PRELIMINARY STUDY OF INSECTS COLONIZING DECOMPOSING DOMESTIC PIGLETS (*Sus domesticus* ERXLEBEN) CARCASSES AT UMUDIKE, SOUTHEASTERN NIGERIA

ABSTRACT

Aim: to determine the Identification and distribution of insects colonizing ground-placed and hung decomposing domestic piglets (*Sus domesticus*) carcasses

Place and duration: at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Study carried out in two seasons: July to September, 2019 (wet), and January to March, 2020 (dry) **Methodology:** Four healthy piglets with average weight of 3.73 kg were sacrificed for the trial by dislocating their cervical vertebrate (to mimic natural death), and put in cages. Two were placed on the ground, whereas the other two were hung on a tree. Data on arthropod populations, temperature, weights and decomposition stages and rates of carcasses were collected. Statistical tests were performed to evaluate insect species distribution and their relationships with the carcasses.

Results: Irrespective of placement, more insects were counted during the dry (655.20) than wet (529.96) seasons but not statistically different. The distribution of insects' taxa showed *Musca* spp. (37.09 %), *Chrysomya* spp. (12.97 %), *Pheidole* spp. (12.09), *Componotus* spp. (9.69 %), *Monomorium* spp. (6.04 %) in seventeen genera, ten families in four orders. The relationship between insects' abundance and mean weight were significantly (P = .05) negative (-0.53) and (-0.96) in the ground-place carcasses in the wet and dry seasons, respectively.

Conclusion: Results show that *Musca* spp. was the predominant species an *Dysdercus* spp. was the least throughout the decomposition period. Higher number of insects were counted from the carcasses in the dry than wet seasons. Insects' abundance increases as the carcasses' weights decreased.

Keywords: Carcass, Colonizing, Forensic science, Insects, Species diversity, *Sus domesticus*

1. INTRODUCTION

Forensic science is the application of scientific principles and techniques to solve crimes and establish evidence in legal proceedings [1]. It encompasses various scientific disciplines such as biology, chemistry, physics, and anthropology to analyze physical evidence found at crime scenes [2]. In the criminal justice system, forensic science is crucial because it offers important evidence and insights that help solve crimes. Forensic experts make a key contribution to upholding justice and preserving the integrity of legal proceedings by merging numerous scientific disciplines. [3].

Forensic entomology in Nigeria is at its infancy as people in Nigeria are finding it difficult to estimate time of death and culprits escape most criminal acts in Nigeria. According to [4] and [5], there is not enough knowledge in forensic entomology in Nigeria. [6] also opined that forensic entomology is not well practiced in Nigeria due to the absence of information. The court system in Nigeria has not fully embraced and accepted forensic science, particularly when it comes to situations involving unnatural deaths [7].

There have been trending reports of suspicious deaths of domestic animals and humans around residential areas [8], and monitoring of insects associated to decomposition has been studied in countries like Australia, USA, Canada, Argentina, Brazil [9]; [10] and [11], [12], and more recently in Nigeria by [13] in Rivers State; [14] in Delta State; [15] in Awka, Anambra State, and [16] in Enugu State. Evidently, no studies on forensic entomology have been documented in Abia State. The objectives of this present study therefore were to determine colonizing arthropod species, and decomposition pattern of *S. domesticus* at Umudike since studies in tropical and subtropical regions [17] on composition and diversity of species varies in relation to geographic region and abiotic factors [18]. This will provide baseline information necessary for PMI determination.

2. MATERIALS AND METHODS

Study Area: Arthropod species colonizing *S. domesticus*, and their decomposition pattern was evaluated at Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, south-eastern Nigeria, located in the tropical rainforest zone on Latitude $05^{0}26'$– $5^{0}25'$N Longitude $07^{0}34'$ – $7^{0}36'$E.

Animal Model: Although human cadavers are favorable for their direct application to actual forensic cases, they were unavailable at the time of this research project due to some ethical considerations and inability to access un-embalmed cadaver with a known time of death. For this research, *S. domesticus* was chosen as a good analogue for studying the decomposition of human cadavers [19], due to the similarities in intestinal flora, skin, tissue and muscle structure, as well as the progression of decomposition [20]; [21]; [18]; [22]; [23]; [24]. Also, [23] researched the decay rates of various animals and found domestic pigs to be the most suitable replacement for humans.

Four healthy piglets (*S. domesticus*) with average weight of 3.73 kg (Table 1) were used for this trial. They were purchased from the piggery farm of MOUAU. Permission was obtained from the Ethical Committee of College of Natural Sciences, MOUAU before the commencement of the field research.

Table 1: Weight of piglets (*Sus domesticus*) used for the study

Specimen	Weight (kg)
Piglet 1	3.4
Piglet 2	2.6
Piglet 3	4.4
Piglet 4	4.5

Total	14.9
Mean	3.7

Experimental Site: A suitable site located at the back of the animal house by the College of Physical Sciences, MOUAU, was carefully selected for this study and the site was cleared using cutlass. The site was characterized of grasses and trees.

Experimental Procedure: Metal cages (120cm x 120cm) were covered with wire (2cm x 2cm meshing size), to allow access to the carcass by insects while preventing scavenger vertebrate and placed in an open location [25]. A sign-post warning passersby about the experiment was tagged at the site to avoid disturbance or removal.

Piglets Killing and Placement: The piglets were transported alive to the study site and sacrificed by severing their cervical (to mimic natural death). Care was taken to prevent external bleeding that might alter the attractiveness of the carcass to flies or provide alternate sites for oviposition or larviposition. After death, the carcasses were immediately placed into the mesh cages to prevent scavenging by large vertebrates. Two of them were hung on a tree (shaded), whereas, the other two were placed on the ground (surface), and they were exposed to natural conditions.

Piglets' Weights: The weights of the carcasses were taken daily using a spring scale.

Arthropod Sampling: The experiment was conducted in two seasons, rainy season (July to September, 2019) and dry season (January to March, 2020). The time of data collection was twice daily (between 6:30 am and 5:00 pm) for two weeks. After two weeks, data were collected once a day either in the morning (6:30am) or in the evening (5pm) until the end of the experiment. During sampling, the sites were approached slowly to minimize the disturbance of flying adult insects.

Data Collection and Analyses

Environmental Data: The temperature was taken using a calibrated mercury thermometer. Internal temperatures of the carcasses were taken via the rectum twice daily [26]: [27] to determine cadaveric cooling. Relative humidity and rainfall were also collected.

Insect Data: Insects were collected from openings on the decomposing carcass such as mouth, ears and eyes. These openings were thoroughly examined for presence of insect species. Flying insects were collected using a hand net, while creeping insects were collected using hand picking forceps, put in vial glasses and weakened using ethyl-acetate. Insects collected were carefully handled to avoid denature and for easy identification. Eggs and larvae present were also collected (when present) and one part was reared to the adult stage for species identification while the other part was transferred to vials containing 70% ethanol for preservation. Identification and taxonomic determinations were made by using current keys [28]; [29]; [30]; [31]; [32]; [33]; [34]. All insects were identified to the generic level. Care was taken to protect the data collectors from any pathogens, pollutants or contaminants by wearing protective clothing.

Data Analyses

Descriptive statistics were used to summarize the succession of insects on *S. domesticus* carcasses. Correlation analyses on insects; abundance, temperatures and weights, and students' *t*-test was used to compare the seasonal variation in insect succession and decomposition of carcasses. Data were analyzed using Statistical Analysis Software (SAS).

3. RESULTS

The recorded mean temperatures and rainy days in wet (630.0 and 17.0), and October (367.3 and 14.0) seasons, 2019. In the dry season, 2020. There were no rains in February (0.0 and 0.0) and March (126.1 and 5.0) (Table 2).

Table 2. Rainfall and rainy days during sampling of insects associated with *Sus domesticus* carcasses at Umudike

Year	Month	Amt. (mm)	Day
2019 (Wet)	Sep	635.0	17.0
	Oct.	367.3	14.0
2020 (Dry)	Feb.	0.0	0.0
	March	126.1	5.0

Source: NRCRI Metrological Station

Ground-placed carcasses (GPC) at different decomposition stages had a daily mean internal temperatures and weights of (31.90℃ and 2.10), whereas the hung carcasses (HC) (30.90℃ and 2.18) (Table 3).

Table 3: Mean Internal Temperature (±se) and Weights (±se) of *Sus domesticus* carcasses at Umudike in Rainy and Dry Seasons

Carcass Placement/Season	Mean (±Standard Error)	
	Internal Temperature (℃)	Weight (Kg)
GPC, Rainy Season, 2019	31.74(±1.81)[a]	2.16(±0.46)[a]
HC, Rainy Season, 2019	31.98(±1.61)[a]	2.09(±0.36)[a]
GPC, Dry Season, 2020	31.99(±1.36)[a]	2.04(±0.37)[a]
HC, Dry Season, 2020	29.82(±1.04)[a]	2.27(±0.29)[a]

p Value	0.54ns	0.96ns

Key: GPC = ground-place carcass; HC = Hung carcass

Insect Populations on Decomposing Piglet Carcasses at Umudike.

Table 4 presents the insects' distribution on decomposing *Sus domesticus* during the 2019 (wet) and 2020 (dry) seasons. Higher population of insects were recorded from GPC in dry (10099), and dry (8315) seasons compared to the hung carcasses in the dry (6281) and wet (4934). *Musca domestica* was most preponderant (37.09%) and the least was *Dysdercus* spp. (0.01 %) (Table 4).

Table 4: Insects' Distribution on *Sus domesticus* Carcasses at Umudike during 2019 (wet) and 2020 (dry) Seasons

Order	Family	Species	Wet Season		Dry Season		Total (%)
			GPC (%)	HC (%)	GPC (%)	HC (%)	
Diptera	Calliphoridae	*Calliphora spp*	79 (0.95)	0	21 (0.21)	0	100 (0.34)
		Calliphora vomitoria	0	46 (0.93)	92 (0.91)	41 (0.65)	179 (0.60)
		Crysomya megacephala	0	0	27 (0.27)	0	27 (0.09)
		Crysomya spp	83 (1.00)	68 (1.38)	221 (2.19)	125 (1.99)	497 (1.68)
		Crysomya sp (Larvae*)*	1725 (20.75)	1643 (33.30)	168 (1.66)	307 (4.89)	3843 (12.97)
	Muscidae	*Fannia spp*	59 (0.71)	0	0	0	59 (0.20)
		Musca domestica (Adult*)*	629 (7.56)	560 (11.35)	888 (8.79)	542 (8.63)	2619 (8.84)
		Musca domestica (Larvae)	2327 (27.99)	1931 (39.14)	3203 (31.72)	3527 (56.15)	10988 (37.09)
		Musca domestica (Pupa)	0	27 (0.55)	0	0	27 (0.09)
	Sarcophagidae	*Sarcophaga spp*	94 (1.13)	0	22 (0.22)	62 (0.99)	178 (0.60)
		Sarcophaga sp (Larvae)	6 (0.07)	0	0	0	6 (0.02)
	Piophilidae	*Piophila casei*	5 (0.06)	0	0	0	5 (0.02)
Coleoptera	Dermestidae	*Dermestes maculatus* (Adult)	5 (0.06)	53 (1.07)	59 (0.58)	13 (0.21)	130 (0.44)
		Dermestes maculates (Larva)	0	146 (2.96)	21 (0.21)	21 (0.33)	188 (0.64)
	Staphylinoidea	*Philonthus* spp	6 (0.07)	0	0	0	6 (0.02)
	Chrysomelidae	*Altica sp*	0	57 (1.16)	232 (2.30)	66 (1.05)	355 (1.20)
		Asphaera sp.	0	0	0	53 (0.84)	53 (0.18)
	Tenebrionidae	*Cynaeus sp.*	0	0	0	8 (0.13)	8 (0.03)
Hemiptera	Pyrrhocoridae	*Dysdercus sp.*	0	0	0	4 (0.06)	4 (0.01)

Hymenoptera	Formicidae	*Camponotus* spp	94 (1.13)	0	2778 (27.51)	0	2872 (9.69)
		Cheliomyrmex andicola	752 (9.04)	0	0	0	752 (2.54)
		Cynaeus sp.	0	0	14 (0.14)	0	14 (0.05)
		Monomorium spp	363 (4.37)	262 (5.31)	620 (6.14)	545 (8.68)	1790 (6.04)
		Pheidole spp	2088 (25.11)	0	1733 (17.16)	-	3821 (12.90)
		Solenopsis sp	0	141 (2.86)	0	967 (15.40)	1108 (3.74)
Total			**8315 (28.06)**	**4934 (16.65)**	**10099 (34.08)**	**6281 (21.20)**	**29629**
MEAN			**332.60**	**197.36**	**403.96**	**251.24**	
P value			**Ns**	**Ns**	**Ns**	**Ns**	

Key: GPC = ground-place carcass; HC = Hung carcass

Seasonal Variations: Carcass Internal temperature vs. weight

In the GPC (wet season), the relationship between insects' abundance and mean weights were significant ($P < 0.05$), but negatively (-0.53) correlated (Table 5). Similarly, in the HC (wet season), the relationship between internal body temp. and mean weights were also significant ($P < 0.05$), but negative (-0.85) (Table 7).

Table 5: Correlation analyses of insects' abundance, internal temperature and weight of ground-placed piglet carcasses in wet season, 2019.

	Insects Abundance	*Internal Temperature*	*Weight*
Insects Abundance	1.00		
Internal Temperature	-0.09 ns	1.00	
Weight	-0.53*	-0.29 ns	1.00

* - Strong positive or negative correlation.

Table 6: Correlation analyses of Insects' abundance internal temperature and weight of ground-placed piglet carcasses in dry season, 2020

	Insects Abundance	*Internal Temperature*	*Weight*
Insects Abundance	1.00		
Internal Temperature	0.12 ns	1.00	
Weight	-0.96*	-0.13 ns	1.00

* - Strong negative correlation.

For the GPC (dry season), the relationship between insects' abundance and mean weights were significant ($P < 0.05$), but negative (-0.96) (Table 6). Also, for the HC (dry season), the relationship between insects' abundance and mean weights were significant ($P < 0.05$) and negative (-0.84) (Table 8).

Table 7: Correlation analyses of insects' abundance, internal temperature and weight of hung piglet carcasses in wet season, 2019.

	Insects Abundance	*Internal Temperature*	*Weight*
Insects Abundance	1.00		
Internal Temperature	-0.18	1.00	
Weight	0.03	-0.85*	1.00

* - Strong positive or negative correlation.

Table 8: Correlation analyses of insects' abundance, internal temperature and weight of hung piglet carcasses in dry season, 2020.

	Insects Abundance	*Internal Temperature*	*Weight*
Insects Abundance	1.00		
Internal Temperature	0.58*	1.00	
Weight	-0.84*	-0.15	1.00

* - Strong positive or negative correlation.

DISCUSSION

This study revealed that five decomposition stages namely: fresh, bloated, active decay, advanced decay and dry/skeletonization were observed in both seasons irrespective of placement method. This result agrees with several researchers [35]; [36], except [14] on *Rattus norvegicus* who observed only three decomposition stages and [6] study on *Sus scrofa* in Delta and Akwa Ibom States in southern Nigeria, who observed four decomposition stages.

It took ten days for decomposition to be complete (fresh to dry/skeleton stage) in the GPC carcasses. This result is similar to the findings of [37]. [38] found that the rate at which mass decreased was slower in the hanging carcass and that each stage of decomposition was prolonged. Since crawling insects would be unlikely to return to the carcass if they fall into the drip zone. Additionally, the action of gravity and movement disturbed the maggot masses causing maggots to fall from the hanging pigs to the drip zones below, thus decreasing the internal maggot masses. The maggot mass within a hanging animal may, therefore, be smaller than those within an animal on the ground, where the maggots are more likely to remain within the body cavity which may affect the rate of decomposition [37].

In the dry season, decomposition lasted longer days for GPC and HC carcasses. The lower internal body temperatures recorded on HC during the dry season might be the main factor that led to longer carcass decomposition duration as recorded in this study. It was also observed that GPC decay faster than those hung under a tree (shade) in both seasons. This also might be attributed to easy access of insects to the carcasses, environmental factor and uninterrupted developmental stages of arthropods faunas and insect abundance, whereas in the temperate zone higher number of decomposition duration such as 118 days had been observed [25].

Insect species abundance and weights of GPC and HC were significant and positively corelated in both seasons. Previous studies by [38] and [39] concluded that correlation between decomposition rate and weight loss.

4. CONCLUSIONS

The information presented in this case study concluded that five stages of decomposition were observed on both surface-placed and hung piglet carcasses. Hung piglet carcasses took longer time to skeletonize when compared to the ground-placed. This study also provides baseline data for PMI determination which can be applied in cases of human death(s) around the study area.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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