

Laboratory colonization of *Lucilia sericata* Meigen (Diptera: Caliphoridae) strain from Hashtgerd, Iran

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ABSTRACT

Background & objectives: The treatment of wounds with live green bottle fly larvae is receiving considerable attention in many countries. Laboratory rearing of *Lucilia sericata* is crucially important for the treatment of wounds.

Study design: The study was carried out for mass rearing of green bottle flies from April to November 2010. Hand catch and net trap baited with beef and cattle liver were used to collect adult flies from the field. The collected samples were placed in appropriately labeled tubes and sent to the laboratory. Adult stage flies reared in the insectary were used for species identification using specific keys.

Results: A total of 89 flies (55 females and 34 males) were collected from Hashtgerd area. In the first generation, 299 flies were produced in the laboratory including 105 (35.12%) males, and 194 (64.88%) females. The female/male sex ratio was 1.61 for parents, whereas it was 1.84, 1.30 for F1 and F2 generations respectively. In total, 432 flies were reared in F3 generation including 173 (40.04%) males, and 259 (59.96%) females, and the sex ratio was 1.49.

Conclusion: Setting up the mass rearing of sheep blowfly at the School of Public Health, Tehran University of Medical Sciences is an important step in producing candidate flies for the treatment of myiasis by maggot therapy in future.

Key words Insectary; Iran; *Lucilia sericata*; maggot therapy

INTRODUCTION

The sheep blowfly *Lucilia sericata* has a long history in medicine and its maggots have been used in wound healing. Some unsuccessful surgical and antibiotic treatments of infections such as temporal mastoiditis and perineal gangrene were treated using maggot therapy. Wounds resulting from cuts, wounds or even trauma resulting from diseases like diabetes, bed sores, gangrene, and burns are the problems of human societies¹. In many of these cases, surgery is the only treatment, sometimes leading to amputation. Leg amputation due to diabetes is frequently reported from around the world. According to World Health Organization, this happens every 30 seconds. Diabetic foot ulcer is responsible in half of diabetic patients' visits to the hospitals. In many of these patients, it may lead to amputation of one or both feet². Treatment by flies' larvae as a suitable alternative method in the treatment of the wounds has introduced³. Up to now, maggot therapy was performed for treating many different kinds of wounds.

Centrally important to maggot therapy is that the larvae used in the treatment of wounds should reduce the infections and improve the nutrition of tissues for rapid improvement of wounds. There are 1100 known species of sheep flies, with 228 species in the Neotropics, Africa and southern Europe. *Lucilia* species has been reported in the countries, namely India, Japan, central America, and southern United States of America⁴.

Considering the significance of maggot therapy, establishing an insectary for mass rearing of larvae to provide hospitals and medical centers with *Lucilia* larvae is important. The aim of this study was mass rearing and maintenance of *L. sericata* (*Phaenicia sericata*) maggot under laboratory conditions at the School of Public Health, Tehran University of Medical Sciences.

MATERIAL & METHODS

Study area

The experimental study was performed from April to

November 2010. Sampling methods used were hand catch by net and bait traps. Sampling was carried out in places including gardens, around livestock, slaughterhouse in Hashtgerd county, Alborz province, northern Iran.

Adult fly collection

The samples were caught from different places of Alborz province and were sent to the insectary of Medical Entomology at School of Public Health, Tehran University of Medical Sciences in appropriately labeled tubes⁵. Beef and Hamburger were used as bait for collecting adult stage of the fly in open area. Information such as place and date of collection, sampling method and weather condition was recorded⁵.

Species identification

Adult flies were identified using standard keys⁶⁻⁸. The diagnosis of larvae is performed based on the respiratory pores and posterior of cephalo-pharyngeal skeleton of larvae, therefore, definitive diagnosis was carried out on dead larvae. In order to identify the live specimens, adults were anesthetized by CO₂, ether or cold shock for a short time. After identification, the adults were transferred to new cages for oviposition.

Maintenance

The flies were maintained in the insectary under controlled conditions of mean temperature of 27±1°C, relative humidity of 80±5%, and daily light /dark period of 16:8 h. The average temperature for larval development was also 27±1°C. The electrical fly killer was hung in the insectary area to kill the free flying flies in the insectary^{9,10}. Layers of red meat with tissue papers in between were placed in the petri dish and, then a cup container with cut edge was placed on the meat¹¹. In order to rear the larvae, sheep blood agar was used because of its simplicity food source^{12,13}. Supervision of rearing cages was essential and larvae were isolated from rearing cages. Upon emerging, the adults were placed in new cages and provided with essential food.

Statistical analysis

The hatching dishes were filled with larval medium and were visited daily according to method of Fleischmann¹³. All values are expressed as the mean ± standard error of the means (SEM).

RESULTS

In all, 89 adult flies were collected from slaughterhouses of Hashtgerd, Alborz province and were transferred

to the Tehran School of Public Health. The life cycle of green flies is shown (Figs. 1–4) and the duration of life cycle of male and female *L. sericata* is given (Table 1). The average number of female parents emerged per eggs batch was 6.1. The time required for the eggs to hatch was 52±2 h. The mean time for larval and pupal development in F1, F2, and F3 generations was 96±2, and 176±2.33 h respectively. The time for emerging from pupae to laying eggs was 200±2.66 h. In F1 generation, 507 maggots age I were reared to 299 adults, hence, a mortality rate during the F1 generation of 41.03% was reported. Mortality rate of *Lucilia sericata* was 53.15% for F2 generation, whereas in F3 generation, it was 27.28%. Number of eggs, larvae and pupae stages I, II, III and adults yielded in F1, F2 and F3 generations are shown (Table 2). Totally, 299 flies were reared including 105 (35.12%) males, and 194 (64.88%) females in the first-generation. They were transferred to spawn cages and nine eggs batches were obtained. In F1 generation, the average number of female flies emerged per eggs batch was 21.55%.



Fig. 1: The batch of eggs of *Lucilia sericata* at the insectary, School of Public Health, Tehran University of Medical Sciences.



Fig. 2: The *Lucilia sericata* maggots at the insectary, School of Public Health.



Fig. 3: The pupa of *Lucilia sericata* at the insectary, School of Public Health.



Fig. 4: The adult of *Lucilia sericata* at the insectary, School of Public Health.

DISCUSSION

The different time periods required for the completion of three generations of *Lucilia sericata* in our study showed that the life stages are affected by temperature and humidity of the Insectary. The first generation of wild flies took longer than their parents to lay eggs. This delay may be justified by the time required for the adaptation to the captivity conditions. Also the first generation took longer to mate. Lysek in 1991 reported the optimal temperature of $37 \pm 3^\circ\text{C}$ for rearing this fly. The duration of larval stage was reported to be 4 to 13 days¹⁴. In our study, the duration of larval stage was 96 ± 2 h with an average temperature of $27 \pm 1^\circ\text{C}$. Sherman and Wyle¹¹; and Wolff and Hansson¹ colonized this species in insectary and reported a cycle from egg to adult of about 1–2 weeks a period which was calculated 8.33 days in our study. In parallel, the egg to egg period was reported as 2–3 weeks¹⁵. In our study, the period from egg to adult was noticeably longer in F2 generation, which might be justified by different diet, nutrition and new environment. Comparison of the results of studies by Sherman and Wyle¹¹; and Wolff and Hansson¹ indicated that in the second generation in our study less eggs were developed to larvae and adults successfully (46.85%). This decrease in the second generation was more than that in the first generation, this fact might be explained by the specific conditions including light, temperature, humidity, food and larvae nutrition. The effect of different kinds of food on larval development of

Table 1. Duration of life stage of *Lucilia sericata* in the insectary 2010

Generation	Adult		Egg period		Larval stage		Pupa stage		Emergence to egg laying (h)	
	Male	Female	Time (h)	Mean \pm SEM	Time (h)	Mean \pm SEM	Time (h)	Mean \pm SEM	Time(h)	Mean \pm SEM
Parents	34	55	–	–	–	–	–	–	–	–
F1	105	194	60 ± 3	52 ± 2	72 ± 1	96 ± 2	192 ± 3	176 ± 2.33	312 ± 3	200 ± 2.66
F2	84	110	48 ± 1		96 ± 3		168 ± 2		168 ± 3	
F3	173	259	48 ± 2		120 ± 2		168 ± 2		120 ± 2	

Table 2. Number of eggs, batch, and larvae stages I, II, III, pupae and adults of *Lucilia sericata* during F1, F2 and, F3 generations in insectary 2010

Generation ration	Egg batch batch (No.)	Larval stage			Pupae	Adults		Total
		I	II	III		Male	Female	
F1	9	507 (100)	431 (85)	375 (87)	330 (87)	105 (35.1)	194 (64.9)	299
F2	11	414 (100)	348 (84.05)	293 (84.19)	214 (73.03)	84 (43.3)	110 (56.7)	194
F3	4	594 (100)	553 (93.09)	509 (92.04)	464 (91.15)	173 (40.1)	259 (59.9)	432

Figures in parentheses indicate percentages.

Lucilia sericata is considerable. The size of larvae fed on hamburger was smaller than those fed on beef and liver. Similar to our results, the growth rate of *Lucilia* larvae fed on beef liver¹⁶ was significantly lower than those grown on the chicken flesh and beef. Protein intake is necessary in the first generation of *in vitro* rearing. The average period between emerging of adults, mating, spawning, and laying eggs in our study was 8.33 days, whereas Sherman and Wyle¹¹; Wallman and Day¹⁷ stated two weeks from emergence to laying of eggs. Mortality rate during the life cycle of *Lucilia sericata* was 41.03, 53.15 and 27.28% in F1, F2, and F3 generations respectively. The mortality rate of gravid female was more than non-gravid and male in F2 generation. The susceptibility of the flies to temperature and humidity was noted in our study. The stages I and II larvae are quite sensitive to dryness and food shortage, whereas the III instar larvae are sensitive to humidity. Establishing a colony of this fly in insectary in Iran is of high importance for undertaking maggot therapy to treat the disease.

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