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First case report of *Calliphora vicina* (Diptera: Calliphoridae) on an outdoor human corpse with an estimation of postmortem interval from Iran

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Abstract
The study of insects on the human corpse has an important role in forensic investigations. Their utility in legal cases has been overlooked in Iran and this report is the first case report on application of forensic entomology in Iran and its potential to estimate the postmortem interval of cadaver. We collected many third-instar larvae of *Calliphora vicina* from a human corpse placed in a 23 meters deep well in Fars Province, Iran. The corpse was in an advanced stage of decay and had a fracture in the neck. The larvae to become pupae and adults were reared on chicken meat in a thermostatic room. The emerged flies were allowed to feed, mate and oviposit in a rearing chamber to monitor the life cycle of this species. Based on the age of *C. vicina* third-instar larvae, minimum postmortem interval was estimated to have been 5-6.5 days prior to the discovery of the body.

Keywords: Postmortem interval, Forensic entomology, Calliphoridae, Iran

1. Introduction
Forensic entomology refers to the use of arthropods in medicolegal investigations. The study of necrophagous arthropods on the human corpse plays an important role in forensic investigations. This can be helpful in determining the time of death or post-mortem interval (PMI) and post-mortem transfer [1]. The important reason for using arthropods in criminal investigations, a science denominated as forensic entomology, resides in the fact that insects are the first ones to detect and to find a cadaver and are present in all stages of body decomposition [2]. The application of the entomological method to determine the time of death consists of two main procedures; the estimate based on the oldest individuals that have developed on the body (minimum PMI) and estimate based on the successional patterns [3]. Depending on species and ambient air temperature this development needs up to several weeks. Insects colonize the corpse in a predictable regularity; as Calliphoridae family are found in the early stages of body decomposition [1]. Therefore, they are useful in the estimation of the minimum postmortem interval (PMI_{min}). Insects are found in various places and there are many differences between insects associated with human corpses in outdoor and indoor conditions [4], high elevation and low elevation [5], and cold and warm seasons [6]. These differences could emanate from the climatic and geographical disparities [7]. When using insects to estimate PMI, it is important to consider the factors (temperature, humidity rainfall, drugs and toxins) which may affect the rates of insect invasion on the body [8, 9, 10]. The first entomology study on the human corpse in Iran was conducted by Keshavarzi who found a beetle species from a human corpse in Shiraz in 2015 [11]. The utility of insects in forensic investigations has been overlooked in Iran and this report is the first case report of application of forensic entomology in Iran.

2. Case study
A 26 years old man was found dead in a 23 meters deep well in Fars province, Iran (29.62° N, 52.53° E) and taken to Shiraz Institute of Legal Medicine on 24th, February 2015. The corpse was in an advanced stage of decay, covered with clothing and had a fracture in the neck (Fig.1). The man in question was addicted to heroin and also had a psychiatric problem. The cause of death was unknown and he had been last seen one week before the discovery of his body. The well was located in an urban area, has been empty of water, but it was wet and

sunlight did not reach the bottom of the well. Ambient temperature on the day of sample collection was 20 ℃ but the temperature of the well was not measured. The large sized third- instar feeding larvae were collected from the corpse during autopsy procedures by the first author at 9:45 am; in addition, the face was widely colonized with maggots at different stages of development. Large sized larvae and other maggots were collected from the face and of these, some immature individuals were killed in hot water and stored in 70% alcohol, while others were transferred to the laboratory of entomology, Shiraz University of Medical Sciences, School of Health, for rearing. The live larvae to become pupae and adults were reared in petri dishes with chicken meat in a thermostatic room with 21 ± 1◦C temperature and 12:12 light and dark period (Fig.2). To provide protection and a source of humidity and water, we introduced a piece of cotton soaked with distilled water.

The emerged flies that were related to third- instar larvae were allowed to feed, mate and oviposit in rearing chambers (8x8x8 inches) at 20 ◦C (ambient temperature on the day of sample collection) to study the life cycle. The colonies were maintained for one generation in this study. After oviposit, they were killed and identified by using valid taxonomic keys [12, 13, 14]. Recording the time required for egg hatching, larval stage developments, pupation and total time for egg-eclosion was performed at every four-hour intervals and every six-seven hour intervals for larva and pupa. Definitive identification of the sample was done in the adult stage [12, 14].

Fig 1: The male human corpse discovered from the bottom of a well in Shiraz, Fars province, south of Iran.

Fig 2: Third instara larvae of *Calliphora vicina*

Fig 3: Adults of *Calliphora vicina*

3. Results and Discussion

For the third-instar larvae, emerged adult flies were identified to be *Calliphora vicina* species. Other maggots at different developmental stages were found to be *Lucilia sericata* species. Genus *Calliphora* (adults) recognized by large size, black thorax, bare stem vein and presence of presutural intra-alar bristles (Fig.3). *C. vicina* species can be distinguished from other species by a yellow basicosta and a genal dilation with reddish ground color on anterior half [12, 13]. *C. vicina* species is a synanthropic species and more frequently present in the cooler months of the year [6]. Temperatures less than 30 °C are necessary for life in this species and at 30°C larvae of this fly failed to become pupae and died [15].

The existence of this species on the human corpse in underground locations such as cisterns and wells have rarely been reported. For example, *C. vicina* eggs and first-instar larvae were collected from a corpse in an underground passage. At this location, very little sunlight reached the discovery site and the walls of the passage were very damp [16]. Puparia of *C. vicina* were collected from two human corpses in a subterranean, dry cistern next to a well over 20 m deep, the temperature was less than 10 °C at this location [17]. Therefore, some of the above-mentioned conditions are also found in our study. PMI can be estimated by analyzing the rate of development of early arrival insect species that colonize the corpse [2, 3]. By this method, *C. vicina* can be used to estimate the PMI_{min} in this case. Anderson studied development rates of *C. vicina* at 20 °C, development rates from oviposition to pupariation were 8.9–9.7 days and development period from oviposition to emergence was 21.5–23.8 days [18]. In our study, development period of *C. vicina* from oviposition to emergence was 19 days in the laboratory. Rearing of the eggs to reach 3rd instars at 20 °C took about 5.7 days (range: 5-6.5 days) and development time from 3rd instars to pupariation took 3.6 days (range: 3-4.2 days). The development rates from oviposition to pupariation (9.3 days) in our study was similar to Anderson report, but the difference in the development rates from oviposition to emergence in our study results and Anderson experience could be explained by the strain of species, different food sources or errors in the measurements of development times. In this case, based on our study, we assume that *C. vicina* reached 3rd instars in 5.7 days and the minimum postmortem interval estimated to be about 5-6.5 days.

In our study, according to the large sized larvae of *C. vicina* it seems that this species was the first fly that attracted to the corpse and similar finding were reported by Keshavarzi [19]. So this species can be helpful in the future for determine the minimum period of time since death in this area.

It should be noted that different body tissues and drugs in the body tissues may change larval development rates [20, 21]. For example, morphine hydrochloride reduced the growth rate of *Lucilia sericata* [22]. For a more accurate estimate in our case it was not possible to determine whether or not heroin had an effect on the development rates of *C. vicina.*

Forensic entomology is a good way to determine the minimum period of time since death. To achieve an accurate estimate, it is necessary to study the life cycle of insects at different temperatures. The insects can be useful tools in investigations on causes and circumstances of death. However, the usefulness of this method depends on how the corpse has been handled before the arrival of the entomologist at the death scene. Ideally, the forensic entomologist should be part of the team of professionals that are first called to the death scene.

The establishment of forensic entomology as a reliable tool in the routine of forensic scientists is neglected in Iran and such

studies can be helpful for the development of forensic entomology. This is the first case report using forensic entomology in Iran and further investigation on insects for use in legal research is needed.

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