The analysis of the artefacts produced by forensically significant blow fly (Diptera: Calliphoridae) activity and the effect on bloodstain pattern analysis.

by

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Abstract

Violent crimes may involve bloodshed resulting in bloodstains spattering on the surrounding crime scene surfaces. Accurate analysis of bloodstains on the scene can provide investigators with insights into the series of events that occurred during the execution of violent crimes. On a crime scene, blowflies feed on bloodstains and deposit artefacts that may cause confusion during crime scene reconstruction.

This dissertation offers background information relating to blood spatter analysis and the role flies may play in altering or complicating blood spatter evidence. A current review of the literature surrounding fly artefacts analysis is also provided. The research conducted as part of this dissertation attempts to describe artefacts on different surfaces caused by fly activity on a crime scene.

Experimental cages of two possible crime scene surfaces (paper to simulate wallpaper and linoleum) were developed such that fly artefacts can be characterized and differentiated from legitimate bloodstains. Pooled bloodstains were created within the experimental cages and blowflies were allowed access to the cages. A total of 10739 and 740 artefacts deposited on paper surfaces and linoleum walls respectively were examined. Unique characteristics of fly artefacts and those that resemble true blood spatter with a possibility of confounding crime scene reconstruction were note.

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Proposal:

The analysis of the artefacts produced by forensically significant blow fly (Diptera: Calliphoridae) activity and the effect on bloodstain pattern analysis

by

Mpasi Pascalis Lesaoana (LSNMPA001)

Research proposal

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Department of Pathology

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Summary

After death or bloodshed, flies are normally attracted to the scene by the scent of a decomposing organism; specifically the volatile chemicals produced as a result of interaction between microorganisms and blood. Blowflies feed on bloodstains by sucking blood on the scene, regurgitating it on the surface and later feeding on the partially digested blood. When blowflies regurgitate and defecate, they leave small spots that look similar to blood spatter produced by force. The study aims to investigate the artefacts in bloodstain pattern analysis which may be the result of entomological activity. This will be achieved by designing forensic entomology cages where blowflies supplied with a pool of porcine blood can be observed. The morphology of fly excrements produced by fifty randomly chosen blowflies and equally distributed into five different cages with a pool of blood at 23° ± 2° C for 24hr with 12:12 hours of light and dark cycle will be analyzed and documented. The whole length of the artefacts will be measured, the tail, body and the width. The study is estimated to run for four weeks starting the first week of July 2015. Some excrements and tear-drop like artefacts that may resemble high impact spatter are expected to be deposited by the blowflies after feeding on blood.

1.1 Introduction and background of the study

Human interactions determine an individual's behaviour. Human behaviour can be aggressive, passive or assertive where the former usually leads to violence among individuals [1]. Violent crimes often involve bloodshed and as a result bloodstains are found at the crime scenes [2]. Normally in homicide cases the accused always opt for lesser weight charges, culpable homicide for instance, on claims that they had no intention to kill the victims. In such situations, forensic experts have to analyze the evidence to prove or refute the accused claims. Analysis of bloodstain pattern can prove or disprove the suspect's intentions to a certain extent when properly undertaken. This assists the courts of law to make informed ruling. Arthropods found at the crime scene help forensic entomologists to estimate the minimum time since death [3].While the presence of arthropods at a crime scene helps in solving forensic cases, they may negatively affect the interpretation of forensic evidence by depositing artefacts that are more likely to confound crime scene reconstruction. For instance, altering the original pattern of bloodstains presented at the scene by the events leading to bloodshed by feeding on legitimate bloodstains, defecating and regurgitating at the crime scene. These have been overlooked. In South Africa and other developing countries, erroneous interpretation of bloodstain pattern evidence by investigators still prevails.

1.2 Literature review

Inasmuch as not all crimes are violent, not all violent actions are crimes. Bloodshed is inevitably a likely consequence of violent crimes. Other bloodstains present at the crime scene may not directly be the result of the violent action, rather an alteration of bloodstains initially present at the crime scene or bloodstains that have been dragged on to the crime scene. However, the bloodstains recovered at the scene in most cases result

from an assault inflicted upon the blood source during a commission of a violent crime [3]. This therefore implies that a proper analysis and interpretation of bloodstain patterns at a crime scene can lead to a reconstruction of events which led to bloodshed [4].

1.2.1 Crime scene and blood evidence

Bloodstains are the most frequently recovered piece of physical evidence in violent crimes [5]. When studied and analyzed by a qualified person, bloodstain patterns at the crime scene can provide investigators with information regarding the sequence of events which led to bloodshed [6], ultimately supporting or refuting the witnesses or suspects' statements. The bloodstain pattern found at the crime scene is determined by the blood's physical properties, contact surface, position of the blood source and the applied force [7, 4]. Information deduced from the analysis of bloodstain patterns at a crime scene can be used to determine the nature of the weapon used, the amount of force applied to inflict an injury or injuries that resulted in bloodstains recovered at the scene and the position the assailant when inflicting the injuries on the victim [8].

1.2.2 Interpretation of blood evidence

Taking into consideration the principles of physics, exposure of blood to the external environment as a result of trauma leads to a predictable bloodstain patterns at the crime scene [5]. This is because the shape, size, and distribution patterns of bloodstains are influenced among others by the amount of force applied to blood source to initiate bloodshed, the size of a free-falling blood drop, the height or distance of a fall and the angle of impact [4]. Uniformly circular stains form when surface tension of a blood drop resists rupture and when a drop of blood impacts a smooth, hard surface [5]. Irregular and distorted stains on the other hand form as a result of a blood falling on a

rough-textured surface which ultimately overcomes the surface tension and cohesive forces of a blood drop thereby causing it to rupture upon impact [5].

The force inflicted upon blood source may be of low-velocity, medium-velocity or high-velocity impact thereby giving rise to a unique bloodstain pattern that can be observed at the crime scene, a regular shaped stain with circular or elliptical shape (spatter stain) or non-spatter stains [9].

1.2.2.1 Low-velocity impact

In low force events, the number of blood drops put in flight is low and the blood drops tend to be larger [9]. The majority of the stains in low force trauma are usually over 4mm in diameter; however, some stains can be smaller [10]. The bloodstains are formed as a result of gravitational force exceeding the exposed blood's surface tension. The resultant velocity of a blood drop upon contact with the surface is usually below 1.5m/s [10, 11].

1.2.2.2 Medium-velocity impact

As the amount of force applied on the blood source is increased, the applied force exceeds the resultant force holding blood together and as such the number of blood drops put in flight increases [10]. Depending on the size of the blood drops generated and the air drag acting against them while in flight, the distance they travel away from the blood source may as well increase [5]. Medium-velocity impact spatter arise at an impact velocity between 1.5 m/s and 7.6 m/s giving rise to blood drops with a diameter of 1 – 4mm [10]. Medium velocity spatter is usually seen in blunt force trauma, stabbings and secondary spatters [4].

1.2.2.3 High-velocity impact

This type of spatter is associated with gunshots, explosions, and high speed collisions, where the impact force at the blood source is measured at velocities greater than 30 m/s [4]. This produces a mist-like spatter with the diameter of the resultant stains of 1mm or less [10]. High-velocity impact spatter are found in close proximity with larger stains in medium-velocity impact stain. Larger drops are heavier and because of their weight, they travel longer distance [5]. High-velocity impact spatter can be easily confused with fly artefacts because of their smaller size, physical and chemical similarities [2].

1.3 Classification of bloodstains

Bloodstains have been categorized according to the force associated with the spatter generation as; low, medium and high-velocity impact spatters [4]. After a noticeable overlap between medium and high-velocity impact spatters, bloodstains were classified based on the mechanism responsible for the staining process [12]. The three types of bloodstain patterns usually found at the crime scene are; passive stains, contact stains and projected spatter [5, 11].

Passive stains arise when blood is forced to leave the body as a result of force of gravity alone [5]. The stains include; drip pattern, flow pattern, splash pattern and pools. They usually result from low-velocity impact force [4].

Contact pattern on the other hand is formed when either a bloodied surface comes into contact with a clean surface or vice versa. The pattern may be repeated several times at the crime scene becoming less intense each time it is being repeated [3]. The pattern may be a swipe, wipe stains or pattern transfer stains.

When enough force is inflicted upon a blood source, blood may be projected through the air as a result of the force applied. This creates bloodstain patterns like arterial spurts, cast-off, exhaled or expirated blood and splash patterns [2].

1.4 Forensic entomology and blood evidence

Apart from salt water habitats, insects are found almost in all habitats with one most important habitat in forensic investigations, a decomposing body [3, 13, 14]. Blowflies are usually one of the first colonizers of a decomposing body on the scene hence they are used as a biological clock, measuring the minimum time since bloodshed or death [14, 15]. This is because blowflies are found at the scene shortly after the incident [3, 15]. Blowflies being diurnal species work more effectively during the day and rest during the night [3]. Thus, bloody scenes or decomposing bodies are more likely to be infested by blowflies during the day and not at night even though they have been noticed in few occasions walking around the carrion in the dark [16].

1.5 Alteration of bloodstain evidence by blowflies at the crime scene

At violent crime scenes where there has been bloodshed, adult blowflies feed in addition to a decomposing organism, on urine, saliva, bloodstains and semen as a source of food [16, 17, 18]. Butler [19] stated that when flies are either feeding or resting, they defecate and leave behind fly artefacts. These are small stains made of the fly's excrements [20]. Flies preferably rest in warm areas such as the light bulbs and on windows where the sun usually strikes [11]. While resting flies deposit the excrements or stains known as fly artefacts. These stains are of forensic importance, they test positive for human blood presumptive testing. Upon visualization with a naked eye of an investigator with no background in fly artefacts and bloodstain pattern analysis as they may resemble true blood spatter [3, 16, 21]. Blowflies at

the crime scene produce artefacts by three mechanisms; when blood-contaminated tarsi come into contact with a clean surface, by regurgitation or vomiting and by defecation [22].

1.5.1 Artefacts caused by blowfly tarsi

Once the flies have located the source of food, they visually locate the best suitable oviposition site and this is achieved by walking over the surface of the carcass [3]. When walking through a pool of blood, the tarsi of the flies may collect traces of blood. These traces may be deposited on surfaces initially not contaminated [3, 16]. As such, the stains may be blood initially deposited at the crime scene, fly vomit or regurgitate, or a mixture of both. These stains may look like bloodstains formed by the incidents leading to the bloodshed [11].

1.5.2 Artefacts caused by regurgitation

When flies suck up or regurgitate blood from a crime scene, they leave or deposit 'spatter-like' stains also known as vomit spots [22]. A number of these stains show evidence of a dimple-like crater formed when flies suck blood from the scene [11]. Vomit spots are usually symmetrical with little or no tail and their size is normally less than 2mm [3, 16, 22].

1.5.3 Artefacts caused by defecation

Fly artefacts or fly spots are stains that can be easily confused with bloodstains caused by a high impact velocity. Their diameter is usually 1mm or less [11]; however, larger faecal spots in the range of 0.5mm to 4mm described as round, light coloured artefacts often with often raised morphological appearance [22]. The location and directionality of these spots is often not consistent with other bloodstains resulting from the events

leading to bloodshed [3, 16]. Stains formed as a result of defecation have also been noted as trails that could reveal directionality; they are shaped like a tear-drop [16].

1.6 Problem statement

While death as a result of violence alone in South Africa is almost five times the world's average with 59935 death reported in the year 2000 amounting to 157.8 per 100 000, South Africa is at least six times the world's average of the rate of homicide of women by intimate partners [23]. Homicide cases involving intimate partners generally happen indoors where there are no eye witnesses to give account of the incidences of the crime [24]. Taking into consideration the fact that offenders especially in the case of intimate partners usually temper with the evidence intentionally to re-direct investigations by simulating events that did not occur, violent crimes in South Africa remain a challenge [25, 26].

Bevel & Gardner [4] stated that interpretation of bloodstain patterns plays a vital role in crime scene reconstruction. However; during analysis of violent crime scenes where bloodshed occurred, investigators need not only assume that the pattern of what appears to be bloodstains on a crime scene is a consequence of an external impact applied to the blood source. The presence of flies on the crime scene is one factor that must be cautiously considered by the crime scene investigators since they can alter the pattern of bloodstains on the scene [21].

During analysis of the bloodstains, flies can create pseudo-spatter that may confound crime scene investigators. In South Africa and many other developing countries, erroneous interpretation of bloodstain pattern evidence by crime scene investigators still prevails [27]. These investigation limitations are associated mainly to lack of resources, money and equipment which eventually negatively affect a basic training for the investigating officers,

thus making proper investigations an impossible task, especially when differentiating between true blood spatter and the artefacts caused by flies during crime reconstruction [28].

1.7 Rationale

While fly artefacts may be easily excluded from legitimate bloodstain by the experts in this field, this is usually a challenge to the investigators from the police departments. The fly artefacts at the crime scene can neither be differentiated from the true blood spatter by presumptive tests performed at the crime scenes nor DNA tests [3, 16]. The effect of fly artefacts on bloodstain pattern found at the crime scenes and how they can eventually affect the reconstruction of a crime scene if misinterpreted as true spatter is known to be one possible source leading to wrongful convictions; however, there are no readily developed methods that can be safely used to differentiate between legitimate blood spatter and blood spatter-like fly artefacts produced by flies in South Africa. Mistaken analysis of these blood-like spots made by the flies as true bloodstains can be interpreted as medium to high velocity blood spatter [3].

1.8 Significance of the study

Failure to recognize the fly artefacts at the crime scene may lead to inaccurate reconstruction of events that allegedly transpired during the execution of the violent crime and thus lead to an invitation of avoidable expenses. The ability to identify fly artefacts from the crime scene will therefore enable the laboratories to save on extra costs that would otherwise emerge as a result of performing unnecessary DNA tests on fly artefacts. Since mistaken analysis of these blood-like artefacts as true bloodstains can be interpreted as medium to high impact velocity

spatter if analyzed by investigators with no proper skills and training in fly artefacts, success of this study will reduce the chances of possible wrongful convictions or violent crimes that would otherwise go unpunished because of misinterpretation of bloodstain evidence [2].

1.9 Research question and assumptions

Blowflies are among the first insects to arrive at the violent crime scene as they are attracted by the smell of blood within minutes since the start of bloodletting assault at the time of violence [29]. The proposed research is based on the following null and alternative hypotheses respectively:

H_0: The presence of blowflies at the crime scene will neither alter nor recreate bloodstain pattern evidence deposited at the crime scene during a physical attack.

H_1: When blowflies feed or walk on the blood and defecate on nearby crime scene surfaces, they produce artefacts that may look like true blood spatter produced by the events of a violent action.

1.10 Aims and objectives

The aim of the study is to investigate the artefacts in bloodstain pattern analysis which may be the result of entomological activity.

The objectives of the research are to:

- Design forensic entomology cages where bloodstain pattern can be observed

- Document the morphology of stains produced by fly activity around blood stains

1.11 Materials and methods

1.11.1 Experimental design

Eight 24x24x24cm fly cages will be used in this study. Blow fly eggs obtained from a set bait (porcine internal organs) will be bred on chicken liver and vermiculite for pupation at 23 ± 2°C at Falmouth building, Forensic Medicine division, level 2 laboratory at the University of Cape Town and the welfare monitoring of animals will be performed by the supervisors (Dr. Marise Heyns with 3 years of experience and Mr Calvin Mole.) and myself (Mr Mpasi Lesaoana). Emerging flies will be transferred to one 24x24x24cm holding cage for 24h after emergence where they will only be fed water and white granulated sugar *ad libitum*. The flies will be moved to a new cage where they will be fed water only for the next 12 hours. This allows for meconium to be deposited outside of the experimental cages and to ensure that flies have deposited all the possible blood out of their crop before they can be introduced into five of the six experimental (24x24x24cm) cages. The five surfaces (top, floor, the two sides and the back part) of the experimental cages will be made of a non-porous Benchkote paper divided into 36 squares drawn, each 4x4cm. The front (24x24cm area) of the experimental cage will not be covered with the Benchkote paper to allow for light to enter the cage, rather a transparent food wrap.

1.11.2 Population and sampling

A pool of blood will be created by adding 4ml of porcine blood on the lid and placed on the floor of each of the six cages. A total of fifty randomly chosen blowflies will

be used in this study. Adult blowflies (36 hours old since emergence) will be equally distributed into the experimental cages with a pool of blood on the surface such that ten randomly chosen flies will be introduced into a cage with a 4ml pool of porcine blood. One cage with blood and no flies will be used as a negative control.

Fresh porcine blood collected from a local abattoir will be used as the sole source of food and water during the experiments. The blood will be carried to the laboratory in sterile containers with no anti-coagulants. The temperature will be maintained at 23° ± 2° C for 24hr with 12:12 hours of light and dark cycle. At the end of the study the flies will be taken out of the experimental cages to one of the 24x24x24cm cages. The flies will then be introduced into the cycle of the present animal in the department since none of the flies will be killed. All the artefacts will be categorized on the basis of colour, morphology and size.

1.12 Data analysis plan

1.12.1 Measurement and calculations

The artefacts will be noted and photographed with and without a scale. The whole length of the artefact will be measured using a calibrated venneir calliper. The length of the tail, body and the width of the artefacts will also be measured and the impact angle which is given by the equation below will be calculated. Where: *w* and *l* are the width and length of the artefact respectively.

$$\vartheta = arc\sin(\frac{w}{l})$$

The processes leading to the formation of the artefacts will be deduced from the morphology of the artefacts. This will be confirmed by comparing the artefacts to the

morphology of the previously determined artefacts whose formation has been visually witnessed or captured on camera from other studies. This will allow for determination of the possible mechanism of deposition which will be noted as well.

1.12.2 Statistical analysis

Before any analysis can be done, raw data will be transformed to the natural logarithm to meet the assumptions of homogeneity of treatment variances. Data will be analyzed by analysis of variance (ANOVA). The variance to mean ratio (VTMR) will be applied to test Complete Spatial Randomness (CSR) of the artefacts to determine whether there is clustering.

1.13 Ethics

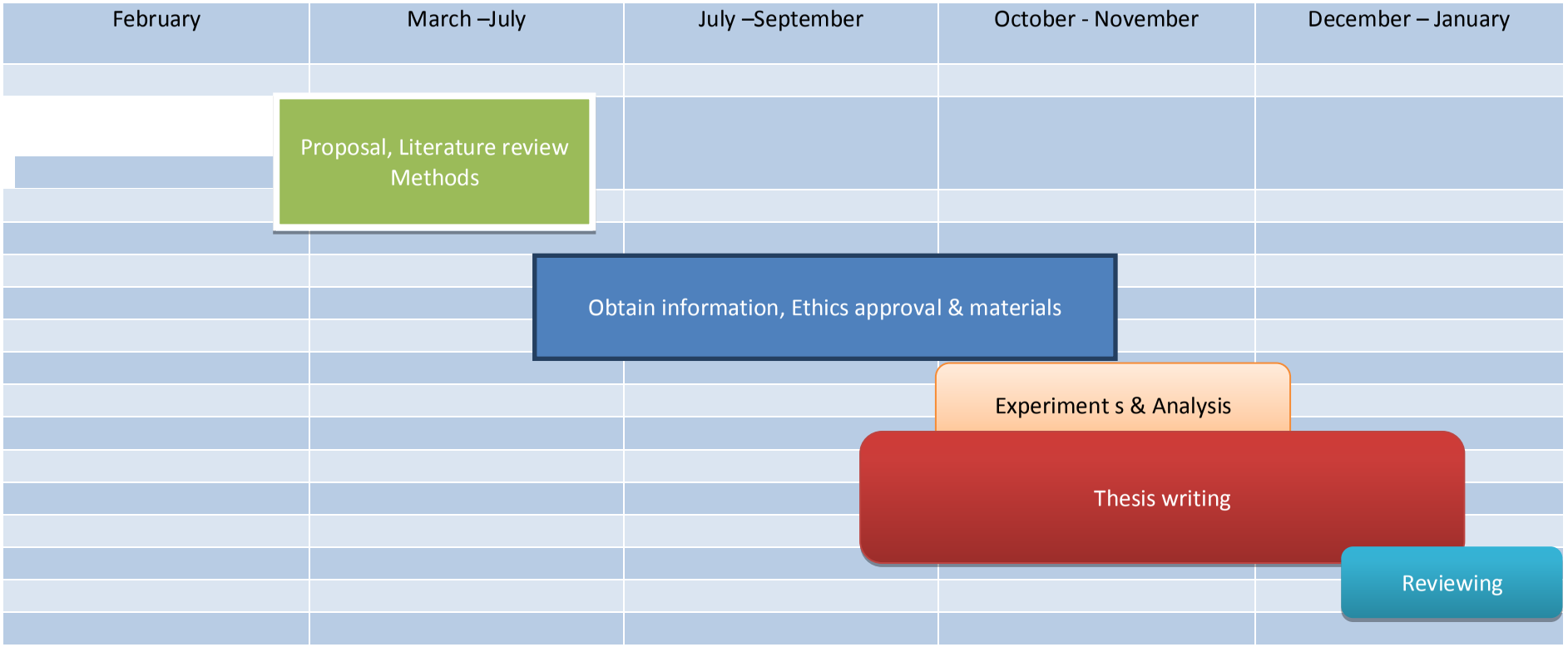
The flies are used because there are no other possible alternatives; however, this study will use a minimal number of blowflies to avoid exposing a large number of flies to stressful conditions unnecessarily. Only as little as fifty blowflies will be used in this study. Throughout the study, except where otherwise impossible, the flies will be treated in a humane manner. Blow fly maggots with access to vermiculite for pupation will be bred on chicken liver. Both the maggots and the adult flies will be reared under environments (23 ± 2°C and ambient humidity) that will ensure their survival and nutrient sources will be provided *ad libitum* throughout the study. The flies will neither be killed nor exposed to hazardous environments in the study; however, some are expected to die of natural causes during the study. The cause of death may not be known. At the end of the experiment the flies will be introduced into the cycle of the present animal in the department. Ethics clearance for this study will be obtained from the animal research ethics committee at the faculty of Health Sciences, University of Cape Town.

1.14 Logistics

Table 1.1: Anticipated budget of the study

Item	Qty	Area (m^2)	Estimated price (ZAR)
White Benchkote paper	1	3	500
Fly cage (24*24*24) cm^3	8		2400
Total			2900

Table 1.2: Anticipated workflow during the course of the study



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Addendum

Several changes were made to the initial proposal after the research was started. This serves as a justification for the changes made to the original proposal.

An additional non-absorbing surface (linoleum) was used so that comparison with an absorbing surface (inner surface of the Benchkote paper) is possible. This also affected the number of blow flies and the cages initially proposed. Additional forty blow flies were used thereby increasing the total number of flies used in this study to ninety.

The amount of blood used to create a pool of blood was increased from the proposed 4ml to 6ml. 4ml was found to be too little and was more likely to dry out quickly. The negative controls were also not included because it was too obvious nothing would to happen from them since the flies would not be put in the cages.

The angle of impact was not calculated since it was determined that it has no bearing on fly artefacts as they are not classified as impact spatter pattern rather a transfer pattern.

Chapter 2: Literature Review

2.1 Introduction

Lewis [1] noted that in a food chain, biological materials are recycled when they decompose or whenever they are eaten by others. While blood is a crucial evidence in violent crimes, unfortunately, it is one biological substance that is utilized by other organisms as a food source once it is available to them. This may potentially degrade the significance of bloodstains evidence in crime scene reconstruction when analyzed by unqualified personnel.

Usually when a violent crime results in death or bloodshed, flies known as blowflies are attracted to the blood source by the smell of volatile chemicals released when microorganisms feed on it. This happens within minutes since the execution of the crime, especially during the day when the flies are active. Upon arrival to the violent crime scene, female blowflies seek for a moist, secure place where it can lay eggs while at the same time staying clear from its predators and then feed on the body as well as on bloodstains found at the crime scene [2, 3]. This makes blowflies the first colonizers of a decomposing body. After feeding on bloodstains, blowflies may defecate and regurgitate on the nearby surfaces. This behaviour introduces new and additional spatter-like fly artefacts at the crime scene that may cause confusion during crime scene reconstruction from bloodstains [4]. These artefacts represent non-existing crime scene events [5].

Thus, adult blowflies are potential crime scene contaminators well known for their notorious behaviour of depositing bloodstain drop-like artefacts on the crime scene.

When force is applied to a victim, the skin and blood vessels may be breached and so bloodshed subsequently becomes a possible consequence of such attacks. In essence, violent crimes may result in a pattern of bloodstain evidence spattering at a crime scene [6]. Recognition and proper analysis of bloodstains recovered at a violent crime scene

may play a critical role in forensic investigations. When properly analyzed and interpreted, bloodstain pattern at a crime scene can help forensic investigators to reconstruct the events that occurred during the commission of a crime [7].

It is therefore important to understand the forensic value of crime scene investigations, the properties of blood, and effects of blowflies on violent crime scenes and appreciate their overall significance in crime scene reconstruction from bloodstain pattern analysis.

2.2 Crime scene investigation

While large amounts of evidential material is found at crime scenes, forensic scientists are faced with the challenge of identifying, analyzing and correctly interpreting probative physical evidence recovered from the crime scene. A crime scene can be a location or an object that an assailant or a victim comes into contact with during the commission of the crime and as such, it may be difficult to interpret some type of evidence especially if it is likely to be affected by the surrounding surfaces or organisms [8]. The exchanged material during contact between any two of the following: perpetrator, victim and a surface during the commission of a crime can be used to supports an assertion and is fit to stand as physical evidence. Thus, when committing a crime, physical evidence sufficient to implicate perpetrators or exonerate the wrongly accused is left behind at the crime scene [9]. This follows the principle postulated by Edmond Locard which states that every contact leaves a trace.

However, it is not every contact that may leave a readily recognizable or an informative trace unless it is analyzed and interpreted by a qualified person; hence crime scene investigation strategies have been set [10]. Even though the standard methods of approaching a scene of

crime have been well defined in order to maximize evidence recovery, the evidential value of the forensic evidence largely depends on investigator's ability to recognize and analyze the evidence [11]. It is impractical to prescribe a specific method of crime scene analysis because every crime scene is unique; recognition, proper documentation, collection, storage and preservation of evidence therefore remain the fundamentals of crime scene investigations [12, 13].

2.3 Violent crimes and blood evidence

When executing a violent criminal act, force or use of force may be imposed upon a blood source, usually a victim. Crimes in which an assailant uses force upon the victim, otherwise known as violent crimes often result in bloodshed [14].

When blood spills at a crime scene, it may result in the formation of various bloodstain patterns that can provide the investigator(s) with insights into the executed crime [2]. Therefore bloodstain pattern analysis is regarded as one of the most crucial aspects in violent crime investigations. This is because when studied and analyzed by a qualified person, the morphology, size, shape and colour of the bloodstains at a crime scene can provide investigators with information regarding the sequence of events during the commission of the crime [6, 15]. Despite the fact that it may be regarded as insignificant by inexperienced investigators, bloodstain evidence can also be used to assist investigators to support or refute allegations made by the witnesses or suspects concerning the actual events that took place during bloodshed [16, 17, 18]. When carefully analyzed, bloodstain patterns can reveal the exact position where a particular violent event took place [19]. Lowe *et al.,* [20] further clarified that even the minimum number of assaults inflicted upon the blood source can be deduced from the bloodstain pattern.

Every crime scene is unique. Therefore an exact crime scene bloodstain pattern replica cannot be reproduced even in laboratory settings [11]. Among others, the pattern of bloodstains found at a crime scene is determined by the physical properties of blood, the contact surface and a combination of other variable factors surrounding an impact that may never be replicated in an exact manner in laboratory setting [10, 21]. It is for these reasons that preservation and careful analysis of the bloodstain evidence must be performed directly at the crime scene. Since the information derived from bloodstain pattern analysis can be used to determine the nature of the weapon used, the relative amount of force applied to inflict an injury or injuries responsible for bloodstains recovered on the scene and the positions of the victim during bloodshed, the artefacts that may confound the crime scene investigator must be identified [16, 22]. Upon analysis of a crime scene, Benecke and Barksdale [23] assumed the reddish material of what looked like bloodstain spatter to be bloodstains produced as a result of impact force applied to the blood source. Determination of the angle of impact led to no possible point of impact and these were later determined to be artefacts deposited by the flies after feeding on blood. Blowflies' excrements, especially after a bloody meal can be easily confused with true bloodstains at a crime scene [24].

A thorough understanding of blood and how it behaves under different conditions therefore must be considered. This includes the physical properties of blood.

2.4 Physical properties of blood

Blood is a liquid connective tissue that transports oxygen and food in the form of glucose, lipids and amino acids from the digestive tract to the cells hence it is consumed by flies as a food source [13, 25]. Generally, at a constant temperature and pressure, the spacing between molecules in liquids remains fixed and as a result liquids take the shape of a container

because it is able to move around freely [26]. Such fluids are said to follow a Newtonian law of motion. However, blood by virtue of its physical properties; viscosity, specific gravity and surface tension, does not follow the same principle and it is described as a pseudoplastic, non-Newtonian fluid, otherwise known as shear thinning fluid [18].

2.4.1Viscosity

When fluids flow, there is an internal force generated which, unlike friction, acts along the direction of a flow. This is known as shear stress and it induces fluids to break into several drops while in motion [26]. The fluid's measure of resistance to gradual change by shear stress is its viscosity. Although the current literature does not clearly indicate how viscosity affects bloodstain pattern formation it is known that an increase in the concentration of sialic acid on the red blood cell membrane increases the viscosity of blood thus making it harder for blood drops to break when in flow [25].

2.4.2 Surface tension

Blood does not readily break into several stains. The neighbouring molecules in blood are held together by cohesive forces that make blood molecules stick together and as a result resist penetration and separation. Unless the molecules are acted upon by an excessive force which overcomes cohesive forces, bloodstains maintain their spherical shape via inter-molecular cohesive forces [7, 21]. However, the generation of blood drops and the resultant impact spatter on the surface are not only determined by the blood surface tension but the overall forces that act on the blood drop [25].

These physical properties may explain why a drop of blood in motion forms a tear drop-like stain or spines when it lands on a hard non-porous surface.

2.5 Interpretation of blood evidence

Usually when a violent crime is committed blood spatters on the nearby surfaces. This produces a distinctive pattern of bloodstains that can be analyzed to interpret the events that led to its formation [6]. The pattern is influenced largely by the amount of force applied to a blood source which ultimately affects the drop size of falling blood. Also influencing the pattern is the height of the fall, the impact angle and the contact surface [10]. Whenever a drop of blood falls on smooth surfaces, it resists rupture and assumes a uniformly circular or oval stain as a result of surface tension and cohesive forces holding the molecules together. Conversely, it is noted that when a drop of blood impacts a hard, rough-textured surface it results in distorted stains [7].

2.5.1 Impact forces

The impact forces may be of low-velocity, medium-velocity or high-velocity forces, and the resultant blood spatter can be related to the applied force associated with their formation [19, 26]. Akin [16] noted an existence of inverse correlation between a bloodstain size and the impact force applied to create them. That was further supported by the findings of Attinger *et al*. [25] that low force impacts produce larger stains whereas high velocity impacts produce very small spatter. However, some inconsistencies or overlaps have been noticed. Also, medium and high impact spatter

stains closely resemble some artefacts produced by flies in terms of shape, size and morphology to an extent that it can confuse crime scene investigators [23].

2.5.2.1 Low-velocity impact spatters

When a small amount of force is applied to the blood source, the number of blood drops put in flight is low and the blood drops always tend to be large, usually over 4mm in diameter [12, 27]. However, very small stains with diameter less than 4mm may be seen to be associated with low velocity impact [28]. The resultant velocity of a blood drop in low force events is usually below 1.5m/s [28]. Although fly artefacts may be as large as 20mm, larger artefatcs usually have irregular shapes and as such, it can be easily differentiated from low velocity impact spatter [24].

2.5.2.2 Medium-velocity impact spatters

As the magnitude of an external force applied to the blood source is increased, the applied force exceeds the adhesion forces holding blood molecules together and so the number of blood drops put in flight increases [25, 28]. Depending on the size and the air drag acting against the blood drops in motion, the distance it travels away from the blood source may increase [25]. The resultant spatter normally form at an impact velocity between 1.5m/s and 7.6m/s giving rise to blood drops with a diameter of 1 – 4 mm [7].

2.5.2.3 High-velocity impact spatters

These are tiny spatter patterns associated with high speed collisions where the impact force at the blood source is measured at velocities greater than 30 m/s. The product is a mist-like spatter with the diameter of the resultant stains of 1 mm or less [9, 28]. This is usually seen in explosions, gunshots and even expirated spray. The artefacts caused as a result of fly excrements at a crime scene after feeding on blood are visually similar to this spatter in the eyes of ordinary investigators with no training in blood spatter analysis [2].

2.5.3 Characterization and classification of blood stain pattern

In any given bloodstain pattern, there is a co-existence of numerous bloodstain spatter of different sizes that can be related to different impact forces, normally an overlap between medium and high-velocity impact spatter is often seen [25]. Because of this overlap, for better categorization, bloodstains are also classified into three main categories, namely: passive stains, altered or contact transfer stains, and projected or spatter stains [10]. This method of classification is based on the mechanisms that led to the blood pattern formation.

2.5.3.1 Passive stains

These are bloodstains formed when exposed blood is forced to leave the body solely as a result of a force of gravity acting on a blood source [10]. Although passive stains cannot generally be associated with low velocity impact forces, the majority of them result from low impact forces. The patterns may include: drip pattern, flow pattern, splash pattern and pools [7, 27].

2.5.3.2 Contact transfer/altered stains

Contact or altered blood patterns are the bloodstains produced when a bloody object comes into contact with unstained surface thereby leaving its pattern [12, 27]. The pattern may be repeated several times at the crime scene possibly becoming less intense each time it is repeated [10]. With regards to staged violent crimes, altered stains may be discovered at crime scenes where there have been attempts to cover up a crime [25]. Bloodied hand or foot prints and crime scene artefacts produced by flies are placed under this category of stains [10]. Altered bloodstains that have been smeared before drying completely may mimic and be confused with some kind of fly artefacts which exhibit dried edges while most of the centered material has been wiped off [24]. Furthermore, artefacts produced by bloodied fly tarsi have also been classified under this category of stains. While the majority of the altered stains like swipe or wipe are well known and more informing to the crime scene investigators, little is known about blood-like fly artefacts and as a result it tends to lead to some level of confusion [22].

2.5.3.3 Projected/spatter stains

During a physical attack, an impact force is applied to the blood source and the blood leaves the source as a result of the applied external force rather than gravity alone [27]. The skin and blood vessels may be breached and large amounts of blood under pressure may land on the target surface giving rise to spattered stains [10]. Projected stains include patterns such as arterial spurts, cast-off, and splash patterns [25]. A special case of projected spatter that is caused by exhaled or expirated blood has been noted. Expirated bloodstains may be easily ignored

during crime scene reconstruction and wrongly considered to be fly artefacts as they may reveal random directionality [22]. When closely inspected however, it can be easily identified and distinguished from the fly artefacts as it may take on a ring appearance due to blood-air mixing [10]. Although fly artefacts are classified as contact stains that can be easily mistaken for projected stains caused by high to medium impact velocity, there has only been a few studies internationally attempting to differentiate between the spatter stains and the fly artefacts and no attempt regarding the artefacts produced by South African fly species.

2.5.4 Height of a fall and its impact on resultant bloodstains

Fly artefacts may range from very tiny circular blood-like spots to large irregular artefacts [24]. The size of the fly artefacts is determined by the amount of the excrement deposited by the flies. Some bloodstain pattern analysts rely on the diameter of a stain to determine the height of the fall [21]. However; fly artefacts may be of various sizes and it may appear similar to legitimate bloodstains [2]. The correlation between height of a fall and bloodstain size is based on the concept that the diameter of the stain increases as the height of a fall increases [25]. This theory however, cannot be relied upon as it assumes an average blood drop (0.05ml) which is not always constant in violent crimes. As blood drops develop from various heights of fall, the number and length of spines emerging from them appear to increase with an increase in a height of a fall [29].

Determination of the height of a fall from the number of spines formed around the parent bloodstains was criticized and deemed unreliable [29]. However; the presence of spines can be used to confirm that the bloodstain is indeed a legitimate bloodstain

and not a fly artefact [30]. Fly artefacts, especially regurgitation spots may exhibit either round, oval or irregular shapes with smooth or rugged edges depending on the type of surface. Legitimate bloodstains on the other hand usually have scalloped edges or spines [10]. Fly artefacts by virtue of being deposited at zero distance do not develop spines around major spots. In a few occasions however, fly artefacts may be surrounded by a very small dark stains that may bear a slight resemblance to spines [31]. Whereas bloodstain spines project from and are often attached to the parent stain, minute satellite-like fly artefacts are not attached to the main artefacts. The presence of spines therefore may be used to differentiate fly artefacts from legitimate bloodstains. However, bloodstains falling from short distances may not show any presence of spines and may appear similar to fly artefacts [10]. The absence of the spines therefore cannot be used as a conclusive proof that the stain is a fly artefact.

Care must be taken while differentiating fly artefacts from the legitimate bloodstains since the surfaces like cloth or paper with tendency to absorb deposited liquids, may not favour the formation of spines upon impact even with true bloodstains.

2.5.5 Directionality of blood stain and droplet size

For a blood drop to land on a crime scene surface, it will be projected towards a certain direction from the source to reach its destination where a bloodstain pattern forms. As shown in figure 2.1, the terminal edge is always on the side of the direction of the travel of a bloodstain. Eckert & James [7] noted that blood drops travelling in a particular direction may show a scalloped edge on the side representing the direction of a travel. Although fly artefacts can also reveal directionality, it has been noted as random, leading to no specific point of convergence [2]. This is one feature that can

be used to differentiate fly artefacts from legitimate bloodstains at a crime scene. However, directionality is not always revealed, especially by the bloodstains that are deposited on surfaces with a tendency of absorbing the deposited liquids. In a purposefully altered crimes scene, it is likely to recover a single isolated bloodstain or a blood-like stain that may reveal directionality. It is necessary to have in place an effective methods of differentiating true bloodstains and fly artefacts at hand in such cases.

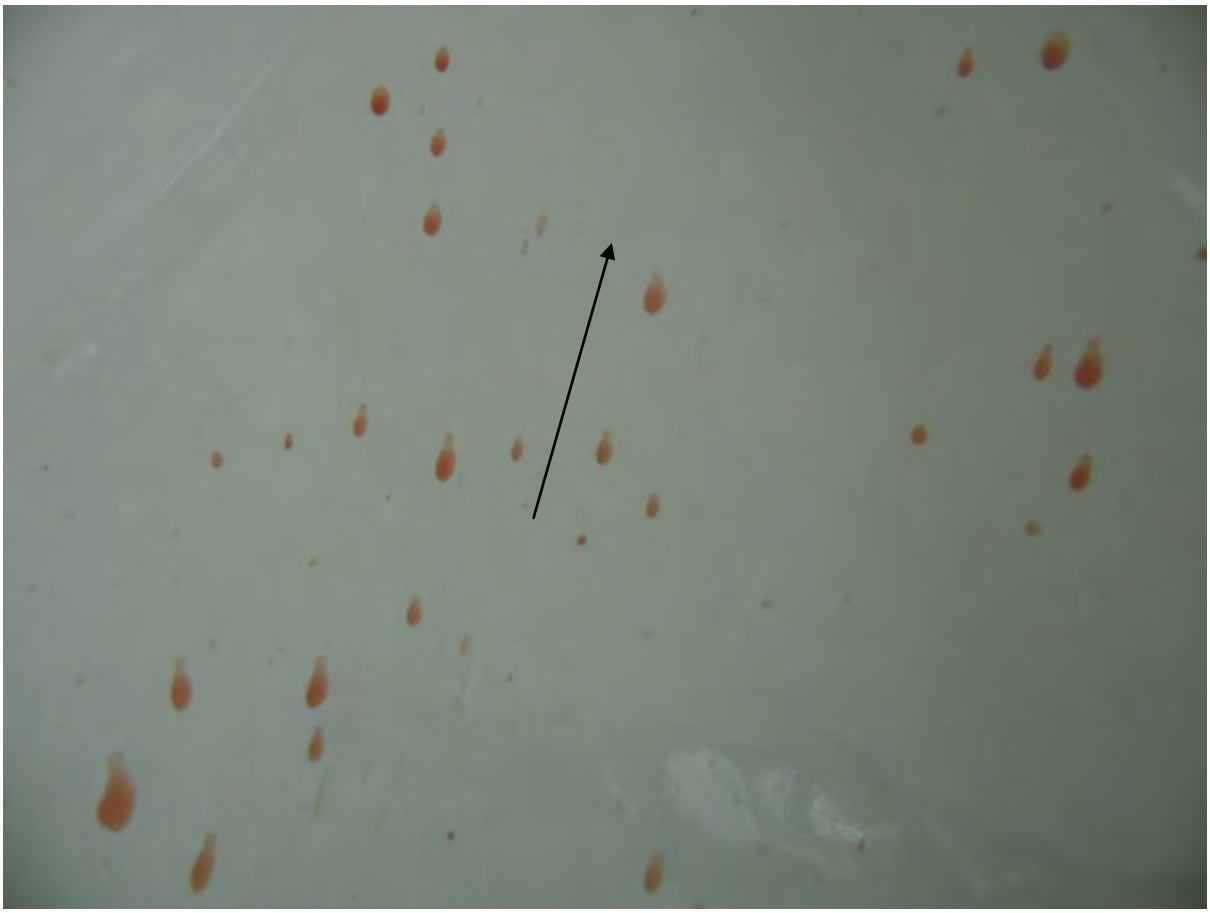

Fig 2.1: Directionality of legitimate bloodstains

2.5.6 Bloodstain size

At violent crime scene, bloodstains of different sizes maybe recovered. While the droplet size is determined by the amount of force applied to the blood source to put bloodstains in flight, the distance travelled by these drops away from the source depends on the amount of force acting against the blood drops in motion and the individual size of the drops [7]. The diameter of the resultant bloodstains decreases as the amount of force used to put the drops in flight increases [27]. The smallest particles are formed when the force applied outweighs the sum of forces holding blood together.

In low force trauma, the distance is shorter than in medium velocity impact and it increases as the amount of force increases. However, larger particles are found furthest from the blood source because of the ability to overcome greater amounts of friction force caused by air during the flight compared to the smaller particles [21]. While the stain size can be used to predict the amount of force used to inflict an injury, there is no particular bloodstain size or size range that can be used to differentiate fly artefacts from true bloodstains.

2.6 Alteration of bloodstain evidence by blowflies at the crime scene

For an organism to survive, feeding and excretion of waste are mandatory. The same behaviour is seen with blowflies. In violent crime scenes, adult blowflies feed on a number of substrates including: carrion remains, urine, saliva, mucus and bloodstains [2, 32, 33]. Crime scene reconstruction from bloodstains at violent crime scenes becomes highly compromised when blood evidence is altered or contaminated. The physical act of

flies feeding on bloodstains changes the actual size of the bloodstain, while fly regurgitation and defecation at the crime scene increase the apparent number of blood-like stains on the scene [2].

Axtell [34] stated that flies normally defecate and leave behind fly artefacts when feeding or resting. These artefacts are discovered in warm areas such as around the light bulbs where the flies usually rest or along the walls where the sun strikes [17, 35]. Fly artefacts are deemed important because it normally test positive for heme-based presumptive blood tests, thus making it highly indistinguishable from true bloodstains by chemical means [36, 37]. While it is clear that fly artefacts are excrements produced by the flies, other artefacts may be produced by flies when wandering about the scene with their blood-contaminated tarsi coming into contact with a clean surface thereby creating small spatter-like spots [4, 24]. Fly artefacts may resemble true bloodstains produced as a result of impact upon a blood source as shown in Fig 2.2.

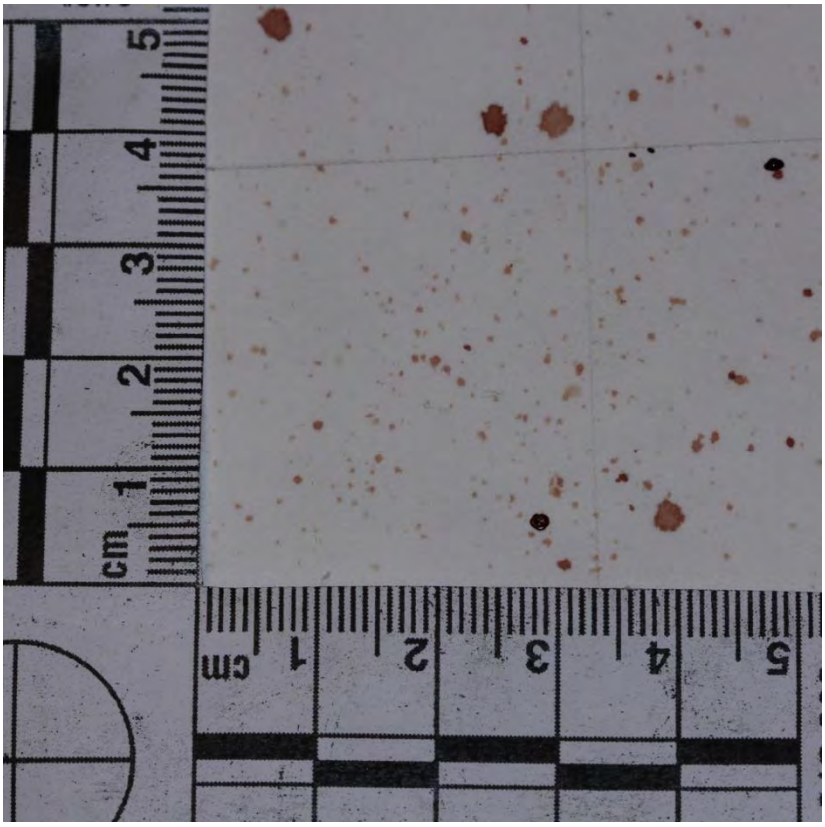

Figure 2.2: Fly artefacts deposited after consuming a bloody meal.

2.6.2 Artefacts caused by a contact between blowfly tarsi and a surface

When the flies have successfully located a decomposing body, a visual search commences for the best oviposition site. This is achieved by using sense receptors on the tarsi when walking over the surface of the carcass [4]. After staying in a pool of blood or feeding from stains of blood, flies randomly walk over surfaces that may not be covered with blood, leaving behind tiny bloodstains of tarsi prints [4]. These stains may originate from pure blood or fly artefacts initially deposited during defecation or regurgitation and it may look like stains formed by the incidents of a bloodletting event [23]. While carpet-based surfaces yielded no supporting information regarding the possibility of contamination by blow fly tarsi, artefacts deposited by fly tarsi have been noted on other surfaces [2]. Durdle *et al.*, [24] observed tiny fly deposits that

were understood to be caused by fly tarsi on surfaces with loosely woven or multidirectional fibers. Mistaken analysis and interpretation of the tarsi marks as true spatter may possibly lead to an inaccurate reconstruction of events since an investigator with no formal training in fly artefacts may be easily misled into thinking it was deposits of high impact velocity spatter [23, 38].

2.6.3 Artefacts caused by regurgitation (vomit spots)

One feeding mechanism adapted by adult blowflies is withdrawing blood using the proboscis and regurgitating it onto a surface to allow for external digestion so that on return, the digested portion can be sucked up [2, 39]. When flies regurgitate food supplements, for example blood ingested from a crime scene, 'spatter-like' stains known as regurgitation spots or vomit spots may be deposited on the nearby crime scene surfaces [6, 31].

According to Byrd & Castner [4], the regurgitated spots accumulate as a medium to large droplet at the tip of the spongy mouthparts of the blow fly. When the regurgitated spot comes into contact with the surface, the result is a small stain, usually less than 1mm exhibiting a symmetrical conformation with little or no tail [2, 4]. In a study conducted by Zuha *et al.* [31], the size of these stains was determined to be in the range of 1mm to 2mm. However, regurgitated spots are not so obvious to the investigating officers with no background in fly artefacts although they may appear lighter than the surrounding true blood spatter [4]. While this discovery was a step in the right direction towards differentiating fly artefacts from true bloodstains, judgment based on brightness differences is a subjective issue and may not be relied upon by the courts of law. Benecke & Barksdale [23] made observations from a number of regurgitation or vomit spots at a crime scene that regurgitation spots may

show evidence of craters. This is one of the criteria which may be used to differentiate fly artefacts from legitimate bloodstains. However, the crater-like feature on true bloodstains has been noticed on several occasions as a result of drying. The presence of crater therefore may not accurately determine whether what looks like a bloodstain at a crime scene is a fly artefact or a legitimate bloodstain.

2.6.4 Artefacts caused by defecation

When a blow fly arrives at the crime scene, it defecates on the nearby surfaces while resting and wandering about the scene. The faecal matter deposited by the blowflies after feeding on bloodstains may, when partially digested, appear to resemble blood [22]. Such stains can be easily confused with bloodstains produced by a high impact velocity as the diameter is usually 1 millimeter or less [23], Zuha *et al.* [31] described fly artefacts as light coloured artefacts in the range of 0.5 mm to 4 mm often with raised morphology. The most worrisome fact about these artefacts is that while it looks like bloodstains deposited at the crime scene, the location and directionality is often inconsistent with other bloodstains resulting from the events leading to bloodshed and as a result it cannot be related to the sustained injuries when reconstructing a crime scene [23]. These stains have been recovered on items like lamp shades, ceilings, and on the body and on the clothes of the victim [7, 23]. Defecated fly artefacts can be described as trails that reveal directionality of a fly's movement, sometimes morphologically similar to an elongated comma shape or tear-drop bloodstains [2, 4].

2.7 Identification of fly artefacts on a crime scene

For proper crime scene reconstruction, an informed identification and clear demarcation between true blood spatter and fly artefacts must be determined. Numerous methods have been developed to positively identify a number of artefacts produced by insect activity at a crime scene. According to Langer & Illes [40], methods of identifying insects stains can be classified into three categories of analysis namely: visual, contextual and chemical. Currently, the developed methods of detection rely mostly on physical properties such as the size of the stains, while some rely on chemical properties. However, these methods of identification cannot be applied to all the artefacts and as such further methods need to be developed [4].

Benecke & Barksdale [23] stated that some fly artefacts have a sperm or tadpole-like structure whose tail's length (Ltl) is greater than the body's length (Lb). The directionality of these stains is random. Although a tail/body length ratio (Ltl/Lb) of 1 or less for a bloodstain has been noted to signify a true blood spatter, a ratio less than 1 does not necessarily confirm that a stain is a true blood spatter since some fly artefacts have been observed to have a Ltl/Lb ratio less than 1 [23].

2.8 Conclusion

Blowflies are attracted to the crime scene where blood has been deposited and upon arrival it deposits excrements which present a potential contamination source. The investigators of violent crimes therefore need to be aware of the possibilities that a crime scene may in fact be staged to redirect the investigations or it may be altered by the flies and other arthropods that feed on the decomposing body itself or on the blood stains. It is

therefore necessary to develop a method that will be able to accurately characterize even a single and isolated bloodstain-like spot as either a true blood spatter caused by application of force or an artefact produced by flies. This will enable investigators not only to accurately reconstruct the events of a crime scene but also to reconstruct arrangement of the bloodied items that may have been moved after the incident in the case of staged crimes.

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Chapter 3: Publication-ready Manuscript

ABSTRACT

Violent crimes involving bloodshed may result in bloodstains spattering on the surrounding surfaces. Accurate analysis of bloodstains at the scene can provide investigators with insights into the series of events that occurred during the execution of a violent crime. At a crime scene, blowflies feed on bloodstains and deposit artefacts that may cause confusion during crime scene reconstruction. Experimental cages of two possible crime scene surfaces (paper to simulate wallpaper and linoleum) were developed such that fly artefacts can be characterized and differentiated from legitimate bloodstains. Pooled bloodstains were created within the experimental cages and blowflies were allowed access to the cages. A total of 10739 and 740 artefacts deposited on paper surfaces and linoleum walls respectively were examined. Clear differences between fly artefacts and legitimate bloodstains were noted and unique characteristics of fly artefacts resembling true blood spatters with a possibility of confounding crime scene reconstruction were distinguished.

Keywords: *Forensic Science; Forensic entomology; fly artefacts; crime scene reconstruction; bloodstain pattern analysis; blowflies, violent crimes.*

Introduction

Violent crimes occasionally involve bloodshed resulting in bloodstains spattering on the surrounding crime scene surfaces [1]. The bloodstains may have come from the perpetrator(s), the victim(s) or both and as such it can be used to provide a link between the perpetrator and the victim of the crime if carefully analyzed and interpreted [2]. While DNA analysis of the bloodstains found at the crime scene can be used to identify and individualize the donors of the biological forensic samples, the physical characteristics of the bloodstains such as the size, colour, shape and the distribution patterns can be used to reconstruct the events that took place during commission of the crime [3]. Bloodstains allow investigators to determine the number of possible assaults inflicted upon the blood source, the likely position of the bloodstain donor during the attack and the type of the weapon used [4]. Accurate analyses of bloodstain patterns at a crime scene may therefore ascertain the offender's motives and give insights into the executed crime, consequently proving to a certain extent, intent to do lethal harm [5].

During bloodshed or immediately after death, blood escapes from the circulatory system and is exposed to the population of microorganisms in the surrounding environment. Microbial interaction with blood yields thousands of volatile chemicals, some of which are natural attractants of blowflies. Incidentally, blowflies have been documented to respond to the decomposition odour originating from up to 63.5 km away [6]. Blowflies by virtue of being attracted to the chemicals produced immediately after death or during bloodshed, ultimately become the first insects to detect and lay eggs on a decomposing biological system. The presence of blow fly eggs or larvae on a crime scene may therefore be helpful in death investigations, specifically when estimating the minimum time since death [7].

Upon arrival at violent crime scenes, female blowflies search for a suitable oviposition site where they lay hundreds of eggs [8]. During such events, blowflies feed on the body as well as on blood present at the crime scene. When insect activity is prevalent, bloodstain pattern analysis and the interpretation of events that occurred during the commission of a crime may become problematic. This is because blowflies feed on both pooled and spattered bloodstains using their proboscis, subsequently regurgitating and defecating on crime scene surfaces [3, 4]. As such, blowflies may alter the original morphological appearance of bloodstain patterns presented by the events of the crime [9]. Furthermore, regurgitation and defecation processes introduce new and additional spatter-like fly artefacts which may complicate the interpretation of the bloodstain evidence. These artefacts may range from 0.5 mm mist-like spatter to about 20 mm large artefacts [10]. Fly artefacts may appear similar to blood spatter produced as a result of application of force to a blood source and as such may result in incorrect conclusions being drawn from a crime scene if mistakenly considered as legitimate bloodstains [8].

Local crime scene investigators often only receive limited training pertaining to the protocol of sample recovery, and often no training regarding the identification of fly artefacts at a crime scene [11]. A further problem associated with fly artefacts is that typical presumptive tests and DNA analysis are not able to distinguish fly artefacts from true blood spatter [3, 12]. Therefore visual inspection, contextual, and chemical analyses of fly artefacts are currently the only conceivable means of differentiating fly artefacts from true blood spatter [6, 13]. However, such tests are still not conclusive. These artefacts can sometimes be found in different rooms or several meters away from the location where the bloodletting event has occurred, thereby making identification even more difficult [3]. Inclusion of fly artefacts in selected bloodstains for crime scene reconstruction may lead to inaccurate reconstruction of the crime scene events.

The consequences of flies at the crime scene and how they can eventually impinge upon the reconstruction of a crime scene if misinterpreted as true spatter, and the role played by the interpretation of bloodstain patterns in crime scene reconstruction are known. However, in South Africa, there are no readily developed methods to conclusively differentiate between legitimate blood spatter caused by force and blood spatter-like fly artefacts. Therefore, mistaken analyses of the fly artefacts as true spatter may be interpreted as medium to high impact velocity blood spatter [8]. The flies' ability to alter bloodstain patterns worsens the already limited investigative skills in crime scene reconstruction from bloodstains. As such, the presence of flies at the crime scene must be cautiously considered by the crime scene investigators [14].

The objectives of the current study were to examine and analyze artefacts produced by fly activities on different substrate surfaces.

Materials and methods

Benchkote paper experimental cage design

Five cages were constructed from Whatmann®Benchkote® paper (Sigma-Aldrich, Johannesburg, South Africa) which is typically used for the protection of laboratory benches. It has a smooth non-absorbing outer surface and a rough absorbing inner surface. Cages were constructed such that flies were in contact with the absorbing side of the material. Each cage consisted of five surfaces (top, back wall, two side walls and the floor) each having dimensions of 24 x 24 cm. The front of each cage was covered in a clear plastic to allow light to enter the cages and to aid in viewing, as such this surface was not included in any analysis. To improve analysis each examined surface was divided into 36 (4x4cm) squares.

Linoleum experimental cages

Four cages were constructed from linoleum sheets. Linoleum is a smooth plastic, non-porous surface often used as flooring in houses. The cages were prepared in the same manner as the Benchkote paper cages.

Specimen collection

Porcine internal organs obtained from a local abattoir were set as bait for blow fly eggs at the Medical Research Council (MRC) in Delft, Cape Town. The collected eggs were placed in a sterile plastic bottle and were transported to the forensic entomology laboratory at the University of Cape Town, Department of Pathology where they were reared at 30 ± 2°C and ambient humidity on chicken liver until they developed into flies.

Emerging flies were transferred to a 24cmx24cmx24cm rearing cage. The flies were fed white granulated sugar and water for 24 hours *ad libitum*. The water was provided to the flies by soaking a paper towel into an 8cm x11cm water filled tub so that flies did not drown. This was done to allow for flies to deposit their creamy meconium before they were introduced into the experimental cages to avoid confusion of fly artefacts with meconium deposition. After 24 hours, the flies were only fed water for the next 12 hours to allow them to clear their crop contents.

Artefact analysis

Ten randomly chosen blowflies were introduced into each experimental cage. The cages were kept in a laboratory incubator at a temperature ranging from 24.1°C to 24.6°C and a humidity ranging from 52.0% to 53.4%. A 12.7cm diameter dish containing 6ml of fresh pooled porcine blood was presented into the experimental cages as the sole source of nutrients. Additives and preservatives were not added to the blood such that it clots normally, thus

mimicking the possible bloodstains on the crime scene. The cages were subjected to 12 hours light-dark cycles and were monitored every six hours. After 24 hours the experiments were ended and the experimental cages were dismantled so that fly artefacts could be categorized, measured and photographed. Mid-range and close-up photos of the fly spots were taken using an EOS 650D canon camera, fitted with a 100mm macro-lens and a canon MR-14EX II Macro Ring Lite. Artefacts on each surface were counted and analyzed according to their morphological characteristics such as size, colour and shape. The Drylac® RAL colour chart was used as a reference standard.

A total of 30 artefacts for each of the seven determined artefacts colours from each cage were randomly selected and measured for their lengths and widths as shown on fig 3.1 shown below. Thus, the dimensions (length and width) of 1050 and 749 randomly chosen artefacts were measured from the five paper cages and four linoleum cages respectively.

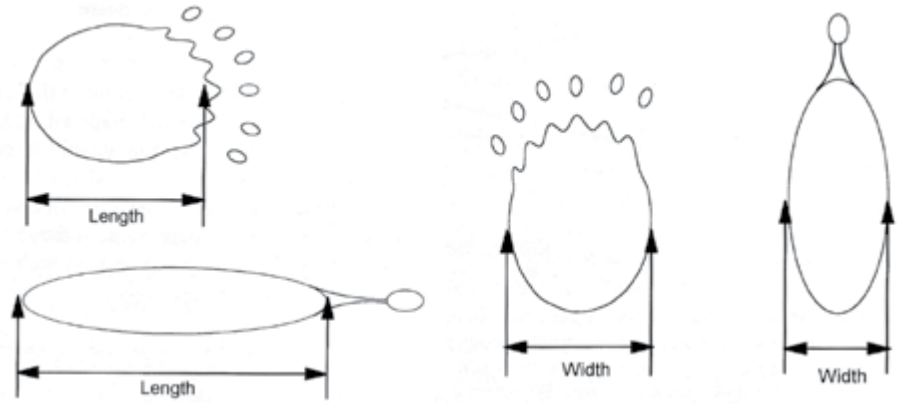

Figure 3.1: Determination of the length and width measurements of the artefacts

Statistical analysis

Shapiro-Wilk test was used to test whether the data followed a normal distribution. When data was normally distributed, ANOVA (analysis of variance) was performed. Bartletts test was performed to test whether the variances are equal. When the variances are equal or not equal, a respective two-sample t-test with equal variances or a two-sample t-test with unequal variances was carried out to determine the significant difference. However, when the data did not follow normal distribution, Kruskal-Wallis equality of populations rank test was used to determine if there are statistically significant differences between two or more groups of an independent variable.

The quadrat method or test was used to determine the complete spatial randomness (CSR) or clustering (Appendix A).

Ethical clearance for this study was obtained from the animal research ethics committee at the Faculty of Health Sciences, University of Cape Town.

Results and discussion

Benchkote paper

A total of 10739 fly artefacts were deposited on the Benchkote paper experimental cages, this included very tiny spots, possible tarsi marks. Defecation spots constituted 94.87% (10188) of the spot sand ranged between 2.02 – 11.30mm in diameter. These artefacts were not raised and exhibited three distinct lighter colours; saffron yellow, red orange and coral red as shown on Fig.3.2.

Figure 3.2: Morphology and colour of defecated blow fly spots A. red orange and saffron yellow, B. coral red spot.

The characteristics of the lighter artefacts matched with the faecal spot characteristics described by Zuha *et al.* [15]. The defecated artefacts were either round, oval or tear drop-like spots with rugged edges.

Regurgitation or vomit artefacts accounted for 5.13% (551/10739) of the total number of spots. Examples of different regurgitation artefacts observed can be seen in Fig. 3.3. These spots often displayed raised or ball-like morphological characteristics while some exhibited a crater roughly in the centre of the artefact. The presence of craters on the raised fly artefact is associated with the sucking activity of the blow flies during regurgitation.

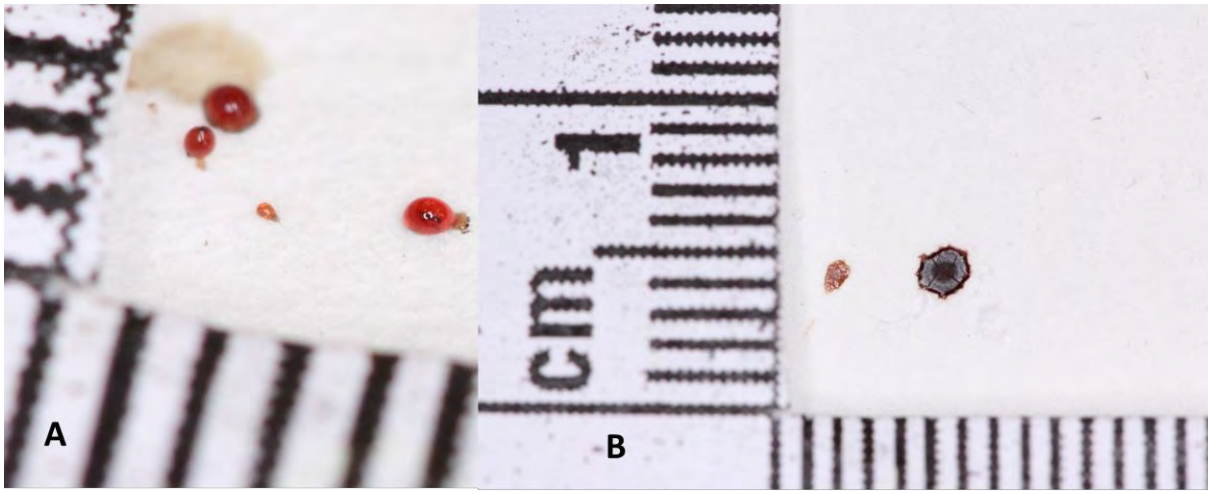

Figure 3.3: Regurgitated ball-like fly artefact (A) and a cratered artefact that may look similar to dried legitimate bloodstain (B)

Some observed stains had a flat perimeter whereas some fly artefacts had thickly raised boundaries with a crater-like structure. Similar stains were also reported by Zuha *et al.* [15]. Cratered bloodstains have also been associated with true blood spatter on surfaces where absorption is limited and this is linked to the drying process [16]. Cratered thickly raised fly artefacts at a crime scene may therefore be easily confused with true bloodstains that possess a cratered or concave centre due to the drying process.

The artefacts on both surface types were not uniformly distributed on the different walls; empty spaces and clusters of artefacts were seen as depicted on Table 3.1 and Figure 3.4 below.

Table 3.1: Complete spatial randomness test for defecated and regurgitated fly artefacts.

Cage type	Surface	VTMR	
		Defecation	Regurgitation
Benchkote	Floor	479 – 2313	18 - 101
	Left	93 – 763	5 – 17
	Right	64 – 293	0 - 36
	Back	160 – 760	0 - 22
	Top	2 – 54	1 - 16
Linoleum	Floor	2 – 76	27 - 92
	Left	2 – 23	9 - 32
	Right	2 – 11	10 - 25
	Back	4 – 7	7 - 15
	Top	0 – 5	1 - 23

*VTMR (variance to mean ratio of the number of spots per quadrat) greater than one indicates clustering

Random distribution was only seen on the top walls of both surface types; on all other walls clustering was noted. The fly artefacts appeared to be clustered on the bottom portion of the left, right and back walls, as well as around the blood source on the floor. This supports the notion that flies randomly disperse its artefacts on the surfaces away from the blood source while they may cause a cluster of artefacts on the nearby surfaces [6]. Fig. 3.4 shows a clustering effect as seen on the different surfaces and a random pattern on the top surface.

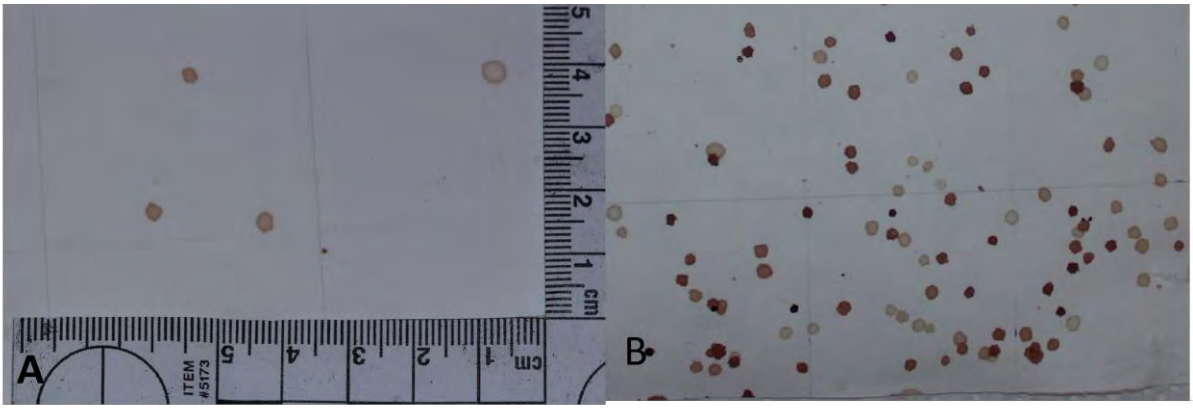

Figure 3.4**:** Spatial randomness of the fly artefacts on different surfaces; a random distribution of fly artefacts on the top surface (A) and a cluster at the back wall (B).

Unlike defecated spots, regurgitation spots were determined by Kruskal Wallis test to be significantly smaller in size ($p<0.001$), ranging between 0.34 – 4.42mm in diameter. Also, a two-sample Wilcoxon rank sum (Man-Whitney) test also revealed a significant difference between the spot sizes on paper and linoleum cages, both in terms of length and width ($p<0.001$) whereby the artefacts on paper cages were significantly larger. Regurgitated artefacts had a raised, smooth and darker perimeter. The colours of regurgitated spots were seen as brown red, wine red, black red and black. Colour determination of the fly artefacts was inevitably subjective; however a reference chart was used to minimize subjectivity (Drylac® RAL colour chart).

Within the Benchkote test cages, floor surfaces had the greatest mean number of both defecated and regurgitated spots (1265 ± 670 and 59 ± 33 respectively) while the top had the lowest mean number of both defecated and regurgitated spots (23 ± 19, 8 ± 5 respectively). Using Kruskal-Wallis equality-of-population rank test, there was a significant difference between the number of defecated spots on different walls (roof, back, floor and the sides) ($p=0.0009$). A significant difference between the number of regurgitation spots on different walls (roof, back, floor, sides) ($p=0.0264$) was also observed. The blood source was placed on the floor. Thus, the floor was the closest surface to the blood source whereas the roof was

the furthest surface. While fly artefacts have been found in rooms away from the room where a violent crime occurred [6], the results imply that it is more likely for the crime scene investigators to find possible fly artefacts on the surfaces adjacent to the blood source than on surfaces very far from the blood source. The sides and the back surfaces possibly by virtue of being closer to the blood source had a higher mean number of fly artefacts than the roof (Table 3.2).

Table 3.2: The number of fly spots on different surfaces of Benchkote paper cages.

Fly artefact	Surface	N	Mean	SD
Defecation spots	Back	5	332	243
	Floor	5	1265	670
	Left	5	256	276
	Right	5	162	95
	Top	5	23	18
Regurgitation Spots	Back	5	10	6
	Floor	5	59	33
	Left	5	15	12
	Right	5	17	16
	Top	5	8	5

There was no significant difference between both the median number of defecation ($p=0.87$) and regurgitation ($p=0.21$) spots between different cages within the Benchkote paper experimental cages.

Linoleum cages

A total of 740 spots were counted from the four experimental cages and 321 (43%) were defecation spots while 419 (57%) were noted as regurgitation spots. Defecation spots had lighter colours (saffron yellow, red orange and coral red) while regurgitated spots had deeper colours (brown red, wine red, black red and black). Defecated spots ranged from 0.22 – 10.00mm while regurgitated spots were generally smaller exhibiting a size range of 0.28 – 5.96mm. Unlike with the Benchkote tests, applying a two-sample Wilcoxon rank sum (Mann Whitney test), this difference was not statistically significant ($p=0.37$). The reason for this may be due to the more liquid consistency of defecation spots in comparison to regurgitation spots [4]. The porous nature of the Benchkote paper thus allowed greater absorption of the defecation spots resulting in a greater size than the less liquid regurgitation spots. Linoleum in comparison does not allow absorption and thus overlap exists in the size ranges of defecation and regurgitation spots [10].

The mean diameter of the spots deposited on linoleum surfaces was determined by Mann Whitney test to be significantly smaller ($p<0.001$) than the spots deposited on paper cages both in terms of mean length and width (Table 3.3). Again, this may be explained by the porous and non-porous nature of the two materials studied.

Table 3.3: Comparison of the sizes of fly artefacts on linoleum and Benchkote surfaces.

Material	Mean length (mm)	SD length(mm)	Mean width (mm)	SD width(mm)
Linoleum	1.11	0.29	0.93	0.28
Benchkote	2.79	0.61	2.35	0.55

The inner surface of the Benchkote paper is capable of absorbing fluids, thus allowing for spreading or diffusion of fluids. It has tiny protruding loosely packed fibers that have a tendency of collecting minute amounts of blood from the bloody items. The absorbency of the Benchkote paper allowed the deposited fly excrement to spread in all directions leading to increased size of the spots. This has previously been noted in other experiments utilizing porous surfaces [10], and demonstrates the importance of considering the type of surface when dealing with bloodstain patterns in crime scene reconstructions. The fly artefacts on paper surfaces, especially the defecated spots, had rugged edges while the artefacts on linoleum surfaces had smooth edges. That is probably because defecation spots are liquid faecal excrement, while regurgitation spots are a bit of a gel-like matter capable of forming raised artefacts that can hardly diffuse in any direction even on an absorbing surface like Benchkotepaper. The rugged edges were possibly formed when the deposited fluid excrements were dissipating at irregular rates in different directions on an absorbing surface.

A large number of fly artefacts were deposited on the paper cages than on linoleum cages. Four cages were made of linoleum surfaces whereas five were constructed out of Benchkote paper, the mean number of fly artefacts on linoleum surfaces per cage (185) was less than the mean number of fly artefacts on paper surfaces (2148). Shapiro-Wilk test revealed a normally distributed data and a variance ratio test demonstrated a significant difference between the number of fly artefacts deposited on paper cages and linoleum surfaces ($p=0.0002$). A two sample t-test with unequal variances showed that there is significant difference between the total number of fly artefacts deposited on paper and linoleum cages. While the same quantity of blood (6ml) was used in both cases, in the case of linoleum cages a pool of blood was created by spreading the blood over a larger surface. This was done to limit the number of flies drowning in the blood pool as was noticed in the Benchkote experiments. The pool of blood created in linoleum cages as a result dried within the first two hours of the experiment.

The flies in linoleum cages consequently had limited access to liquid blood hence fewer artefacts were deposited in the cages.

While possible tarsi marks were noticed in the paper cages, none were seen in the linoleum cages. In a study conducted by Striman *et al.* [4], no evidence of blood tracking by fly tarsi was seen and it was suggested that the tarsi could not break the surface tension of the blood. The possible tarsi marks in the paper cages therefore may have been formed when a bloodied part of the flies come into contact with the loose protruding fibers on the surfaces of the paper cages.

Swiping stains are fly artefacts that exhibit a distinguishable head and tail (Figure. 3.5). It may have a flat or a raised perimeter and it is formed by fly activities, either defecation or regurgitation [15]. Twelve artefacts (1.2%) on linoleum surfaces and 13 (0.12%) spots from the Benchkote paper cages were swiping. All of the swiping stains on linoleum surfaces had the Ltl/Lb ratio greater than 1. An Ltl/Lb ratio less than 1 has been used to indicate true blood spatters [6], however six swiping stains from Benchkote paper had Ltl/Lb ratio less than 1. The Ltl/Lb< 1 therefore should not be used as a conclusive proof that a stain is legitimate blood spatter produced by impact force. Three of the swiping stains from linoleum surfaces had the raised morphology indicative of regurgitation spots; raised borders with a crater or a dimple-like structure in the middle of the spot. The dimples are the result of the sucking activity of the flies [4, 8, 15].

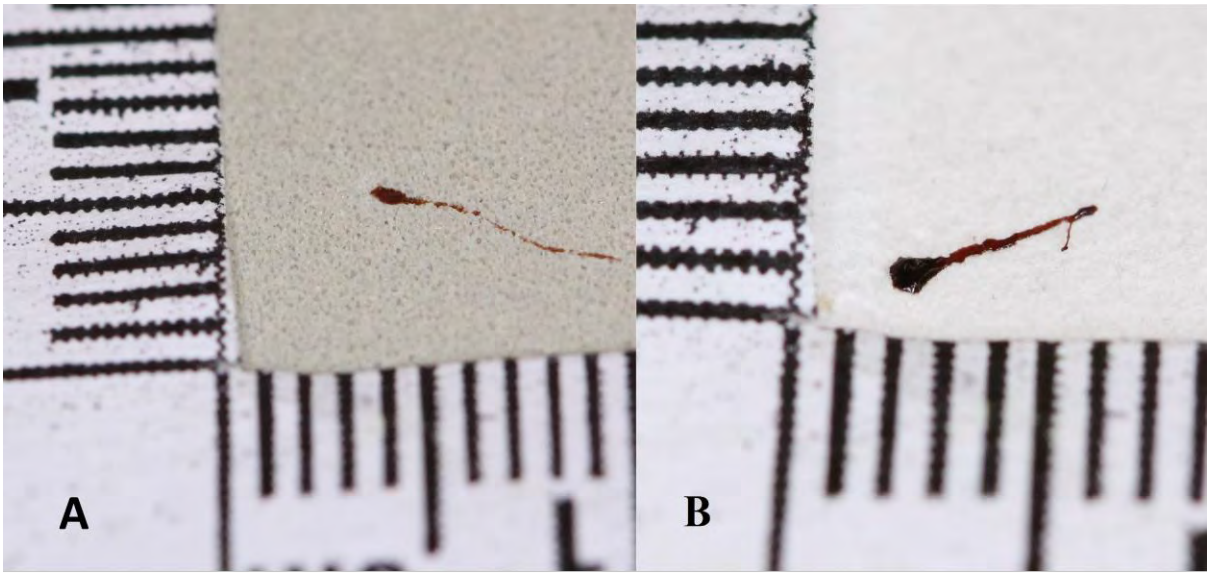

Figure 3.5: Sperm-like swiping stains caused by defecation (A) and regurgitation (B)

The tear-drop like stain was seen in all cages and it appeared to be true blood spatter revealing a possible direction of flow. When true bloodstains are inspected as a group, it reveals directionality and a possible point of impact or convergence [9]. However, the directionality of the fly artefacts was random and led to no possible point of convergence (Fig. 3.5). Random directionality with expirated spatter has however also been noted and should be examined carefully not to be confused with fly artefacts [4].

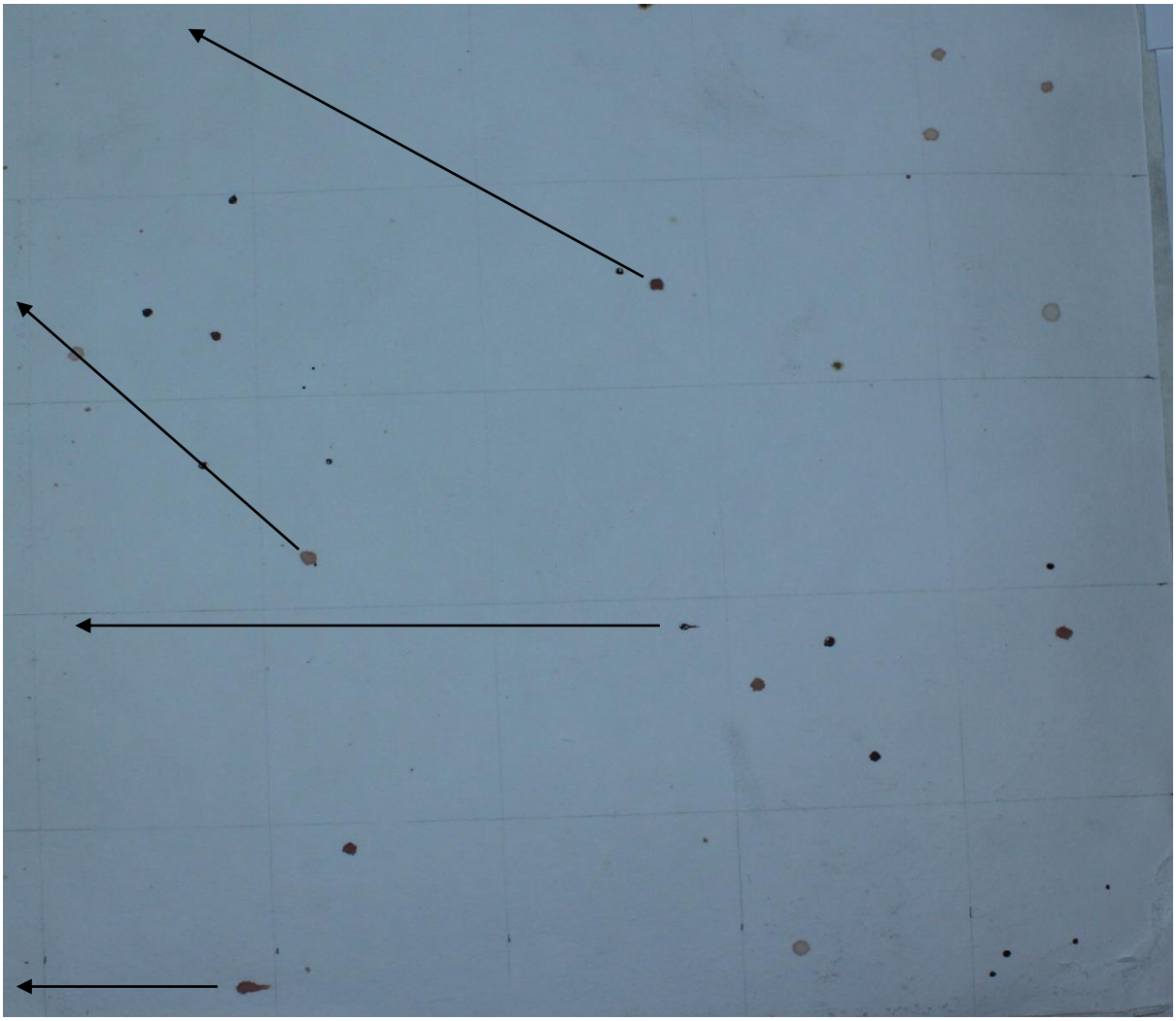

Figure 3.5: Random directionality with no point of convergence as revealed by fly artefacts.

Limitations and suggestions for further research

The current study utilized only two different surfaces; however every surface type may exhibit fly artefacts in different ways. It is therefore important to study more surfaces in future studies. The authors chose a mix of different species without focusing on the artefacts produced by a single type of species as has been done in previous research. The reason for this is that it is highly improbable that a single species of blow fly would be present at a crime scene. Comparisons between the Whatman®Benchkote paper are linoleum experiment

are however invalidated. Mis-categorization may have affected the defecation and regurgitation percentages which are seemingly unbalanced.

Conclusion

Blowflies feed on a pool of blood and deposit artefacts that vary in morphological appearance, size and colour. The majority of observed artefacts had a flat perimeter whereas some fly artefacts had thickly raised boundaries. A total of 11749 fly artefacts were characterized. While defecated artefacts displayed lighter colours (saffron yellow, red orange and coral red) that can be easily distinguished from legitimate bloodstains, regurgitated artefacts were darker and are more likely to be confused with aged bloodstains. Bloodstains therefore must be analyzed in the context of the crime scene before it can be regarded as individual possible spatter. This will eliminate the possibilities of mistakenly including fly artefacts in the number of bloodstains to be used for crime scene reconstruction.

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Chapter 4

Appendices

Appendix A

Variance-to-mean ratio (VTMR) and complete spatial randomization CSR calculations.

The total number of squares on each surface (n) is 36 and the diameter of the lid is 12.7 cm. Each square on the surface is 4cm x 4cm.

Area covered by the lid:

$$A = \pi r^2$$

$$A = 6.35^2\pi$$

$$\boldsymbol{A = 126.6cm^2}$$

The approximate total number of squares covered by the lid (N_L)

$$\boldsymbol{N = A/16cm^2}$$

$$\boldsymbol{N = 7.91}$$

$$\mathbf{N_L \approx 8}$$

Therefore the number of squares on the floor surfaces (N_F) will be given by subtracting the total number of squares on the floor or any surface minus the approximate number of squares covered by the lid (N_L).

$$\mathbf{N_F = 36 - N_L}$$

$$\mathbf{N_F = 36 - 8}$$

$$\mathbf{N_F = 28}$$

Variance (σ^2):

$$\sigma^2 = \frac{\sum x^2 - (\frac{\sum x}{n})^2}{n-1}$$

Where n is the number of squares on the surface.

Mean number of artefacts per quadrat (μ):

$$\mu = \frac{Total\ number\ of\ spots\ on\ the\ entire\ surface\ (X)}{Available\ number\ of\ the\ squares\ on\ the\ surface}$$

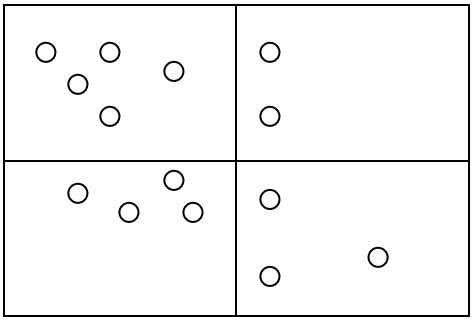
Variance-to-mean ratio (VTMR) (D)

$$D = \frac{\sigma^2}{\mu}$$

A VTMR greater than one indicates clustering

Example: Consider the random area below. The area is divided up into four quadrats as seen.

Visually it appears that there may be clustering toward the left of the area.



$$\sigma^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}$$

$$\sigma^2 = \frac{\left(5^2 + 2^2 + 4^2 + 3^2 - {13^2}/{4}\right)}{4 - 1}$$

$$\sigma^2 = 3.92$$

Mean number of artefacts per quadrat (μ):

$$\mu = \frac{Total\ number\ of\ spots\ on\ the\ entire\ surface\ (X)}{Available\ number\ of\ the\ squares\ on\ the\ surface}$$

$$\mu = \frac{13}{4}$$

$$\mu = 3.25$$

Variance-to-mean ratio (VTMR) (*D*)

$$D = \frac{\sigma^2}{\mu}$$

$$D = \frac{3.92}{3.25}$$

$$D = 1.2$$

This indicates a weak level of clustering.

Interpretation of VTMR calculations:

VTMR = 0 Not dispersed

0 < VTMR < 1 Under dispersed

VTMR > 1 Over-dispersed

Appendix B

Instruction to Authors

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Abstract and Keywords

Abstracts should be no more than 150 words. This journal uses unstructured abstracts; however, the abstract should include the following – background, brief description of methods and results (give specific data and their statistical significance, if possible), and

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Authors should provide a minimum of six keywords that will assist indexers in cross-indexing the article and that may be published with the abstract. The **first keyword must be forensic science**; the second and subsequent words should assist abstracters in properly categorizing the work so that it will be found in journal article data bases by interested researchers. Frequently, the second keyword represents a subfield of forensic science, e.g. forensic anthropology, forensic pathology, or DNA typing.

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Statistics

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals).

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Acknowledgements

The Acknowledgements section immediately precedes the Reference list. Here, specify contributions that need acknowledging but do not justify authorship, such as general support by a department chair or acknowledgments of technical help. Persons who have contributed intellectually to the paper but whose contributions do not justify authorship may be named and their function or contribution described – for example, 'scientific adviser,' 'critical review of study proposal,' 'data collection,' or 'participation in clinical trial.' Such persons must have given their permission to be named. The Acknowledgements header should be italicized.

Authors are responsible for obtaining written permission from persons acknowledged by name, because readers may infer their endorsement of the data and conclusions. Technical help should be acknowledged in a paragraph separate from those acknowledging other contributions.

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Number references consecutively in the order in which they are first mentioned in the text. Identify references in tables, and legends by Arabic numerals. References cited only in tables or legends should be numbered in accordance with a sequence established by the first identification in the text of the particular table or figure. Within the text, tables or figures, cite references by Arabic numeral in parentheses. Within the reference list, number the references 1., 2., 3., etc.

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Articles in Journals

1. Standard journal article (List all authors, but if the number exceeds six, give six followed by et al.)

> You CH, Lee KY, Chey RY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980; 79(2):311-4.

As an option, if a journal carries continuous pagination throughout a volume, the month and issue number may be omitted.

> You CH, Lee KY, Chey RY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980; 79:311-4.

> Goate AM, Haynes AR, Owen MJ, Farrall M, James LA, Lai LY et al. Predisposing locus for Alzheimer's disease on chromosome 21. Lancet 1989; 1:352-5.

2. Organization as author

> The Royal Marsden Hospital Bone-Marrow Transplantation Team.Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977;2:742-4.

3. No author given

> Coffee drinking and cancer of the pancreas [editorial]. BMJ 1981;283:628.

4. Article not in English

Massone L, Borghi S, Pestarino A, Piccini R, Gambini G. Localisationspalmairespurpuriques de la dermatiteherpetiforme. Ann DermatolVenereol 1987;114:1545-7.

5. Volume with supplement

Magni F, Rossoni G, Berti F. BN-52021 protects guinea-pig from heart anaphylaxis. Pharmacol Res Commun 1988;20Suppl 5:75-8.

6. Issue with supplement

Gardos G, Cole JO, Haskell D, Marby D, Paine SS, Moore R.The natural history of tardive dyskinesia.JClinPsychopharmacol 1988;8(4 Suppl):31S-37S.

7. Volume with part

Hanly C. Metaphysics and innateness: a psychoanalytic perspective. Int J Psychoanal 1988;69(Pt 3):389-99.

8. Issue with part

Edwards L, Meyskens F, Levine N. Effect of oral isotretinoin on dysplastic nevi. J Am AcadDermatol 1989;20(2 Pt 1):257-60.

9. Issue with no volume

Baumeister AA. Origins and control of stereotyped movements.Monogr Am AssocMentDefic1978;(3):353-84.

10. No issue or volume

Danoek K. Skiing in and through the history of medicine. Nord MedicinhistArsb 1982;86-100.

11. Pagination in roman numerals

Ronne Y. AnsvarsfallenBlodtransfusion till fel patient.Vardfacket 1989;13:XXXVI-XXVII.

12. Type of article indicated as needed

Spargo PM, Manners JM. DDAVP and open heart surgery [letter].Anaesthesia 1989;44:363-4.

13. Article containing retraction

Shishido A. Retraction notice.Effect of platinum compounds on murine lymphocyte mitogenesis [Retraction of Alsabti EA, Ghalib ON, Salem MN. In: Jpn J Med SciBiol 1979;32:53-65]. Jpn J Med SciBiol 1980;33:235-7.

14. Article retracted

Alsabti EA, Ghalib ON, Sale MN. Effect of platinum compounds on murine lymphocyte mitogenesis [Retracted by Shishido A. In: Jpn J Med SciBiol 1980;33:235-7]. Jpn J Med SciBiol 1979; 32:53-65.

15. Article containing comment

Piccoli A, Bossatti A. Early steroid therapy in IgA neuropathy: still an open question [comment] Nephron 1989;51:289-91. Comment on: Nephron 1988;48:12-7.

16. Article commented on

Kobayashi Y, Fujii K, Hiki Y, Tateno S, Kurokawa A, Kamiyama M. Steroid therapy in IgA neuropathy: a retrospective study in heavy proteinuric cases [see comments]. Nephron 1988;48:12-7. Comment in: Nephron 1989;51:289-91.

17. Article with published erratum

Schofield A. The CAGE questionnaire and psychological health [published erratum appears in Br J Addict 1989;84:701]. Br J Addict 1988;83;761-4.

Books and Other Monographs

18. Personal author(s)

Colson JH, Armour WJ. Sports injuries and their treatment. 2nd rev. ed. London: S. Paul, 1986.

19. Editor(s), compiler as author

Diener HC, Wilkinson M, editors.Drug-induced headache. New York: Springer-Verlag, 1988.

20. Organization as author and publisher

Virginia Law Foundation.The medical and legal implications of AIDS. Charlottesville: The Foundation, 1987.

21. Chapters in a book

Weinstein L, Swartz MN.Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editors. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders, 1974;457- 72.

22. Conference proceedings

Vivian VL, editor. Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference on Child Abuse and Neglect; 1984 Mar 30-31; Chicago. Chicago: American Medical Association, 1985.

23. Conference paper

Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB, Kaye SV, editors.

Indoor air and human health.Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (Ml): Lewis, 1985;69-78.

24. Scientific or technical report

Akutsu T. Total heart replacement device. Bethesda (MD): National Institutes of Health, National Heart and Lung Institute; 1974 Apr. Report No.: NIH-NHLI-691 218514.

25. Dissertation

Youssef NM. School adjustment of children with congenital heart disease [dissertation]. Pittsburgh (PA): Univ. of Pittsburgh, 1988.

26. Patent

Harred JF, Knight AR, McIntyre JS, inventors.Dow Chemical Company, assignee.Epoxidation process. US patent 3,654,317. 972 Apr 4.

Other Published Material

27. Newspaper article

Rensberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7; Sect. A:2 (col. 5).

28. Audiovisual

AIDS epidemic: the physician's role [videorecording]. Cleveland (OH): Academy of Medicine of Cleveland, 1987.

29. Computer file

Renal system [computer program].MS-DOS version. Edwardsville (KS): MediSim, 1988.

30. World Wide Web address or URL

http://www.uocf.edu/pharmacy/depts/drugdose/barbitutuates/index.html

31. Legal material

Toxic Substances Control Act: Hearing on S. 776 Before the Subcomm. on the Environment of the Senate Comm. on Commerce. 94th Cong., 1st Sess. 343 (1975).

32. Map

Scotland [topographic map]. Washington: National Geographic Society (US), 1981.

33. Book of the Bible

Ruth 3:1-18. The Holy Bible. Authorized King James version. New York: Oxford Univ. Press, 1972.

34. Dictionary and similar references

Ectasia. Dorland's illustrated medical dictionary. 27th ed. Philadelphia: Saunders, 1988;527.

35. Classical material

The Winter's Tale: act 5, scene 1, lines 13-16. The complete works of William Shakespeare. London: Rex,1973.

Unpublished Material

36. In press

Lillywhite HD, Donald JA. Pulmonary blood flow regulation aquatic snake. Science.In press.

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Tables should be submitted as separate files. Do not submit tables as photographs. Number tables with Arabic numerals consecutively in the order of their first citation in the text and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all nonstandard abbreviations that are used in each table. For footnotes use the following symbols, in this sequence: *,†,‡,§,((,¶,**,††,‡‡.

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Appendix C

Fly artefacts deposited after interaction with bloodstains

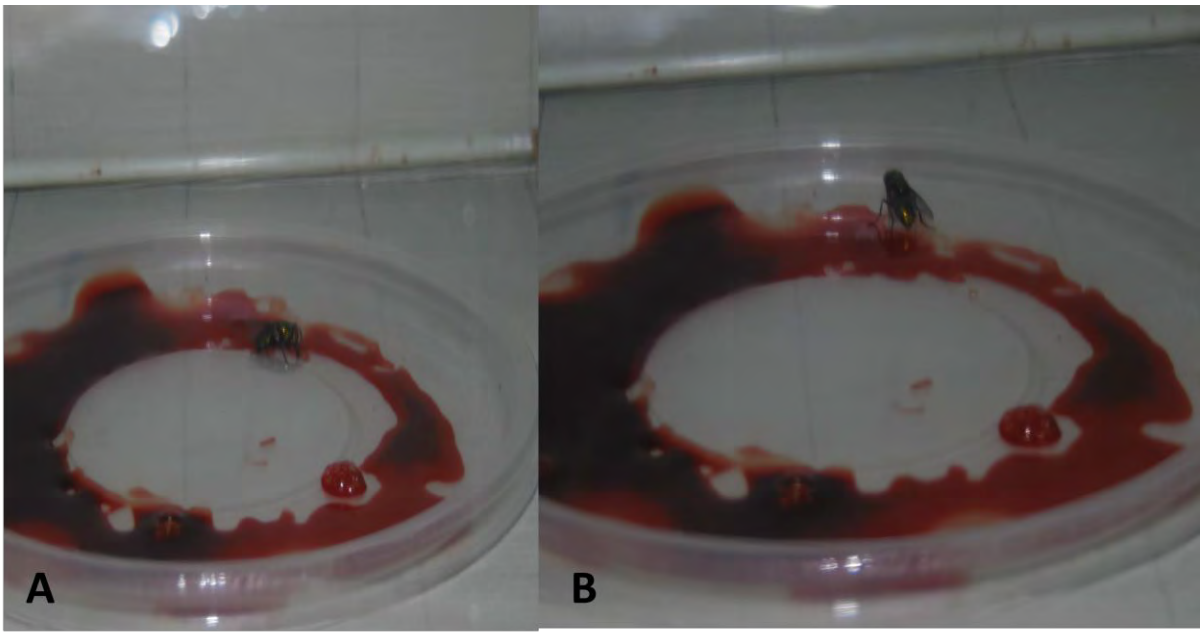

Figure 4.1: Blowflies feeding (A) and walking on a pool of blood (B)

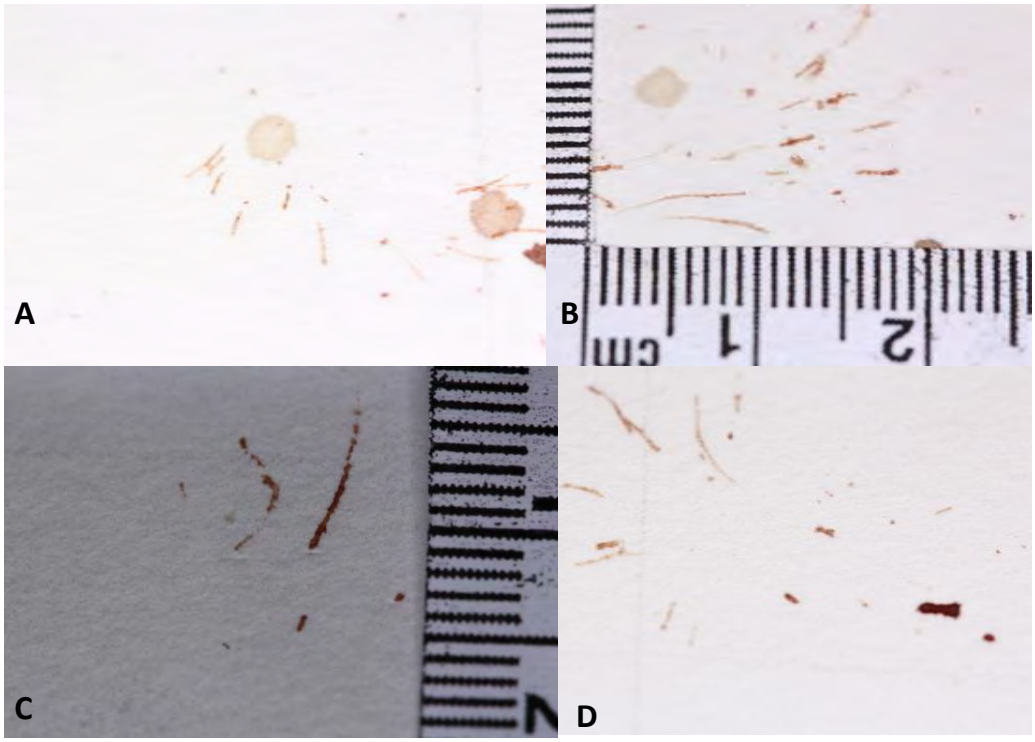

Figure 4.2: Drag marks produced by blowflies after interaction with a pool of blood

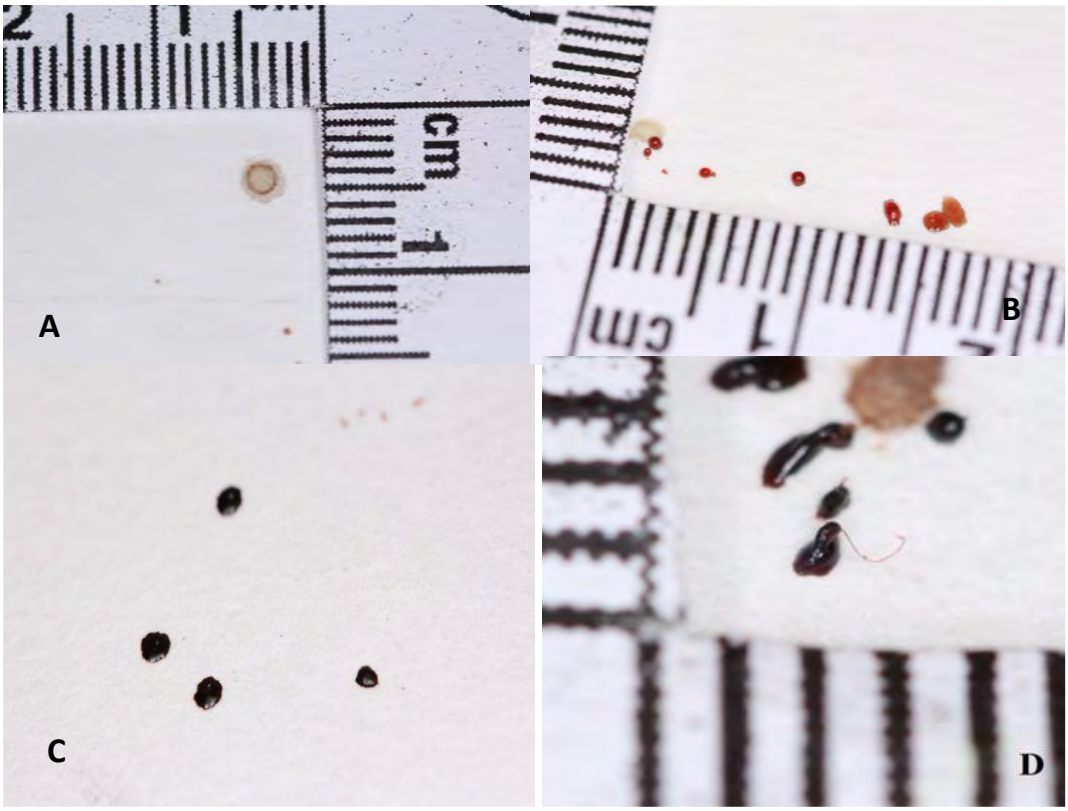

Figure 4.3: Regurgitated blow fly spots after ingesting blood

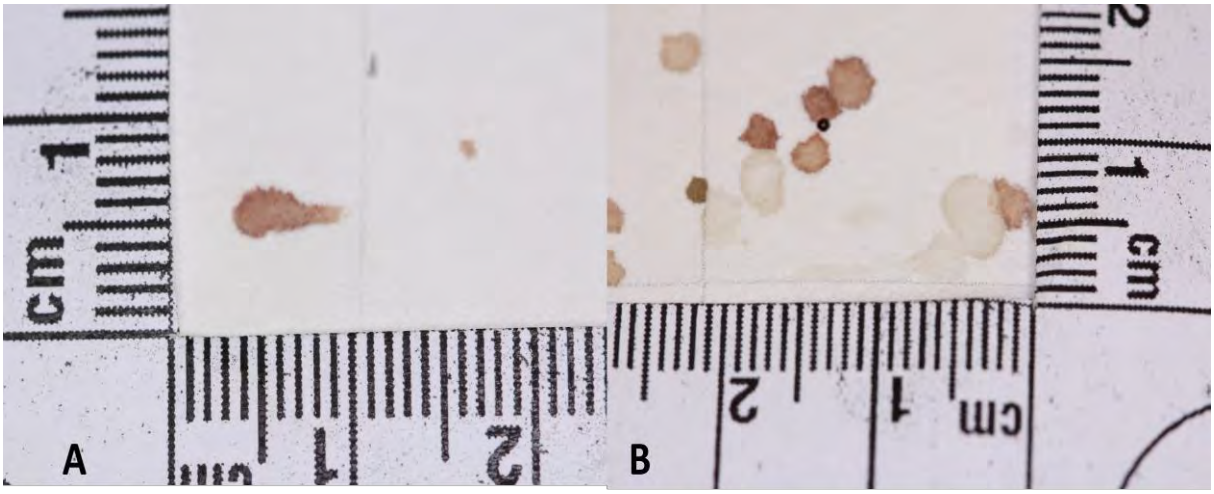

Figure 4.4: Defecated flyspecks

Appendix D

Ethics Approval

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences Animal Ethics Committee
Room E53-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 6411
e-mail: nosi.tsama@uct.ac.za
http://www.health.uct.ac.za/fhs/research/animalethics/forms

23 November 2015

Mr M Lesaoana
C/o Dr M Heyns
Forensic Medicine & Toxicology
Clinical Lab Sciences
Falmouth Building

Dear Mr Lesaoana

PROTOCOL TITLE: THE ANALYSIS OF ARTEFACTS PRODUCED BY FORENSICALLY SIGNIFICANT BLOWFLY (DIPTERA: CALLIPHORIDAE) ACTIVITY AND THEIR EFFECT ON BLOODSTAIN PATTERN.

FHS AEC REF NO: 015/021

Thank you for submitting your protocol to the Faculty of Health Sciences (FHS) Animal Ethics Committee (AEC) for review.

I am pleased to inform you that the FHS AEC has **authorised** your protocol; this authorization is of limited duration and will terminate on **30 November 2018.** If the project is to continue beyond that date, it must be reviewed not less than on an annual basis and in accordance with AEC policy.

Any modification to the study that affects or alters the use of animals or otherwise departs from the approved version of the protocol must receive prior approval from the AEC as an amendment of protocol.

Number of animals & species: 50 Blowfly

Please quote the FHS AEC REF NO (above) in all future correspondence.

Please note that the authorisation of this protocol imposes the following obligations on the (PI) principal investigator:

1. To submit an annual mandatory progress report. The first annual report for this protocol is due on **29 February 2016.** The forms can be accessed from http://www.health.uct.ac.za/fhs/research/animalethics/forms

2. To submit a final mandatory report on the **30 November 2018**, please access the final report form from: http://www.health.uct.ac.za/fhs/research/animalethics/forms
3. To ensure that all study participants perform within the confines of the procedures and experimental design of the protocol as authorised, or as amended.
4. Ensuring that all study participants comply with all applicable national legislation, UCT policies, FHS AEC policies and standard operating procedures (SOPs) and national standards (SANS 10386: 2008).
5. To ensure in your capacity as the PI (principal investigator) that you immediately alert the FHS AEC to any event involving the welfare of the animals which has occurred during the course of the study, as well as the actions that were taken to respond to these events.
6. To ensure in your capacity as the PI (principal investigator) that you alert the FHS AEC to any new or unexpected ethical issues that arose during the course of the study, and how these issues were addressed.
7. To ensure that research is conducted in duly registered facilities in accordance with the South African Veterinary Council Rule 32 (as applicable) and that all key personnel are registered with and/or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals, or will be performing the procedures under the direct and continuous supervision of SAVC-registered veterinary professionals or SAVC-registered para-veterinary professionals.
8. To report any instance of an animal discovered to be dead to the RAF on the appropriate form: http://www.health.uct.ac.za/fhs/research/animalethics/forms
9. To report any instance of an animal found in distress to the RAF on the appropriate form.
10. To consult with the AEC in regard to any confusion or uncertainty about how to respond to any of the obligations mentioned herein, how to deal with any of the issues mentioned herein, or otherwise conduct animal research responsibly and in a manner consistent with applicable UCT policies.

My best wishes for a successful research and /or teaching endeavour.

Yours sincerely Signed by candidate

Signature Removed

PROF PJ COMMERFORD
CHAIR, FHS AEC