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Research

Retention of Human Body Fluids in Adults of *Calliphora vicina* (Diptera: Calliphoridae)

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Abstract

Foraging by *Calliphora vicina* Robineau-Desvoidy often leads to a period of bubbling behavior, followed by either deposition of the regurgitate onto surfaces or reuptake of the bubble. Eventually, the partially or undigested food is passed in the excreta forming fecal or defecatory stains on surfaces in which deposition occurs. This study examined the digestive artifacts (i.e., regurgitate and defecatory stains) formed following consumption of human blood and semen by adult flies in an attempt to determine the length of time the meal was retained in the crop. The morphological appearance of either type of stain appeared consistent with the color of blood or semen for 10–20 d after feeding. When tested with ABA Hematrace immunochromatographic strip assays, blood was detectable in at least 33% of fly artifacts 25 d after the initial consumption of blood. Similarly, semen was detected in nearly 34% of digestive artifacts 30 d after feeding on human semen when using ABA p30 cards. Human body fluids were also detected in fly artifacts when using RSID lateral flow assays, but a much lower percentage of artifacts tested positive for blood (4.9%) and semen (4.6%) 25-d postfeeding in comparison to ABA strip assays. The difference between the types of lateral flow assays appeared to be due to extraction efficiencies of the buffers used for isolation of blood or semen from the fly artifacts. The implications of these observations in reference to seasonal adaptations and to bloodstain pattern analysis at crime scenes are discussed.

Key words: dipteran crop, regurgitation, insect artifact, fly stain, lateral flow assay

Adult flies routinely leave evidence of foraging at crime scenes in the form of artifacts or stains. Typically, the artifacts are created through modification of existing stains, distortion of the shape of fluids before they have dried, or through the deposition of secretions/excretions from the oral (regurgitate) or anal (feces or defecate) openings (Rivers and Geiman 2017). Alterations of wet stains are the result of adult flies interacting directly with pooled blood or other body fluids, such as walking or dragging a body part through the liquid, potentially changing the shape of the fluid, and, in turn, transferring droplets of blood to another location via transference. Transfer patterns are most commonly observed as tarsal tracks or fly 'footprints', but also appear as asymmetrical lines of varying length (Parker et al. 2020, Rivers et al. 2020). Cast-off patterns are less frequent and result from flinging fluids from the body or flapping blood covered wings. Flies will forage on wet or dried stains, altering both the shape and color (intensity) of a stain due to feeding. For example, adults consuming a portion of a dried bloodstain may create a cratered appearance due to the sucking action of the proboscis (James and Sutton 1998). This activity in turn yields a stain that is lighter in color than the originally deposited blood droplet.

The frequency of altered or modified stains is relatively low by comparison to the number of digestive artifacts produced by adult flies (Rivers et al. 2018). Digestive artifacts form as a result of regurgitation or defecation and, to a much lesser extent, vomiting. These stains are generally larger than those created by transference, are more likely to have a rounded or circular appearance, and at times may possess tails that reflect information about the mechanism of production (Rivers and McGregor 2018, Rivers et al. 2020). Morphological characteristics of digestive stains are influenced by the porosity and topography of the surface material deposited upon. Under some conditions, artifacts produced by flies can be challenging to distinguish from human body fluid stains (Rivers et al. 2019), especially some forms of bloodstains (Benecke and Barksdale 2003, Durdle et al. 2013a).

Regurgitation is commonly associated with bubbling behavior, in which the contents of the crop are forcibly expelled through the

foregut to the opening of the mouth, where it hangs from the tip of the proboscis (labellum). In some species, the bubble or regurgitate is rarely released and, instead, is reingested to move back to the crop or directly into the midgut (Hendrichs et al. 1992, Stoffolano et al. 1995). For others, regurgitate is frequently deposited onto a substrate, where it may be reconsumed or used to digest solid food. Adults of some species of tephritids and muscids appear to release regurgitate as a means to decrease crop volume or in response to being disturbed by other flies (Coleman 1984, Hendrichs et al. 1992). In the laboratory, adults of *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) respond to stimuli like solid sucrose or 'meat' by regurgitation of the crop contents (Bay 1978). This behavior appears to also occur in response to the presence of carrion or a human corpse, as digestive artifacts are commonly deposited on the surfaces of a decedent (Viero et al. 2019, Parker et al. 2020). The process of regurgitation is thought to evacuate the contents of the crop for many fly species (Wang et al. 2017). However, this is not the case under all circumstances or for all species. For example, the estimated volume of a regurgitate bubble determined for several species of calliphorids and sarcophagids appears to be less than the volume capacity of the crop for many fly species (Stoffolano and Haselton 2013, Rivers and McGregor 2018). This is supported by recent observations that have demonstrated retention of human blood in the crop of *C. vicina* 2–3 d after consuming the meal despite continuous production of regurgitate and fecal stains (D.B.R., personal observations). Similar observations have been made for larvae of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) feeding on semen (Nutton 2017), suggesting that artifacts containing human body fluids could be deposited several days after the initial consumption of the tissues.

In this study, we examined the ability of *C. vicina* to retain human body fluids in the crop by characterizing the morphology and chemical composition of digestive artifacts produced following feeding on human blood and semen. Lateral flow assays that detect human blood and semen were used to detect the presence of food in digestive artifacts. An immunoassay specific to fly digestive artifacts was also used to detect stains produced by regurgitation and defecation (Rivers et al. 2018). The implications to forensic investigations involving trace evidence present at crime scenes are discussed.

Materials and Methods

Fly Rearing

A colony of *C. vicina* was established from larvae collected in Baltimore, MD (47.306732, 4.260684) in April 2018. Field collected larvae were fed fresh beef liver that was placed on sand in plastic containers (30 × 20 × 10 cm) and maintained at 25°C, 70–75% RH under a long-day (LD 15:9 h) photoperiod in environmental chambers (Model 130BLL, Percival Scientific, Boone, IA). The established colony was supplemented with field collected larvae (same location) in March 2019 and April 2020. Specimens were identified using the dichotomous keys of Marshall et al. (2011) and Jones et al. (2019). Voucher specimens from all collections are maintained in the Forensic Entomology Research Laboratory in the Department of Biology, Loyola University Maryland, Baltimore, MD.

The laboratory colony of *C. vicina* was maintained as previously described (Rivers and McGregor 2018). Adults were reared in wire mesh cages (30 × 30 × 30 cm) at 25°C, 70–75% RH under a long-day (LD 15:9 h) photoperiod, and fed beef liver, water, and sugar cubes ad libitum. Fresh liver was provided to adults for oviposition. Larvae were fed fresh beef liver throughout development under the same conditions as adults in environmental chambers.

Postfeeding Retention of Human Body Fluids

To examine the length of time adult flies retained human body fluids in the digestive tract after feeding, digestive artifacts were tested for the presence of human blood or semen at 24-h interval postfeeding. Individual flies (4–8 d after adult emergence from puparia at 25°C) were isolated into single Petri dishes (60 × 100 mm) lined with Whatman #4 qualitative disc filter paper (110 mm Ø, GE Healthcare, Buckinghamshire, United Kingdom). Prior to isolation, flies were allowed to fed ad libitum on sugar cubes, dry powdered milk, and water. For the experimental assays, isolated flies were offered one milliliter of either human blood (AB^+) or human semen (neat) along with sugar for 24 h under a long-day photoperiod (15:9 h light: dark) at 25°C, 70–75% RH. Preliminary experiments demonstrated that this experimental set up permitted flies to easily access body fluids and sugar and to subsequently deposit digestive artifacts onto filter paper. After exposure to a given body fluid, each fly was transferred to an identical clean Petri dish with the exception that only sugar and water were available for consumption. The filter paper was removed from the Petri dish and transferred to a small paper bag, labeled, and stored at room temperature in total darkness until used for visual inspection or testing. On each subsequent day, flies were transferred to clean Petri dishes lined with filter and containing sugar and water. Artifacts were collected from each fly every 24 h until the fly died. Ten adult flies were tested for each type of body fluid, and each experiment was replicated five times for a total of 50 flies per food source.

Artifacts dried rapidly once deposited and diffusion was minimal before drying. Newly deposited and dried artifacts were essentially identical in terms of shape and color. Regurgitate, defecate, or transfer patterns on filter discs resulted in diffusion stain boundaries of >0.1 mm (n = 750 artifacts examined following blood or semen consumption) in any direction at 25°C. Thus, the dried stains were representative of wet artifacts in terms of 2D morphology.

Whole human blood (AB^+) and human semen (neat) were purchased from BioChemed Services (Winchester, VA), aliquoted, and then stored frozen at –80°C until use. Samples used for artifact collections experienced only one freeze–thaw cycle.

Morphological Assessments of Fly Artifacts

To examine postfeeding effects on human blood and human semen, the morphology of digestive artifacts was characterized on each day of deposition. Artifacts deposited by flies fed either blood or semen were characterized by morphology (i.e., size, shape, and color), stain type (digestive [regurgitate, defecatory] and transfer patterns [translocation and tarsal tracks]), and total number of artifacts deposited. All artifacts were examined using a Nikon color camera (DSRi2) mounted on a stereo-dissecting microscope (Nikon SMZ1270, Tokyo, Japan) connected to a Hewlitt Packard Z2 workstation. Images were captured using Nikon Elements D image analysis software (v. 5.11.01, Tokyo, Japan). All size measurements of artifacts were made use Nikon Elements D image analysis software and were performed on captured images. As individual flies deposited very few artifacts per 24-h interval, with the exception of the initial day of body fluid consumption, random sampling of fly artifacts was not possible. Consequently, all artifacts were analyzed for each fly on each day postfeeding.

Lateral Flow Assay Detection of Human Blood and Semen

To detect the presence of human blood and human semen in digestive artifacts, lateral flow assays designed for forensic analysis were

used. Individual artifacts were tested by using surface sterilized scissors (soaked in 10% bleach for 30 s followed by a rinse in 70% ethanol, and then blotted dry) to cut out each fly stain from filter paper and then placing each in appropriate extraction buffer supplied by the manufacturer using surface sterilized fine forceps. All instruments were surface sterilized between each sample preparation. For detection of human blood in artifacts, ABA Hematrace and RSID blood cards were used as confirmatory tests following the manufacturer's instructions and using the modifications of Johnston et al. (2003) to enhance detection of low concentrations of blood in evidentiary samples. Human bloodstains (AB^+) and extraction buffer on filter paper and untreated filter paper served as controls. In parallel experiments, ABA p30 and RSID semen cards were used for detection of human semen in fly digestive artifacts. Semen stains and extraction buffer on filter paper and untreated filter paper served as controls. Ten adult flies were tested for each type of body fluid, and each experiment was replicated five times for a total of 50 flies/food source.

Comparisons between each type of lateral flow assay also were made in terms of extraction efficiencies of body fluids from digestive artifacts. Artifacts were collected as described earlier and artifact deposition monitored continuously using digital recordings (Canon XA20 Professional Camcorder, Tokyo, Japan) at 25°C. Captured images from video playback were analyzed using Nikon Elements D image analysis software to identify digestive artifacts on filter paper that visually contained food (presumed to be either blood or semen). In turn, each selected artifact was assayed using the lateral assays for blood or semen. Percentage extraction efficiency was considered equal to percentage detection by a given lateral flow assay.

Immunoassay Detection of Digestive Artifacts

To confirm the type of fly artifact deposited by *C. vicina*, dot blot assays were used for detection of cathepsin D-like proteinase in fly regurgitate and feces (Rivers et al. 2018). This technique has been previously shown to distinguish between fly digestive artifacts and various types of human body fluids, with no false positives or false negatives being evident (Rivers et al. 2019). Artifacts produced by adult flies following feeding on either human blood or semen were used in the dot blot assays. Individual artifacts were isolated as described earlier as single stains per filter paper discs and then placed into individual wells of 24-well cell culture plates (Costar). Binding with anti-md3 serum was performed as described in the Promega Technical Manual for ProtoBlotII AP System with Stabilized Substrate (Promega). Detection of antisera binding to artifacts was visualized colorimetrically using goat anti-rat IgG antibody conjugated to alkaline phosphatase. All membranes were digitally captured using a Bio-Rad Gel Doc imager (Bio-Rad) equipped with Quantity One 1-D analysis software (v. 4.6.7, Bio-Rad). All artifacts deposited by the flies were examined by dot blot assays. Ten adult flies were tested for each type of body fluid and each experiment was replicated five times for a total of 50 flies per food source.

Statistical Analysis

Percentage data were arcsine transformed before analyses to yield normal distributions. One- and two-way analyses of variance were performed using GraphPad Prism statistical software (Macintosh, GraphPad Software, San Diego, CA). Means for binding selectivity to each type of artifact and stain were compared using Student–Newman–Keuls multiple comparisons tests with $\alpha = 0.05$.

Results

Morphological Characteristics of Fly Artifacts

Adult flies offered human blood or semen as a food source deposited four main types (regurgitate, defecatory, translocation, and tarsal tacks) of artifacts within 24 h after feeding. For either food source, regurgitate stains were the most prevalent (X ± SEM = 66.2 ± 3.2%, n = 16,870 total artifacts produced), followed by defecatory stains (27.1 ± 1.8%), and tarsal tracks (5.8 ± 1.7%). Very few translocation stains (0.9 ± 0.1%) were produced by the flies. This pattern of artifact deposition changed dramatically by day 2 postfeeding (Fig. 1), as the total production of artifacts dropped significantly (F = 87.2; df = 4, 2675; $P < 0.001$) and remained low until the flies died, regardless of whether flies consumed blood or semen (Fig. 2). The type of artifact deposited also changed as only digestive artifacts (i.e., regurgitate and defecatory stains) were produced by adult flies on all days following the initial feeding on blood or semen. The presence of regurgitate and defecatory stains was confirmed via positive tests using dot blot assays to detect cathepsin D-like proteinase in the artifacts.

On the initial day of artifact deposition, fly stains typically appeared the same color as the food source (Figs. 3 and 4). However, over time flies deposited digestive artifacts that were lighter in color by comparison to those from earlier time points, especially for regurgitate and defecatory stains produced after blood consumption (Fig. 3), and were round or nearly so in shape, lacking tails (Figs. 3 and

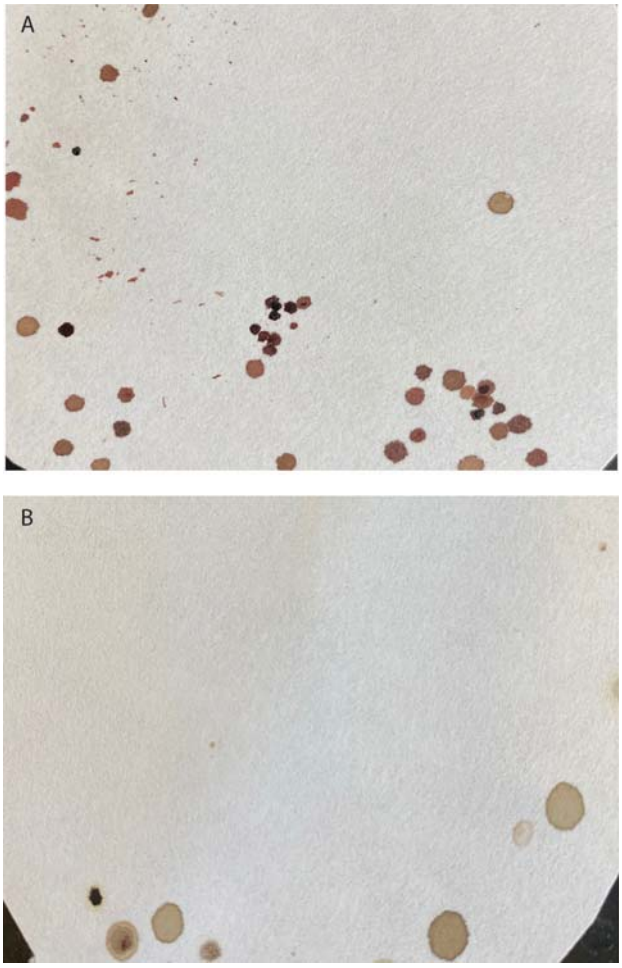

Fig. 1. Representative artifacts produced by *Calliphora vicina* at (A) 24 h and (B) 48 h at 25°C after consuming human blood.

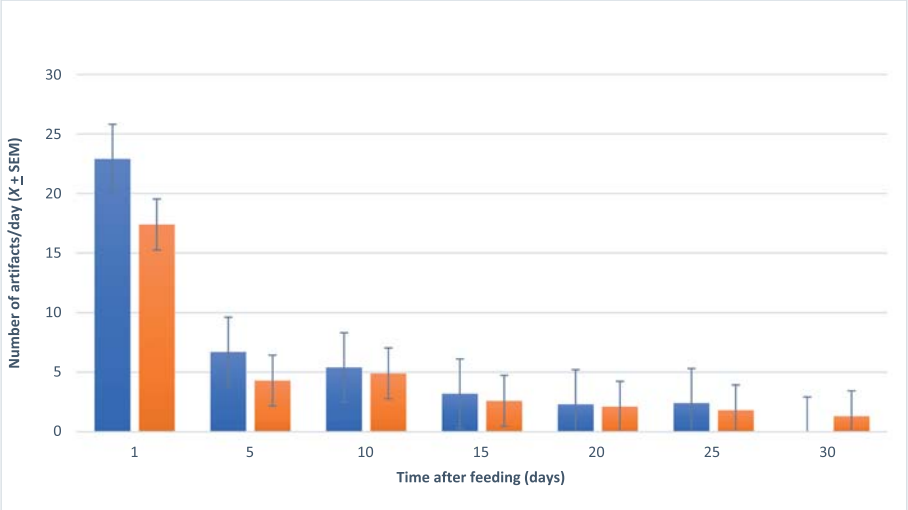

Fig. 2. Changes in the number of digestive artifacts deposited by *Calliphora vicina* each day following consumption of either human blood or semen. The blue or dark bars represent blood, the lighter shaded represent semen.

4). By 25 d postfeeding, digestive artifacts were difficult to contrast from the surface material used for deposition and appeared nearly identical in shape and color, regardless of whether blood or semen had been ingested by the flies (Figs. 3 and 4).

Lateral Flow Assay Detection of Blood and Semen in Fly Artifacts

Digestive artifacts tested positive for human blood using RSID Blood and ABA Hematrace lateral flow assays. Blood was detected in artifacts deposited up to 25 d following the initial consumption of the food source (Table 1). After which time, flies stopped depositing artifacts or had died. Once flies stopped producing digestive artifacts, they typically died within 1–2 d at 25°C. There was no evidence of any differences in detection of blood between regurgitate and defecatory stains using either form of immunochromatographic strip assay. However, the assays were not equally effective in overall blood detection in digestive artifacts. For example, signal detection (i.e., formation of bands in the test [T] area) with ABA Hematrace strips (Figs. 5C–F and 6E–F) was much stronger at all time points tested by comparison to RSID Blood assays (Fig. 6B and C), and significantly more digestive artifacts (F = 53.9; df = 12, 9834; P < 0.001) tested positive for human blood using ABA Hematrace strips versus those detected by the RSID Blood assays (Table 1). Both types of assays tested positive when human blood served as a control (Figs. 5A and 6A and D), and negative for nonblood fly artifacts (Fig. 5B) or when extraction buffer alone was tested (data not shown).

Similar trends were observed with human semen in that the body fluid was detected in fly digestive artifacts using either ABA p30 cards or RSID Semen assays, and the two immunochromatographic assays were not equal in effectiveness (Table 1). For example, positive signal detection was weaker (lighter) with RSID Semen assays than ABA p30 cards, and the percentage of digestive artifacts testing positive for semen was significantly (F = 105.6; df = 12, 10,270; P < 0.001) higher with ABA p30 assays at all time points from 5-d postfeeding and beyond (Table 1). Semen was also detected in digestive artifacts longer (up to 33-d postfeeding at 25°C) after the initial consumption of the food source using ABA p30 strip assays than RSID Semen cards (Fig. 7D–H, Table 1). Like with blood, there was no evidence of any differences in detection of semen between regurgitate and defecatory stains using either form of immunochromatographic strip assay. For both types of assays, human semen tested positive when used as a control (Fig. 7A, only data for ABA p30 cards is shown), and negative for nonsemen fly artifacts (Fig. 7B) or when extraction buffer alone was tested (Fig. 7C).

Extraction Efficiency of Digestive Artifacts Using Lateral Flow Assay Buffers

Differences between lateral flow assays in detection of blood or semen in fly artifacts appeared to be linked to extraction efficiency of the respective extraction buffer associated with each test (Table 2). For example, digestive artifacts observed through digital recordings to contain food when deposited on filter paper were more likely to test positive for blood with ABA Hematrace cards than RSID Blood assays at all time points 10 d postfeeding or longer (Table 2). The same trend was true for semen detection in digestive artifacts using ABA p30 assays versus RSID Semen cards. In the case of either RSID assay, the same universal buffer was used for extractions, which is reflective of the nearly identical percent extraction efficiencies between RSID Blood and Semen assays (Table 2).

The presence of digestive stains detected by digital recordings was confirmed via positive tests using dot blot assays to detect cathepsin D-like proteinase in the artifacts (Fig. 8). Human semen (Fig. 8.1a and 8.2a) and blood (Fig. 8.3a and 8.4a) were used as controls, and they did not react with the anti-md3 serum.

Discussion

Adults of *C. vicina* fed human blood or semen produced secretory (regurgitation) and excretory (defecatory) stains that contained undigested or partially digested food material for several days after initial consumption. This was evident by the morphological appearance of digestive artifacts and through detection of blood or semen using lateral flow assays. Flies produced digestive artifacts continuously (meaning each day) for over 3 wk, with some individuals depositing artifacts containing blood or semen for as long as 25–33 d, respectively. These observations indicate that the adult flies retained portions of the initial meal for an extended period of time because blood or semen were offered only once for consumption and that complete emptying of the crop did not occur with regurgitation or passing food into the proventriculus to the midgut. Initial

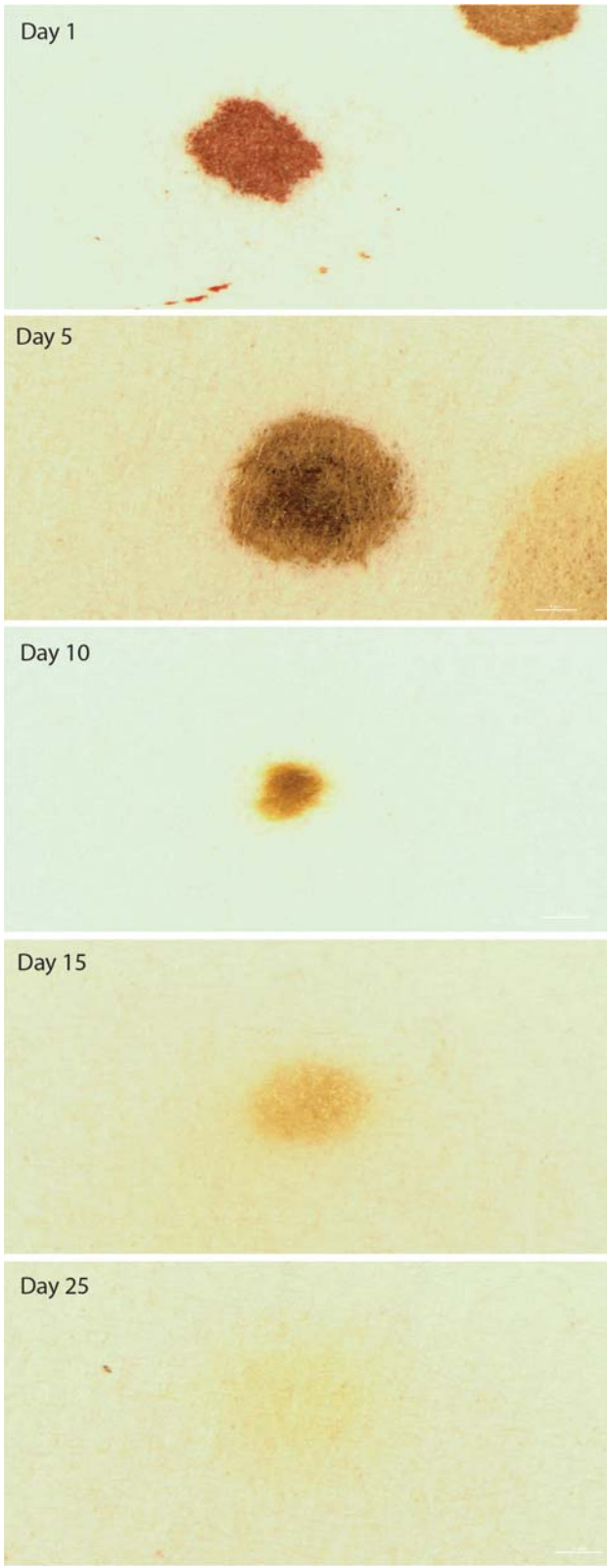

Fig. 3. Deposition of digestive artifacts by *Calliphora vicina* on days 1, 5, 10, 15, and 25 after consumption of human blood. The bar in each panel represents 1 mm at 20× magnification.

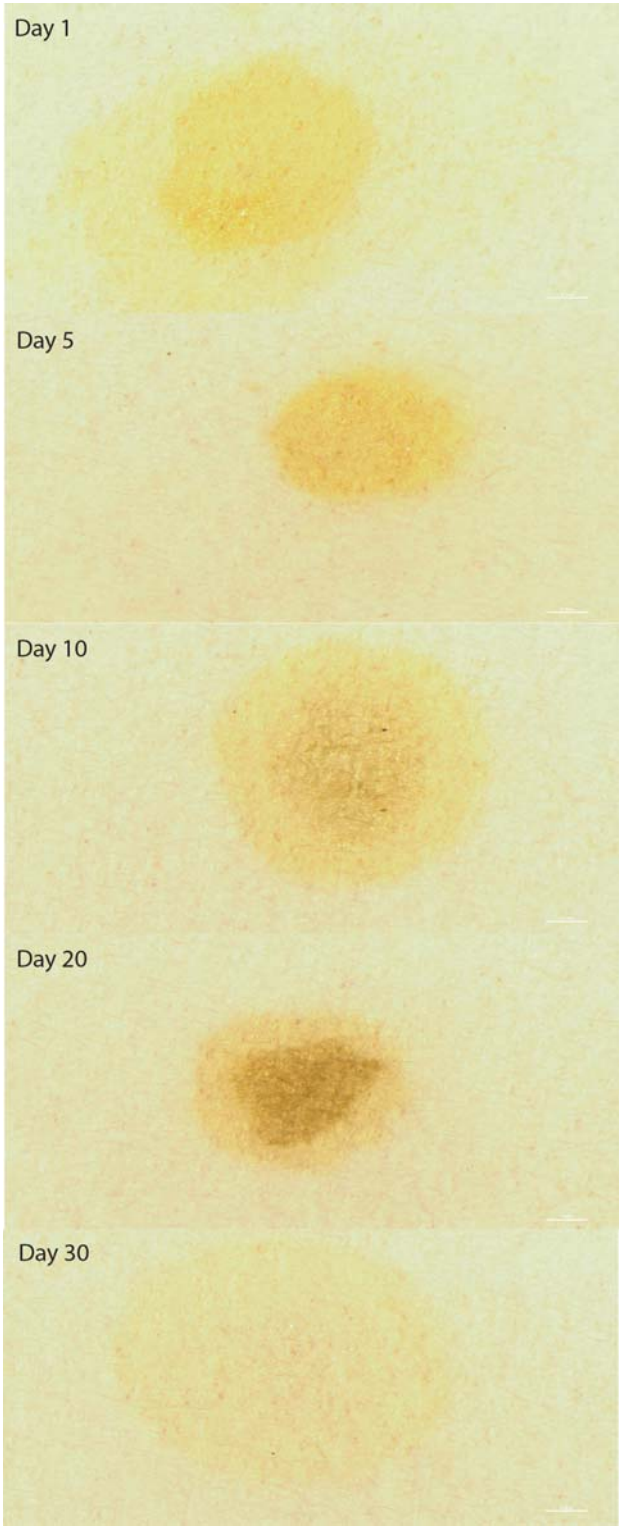

Fig. 4. Deposition of digestive artifacts by *Calliphora vicina* on days 1, 5, 10, 20, and 30 after consumption of human semen. The bar in each panel represents 1 mm at 20× magnification.

regurgitation under the conditions of this study most likely decreased crop volume, thereby reducing the rate of bubbling behavior and regurgitation on subsequent days, as has been demonstrated in other fly species when the crop is no longer full (Hendrichs et al. 1992; Stoffolano et al. 2008, 2014). Protein was only available to the flies via consumption of either blood or semen. On all subsequent days, adults fed only on water and solid sugar, which may not have induced sufficient crop stretching or served as appropriate

Table 2. Efficiency of extraction of digestive artifacts produced by *Calliphora vicina* using lateral flow assay buffers

	% detection (X ± SEM)[a]					
	Time after feeding (d)					
Food source	1	5	10	20	25	30
Blood						
ABA hematrace	98.4 ± 2.5a	94.9 ± 3.1a	88.1 ± 3.6b	90.2 ± 3.9b	86.3 ± 4.0b	85.2 ± 4.6b
RSID blood	92.4 ± 3.7a	89.2 ± 3.3a	80.0 ± 4.5c	73.8 ± 4.2d	56.9 ± 3.5e	60.4 ± 4.0e
Semen						
ABA p30	99.4 ± 1.4a	97.3 ± 2.6a	96.8 ± 3.0a	91.9 ± 3.9b	90.3 ± 2.2b	84.3 ± 3.8b
RSID semen	88.3 ± 2.9b	83.1 ± 4.6b	77.5 ± 3.2c	70.1 ± 4.2c	62.0 ± 3.8d	63.9 ± 4.3d

[a]Extraction efficiency was determined by using an immunoassay specific for digestive artifacts (Rivers et al. 2019). Percentage detection is equal to % extraction efficiency. Extraction efficiencies were determined by assaying individual digestive artifacts in extraction buffers associated with either ABA Hematrace and ABA p30 extraction buffers or RSID Universal buffer (for blood and semen). Values in the same column (for specific lateral flow assays) followed by a different letter differ from each other at $P < 0.05$.

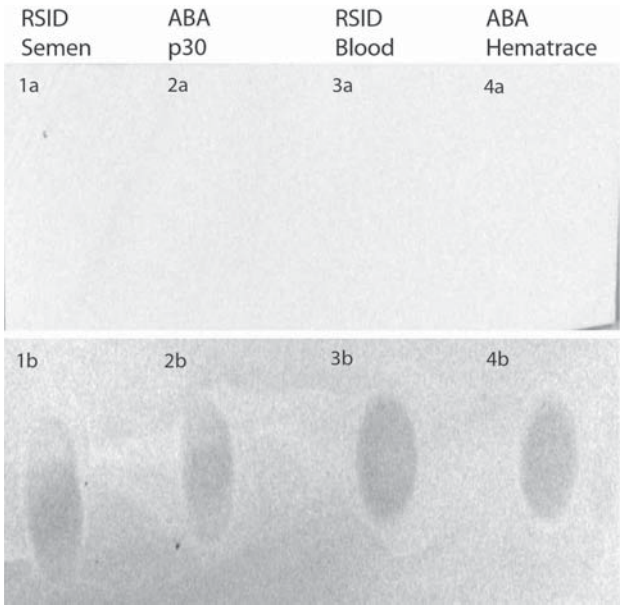

Fig. 8. Immunoassay detection of digestive artifacts after feeding on human semen (1 and 2) or blood (3 and 4) and followed by extraction in RSID universal buffer (1 and 3), ABA p30 (2), or ABA Hematrace (4). Controls were 2 µl of human semen (1a and 2a) and human blood (3a and 4a).

Retention of food in the crop of *C. vicina* and other species of flies may have implications for forensic entomology. Adult flies are known to deposit regurgitation and defecatory stains at crime scenes (Viero et al. 2019). The general assumption is that the artifacts are produced after feeding on the corpse or exuded fluids present at the crime scene. However, as noted by Kulstein et al. (2015), flies often gain access to indoor crime scenes when the investigators arrive, leaving doors propped open as forensic units and law enforcement move in and out of the premises. Under these conditions, the possibility exists that adult flies deposit artifacts derived from feeding at another location, including on trash and human refuge that may contain human DNA. Very little attention has been given to the potential of necrophagous flies to serve as vectors of human DNA to and from crime scenes, despite compelling evidence that secondary DNA transfer can occur through numerous mechanisms (Goray et al. 2010, Fonnelop et al. 2015, Cale et al. 2016, Taylor et al. 2017). In the few studies that have examined viability of human DNA in insect stains, sufficient viable DNA to permit genotyping using standard profiling kits and allele matching to the corpse or source fluid (i.e., victim or donor) have been observed with single artifacts produced by *C. vicina* and *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) (Durdle et al. 2009, 2013b; Kulstein et al. 2015). Depending on the fluid consumed (i.e., blood, semen, or a mixture), viable DNA could be quantified and genotyped from artifacts produced by *C. vicina* and *L. cuprina* for 300 and 750 d, respectively, after deposition on plastic surfaces (Durdle et al. 2013a; Kulstein et al. 2015). This is an indication that human DNA is not digested quickly in the adult fly gut and remains stable in insect fluids for long periods after deposited onto a surface. Functional human profiles have also been generated from DNA isolated from larvae, pupae, and puparia of *C. stygia* and *C. augur* fed semen as immatures, demonstrating that viable DNA was present in the fly gut for at least 5–6 d (Powers et al. 2019). Similar postdigestion influences on artifact production and composition, including human DNA content, have never been examined in adult flies. However, the findings from this study demonstrate a need for further research examining the viability of human DNA in the gut of flies like *C. vicina* that can retain a meal for a long period of time.

Two types of immunochromatographic stripes or lateral flow assays were used to assess whether human blood or semen were present in fly artifacts. All of the lateral flow assays used depend on capture and detection antibodies specific for a unique antigen associated with human blood (either hemoglobin [ABA Hematrace] or human glycophorin A [RSID Blood]) or semen (p30 [ABA p30] or semenogelin [RSID Semen]). In the case of blood detection, both the ABA Hematrace assay and RSID Blood assay have been used successfully to detect human blood in fly artifacts (Durdle et al. 2015) and gut contents of several hematophagous arthropods (two species of reduviids and argasid ticks; Beatty et al. 2017, 2019), respectively. ABA p30 cards have been used to detect semen in digestive artifacts produced by *L. cuprina* (Durdle et al. 2015), but we are not aware of any study using RSID Semen assays in conjunction with insect testing. In this study, the effectiveness of the immunochromatographic strips was not equal in detection of blood or semen in terms of percentage of artifacts testing positive for either blood or semen. These findings suggest the possibility that differential digestion of the antigen specific to each assay occurs in the fly gut. However, very little is known about the digestive physiology of a blood or semen meal in the foregut or midgut of adult necrophagous flies. More research is needed to understand how human body

fluids are digested in necrophagous flies and to identify factors that contribute to reduced or delayed protein digestion in the crop and/or midgut before any conclusions can be drawn.

An alternative explanation is that the extraction buffers used were not equally effective in separating blood and semen from fly stains. This was evident in comparison of the extraction efficiencies of the buffers supplied by the manufacturers of each type of assay. Additionally, the RSID Semen and Blood Assays utilized the same universal buffer and, correspondingly, demonstrated similar percentages of digestive artifacts testing positive for either semen or blood.

The presence of fly artifacts at crime scenes reflects trace evidence resulting from the interactions of flies with human remains. How frequently the artifacts are encountered is unknown, as this type of evidence is typically not recorded or collected during processing of a crime scene. However, it is clear that this practice should change. Fly stains may contain blood, semen, or other biological components from feeding on human tissues long after the initial interaction. The resulting artifacts generated through regurgitation and fecal elimination are thus potential sources of valuable information that may aid or potentially confound an investigation. More research is needed to decipher the potential utility of artifacts produced by flies and other insects in forensic investigations.

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