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Profiling the scent of weathered training aids for blood-detection dogs

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ABSTRACT

At outdoor crime scenes, cadaver-detection and blood-detection dogs may be tasked with locating blood that is days, weeks or months old. Although it is known that the odour profile of blood will change during this time, it is currently unknown how the profile changes when exposed to the environment. Such variables must be studied in order to understand when the odour profile is no longer detectable by the scent-detection dogs and other crime scene tools should be implemented. In this study, blood was deposited onto concrete and varnished wood surfaces and weathered in an outdoor environment over a three-month period. Headspace samples were collected using solid phase microextraction (SPME) and analysed using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC × GC–TOFMS). The chemical odour profiles were compared with the behavioural responses of cadaver-detection and blood-detection dogs during training. Data interpretation using principal component analysis (PCA) and hierarchical cluster analysis (HCA) established that the blood odour could no longer be detected using SPME–GC × GC–TOFMS after two months of weathering on both surfaces. Conversely, the blood-detection dogs had difficulty locating the blood samples after one month of weathering on concrete and after one week of weathering on varnished wood. The scent-detection dogs evaluated herein had not been previously exposed to environmentally weathered blood samples during training. Given that this study was conducted to test the dogs' baseline abilities, it is expected that with repeated exposure, the dogs' capabilities would likely improve. The knowledge gained from this study can assist in providing law enforcement with more accurate training aids for blood-detection dogs and can improve their efficiency when deployed to outdoor crime scenes.

1. Introduction

Blood has significant value when located at a crime scene. It can provide information to help identify: 1) the location of a primary or secondary crime scene; 2) persons of interest who were present at the crime scene; 3) potential murder weapons involved; or 4) other valuable evidence items. Blood evidence found at crime scenes is often present in small amounts and therefore may not be visible to the human eye; this is referred to as latent blood. As a result, appropriate detection techniques are required to locate latent blood present at a crime scene, often within a large search area. The technique used must also ensure the preservation of the evidence to allow for extraction and identification of DNA.

Luminol is a common chemical that has been used for years as a presumptive test in blood-detection; however, it requires direct contact with blood for chemiluminescence to occur, and a dark room to enhance this luminescence. This makes it inappropriate for outdoor settings where the search area is large or the location of the blood is unknown, or in cases where the luminol is likely to react with other interfering compounds present, such as bleach in a bathroom. It has also been treated with caution when used at crime scenes due to its health and safety risks such as being an irritant to eyes, respiratory systems and skin [1]. In recent years, Australia, Italy and the United Kingdom have substituted chemical blood-detection techniques with blood-detection canines, a specialised group of cadaver-detection canines trained to detect the scent of latent blood.

Scent-detection canines are employed by many law enforcement agencies to detect a specific class of contraband such as illicit drugs,

Abbreviations: BOM, Bureau of Meteorology; EDTA, ethylenediaminetetraacetic acid; F_{crit}, critical value; PC-1, first principal component; GC–MS, gas chromatography – mass spectrometry; GC × GC–TOFMS, comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry; HCA, hierarchical cluster analysis; K_3EDTA, tripotassium ethylenediaminetetraacetic acid; NIST, National Institute of Standards and Technology; PCA, principal component analysis; PDMS/DVB, polydimethylsiloxane/divinylbenzene; RSID™- Blood, Rapid Stain Identification of Human Blood; S/N, signal-to-noise ratio; (PC-2), second principal component; SPME, solid phase microextraction; TIC, total ion current; VOCs, volatile organic compounds

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firearms, explosives or accelerants, through tracking and identifying the substances' characteristic odour [2–6]. They are a desirable tool as they are minimally destructive and are highly sensitive, allowing them to easily reduce a search area even when only minute concentrations of the target odour are present [7,8]. As well as being highly sensitive, canines have high selectivity, being able to isolate and detect odours of trace amounts even in the presence of many other extraneous odours such as those naturally present in the environment [3,4,7–10]. This is particularly useful in outdoor scenes, such as bushland or other difficult terrain, where the canines can reduce the target area for further processing using more confirmatory (and costly) techniques such as Rapid Stain Identification of Human Blood (RSID™- Blood).

The canines detect their target odour through recognising its unique chemical makeup. All odours are comprised of different compounds known as volatile organic compounds (VOCs). As the canine sniffs, the air passes through the receptor cells in their nose where the VOCs bind, sending a chemical signal to the canine's brain to register the compounds present [8,11]. Canines have 20–60 times more receptor cells than humans, giving them the ability to detect a higher number of compounds at any one time, as well as those present in trace concentrations (e.g. in cases where a perpetrator may have washed away all visible blood) [4,5,12,13].

Cadaver-detection canines are primarily used to locate human remains, with some also trained to detect blood and other tissue samples. Blood-detection canines are a specialised group of cadaver-detection dogs, trained and employed to detect odours associated with human blood at both indoor and outdoor crime scenes [4,9]. At present, the main training aid used in the development of blood-detection dogs is fresh human blood deposited on bandages, soil or directly in tin cans [12]. Blood is advantageous as a training aid as it accurately represents the target odour and is able to be donated consensually, thus reducing ethical and legislative concerns [9,12].

Although they are primarily trained directly on their target odour, rather than synthetic training aids, blood-detection dogs are treated with caution when referred to in court, due to the insufficient research surrounding their abilities and limitations [14]. This is often the case when confirmatory tests are unable to validate the dog's findings. Anecdotal evidence suggests that the dogs are far superior to current technology but data to support this is currently not available in the literature. Therefore, in order for blood-detection canines to be valued in the forensic and law enforcement communities, further research is required to better understand both the VOCs liberated from blood under varying conditions and the corresponding canine response to the chemical composition of the sample, thus improving our understanding of the compounds and concentrations the canines are able to detect.

Previous research in the literature has demonstrated the consequences of utilising training aids that do not cover the full spectrum of blood odour a cadaver-detection or blood-detection dog is likely to encounter in the field [15]. By exposing cadaver-detection dogs to blood samples of varying ages, Degreeff et al. [15] demonstrated the difficulty of dogs trained exclusively on aged blood to locate fresh blood, with their accuracy increasing as the blood was aged. It has been theorised in other studies that the increase in complexity of the blood odour profile as it ages and degrades may influence the canine's ability to track and locate both fresh and degraded blood operationally [5]. The difficulty the canines experienced in locating fresh blood in the study by Degreeff et al. [15] is likely due to a difference in the odour profile of fresh and aged blood [5], and as a result, due to gaps in the canine's training.

Very little research has investigated the odour profile of blood in a forensic context, and even less research has focused on the effects of the outdoor environment. Previous studies have established that blood has a distinct odour compared to other human tissues [8], and that ageing and storage conditions also have a significant impact on the overall odour profile [5,8]. Although these studies are helpful in enhancing current training aids, and therefore increasing the dog's ability to recognise relevant VOCs, most research has been performed in a closed environment and the effects of the outdoor environment are still unknown [5,6,9]. It is important to study these environments as blood-detection canines can be employed in outdoor crime scenes and as a result, their training aids should be an accurate representation of potential casework environments.

Previous studies have collected the VOCs present in the blood odour profile from the headspace of samples using solid phase microextraction (SPME). SPME is a simple technique with minimal sample preparation and requires no contact with the sample making it ideal for extracting VOCs from biological materials. Once collected, the preferred analytical technique is gas chromatography – mass spectrometry (GC–MS), however, this technique often has insufficient resolution capabilities when analysing complex mixtures such as blood. In recent years, comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC × GC–TOFMS) has emerged as the preferred analytical technique as it provides improved separation due to its high peak capacity, allowing for a more comprehensive profile to be discovered in these complex matrices [5,16,17].

This study aimed to bridge the gap in research by identifying the effects of an outdoor environment on the odour profile of blood. The study identified VOCs present in the odour of blood deposited on concrete and varnished wood samples that were weathered in an outdoor environment over a three-month period using headspace SPME and GC × GC–TOFMS. The results from the chemical trials were compared with those from dog trials whereby cadaver- and blood-detection dogs were exposed to fresh and weathered blood on concrete and varnished wood surfaces for the first time in order to establish insight into their baseline abilities and limitations. The knowledge gained from this research will help to establish training aids that more closely replicate the odours the dogs are likely to encounter in the field, increasing their accuracy and reliability.

2. Materials and methods

2.1. Experimental design

Blood was collected following human ethics approval (HREC# 2013000132), and was collected from a single donor to eliminate the possibility of inter-subject variation across samples. The donor was a 22-year-old female who was not taking any medication and had no ongoing health issues at the time of collection. A regular routine of hygiene and diet was maintained by the donor prior to collection to ensure an accurate representation of natural biological conditions.

A qualified phlebotomist aseptically collected blood via venipuncture into BD Vacutainer® Tubes with Lavender BD Hemogard™ caps (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). BD Vacutainer® Tubes contain glass and spray-coated tripotassium ethylenediaminetetraacetic acid (K_3EDTA), which is used to prevent blood clots, allowing for an easier transfer of the blood onto the surfaces throughout the study [18,19]. The effect of K_3EDTA on the blood VOC profile has been previously tested and determined to have no significant effect above background VOC levels [5]. The vacutainers were inverted several times after blood collection to ensure adequate mixing of the EDTA and blood for effective anticoagulation [19]. Studies have shown that EDTA has no significant effect on blood cells if removed from the tubes 2–3 h after collection [19]. Therefore, the blood was transferred from the BD Vacutainer® Tubes within 1 h of collection.

Two weathering trials were performed during this study: one with blood deposited on concrete (Brighton Charcoal Concrete Pavers; Bunnings, Australia) and the other with blood deposited on varnished wood (Tarkett Bamboo; Bunnings, Australia). The local police dog unit recommended these surfaces, as they are both commonly encountered at outdoor crime scenes where blood is likely to be located. Tarkett bamboo wood was chosen as an alternative for hickory, the typical wood type used in hammer and shotgun handles, as it was accessible,