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APPLICATION OF FORENSIC ENTOMOLOGY TO ESTIMATE POST-MORTEM INTERVAL (PMI) IN MAMMALIAN MODELS IN BANGLADESH

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ABSTRACT
Forensic entomology is used as an important tool to estimate the post-mortem interval (PMI) or time elapsed since death, which is crucial for linking a victim with a suspect in suspicious death case investigations. This research work included two case studies, in which estimates of the PMIs were performed using accumulated degree-hours (ADH) method. In both case studies, the PMI estimations were based on the biology of sheep blow fly, *Lucilia sericata* in two mammalian models- mongoose and mouse in Jahangirnagar university campus, Bangladesh. In the first case study, the calculation suggested the death of the mongoose at around 15.49 hours on 19th February 2018 and the calculated PMI based on the 3rd instar larvae was 3.65 days. The skeletal observations revealed that some of the bones particularly in the vertebral column of the cervical area were found displaced giving the proof of the death of the animal by traffic accident because the location of the death spot was a busy road. In the second case study with a mouse corpse, the estimation demonstrated that the death of the mouse occurred at around 01.08 hours on 2nd August and the calculated PMI based on the 3rd instar larvae was 2.62 days. Both case studies offer potentials and necessary tools in estimating PMI in case of the death for any kind of animals and particularly in the medico-legal investigations of homicide cases or any unauthorized killing of wild lives or domestic animals in Bangladesh.

KEYWORDS: Forensic entomology, post-mortem interval (PMI), mammals, *Lucilia sericata*, ADH method.

INTRODUCTION
Forensic entomology is the study of the application of insects and other arthropods to legal issues, especially in a court of law.[1] It examines the insects involved in the decay of corpses, the collection of insect evidence and its use in predicting the post-mortem interval (PMI). The most important aspect of forensic entomology is the study of insects associated with a human corpse in an effort to estimate elapsed time since death. In the medico-legal investigation of death, one of the most essential questions is finding out the time of death. The precise determination of the PMI or the period from death to the discovery of corpse is related to any homicide case because such knowledge can narrow the field of probable suspects in the crime.[2]

Although there are several methods for estimation of PMI involving the early post-mortem changes, such as livor mortis, rigor mortis, and body cooling, using insects is important for late post-mortem changes.[3] Forensic entomology provides evidence of the amount of time during which a corpse or carcass has been exposed to colonization by insects, which helps to estimate the minimum PMI. The application of forensic entomology is recommended by the United Nations (UN 1990) and is used in different countries as a scientific tool that can aid in criminal investigations, particularly during the advanced state of decomposition of the corpse, which deters the estimation of PMI by using traditional methods. However, forensic entomology provides essential clues to the determination of time of death, which is the main and most important goal of this scientific study.[4]

In general, forensically important Dipteran flies include the families of Calliphoridae (blow flies), Sarcophagidae (flesh flies), Muscidae (house flies), and Piophilidae (cheese flies) but the Blow flies under the family of Calliphoridae is the taxon of greatest interest in forensic entomology because they are the first comers at a carcass.[5] One of the blow flies, the Sheep blow fly, *Lucilia sericata* (Diptera: Calliphoridae) is a necrophagous fly that is important in forensic investigations because it is used as a biological indicator in estimating PMI.[6] In forensic entomology, the larvae of this fly help to estimate the period of insect colonization because it relates to the time of death facilitating law enforcement in investigations. The larvae and pupae of blow fly species are frequently used in PMI estimation, their age indicating minimum time since death and most of the studies have considered age estimation of larvae, neglecting the study of pupae. [7]

Though entomological knowledge can be used to determine a wide variety of aspects in forensic science like the manner or location of death, it is most frequently used to estimate the elapsed time since death. The knowledge of the insect species found on exposed human remains and their succession patterns has been used by forensic entomologists in PMI estimation in cases of homicide, suicide, accident, and unattended death due to natural causes. Based on the identification of specific insects present in the dead body and examination of the developmental stages of the fly larvae, investigators can approximate how long a body has been left exposed. Accordingly, the correct typing of insect species is a crucial requirement in the estimation of PMI.[8]

Since different forensically-important insects are associated with the human body after death, they are always a potential candidate of evidence in case of murders or suspicious deaths.[9] In spite of these huge potentials, the field of forensic entomology remains unexplored in Bangladesh, largely because of lack of awareness of the benefits and applications. Moreover, forensic entomology requires extensive information on the local insect population, which is subject to many environmental factors including temperature and they need access to the bodies.

The mammalian models provide useful tools and ability in estimating PMI of suspicious or unnatural deaths in all kind of animals particularly in humans because human data are considerably limited and inconclusive. The results of this study might strongly support the feasibility of developing predictive PMI equations for humans as well as in other samples. Further, it can be used for samples collected anywhere in the world including Bangladesh without specialized knowledge of insect fauna. Therefore, the study applied the common blow fly, *L. sericata* larvae or maggots to determine the PMI in mammalian models in Bangladesh perspective.

MATERIALS AND METHODS

Study Site

The study was conducted in the garden and Medical and Forensic Entomology laboratory of Department of Zoology in Jahangirnagar University and in this university campus at Savar, Dhaka-1342 in Bangladesh, 2018.

Experimental Animals

Since the main target of this study was to understand and determine the estimation of post-mortem interval for homicide or suspicious death cases of human beings, two mammals were selected as experimental animal models for the experiment of post-mortem interval experiments.
One of them was *Herpestes* sp. (Mongoose), and the other was *Mus musculus* (Laboratory mouse).

Experimental Insects

The larvae of blow flies were collected and reared on their natural diets. The development time of *Lucilia sericata* (Meigen) from egg to pupae were observed at temperature of 23± 1°C and 31± 1°C respectively because of the prevailing temperature of that particular time of the study sites and the recorded data were utilized to calculate accumulated degree hours (ADH) for the estimation of PMI. The insect samples were collected from the corpse and preserved in 80% ethanol for identification.

Determination of developmental stages of *L. sericata*

The developmental stages, particularly the instars were determined through the studies of their size and the number of slits present in the posterior spiracles.

Identification of the adults of *L. sericata*

The adults were identified by studying their morphological characteristics under light microscopes.

Accumulated degree hours (ADH) for PMI Calculations

The ADH for *L. sericata* life cycle were calculated by applying the following formula:
ADH = Time (Hrs.) × (Average temperature – Minimum development threshold temperature).

Case study: 1. Estimation of PMI of a mongoose

A dead body of mongoose was found at around 9 am on 20th February 2018 on an adjacent road of a bushy area. Some parts of the innards of the body were out with the presence of blow flies on the body. The corpse was put into a suitable bag for transferring it to forensic cage installed at a garden as a mammalian model for the estimation of PMI. PMI was calculated by using the developmental stages of the blow flies, *L. sericata*. The developmental stages were observed carefully and identified by using the key characteristics to the different larval instars of this species. Temperatures of the study area were collected from the local meteorological station during observations. Photographs of different decompositional stages of the corpse and the developmental stages of *L. sericata* were taken. Based on the time of collection of 3rd instar larvae from the corpse, PMI and time of death of the mongoose were estimated. By analyzing the post-decay stage of this corpse, the possible reason of the death was also determined.

Case study: 2. Estimation of PMI of a mouse

A dead body of a mouse was found with the presence of blow flies on the body in the afternoon on 2nd August 2018 in a garbage place. The corpse was collected to use as another mammalian model to estimate the post-mortem interval. In order to determine the PMI of the dead body, similar to the 1st case study, this corpse was also taken to the garden and exposed to the insects by using forensic study cage. The developmental stages of *L. sericata* were observed carefully and identified as the 1st case study. Temperatures of the study area were collected from the local meteorological station during observations. Photographs of different decompositional stages of the corpse and the developmental stages of *L. sericata* were taken. On the basis of collection time of 3rd instar larvae, PMI and the time of death of the mouse were estimated.

RESULTS

The study included two case studies on mammalian models, in which the estimation of PMIs were performed by using accumulated degree-hours (ADH) method. In both cases, the PMIs were calculated through the studies of the developmental stages of the sheep blow fly, *L. sericata* in two mammalian models i.e., mongoose and mouse. The study involved the analysis of the developmental time of forensically-important blow fly, *L. sericata* to estimate the PMI of the experimental animals as mammalian models because of their high potentiality for this purpose in Bangladesh perspective. Although many insects arrived at the carcasses in succession on the course of the study, not all of them were useful in forensic entomological studies because they were only opportunistic visitors.

Identification of blowfly

The development of blow fly, *L. sericata* (Meigen) included eggs, three larval instars, and the pupae. The eggs were ball like structures measuring from 1.0-1.5 mm with yellowish or whitish colour and around 5-6 mm in length. The 1st instar larvae were distinguished by having one slit (two merged into one) in the posterior spiracles. The 2nd instar larvae were identified by measuring them with a scale which was around 10-11mm long followed by observation of the slits in the posterior spiracles. Two slits were seen in the posterior spiracles. The 3rd instar larva was also preliminarily identified by measuring it (14-15mm long) with scale (Fig. 1). Furthermore, the number of slits in the posterior spiracle was also studied and it was three which again confirmed it as third instar larva (Fig. 2). In the posterior spiracles of 1st instar, 2nd instar and 3rd instar larvae, the numbers of slits were 1, 2 and 3 respectively.[10][11][12]

1 2

Fig. (1-2). Measurement of the third instar larva of *L. sericata* and **slits in the posterior spiracles of the third instar larva of *L. sericata***

The adults of *L. sericata* were identified by using the key morphological characteristics described in different kinds of literature. The colour of the fly was metallic green, the ridge was just above the squama and the basicosta was yellowish in colour suggesting prominent characters of *L. sericata*.[12] The number of para-vertical setulae or occipital bristles was 4 in number (two in each of the sides), the colour of the fore femora was dark metallic blue, the meta-sternal area was found hairy, which altogether confirmed that the blow fly species was *L. sericata*.[13]

Estimation of post-mortem intervals (PMIs)

In this study, the PMIs of mongoose and mouse (mammalian models) were estimated by observing the developmental stages of *L. sericata* in the different stages of decomposition stages on the dead bodies of mongoose (Fig. 4) and mouse (Fig. 5) through the application of ADH method.[4][24]

PMI of Mongoose: Since the temperature of the study area during the days of study was 23±1°C, reference values (24°C) of durations of developmental stages were used for the estimation of PMI (Table 1)[14]. The ADH for *L. Sericata* life cycle from 24th to 19th February 2018 was calculated (Table 2). Finally, by applying ADH method, the time of death and PMI of the mongoose were determined (Table 3).

Table 1. Development time (in hours) required for *L. sericata* at 24°C and 30°C [14].

Range of developmental stages	Average development cycle time in hours at 24°C	Average development cycle time in hours at 30°C
Oviposition to egg	15.8	11.2
Egg to third instar larva	42.3	28.8
Third instar larva to pupa	85.0	85.0
Pupal stage to adult emergence	185.5	143.0
Oviposition to adult emergence	328.7	268.1

Table 2. Determination of Accumulated Degree Hours (ADH) for *L. sericata* from 24th to 19th February 2018.

Date	Temperature in °C			Threshold Temp. °C	Degree Day Value (DD) (Average.Temp. - Threshold Temp.)°C	Accumulated Degree Hours (ADH)
	Max.	Min.	Average			DD x 24 hours
19/2/2018	30	17	23.5	10	13.5	324
20/2/2018	32	19	25.5	10	15.5	372
21/2/2018	32	20	26	10	16	384
22/2/2018	33	21	27	10	17	408
23/2/2018	33	20	26.5	10	16.5	396
24/2/2018	33	21	27	10	17	408

Table 3. Accumulated Degree Hours (ADH) method for determining the Post mortem Interval (PMI) of dead body.

Accumulated Degree Hours method	
Collection of 3rd instar larvae	at 07.00 hours on 23rd February 2018
The reference Accumulated Degree Hours (ADH) taken by *L. sericata* to reach the third instar stage[14] (Table 1).	(15.8 hrs + 42.3 hrs) × 24°C =1394.4 ADH
Total ADH from 23rd February to 20th February (Table 2)	ADH = Development time (hrs) × Growing Degree Day value (DD) (°C) (7 hrs ×16.5°C)+(24 hrs ×17°C)+(24 hrs ×16°C)+(24hrs×15.5°C)= 1279.5ADH
Difference between Accumulated Degree Hours (ADH) taken by *L. sericata* species to reach the third instar stage at 24°C - Total ADH of 20th, 21th ,22nd, 23rd February 2018	(1394.4 -1279.5) ADH=114.9 ADH
Dividing 114.9 ADH by the Growing Degree Day value(DD) of 19th February 2018 (13.5) [Table 2]	114.9 ADH /13. 5°C =8.51 hrs
Determination of oviposition and PMI	backward counting of 8.51 hrs from 19th February 2018 revealed that the female *L. sericata* species might have laid its eggs on the dead body at around 15.49 hrs on 19th February. Total hours = (23rd February: 7 hrs+ 22nd February: 24 hrs+ 21st February: 24 hrs+ 20th February: 24 hrs+ 19th February: 8.51 hrs)= 87.5 hrs= 3.65 days
Thus the death of the mouse might occur at about 15.49 hrs on 19th February 2018 and the calculated PMI after obtaining the third instar larvae was 3.65 days.(Fig. 3)	

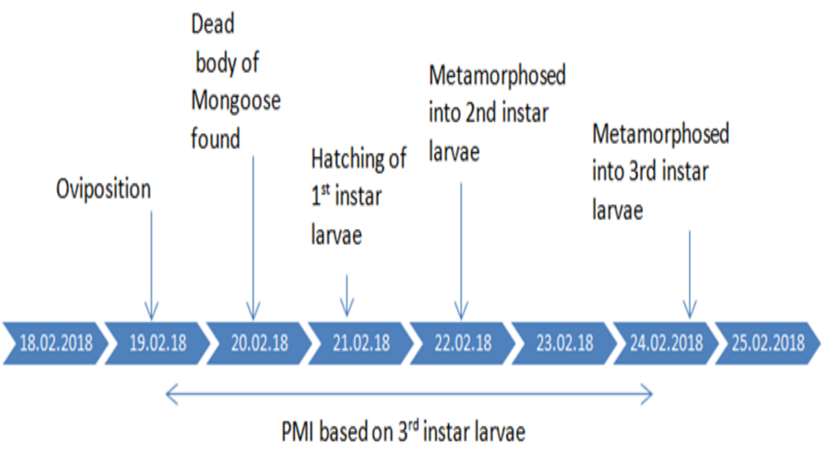

Fig. 3. The timeline indicating the main events in the dead body of the mongoose.

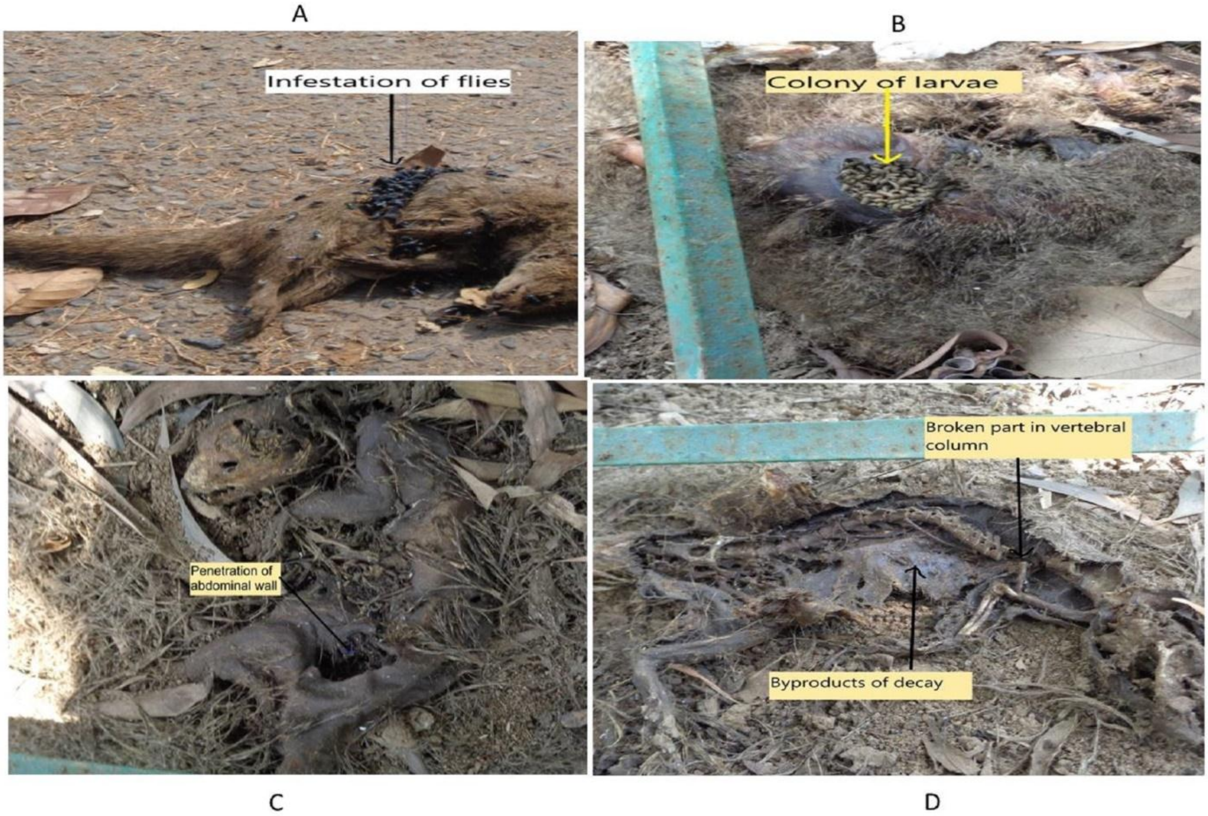

Fig. 4. Images of different stages of decomposition of the mongoose carcass: A. Fresh stage; B. Bloated stage; C. Decay stage; D. Post-decay stage

PMI of Mouse

Similar to the 1st case, as the temperature of the study area during the days of study was 31±1°C, reference values (30°C) of durations of developmental stages were used for the estimation of PMI (Table 1)[14]. The ADH for *L. sericata* life cycle was calculated from 4th August to 3rd August 2018 (Table 4). Finally, by applying ADH method, the time of death and PMI of the mouse were determined (Table 5).

Fig. 5. Mouse carcass. A. Adult *L. sericata,* eggs and larvae; B. Decomposition of carcass with larvae.

Table 4. Determination of Accumulated Degree Hours (ADH) for *L. sericata* from 31st July 2018 to 4th August 2018.

Date	Temperature (°C)			Threshold Temp. (°C)	Growing Degree Day Value (DD) (Avg. Temp-Threshold Temp.)°C	Accumulated Degree Hours
	Max.	Min.	Average			DD × 24 hours
31/7/208	32	27	29.5	10	19.5	468
1/8/2018	32	27	29.5	10	19.5	468
2/8/2018	30	27	28.5	10	18.5	444
3/8/2018	32	26	29	10	19	456
4/8/2018	33	27	30	10	20	480

Table 5. Determination of the Post-mortem Interval (PMI) of a mouse corpse.

Accumulated Degree Hours Method	
Collection of 3rd instar larvae	at 16 hours on 4th August 2018
Accumulated Degree Hours (ADH) taken by *L. sericata* to reach the third instar stage at 30°C[14] (table- 1)	(11.2 hrs+28.8 hrs) × 30°C =1200 ADH
Total ADH from 4th August to 3rd August (table-4)	ADH = Development time (hrs) × Growing Degree Day value(DD) (16 hrs×20°C)+ (24 hrs×19°C)= 776 ADH
Difference between Accumulated Degree Hours (ADH) taken by *L. sericata* species to reach the third instar stage at 30°C – Total ADH of 4th and 3rd August.	(1200-776) ADH= 424 ADH
Dividing 424 ADH by the Growing Degree Day value (DD) of 2nd August (18.5) (table- 4)	424 ADH/18.5°C = 22.92 hrs
Determination of oviposition and PMI	22.92 hrs backward counting from 2nd August 2018 revealed that the female *L. sericata* species might have laid its eggs on the dead body at around 01.08 hrs on 2nd August 2018. Total hours = (4th August: 16 hrs+ 3rd August: 24 hrs+ 2nd August: 22.92 hrs)= 62.92 hrs= 2.62 days
Thus the death of the mouse might occur at about 01.08 hours on 2nd August 2018 and the calculated PMI after obtaining the third instar larvae was 2.62 days.(Fig. 6)	

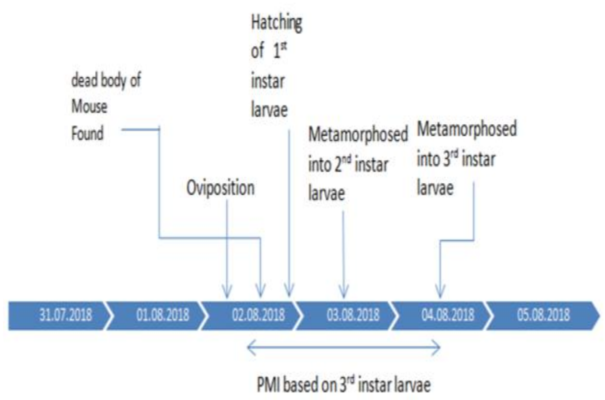

Fig. 6. The timeline indicating the main events in the dead body of the mouse.

DISCUSSION

The PMIs estimated for a mongoose and a mouse by using this necrophagous species of blow fly, *L. sericata* demonstrated its potentials for forensic interest in estimation of PMIs in Bangladesh. In the first case study, the calculation revealed that its death occurred at around 15:49 hrs on 19th February 2018 and the PMI based on the third instar larvae was 3.65 days. The skeletal observation revealed that some of the bones particularly the vertebral column in the cervical area were found broken (Fig. 4) giving the evidence of the death of the animal by a traffic accident. In the second case, *L. sericata* laid eggs on the mouse corpse on 2nd August in 2018 at about 01.08 hrs and the calculated PMI after obtaining the third instar larvae was 2.62 days.

Usually, when homicide or suspicious death, as well as wildlife poaching cases are discovered, it is difficult or impossible to determine the time of death[15]. Without this evidence, it is extremely difficult to pursue a case, so many cases are not followed through. Therefore, such criminals or poachers remain at large, and more animals are killed around the world including Bangladesh.

Here two mammalian animals were used to use this knowledge in future for investigation of homicide and other suspicious death cases. However, the principles are the same. Insects are attracted to a dead animal in the same way that they are attracted to a dead human. Recent work in British Columbia, Canada has also shown that they colonized a bear carcass in the same way and at the same time as they would a pig carcass, the usual model for human decomposition[15].

The cases studied here was a simple determination of time since death based on blow fly, *L. sericata*. The reason of using this blow fly was that estimations of PMI often rely on blow flies (Diptera: Calliphoridae) because they are generally among the first colonizing insects of cadavers, frequently within hours or even minutes after death[16]. The colonization by blow flies starts when a female lays her eggs on the body; once the embryonic development is complete,[17] the larvae will hatch and feed on the dead soft tissues, passing through three larval instars[18].

Since insects are poikilothermic organisms, the developmental rates of different life stages are highly regulated by the environmental temperature. Therefore, a PMI can be accurately estimated on the basis of temperature information from the forensic scene and available development data for the pertinent species and the oldest developmental stage was collected [16]. Developmental studies of the intra-puparial period are very much important in forensic entomology because it involves more than 50% of the duration required for the development of the blow fly[19].

Several methods are used in the estimation of PMI, including the phenomenon of succession and use of accumulated degree-hours or degree-days ADH/ADD[20]. The calculated ADH/ADD values depend on the thermal history of the immature stages of the insect of interest which must be examined together with the temperature at the crime scene before the body was discovered as well as temperature from the nearest weather station[21]. The forensic entomology-based PMI estimation has been performed by using accumulated degree-hours (ADH) or accumulated degree-days (ADD) by many researchers. The ADH or ADD of a particular insect species can be a measure of thermal energy required for larvae to reach a specific life stage. The ADH can be applied to determine the approximate time since death upon the colonization of insects [1] [19] [22]. In Kaduna, Nigeria PMI of a mammal, *Cavia porcellus* (guinea pig) was estimated with the life cycle of *Phaenicia sericata* from the difference between the total development time of the species and the time it required to become adult in the laboratory. The estimated minimum post-mortem interval was 13.8 days coinciding with the actual time since death of the animal[23].

In India, the PMI of a headless corpse of a (male) foetus was estimated with the help of ADH Method based on the developmental period of 2nd instar larvae of blowfly *Chrysomya rufifacies* (Macquart). The PMI of the male foetus was determined to be 3.5 days based on forensic entomology while autopsy surgeon gave a PMI of 3 to 5 days where there was a large gap[24]. In another case study, the PMI of a female body was estimated as 9.6 days, whereas the autopsy surgeon estimated a PMI of 10 to 12 days[2].

In Thailand, approximately 30 forensic entomology cases were examined and reported where *Chrysomya megacephala* and *Chrysomya rufifacies* were the most common species found in the ecologically varied death scene habitat[25].

In Columbia, the post-mortem interval of a human corpse was estimated on the basis of previously published data of *Chrysomya albiceps* (Diptera, Calliphoridae) and was verified through accumulated degree hours (ADH) and intra-puparial development. The estimated minimum post mortem interval was 9.5 days or 229 hrs and the death occurred at around at 21.87 hrs [4].

It is very essential to conduct experiments like the above ones to estimate correct PMI in Bangladesh as this study is the first of its kind regarding the developmental stages of forensic flies in the country. The present study on post-mortem interval revealing the time of death of mammalian models using blow fly, *L. Sericata* offers potentials for future use in medico-legal investigations in Bangladesh.

CONCLUSION

In this study, the PMIs of the dead body of a mongoose and a mouse were accurately determined. The death of the mongoose occurred at around 15:49 hours on 19th February 2018 and the PMI based on the third instar larvae was 3.65 days. Some of the displaced bones particularly the vertebral column in the cervical area (Fig.4-D) gave the presumption of the death of the mongoose by traffic accident. The death of the mouse occurred at about 01.08 hours on 2nd August 2018 revealing the PMI as 2.62 days. As it is a pioneering study in forensic entomology, it will be of great importance in medico-criminal investigations in Bangladesh.

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