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Original Article

Colonization and Biology of *Phlebotomus papatasi*, the Main Vector of Cutaneous Leishmaniasis due to *Leshmania major*

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Abstract

Background: Laboratory bred sand flies are essential for the study of different biological phenomena including the transmission dynamics of *Leishmania*. The aim of the study was to determine the suitable situation for colonization and maintenance of Iranian strain of *Phlebotomus papatasi* at laboratory conditions from an endemic focus of cutaneous leishmaniasis due to *Leishmania major*.

Methods: One hundred and thirty *P. papatasi* were collected by CDC miniature light traps and aspirator from indoors in Badrood, central Iran. The fly was maintained by the procedures of Modi & Tesh (1983) with minor modifications for 7 generations.

Results: Minimum and maximum rate of productivity was calculated to be 8.5 and 56.1 in F7 and F3, respectively and significant difference was observed among productivity of some generations ($P< 0.001$). The sex ratio ranged between 70(F1) and 101.8(F6). The mean duration of egg to adult emergence varied between 47.21±4.46 and 52.6±7.85 days. The life cycle was completed in 34.4 to 60 days at 26±1 ºC.

Conclusion: *P. papatasi* was colonized and maintained successfully for the first time as a laboratory strain. Using larval diet without liver powder is recommended. The blood of white hamster was preferred to golden hamster and guinea pig for the sand fly vector blood feeding at the insectary.

Keywords: Phlebotomus, *Sand fly, Leishmaniasis, Arthropod vectors, Iran*

Introduction

The establishment of laboratory colonies of blood-sucking insect vectors is essential in elucidating many aspects of their life cycle and in understanding the dynamics of disease transmission under controlled conditions. Although the presence of sand flies in Iran was first recorded by Adler, Theodor and Lourie in 1930 but our knowledge on their biology is scant (1). This situation is partly due to difficulties, which have been encountered in rearing large numbers of flies for experimental purposes. Rearing and colonization of sand flies are highly specialized undertaking. The delicate nature of them, their narrow limits of tolerance to environmental hosts and difficulties of copulation are some of the problems faced with colonization of sand flies.

Phlebotomus papatasi has a wide distribution in the Old World, it occurs through most of the Mediterranean basin, the Middle East, and the Indian subcontinent (2). A high natural *Leishmania major* infection rate of this species (15.6%) has reported from the rodent burrows of Badrood, central Iran (3). This species is the main and proven vector of *L. major* transmission to man in Turkmenistan, Uzbekistan, Saudi Arabia, Iran, southern Morocco and central Tunisia (4, 5). After the first attempts for rearing of this species (6), it has been colonized in different countries. Iranian researchers gave a try for rearing of *P. papatasi* during 1963-1964 but it was failed after three generations (7), and to our knowledge there are no reports on long-term rearing of any sand fly species in the country.

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The present work deals with the successful laboratory colonization and some life-table data of Iranian strain of *P. papatasi* from Badrood, central Iran.

Materials and Methods

Adults of *P. papatasi* were collected by CDC miniature light traps and aspirator from indoors (bedrooms, stables, storerooms, toilets) in the village of Abbasabad, rural district of Badrood (33° 44′ N, 52° 2́ E) 4 km from the city of Badrood, Natanz county, central Iran in September 2002. The collected flies were transferred to wooden cages (20×20×20cm), covered with a moist towel and then transferred to the Sand fly Insectary of School of Public Health, Medical Sciences/University of Tehran. The gravid and engorged female sand flies were tubed individually in small glass vials (42.39 cm^3 capacity, 6 cm height, 3 cm diameter) which were plastered inside with a layer of Paris poured (8). After oviposition, each female was dissected, unlaid eggs were counted, then the head and terminal of abdominal segments were mounted in Puri's medium (9), which was manufactured in leishmaniasis laboratory and identified after 24 h using the related key (10).

Sand flies were reared under laboratory condition at 24-27 ˚C, 80% RH and 14:10(L: D), following the method of Modi & Tesh for mass rearing of *P. papatasi* by some modifications (11). Emerged adults were released in 30 cm cubic cages which had constant access to a diet of fresh 30% sucrose solution. For rountine blood feeding, females aged 4-5 d were starved for 24 h prior to being permitted to feed directly upon a white hamster, golden hamster or a guinea pig which were anesthetized with ketamine hydrochloride (1 ml/kg) for 1 h. Adult longevity was determined by transferring males, unfed and blood fed females into 42.39 ml individual tubes, under the insectary conditions. Larval diet was prepared according to the method of Young et al. (12) and also, Modi & Tesh by mixing and grinding rabbit pellets, rabbit faeces and liver powder (11).

Statistical analysis was conducted by SPSS11.1, One-Way ANOVA, X^2 and Dunnett's test for comparing the different factors.

Results

A total of 130 *P. papatasi* were caught from indoors in two times during September 2002. Females were transferred to the oviposition pots 24-48 h after blood feeding and laid their eggs during 7-20 d. A total of 9278 sand flies were produced during 7 generations. Minimum and maximum rate of productivity were calculated to be 8.5 and 56.1 in F7 and F3, respectively. Significant difference was observed among productivity of some generations ($P< 0.001$). The sex ratio, i.e. number of males per 100 females of *P. papatasi* ranged between 70 and 101.8 in F1 and F6, respectively (Table 1).

Table 2 indicates the ranges of minimum development times (in days) of *P. papatasi* over 7 generations. The mean duration of egg to adult emergence ranged from 47.21±4.46 to 52.6±7.85 d in F4 and F3, in that order. The One-Way ANOVA tests showed significant differences among pre-oviposition, eggs to male and female adult periods in seven generations ($P< 0.0001$). Non-significant difference was observed among the first stage larval development, 2-4 larval duration and duration of pupation in 7 generations. This colony produced 3.5-4 generations per year and the period from emergence of parent to emergence of offspring adult differed from 63-71 d over 7 generations. The life cycle (from egg deposition to 1st adults) at 26±1˚C was completed in 34.4 to 60 d, depending on the sex and type of larval nutrition (Table 3). In F2, the mean duration of 2-4 larvae in diet without liver powder (19.5±2.1) was higher than with liver powder (16.6±1.1) and a significant difference was observed ($P< 0.05$). In F6, the mean duration of first instar larvae in diet without liver powder (8.04±1.53) was significantly ($P< 0.05$) more than with liver powder diet (6.66±2.03). It should be mentioned that non significant difference observed among other generations.

Assessment of blood meals on the biological cycle of *P. papatasi* showed significant difference from egg to male and female duration only in F6 generation. Based on our results, the duration from egg to male adult was significantly higher in golden hamster (54.4±12.7 d) than guinea pig (53.2±9.4 d) and white hamster

(42.09±6.1), $P< 0.05$. The duration from egg to female adult was also significantly higher in golden hamster (55.2±12.7 d) than guinea pig (46±17.07) and white hamster (42±5.53), $P< 0.05$. The longevity of adults in F6 was calculated to be 16.5, 11.1 and 26.5 d in males, unfed females and blood-fed females, respectively.

Table 1: Some biological aspects of laboratory reared *P.papatasi*, Badrood strain, Iran

Generation / Factor	F1	F2	F3	F4	F5	F6	F7
No. of blood-fed females	60	68	155	385	694	870	516
No. of eggs/generation	660	1480	1570	5017	9873	10977	4693
No. of 1st instar larvae	232	649	1015	2173	3761	4182	1439
No. of pupae	126	636	991	1870	3410	3016	711
No. of adults	119	604	880	1519	3005	2751	400
No. of males	49	281	416	765	1382	1388	196
No. of females	70	323	464	754	1623	1363	204
Productivity	18	40.8	56.1	30.3	30.4	25.1	8.5
Sex Ratio	70	87	89.7	101.5	85.2	101.8	96.1

F1-F7: First to 7th generations;
Sex ratio = Males/100 females;
Productivity = No. 100 Adults/eggs

Table 2: Mean development times (in days) of laboratory-reared *P.papatasi* over seven generations, Badrood strain, Iran

Generation	Pre-oviposition	Egg duration	1st star of Larval duration	2-4 larval duration	Pupation	Adults* emergence
F1	13±6.12	5.33±2.33	10.5±2.42	16.5±7.34	16±4.14	48.3±13.54
F2	12.94±2.52	6.65±3.1	8.18±2.59	18.28±2.77	14.76±2.97	47.89±6.66
F3	9.6±1.79	5.75±2.67	7.6±2.54	16.95±6.01	22.3±3.89	52.6±7.85
F4	10.85±2.83	5.14±2.54	6.78±3.05	17.85±2.13	17.42±3.67	47.21±4.46
F5	13.37±1.95	5.77±1.77	7.98±1.9	18.04±3.55	16.67±2.7	48.47±5.39
F6	15.97±3.43	7.81±2.47	7.71±1.71	17.21±3.3	17.47±4.14	49.81±6.02
F7	13.5±2.05	6.2±1.93	8±1.81	16.43±2.7	16.86±3.14	47.5±3.98

F1-F7: First to 7th generations; Measures given as mean± standard error
* Duration of eggs to adult emergence

Table 3; Effects of larval diet on biological cycle of laboratory reared *P.papatasi*, Badrood strain, Iran

Generation	Larval diet	Larvae 1 duration		Larvae 2-4 duration		Pupae duration		Egg to male adult		Egg to female adult	
		Mean±SD	***P***	Mean±SD	***P***	Mean±SD	***P***	Mean±SD	***P***	Mean±SD	***P***
F1	A	10.5±2.4	-	16.5±7.3	-	16±4.1	-	34.8±17.2	-	41.3±4.1	-
	B	-		-		-		-		-	
F2	A	8.5±2.88	0.077	16.6±1.1	0.05 *	16.2±4.03	0.480	52.3±7.6	0.289	50.4±4.3	0.289
	B	4.5±1.32		19.5±2.1		19±3.1		45.5±8.2		45.9±7.2	
F3	A	8.18±2.5	-	18.2±2.7	-	14.7±2.9	-	49.6±9.5	-	50.8±9.5	-
	B	-		-		-		-		-	
F4	A	7.6±2.54	-	16.95±6.01	-	22.3±3.8	-	49.7±12.3	-	52.1±11.2	-
	B	-		-		-		-		-	
F5	A	7.93±1.8	0.760	17.5±3.03	0.493	16.5±3.02	0.632	55.3±16.5	0.774	58.4±11.3	0.679
	B	8.07±2.1		19±4.4		16.8±2.2		58.1±10.47		60±10.1	
F6	A	6.66±2.03	0.043	17.5±3.21	0.712	15.8±2.5	0.162	47.8±11.03	0.408	40.55±16.5	0.07
	B	8.04±1.53	*	17.2±3.4		17.8±4.4		51.7±11.8		52.03±11.8	
F7	A	9±2.2	0.135	17.2±3.3	0.636	17.7±4.3	0.462	51.4±9.07	0.053	34.4±32.3	0.739
	B	7.3±1.4		15.6±2.1		16.6±2.6		40.3±9.7		41.05±9.6	

F1-F7: First to 7th generations;
A: With liver powder;
B: Without liver powder;
*****: Significant difference

Discussion

Pervious attempts to colonize *P.papatasi* were failed in Iran (7). This is the first successful colonization of this species for consecutive generations in the country. The life cycle of *P. papatasi* during 7 generations was affected by pre-oviposition period. Difference in pre-oviposition period (blood feeding to oviposition) is not related to temperature, because all females were reared in the same conditions. Wild parents of the first generation had a longer pre-oviposition period than F2-4 generations due to their environmental changes in comparison with the field situation. Maroli et al. obtained the same results in rearing *P. perniciosus* under laboratory conditions (13). This period was increased during F4-6 generations and then dropped in F7. Reduction in pre-oviposition and first larval periods in 2-4 generations and therefore increasing in the population of 4th generation probably is due to the number of females released in mass rearing tubes, more production of oviposition pheromone and kind of plaster used in tubes. In F1 generation we had a problem with oviposition of gravid sand flies. After changing the plaster and transferring them into the large 500 ml tubes, they laid their eggs in few days. The same results were obtained by *Lutzomyia migonei* indicated preference of this species to oviposit on irregular horizontal surfaces in response to thigmotropic behavior (14).

The productivity was increased from F1 to F3 generations but decreased from the generation of F4 to F7. It seems to be due to the bottleneck phenomenon and gene-pool reduction. The same results were obtained by other researchers on the rearing of *Lu. longipalpis* (15,16). Comparison the methods of individual and by mass rearing of *P. papatasi* showed that mean of eggs/females was higher by mass rearing. Due to the low cost of mass rearing and providing more eggs in short time, this method was used in the present study. The results obtained with *Lu. migonei* using groups of females or individually isolated females in oviposition pots of different sizes, indicated that physical space is not an important factor con-

trolling oviposition behavior in laboratory conditions for this species (14). In rearing of *Lu. evansi*, mean number of eggs laid/female in mass rearing method was lower than individual tubes (17), but in our colony the result was in contrast. It could be in consequence of different laboratory conditions, species and oviposition pheromone of *P. papatasi*. Although there were differences in the duration of 2-4 and first instar larvae in F2 and F6 generations but using larval diet without liver powder is recommended.

The study demonstrates that white hamster is preferred for vector blood feeding at the insectary. Our serious problems have been fungi such as *Mucor* sp., *Cladosporium* sp. and *Aspergillus* sp., bacteria such as *Pseudomonas* sp., *Salmonella* sp., *Diphteroide* sp. and mites (Order Mesostigmata). In the case of fungi and bacteria prevention, routine autoclaving of containers and food and the use of sterile instruments held these agents to manageable level. Regarding mites, which were attacking to the eggs and 1st instar larvae, they were killed mechanically by sterile needles and greasing the around of rearing containers, and rearing pots and the legs of racks, which was effective in preventing and their entrance or spread.

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