

**Effect of the intensity of *Hepatozoon gracilis* parasitaemia on some biological aspects of *Culex (Culex) pipiens* (Diptera: Culicidae)**

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**ABSTRACT**

The bean skinks were collected manually from Abu.Rawash, Giza, Egypt, separated according to size and investigated for *H. gracilis* infection. Intensity of parasitaemia was determined as slight ( $\leq 2\%$  per 1000 counted erythrocytes), moderate (3-10% per 1000 counted erythrocytes) and high (11-20% per 1000 counted erythrocytes). *Cx. (Cx.) pipiens* L were reared under controlled laboratory conditions ( $20 \pm 1$  °C, 60–70% RH and 10L: 14D photoperiod). Four groups of 3-4-day-old females, 50 individuals each, were starved 12 h prior to feeding. The first group was used to feed on parasite-free bean skink while the other groups were offered blood meals from slightly, moderately and highly infected lizards. The fully engorged females from each group were transferred separately to oviposition tubes and observed daily for longevity and reproductive capacity. Data were statistically analyzed by ANOVA using SPSS software, Ver., 15.0.

The present study declared that the majority of naturally infected bean skinks were slightly infected. Generally the parasitic infections were costive to their host vector and this cost was manifested as a reduction in reproductive and transmission capacities. Preoviposition period was reduced by 15% and increased by 55% in case of mosquitoes fed on highly and moderately infected skinks, respectively, as compared to control. Fecundity was reduced by 48, 37 and 18% and fertility was reduced by 8, 3 and 6% in case of mosquitoes fed on highly, moderately, slightly infected skinks, respectively, as compared to control. No significant difference in the egg incubation period was observed between control and infected groups. Adult longevity was reduced by 40% in case of mosquitoes fed on highly infected skinks and increased approximately 2 folds in case of mosquitoes fed moderately and highly, infected skinks as compared to control.

The present study suggested a decreased competence of *Cx. (Cx.) pipiens* in the hepatozoonosis disease circulation in nature when the infection intensity was high or moderate. On the other hand, the vector is a very strong candidate in the disease circulation in nature in case of slight intensity of infection.

**Keywords:** *Hepatozoon gracilis*, *Culex pipiens*, mosquito, biology, parasitaemia, reproductive capacity.

**INTRODUCTION**

Apicomplexan parasites of the genus *Hepatozoon* have been described in many groups of terrestrial vertebrate and are especially prevalent in rodents and many families of carnivores (Smith, 1996). Numerous species of *Hepatozoon* have been reported to encompass a broad range of clinical disease in both wild and domestic animals (Rubini *et al.*, 2006, Johnson *et al.*, 2007, Breshears *et al.*, 2009). The clinical syndrome resulted from *Hepatozoon* infection is known as hepatozoonosis. Hepatozoonosis is an arthropod borne infection that affects numerous species

including amphibians, reptiles, birds, and mammals (Smith, 1996). Hepatozoonosis syndrome varies clinically in different species; however, all share a common life cycle. This includes sexual development and sporogony within a hematophagous arthropod vector, ended by transmission to a vertebrate host and asexual development with merogony and gamontogony (Smith, 1996). Hepatozoonosis was diagnosed in many wild and domestic animals (Baneth *et al.*, 2003, Potter *et al.*, 2010).

Transmission of *Hepatozoon* spp. to vertebrate intermediate hosts usually occurs by ingestion of sporulated oocysts, which develop in the invertebrate definitive host. After confirmation of *Ambylomma maculatum* as the definitive host for *Hepatozoon americanum* (Mathew *et al.*, 1998; Ewing *et al.*, 2002) and determination of host preferences for immature *A. maculatum* (Barker *et al.*, 2004), Ewing and Panciera (2003) suggested predation as a means of transmission of American canine hepatozoonosis. Forlano *et al.* (2005) demonstrated that *A. ovale* can be a vector of *Hepatozoon* spp. in Brazil. In addition, it was suggested that *H. americanum*, *H. canis*, as well as *H. canis-like* organisms were present and might cause disease in dogs in the southern U.S. (Johnson *et al.*, 2008). However, infection via ingestion of cystozoites for *H. americanum* has only recently been documented supporting the concept of predation as a means of natural transmission (Johnson *et al.*, 2008).

Because mosquitoes were considered as the most important medical insects, their biological aspects were investigated long time ago by several authors (Howlett, 1910, Galal, 1998, Dixit, 2001, Vinogradova, 2003). *Culex pipiens* was considered as cosmopolitan species, distributed all over the world all over the year. The efficiency of *Cx. pipiens*, as a transmitter of different human and animal pathogens, greatly depends upon their adaptability to harbor these pathogens without gaining detrimental effects.

The main objective of the present work was to evaluate the impact of intensity of *Hepatozoon* parasitemia on some biological aspects of the adult *Cx. (Cx.) pipiens*.

## MATERIALS AND METHODS

### 1- Field collection

The bean skink, *Mabuya quinquetaeniata quinquetaeniata* (Family: Scincidae), was collected manually from Abu.Rawash, Giza, Egypt. Collected lizards were separated according to size and then transferred as soon as possible to the laboratory and maintained at room temperature in small glass cages (35 x 35 x 35 cm) with sand beds and wire gauze tops. Water and small insects were supplied as food source for the lizards. The lizards were identified according to Mostafa (1997).

### 2- Parasite detection

To detect the presence of blood parasites, thin blood film from the peripheral blood of each lizard was taken by clipping off tail tips or leg according to the method described by Bashtar *et al.* (1984). The blood films were air dried, fixed in absolute methanol for 5 min and stained with 3% Giemsa's solution in phosphate buffer saline pH 7.3. The slides were washed in phosphate buffer saline pH 7.3 and examined under research light microscope (Olympus Optical Corp., Tokyo, Japan). Intensity of the parasitaemia was defined according to the actual number of erythrocytes examined (actually counted, not estimated from extrapolated partial counts or from the number of fields of view examined). Slight parasitaemia was defined as:  $\geq 2\%$  infected erythrocytes per 1000 counted erythrocytes, moderate parasitaemia was defined as: 3-10% infected erythrocytes per 1000 counted erythrocytes and high parasitaemia was defined as: 11-20% infected erythrocytes per 1000 counted erythrocytes.

### 3- Rearing technique of *Cx. (Cx.) pipiens* L

*Cx. (Cx.) pipiens* L was supplied from the Medical Entomology Institute, Dokki, Giza, Egypt and colonized in the insectary of the Department of Entomology, Faculty of Science, Cairo University since July 2007 till now. Stock colony of the adult mosquitoes was maintained under laboratory conditions ( $20 \pm 1$  °C, 60–70% RH and 10L: 14D photoperiod) for supplying adults of known ages. According to the method described by Adham *et al.* (2003), adult mosquitoes were reared in 35 x 35 x 35 cm cages. Each cage was provided with 10% sugar solution in a small Petri dish containing a piece of cotton soaked for feeding both newly emerged males and females. Sucrose solution was replaced daily to avoid fungal contamination. Blood meals were ensured by feeding upon domestic pigeon (*Columba livia domestica*).

Mosquitoes (3-4-days-old) were starved 12 h prior to the 1<sup>st</sup> blood meal after adult female emergence. The gravid females were provided with plastic cups (7 cm diameter and 6 cm high) half filled with tap water for oviposition. Egg rafts were collected daily and transferred carefully to enamel pans (25 cm diameter and 9 cm depth) half filled with tap water and kept until the egg hatching. On hatching, 300 newly hatched larvae were transferred to a larger white enamel pan (40 cm diameter and 9 cm depth) containing about 500 ml tap water to avoid overcrowding. Larvae were fed daily on dry active yeast. Dead larvae, scum and exuviae were removed daily. Pupae were collected from breeding pans and transferred to the adult breeding cages. No attempts were made to control the light in the insectary room. However, during the working hours overhead fluorescent lamps were lighted.

### 4- The effect of the level of *H. gracilis* parasitaemia on some biological aspects of females *Cx. (Cx.) pipiens* L

#### a- Reproductive capacity

Four groups of 3-4-day-old females, 50 individuals each, were starved 12 h prior to feeding. The first group was used to feed on parasite-free bean skink while the other groups were offered blood meals from slight, moderate and high infected lizards. The fully engorged females from each group were transferred separately to oviposition tubes (2 cm diameter and 5 cm depth) containing 1.5 ml of water and examined daily for oviposition. The preoviposition period, the number of eggs per female (fecundity), the incubation period of eggs and fertility (number of larvae hatched from each raft) were calculated. Each experiment was repeated four times under laboratory conditions during winter ( $20 \pm 1$  °C, 60–70% RH and 10L: 14D photoperiod).

#### b- Adult longevity

Four groups of 3-4-day-old females, 50 individuals each, were used. Females were starved 12 h prior to feeding. The first group was allowed to feed on parasite-free bean skink while the other groups were offered blood meals from slightly, moderately and highly infected lizards. The fully engorged females from each group were transferred separately to vials and examined daily for survival. Dead females were removed and the number of survived females was recorded daily. The experiment was repeated four times under laboratory conditions ( $20 \pm 1$  °C, 60–70% RH and 10L: 14D photoperiod).

### 5- Statistical analyses

Statistical analyses for biological data were carried out using SPSS computer software for Windows, Version 15.00 (Install Shield Corporation, Inc.). One way analysis of variance (ANOVA) and subsequent multiple comparison tests (LSD) were done to calculate the significance level between means.

## RESULTS

### Field studies

Out of 557 collected bean skink during 2009 from Abu.Rawash, Giza through 21 field trips, only 63 bean skinks (11%) were found infected with the blood parasite, *H. gracilis*. Out of 63 infected skinks, 18 were highly infected, 13 were moderately infected and 32 were tested as slightly infected skinks.

### The effect of the intensity of *H. gracilis* parasitaemia on some biological aspects of *Cx. (Cx.) pipiens* L

#### Reproductive capacity

##### Preoviposition period

Table (1) shows that the preoviposition period was reduced by 15% and increased by 55% for mosquitoes fed on highly and moderately infected skinks, respectively, as compared to control. One way analysis of variance indicated over all significant difference between groups ( $df = 3$ ,  $F = 45.03$ ,  $P = 0.00$ ). Multiple comparison tests revealed significant differences in the preoviposition period in case of mosquitoes fed on highly and moderately infected skinks as compared to control ( $P < 0.01$ ). Meanwhile, no significant difference was observed in case of mosquitoes fed on slightly infected skinks as compared to control ( $P > 0.05$ ). In addition, the difference in preoviposition period was significant between all infected groups as compared to each others ( $P < 0.01$ ).

##### Fecundity (no of eggs/ female)

Table (1) showed that the number of eggs per female was reduced by 48, 37 and 18% in case of mosquitoes fed on highly, moderately and slightly infected skinks, respectively, as compared to control. One way ANOVA indicated over all significant difference between groups ( $df = 3$ ,  $F = 5.39$ ,  $P = 0.009$ ). Multiple comparison tests revealed a significant difference in the fecundity of mosquitoes fed on highly, and moderately infected skinks as compared to control ( $P < 0.01$ ). Meanwhile, no significant difference was observed in the fecundity of mosquitoes fed on slightly infected skinks as compared to control ( $P > 0.05$ ). In addition, the fecundity of mosquitoes fed on highly and slightly infected skinks was significant ( $P < 0.05$ ).

##### Incubation period

The data in Table (1) shows that the incubation period of eggs was not changed in case of mosquitoes fed on highly, moderately and slightly infected skinks, respectively, when compared to control. One way ANOVA indicated no significant difference between the three groups ( $df = 3$ ,  $F = 0.00$ ,  $P = 1$ ). Multiple comparison tests revealed no significant differences in the incubation periods of eggs deposited by mosquitoes fed on highly, moderately, slightly, infected and control skinks ( $P < 0.05$ ).

##### Fertility

The data in Table (1) shows that the fertility of mosquitoes fed on highly, moderately and slightly infected skinks was reduced by 8, 3 and 6%, respectively, as compared to control. One way ANOVA indicated an overall significant difference between all groups of mosquitoes ( $df = 3$ ,  $F = 4.004$ ,  $P = 0.015$ ). Multiple comparison tests revealed significant reductions in the percentage of egg hatchability in case of mosquitoes fed on highly and slightly infected skinks as compared to control ( $P < 0.05$ ). Meanwhile, no significant difference was observed in case of mosquitoes fed on moderately infected skinks as compared to control ( $P > 0.05$ ). In addition, the difference in percentage of hatching of mosquito eggs fed on highly and moderately infected sinks groups was significant ( $P < 0.05$ ).

Table 1: Effect of the intensity of *H. gracilis* parasitaemia on some biological aspects of *Cx. (Cx.) pipiens*.

Level of parasitimea	Longevity	Preoviposition period	Fecundity	Incubation period	Fertility
Control	10.71 ± 1.73 <sup>a*</sup> (1.47)	7.0 ± 0.28 <sup>a</sup> (5.8)	114.33 ± 14.52 <sup>a</sup> (67.155)	4.60 ± 0.18 <sup>a</sup> (4.5)	99.6 ± 0.64 <sup>a</sup> (94.100)
High	6.62 ± 0.49 <sup>b</sup> (1.22)	5.91 ± 0.19 <sup>b</sup> (4.11)	59.6 ± 4.19 <sup>b</sup> (47.73)	4.62 ± 0.18 <sup>a</sup> (4.5)	91.47 ± 2.45 <sup>b</sup> (80.8.96.8)
Moderate	20.14 ± 1.5 <sup>bc</sup> (1.40)	10.83 ± 0.44 <sup>bc</sup> (5.15)	72.4 ± 8.03 <sup>b</sup> (51.93)	4.61 ± 0.18 <sup>a</sup> (4.5)	97.37 ± 0.86 <sup>a</sup> (94.1.100)
Slight	23.17 ± 2.06 <sup>bc</sup> (3.42)	6.96 ± 0.29 <sup>a</sup> (5.13)	93.4 ± 10.0 <sup>a</sup> (60.115)	4.63 ± 0.18 <sup>a</sup> (4.5)	93.7 ± 2.4 <sup>bc</sup> (80.100)

\* Different letters indicate significance between groups (P < 0.05).

### Adult longevity

The data in Table (1) shows that the adult longevity was reduced by 40% in case of mosquitoes fed on highly infected skinks as compared to control. Longevity was increased approximately 2 folds in case of mosquitoes fed on moderately and slightly infected skinks as compared to control. One way ANOVA indicated an overall significant difference between all groups of mosquitoes studied (df = 3, F = 34.79, P = 0.00). Multiple comparison tests revealed that longevity of adults fed on highly infected skinks was significantly reduced as compared to control mosquitoes (P < 0.05). Meanwhile, longevity was significantly increased in case of mosquitoes fed on lightly infected skinks as compared to control and mosquitoes fed on highly infected skinks (P > 0.05). No significant difference was observed between mosquitoes fed on slightly and moderately infected skinks (P < 0.05).

### Percentage mortality

Fig. (1) shows the daily mortality rate of female mosquitoes fed on slightly, moderately and highly infected skinks and control females.

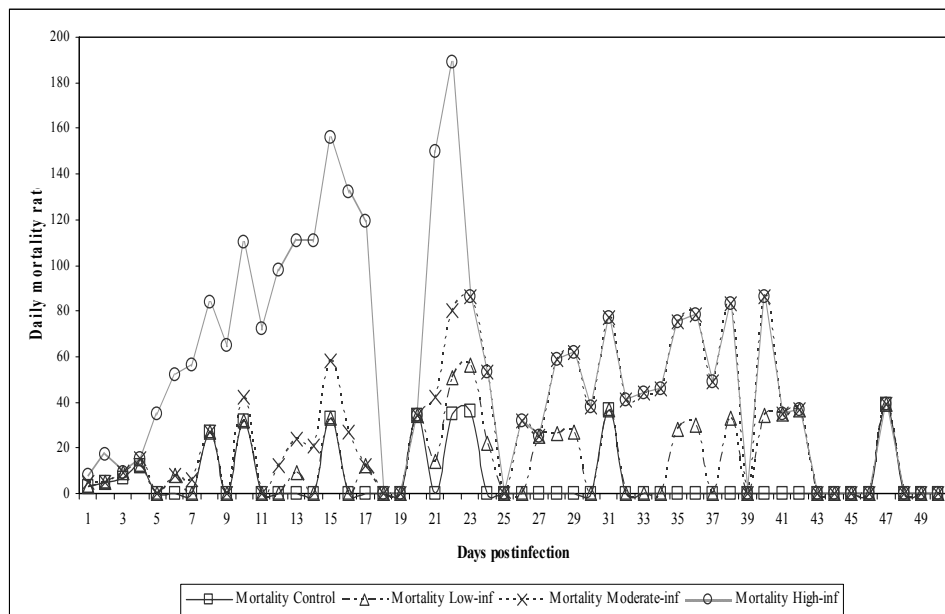


Fig. 1. Effect of the intensity of *H. gracilis* parasitaemia on cumulative daily mortality rate of *Cx. (Cx.) pipiens*.

It was observed that the daily mortality rate increases gradually with the increase in the level of parasitaemia. The highest peak of mortality was observed by the 5<sup>th</sup> (32%), 14<sup>th</sup> (36.5%), 6<sup>th</sup> (21.6%) and 8<sup>th</sup> (69%) days post infection in mosquitoes fed on highly, moderately, slightly infected and control mosquitoes. Daily mortality curves of mosquitoes fed on highly and moderately infected skinks were reserved in higher rate than in mosquitoes fed on slightly and control skinks till the mortality of all females by the day 47 post feeding (Fig. 1). Mortality of mosquitoes fed on highly infected skinks started early by the 1<sup>st</sup> day post feeding, reaching 40% by the 6<sup>th</sup> day post feeding. The death rate fluctuates smoothly to 79% by the day 12 post feeding until all females diminished by the day 22 post feeding. Although the daily mortality of control females started by the 1<sup>st</sup> day post feeding, a smooth curve was observed until all control females were diminished by the 47<sup>th</sup> day post feeding. It was obvious that the death rate of mosquitoes fed on slightly infected skinks was more or less similar to that of the control ones (Fig. 1).

## DISCUSSION

The present study suggested that the majority of naturally infected bean skinks were slightly infected. Little has been published on the infection rate of the lizard, *Mabuya quinquetaeniata quinquetaeniata* with the blood parasite *H. gracilis*. 40 and 32% infection rates of the bean skinks with *H. gracilis* were previously recorded in Egypt (Bashtar *et al.*, 1987 and Issa *et al.*, 2000). This difference in the rate of infection may be attributed to the environmental conditions in the other localities (Issa *et al.*, 2000, Nadler and Miller, 1984) or to the dissemination of the vector (Ball, 1967). The most probably controlling factor during this study was the abundance of the vector in the same vicinity of the host.

Generally parasitic infections are costive to their host vector and this cost is often manifested as a reduction in reproductive and transmission capacities. No significant differences were found in most parameters tested in case of mosquitoes fed on slightly infected skinks and control. Reproductive capacities of mosquitoes fed on highly and moderately infected skinks were significantly affected when compared to control. Significant reductions in fecundity, fertility and longevity were observed as compared to control. Combining these results with the results of Galal (1998), Adham *et al.* (2003) and Ebraheem *et al.* (2006 and 2006), revealed that the decrease in fecundity was not caused by a reduction in blood meal size but mostly was a result of protozoon/ insect association. These results run parallel to the results presented by Hacker (1971), Hurd (1990) and Ferdig *et al.*, (1993) who examined protozoon/ insect association in relation to fecundity for six strain of *Aedes aegypti* infected with *Plasmodium gallinaceum*. The decrease in egg production might be attributed to the mean speed of oogenesis that was significantly retarded in infected mosquitoes (Carwardine and Hurd 1997). Also follicle resorption is one of the main factors contributing to malaria induced fecundity reduction in mosquitoes (Hopwood *et al.*, 2001, Ferguson and Read 2002). However this relationship was contradicted by (Hammad, 1997 and Catarina *et al.*, 2003) for *Cx. (Cx.) pipiens* and *Cx. quinquefasciatus* infected with *Wuchereria bancrofti* microfilaria as well as Ferguson *et al.* (2003) who reported that the mosquitoes fed on infected mice were more fecund.

No significant difference was found between the incubation periods of eggs of both control and infected females. Similar results were reported by Galal (1998) for *Cx. (Cx.) pipiens* infected with *H. matorhensis* and Adham *et al.* (2003) for *Cx. (Cx.)*

*pipiens* infected with *H. gracilis*.

Fertility was significantly reduced in case of mosquitoes fed on highly and slightly infected skins as compared to control. Similar results were presented by Andreadis and Hall (1979) for *Cx. salinarius* infected with *Amblyospora* sp. Contradictory results were presented by Sweeney *et al.* (1989) who reported that there were no differences in fertility and fecundity of infected and control females. In another study, the authors didn't observe any effect of *H. maturhensis* on *Cx. (Cx.) pipiens* (Galal, 1998).

On comparing the longevity of control and infected groups we can conclude that the high infection greatly reduced longevity of mosquitoes (40%). However, moderate and slight infection resulted in 2 folds increase in adult longevity as compared to control. Inconsistent results were previously reported for *An. albimanus* infected with *P. vivax* (Chan *et al.*, 1994) and for *An. Gambiae* infected with *P. falciparum* (Hogg and Hurd, 1997).

Consequently, mortality rates of mosquitoes fed on highly and moderately infected skins were different as compared to slightly and control ones. These results are in agreement with those obtained by Klein *et al.* (1982) who stated that early increased mortality rate of the infected mosquitoes; *An. dirus* might be due to the rupture of the oocysts of *P. gynomolyi*. Findings confirming our results were reported for *Glossina morsitans* harboring *Trypanosoma vivax*, *T. congolense* and *T. brucei* by Moloo and Kutuza (1985), for *Pseudolychia canariensis* infected with *Haemoproteus columbae* by Rashdan (1991). The recorded increase in mortality is probably due to decreased efficiency in obtaining blood and increased feeding (Anderson *et al.*, 2000). This relationship was disrupted by reports stating that mortality rate of infected mosquitoes are not differed from that of control ones (MacDonald, 1957). Furthermore, no significant difference in parasite burdens of dead mosquitoes was found. This finding could not detect a significant effect of infection on survivorship (Catarina *et al.*, 2003).

## CONCLUSION

Conclusively, this study described natural infection rate of the bean skink, the level of parasitaemia and its effect on some biological aspects of *Cx. (Cx.) pipiens*. The protozoan infection was, in general, costive to its mosquito vector. The reduced reproductive capacity, in addition to decreased adult longevity and increased mortality (in high and moderate infection) suggested decreased competence of the mosquito vector, *Cx. (Cx.) pipiens*, to share in the disease circulation in nature when the infection intensity was high or moderate. On the other hand, the vector is a very strong candidate to share in the disease circulation in nature in case of slight intensity of infection. This hypothesis could be strengthened by the previously obtained prepatent period ( $32 \pm 3$  days) after which the vector bite is infective as related to the vector longevity.

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## ARABIC SUMMARY

تأثير شدة الإصابة بطفيل الدم هيباتوزون جراسيليس على بعض الجوانب البيولوجية لبعوضة كيولكس (كيولكس) ببينز (ثنائية الأجنحة: كيوليسيدى)

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تم جمع سحالي الفول يدويا من منطقة أبى رواش، الجيزة ، مصر ، وتم فصلها وفحصها على أساس شدة الإصابة وكثافة الطفيل فى الدم . و اعتبرت الإصابة طفيفة إذا كانت الإصابة (أقل من أو تساوى ٢ ٪ فى ١٠٠٠ من كريات الدم) ، معتدلة إذا كانت الإصابة (٣-١٠ ٪ لكل ١٠٠٠ من كريات الدم) ، وشديدة إذا كانت الإصابة (١١-٢٠ ٪ لكل ١٠٠٠ من كريات الدم). كما تمت تربية بعوضة الكيولكس (كيولكس) ببينز تحت ظروف المختبر (٢٠ ± ١ م° ، ٦٠-٧٠ ٪ رطوبة نسبية و ١٤:١٠ فترة ضوئية). تم تقسيم أربع مجموعات من الإناث (عمرها ٣-٤) ، كل مجموعة تتكون من ٥٠ فرداً ، وتم تجويعها قبل ١٢ ساعة من التغذية. واستخدمت المجموعة الأولى لتغذى على سحالي خالية من الطفيل ، بينما تم تغذية المجموعات الأخرى على وجبات دم من سحالي طفيفة ، و معتدلة ، شديدة الإصابة بالطفيل . وبعد تمام عملية التغذية تم نقل كل أنثى على حدة لأنابيب وضع البيض ، من كل المجموعات ، ولوحظت يومياً لتحديد طول العمر والقدرة الإنجابية . وقد تم تحليل البيانات إحصائياً باستخدام برمجيات SPSS ، وأسفرت الدراسة عن أن غالبية السحالي المصابة كانت إصاباتها طفيفة. وعموما فقد كانت العدوى الطفيلية مكلفة للعائل المضيف (البعوضة) ، وتجلت هذا في تخفيض القدرة الإنجابية والقدرة على نقل العدوى. وكان هذا ممتثلاً فى تخفيض فترة ما قبل وضع البيض بنسبة ١٥ ٪ وزيادتها بنسبة ٥٥ ٪ فى حالة كل من البعوض الذى تغذى على سحالي شديدة و معتدلة الإصابة ، على التوالي ، بالمقارنة مع التجربة الضابطة. هذا وقد انخفضت الخصوبة بنسبة ٤٨ ، ٣٧ و ١٨ ٪ ، كما انخفض معدل الخصوبة بنسبة ٨ ، ٣ و ٦ ٪ فى حالة البعوض المتغذى على سحالي شديدة ، و معتدلة ، و طفيفة الإصابة بالطفيل ، على التوالي ، بالمقارنة مع التجربة الضابطة. ولم يلاحظ أي اختلاف معنوي في فترة حضانه البيض بين التجربة الضابطة والمجموعات المصابة. و قد لوحظ انخفاض فترة طول العمر بنسبة ٤٠ ٪ فى حالة البعوض المتغذى على سحالي شديدة الإصابة ، وزيادتها لما يقرب من الضعف فى حلة البعوض المتغذى على سحالي معتدلة ، و طفيفة الإصابة ، بالمقارنة مع التجربة الضابطة. وقد أوضحت النتائج انخفاض قدرة بعوضة الكيولكس على نقل الطفيل بين عوائله المختلفة فى الطبيعة فى حالة الإصابة الشديدة و المعتدلة. و من ناحية أخرى ، فقد اعتبرت بعوضة الكيولكس من أقوى الناقلات المرشحة فى تداول الطفيل فى الطبيعة فى حالة الإصابة الطفيفة.