for *C.pipiens* and*C.quinquefasciatus*, respectively. While skewness moments for oocysts were g = 3 .28 , 38 % for *C. pipiens* and g = 1 . 9 , 36 % for *C. quinquefasciatus* .

Table (1): Hepatozoon sp. density and infection prevalence in *C. pipiens* and *C .quinquefasciatus*

Mosquito species	Zygote density			Oocyst density			Zygote mortality (K)	Zygote infectioon prevalence (%)	Oocyst infectioon prevalence (%)
	Min.	Max.	Mean.	Min.	Max.	Mean			
C.pipiens	41.1	341.3	119.2	0.9	3.9	3.2	1.57 (37 fold)	89.3	42.0
C.quinquefasciatus	24.2	380.1	150.8	0.7	3.2	2.9	1.72 (52 fold)	80.6	29.1

$K = \log_{10}$ (zygote) - $\log_{10}$ (oocyst)

Table (2):*Hepatozoon sp.* oocyst and sporozoite infection rate in *C. pipiens* and *C. quinquefasciatus*

Mosquito species	Oocyst prevalence on day 10 (%)	Oocyst prevalence on day 20 (%)	Sporozoite prevalence per oocyst-infected mosquito on day 20
C.pipiens	92	100	52
C.quinquefasciatus	43	56	46

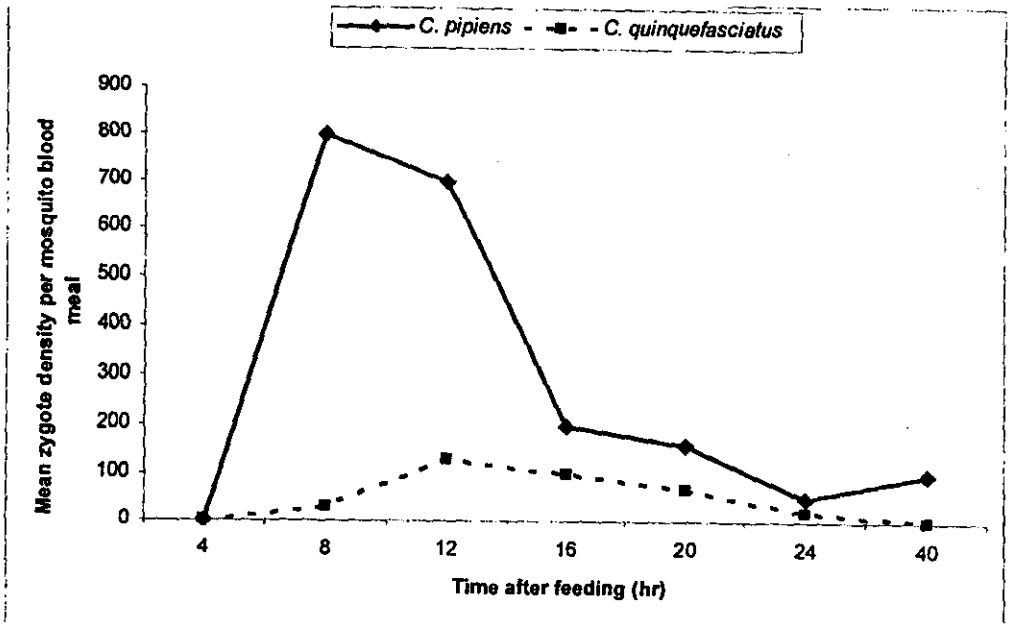

Fig. (1) : The development of *Hepatozoon sp.* zygote in the blood meals of *C. pipiens* and *C. quinquefasciatus* fed on a single gametocytemic lizard.

DISCUSSION

Kinetics of the *Hepatozoon* zygote development differed between the two mosquito species (Fig. 1). In *C.pipiens* the sharp peak of the zygote abundance occurred after 8 hrs , indicating rapid synchronous development . Likewise the rapid decline in zygote abundance after 12 hrs indicates that they might forced their way out into the myxocoel while the peritrophic membrane was still soft and thin (Freyvogel and Staubli , 1965 ; Gad et al., 1996 & Romoser et al., 2000) i. e . a synchronous exodus from the midgut . This results agreed with that recorded by Paperna and Lainson (2003) for *Hepatozoon* sp. and *C.quinquefasciatus* and by Seiber et al . (1991) for Plasmodium gallinaceum and Aedes aegypti and Vaughan et al., (1994 a) for Plasmodium yoelli and three anopheline mosquito species . In case of *C.quinquefasciatus* the zygote development was more protracted with a relatively high density , the reason for this was unknown . It might be a physiological action . The prolonged presence of zygotes in the *C. quinquefasciatus* gut might be due to asynchronous development as a reason of a relatively rapid formation of the peri-

trophic membrane in *C .quinquefasciatus* (Hardly et al . , 1983 ; Beerntsen et . al . , 2000 and Okuda et al . , 2002) that might prevents the parasite from exiting the blood bolus making their way out to the myxocoel . Similar results were reported by Gass and Yeates, 1979 and Seiber et al., 1991 for *Plasmodium gallinaceum* and *Aedes aegypti* ; Vaughan et al . , 1994 a for *Plasmodium yoelii* and *Anopheles albimanus* and Vaughan et al., 1994 b for *Plasmodium falciparum* and *Anopheles dirus* .

The successful conversion of zygotes to mature oocysts differed between the two mosquito species. The conversion was higher in *C. pipiens* than that observed in *C.quinquefasciatus* indicating the more ease of zygote to traverse the midgut .

The absence of melanotic encapsulation indicates that *Hepatozoon* zygote or oocyst did not elicit an immune response from either *C . pipiens or C . quinquefasciatus* .

Only half of the oocyst - infected females in both mosquito species produced sporozoite - infected ones . *Culex pipiens* showed sporozoite - positive glands . Similar results were observed by Adham et al . (2003) and Rashdan et al., (2006 a & b) . While *Culex quinquefasciatus* showed sporozoite - negative glands . This results agreed with that reported by Rosenberg (1985) ; Touray et al . (1992) and Vaughan et al . (1994 b) who stated that specificity of sporozoite - gland recognition depends on the species of plasmodia and mosquitoes involved . While Vanderberg and Yoeli (1966) ; Wery (1968) and Carter and Diggs (1977) stated the reason for poor gland invasion by Plasmodium sporozoites to be the suboptimal temperature at which infected mosquitoes were maintained that affect the viability of sporozoites .

Zygote and oocyst populations exhibited clumped distribution patterns within the two mosquito species (Fig. 2) . Clumped distributions are typical of parasite populations among their hosts (Crofton , 1971) and have been recorded for oocyst populations of many plasmodial species within a variety of mosquitoes (Eyles , 1951 ; Beasley , 1972 ; Medley et al . , 1993 and Vaughan et al . 1994 a & b) .

Considering *C . pipiens* a natural vector of the *Hepatozoon sp* . infecting *Uromastyx microlepis* (Rashdan et al . , 2006 a) was confirmed in this research . While further investigation will be undertaken to detect if *C. quinquefasciatus* has a role in the transmission of the same parasite .

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مقارنة قابلية بعوضتى " الكيولكس ببينز " والكيولكس كونكيفشياتس للإصابة بطفيل " الهيباتوزون " الذى يصيب السحلية السعودية " يوروميستبكس ميكروليبس "

نجوى رشدان

قسم الحشرات - كلية العلوم - جامعة القاهرة

جرى فحص أنثى البعوضتين " الكيولكس ببينز " والكيولكس كونكيفشياتس" المصابتين بطفيل الهيباتوزون المتواجد فى السحلية السعودية "يوروميستكس ميكروليبس ".

وقد بل الفحص على تكوين الزيجوت على توالى بعد ثمانية ساعات فى بعوضه الكيولكس ببتيز فى حين يتأخر تكوينه فى بعوضه الكيولكس كونكيفشياتس ويبلغ ذروته بعد أثنى عشر ساعه. أوضحت النتائج أن خلال التحور من مرحله الزيجوت إلى مرحله الاواوسيست أن الفقد في انتشار أو كثره الطفيل تعد غير معنوى فى بعوضه الكيولكس ببينز عنها فى بعوضه الكيولكس كونكيفشياتس ($P>0.05$).

كذلك لم يلاحظ تحوصل الاواوسيست فى كلا البعوضتين. وقد لوحظ وجود الإسبوروزيت الحر (الطور المعدى للطفيل) فى الغدد اللعابية لبعوضه الكيولكس ببينز وعدم وجوده فى حالة الكيولكس كونكيفشياتس. كما لوحظ أن حوالى نصف عدد اللبعوض الذى يحتوى على الاواوسيست الناضج ينتج عنه الطور المعدى للأسبوروزيت. الاهلات الطفيلية لاتعد نورعه بطريقة طبيعية فى أى من هاتين البعوضتين ولكنها توصف بالمعادلة السلبية ذات الحدين.