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Experimental colonization and Life Table of the *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae)

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Abstract
The urban bluebottle blow fly, *Calliphora vicina* (Robineau-Desvoidy), is considered a valuable tool in forensic entomology. The adult flies collected from Tehran, Iran. The colonies were established. The rearing process was continued until fifth generation. The life table of *C. vicina* was evaluated with two diets in laboratory. For the life table study from fifth generation, the eggs were collected from cage and put on cow meat and artificial food as two food regimens. The duration of life cycle of *C. vicina* was determined based only on cow meat in five consecutive generations (F2 to F6). Results of this research showed significant differences between two diets in mortality rates. It is cleared that cow meat is more efficient in the developmental process of all the life cycle stages of *C. vicina.* Also the overall development time from egg to adult stage of *C. vicina* was 384±20 hours.

Keywords: *Calliphora vicina*, colonization, life table, survival rate, Iran.

1. Introduction
Calliphoridae (blowflies) which is a family of Diptera order with 1000 known species is found throughout the world [1]. Calliphoridae is known as blowflies or blue, brown or green bottle flies. Some members of the family show medical, veterinary and forensic importance [2].
Blowflies can cause infestation of a live mammal typically from an infected sore called myiasis [3]. Either adults or larvae of blowflies may transmit pathogenic microorganisms and act as vector [4, 6]. Many species of blowflies are attracted and feed on dead tissues and could be used for maggot therapy [7]. Blow fly larvae fulfill an important ecological function in the decomposition of animal remains, therefore, considered as a valuable tool in forensic entomology to determine post mortem interval (PMI) [8].
One of the major purposes for laboratory rearing is to more clearly define the PMI, since the rearing of subsequent life stages provides a more precise estimation of the amount of development the insects had undergone at the time of collection [9].
Blow fly are generally autogenouse, means that adult females require protein meals for ovarian development [10]. The female blow fly typically lays 150-200 eggs per batch, and usually is iteroparous, laying about 2000 eggs during the life [1]. Larvae of the blowflies are necrophagous or polyphagous and the adult feed on sugars (e.g. vegetal nectar, ripe fruit juices, confectionary, aphid honeydew) and proteins (e.g. carcasses of animals) [11, 12].
Calliphora vicina (Robineau-Desvoidy) is a member of Calliphoridae family, which is known as a blue bottle fly due to the metallic blue-gray coloration of the thorax and abdomen. *C. vicina* is a large fly, usually ranging from 10 to 14 mm long [13]. *C. vicina* is common primary colonizing species on human and animal dead bodies [9].
This species generally benefits from the presence of human populations and is sometimes called the urban bluebottle blow fly [14]. In nature, *C. vicina* is an important decomposition agent that facilitates rapid degradation of dead animals. It is a long- rang flyer and actively searches for carcasses to breed, because of its swift detection of dead organisms, it is commonly used in forensic investigation to estimate the time of death [15].

Life tables provide data on survivorship and fecundity of individuals within a population. A standard method is to collect data on a cohort, or group of individuals all born in the same time period.
Life tables constructed this way are called cohort life tables. They can then be used to determine age or stage-specific fecundity and mortality rates, survivorship, and basic reproductive rates. A life table developed from field data may be used to estimate fitness of a population as influenced by various biotic and abiotic factors. Unfortunately, field life tables are often difficult to construct because tracing population survival and reproduction in the open field under variable environmental conditions is exceedingly difficult. A vertical life table is used to analyze survival or mortality in different stages of life [16]. The aim of this study was to find the best diet for successful laboratory colonization and producing life-table data on Iranian strain of *C. vicina* for the first time.

2. Materials and Methods
2.1 Sample collection
The adults *C. vicina* were collected from three sites in Tehran during September 2012 including: a sheep breeding place near Kan River at 35º 45' N, 51º 17' E with 1376 m altitude, around a temporary slaughter house in Azadegan highway at 35º 39' N, 51º 14' E with 1146m altitude and Laleh forest Park at 35º 42' N, 51º 23' E with 1241m altitude. The samples were collected by entomological net and transported to the Department of Medical Entomology and Vector Control of Tehran University of Medical Sciences for exact identification using relevant keys [17, 18]. Each female was placed in 200ml Plastic cups covered with nylon netting. A piece of fresh cow meat (muscle tissue) (10 g) was placed for egg laying in each cup.
Deposited eggs were transferred to a bigger cylindrical jar containing fresh cow meat (muscle tissue). Grown 3rd instar larvae were transferred into another plastic container with dry wood chips in it for pupation. The pupae were transferred to big cages (40× 40 × 40 cm) for rearing of newly emerged adults. The adults were sexed and placed at least 20 pairs in each cage. The colonies were maintained continuously at insectary room with 24 ± 1 ˚C temperature, 16:8 light and dark period and 60 ± 5% of relative humidity. A cotton pad soaked in a sugar solution and a piece of hen liver was placed in cages for feeding of adult flies. A piece of fresh cow meat (muscle tissue) was placed in each cage as a substrate for egg laying. This rearing process was continued until the fifth generation.

2.2 Diets
Two diets were evaluated in this study. Diet 1 consisted of chicken liver and cow meat for feeding of adults and larval stages respectively. Diet 2 which has been proposed by [19], consisting of whole milk powder (50 g), dried yeast (50 g), wheat germ (50 g), agar powder (14 g) and water (1000 ml). This diet used for feeding of both adults and all larval instars.

2.3 Vertical Life table study
This research was conducted in the laboratory from September 2012 to May 2013. For the study of life table of *C. vicina*, 1000 cohort eggs were collected from cages and arranged in two sets of 5 replicates (100 eggs in each replicate). Each set was placed on each of two food regimen. Number of individuals which passed one life stage to another were counted and recorded two times in a day until the emerging of adults. Sex ratio of emerging adults was calculated. Mortality rates and survival curves (Proportion alive (lx), the proportion of survivors at the beginning of age interval (dx), mortality rate during age interval x (qx), the force of mortality (kx), were calculated and recorded based on Slud 2012 [20].

2.4 Statistical analysis
The results were developed with the 14.0 version of the SPSS program. The analysis of variance (ANOVA) was used to compare the data obtained from the two diets.

3. Results
3.1 Determination of life cycle of *C. vicina*
Life cycle duration of *C. vicina* was determined based on diet 1 in five consecutive generations (F2 to F6) (Figure 1). Life cycle from egg to adult stage of *C. vicina* were 16±.83 (384±20 hours) days. The average time for the different stages of *C. vicina* is given in (Figure 2).

Fig 1: Life cycle of *C. vicina:* 1: Egg, 2: Larvae I, 3: Larvae II, 4: Early 3th larval stage, 5: Late 3th larval stage, 6: Pupae and 7: Adult

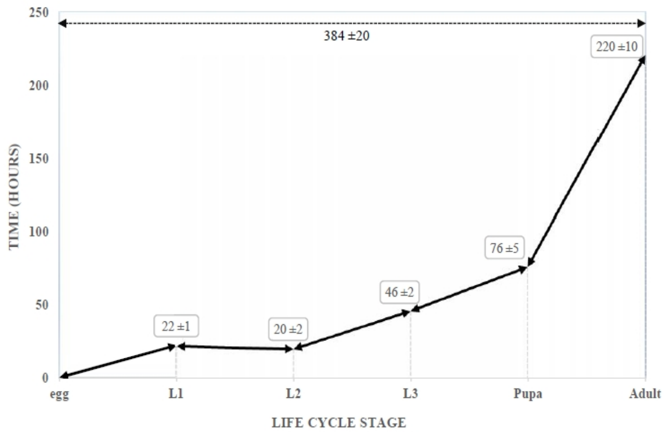

Fig 2: Life cycle of *C. vicina* under laboratory conditions (Time ± SD)

3.2 Analysis stage-specific mortality on both diets
Life table components of *C. vicina* for two food regimens have shown in table 1. The total of 185 and 114 males and 156 and 84 females were obtained from diet 1 and 2 respectively. The number of alive individuals (ax) and proportion of alive individual (lx) at the beginning of each age interval of life cycle of *C. vicina* were higher for diet 1 (P value=0.000). The proportion of survivors at the beginning of age interval x (dx) and mortality rate during age interval x (qx) of diet 1 as compared to diet 2 was very low. The force of mortality (kx) which indicates the relative strengths of the various factors on the total mortality rate within reared larvae on diet 2 was higher than diet 1(P value=0.000).

Generally, the survival of larval stages on diet 1 and diet 2 was 92.2%±3.7, 74.4%±4.22 respectively. Furthermore total survival from egg to adult stage was 68.2±6.3, 39.6%±4.6 for diet 1 and diet 2 respectively.

Table 1: Vertical life table for *C. vicina* on the two diets

x	Diet	ax	lx	dx	qx	Kx
Egg	1	500	1	0.078	0.08	0.035
	2	500	1	0.256	0.26	0.128
Larvae I*	1	461	0.92	0.068	0.07	0.033
	2	372	0.74	0.166	0.22	0.110
Larvae II*	1	427	0.854	0.058	0.07	0.031
	2	289	0.578	0.062	0.11	0.049
Larvae III*	1	398	0.796	0.038	0.05	0.021
	2	258	0.516	0.04	0.08	0.035
Pupae*	1	379	0.758	0.076	0.10	0.046
	2	238	0.476	0.08	0.17	0.080
Adult*	1	341	0.682			
	2	198	0.396			

x = Life cycle stages, ax = Number of individuals observed in each stage, lx = Proportion alive, dx =the proportion of survivors at the beginning of age interval x, qx = Mortality rate during age interval x, kx = Force of mortality.
* Significant differences (P<0.05)

3.3 Survival curves of *C. vicina*
The survival curves of *C. vicina* for two diets which have been shown in Fig 3 can be categorized as type II according to ecological classification because of relatively constant survivorship or mortality throughout life. Diet 2 showed more mortality than diet 1 (P value=0.000).

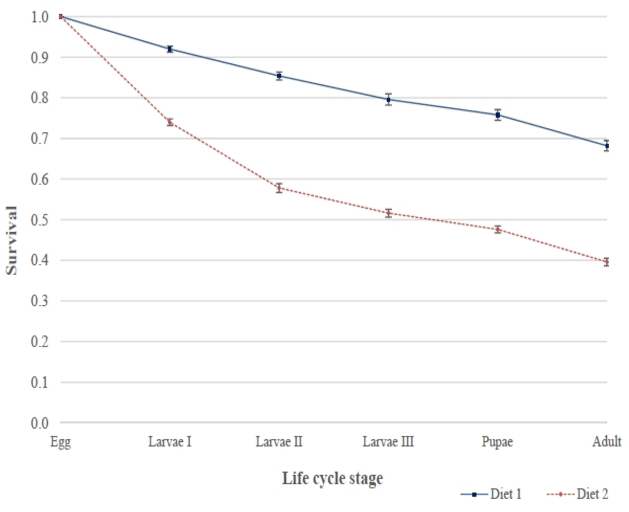

Fig 3: Survival curve of *C. vicina* obtained from the two diets (lx ± SD)

4. Discussion
C. vicina is considered to be the most widely distributed blow fly in the world with forensic and ecological importance as colonisers of carcasses, which plays a crucial role in corpse colonization during the cold seasons [21]. It can be concluded that life table data of *C. vicina* is an integral part in post mortem interval determination [22].
The food consumed by blowflies is a key factor for biological developmental and population dynamics [23]. The larvae of many fly species of forensic importance can be easily reared on several different substrates such as beef, chicken and pork [9].
In this assay we tried to compare the conventional food with the diet which has recommended by [19].The results showed that the diet 1 (conventional food regimen) was more efficient than diet 2 in mass rearing of *C. vicina*. However the main problem of the traditional food regimen is stinky odor which may cause the rejection of that efficient food.
Less reports are available on life tables of *C. vicina* under laboratory condition. In life table studies under field conditions, many factors can influence on their survival and developmental rates [24]. Therefore calculation of life table of this species seems to be necessary.
Our findings are in parallel with previous study that indicates between two diets there are significant differences in mortality and survival rate [25].
Our results are similar to results of previous research that have been conducted by Kaneshrajah and Turner (2004). They found that different diets (various body organs of pig) may have significant effect on larval growth of the blow fly, *C. vicina* [26].
According to the results of the present study, the time for pupation and emergence of the adults of *C. vicina* was 7 days and 16 days, respectively. These factors for *L. sericata* have been estimated as 6.5 and 14 days [25]. Results of two similar studies that have been performed on *Chrysomya albiceps* showed that times from oviposition to pupation and oviposition to emergence of the adults were 8 days and 14 days, respectively [27, 28]. In two similar studies the average time from egg to pupae for *L. sericata* was 12-15 days (at 22 ºC and 50% relative humidity) [29] and 14 days (at 27ºC) [30].
Results of this study showed significant differences for mortality in larval stages in comparison of the vertical life table values for two diets (P value <0.05). The survival percentage was higher in the diet 1, while mortality parameters were higher values in the diet 2. Thus it can be suggested that using diet 1 is more efficient in the developmental process of all the life cycle stages of *C. vicina*.
This information can provide a clue for rearing and studying on biological aspects of *C. vicina* which is useful for forensic entomology and so on. It is necessary to carry out further research on life table of *C. vicina* in different environmental conditions.

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