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Distinction of Fly Artifacts From Bloodstains in Crime Scenes: A Brief Literature Review

تشخيص آثار الذباب من بُقع دماء في مسارح الجرائم: دراسة بحثية مختصرة

Somasish Ghosal[1], Soumeek Chowdhuri[2,*], Saurabh Chattopadhyay[3], Achintya Biswas[2], Abhishek Das[2], Subhankar K. Sarkar[1]

[1] Entomology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.
[2] Department of Forensic and State Medicine, Calcutta National Medical College & Hospital, Kolkata, West Bengal, India.
[3] Department of Forensic Medicine and Toxicology, N.R.S. Medical College & Hospital, Kolkata, West Bengal, India.

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Abstract

Insect artifacts produced by necrophagous adult flies over a variety of depositional surfaces are problematic in crime scene investigations. Assessment of bloodstains through morphological features combined with contextual and presumptive chemical analysis, as traditional practices, are often inconclusive. Absorbance of bloodstains and substantial wicking on fabrics due to underlying texture and repeated stitch loops in the fabric makes the diagnosis even more difficult than stain morphology.

Literature related to the application of modern techniques in the diagnosis of fly artifacts was critically reviewed and presented along with working efficacies and challenges. Apart from the traditional morphological comparison of bloodstains, findings on immunoassay, scanning electron microscopy and DNA-based molecular discrimination have been considered more confirmatory differential diagnosis in crime scenes. Scanning electron microscopic methods reveal distinctive features of small crystal-like deposits with fly artifacts absent in bloodstains. The immunodetection method tested positive with antiserum (anti-md3) against defecatory and regur-

Keywords: Forensic Sciences, Forensic Entomology, Blood Stains, Criminology, Fly Artifacts.

المستخلص

تعد آثار الحشرات الناتجة عن الذباب البالغ الذي يتغذى على الجثث من خلال مجموعة من الأسطح المترسبة مشكلة عند تنفيذ التحقيقات في مسارح الجرائم. فتقييم بُقع الدماء من خلال اتباع ممارسات تقليدية مثل فحص السمات الشكلية المقترن بتحليل كيميائي قريني وافتراضي، لا يشكل في أغلب الأحيان دليلاً قاطعًا. حيث يؤدي امتصاص الأقمشة لبقع الدماء والفتائل الكبيرة بسبب النسيج الذي يشكل تلك الأقمشة وعقد الخياطة المتكررة الموجودة بها إلى زيادة صعوبة التشخيص إذا ما قورن بشكل البُقع.

تمت المراجعة النقدية لإحدى الدراسات المتعلقة باستخدام التقنيات الحديثة في تشخيص آثار الذباب، مع إبراز جوانب الفعالية التي حققتها والتحديات التي واجهتها أثناء العمل. وبغض النظر عن المقارنة الشكلية التقليدية لبُقع الدماء، اعتُبرت النتائج المتعلقة بالمقايسة المناعية، والمجهر الذي يقوم بالمسح من خلال الإلكترونات والتفريق الجزيئي اعتمادًا على الحمض النووي DNA بمثابة تشخيص تفريقي تأكيدي بشكل أكبر في مسارح الجرائم. حيث كشفت طرق المسح المجهري من خلال الإلكترونات عن خصائص مميزة ممثلة في ترسيبات صغيرة مشابهة للبلور مع غياب لآثار الذباب في بُقع الدماء.

كما أظهرت طريقة الكشف المناعي أنها إيجابية من خلال مصل مضاد (anti-md3) مقارنة ببقع التغوط والتقيؤ. علاوة على ذلك، يعد

الكلمات المفتاحية: علوم الأدلة الجنائية، علم الحشرات الجنائي، بُقع الدماء، علم الإجرام، آثار الذباب.

* Corresponding Author: Soumeek Chowdhuri
Email: smk.kgp@gmail.com
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gitate stains. Moreover, mitochondrial DNA cytochrome c oxidase I (CO1) gene-based molecular diagnosis hold promises in discrimination. However, these modern techniques may be applied along with traditional methods to overcome confusion as per suitability of sampling, following conservative and non-conservative approaches that may offer a real help to crime scene investigators.

التشخيص الجزيئي المعتمد على جين أول سيتوكروم سي أوكسيداز I للحمض النووي الميتوكوندري طريقة واعدة للتفريق. من ناحية أخرى، يمكن استخدام هذه التقنيات الحديثة بجانب الوسائل التقليدية للتغلب على أي تشوش تبعًا لملاءمة العينات، بعد اتباع الأساليب التحفظية وغير التحفظية، التي يمكن أن تشكل بدورها وسائل مساعدة حقيقة عند إجراء التحقيقات في مسارح الجرائم.

1. Introduction

Bloodstain artifacts are an artifactual spatter that has no link to a crime and can mislead crime scene investigators. These bloodstains are subjected to an alteration from their original appearance and categorized as altered bloodstain patterns [1].

Due to their typical foraging behavior, insects that are necrophagous dipterans contaminate bloodstains at a crime scene thereby producing unique stain pattern that may be categorized as postmortem artifacts [2-4]. The Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) coined the term “Insect Stain” under their recommended terminology. This is variably referred to by several authors as fly artifacts, insect artifacts, fly specks, fly spots, and is defined as “a bloodstain resulting from insect activity” [2, 5-7]. Earlier, visual methods based on morphology of fly artifacts such as shape, size, color, tail-to-body ratio, and reflectance etc., were given more preference to differentiate between actual bloodstain pattern and insect artifacts bloodstains, as little information was available on other differential diagnosis methods [6-9]. Both visual and contextual analysis with respect to the crime scene and heme-based presumptive chemical analysis often remained inconclusive with false positive results [10-12]. Though some authors have reviewed the morphological characterization of fly artifacts, no empirical method for reliable distinction between fly artifacts and human bloodstains was presented in those studies; they were predominantly based on comparative morphologies of stain analysis [9,10,13]. Recently, the challenging field of differentiating fly artifacts from actual bloodstains more accurately has made commendable progress. Therefore, a review on these newly developed techniques to identify fly artifacts from forensic aspects will be of immense practical importance for researchers and forensic investigators.

2. Sources and Method of Literature Search

Two electronic databases, PubMed and Google Scholar, were searched with keywords related to the aim of study. The keywords included in the search string were “bloodstain” and “fly artifacts”, within a range from 2018 to 2021. Following inclusion criteria, nine full text papers relevant to the recent development on various confirmatory methods in discrimination of fly artifacts from parent human bloodstains were prioritized and extensively reviewed. Papers not meeting the criteria of an original article in the relevant field were excluded.

3. Methods of Detection

In recent years, the forensic diagnosis of fly artifacts has evolved rapidly from morphological comparison to newer DNA-based molecular discrimination. The methods of diagnosis, their efficacies for actual casework, limitations and challenges are enumerated in following sections.

3.1 Visual methods

Rivers & McGregor reported the regurgitate stain (70.9 ± 2.4%) as the most frequent type of in-

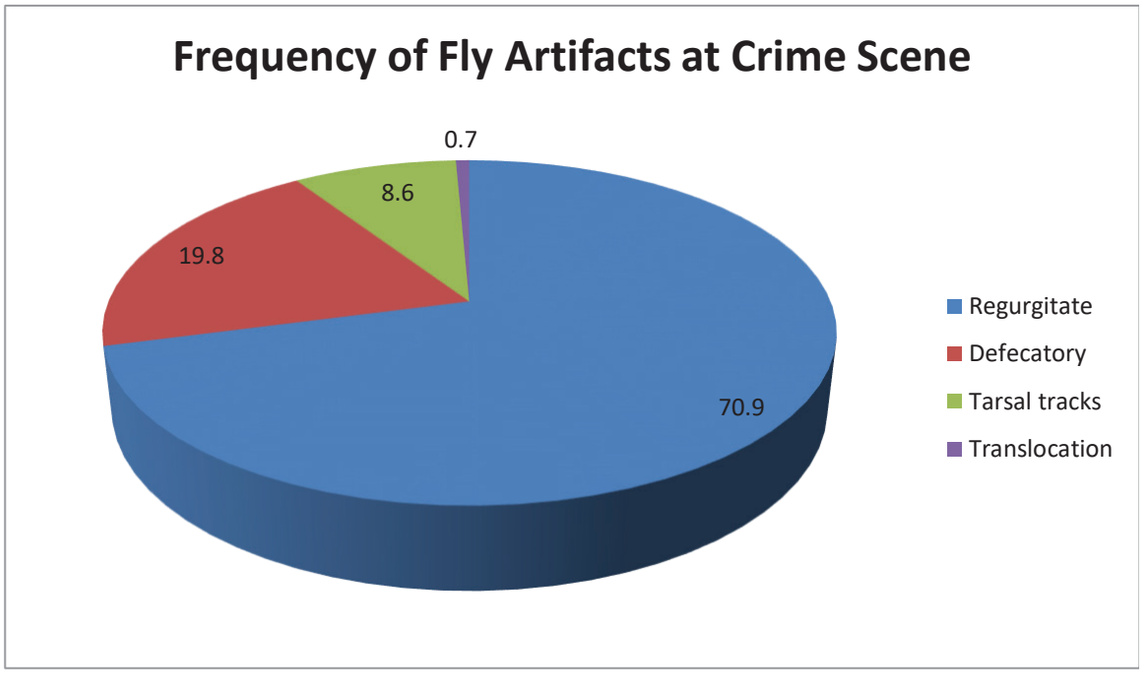

Figure 1- *Pie chart representation for the frequency distribution of fly artifacts observed by Rivers & McGregor in their experiment [13]. Regurgitate stains are most frequently observed in fly contaminated crime scene.*

sect stain, followed by the defecatory stain (19.8 ± 4.0%), tarsal tracks (8.6 ± 1.2%) and translocation (0.7 ± 0.1%) (Figure-1) [13]. The study was carried out with five necrophagous species (*Calliphora vicina, Sarcophaga bullata, Phormia regina, Chrysomia rufifacies* and *Ch. megacephala*). Artifacts were collected after feeding them with a wide array of adult diets e.g., fresh tissue (bovine liver), liquid blood (bovine and human), powdered milk and mouse carcass [13]. Among all the species, *Calliphora vicina* and *Sarcophaga bullata* contributed to most artifacts, as compared to *Chrysomia rufifacies* (21.2 ± 2.2%) and *Ch. megacephala* (21.4 ± 2.5%) that mainly produced tarsal tracks after feeding on human blood. Unique features of the defecatory stain supported by Ltl:Lb (length of tail: length of body) exceeding 1 were shown to be similar with *Ch. Megacephala*, as argued by Benecke and Barksdale [2]. However, the criteria were not met in cases of *Sarcophaga bullata* that fed on mouse carcass (0.92 ± 0.7), *Calliphora vicina* that fed on bovine blood (0.69 ± 0.3) and *Chrysomia rufifacies* that fed on bovine blood (0.89 ± 0.3) [13]. Moreover, *Phormia regina* that fed on all the adult diets mentioned above did not show the uniqueness for the defecatory stain. As suggested by Rivers & McGregor, these variations were influenced by the type of diet and depositional surfaces. The study carried out on morphological dimensions of approximately 3,000 collected artifacts showed that more accurate methods are necessary to discriminate such confounding artifacts that readily vary between different species and conditions without relying on morphological dimensions alone. Durdle et al. (2018) demonstrated that fly artifacts (*Lucilia cuprina*) are common to the proximity of food and light sources and depend on ambient temperature with free availability of human blood, as in cases of indoor crime scenes [14]. In a recently published report, Rivers et al. (2020) have documented the patterns of fly artifacts (*Calliphora vicina*) on an array of textiles and shirt fabrics [15]. In general, bloodstains are likely

to get smudged or distorted or remain latent based on color, knitting texture and repeated stitch loops on different types of fabrics compared to smooth and non-porous surfaces. So, these morphological methods are very challenging in forensic discriminations between bloodstain and fly artifact. Highlights of the findings of these works are presented below:

a) Satellite stains out of fly artifacts remain absent on any fabric under experimental conditions. However, stain production relied on low-velocity passive drop mechanisms or occurred from direct interaction with fabric surfaces [15]. b) Unique three-dimensional defecatory stains were noticed in adult flies that neither wetted nor wicked and were distinct from spherical human bloodstains striking any textile surfaces [15]. c) The absorbency and wicking properties of cotton and polyester knit fabrics influenced the morphological appearance of parent and secondary stains that originated as defecatory artifacts [15]. d) The pattern of stains that originated from digestive artifacts and tarsal tracks could be discriminated on fabrics [15]. e) Based on morphology, fly artifacts are most likely to be discriminated from human bloodstains on white-light colored fabric materials. However, in cases of substantial wicking, the absence of satellite stains on less bright colored fabric makes the identification of fly artifacts is very challenging [15].

3.2 Scanning electron microscopy (SEM)

Pelletti et al. (2019) were the first to introduce the application of scanning electron microscopy (SEM) for differential diagnosis of fly artifacts [16]. Artifacts produced by *Sarcophaga carnaria* on on five different depositional surfaces e.g., A4 porous paper, porous cardboard, non-porous glossy photo paper sheet and non-porous transparent plastic film, were analyzed along with control samples (fresh human blood with no-fly activities) under SEM. The visual morphologies of artifacts (color, tail, surface, shape and edges) were compared with SEM images. Distinctive features of glomerular and small crystal-like deposits were visible under low magnification (40X to 300X), which were absent in controls. Under high magnification (600X to 1200X), features of clustered amorphous material and micro-crystals were observed. In contrast, red blood cells (RBCs) appeared as clear biconcave discs, maintained their own shape and appeared stacked in “rouleaux”. RBCs were absent in fly artifacts. In their subsequent studies, artifacts from *Calliphora vomitoria* were analyzed on glass, metal, plaster, cotton and polyester [17]. Amorphous crystals, and/or micro-crystals whose morphologies are similar to uric acid and cholesterol are confirmatory observations with FA. However, these observations were not clearly confirmed on cotton fabrics. SEM analysis on fabrics are generally cumbersome. Complete absorption of bloodstain on fabrics often leads to inconclusive result where biological residues could not be observed either for features of RBCs or amorphous crystal like material. This may confound to distinguish between bloodstain and FA. Regarding the limitations of the study, SEM should be performed as the last diagnostic tool for its non-conservative approaches in actual forensic casework.

3.3 Immunodetection/ immunoassay

Rivers et al. (2018) introduced the immunodetection method (dot blot assay) with polyclonal anti-md3 serum based on unique digestive cathepsin D (antigen) found in dipteran flies [18]. In their study, among the three polyclonal antisera developed against cathepsin D aspartic proteinase, the highest selectivity was noted with antiserum (anti-md3) generated against the synthetic peptide for amino acid residues 148-163 of the mature fly enzyme, designated as md3. The method was tested on defecato-

ry and regurgitate stains produced by *Protophormia terraenovae* (necrophagous flies). High degree of specificity was observed and it did not bind either with translocation, tarsal tracks or other mammalian blood. However, artifacts having the level of antigen (digestive enzyme) below the lower threshold needed for antisera recognition resulted in a false negative. In their subsequent studies, dot blot assay was validated over 27 species of flies representing 9 families [19]. However, artifacts from 4 fly species did not bind the antiserum, which was explained by the lower antigenic concentration. Surprisingly, two species of flies from non-necrophagous or saprophagous (e.g., *Anthomya illocata* and a *dolichopodid*) did react with antisera, having antigenic sharing. As an additional advantage, this detection method are applicable for age old stains deposited 7 years back. It was confirmed with artifacts produced by adult *Sarcophaga bullata* and hoped to help the process of crime scene investigation on old stains.

In 2020, with a little modification, artifacts deposited on household materials (plush carpet, cotton t-shirt, unfinished dry wall, ceramic tile and untreated wood block) were transferred to filter paper via lift technique for subsequent dot blot analysis [20]. Antisera (anti-md3) based diagnosis was confirmed on artifacts produced by *Calliphora vicina*, *Cynomya cadaverina, Sarcophaga bullata,* and *Protophormia terraenovae*. The majority of artifacts (94.1± 3.7%) that were assayed regardless of fly species and human body fluid tested positive (reacted with anti-md3 serum). This statistical trend increases the reliability of the method for confirmatory diagnosis. On the other hand, tested human fluids (e.g., blood, feces, saliva, semen and urine) did not react with the antisera, negotiating chances of false positivity [20]. The authors added the fact that binding of anti-md3 was proportionate to the optimum concentration of the antigen in digestive artifacts and therefore needed to be validated for actual forensic casework [20].

3.4 Molecular methods

Recently, Bini et al. (2021) applied the mitochondrial DNA cytochrome c oxidase subunit I (MT-CO1) gene-based molecular method for diagnosis of artifacts likely to be regurgitated and digestive defecated types deposited by *Calliphora vomitoria* on wooden and glass wall (fly box) under experimental conditions [21]. The major percentage (94%) of fly artifacts showed positive results against newly designed primer pair to amplify the CO1 gene, which contains species-specific single base variation. Primers designed for their studies are as follows: a) forward: primer C1-J-1751 having sequences 5´GGATCTCCTGATATAGCTTTCCC 3´and length 23. b) Reverse: primer C1-N-2191 having sequences 5´CCCGGTAAAATTAAAATATAAACTTC 3´and length 26 bases. Despite the fact of high sensitivity, authors observed only a few artifacts with false negatives but with no human DNA contamination and were therefore considered as a confirmatory test. MT-CO1 sequence on artifacts produced from other biological fluids deposited on an array of substrates at different sampling intervals were further suggested.

The summary of results on diagnostic methods are presented in Table-1. A yearly publication trend in the field is presented in Figure-2.

4. Further Developments

Bloodstain analysis is an important medico-legal aspect with wide applications in civil and criminal cases. Identification and proper interpretation of the source of questioned bloodstains precedes crime scene reconstruction.

Fly artifacts are confounding to scene reconstruction as they interfere with bloodstains. It is not always

Table 1- *Recent developments in various methods for forensic identification of fly artifacts.*

Reference	Country	Year of study	Methods applied	Species specificity	Deposition surface analyzed	Study highlights
Rivers & Mc-Gregor	USA, Baltimore	2018	Visual/ morphological	*Calliphora vicina* *Sarcophaga bullata* *Chrysomya rufifacies* *Ch. megacephala* *Phormia regina*	Porous filter paper	Morphology of stain / visual methods alone are not confirmatory to distinguish
Durdle et al.	Australia	2018	Visual/ morphological	*Lucilia cuprina*	Simulated indoor crime scene	Ambient temp. & proximity of light and food sources impacted on fly stain
Rivers et al.	USA, Baltimore	2018	Immunodetection/ Immunoassay	*Protophormia terraenovae*	Laboratory experiments	Development on Immuno diagnosis of anti-md3 reactivity against fly stain attempted
Rivers et al.	USA, Baltimore	2018	Immunodetection/ Immunoassay	Wide ranges of flies (27 spp.. & 9 families) *Musca domestica* *Protophormia terraenovae* *Sarcophaga bullata*	Laboratory experiments	A diagnostic approach (immunodiagnosis) of anti-md3 for detection of fly artifacts was developed
Pelletti et al.	Italy	2019	Scanning electron microscopy (SEM)	*Sarcophaga carnaria*	Different depositional surfaces under experimental conditions	SEM analysis considered as an useful differential diagnosis of fly artifacts irrespective of their type
Rivers et al.	USA, Baltimore	2020	Immunodetection/ Immunoassay	*Calliphora vicina* *Cynomya cadaverina* *Sarcophaga bullata* *Protophormia terraenovae*	Transfer of fly artifacts from household test materials to filter paper	Confirmed using anti-md3 serum for detection of fly artifacts
Rivers et al.	USA, Baltimore	2020	Visual/ morphological	*Calliphora vicina*	Characterization on textiles/ fabrics	Discrimination although challenging can be made on white/ light colored fabrics. Wetted, wicking must be carefully observed.
Bini et al.	Italy	2021	Molecular method/ DNA based	*Calliphora vomitoria*	wooden & glass surface walls under experimental condition	Fly DNA-based approach (COI) considered as confirmatory test
Pelletti et al.	Italy	2021	Scanning electron microscopy (SEM)	*Calliphora vomitoria*	Hard surfaces and fabrics common to crime scene under experimental condition viz. metal, glass, plaster, cotton and polyester.	SEM analysis considered as differential diagnosis for hard surfaces only but inconclusive on fabrics

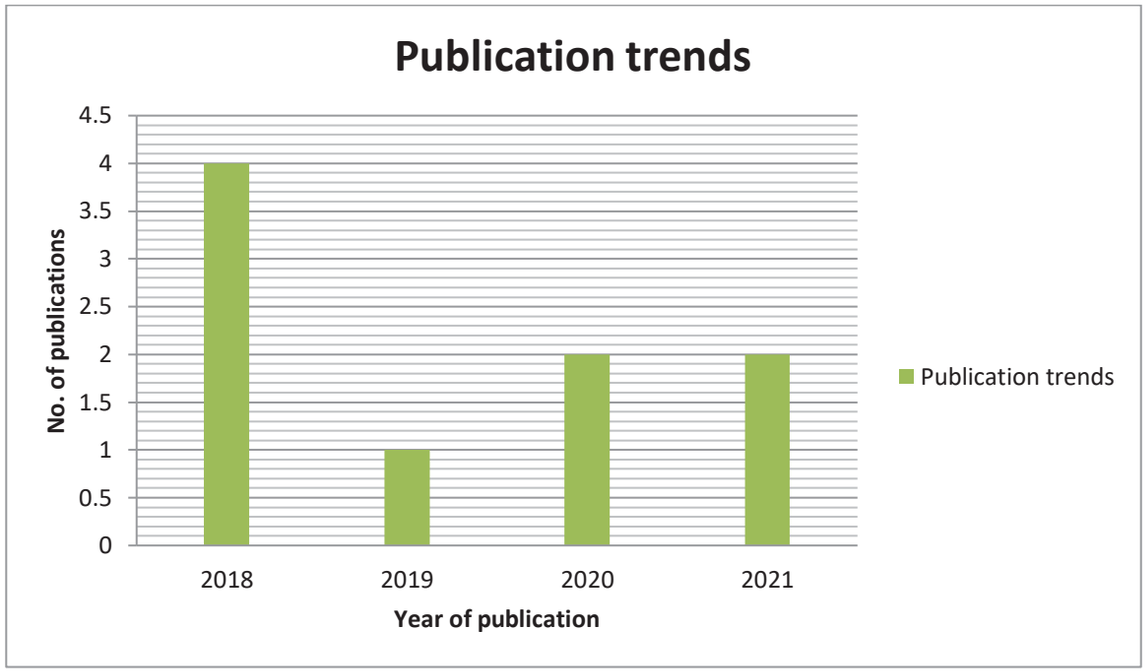

Figure 2- *Publication trends in recent years.*

possible for a bloodstain analysis expert or trained forensic pathologist to be present at the crime scene. Actually, there is no standardized and reproducible methodology available for existing visual/stain morphology assessment. Therefore, chances for inter-observer differential human error are obvious, despite skills. So, reliable scientific refinements on methods for accurate differential diagnosis are necessary.

Recently, the field has evolved rapidly with scientific investigations on development of advanced diagnostic methods in forensics. Though the differential diagnosis between human bloodstains and fly artifacts still remains a debate that demands more scientific refinements, at the present there are various options left, which are discussed in this review.

5. Conclusion

It is hoped that this review might help crime scene investigators or forensic experts with comprehensive knowledge on updated methods in proper recognition of questioned bloodstains suspected as fly artifacts. Moreover, visual or contextual methods may be applied in addition to modern techniques, e.g., SEM analysis, immunoassay, molecular methods, as per suitability of sampling, following conservative and non-conservative approaches.

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Conflict of interest

The authors declare no conflict of interest.

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