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Analysis of the effect of methadone and temperature on the development rate of *Calliphora vicina* (Diptera: Calliphoridae): A forensically important fly

DAWOUD KESHAVARZI[1], YAVAR RASSI[1,♥], KOUROSH AZIZI[2], MOHAMMAD ALI OSHAGHI[1], SAYENA RAFIZADEH[3], ALI MOHAMMAD ALIMOHAMMADI[4], MARZIEA SHAHRIARI NAMADI[2], SEYEDEH ZAHRA PARKHIDEH[1]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences. Tehran, Iran.
♥email: rassi184@gmail.com, fereidoonim25@gmail.com
[2]Research Center for Health Sciences, Institute of Health, Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences. Shiraz, Iran
[3]Ministry of Health and Medical Education. Tehran, Iran
[4]Tehran Legal Medicine Organization. Tehran, Iran

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Abstract. *Keshavarzi D, Rassi Y, Azizi K, Oshaghi MA, Rafizadeh S, Alimohammadi AM, Namadi MS, Parkhideh SZ. 2020. Analysis of the effect of methadone and temperature on the development rate of* Calliphora vicina *(Diptera: Calliphoridae): A forensically important fly. Nusantara Bioscience 12: 87-91.* The development rate/time of flies is a scientific method to estimate the minimum time elapsed after death. Several studies have shown that opioids and temperature affect maggot growth rates. However, there are few published data that investigate the effect of ante-mortem methadone use on larval length of *Calliphora vicina* (Robineau-Desvoidy). Therefore, the purpose of this research was to investigate the effect of methadone and temperature on the development rate/time of this species. During this study, four rabbits were administered 0.10, 0.50, 1.0, and 10 mg/kg of methadone via gavage over a period of 14 days, and a five rabbit, which did not receive methadone, was used as a control. The rabbits' tissues were separated and exposed to maggots for rearing. Minimum developmental times of *C. vicina* life stages at six constant temperature regimes were provided. From hours 48 to 96, larvae feeding on tissues containing 10 mg/kg methadone developed more rapidly than those feeding on tissues containing 0.1, 0.50, and 1 mg/kg and also from the control. In the present study, development rate of *C. vicina* was linearly related to temperature (R2 = 0.96, p =0.02) between 16 and 32°C. The results revealed that the differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 24 h.

Keywords: Forensic entomology, insect development rate/time, methadone

INTRODUCTION

Synthetic opioid deaths continue to rise in different parts of the world (Akhgari et al. 2018; Concheiro-Guisan et al. 2018). There are more than 42,000 deaths due to opioid overdose in the United States (Concheiro-Guisan et al. 2018). Methadone is a synthetic opioid drug with a high attraction to μ receptors that prescribed for the treatment of opioid addiction (Hsieh et al. 2018; Wolff 2002). This is a widely used drug in Iran and 1274 related deaths were reported from Tehran during 2009-2015 (Akhgari et al. 2018).

Forensic entomotoxicology, as a new branch of forensic entomology, studies the use of insects as alternate toxicological specimens and the effects of drugs on insect physiology (Fathy et al. 2008; Amendt et al. 2007). Necrophagous species include dipteran and coleopteran species are the most important orders for PMI determination in different stages of decomposition (Watson 2004). The degree of development of insects is the main technique for estimating PMI. In this technique, the investigator calculating the maggot age by measurement of the larval length, actually the maggot age indicates the time when flies first laid their eggs on the cadaver (Amendt et al. 2007; Fathy et al. 2008). The ages of maggots found on a corpse can provide evidence for the estimation of a minimum PMI ranging from 24 hr. up to more than 5 weeks, depending on the fly species involved and the climatic conditions at the death scene (Donovan et al. 2006). Flies are ectothermic and they are thus specifically susceptible to climatic changes. Development rates are affected by weather conditions, temperature, relative humidity, food sources, body decomposition stage, and the presence of drugs in the tissues as food (Mann et al. 1990; Turchetto and Vanin 2004).

Some species of calliphorid flies are attracted to carcasses of animals (Keshavarzi et al. 2019). The imago will feed on any secretions, including blood, and gravid females will rapidly lay their eggs on the body. In the case of *Calliphora vicina* (Robineau-Desvoidy) as a cold friend species, at temperatures between 14-16ºC the eggs hatched after about 24 h, whereupon the larvae begin to feed on the body tissues. After growth is complete, the post-feeding larvae usually migrate away from the remainder to pupariate (Hans et al.2019; Ody et al. 2017). *Calliphora vicina* is widely distributed throughout Middle East as well

as in Iran (Akbarzadeh et al. 2015), so, it is important to study the life cycle of this species.

The effects of drug/toxin on body weight and body length as well as the rate of development of insects have been investigated in several, for example; heroin, codeine, and methamphetamine decrease larval development time and increase the larval length (Fathy et al. 2008; Goff et al. 1991; Goff et al. 1997). In most above-mentioned studies, the animal model was killed immediately after the substance injection. But in the present study, the animal becomes addicted to the substance and exposure to the substance for a longer period of time. Hence, the current study described here attempts to determine whether ante-mortem use of methadone impact on the development rate of *C. vicina*, and also examined the larval length of this species at temperatures of between 15°C and 32°C, under controlled laboratory conditions.

MATERIALS AND METHODS

Study sites

The study was conducted in winter 2019 in the Research Station of Kazerun (29°37′10″N 51°39′15″E), a research facility of Tehran University of Medical Sciences located in the county town of Kazerun, Fars Province, southern Iran. Kazerun is 860 m above sea level and the climate in the region is a subtropical steppe type with an average annual temperature of 21.6°C and precipitation rate of 257 mm.

Carcasses and methadone dosing

Four rabbits (≈ 1.8-2.3 kg) obtained from the Animal Lab at Shiraz University. Four animals were administered methadone before euthanasia and one was used as controls. Trial rabbits received 0.10, 0.50, and 10 mg/kg/day of methadone via gavage over a period of 14 days. At the end of the period, blood samples were taken from the ear vein of rabbits and were analyzed for methadone by one step methadone test stripe (Abon®, China) At the end of the treatment period, the animals were killed by chloroform (2 ml/kg) and their tissues were separated and exposed to *C. vicina* larvae for rearing. The Ethical Committee of the Tehran University of Medical Sciences, Iran has granted permission to use rabbits as a research animal for this study.

Source of larvae and rearing

Adults of *C. vicina* were collected from chicken liver in the county town of Kazerun. Adults were identified using morphological character described by Akbarzadeh et al. (2015). The flies were held in an insectarium at 22 ºC +/-2 with 60% relative humidity and a photoperiod of LD 16:8 h. About 85 flies were kept in a net (40 cm * 40cm * 40 cm) and fed with water, sugar, and also rabbit blood to allow egg maturation (Figure 1). After the oviposition, eggs were transferred into 3 plastic jars (12 * 15 * 22 cm) containing rabbit tissues with different methadone concentrations. This procedure was repeated 3 times for each concentration. The bottom of each jar was covered with 2cm thick sawdust, to provide a dry place for pupation. Few drops of water were sprayed daily to keep the substrates moist. Following hatching, 15 larvae at each growth stage were randomly selected from each plastic container and fixed in boiling water for 3 minutes, and then maintained in 70% alcohol and their lengths measured within 1 h using a ruler and also an eyepiece micrometer on a light microscope. The developmental time/rate was monitored every 12 h during photoperiod.

Growth under different constant temperature regimes

Fresh rabbit tissues were provided as a suitable source for oviposition at 22°C. The oviposition time was recorded, and eggs were removed and transferred into plastic jars. The jars were then placed into a precision incubator at one of six desired temperature regimes (16, 20, 24, 28, 32°C). This procedure was repeated three times for each temperature regime (n = 3), and approximately 200 eggs were used in each replicate. Larval length in each temperature regime measured as mentioned before. Lower temperature threshold for development was estimated from the linear regression of the developmental rates (y = 1/developmental time) on constant temperature (x) (Campbell et al. 1974).

Statistical analysis of the data was conducted using SPSS v12.0.1 and a p value ≤0.05 was considered significant for all of the following analyses. Data normality was inspected using Kolmogorov–Smirnov normality tests. One-way ANOVA test was used to investigate differences between treatment groups.

RESULTS AND DISCUSSION

The mean time of development from oviposition to pupariation and from oviposition to adult eclosion at each of the six studied temperature regimes is specified in Table 1. We observed that few eggs hatched at 32°C and development time was not completed at 32°C and all pupae died. The rate of maggot development (from oviposition to pupariation) increased with temperature, with development rates of 0.08, 0.12, 0.14, 0.16, and 0.20, at 16, 20, 24, 28, and 32°C, respectively. In the present study, development rate of *C. vicina* was linearly related to temperature (R2 = 0.96, p =0.02) between 16 and 32°C. The lower threshold temperature for this species was 1.8°C. The mean larval length from first instar to pupariation at each of the six studied temperature regimes is provided in Figure 2. The duration of each developmental stage under all temperature regimes provided in Figure 3.

Table 1. Average developmental times of *C. vicina* life stages at five constant temperature regimes.

Temperature (°C)	Days from oviposition to:	
	Pupariation	Emergence
16	12.5+/-0.10	29.7+/-0.36
20	8.2 +/-0.12	19.8+/-0.21
24	7.0+/-0.28	16.2+/-0.24
28	6.2 +/-0.18	15.0+/-0.40
32*	5.1+/-0.13	Not emerged

Note: *: Few number of eggs hatched. (R2 = 0.96, p= 0.02)

Figure 1. Sampling process, rearing and measuring of the larval length

In the present study, from hours 48 to 96, larvae feeding on tissues containing 10 mg/kg methadone developed more rapidly than those feeding on tissues containing 0.1, 0.50 and 1.0 mg/kg and also from the control. The mean larval length from first instar to pupariation in each of the four studied treatments is given in Figure 4. One-way ANOVAs showed no statistically significant differences between the first three concentrations (0.1, 0.50, and 1 mg/kg) in length ($p = 0.21$). While, larvae feeding on tissues containing 10 mg/kg methadone developed more rapidly than those feeding on tissues containing 0.1, 0.50, and 1 mg/kg and also from the control and this difference was statically significant ($p = 0.041$).

With respect to larval growth rates, this study determined that the development of *C. vicina* is affected by methadone only in high concentrations. This finding is similar to a previous study that used different species and feeding substrates (Gosselin et al. 2011; Strehler 2008). Gosselin et al. (2011) identified methadone and its metabolite (EDDP) in *Lucilia sericata* pupae only at high concentrations (8.4 µg/g). The development time in the control group and low concentrations was similar, it could be related to the degradation power of methadone by *C.vicina* fat bodies (Gosselin et al. 2011). In another study, methadone increased the length of the larvae and reduced the overall development time by 15 hours (Hecht et al. 2007). Contrary to our study, Strehler et al. (2008) reported that methadone at concentrations of 0.5 and 0.1 mg/kg had a significant effect on *C. vicina* larval length on days 5 and 6 and reducing larval length.

The study conducted by George et al. (2009) showed that growth rates of *Calliphora stygia* fed on morphine spiked mince did not differ significantly from those fed on control mince. Another study on the effect of morphine in rabbit tissues on the rate of development of *Lucilia sericata* showed that morphine affects the growth rate of the species and reduces the length of the larvae and increases the duration of larval development. But it does not affect the length of the pupae. At doses of 12, 25, and 50 mg, morphine could change the estimated death time from 24 to 162 hours in rabbits (Bourel,1998). According to Goff et al. study (1991), heroin shortened the duration of the larval period. But it increases the length of the pupal period. Heroin was also negative in larvae fed on morphine-containing tissue at 12 or 18 mg concentrations in the first 24 hours of heroin testing, but it was positive at 48 hours. The results of our study revealed that the differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 24 h.

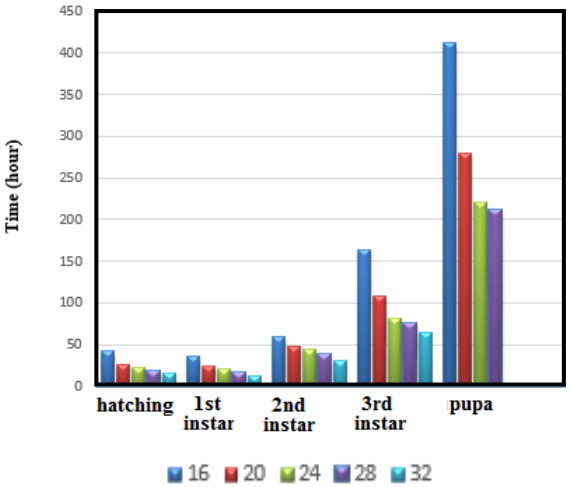

Figure 3. Development time of *Calliphora vicina* life stages from hatching to pupation at five different constant temperature regimes

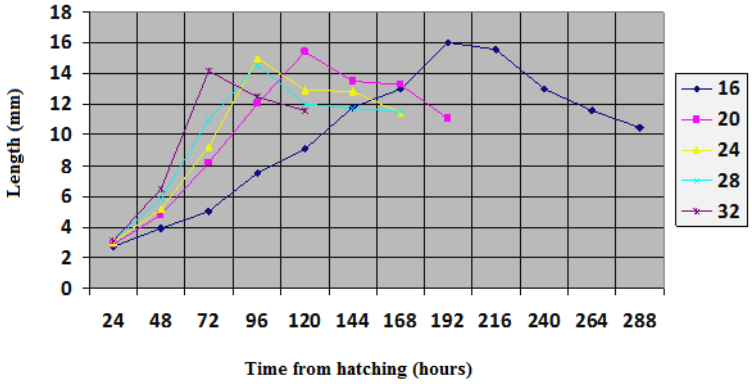

Figure 2 Average length of *Calliphora vicina* from hatching to pupation at five different constant temperature regimes.

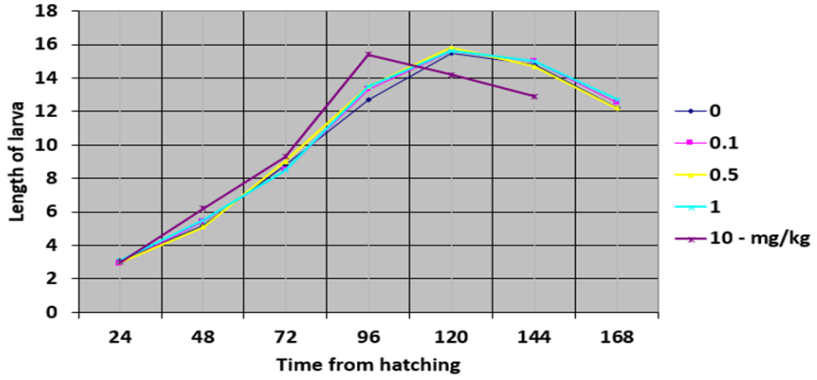

Figure 4. Average length of *Calliphora vicina* larvae at different concentration of methadone

In the present study, development of *C. vicina* was linearly related to temperature between 16 and 32°C and the lower threshold temperature was 1.8°C. Marchenko (2001) reported that threshold temperature was 2°C. In the present study, we observed that few eggs hatched at 32°C and development time was not completed at 32°C and all pupae died. It has been reported that the highest and lowest temperatures (15°C and 28°C) had a deleterious effect on *C. vicina* (Defilippo et al. 2013).

In the present study, development time from oviposition to pupariation and to adult eclosion was similar to Defilippo et al. (2013) study. But this time was different from Marchenko study (2001), where, at 20°C, development times from oviposition to pupariation were 10.5 and from oviposition to emergence was 21.6. While in this study, development times were 8.2 days for pupariation and 19 days for adult eclosion.

We conclude that the development time of *C. vicina* varied at different temperatures and high concentration of methadone could also reduce its development time. Therefore, the presence of this substance in high concentration in cadaver could have an effect on the PMI estimation based on the development time. The current study supplied a database in the field of medico-legal entomology, these data could be useful or estimating minimal postmortem intervals.

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