**Abstract**

One of the most important applications of forensic entomology is Postmortem Interval (PMI) estimation. Insects are of significant importance in case of badly decomposed and unidentified remains and with an undetermined time of death. Insects are attracted to a body immediately after death, often within minutes. In this study an effort has been made to calculate Postmortem Interval of mummified body of a female by utilizing insect evidence. The body was recovered from the rice field of village Kakrala, Police Station Ghagga, District Patiala, Punjab, India on 26 October 2014. Blow flies of family Calliphoridae i.e. *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart), their immature stages (eggs and pupae) and adult beetles of family Dermestidae i.e *Dermestes maculatus* were collected from the body. Average temperature and relative humidity was calculated from the Meteorological Department of Punjabi University, Patiala. Identification of insects was carried out in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala. Postmortem Interval is calculated by utilizing ADH method.

**Keywords:** Forensic entomology; Postmortem interval; Blowfly; Calliphoridae; Pupae; ADH

**Introduction**

Forensic entomology or medicolegal entomology can be defined as the study of insects associated with a human corpse in an effort to determine elapsed time since death [1-3]. In the medicolegal investigation of death, one of the most critical question is when did the death takes place? Accurate estimation of the Postmortem Interval (PMI), the period from death to discovery of corpse, has special relevance in a homicide case because such knowledge can narrow the field of possible suspects in the crime [4].

Insects mostly involved in the forensic investigations are true flies of order Diptera. The predominant species in this order are Calliphoridae (blow flies), Sarcophagidae (flesh flies) and Muscidae (house flies). Calliphoridae (blow flies), Sarcophagidae (flesh flies) may arrive within minutes after death. Muscidae (house flies) delay colonization until the body reaches bloat stages of decomposition. Insects present on the dead body can provide evidence for the estimation of PMI up to one month or more [5]. Correct species identification is the initial step for calculation of PMI. Different species differ in their growth rate and maturation. The rate of development of insects is temperature dependent. Each stage of development has its temperature requirement hence each species has its own defined number of accumulated degree days (ADD) or accumulated degree hours (ADH) to complete its development. Once the thermal history is obtained, it can be compared with temperatures at the death scene and PMI can be estimated.

*Chrysomya megacephala* and *Chrysomya rufifacies* are forensically important blow flies and are commonly available in northwest part of the country. *Chrysomya megacephala* (Fabricius, 1794) is commonly found in cadavers in many parts of the world [6-8], and is used in forensic investigations to determine Postmortem Intervals [9-13].

In the present study PMI is estimated by utilizing pupae of *Chrysomya megacephala* by using accumulated Degree hours (ADH) method.

**Material and methods**

On 26th October, 2014 a corpse of 23 years old female was...
recovered from the rice field of village Kakrala, District Patiala, Punjab, India. The body was in mummified stage of decomposition. Finger and thumbs of both the hands were missing. Lower part of chest cavity and whole of its abdomen along with all thoracic and abdominal organs were missing. Lower vertebral column was visible (Figure 1). Skull was devoid of soft tissues (Figure 2). Pupae of *Chrysomya megacephala* and *Chrysomya rufifacies* were collected from torn clothes (Figure 3) Adult flies of *Chrysomya megacephala* and *Chrysomya rufifacies* and Beetles of family Dermestidae i.e *Dermestes maculatus* were also collected from clothes and skull of the body.

The Pupae, adult flies and beetles were transferred into a glass vial and preserved in 80% ethanol. Identification of insects was carried out in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala, with the help of taxonomic keys. Temperature data was taken from Meteorological Department of Punjabi University, Patiala. ADH method is utilized for calculation of PML.

**Results**

The insect samples were collected from corpse and preserved in 80% ethanol. Pupae of blow flies (Diptera: Calliphoridae) were collected and identified. Temperature data from 17th October to 26th October were collected from Meteorological Department of Punjabi University, Patiala (Table 1). The development time of *Chrysomya megacephala* from egg to pupae at temperature of 25± 1°C was utilized to calculate Accumulated Degree Hours for estimation of PMI [14] (Table 2). *Chrysomya megacephala* takes 6460.8 hrs at 25± 1°C from egg stage to pupal stage. Accumulated Degree Hours for *Chrysomya megacephala* life cycle was calculated according to temperature obtained from Meteorological Department Punjabi University, Patiala from 17th October to 26th October 2014 and total ADH was 3489.6 (Table 3). ADH value represents certain number of "energy hours" that are necessary for the development of insect larvae. The formulae for calculating ADH is:

\[
ADH = \text{Time (hrs.)} \times (\text{Average temp. - Minimum development threshold temperature})
\]

Whole formulae for PMI calculation by using ADH method is depicted in Table 4. The PMI estimated was 9.6 days and her death may occur on 17th October 2014 (Table 4). The autopsy was performed 2 days after recovery of corpse. According to autopsy surgeon the PMI of corpse is 10 to 12 days without intimating any known reason of death. This case study illustrates the importance of using insect evidence to estimate minimum Postmortem Interval and to reconstruct a possible scenario of the events.
Table 1: Meteorological data from 17/10/2014 to 26/10/2014 for maximum, minimum temperature and relative humidity. (Meteorological Department Punjab University, Patiala)

| Date       | Temp °C | Temp °C | Relative Humidity % (8:30) | Relative Humidity % (17:30) |
|------------|---------|---------|---------------------------|-----------------------------|
|            | Max.    | Min.    | I                         | II                          |
| 17/10/14   | 30      | 15.6    | 80                        | 55                          |
| 18/10/14   | 30      | 16.5    | 83                        | 50                          |
| 19/10/14   | 30.7    | 16.6    | 76                        | 46                          |
| 20/10/14   | 31.5    | 18.4    | 74                        | 49                          |
| 21/10/14   | 31.6    | 18.1    | 71                        | 54                          |
| 22/10/14   | 32.8    | 19.6    | 91                        | 57                          |
| 23/10/14   | 32      | 17.2    | 87                        | 64                          |
| 24/10/14   | 31.3    | 18.4    | 89                        | 65                          |
| 25/10/14   | 30.6    | 18.6    | 91                        | 64                          |
| 26/10/14   | 30.8    | 20.7    | 75                        | 66                          |

Table 2: Development time (hrs.) of *Chrysomya megacephala* at constant temperature of 25±1 °C (Bharti et al. [14]).

| Development Stage of *Chrysomya megacephala* | Development Time (hrs.) of *Chrysomya megacephala* at 25±1 °C |
|---------------------------------------------|---------------------------------------------------------------|
| Egg Stage                                   | 16.8                                                          |
| 1<sup>st</sup> instar larvae                | 13.2                                                          |
| 2<sup>nd</sup> instar larvae                | 22.7                                                          |
| 3<sup>rd</sup> instar larvae                | 40.3                                                          |
| Prepupae period                             | 76.2                                                          |
| Pupal period                                | 100                                                           |

Table 3: Determination of Accumulated Degree Hours (ADH) for *Chrysomya megacephala* life cycle from 17/10/14 to 26/10/14.

| Date       | Temperature | Threshold Temp °C | Growing Degree Day Value (DD) (Avg. Temp- Threshold Temp.) | Accumulated Degree Hours DD x 24 hours |
|------------|-------------|-------------------|------------------------------------------------------------|--------------------------------------|
|            | Max °C      | Min °C            | Avg. °C                                                    |                                      |
| 17/10/14   | 30          | 15.6              | 22.8                                                      | 10                                   | 12.8                                  | 307.2                                   |
| 18/10/14   | 30          | 16.5              | 23.25                                                     | 10                                   | 13.25                                 | 318                                     |
| 19/10/14   | 30.7        | 16.6              | 23.65                                                     | 10                                   | 13.65                                 | 327.6                                   |
| 20/10/14   | 31.5        | 18.4              | 24.95                                                     | 10                                   | 14.95                                 | 358.8                                   |
| 21/10/14   | 31.6        | 18.1              | 24.85                                                     | 10                                   | 14.85                                 | 356.4                                   |
| 22/10/14   | 32.8        | 19.6              | 26.2                                                      | 10                                   | 16.2                                  | 388.8                                   |
| 23/10/14   | 32          | 17.2              | 24.6                                                      | 10                                   | 14.6                                  | 350.4                                   |
| 24/10/14   | 31.3        | 18.4              | 24.8                                                      | 10                                   | 14.8                                  | 355.2                                   |
| 25/10/14   | 30.6        | 18.6              | 24.6                                                      | 10                                   | 14.6                                  | 350.4                                   |
| 26/10/14   | 30.8        | 20.7              | 25.7                                                      | 10                                   | 15.7                                  | 376.8                                   |
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Accumulated Degree Hours (ADH) taken by Chrysomya megacephala to reach the pupal stage at 25± 1°C

Y = 29712 / 307.2 = 9.6 Days

Thus Chrysomya megacephala laid eggs on corpse on 17th October at 6 pm.

Discussion

The estimate of Postmortem Interval based on entomological evidence was in agreement with the PMI obtained by standard means, provided that all evidence from the death scene is taken into consideration, such as the delayed arrival of flies to a corpse when in enclosed environments. The application of the entomological method requires extensive knowledge of the mechanical and environmental factors that can interfere with the processes of colonization, the development time and the decomposition of the corpses by insects. The forensic entomologist has to do careful measurements of the temperature which the immature insect was submitted to, in order to determine which temperature should be used for obtaining a more precise calculation.

Sukontason et al. [7] examined approximately 30 forensic entomology cases in northern Thailand. The flies obtained from corpses were mostly from the family calliphoridae, which refer to Chrysomya megacephala, Chrysomya bezziana, Chrysomya nigripes, Lucilia cuprina, Hemiopyrella ligurries. Both Chrysomya megacephala and Chrysomya rufifacies were the most common species found in the ecologically varied death scene habitat in Thailand [7]. Sukontason et al. [15] reported entomological evidence from the floating corpse in Thailand. Numerous third-instar larvae of Chrysomya megacephala and Chrysomya rufifacies were collected and used to estimate Postmortem Interval. This is the first report of Chrysomya megacephala as forensic important fly species in Thailand. Goff [16] found that Chrysomya rufifacies is one of the most common species of blow flies found on dead bodies, often arriving within 10 minutes of death.

Introna et al. [17] presented three cases of forensic interest regarding the estimation of Postmortem Interval (PMI) by entomological data. The PMI estimation was based on comparison of data from autopsy reports (rate of decay), local environmental conditions (temperature, humidity, rainfall) and development times for the immature stages of each species of local arthropod and succession pattern. Babu et al. [10] estimate post mortem interval (PMI) of a headless corpse of a male fetus of 9 months found in the forest nursery at jagdalpur, India. The PMI was determined on the basis of developmental period of 2nd instar larvae of blowfly Chrysomya rufifacies. Cheong et al., Lee et al., Kumara et al., Kavitha et al. [19-22] reported forensic cases from Malaysia and found that both Chrysomya megacephala and Chrysomya rufifacies are flies commonly associated with human death scenes both inside human dwellings and in open environment in urban, suburban, rural and high elevations areas.

In the present study the PMI of female body was estimated 9.6 days, whereas the autopsy surgeon estimated a PMI of 10 to 12 days. The results of ADH calculation were consistent with the result of police investigation, and were more precise than the medical examiner’s statement.

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