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Life table characteristics of the female sandfly, *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) under three food regimes

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ABSTRACT

Background & objectives: In Egypt, *Phlebotomus papatasi* is the main vector of cutaneous leishmaniasis. In nature, *P. papatasi* feeds on blood from different hosts and sucrose (other sugars) mainly from fig fruits. The aim of this study was to examine the effects of three food regimes on the life table parameters of females mainly the life expectancy as a factor determining the fly's capability for *Leishmania* transmission.

Methods: Females maintained on different diets (30% sucrose solution, Guinea pig blood and sucrose followed by blood) under laboratory conditions were observed for offspring emergence to examine the survival period expressed as the median emergence time (E_{50}) and female fecundity (females/female). Life table was constructed including the mean life expectancy at emergence (e_0) as a measure of longevity and the mortality rate per day (qx).

Results: Females fed on sucrose-blood has the highest fecundity and the shortest E_{50} compared to those fed on other diets. The mean life expectancy at emergence (e_0) differed significantly with the highest value being for females fed on sucrose.

Interpretation & conclusion: The calculated expectancies for female life beyond the infective age (8 days) indicated that more flies would survive to become infective when fed on sucrose-blood meals than those offered blood alone which increases its capability for *Leishmania* transmission.

Key words Daily mortality rate; life table characteristics; *Phlebotomus papatasi;* sandfly; survival curves

INTRODUCTION

Cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is an important health problem in many parts of the world, especially the Mediterranean and Middle East countries[1, 2]. In Egypt, it is a well-documented disease[3]. As in several parts of the world, *Phlebotomus papatasi* in Egypt is the main and proven vector based on finding naturally infected flies in north Sinai[4]. It is reported that laboratory breeding of sandflies is essential for the study of its different biological phenomena including the transmission dynamics of *Leishmania*. In spite of several biological studies concerning that important sandfly vector[5, 8], few studies were carried out to examine the life table characteristics specially those of Turkish populations[9, 10].

The reproductive and survival parameters included in the life table are important factors determining the vectorial capacity of such important disease vector. Therefore, this study was planned to examine the effects of three food regimes on the life table parameters of *P. papatasi* most importantly the life expectancy (survival) of female flies as a factor determining the fly capability for *Leishmania* transmission.

MATERIAL & METHODS

Sandflies

Sandflies *(P. papatasi)* used in this study were obtained from a laboratory colony maintained at 27 ± 2°C, 75 ± 5% RH in the Research Institute of Medical Entomology, Dokki, Giza, Egypt, following the techniques of Modi and Tesh[11]. Three groups of 2-day old adults (males and females) each were kept in a separate wooden cage (20 × 20 × 20 cm) and offered different diets as follows: Males of the three groups were fed on sucrose solution 30% w/v, females of I group were fed on sucrose solution, females of the II group were fed on blood of Guinea pig which was anesthetized with ketamine hydrochloride[7] and females of the III group were first fed on sucrose solution then 24 h later they were fed on blood (sucrose-blood fed females). The maintenance and care of the experimental animals is as per "the guidelines for use of laboratory animals in research" specified by the Ethics

Committee of the General Organization for Teaching Hospitals and Institutes, Ministry of Health, Egypt.

Methods

From each group four replicate cohorts of 25 females and 25 males were aspirated and placed as pairs (to ensure mating) in a coded polystyrene vials (6 cm height × 3 cm diameter) half filled with moistened plaster of Paris[12], provided with a small quantity of larval food prepared by mixing and grinding rabbit faeces and cow blood (from a slaughter house)[13] and covered with muslin netting.

Females were observed for offspring emergence to examine: (i) the survival period or the median emergence time (E_{50})[14]; and (ii) the total number of living females produced per single female per generation (fecundity or female reproduction potential). The daily mortality was recorded till all females died and life table was constructed[15] including the mean life expectancy at the female zero age day (i.e. at emergence or e_0) as a measure of mean life time or longevity and the mortality rate per day (qx).

Statistical analysis

Means and standard deviation(s) were calculated and compared by the one-way ANOVA. If ANOVA showed significant inequality of the means, they were exposed to pair-wise comparisons based on Tukey's HSD test. The relation of (qx) to the female age (x) was examined by simple regression of the form $qx = a + bx$, where, a = constant, and b = the regression coefficient (slope). The SPSS software (Version 11 for windows, SPSS Inc., Chicago, IL) was used for statistical analysis. Whatever the probability level, it was restricted to a maximum of 1%.

RESULTS

Fecundity

Females fed on different diets had significantly different productivity (p <0.05, ANOVA; Table 1). Females fed on sucrose-blood have the highest yield (mean = 30.50 females/female) than those fed on blood (p <0.05, Tukey's test) or those fed on sucrose (p <0.01, Tukey's test).

Survival period of female off springs (E_{50})

Insignificantly different E_{50} times (p >0.05, ANOVA; Table 1) were observed for offsprings produced by females offered three nutrients (mean = 26.24 – 29.15 day).

Life expectancy

The mean life expectancy at emergence (e_0) as an expression for adult survival was calculated for females fed on three diets (Table 1). The obtained means differed significantly (p <0.01, ANOVA) with the highest value (Tukey's test) for females fed on sucrose (mean = 17.05 days) followed by that of females fed on sucrose-blood (p <0.05) and that of females fed on blood (p <0.01). The survivorship curves for females fed on different nutrients are presented in Fig. 1.

Table 1. Biological attributes of *Phlebotomus papatasi* females-fed on sucrose (S), blood (B) and sucrose followed by blood (S-B)

Attribute	Mean(s)[1]/Diet			$F^2_{(d.f.=2, 9)}$
	S	B	S-B	
Fecundity (Females/female)	8.70[a] (7.38)	15.60[b] (12.24)	30.50[c] (6.35)	6.13*
Offspring survival period (E_{50}) day	29.15 (5.13)	27.81 (6.16)	26.24 (4.62)	0.30
Life expectancy at emergence (e_0) day	17.05[a] (3.80)	10.07[b] (0.32)	12.54[b] (2.03)	9.94+

[1]Mean of 4 replicates each of 25 females, Horizontally, means with similar superscript letters are not significantly different (Pair-wise comparison by Tukey's HSD test, p >0.05); [2]*Significant at 5% level; +Significant at 1% level.

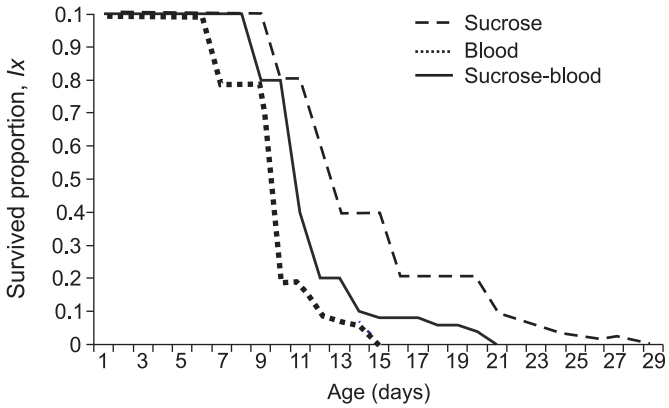

Fig. 1: Age-specific survivorship curves for *P. papatasi* females-fed on sucrose, blood and sucrose followed by blood.

Mortality-age relationship

Regression analysis revealed that mortality (qx) is positively correlated with the female age (x) for sugar fed (b = 0.02, r = 0.59, p <0.01), for blood fed (b = 0.02, r = 0.52, p <0.01) and for sugar-blood fed females (b = 0.36, r = 0. 44, p <0.05).

DISCUSSION

It was observed that females fed only on sugar also produced offsprings indicating autogeny in this Egyptian

species as previously reported[16, 17] depending on the availability of a suitable blood meal similar to the Tunisian and other strains[18, 19], however, oocyte maturation is usually concordant with the digestion of a blood meal. Moreover, it was demonstrated that the autogenous females produced fewer progeny than the blood fed flies. In the present study, nutrients affected the female fecundity (p <0.05) in terms of female offspring produced from a single female. Females fed on only sucrose produced fewer number of offsprings (mean = 8.70 females/female) in comparison to females fed on blood (p <0.05) or sugar-blood (p <0.01). Similarly, in an another study in Egypt[5], *P. papatasi* fed on Guinea pig blood gave a higher productivity (30.37 adults/female) in comparison to sucrose. The results presented here indicated that the estimated fecundity for females fed on blood (15.6 females/female) is comparable to 18.1 and 12.3 females/female estimated for Egyptian Sinai and Aswan strains, respectively[20]. If the number of produced females is taken as a rough estimate for the net reproductive rate then the population would increase by *ca* 31, 16 and 9 folds if the parent females were offered sucrose-blood, blood and sucrose, respectively. El-Kordy *et al*[5] estimated that *P. papatasi* population increased by *ca* 15 and 11 folds when females were fed on blood and sucrose, respectively. The results indicated that the three diets had no effect on the time required for female emergence (p >0.05). It was observed[5] that within the generation time**,** nutrients affect the female pre-oviposition and egg hatching periods.

Females tend to be with higher survival when fed on sucrose solution (mean e_0 = 17.05 days), while those given blood either alone (p <0.01) or alternated with sugar (p <0.05) seemed to have shorter longevity, This agrees with other report[5] where the highest e_0 value (14.98 days) was obtained for sucrose fed females and the shortest e_0 (p <0.05) for sucrose-blood fed females (9.17 days). It was suggested that blood serves principally for ovarian development while sugar serves as energy source for the insect normal activities[21]. Plotting the proportion of survived females (I_x) against age (x) for females fed on the three nutrients resulted in curves that resemble the type II of Slobodkin[22] which indicates that mortality is more in the old individuals similar to the other insects, e.g. mosquitoes[23, 24]. This was supported by regression analysis which revealed a positive correlation of female mortality and its age for the three nutrients.

As a vector transmitting *L. major*, *P. papatasi* females must survive for *ca* 6 days after imbibing an infected blood meal[25] under temperature and humidity similar to those used in the present study. Assuming that the blood meal is usually taken 2–3 days after female emergence[26], then the potentially dangerous females will not be <8–9 days of age (in case that its first blood meal is infected). The estimated life expectancy at 8 days was *ca* 4 (3–6) and 2 (1–3) days for females offered sucrose-blood and blood, respectively. This indicates that more flies would survive to become infective when fed on sucrose-blood meals which increase its capability for transmission. It was previously observed that the calculated expectancies for female life beyond the infective age indicated that sucrose-blood fed females have higher capability of transmission than those fed on blood-sucrose or blood alone[5]. It has been found that flies, under laboratory conditions, seldom take the second blood meal[5, 8]. The fact that the female sandflies die while oviposition or shortly after a common obstacle in the laboratory maintenance of this species[27], and interfere in the successful experimental infection and transmission of diseases. However, in Iran[6], it was successful for the first time to colonize and maintain *P. papatasi* colony for seven generations using larval diet without liver powder. In nature, the probable capability of *P. papatasi* to have multiple blood feedings within a single gonotrophic cycle may enhance its vectorial capacity in disease transmission.

CONCLUSION

The present study demonstrates that nutrients affect female fecundity (female offspring produced per single female) and longevity. Females fed on sucrose followed by Guinea pig blood produced higher number of females and consequently increased the population by *ca* 31 fold while sucrose tends to be essential for longer survival period. Based on the calculated expectancy for female life, more flies would survive to become infective when fed on sucrose-blood meals which increase its capability for *L. major* transmission.

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