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The Effect of Embalmming and Insect Activity on the Decomposition Timeline of Carrion in a Tropical Nigerian Climate

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Abstract

The need to determine PMI is very crucial in clandestine deaths, and insects play quite a significant role is this estimation. After death, a sequence of naturally occurring post mortem changes are immediately activated first, by the actions endogenous microorganisms and later by exogenous necrophagous insects with temperature being a crucial determinant factor. Although these changes proceed in a relatively orderly fashion, a variety of external factors like topography and chemical substances like poisons and in most cases embalming fluid pose a great challenge for Taphonomist in PMI estimations. The present study was conducted to examine the effect of embalming fluid and insect succession patterns on the decomposition timeline of pigs (*Sus Scrofa*). The study was carried out on two six week old Pigs (*Sus scrofa*) that were kept on the surface and exposed to varying degrees of environmental factors. One was embalmed and the other was not embalmed. Observations and temperature data were recorded daily throughout the 30 day study period. Insects & larvae were captured and stored in 70% ethanol and were later identified by a Forensic Entomologist. Observation from the study showed that embalmment significantly delays the natural process of decomposition. The embalmed pig did not follow the decomposition timeline.

It took just seven days for the unembalmed pig to reach skeletinization/mummification while the embalmed carrion remained fixed throughout the study period. It was also observed that insect activity varied significantly between the specimens. The unembalmed carrion attracted more muscidae, formicidae and sacrophidae species while histeridae, scarabidae and cleridae were mostly found around the embalmed carrion. Conclusively succession patterns showed that dipterian species were mostly associated with early stages of decomposition while coleopteran and dermastids were associated with advanced stages. Our tropical decomposition timeline confirms the assertion that decomposition is highly variable from one micro climate to another and data from one geograpical region cannot be used for post mortem interval calculations in another geographical location.

Keywords: Decomposition; Taphonomy; Forensic Entomology; Embalmment; Post Mortem Interval

Introduction

Decomposition is the degradation process of living things into basic respective constituents macroscopically and microscopically by action of microorganisms, arthropods and scavengers [1]. The decomposition of a body is a mixed process that varies from cellular autolysis by endogenous chemical destruction to tissue autolysis, by either the release of enzymes or external processes, resulting from the bacteria and fungus in the intestines or from external environment [2]. After death, a sequence of naturally occurring post mortem changes are immediately activated first, by the actions of endogenous microorganisms and later by exogenous necrophagous insects with temperature being a crucial determinant factor. These postmortem processes aside reducing animal remains to basic organic constituents are heavily relied upon by forensic experts in the estimation of the Postmortem Interval (PMI) [3]. Although these changes proceed in a relatively orderly fashion, a variety of external factors and intrinsic characteristics may accelerate or retard animal decomposition. These factors include; climate, topography, mode of death, insect succession patterns and chemical substances like poisons and in most cases embalming fluid. Studies have demonstrated that embalming greatly slows down the decomposition process and can be very detrimental in PMI calculations thereby making estimation very difficult.

Furthermore, it is important to state at this point that a lot of taphonomic studies have been carried out in Europe, America, Asia and Australia [4-6] with only a few studies from Africa [7,8] and because it is an already established fact that data from one microclimate cannot be successfully applied to a different microclimate owing to the unique fauna, flora and temperature variations. To solve this problem, model studies like ours will help generate baseline data for our tropical climate and also aid forensic experts in knowing to what extent embalming fluid influences the decomposition timeline and its resultant effect in the estimation of PMI.

Method

The study was conducted at the Department's Forensic Anthropology Research Facility. The topography of the area where the research was conducted is a combination of hills and lowland areas. It has an area of 972km2 65 and an estimated population of 171,901. It is located in Southern Nigeria between latitude 06º39'30'N to 08º47'57'E with an elevation of 96m above sea level. The study was carried out on two six week old male Pigs (Sus scrofa). One was embalmed and the other was not embalmed. The weights of the pigs were 5.2Kg for the embalmed pig and 5Kg (11Ibs.) for the unembalmed pig. Both pigs were confirmed healthy by a vet from where there were purchased. Length and girth measurement around its chest and waist were all recorded using a digital body thermometer and tape respectively. After which the pigs were euthanized by suffocation at about 09:27a.m local time. Once death was confirmed both experimental animals were bagged and taken to the site where they were placed in wire mesh cages for observations to commence.

The cages allowed insect activity to occur without hindrance but prevented the pig carcasses from being disarticulated or dragged away by larger predators. The carcass was visited thrice daily for the first week, twice daily for the second week and once daily for the third and fourth week. Ambient daily temperatures were recorded from the onsite atmospheric thermometer during each visit to the site. The study was carried out between the months of May and June 2018. This period is usually characterized by moderate amounts of rainfall and humid temperatures. Another study has been designed for the dry harmattan season.

Methodology Employed in Unembalmed Pig

The animal was immediately bagged and taken to the study site which was about 15 minutes away. The carrion was placed on the ground surface and secured with metal wire mesh buried deep inside the earth and held in position with cement. The cage was needed to protect the decomposing carrion from scavengers.

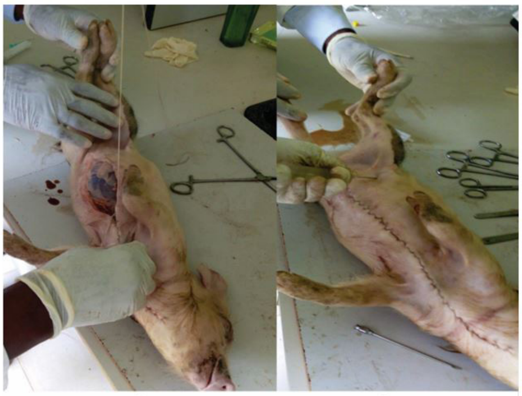
Methodology Employed in Embalmed Pig

All embalming equipment used was provided by the Department's Morbid Anatomy unit. Embalmment was carried out in the unit by an experienced embalmer who had over 15 years of experience in the field. The pigs were embalmed in a manner that would be performed on a human comparable in size and weight. A vascular (arterial) method was chosen along with the cavity method since these two are routine in the regular embalming of the human body. The method was adopted from [9]. The pig was slit from below the chin to below the sternum. The carotid artery was then located. A 60ml Kendall Monoject™ Luer Lock syringe was used to regulate the rate of pressure and rate of flow of embalming fluid injected. Once the fluid had been injected, pink foam began escaping from the nostrils of the pig. This is usually a sign that the body has been thoroughly penetrated by the embalming fluid. The incision was then closed using a circular curved needle and ligature twine.

All four points were located on the abdomen, and the syringe was shifted back and forth as injection was taking place in order to ensure that the cavity fluid permeated internal organs. The embalmed pig was then bagged and taken to the site and placed on the surface in a separate cage far away from the unembalmed pig for observations to commence (Figures 1 & 2).

A B

Figure 1: Embalming Procedure Photographs **(A)** Locating major arteries and veins. **(B)** 105 Injecting formalin with a syringe into the arch of aorta



C D

Figure 2: Embalming Procedure Photographs **(C)** Pig sutured with ligature. **(D)** Four point 109 cavity injection of embalming fluid with a syringe.

Insects associated with the decomposing pig carrion were collected with either blunt forceps or sweep nets for trapping flies. Collected insects and their larvae were preserved in specimen tubes containing 70% ethanol. On completion of the 30 days observational period, all necrophagous insects and larvae obtained from the site were identified by a Forensic Entomologist. The daily ambient temperature of the study area were recorded using general purpose 'glass rod'mercury thermometer and portable digital thermo hygrometer. Photographs were also taken during each day of this study, starting from the first day of the research to its final day to document appropriately the stages of decomposition and the succession of fauna.

Results

The results presented are a compilation of visual observations obtained from visits to the research site and weather data recorded daily. Visual observations serve to describe the state of decomposition and examine insect succession on the carrion.

Observations for Unembalmed Pig

Day 1: May 29, 2018 (Figure 3)

Figure 3: Fresh Stage of *Sus Scrofa* (Day 1, May 29, 2018)

The fresh stage of decomposition began on this date (Day 1) and continued until Day 2 when the carrion began to bloat. The weather conditions on the day of placement were hot and dry with an ambient temperature of 27^{o} 133 C. Dipterans of the family of Muscidae were the first flies attracted tothe carrion immediately upon deposition and other insects present included Hymenoptera (*F.Campanotus preiisi, and F. Dorylus affinis*), which was noticed around the anus and could also be seen feeding on the eyes.

Day 2: May 30, 2018 (Figure 4)

Figure 4: Bloat Stage of *Sus Scrofa* (Day 2, May 30, 2018)

Day 2 marked the end of the fresh stage and the beginning of the early decomposition stage. This stage was characterized by the discoloration of the body and bloating with purging of decompositional fluid from the mouth, nose, and anus. The skin was pink and marbled and the gravitational pooling of blood (lividity) on the dependent body parts was observed. Diptera were prevalent especially around the mouth, nose, and anal regions. Calliphoridae could also be seen depositing eggs on the orifices around the head and anus. Odor associated with this stage was mild and characteristic of the natural scent of the animal and fecal materials.

Day 3: May 31, 2018 (Figure 5)

Figure 5: Early Decay Stage of *sus scrofa* (Day 3, May 31, 2018)

By Day 3, the carrion began to deflate and entered the active decomposition 157 stage. This stage was characterized by greenish/greyish discoloration of the skin around the ventral aspect of the thorax and abdomen. There was also signs of visible skin slippage and leaching of fluids from the mouth and anal orifices. Maggot activity began and was extensive, concentrating on the open orifices of the eyes, ears, nose, mouth and anus. Other regions that exhibited extensive maggot activity included the neck, back of the head, and stomach region. These maggots were found out to be in the first instar larvae stage.

Day 4: June 1, 2018 (Figure 6)

Figure 6: Active Decay of *Sus Scrofa* (Day 4, June 1, 2018)

This stage shows an increase in maggot activity as they actively devour carrion. It was characterized by sagging of flesh caving-in of the abdominal cavity. There was also an increase in maggot size. Odor emanating from the carrion was severe and pungent and could be perceived from over 50 yards away. Insects that actively participated during this stage include Calliphoridae (*Chrysomya* and *Phormia species*), Histeridae (*Histamonoton*) and Scarabidae (*Strategus antacus*).

Day 6: June 3, 2018 (Figure 7)

Figure 7: Advanced Decay of *Sus Scrofa* (Day 6, June 3, 2018)

Advanced decomposition began on Day 6, June 3, 2018 and lasted until skeletonization. This stage was characterized by the complete breakdown of soft tissue, greasy appearance of the now exposed bones of the head and limbs and *Hymenopterans* making beds around the carrion. The black stain resulting from the leaching and seepage of fluids increased in size to approximately 35cm surrounding the entire carcass. A sign of mummification around the thoracic region was equally observed as dark-brown dry skin was seen to adhere strongly to the bones of thoracic region and some parts of the skull. Large maggots were observed moving away from the carrion to burrow into the soil to pupate. This activity was not observed to occur in maggot masses but individually (Figure 8).

Figure 8: (A) Mummification/Skeletal Stage of *Sus Scrofa* (Day 20, June 17, 2018). **(B)** Skeletal Stage of *Sus Scrofa* (Day 30, June 27, 2018)

The carrion continued to darken and dry out making the bones more visibly seen. The most notable areas of drying included the ears, mouth, nose, and eyes. The maggot activity drastically reduced and new succession of insects, which were mostly beetles, were the ones now seen feasting on the now dry and partially mummified skin. The carrion remained in the skeletal stage following its transition from Mummification/skeletal stage for the remainder of the surface study. The already disarticulated bones were now very dry with very minimal insect presence. At the end of observations, the study site was carefully swept all puparal cases that were found present were collected. The bones collected were taken to our Histology Laboratory, for cleaning and bleaching to be used for comparative studies.

Observations for Embalmed Pig

Upon placement of the embalmed *sus scrofa* in the field, it was immediately observed that very few insects visited the carrion and the few that came died on prolong contact due to what we noticed was the toxic embalming fluid. Dipterans were the first flies attracted to the carcass 8hours upon deposition. The first stages of decomposition were totally altered dew to the replacement of arterial fluid with embalming fluid. The primary signs of death such as *rigor, algor* and *livor mortis* which usually occurred at predictable intervals in the early postmortem period and the even the progressive decomposition timeline of autolysis, putrefaction, bloating and skeletonization were totally absent as the body was fixed. The only significant observation that was made during the first 3 days was a foamy substance that poured out from the nostrils of the carrion (Figure 9).

Figure 9: Day 2 & 3 - foamy substance coming out through the nostrils and mouth

We observed on day 5 & 6 that the head region began to enter the active decay stage, though the remainder of the pig's body remained relatively fresh. Insects were finally able to feast on the soft tissues of the head without being killed by the now fading embalming fluid. Diptera were the only taxa noticed on the carcass and its larvae was also seen at the neck region (Figure 10).

Figure 10: Day 5 & 6 – insects noticed only at the neck region.

By day 7 the torso was still being avoided by insects and the few flies that 228 dared come near only perched and immediately flew away indicating that the embalming fluid which saturated the abdomen was still repulsive and therefore hampered insect activity around the thorax and abdomen. In contrast, the skin around the head was undergoing color changes and early signs of mummification. The mummification around the head region kept on advancing till day 11 when it was observed that decomposition had gradually reached the lower neck as seen from the cluster of maggots at neck region. A mild odor could also be smelt from a few yards away indicating that the embalming fluid was beginning to wear off and that putrefaction had finally set in. events stayed that way until day fifteen, when we noticed that the limbs began to change from a black leathery appearance to a dry mummified skin. Insect activities were also on the increase with *Hymenoptera* and Coleoptera the major ones actively feeding on the decomposing carrion. Between day 16 and day 20, bulk of the skin around the head, neck and limbs were completely mummified while the skin around the trunk above the ground was beginning to show signs of mummification. The only insects that could be found were mostly beetles and ants that burrowed the soil around the carrion (Figure 11).

Figure 11: Day7, 6:56PM -mummification set at the nose eye and lips

The final 10 days of observations from Day 20 through Day 30 showed minimal changes in the head and limbs with most changes occurring at the trunk. Though there was little bloating observed, the hair on the skin of the trunk sloughed off and the skin immediately 249 entered a dry mummified state (Figure 12 & 13).

Figure 12: Day 11&19 Small maggot mass at the neck & increase in mummification at limbs and torso.

Figure 13: Day 21-30 Carrion partially buried as result of the action of burrowing insects & Excavated remains of the embalmed carrion on the last day of observation

Post Observation Dissection

Our study was designed to last for 30 days so on completion of observations in the field, we were curious to find out the extent of decomposition of the internal organs given the fact that the carrion did not fully bloat and as such there was no caving-in of the 262 abdominal cavity. The carrion was taken to our anthropology lab where it was dissected. To our surprise, the entire abdominal viscera were still intact and traces of blood were still noticed in some areas. The stomach and intestines were cut open and everything was sterile with no maggot or insect activity noticed, in fact the food that was last eaten by the carrion was still in the stomach implying that the embalming fluid was still able to preserve viscera even after 30 days! (Figure 14).

Figure 14: Dissection of embalmed carrion. Note the fresh internal viscera and dissected stomach with food left intact.

Temperature Data (Figure 15)

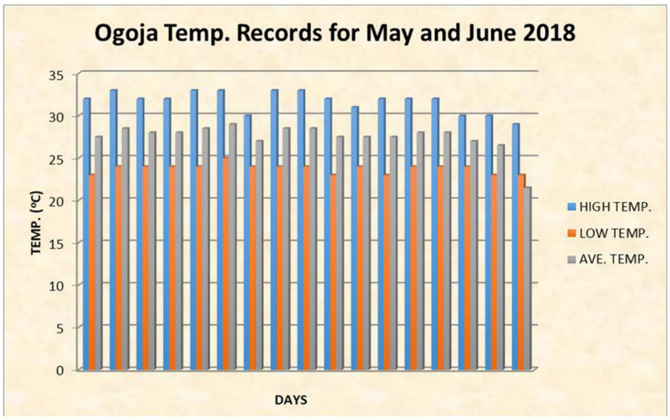


Figure 15: Daily High-Low and Average Temperatures for May and June 2018

Necrophagous Insect Succession Data

All insect identifications were carried out by renowned forensic entomologist (Table 1-3).

Order	Family	Genus Species
	Muscidae	*Musca Species*
	Sarcophagioae	*Fannia Species*
	Calliphoridae	*Calliphora Species*
	Calliphoridae	*Chrysomya*
Diptera	Muscidae	*Hydrotaea chalcogaster*
	Calliphoridae	*Phaecia Species*
	Phoridae	*Megaseliascalaris*
	Calliphoridae	*Phormiaregina*
	Fannidae	*Fannia species*
	Muscidae	*Atherigonaorientalin*
Coleoptera	Scarabidae	*Strategus Antacus*
	Histeridae	*Hister Species*
	Histeridae	*Histamonoton*
	Dermestidae	*Dermestis species*
	Silphidae	*Saprinus species*
	Cleridae	*Necrobia species*
	Anobiidae	*Troxsuberosus*

Table 1: Insect taxa associated with EMBALMED carrion

Date Collected	Time		Insects
	AM	PM	
29/05/2018	X	X	*Musca Species*
	X		*Fannia Species*
		X	*Campanotus preiisi* (Formicidae)
	X		*Dorylus affinis* (Formicidae)
		X	*Phormia regina Crematogarter lineolata* (Formicidae)
30/05/2018		X	*Strategus Antacus* (Coleoptera)
		X	*Hydrotaea chalcogaster* (Muscidae)
		X	*Pheidole species* (Formicidae)
	X		*Chrysomya megacephala*
31/05/2018		X	*Phormia Species*
		X	*Hister Species* (Coleoptera)
	X		*Campanotus maculatus* (Formicidae)
		X	*Hista monoton* (Coleoptera)
		X	*Megaselia scalaris*
		X	*Grylloblata species*
1/6/2018		X	*Chrysomya species*
	X		*Phormia regina*
	X		*Dermestis species* (Coleoptera)
		X	*Megaponera foetens* (Formicidae)
	X		*Strategus antacus* (Coleoptera)
	X		*Hista monoton* (Coleoptera)
2/6/2018	X		*Grylloblata species*
		X	*Agathis aciculatus*
3/6/2018		X	*Saprinus species* (Coleoptera)
	X		*Megaselia scalaris*
	X		*Chrysomya*
		X	*Hista monoton* (Coleoptera)
		X	*Glymmatophora dejoneki*
4/6/2018	X		*Necrobia species* (Coleoptera)
	X		*Trox suberosus* (Coleoptera)
8/6/2018	X		*Fannia species*
17/06/2018	X		*Atherigona orientalin*

Table 2: Distribution of Insects Associated with the UNEMBALMED carrion and the specific dates there were collected

Decomposition Stage	Associated Insect	Developmental Stage
Fresh	Muscidae (Diptera)	Adult
	Formicidae (Hymenoptera)	Adult
	Sarcophagioae (Diptera)	Adult
	Calliphoridae (Diptera)	Adult
Bloated	Calliphoridae (Diptera)	Adult and Larvae
	Muscidae(Diptera)	Adult and Larvae
Decay	Calliphoridae (Diptera)	Adult and Larvae
	Histeridae (Coleoptera)	Adult
	Phoridae (Diptera)	Adult
	Formicidae (Hymenoptera)	Adult
	Scarabidae (Coleoptera)	Adult
	Dermestidae (Coleoptera)	Adult

Decomposition Stage	Associated Insect	Developmental Stage
Mummification/Skeletal	Silphidae (Coleoptera)	Adult
	Braconidae (Hymenoptera)	Adult
	Calliphoridae (Diptera)	Adult and Larvae
Dry	Cleridae (Coleoptera)	Adult
	Anobiidae (Coleoptera)	Adult
	Dermestidae (Coleoptera)	Adult and Larvae

Table 3: Distribution of Insects Associated with each Stage of Decomposition for UNEMBALMED carrion in a tropical Nigerian climate

Discussion

The need to determine PMI is very crucial in clandestine deaths, and insects play quite a significant role is this estimation. The present study was conducted to show the pattern of decomposition and the effect of embalming fluid on domestic pigs (*Sus Scrofa*) that were kept on the surface and exposed to varying degrees of environmental factors. Estimating the postmortem interval can be a daunting challenge for criminal investigators, forensic scientist and emergency workers who attempt to identify victims of mass disasters. It has been well recorded that several factors are responsible for the inaccuracies that abound in PMI estimations. Chief among these factors are temperature, necrophagous insects associated with decomposing carrion and chemicals and other xenophobic substances that the carrion was exposed to pre mortem, peri mortem and post mortem. The use of chemicals like formalin have been shown to delay the onset of decomposition making PMI estimates less accurate on such bodies. Such malpractices are usually carried out for criminal concealment, when for instance the perpetrator needs more time to bury the victim.

When such cases are found, it is necessary that such factors be taken into consideration by the forensic experts handling the case. As observed in our study and confirmed by Bass [10], embalmment greatly delays the natural process of decomposition. After the death and placement of the specimen in our anthropology facility, there were contrasting findings when comparing the embalmed and unembalmed remains. Primary postmortem changes were immediately observed on the unembalmed pig in patterns similar to those that have been reported by [11,12]. The noticeable changes included; absent of pupillary reflex, body temperature began to drop. Lividity was clearly seen to be formed following the pooling of blood in the capillary bed to the more dependent side on which the animal was placed. Few hours later, rigor mortis started developing around smaller muscles of the limbs and jaw. In contrast the embalmed carrion did not show any of the primary signs of decomposition due to formalin fixative. With regard to the stages of decomposition, it was highly variable between the embalmed an unembalmed pig.

The embalmed pig did not follow the decomposition timeline talk more of reaching skeletinization. It took 20-30 days for the unembalmed pig to undergo mummification and skeletisation. We discovered after the completion of the study that carrion decomposition for the unembalmed pig followed the already established patterns laid down by several authors whose taphonomic studies were carried out in other geographical regions [1,13-15]. The decomposition stage usually follows in four stages which are fresh, bloat, active decay and dry/skeletal. But in this study we observed that a later stage surfaced; mummification/skeletal stage. This stage occurred during the transition from the active decay stage to dry/skeletal stage. Therefore, we observed the following stages: Fresh, Bloat, Active Decay, Mummification/Skeletal and Dry/skeletal. This transition may be due to the inconsistency in the climatic conditions because high temperatures sharply contrasted with huge amounts of rainfall throughout the study duration.

Insect activity varied greatly between the two study animals. Insects were 361 not equally attracted to the specimens as it took just a few minutes for insects to swamp on the unembalmed specimen while it took a massive eight hours for any insect to come near the embalmed pig; the few that attempted to perch on the embalmed carrion died on contact. It was not until the fourth day, seventy two hours after deposition that minor insect activity was noted on the embalmed carrion. Omil [16] and McQuinn [9] had similar observations. Insect presence appears to be an important factor that hastens the rate of decomposition. The unembalmed carrion that received more insect visitation spent fewer days in each of the stages of decomposition than the embalmed specimen that was rarely visited by insects. This observation is supported by findings made by [10,17,18]. All the insect families recorded in this work have been known to be associated in carcass decomposition. For instance, five out of the twenty eight insect families (Formicidae, Calliphoridae, Sarcophagidae, Muscidae and Cleridae) recorded in the tropics where our work was carried out were also reported to have been associated with carcass of pigs in Brazil [19] and decomposing human remains in India [20].

More flies and maggots were observed and captured from the unembalmed pig while more beetles and ants were captured from the embalmed pig. This can be attributed to the diet of both types of insects. Beetles possibly benefitted more from the embalmed pig since they tend to feed on hide and dried tissues. Since the embalming cavity fluid, coupled with high temperatures and exposure to sunlight, essentially mummified most of the remains of the embalmed carrion, the beetles and ants had a larger food source there. This could have huge implications for PMI estimates as insect data may not be able to give accurate predictions in cases where bodies have reached more advanced stages of decomposition. Of all the insect taxa identified, it was observed that the succession patterns followed predictable patterns of colonization as previously documented by [21]. In comparing the insects found in our study with those recorded by elsewhere, we discovered that there were certain species that we found here that were not found in a similar study carried out in Kentucky, USA by [9].

This knowledge gives credence to the already established fact that fauna found in a different geographical location cannot be used by researchers elsewhere to estimate PMI. Our discovery of these new insect taxa should be added to the entomological database as it will assist in subsequent insect succession studies. In assessing temperature and its effect on decay rate, it was found that low temperatures resulted in fewer numbers of insect; in contrast it was found that the decomposition of carrion was slower on cool, doudy days. The opposite was recorded on warmer days since high temperatures intensified insect activity, resulting in a rapid depletion of the carrions. Temperature was found to be an important climatic factor that influenced insect abundance on the unembalmed pig. Environmental factors thus significantly influence the succession and colonization of insects on carrions and are unique to different regions and can even vary from one animal to another [22,23].

Subsequent studies are already underway to test the validity of our baseline data by making use of higher sample sizes and will also focus on using Accumulated Degree Days (ADD) and Total Body Scores (TBS) to develop predictive PMI equations for our tropical Nigerian climate.

Conclusion

This pilot study employed the use of one embalmed and one unembalmed pig (sus scrofa) to study the decomposition pattern and the necrophagous insects involved in the decomposition of carrion in a tropical climate. It was observed that unembalmed surface remains attracted more flying insects in contrast to embalmed carrion which attracted mostly crawling insects like ants and beetles. This pilot study though limited by a small sample size has provided an insight on the progression of decomposition in a tropical Nigerian setting and demonstrated how preservative chemicals like formalin greatly affect the decomposition timeline.

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