Introduction

In ideal conditions, decomposition of a human cadaver follows a predictable exemplar and aids in determining a post mortem interval (PMI) [1-3]. However, there are many factors that can influence this process by changing the pattern or rate and therefore the predictability of the PMI. These factors can be intrinsic (e.g. bacteria, weight or drugs in the system) or extrinsic to the carcass, such as ambient temperature, insect activity or the covering of a carcass [2-13]. Previous quantitative comparisons of decomposition affecting variables show that insect activity and temperature are the most influential on the rate of decomposition [5,7,9,12].

Once death occurs, decomposition gases created within the internal organs are released from the carcass through its natural orifices such as the mouth, nose, anus and genitalia [10,14,15]. Female flies oviposit within these openings, within limb creases and in positions where the carcass is in contact with the ground, due to the accumulation of decomposition fluids and for protection from weather [16,17]. Once the eggs hatch, large maggot masses feed on the soft tissue of the cadaver. Insects, therefore are the largest destructor of soft tissue during decomposition. Previous literature states that without insect access the decomposition rate is significantly decreased [5,7,12]; if temperatures are too low for insects to be present, decomposition is almost halted showing insect activity is dependent on temperature [5-7]. However, temperature alone also has a great effect on the rate of decomposition, as bacteria grow at optimum conditions of 25-35 °C and aid in breaking down a carcass [10]. At higher temperatures decomposition rate is increased [2,18]. A partial covering, such as a plastic bag covering the head of a cadaver would have an effect on insect access as well as temperature.

Whilst Jones *et al.* [19] report that only 0.24 out of 100,000 deaths per year involve plastic bag asphyxia, there are numerous case studies to be found in the literature dating back to the 1960s [20]. Retrospective studies on asphyxia and plastic bag suffocation are multiple, especially concerning accidental autoerotic asphyxia; hereby the individuals involved are mostly male ranging from young adults to middle age [21-23]. Other accidental deaths occur with solvent abusers or children playing [24]. Suicide has also been reported often [19,25,26], along with the mentioning of Derek Humphry's 1992 [27] novel, a guidance to ending one's own life in which this method is referenced. Though rates of suicide by this method increased after the publication, the overall rate of suicide did not [(26,28)]. Ages of individuals committing suicide by this method range from young adult to the elderly [(24,28)]. Due to the different manners associated with plastic bag suffocation and the broad age group that is involved, the need for understanding the effect on decomposition is essential. Though many case reports exist, a quantification of the stage of decomposition has not been given and whether there are pattern differences seen between the head and other areas of the body is not discussed.

In this study the effect on decomposition of a plastic bag covering the head is examined. By introducing a partial covering to the carcass a significant difference in insect activity and temperature was expected; three potential effects on decomposition required consideration: (1) Decomposition gases cannot escape from the head orifices and attract insects, (2) insects are denied access to the head and are expected to oviposit elsewhere on the carcass and (3) the temperature within the bag is higher than ambient temperature. Currently there is no literature on the effect of regional insect absence with accessibility given to the rest of the carcass. Furthermore, once decomposition progresses at the neck area insects may breach the bag and acquire access to the head. Due to gases being released from the anus, a higher colonisation near the rump of the carcass was expected.

If the bag covering the head was airtight, the temperature within the bag would be expected to be significantly higher than ambient temperature. Together with the greenhouse effect of the plastic bag, the head would be within a warmer climate compared to the rest of the carcass and when comparing to the heads of the control group, increasing the decomposition rate of the sample. This study is a preliminary research into the effects of plastic bag suffocation on decomposition, utilising the method put forth by Megyesi *et al.* [(2)]. This study focuses not only on differences in rate and pattern of decomposition between the sample and a control group, but differences within the groups as well.

The importance of this project is threefold. Firstly, there have been no quantitative decomposition studies done on plastic bag suffocation or any partially covered carcasses, therefore the effect of partial covering on the pattern and the rate of decomposition of an entire carcass has not been identified. Second, due to the nature of suicide, homicide and especially accidental sexual asphyxia, these acts are done in private and secluded areas, leading to advancement in decomposition more often than finding fresh corpses in this state. And thirdly, the nature of such acts is, to some relatives, highly embarrassing and may lead to removal of the plastic bag once found [(28)]. Therefore, it is of great importance when attending a scene of such an incident to include all relevant observations and possible paraphernalia present at the scene [(28)], before deciding on a correct manner and cause of death as well as how to establish a correct PMI. The aims of this project were to determine the influence of a plastic bag covering the head on the rate and pattern of decomposition. The influence on PMI estimation was considered.

METHODOLOGY

The decomposition study was performed during the summer months of 2015 (June until July) at an outdoor research site, belonging to the University of Central Lancashire (UCLan) in the North-West of England, Lancashire, United Kingdom. Twenty domestic pigs, *Sus scrofa domesticus,* were utilised as models for human decomposition, with the control group ('C') and the sample group ('S') consisting of ten carcasses each. The weights of the carcasses ranged from 38.3kg to a maximum of 57.85kg for the sample group, while the carcasses of the control group weighed between 32.95kg and 52.6kg. The mean weight of the sample group was 48.96kg, while the control group mean was 44.52kg. Matuszewski *et al.* recommend weights of above 40kg to account for a wide range of carrion insects. Only one carcass of the sample group was below the recommended weight (38.3kg) and two of the control group weighed in below 40kg (32.95kg, 38.85kg).

After ethical approval by UClan's Animal Projects Committee, the animals were humanely dispatched, by a licensed individual, with the use of a captive bolt apparatus. Each carcass within the sample group had a plastic bag placed over the head and secured by a rubber band at the neck (ears within the bag) (Figure 1), with the control group not being modified. The carcasses for each group were selected at random and placed out in a field of open grassland, each ten meters apart from the next, each covered by a mesh-lined cage to allow for insect access [(29)]. The carcasses were acquired from a near-by commercial farm, leading to placement shortly after death (< 5 hours).

Megyesi *et al.* [2] created a scoring system to allow for a quantitative method of scoring decomposition. To utilise this methodology a total body score (TBS) is allocated. This is calculated by summing the three regional scores assigned to the head/neck, trunk and limbs. Each region is visually assessed and based on the criteria developed by Megyesi *et al.* [2] a score is assigned. The scores start at one for each region and go up to a maximum of 10, 12 and 13, for the limb, trunk and head scores respectively. The TBS directly after death would be given a score of three, with the final stage of decomposition being dry bone for all regions and being scored with a TBS of 35. The separation of TBS into regional scores makes comparison between regions possible and aids in determining a pattern. Though the methodology was developed utilising case reports of human decomposition, the system is applicable to *Sus scrofa domesticus* (domestic pig) studies, as the domestic pig decomposition is comparable to human decay [10,18,30,31, Keough et al 2017, Matuszewski et al 2019].

During the project period, from June 9th to July 30th 2015, data were collected on TBS, regional scores and temperature, while leaving each specimen undisturbed throughout the entirety of the project. The site was attended twice weekly (ca. every 50 ADD). On these days all 20 carcasses were photographed and the regional scoring of the head/neck, trunk, limbs, and TBS were recorded. These scores were given based on soft tissue modifications of the carcass [2]. The three regions were scored separately due to possible differences in decomposition rates between the regions and to determine a decomposition pattern. The overall TBS aided in determining differences in rate of decomposition between groups ‘C’ and ‘S’.

To record temperature data, all 20 carcasses had internal data loggers, inserted rectally before placement in the field. In addition, sample subjects (‘S’) had a data logger placed within the bag, recording the internal temperature thereof. Twelve loggers were distributed around the site to record ambient temperature. All 42 data loggers recorded temperature in degrees Celsius (°C) every six hours. Together with the temperature from the ambient loggers and the local weather station the accumulated degree days (ADD) were calculated.

All data collected were analysed with the R Studio analysis package, Version 3.1.1 [32]. The weights of each group were compared within and between the two groups, to detect any weight differences and possible outliers for exclusion. To test for differences within each group a Shapiro test was performed for each group separately. A comparison of weights between the two groups was performed by creating a linear model of the data; with the explanatory variable being the group and the response variable being the weights of the carcasses. After normal distribution of the residuals and equal variance of the data was shown, a Student's t-test was performed. To check for a statistical relevance of weight, to whether insects had gained access to the bag or not, a linear model was created and an ANOVA test was performed on the data.

To determine whether there was a difference between the rates of decomposition of the sample and control groups a mixed-effects linear model of the data was created. While the group (sample/control), together with ADD, acted as the explanatory variable and TBS as the response variable, the carcass was included as a random variable. The residuals were not normally distributed, leading to the transformation of ADD to $\log_{10}$ADD.

Differences in pattern of decomposition were determined between the two groups as well as within the groups, between regions (head/neck, trunk and limbs). Again a mixed-effects linear model was created with the data, utilising regional scores as the response variable, and the region within the group in combination with $\log_{10}$ADD as the explanatory variable. The specific carcass acted as the random variable. A final ANOVA test was performed on the data.

Temperature data collected during the course of the study were compared utilising a linear model and performing a Tukey test. Due to the prior Q-Q Norm plot showing the data to be not normally distributed, all temperature data were transformed to the power of -1. The following Tukey test showed the statistical significance.

RESULTS

On placement of the animals at the site, the TBS of each carcass within both groups was given a score of three. The carcasses had been placed shortly after death and appeared fresh. Though some rigor and lividity was present, this falls within a TBS of three. By the final day of the project the carcasses had been exposed for 790.9 ADD, 52 days. The highest TBS recorded for the sample group was 31, with a mean of

25.2. The highest TBS recorded for the control carcasses was 27, the mean TBS for this group was 24.4.

The results for weight differences, showed no statistical weight difference between specimen carcasses within each group (Sample: p=0.13, Control: p=0.94), leading to no exclusion of any specimen. When comparing the weights between the control and sample carcasses, no statistical significance was found (t=-1.48, df=18, p=0.16). (Figure 2).

Due to 60% of the bags being breached by insects by the end of the study, the weight of the pigs was tested, against whether or not insects had gained access. The following tests showed that insects gaining access was not significant to the weight of the carcass (ANOVA: $F_{1,8}$=0.06, p=0.8). Only whether or not insects had obtained access by the final data collection day of the study was included in the data.

Results of determining differences between rates of decomposition were as follows: no statistical difference was found when comparing both groups without the inclusion of ADD (t=1.19, p=0.07). However, when the interaction of the group (sample/control) and ADD was used to compare the TBS of both groups a statistical difference between rates of decomposition was found (t=-2.2, p=0.02). Figure 4 shows the regression of TBS vs $\log_{10}$ADD for both groups.

When looking at the differences in the pattern of decomposition, the results showed a statistical difference of decomposition rate between the heads and trunks of the two groups ($F_{2,875}$=24.62, p<0.0001 and $F_{1,278}$=7.08, p=0.008 respectively). Limbs, however, decomposed at a similar rate for both groups ($F_{1,278}$=0.17, p=0.68). The heads of the sample group were initially assigned higher scores than the control group, however by the end of the study the heads of the control group had higher scores assigned, compared to the sample. The opposite effect was seen for the trunk scores. Insect egg masses were first found on the control heads and limb creases after 127.6 ADD, while the sample carcasses showed egg masses around the neck/rubber band, upper limb and anus/back at this time. Only when the bags were breached, maggots were also found on the sample heads, initially seen at 251.8 ADD on two carcasses. In cases where the bags were not breached by maggots, feeding was only seen at the trunk and limb sites.

In comparing each region (head/neck, trunk, limbs) within each group, it was found that the head decomposed at a faster rate than the trunk and limbs, with the trunk decomposing quicker than the limbs. Therefore, the rates can be described as: head>trunk>limbs in descending order of decomposition rate. This was found to be true for both groups. Figure 5 shows the descending order of decomposition rates for the regions as well as the comparison of regional scores between groups and their regression.

Regarding the temperature data collected, the outcome of the statistical evaluation showed the control ($p<0.001$), sample ($p<0.001$) and bag temperature ($p<0.001$) to be significantly different to the ambient temperature. However, there was no statistical difference found between the internal carcass temperatures of the groups ($p=0.99$), nor between the internal carcass temperatures of each group and the bag (control: $p=0.6$ and sample: $p=0.44$). Figure 3 shows a graph of the temperature data by day, taken during the course of the project (52 days).

Discussion

The plastic bag covering the head of the sample carcasses had multiple effects on the decomposition rate and pattern of and between the two groups. The overall rate of decomposition between the two groups differed significantly, with the sample group initially having higher TBS than the control group; however, as seen in Figure 4, the control group has a higher overall rate of decomposition. The study ended with the controls being scored with higher TBS compared to the sample group. The crossover point at which the TBS of the controls overtook the sample group was at ca. 225 ADD. It can be said, that the bag slowed down the overall decomposition rate of the sample; however, if a cadaver is found within the first 225 ADD, the rate of decomposition is initially increased. This should be taken into consideration when determining a post-mortem interval. The initially high TBS of the sample group carcasses are due to the high regional score of the head at the start of the study. After the first 50 ADD, at the first data collection day, the bags around the heads had collected ca. 200 ml of dark fluid (Figure 6). This gave a comparatively increased regional score of 5 for all sample carcasses; compared to the control heads with scores of 1-3.

It can only be hypothesised as to the origins of the liquid within the bag, as no chemical analysis was done. However, due to the early appearance of the liquid (within 48 hours after placement) it is most likely a combination of factors such as: purging from the head wound, chest or abdominal cavity via the mouth and nose and condensation forming due to the air in the bag and the initial warmth of the carcass cooling. The liquid is, however, not likely to be purging of decompositional fluids due to accelerated decomposition of the internal organs. During the course of the study the bags collected further fluid (ca. 500ml), this being attributed to decomposition of internal organs. Although decompositional purging from the head orifices is expected at a later stage of decomposition [(2)], initial purging as seen in this study, has yet to be discussed in previous literature; this may be due to it not being collected and therefore not being identified. Furthermore, the carcasses were placed on their sides; in cases with individuals found in an upright position, liquid would not be excreted into the bag.

The arising pattern of decomposition of the head>trunk>limb regional scores was seen in both groups (Figure 5). However, the head and trunk regions decomposed at different rates when comparing both groups, while the limbs showed no statistical difference between groups. A difference for the head decomposition was expected, as the treatment of the groups varied at this region. Nevertheless, a trunk difference in decomposition rate shows the influence of partial carcass covering on regions that are exposed. As seen in Figure 5, both the head and the trunk decomposition rate regression lines crossover throughout the project. While the head scores of the sample are initially higher than the control group, the trunk scores of the control group start off higher whereas the sample scores end the project with higher scores. The regression lines of the head crossover at approximately 200 ADD, while the trunk crossover point is at around 120 ADD. These crossover points are due to two separate occurrences. The initially high head scores of the sample group did not change for the first two data collection days, giving the control group time to decompose to the point of passing the sample group in decomposition on the third collection date. Throughout the rest of the study the sample heads without insect access did not show as much decomposition increase, ending with a score of 7, compared to the control group with scores of 9-11. Meanwhile the trunk regional scores for both groups started off at similar scores with the rate of decomposition of the sample group being significantly higher by the end of the study. This could be due to insects not having access to the head and therefore oviposition and feeding commenced at other areas around the trunk, leading to greater feeding at this area compared to the control group, and thus higher regional scores.

During the course of the study, insects gained access to six of the bags covering the ten sample carcasses' heads. The carcasses with and without insect access were not considered separately as this shows the overall variability which would be expected in human scenarios. The bag and rubber bands were still intact at the end of the study, were tight around the remaining soft tissue, and did not dry out during the summer months. It is not possible to say what factors influenced whether or not insects acquired access to the bag. Weight was not statistically relevant to whether or not insects gained access, although this was thought to be one possibility, as heavier carcasses' heads filled out the bag more making the rubber band tighter. Once insects had accessed the bag, the heads decomposed rapidly being almost fully skeletonised by the end of the study, due to the insects eating away the soft tissue and being protected from the elements. These heads were surrounded by liquid, with the bones covered in a dark, oily film. This was not the case for any control carcasses with the heads showing mummification and dry bone exposure. The difference between the heads is due to the bag inhibiting the desiccation of the head, whilst protecting the feeding insects within. Due to the higher than ambient temperatures within the bag, a warmer, moist environment was created. Figure 7 shows S6 with maggots within the bag and S8 without maggots, at ca. 380 ADD.

Insect access showed to be the most destructive factor in soft tissue removal, as mentioned in previous literature [7,12]. Due to the plastic bags covering the head, oviposition took place at other sites, including within the lining of the bag, near the rubber band. This is either due to this site being warm and protected from weather, the rubber band having a similar morphology to skin creases i.e. limb attachments or could be due to decompositional gases from the head leaking through the bag and attracting insects nearer to the head. Though a heavier colonisation was expected at the anus, as this was the only other site of decompositional gases being expelled, there were no comparatively larger masses than on the controls within this area. Other sites of oviposition were the limb creases and the back, near the ground, as these were areas protected by the carcass and surrounding grass from weather. This may have contributed to higher trunk decomposition rates within the sample group, as the head was not available for oviposition. In terms of insects being attracted to the sample carcasses later, due to the head covering limiting decompositional gases escaping, this cannot be quantified. At the first sight of insects at ca. 50 ADD, they were present on carcasses of both groups. Egg masses and small maggot masses were found on both groups at the next data collection day (ca. 125 ADD). However, due to the author only attending the site approximately every 50-75 ADD, it may very well be possible that insects had been attracted to and had ovipositioned on the control carcasses before the sample carcasses were subjected. More frequently monitoring the groups can show whether this phenomenon is true.

As the overall rate of decomposition of the sample group was decreased in comparison to the control group, the calculation for determining PMI was adjusted. Megyesi *et al.* (2) created a calculation from the regression line of TBS vs ADD, to estimate ADD from a visual TBS scoring. Knowing how much heat has been placed on a carcass may allow for an estimation of a time since death, when temperature records of the area are available. Due to the significant difference in overall rate of decomposition between the two groups a new calculation for ADD was determined for the sample group:

Megyesi *et al.* (2) calculation: $ADD = 10^{(0.002 \times TBS \times TBS + 1{,}81)}$

New calculation: $ADD = 10^{\frac{TBS+17.25}{14.32}}$

Temperature measurements were taken: internally for both group carcasses, within the bag of the sample group and ambient temperature of the site. All three data loggers associated with the carcasses showed significantly higher temperatures than ambient temperature. It is known that maggot masses can create heat of more than 20°C greater than ambient temperatures while feeding (16). It is uncertain, however, whether the similar heat within the bag to the internal carcass heat was due to maggots feeding from within the bag or head, or whether the plastic bag created the significant amount of heat to compare to feeding larvae. As temperature was not taken near or within the control carcass heads, there is no quantifiable difference between a head with and without a plastic bag, in terms of temperature. In Figure 3 it can be seen that the temperature within the bags is slightly higher than the other three measurements for the first half of the study, while being comparable to the internal temperatures for the final half. As insects did not fully access the bags until later on in the study, it may be inferred that the initial higher temperatures were indeed caused by the plastic bag, while later heat was due to insect activity.

Though Megyesi *et al.*'s scoring method has been criticized in recent years, with adjustments being proposed (Moffatt et al, 2016, Gelderman et al, 2017, Marhoff-Beard et al, 2018), at the time of the study this was the most appropriate method. Utilising an adjusted method retrospectively on the study, would introduce sources of error, as an observer could only utilise pictures for the scoring of TBS. Though making use of photographic images has been found in many research papers, including Megyesi *et al.,* Dabbs *et al.*(2017) have mentioned issues with scoring based solely on photographs, with the importance of full documentation of the regions, overall body, features of interest and importantly, proper color representation. As the aim of this study was to score TBS *in-situ*, the photographs taken cannot be seen as appropriate for scoring.

There are further limitations to the study, which need consideration in future research. Firstly, as Sauvageau and Racette [(23)] discussed in their 2006 paper, most deaths involving plastic bags occur indoors. Though this study was conducted in an open field, the results between a comparison of a control and sample group are expected to be similar indoors. Insects are found quite readily indoors and will find their way to carcasses, if there is any opening to the room [(33)]. Anderson [(33)] found a delay in insect access and therefore decomposition between indoor and outdoor carcasses. This would be expected in an indoor setting for both a sample group with a plastic bag covering the head and a control group without a covering, with additional delay in decomposition for the sample group, due to the covering of the head.

In numerous case reports, [(23,24,28)] especially involving accidents of solvent abusers and in many cases of suicides, individuals have been found to have high blood alcohol levels or other drugs in their system. The current study shows the effect of the plastic bag on the rate and pattern of decomposition, however, fails to include the effect of any solvents, whether intrinsic to the overall carcass or gaseous, within the bag. The exact effect of drugs of abuse on the rate of decomposition is currently unknown, however, this may have a great impact on decomposition in its own right and in combination with a plastic bag head covering. No quantitative study has been done on these effects. Though this should be considered in future research, ethical implications are substantial.

Though the plastic bag was clear, visual observations of the sample heads was difficult at times. Condensation, foaming, fluids, insect activity and clouding of the bag all contributed to difficulties in scoring the heads. Though this was a slight inconvenience, nothing could have contributed to better visualisation, without movement of the bag, introducing possible insect activity or liquid loss. Though there were times the scoring presented difficulties, a score could always be determined by the author: the fluids were never fully covering the heads, the insects could be slightly moved by using the bag to swipe the insects to the side for better visualization. A further observer was not utilised as there were no means to do so, however both Dabbs *et al*. (2016) and Nawrocka *et al* have since concluded that the Megyesi *et al*. scoring method has a high inter-observer reliability.

Finally, the new calculation suggested for use is based only on the small sample size of 10 carcasses. This should be considered and a larger margin of error expected.

CONCLUSION

During this study the effect of a plastic bag covering the heads of ten *Sus scrofa domesticus* on the rate and pattern of decomposition was determined. A significant difference was found in overall rate of decomposition between the sample group and a control group, without a head cover. After an initial high rate of decomposition of the sample group, an overall delay in decomposition could be determined by the end of the study. The pattern of decomposition rate was found to be the same between the groups: head>trunk>limbs in order of decreasing decomposition rates. Differences were determined between the head and trunk regions when comparing rates between the groups; with the heads of the control group decomposing at an overall greater rate than the sample group, while the trunk of the sample group decomposed quicker than the controls. Both of these results were deemed due to the plastic covering of the head, which slowed down overall decomposition of the sample group, while also speeding up decomposition of the trunk, due to heightened insect activity at this area.

There were multiple limitations of the study, including visibility within the bag, a small sample, and case reports highlighting victims being indoors, with many having alcohol and drugs present in their system. Furthermore, only one observer was used for scoring the decomposition. Most importantly, however, the scoring system should be considered and compared to adjusted versions, such as Gelderman *et al.*(2017), as well as a required update in the statistical analysis according to Moffatt *et al.* (2016). Consideration of these aspects is needed in further study of the effects of a plastic bag covering the head. Though this study has a relatively small sample size, comparisons between the two groups could be made and quantitatively discussed. Due to the statistically significant results and the rise in plastic bag related deaths [28], future research into the effects of plastic bag suffocation or partial covering of a carcass is warranted.

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