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ORIGINAL ARTICLE

Examination of forensic entomology evidence using computed tomography scanning: case studies and refinement of techniques for estimating maggot mass volumes in bodies

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Abstract A new technique has recently been developed for estimating the volume of maggot masses on deceased persons using post-mortem CT scans. This allows volume to be measured non-invasively and factored into maggot mass temperature calculations for both casework and research. Examination of admission scans also allows exploration of entomological evidence in anatomical areas not usually exposed by autopsy (e.g. nasal cavities and facial sinuses), and before autopsy disrupts the maggot distribution on a body. This paper expands on work already completed by providing the x-ray attenuation coefficient by way of Hounsfield unit (HU) values for various maggot species, maggot masses and human tissue adjacent to masses. Specifically, this study looked at the HU values for four forensically important blowfly larvae: *Lucilia cuprina*, *L. sericata*, *Calliphora stygia* and *C. vicina.* The *Calliphora* species had significantly lower HU values than the *Lucilia* species. This might be explained by histological analysis, which revealed a non-significant trend, suggesting that *Calliphora* maggots have a higher fat content than the *Lucilia* maggots. It is apparent that the variation in the x-ray attenuation coefficient usually precludes its use as a tool for delineating the maggot mass from human tissue and that morphology is the dominant method for delineating a mass. This paper also includes three case studies, which reveal different applications for interpreting entomological evidence using post-mortem CT scans.

Keywords Forensic entomology · Maggot mass · Computed tomography · Volume · X-ray attenuation coefficient

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Introduction

Forensic entomologists usually estimate the minimum post-mortem interval based on the oldest insect specimens that have developed on the body [1]. The developmental time of a maggot is dependent upon both its species and the temperature to which the maggot has been exposed [1]. After the discovery of a body, temperatures measured by the nearest weather station should be used, in conjunction with a temperature logger at the death scene, to reconstruct ambient air temperatures using regression analysis [2–4]. These temperatures are often considered to be those that the maggots experienced during the period of interest. While this technique is effective for predicting ambient temperatures [4], it is likely that the temperatures to which maggots are exposed are actually greater than ambient temperatures if they are involved in massing.

When maggots mass together they can generate temperatures more than 30°C above ambient [5–8]. This can be problematic for entomologists because these elevated temperatures may cause increased growth rates of the individual maggots [7]. If a mass heats sufficiently, it is also possible for the maggots to continue feeding and growing within a mortuary refrigerator prior to autopsy. This would not only affect the entomologist's estimates of the post-mortem interval, but may also lead to the destruction of physical evidence present on the body as the maggots continue

consuming the flesh [8]. It is therefore important that corpses with heavy infestations of maggot be prioritised for autopsy and that mass temperatures are monitored [8].

The first systematic study of maggot massing, which was carried out by Slone and Gruner [9] in the USA, found a range of elevation above ambient of between approximately 0 and 23°C. Temperature elevation may have significant implications for estimating minimum post-mortem interval, yet, until recently, there has been very little research into the factors influencing the temperatures generated by maggot masses [9–11]. It has become clear that the volume of a maggot mass plays a key role in producing this heating effect, but measuring mass volume is challenging. Slone and Gruner [9] were unable to measure many of the masses occurring in their study due to the inability to adequately access them within their experimental carcasses, while Charabidze et al. [11] conducted laboratory experiments and controlled the mass volume to some extent by allocating a set weight of maggots to each treatment. However, they were unable to ascertain the volume of their masses at any one time; this is changeable because masses can divide into multiple groups and can reduce their density by increasing individual separation, or can increase it by packing together. Due to this inherent difficulty, and the uncertainty in predicting maggot mass temperature, it is crucial that further study into this phenomenon is carried out. In the meantime, forensic entomologists should endeavour to measure temperatures of all maggot masses involved in casework [3, 12].

Recent studies by Johnson et al. [13] utilised a computed tomography (MDCT) scanner for volumetric analysis of maggot masses. This technique has proven successful for both experimental masses and actual casework. This has enabled incorporation of non-invasive determination of mass volumes into research on mass heat production (currently ongoing at the Victorian Institute of Forensic Medicine (VIFM)). It has also allowed Victoria's forensic entomologist (MA) to comment routinely on the entomological features of CT scans in casework (under supervision of an experienced post-mortem radiologist: CO'D).

Maggots contain large fat stores within their body, which they accumulate in preparation for pupation. Thus, the HU values associated with maggots should reflect this lipid accumulation. While Johnson et al. [13] found that the HU values for *Chrysomya rufifacies* (Macquart) and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) lie between −50 and −250, it is unknown whether this reported range varies between species, perhaps reflecting differing body fat content.

Even if individual maggot x-ray attenuation does parallel that of human fat (−50 to −100 HU), it is unknown if this reflects the mean x-ray attenuation of a maggot mass. A maggot mass is a heterogeneous environment, which, while predominantly comprising maggots, also contains trapped pockets of air between maggots and flesh that is both in its original state and following digestion by maggots. It would therefore be expected that this mixture of substances would display a much wider range of HU values, with air pockets pushing the mean values further into the negative and soft tissue pushing them into the low positive range.

This paper builds on the work of Johnson et al. [13] by ensuring that the approach to CT mass volume estimation applies equally well to maggots of different species that are commonly encountered in forensic cases in south eastern Australia. In particular, the focus of this study is to distinguish human tissue from maggot masses using the x-ray attenuation coefficient by way of HU calculations. By obtaining a clearer understanding of the HU values associated with maggot masses, it may be possible to use these calculations to separate maggot masses from human tissues. The current paper also presents three recent case studies taken from the Victorian forensic entomology practice that show different applications of CT to the analysis of entomological evidence.

Methods

Maggot culture

Four different species of blowflies (Diptera: Calliphoridae) were kept in culture for this study: *C. stygia* (Fabricius), *C. vicina*, *L. cuprina* (Wiedemann) and *L. sericata* (Meigen). Culture methods were followed as per Johnson et al. [13]. Flies were maintained at 20°C with a 12:12 light/dark cycle in the Forensic Entomology Laboratory at the VIFM. Adult flies were maintained on water and sugar ad libitum, and females were provided with kangaroo mince (Gourmet Game Kangaroo Mince, Macro Meats, Athol Park, SA, Australia) to facilitate ovarian maturation. Flies oviposited onto kangaroo mince covered with a thin layer of cotton wool. Once eggs hatched, maggots were removed with forceps and allocated to experimental replicates.

CT scanning details

The same CT scanning machine and scanning parameters were utilised as per Johnson et al. [13]. Images were acquired using a 16-channel multidetector computed tomography (MDCT) scanner (Aquilion16®, Toshiba Medical Systems, Minato-ku, Tokyo, Japan). Scan data were stored on a picture archiving and communication system (PACS) (IMPAX®, Agfa HealthCare NV, Mortsel, Belgium). MDCT scanning protocols were identical to those used for coronial cases admitted to the VIFM. Scans were performed by mortuary technicians accredited by the Radiation Safety Section of the Victorian Department of Health to perform radiographic procedures. For each scan, contiguous 0.5-mm

collimated slices were obtained and reconstructed into 1/0.8-mm overlapping axial slices with soft tissue and bone convolution kernels. We collected data from multiplanar reformatted (MPR) and 3D volume rendered (VR) images in the axial plane using a Vitrea® 2 (Vital Images, Inc. Minnetonka, MI, USA) workstation (for volumetric analysis and calculation of HU densities). We also used Aquarius Net Viewer V 4.4.1.4 (TeraRecon, San Mateo, CA, USA) thin client desktop software for calculation of HU densities of individual maggots and for some analyses of coronial cases.

Interspecific variation in the x-ray attenuation coefficient of maggots

We determined whether there were any differences in the x-ray attenuation coefficient of maggots of four forensically important blowfly species within south eastern Australia: *C. stygia*, *C. vicina*, *L. cuprina* and *L. sericata*. Three replicates were established for each species, with 30 maggots in their second larval instar (2 days old at 20°C) placed onto 30 g of kangaroo mince within 70-mL pathology specimen jars. A square of fine mesh tulle was placed over the top of each jar and affixed with an elastic band. A unique plastic letter code was taped to each specimen jar to identify individual replicates when they were CT scanned together. All replicates were then placed in a temperature-controlled cabinet (Thermoline Scientific, Smithfield, NSW, Australia) at 25°C in darkness for 24 h to allow them to settle. They were then removed for CT scanning and immediately killed and fixed in hot water and preserved in 80 % ethanol. The length of preserved maggots was measured.

The scans were viewed in the axial plane using Aquarius Net Viewer V 4.4.1.4 (TeraRecon, San Mateo, CA, USA) and the best viewing settings established (as per Johnson et. al [13]). Between five and 15 individual maggots per cup were located within the CT image, and one small circular region of interest 1.75 (±1.25) mm^2 was then drawn at random levels over the lateral regions of each visible maggot in the region of the sub-cuticular fat stores, while avoiding the central gut area. Each circle provided the mean, SD, minimum and maximum HU value for that area.

The mean HU values were analysed using the SPSS statistical package (IBM Corporation, Armonk, NY, USA); a mixed model was run to test for a significant difference between the HU densities of each species, and a post hoc pairwise comparison statistic with Bonferroni correction was used to determine where the differences occurred.

Histological investigation of differences in the x-ray attenuation coefficient

Histological sectioning of maggots was used to investigate whether any differences in the HU values of each species were related to the size of their fat stores. Two maggots from each of the above replicates (six per species in total) were placed into histology cassettes and processed on a Tissue Tek 5 (Table 1).

After processing, the cassettes were removed and embedded in paraffin wax using the Tissue Tek TEC embedding centre (Sakura Finetek, USA). Using a Reichert-Jung 2030 Microtome (Leica Biosystems Nussloch GmbH, Germany) and Shandon disposable blades (Thermo Fisher Scientific Inc., USA), cassettes were then carefully trimmed to approximately one-third of the way through the specimens. Four longitudinal parasagittal sections were taken 24–30 μm apart before trimming deeper until approximately half way through the specimens (medial). As before, four levels were taken here, and trimming then proceeded deeper until reaching the final third parasagittal section of the specimen, where another four levels were cut (a total of 12 sections). Each section was 4 μm thick.

The sections were individually floated onto a water-bath (with 5 % gelatine added) at 55°C and picked up on Menzel-Glasser Superfrost HDS glass microscope slides (Gerhard Menzel GmbH, Germany). These were then allowed to rest on a hot plate at 50°C before being put into a rack and stained with haematoxylin and eosin on the Leica ST 5020 staining machine (Leica Microsystems Pty Ltd., Germany). Application of cover slips was done on the Leica CV 5030 cover slip machine using 24×50-mm HDS cover slips and Leica CV mounting media.

Table 1 Protocol used for the histological processing of maggots showing fixatives, clearing and embedding agents

Station	Process	Time agitation (h)	Temperature (°C)	Pressure/ vacuum
1	Formalin	1	40	On
2	70 % alcohol	2	40	On
3	100 % alcohol	1	40	On
4	100 % alcohol	1	40	On
5	100 % alcohol	1	40	On
6	100 % alcohol	2	40	On
7	100 % alcohol	2	40	On
8	Xylene	2	40	On
9	Xylene	2	40	On
10	Xylene	2	40	On
11	Wax	1	60	On
12	Wax	1	60	On
13	Wax	1	60	On
14	Wax	1	60	On

The third slice of the medial section of each maggot was chosen for analysis because this contained the most trophocyte (fat) cells. A photograph within the last three abdominal segments of each histology slice was taken at 4× magnification using a Leica DM2000 light microscope with Leica Application Suite 3.8 software. Using this histological level avoided large digestive structures, such as the crop and mid gut, and also represented the region that included the largest fat store. The areas of exclusive fat, as well as total maggot area within each field of view (1,600×1,200 μm), were then measured using the software 'free draw' tool (Fig. 1), and the percentage of fat was calculated for each maggot. The average percentage area of fat was then calculated for each species and compared with SPSS using an ANOVA.

Identifying maggot masses within body tissues

We examined the difference in the x-ray attenuation coefficients between maggot masses and surrounding tissue in decomposing bodies to determine whether HU values could be used to more effectively delineate masses from human tissue. Admission CT scans of eight maggot-infested bodies were selected from the VIFM database based on the fact that large and well-formed masses were seen while the bodies were in the mortuary. Details of each case were obtained from the mortuary admissions data (Table 2). Samples of maggots were collected from each mass examined with CT, and at least 20 maggots were killed and fixed in hot water for 30 s and transferred to 80 % ethanol, while the remainder were reared to adulthood to assist species identification. Three distinct masses were chosen from each body, where possible. Each case was examined on a Vitrea® 2 workstation, and a small circular area 200 (±75) mm^2 was drawn in an area of the maggot mass, and then in an area of adjacent tissue surrounding the mass (within 2 cm of its boundary). The mean HU values of these areas were then recorded for comparison. Ten HU measures each were taken from maggot masses and surrounding flesh for every mass investigated per body. The tissue type surrounding each mass was also recorded to aid in determining any tissue types that may be more or less difficult to differentiate from maggots. A nested ANOVA was carried out using the JMP 7 (SAS Institute Inc.) statistical package to look for significant differences between the HU values of the mass and the corresponding flesh.

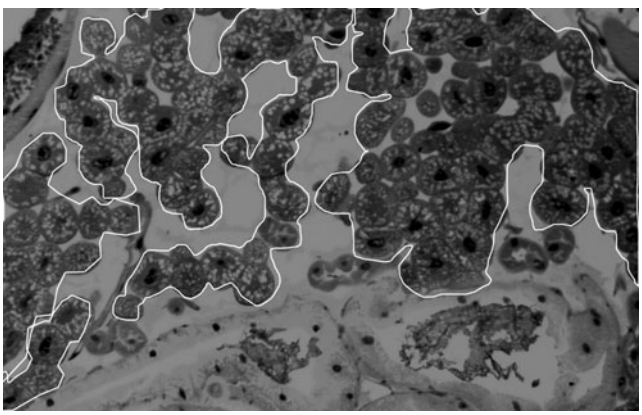

Fig. 1 Sample of photographs taken of histology slides for *C. vicina*, with outline of fat cells (trophoblasts) in white

Results and discussion

Interspecific variation in the x-ray attenuation coefficient of maggots

All larvae preserved after CT scanning were in their third instar and the mean (±SD) length of each species was 13.7 (±0.9) mm for *L. cuprina*, 13.5 (±0.7) mm for *L. sericata*, 13.9 (±1.7) mm for *C. stygia* and 15.8 (±0.5) mm for *C. vicina*. A mixed model analysis revealed significant difference in mean HU values between all species combined ($F_{3,15}$=32.75, P<0.0001). A post hoc comparison (with Bonferroni correction) separated the species according to genera, with *L. cuprina* being significantly different from both *C. stygia* and *C. vicina*. Similarly, *L. sericata* was also significantly different from both *C. stygia* and *C. vicina*. No significant difference was seen between species of the same genus (i.e. between *L. cuprina* and *L. sericata* or between *C. stygia* and *C. vicina*) (Fig. 2). The decrease in HU density for *Calliphora* species suggests an increase in the fat present in the maggot (perhaps reflected as a non-significant trend in the histology results shown in the next section). It should be noted that, although differences between species do occur, the range of HU values for maggots is still within the previously established maggot HU value range [13]. Therefore, this pre-existing range can be used to assist in distinguishing maggot from other tissue types which lie outside this range (e.g. brain tissue).

Histological investigation of differences in the x-ray attenuation coefficient

Analysis of the histology data showed no significant differences in the percentage of fat present between species ($F_{3,10}$=2.12, P=0.19). While the percentage of fat was highly variable between each maggot, a trend towards a higher level of fat in the *Calliphora* species compared to the *Lucilia* species can perhaps be seen (Fig. 3), and this would correlate well with the differences seen in HU values between these two genera. This difference may indicate that *Calliphora* species have a greater ability to store fat compared with *Lucilia* species or that the *Calliphora* species simply acquire fat stores earlier in development than *Lucilia* species. It is likely that these differences in fat stores are the

Table 2 Details of masses from de-identified cases

Case number	Deceased's		Month of death	Maximum death time (time since last sighting) (days)	Location	Mass number	Location on the body	Tissue type	Species of maggot	Maggot instar	Significant difference ($P < 0.05$)
	Age (years)	Sex									
1	71	M	Nov.	21	Outdoors	1	Perineum	Fat/muscle	*C. augur* Sarcophagidae *C. stygia* *Ch. rufifacies*	3rd 3rd 3rd 2nd	No
						2	Right mid tibial region	Muscle	*C. stygia* *Ch. rufifacies*	3rd 3rd	No
						3	Buccal cavity	Muscle	*C. augur* *C. stygia* *Ch. rufifacies*	3rd 3rd 3rd	Yes
2	57	F	Jan.	6	Indoors	4	Umbilicus	Fat	No sample		No
						5	Right breast	Fat/ connective tissue	*C. augur* *L. sericata*	2nd & 3rd 2nd & 3rd	No
						6	Right supraclavicular region	Muscle	*C. augur* *L. sericata*	3rd 3rd	Yes
3	69	M	Jan.	19	Outdoors	7	Over left acetabulum	Fat/muscle	Sarcophagidae *Ch. rufifacies*	3rd 3rd	Yes
						8	Antero-lateral neck, hyoid level	Muscle	Sarcophagidae *Ch. rufifacies*	3rd 3rd	Yes
						9	Posterior neck	Fat/muscle	Sarcophagidae *Ch. rufifacies*	3rd 3rd	No
4	35	M	Feb.	3	Indoors	10	Posterior neck	Fat/muscle	*Ch. rufifacies*	3rd	Yes
						11	Right orbit	Orbital contents	*Ch. rufifacies*	3rd	No
5	69	M	Jan.	3	Outdoors	12	Left ear pinna and canal	Cartilage, connective tissue and muscle	*Ch. rufifacies*	2nd & 3rd	No
						13	Posterior neck	Fat and muscle	*Ch. rufifacies*	2nd & 3rd	No
						14	Perineum	Fat and muscle	*C. stygia* *Ch. rufifacies*	3rd 2nd & 3rd	Yes
6	28	M	Apr.	12	Outdoors	15	Right anterior chest, mid-level	Fat and muscle	*C. stygia*	3rd	Yes
						16	Left lateral thigh	Fat and muscle	*C. stygia*	3rd	Yes
						17	Between left arm and lateral chest	Fat and muscle	*C. stygia*	3rd	Yes
7	63	F	Mar.	18	Outdoors	18	Lower leg	Fat and muscle	*C. stygia* *Ch. rufifacies*	3rd 3rd	No
						19	Upper axilla	Fat and muscle	*C. stygia* *Ch. rufifacies*	3rd 3rd	No
						20	Upper chest/anterior neck	Fat and muscle	*C. stygia* *Ch. rufifacies*	3rd 3rd	Yes

Table 2 (continued)

Case number	Deceased's Age (years)	Deceased's Sex	Month of death	Maximum death time (time since last sighting) (days)	Location	Mass number	Location on the body	Tissue type	Species of maggot	Maggot instar	Significant difference ($P<0.05$)
8	62	M	Mar.	9	Indoors	21	Anterior surface of left shoulder, level of glenohumeral joint	Fat and muscle	*Ch. rufifacies*	3rd	Yes
						22	Posterior surface of left shoulder, level of glenohumeral joint	Fat and muscle	*Ch. rufifacies*	3rd	Yes
						23	Left inguinoscrotal region	Fat and muscle	*Ch. rufifacies*	2nd & 3rd	No

main factor driving the differences seen in the x-ray attenuation coefficient of each species, although it is not possible to rule out parameters such as the size of the gut and the density of its contents, or the density of haemolymph. Effort was made to avoid gut structures when measuring HU values because this would reflect mainly the density of food in the maggot's gut, rather than the maggot itself. However, it is impossible to eliminate these influences entirely. Haemolymph-filled coelomic space is also likely to have been included in the calculations of the mean HU values, and the amount of this space probably varies between species. However, we did not consider it valid to measure this histologically due to concerns about distortion of the body wall during preparation: the amount of apparent empty space within a maggot slide section is likely to be profoundly affected by this.

Identifying maggot masses within body tissues

Assessment of the x-ray attenuation coefficient of maggot masses and surrounding flesh in coronial cases revealed that values in the maggot masses ranged from −526 to −43 HU, while the surrounding flesh ranged from −805 to 729 HU (thus encompassing the range of mass densities). This shows that despite varied HU values of the surrounding environments, maggot mass HU values were always negative for the present study, and this is likely due to the inclusion of air pockets and the negative values of the individual maggots. A nested ANOVA also revealed a significant difference overall in HU values between the maggot mass and adjacent flesh for each mass ($F_{23,\ 445}=25.88$, $P<0.001$), but a post hoc Tukey test revealed that a significant difference in HU values between the maggot mass and surrounding tissue actually occurred in only 12 of the 23 masses.

There do not appear to be any identifying factors that allow prediction of where these significant differences will occur. The masses that were analysed came from various regions of the body, within various tissue types. The cases also varied in the time since death, the season and the location of death (Table 2). Significant differences also did not depend on tissue type, mass location on the body or species or age of infesting maggot. Similarly, there was no way of predicting whether it would be the maggot mass or

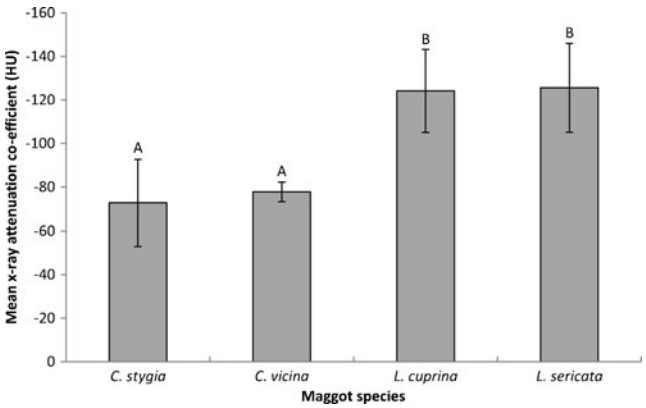

Fig. 2 Mean (±SE) Hounsfield unit values of four different species of forensically important blowfly maggots of equivalent age. Bars topped by different letters are significantly different

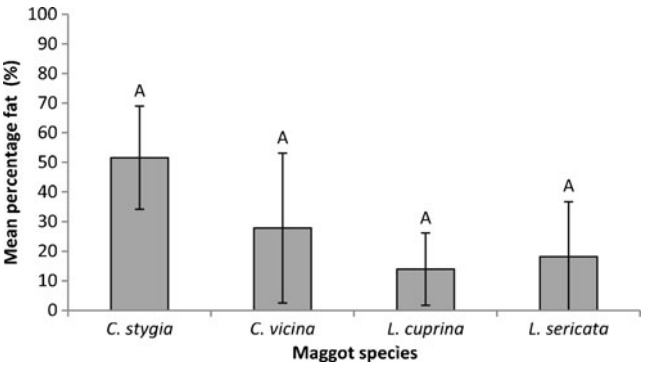

Fig. 3 Mean (±SE) percentage fat found in histology sections of four different species of forensically important blowfly maggots. Bars topped by different letters are significantly different

surrounding flesh that would have the higher HU value. The density of the mass may be regulated by features such as: 1) the size and age of the mass; 2) the age and species of the maggots, affecting the fat and gut content; and 3) the temperature and level of disturbance of a mass, which will impact on how the maggots position themselves within the mass and how many air pockets exist within the mass. The density of the tissue in a decomposed body is regulated primarily by tissue type, temperature during decomposition and time since death (the latter two variables especially affect the pattern of post-mortem gas distribution in the body, which is a major determinant of regional x-ray attenuation coefficients).

The range of −43 to −526 HU for maggot masses found in this study is a much larger range than previously recorded (−50 to −250 HU by Johnson et al. [13]). This increased range may be due to the inclusion of a greater range of species, as the previous study was limited to *Ch. rufifacies* and *C. vicina* [13]. Despite this increased range, the HU values of maggots were always negative in the current study. Regardless of the variety of maggot species, and the various feeding substrates on which the maggots were found, all masses could still be successfully identified by multiple observers (AJ, JW, LS and MA) and delineated from their surrounding tissue using morphology as the predominant discriminator.

Case studies

The following case studies were coronial cases admitted to the VIFM and referred for a forensic entomology opinion. Specific details provided about each case have been minimised to preserve privacy of the deceased. All entomological examinations and CT analyses of entomological evidence were carried out by the forensic entomologist (MA), and all radiological interpretations were corroborated by the forensic and clinical radiologist (CO'D). We believe that an entomologist with no medical or anatomical training could learn to visualise entomological evidence on a CT scan and put it in context with their findings on physical examination. However, no matter how practiced the entomologist becomes, they should never report interpretations without help from a medical colleague experienced in post-mortem radiology.

Case 1

The body of a middle-aged man was found in his home, approximately 5 days after the last sighting of him alive. The cause of death was a combination of incised injury and blunt force trauma. Few insect specimens were located at the scene, despite a careful search. The only insects present were <50 maggots under the anterior chest of the prone body. Additional samples were collected at autopsy from the body itself within 6 h of recovery from the scene.

Larvae on the body were identified as third-instar *C. augur* (Fabricius) (Diptera: Calliphoridae), as well as first- and second-instar *L. sericata*, *C. vicina* and *C. augur*. There were estimated to be <1,000 maggots on the body in total, the majority of which were in their second instar. There were minimal signs of previous maggot feeding focussed within incised wounds to the neck and anterior chest, and in particular, the facial orifices showed no sign of previous infestation.

An analysis of the admission CT scan was performed to reconstruct the maggot mass distribution and to estimate mass volume prior to the disturbance rendered by autopsy. Maggot masses viewed in the CT image were small, which was in keeping with the low numbers of maggots collected from the body. While there would have been some disruption of the mass when moving the body, it was assumed that maggots had re-aggregated by the time of the admission scan because there was no radiological evidence of maggots scattered over the body surface. CT images confirmed that maggot masses were centred in the pre-existing injuries seen on physical examination. The first mass was immediately overlying the manubrio-sternal joint and was estimated using Vitrea®2 workstation to have a volume of 4.4 cm^3. A second mass was noted at the surface of the anterior neck, immediately caudal to the level of the hyoid; this mass was estimated on CT to have a volume of 1.4 cm^3. This corresponded with an area of superficial maggot feeding noted in the entomological examination. A third mass was identified in the nasal cavity, adjacent to the left inferior nasal concha (inferior turbinate), with an estimated volume of 1 cm^3 (Fig. 4). No corresponding external sign of this mass was

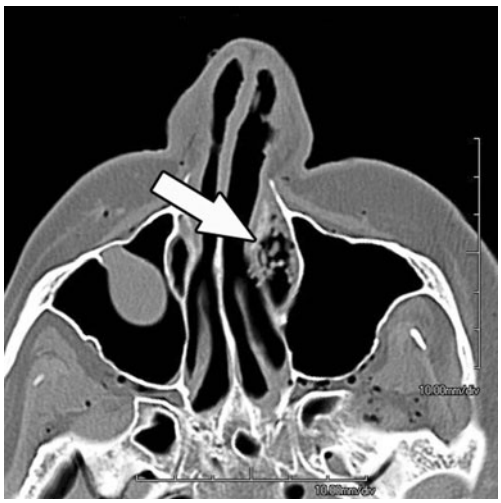

Fig. 4 CT image viewed in the axial plane showing a small maggot mass (arrow) in the left nasal cavity, within the inferior concha (estimated volume of 1 cm^3)

seen during physical examination because this cavity is not exposed by autopsy.

The admission CT scan was used successfully to reconstruct the distribution of maggots prior to their disturbance at autopsy, and it confirmed the findings of the physical examination. This application has proven especially useful in cases where the entomologist has been unable to inspect the body until after autopsy, and has provided the entomologist with a valuable aid to understanding how the mass volume could affect the temperature to which the maggot has been exposed. In this case, it could be said that the small mass sizes would produce heating of a few degrees above ambient at most. The CT findings also added new information about a maggot mass that was not visible on inspection, and this would allow an entomologist to target their examination to locate this mass if necessary.

Case 2

A young male disappeared under suspicious circumstances. The deceased had last been seen 14 days prior to discovery of his remains in bushland. The cause of death was not determined.

Entomological evidence was collected at the site of body discovery, and further samples were collected at autopsy 12 h later. The remains received sun exposure during the entomological examination, and hundreds of adult *Australophyra rostrata* (Robineau-Desvoidy) (Diptera: Muscidae) were active over the remains. Additional invertebrate activity included adult and larval *Ptomaphila lacrymosa* (Schreibers) (Coleoptera: Silphidae) beetles, approximately 20–30 adult *Creophilus erythrocephalus* (Fabricius) (Coleoptera: Staphylinidae) beetles, thousands of dolichoderine (Hymenoptera: Formicidae) ants, a small number of first- and second-instar larvae of *A. rostrata*, and a single *Ch. varipes* (Macquart) (Diptera: Calliphoridae) pupa.

Extensive masses of feeding and prepupal maggots were noted on and under the deceased at the site of body discovery. The majority of maggots were *C. stygia*, and >1,000 of these maggots were feeding in the inguino-scrotal and pelvic region in a bilateral distribution; this mass had a mean (±SE) temperature of 36 (±0.3) °C compared with an ambient temperature of 17–18°C. The mass extended deep into the soft tissues of the thighs and combined with another mass located on the ground between the thighs and lower legs. There were also>1,000 maggots feeding underneath the tee shirt of the deceased and massing over the anterior chest and left and right upper limbs, with a maximum temperature of 33 (±0.3) °C. A mass of>1,000 pre-pupal maggots were active in the soil underneath the remains. An additional mass of third-instar *Ch. varipes* was also present within the material of the right tracksuit leg.

Inspection of the admission CT scan confirmed that radiological mass positions were in accordance with those

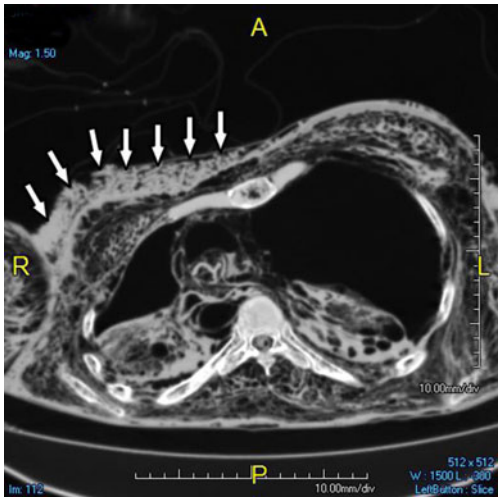

Fig. 5 CT image viewed in the axial plane showing extensive massing underneath the tee shirt of the deceased over the anterior chest (level of 5th thoracic vertebra). This mass was recorded to have a maximum temperature of 33°C in the field (ambient temperature of 17–18°C). Note the varying density of the mass from right to left, even within this single slice

seen on external examination at the site of body discovery. There were multiple masses of over 100 mm^3, suggesting that masses were most likely to be mainly or wholly regulating their own temperature (as per Slone and Gruner [9]). This was supported by temperatures recorded at the scene. Extensive masses were seen on CT, mostly superficially over the anterior chest (Fig. 5), upper limbs and lower legs. Additional masses were also identified within the deep tissues of the pelvic area and thighs. Further maggots were evident in the facial tissues, left and right orbits, and buccal

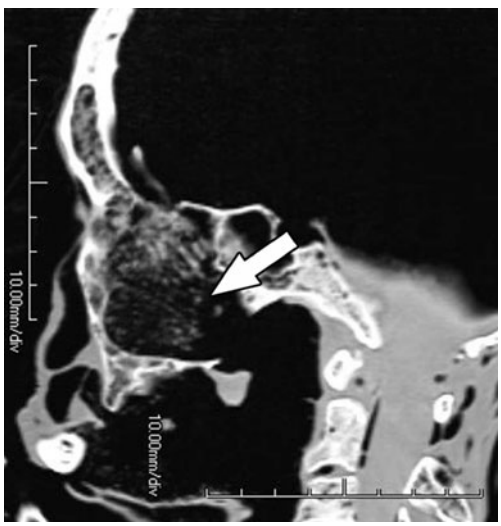

Fig. 6 CT image reconstructed in the sagittal plane showing areas of heterogeneous HU density within the air spaces of the left nasal cavity and frontal sinus of the deceased. The numerous clustered ellipsoid objects 1–3 mm in size were revealed to be sphaerocerid pupae and puparia

and nasal cavities, though there was no evidence of massing or extensive maggot invasion into the cranial cavity.

In this case the entomologist was able to attend the body discovery site, and maggot mass temperatures and visual inspections were sufficient to describe the infestation. There was no need to measure the maggot mass volume with precision. However, the correlation between the visual inspections at the scene and the radiological observations shows the usefulness of the technique and particularly how valuable it could be when the entomologist is unable to attend the scene or autopsy.

Case 3

The remains of an elderly female were discovered within a closed house and were alleged to have been in situ for some months. The cause of death was unascertained, but there was evidence from the previous medical care of the deceased that death was due to a natural cause. A full Scenes of Crimes investigation was carried out mainly because there were concerns that another occupant of the house had failed to report the death.

Invertebrates present were collected at the site of body discovery and at autopsy approximately 12 h later. There were large numbers of calliphorid puparia at the site of body discovery distributed in a radius around the remains, which is typical of remains that lie undisturbed throughout soft tissue decomposition [14]. Examination of the deceased by the entomologist (MA) revealed a low level of insect activity. There were <50 each of adult and teneral sphaerocerid (Diptera: Sphaeroceridae) flies and only a single live sphaerocerid maggot. Eclosed sphaerocerid puparia and uneclosed pupae were scattered over the head, neck and torso, and were clustered in the pinna of the left ear. There was evidence of limited previous blowfly maggot feeding on the remains, which would have occurred during the initial stages of decomposition, and there were calliphorid puparia scattered over the remains. The appearance of residual soft tissues indicated previous maggot mass activity in the mouth, nostrils, right ear and anterior neck.

Examination of the admission CT scan confirmed that there was no current feeding maggot mass, thus supporting the findings of the physical examination. However, the nasal cavities and frontal sinuses revealed areas of heterogeneous HU values within the air spaces, especially on the left. These appeared as numerous 1–3-mm ellipsoid and elongated objects that were clustered within the air spaces (Fig. 6). Re-examination of the nostrils of the deceased post-autopsy with a torch revealed that hundreds of sphaerocerid puparia were in situ and were barely visible deep within the nasal passages. These were likely to have extended throughout the nasal cavities and frontal sinuses, giving rise to the heterogeneous appearance on CT.

There were no maggot masses for volume estimation in this case, but radiological examination led to the discovery of large numbers of additional puparia within the nostrils and nasal cavities of the deceased, which would otherwise have been missed. This would not have changed the entomological opinion in this case because numerous spharocerid puparia had been collected from multiple body regions. However, it provides a cautionary note for future cases where a taxon of interest may only be located within cavities of the skull not usually reached by autopsy or external examination. It also demonstrates that CT evidence is most powerful as a correlate and extension of the physical examination.

Conclusion

The current study concludes that the morphology of maggot masses is the best way to identify their boundaries because the mean x-ray attenuation coefficients of masses vary from case to case and are not always significantly different from the surrounding tissue. Calculations of HU values may still be used to differentiate maggot masses when they are within a substrate lying outside the maggot mass HU value range. For example, brain tissue has a positive HU value (20–40 HU), and since the maggot masses are usually HU negative, their density is often useful in determining the boundary between maggot mass and brain tissue (personal experience of authors).

The results of the present work, along with the three case studies, confirm that CT scanning represents a rapid and accurate method for identifying and estimating the size of masses in forensic cases and for assisting with research into maggot mass thermogenesis and behaviour. Such information is essential for accurately estimating the effects of maggot massing on the developmental time of individual maggots and thus the post-mortem interval of the corpse in which they are found. Our case studies indicate some of the variety of applications for CT scanning in forensic entomology casework. This technique is very new and has only been used in our casework since late 2010. It is therefore inevitable that new applications and refinements of this methodology will continue to be discovered.

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Ethical standards Ethics approval for the use of de-identified CT scans of deceased was granted by the Ethics Committee of the Victorian Institute of Forensic Medicine.

Conflicts of interest The authors declare that they have no conflict of interest.

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