NECROPHAGOUS ORGANISMS ASSOCIATED WITH CHICKEN (*GALLUS GALLUS DOMESTICUS*) CARRION IN ABRAKA, DELTA STATE, NIGERIA.

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Abstract

Necrophagy is the feeding behaviour of organism that feed on carrion. The decaying flesh of dead animals is an important food source for necrophagous organisms in most ecosystems. The time of arrival and the growth rate of organisms inhabiting carrion are used to determine circumstances surrounding deaths. Studies on necrophagous organisms associated with *Gallus gallus domesticus carrion* provide useful data to estimate the post-mortem interval in forensic cases. The present study was undertaken to determine the abundance of some forensically important organisms on the carrion of chicken buried at different depths namely, 0.6 m, 1.21 m and on the soil surface in six successions in Abraka, Delta State Nigeria. Entomological, parasitological, and microbiological analyses were carried out on the carrion by standard procedures. Site climatic factors such as temperature and relative humidity were recorded using a digital thermo-hygrometer. A total of 417 necrophagous organisms were isolated from the carrion. Among the insects, three orders comprising nine families and sixteen species were encountered. The highest number of insects were observed among members of the *Saprinus chalcites* 103.0(24.70%), followed by *Streblognathus aethiopicus* 71(17.03%), *Xerosaprinus* species 58(13.91%), *Musca domestica* 35(8.39%), *Onitis* 33(8.0%), *Bryocharis* 24(5.76%), *Piesocorynus lateralis* 22(5.38%), *Chrysomya* species 20 (4.80%), Heteronychus *arator* 17(4.08%), *Solenopsis* species 16 (3.84%), *Trochosa* species 5 (1.20%), *Diaparis* species 3(0.72%), *Marganinotus feedatus* 3(0.72%), *Phalacridae* species 3(0.72%), and *Eparea* species 1(0.24%). Statistically, there was a difference ($p<0.05$) in the relative abundance of the necrophagous arthropods collected. There was no significant difference in the relative abundance of the organisms collected on the surface soil and the different soil depths. Relative humidity had a significant effect on the abundance of fauna obtained as it was correlated positively with the relative abundance of the organisms ($r=0.941$). The parasites collected were *Hymenolepis nana, Capilaria contorta, Ascaris gallus, and Eimeria tenella*. The bacteria isolated were *Klebsiella Pneumoniae, Streptococcus pneumoniae, Escherichia coli, Staphylococcus auerus, Pseudomonas aeruginosa, and Bacillus cereus.* The fungi isolated from the carrion were *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Aspergillus fumigatus, Rhizopus, Trichosporon asahii Scedosporium aurantiacum, Chrysosporium tropicum and Lecytophora* sp. It is hoped that the data in this will contribute to postmortem and forensic investigation in our locality.

Keywords: Necrophagy, *Gallus gallus*, postmortem, necrophagous organisms, carrion.

Introduction

Necrophagy is the feeding behaviour of an organism that eats carrion from another animal that it did not kill (Hovenden, 2010). The role played by necrophagous organisms such as arthropods, bacteria, fungi, and parasite is also of immense significance from ecological perspectives (Amendt, *et al*., 2004). Detritivores influence the decomposition of detrital resources in virtually all natural systems, with broad consequences for community structure and ecosystem function (Moore *et al*. 2004), by shredding, consuming and transforming organic detritus, detritivores not only accelerate microbial decomposition but also helps in nutrient cycling (Amendt *et al*., 2004). According to Hovenden, (2010) carrion is the decaying flesh of dead animals, including human flesh. Carrion is an important food source for large carnivores and omnivores in most ecosystems.

Carrion is a microhabitat characterized fundamentally by its rapid ecological successions, being an extremely transient micro-ecosystem that is rapidly degraded by the action of the organisms that colonize them. It represents not only a rich source of energy, but also a very specialized habitat that is exploited by necrophagous organisms. Galante and Marcos-Garcia, (2004) reported that the organisms involved in succession vary according to geographical areas, even in places with similar climates. Also, the number of individuals and the species colonizing these microhabitats vary enormously from one place to another, and through time.

Every living organism will eventually die, and the body will undergo decomposition process (Phillipps and Phillipps, 2016). Decomposition is a process of chemical breakdown of organic matter into its constituents by the action of decomposer. Some arthropods play important roles as decomposer beside bacteria and fungi. The type and composition of taxa that are attracted to a carcass usually change in a predictable pattern as decomposition progresses through different stages (Madinah and Nor-Aliza, 2018).

Decomposition of animal carrion is achieved primarily through the activities of invertebrates, such as flies and beetles, and large scavengers, generally other vertebrates such as opossums, raccoons, and vultures. The processes of decomposition begin within a few minutes of death (Vass *et al*., 2002). Decomposing remains offer a temporary, changing site of concentrated resources which are exploited by a wide range of organisms. Of these organisms, arthropods are often the first to arrive and the predominant exploitive group. Arimoro, (2013) reported that necrophagous insects are key players in the decomposition process which is associated with decaying human and animal

remains and utilized by organisms as their micro-niches, thus, forming diverse micro-communities. Insects proceed through a predictable sequence of stages when decomposing vertebrate remains. According to Gruner *et al*. (2007), a diverse fauna of necrophagous species that form this important community belong to the groups Coleoptera, Diptera, Hymenoptera and Arachnida.

Microbes, such as bacteria and fungi, are important for carrion decomposition (Bass and Jefferson, 2003). These microbes largely influence the flow of energy and matter throughout an ecosystem. Bacteria from the carcass and surrounding environment, such as soil bacteria, are essential for the decomposition of animal remains into organic matter and nutrients.

According to Cardoso *et al*. (2017), the most important parasites in Forensic Science are helminths specifically schistosomes. Motomura *et al*. (2016) unexpectedly detected in an autopsy case S. japonicum eggs in the liver tissue where the presence of the eggs was not related to the cause of death (Berens et al., 2009).

Recinos-Aguilar *et al*., (2019) stated that the decomposition of a living being involves a series of changes produced by a number of interacting abiotic and biotic factors. Rofela, (2017) also revealed that many species of insects are known to be associated with carrion and impacts greatly on the decay rate as well as provide useful data on the mode and time taken for death of that organism. There is paucity of information on the biodiversity associated with Gallus gallus domesticus carrion in our locality. This investigation apart from providing baseline of information on the biodiversity will provide data which will be useful in postmortem intervals and forensic investigation.

Materials and Methods

Study Area/design: The study was conducted during the wet and dry season in Abraka, Delta State Nigeria (5°47'21.96"N, 6°6'8.45"E). A total of fifty-four Chicken (Gallus domesticus), with weights ranging from 115-125g were acquired from the farm and killed aseptically according to Animal Welfare Act (1966). They were both sexes within 4-5 weeks old. Each month, nine (9) chickens were killed and buried in replicates at three different soil depths namely, 0.6m and 1.2m and on the surface soil. The decaying carrion was observed daily for assessment of the decomposition to monitor the decaying processes/stages of the carcass as well as collection of necrophagous organisms.

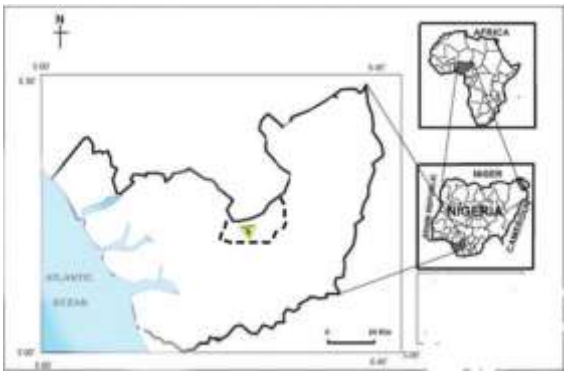

Fig. 1 Map of Delta State Showing the Area of Study

Sampling Procedure: The collection of necrophagous arthropods were conducted daily. The actively flying insects were collected with an aerial net. Insects not captured with the aerial net were handpicked from the carrion and placed in labelled vials containing Ethyl acetate for preservation. All organisms were taken to the laboratory and examined using hand lens and binocular microscope. They were sorted out into species using taxonomic keys (Grassberger, 2004). The collections were conducted with low harmful impact on the fauna, particularly on the immature specimens. Specimens for parasitology investigation were collected from the rectum within the first three days, preserved in formalin and examined using concentration method and direct smear method (Cheesbrough, 2010). The microbial community composition of the GIT, liver, and heart were sampled with a sterile swab and taken to the laboratory for culturing and biochemical tests (Cheesbrough, 2010). The meteorological parameters namely, temperature, and humidity were recorded daily.

Statistical Analysis: The data obtained from the study were analyzed using the PAST statistical package and word excels 2013 by T-test and Analysis of Variance (ANOVA). Values of $P \leq 0.05$ were considered significant.

Results

The necrophagous fauna associated with 3 decomposing stages of *G. gallus domesticus* decomposition are presented in Table 4.1. The Table showed that the bloated stage had the highest number of organism's assemblages 152(36.50%) while, fresh stage had moderate abundance 134 (32.1%) and active stage had the least of 131(31.40%). Statistically, these differences were not significantly different ($P>0.05$). The fresh stage had all assemblages of necro-fauna except *Epareae,* and *Diaparis* which was absent in the bloated stage (Table 4.1). Three species, *N. rufipes, Phalacridae, M. feedatus* were absent in the active decay stage of *G. gallus domesticus*.

Table 4.1: Necrophagous insects associated with each stage of decomposing chicken (*Gallus gallus domesticus*)

Stages / Insects	Fresh Day 1-2 (%)	Bloated Day 3-5 (%)	Active Day 6-8 (%)
Streblognathus aethiopicus	36(26.86)	9(5.92)	26(19.85)
Xerosaprinus sp.	20(14.93)	26(17.10)	12(9.16)
Necrobia rufipes	1(0.75)	1(0.66)	0
Bryocharis sp.	8(5.97)	6(3.95)	10(7.63)
Heteronychus arator.	9(6.71)	5(3.30)	5(3.82)
Onitis sp.	6(4.50)	20(13.16)	6(4.60)
Saprinus chalcites	33(24.62)	38(25.00)	32(24.43)
Phytophthora. Lateralis	7(5.22)	9(5.92)	6(4.60)
Magrinotus. Feedatus	1(0.75)	2(1.32)	0
Musca domestica	1(0.75)	15(9.87)	19(14.50)
Trochosa sp.	1(0.75)	3(1.97)	1(0.76)
Solenopsis sp.	1(0.75)	11(7.24)	4(3.05)
Diaparis sp.	2(1.50)	0(0.00)	1(0.76)
Phalacridae sp.	1(0.75)	2(1.32)	0
Chrysomya sp.	7(5.22)	5(3.30)	8(6.11)
Epareae sp.	0(0.00)	0(0.00)	1(0.76)
Total	134(100%)	152(100%)	131(100%)
Grand total	417		

Table 4.2: Decay stages, Order, Families and Species composition of necrophagous insects of *Gallus gallus domesticus*

Days	Stage	Orders	Families	Species
1-2	**Fresh**	Coleoptera	Histeridae	*Saprinus chalcites, Xerosaprinus* species
		Hymenoptera		*Diaperis* species
			Tenebrionidae	*Sole*nopsis species
		Diptera	Formicidae	*Chrysomya* species
			Calliphoridae	*Bryocharis* species
			Staphylinidae	*Streblognathus aethiopicus*
			Formicidae	*Necrobia rufipes*
			Cleridae	
3-5				
	Bloated	Diptera	Calliphoridae	*Chrysomya* species
			Muscidae	*Musca* domestica
		Coleoptera	Scarabaeidae	*Onitis* species, *Heteronychus* arator,
				Streblognathus aethiopicus. *Xerosaprinu*
		Hymenoptera	Formicidae	species, *Magrinotus feedatus*
				Phytophthora lateralis
			Histeridae	*Trochosa* species
			Anthribidae	
			Lycosidae	
6-8				
	Active	Coleoptera	Anthribidae	*Phytophthora lateralis*
		Hymenoptera	Formididae	*Streblognathus aethiopicus, Solenopsi* species
			Histeridae	*Xerosaprinus* species
			Muscidae	*Musca domestica*
			Scarabaeidae	*Onitis* species
				Saprinus chalcites
				Diaperis species
				Heteronychus arator

The decomposition stages as well as taxonomic characterization of necrophagous fauna associated with *Gallus domesticus* are presented in Table 4.2. Three (3) orders (Coleoptera, Diptera and Hymenoptera) comprising of nine (9) families and sixteen (16) species were encountered in this study. Family Gyrinidae was common in all decomposing stages (Fresh, bloated, and Active) of *G. g. domesticus*. Family Muscidae, Scarabaeidae, Hycopcidae was only observed in the bloated stage of decay. (Table 4.2). The daily percentage distribution of necrophagous insect fauna associated with *Gallus gallus domesticus* Carcasses over six months period are shown in Table 4.2. Day 1-3 had the highest percentage composition of necrophagous fauna, while the least was observed in day 5.

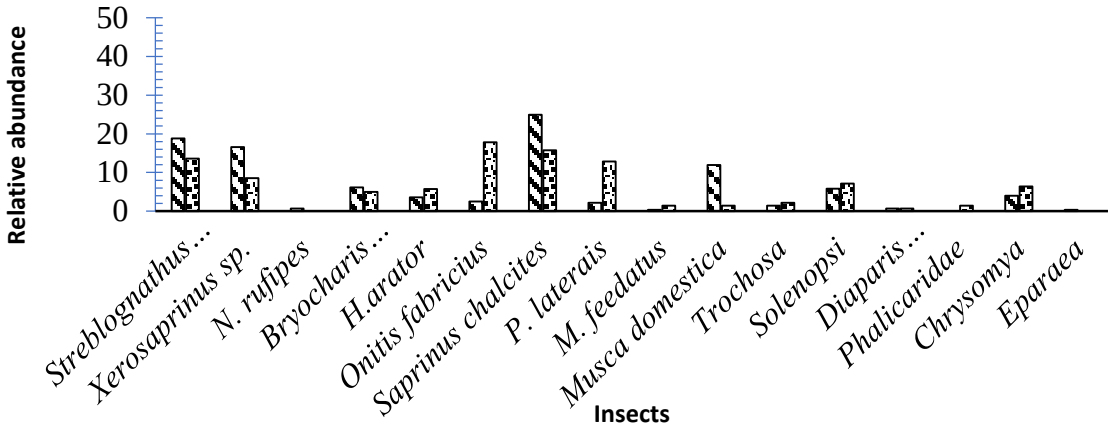

Fig.2: Relative Abundance of Necrophagous insects associated with *Gallus gallus domesticus* Carcasses in Dry and Wet seasons.

Comparatively, the relative abundance of insects were higher in the dry months than the wet months. *Saprinus chalcites* had the highest relative abundance in the dry months with a value of 24.91% (Fig. 2), while *Onitis fabricius* exhibited similarly high values in the wet months with a value of 17.85%, closely followed by *Saprinus chalcites* with 15.71%. The least abundance in terms of seasonal variation was observed among the *Phalacridae* in the dry months. The wet season had its least abundance exhibited in by *Epareae* species (fig2) …

Table4.6: Monthly Mean Environmental Variables and percentage Abundance of Necrophagous fauna associated with *Gallus gallus domesticus* Carcasses over six months period of observation (Jan. – June 2021)

Environmental Factors and % abundance	MONTHS					
	Jan	Feb	Mar	Apr	May	June
Temperature (^{0}C)	28 ^{0}C	28^{0}C	28^{0}C	27^{0}C	27^{0}C	27^{0}C
Relative Humidity (%)	79%	74%	80%	82%	87%	81%
Percentage Abundance (%)	22.30%	18.22%	25.90%	13.90%	9.11%	10.55%

The environmental factors (Temperature and humidity) and the monthly abundance of arthropods are shown in Table 4.6. The highest percentage abundance of necrophagous insects were observed in the month of March 25.90%, January 22.30%, and February 18.21%. The least percentage abundance was obtained in the month of June with 5.76. Relative humidity had a significant effect on the abundance of fauna obtained as correlated positively with the relative abundance (r=0.941). The data on the relative humidity (R.H) showed that, the months with lower relative humidity had the highest number of arthropods abundance.

Table 4. 7: Parasitic Composition of Decaying *Gallus gallus domesticus* in the Study Area

Parasites	Depth		
	Surface	0.6m	1.2m
Hymenolepsis nana	+	-	-
Ascaris gallus	-	+	-
Capillaria contorta	+	-	-
Eimeria tenella	+	-	-

A total of four parasites were encountered in the study as presented in Table 4.7. More parasites (*Hymenolepsis nana, Capilaria contorta, Eimeria tenella*) were recorded at the surface of the carcass of *Gallus.* However, no parasites were found at 1.2m depth (Table 4.7)

Table 4. 8: Composition of Bacteria in various Decay Stages *Gallus gallus domesticus* in the Study Area

Days	Stage	Bacteria
1-2	Fresh	*Escherichia coli, Klebsiella pneumoniae Staphylococcus aureus, Bacillus cereus*
3-4	Bloated	*Klebsiella pneumoniae, Streptococcus pneumoniae, Escherichia coli, Staphylococcu aureus, Pseudumonas aeruginosa,* and *Bacillus cereus.*
5-6	Decay	*Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Streptococcus pneumoniae* and *Escherichia coli.*
6-8	Active decay	*Escherichia coli, Bacillus cereus Streptococcus pneumomae,* and *Staphylococcus aureus.*

Table 4.8 showed the bacterial composition found in the *Gall*us carrion in the study area, these are *Klebsiella pneumoniae, Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus* (Table 4.8). *Streptococcus pneumonia* was not found in the fresh stage of *G. g. Domesticus* but was isolated in the bloated, decay and active stages. (Table 4.8).

Table 4. 9: Checklist of Fungi associated with *Gallus gallus domesticus* decomposition stages in the Study Area.

Days	Stage	Fungi
1-2	Fresh	*Lecytophora* species, *Aspergillus niger*, *Penicillium* species, *Aspergillus fumigatus, an Rhizopus* species.
3-5	Bloated	*Rhizopus* species, *Scedosporium aurantiacum, Chrysosporium tropicum, and Aspergillu niger,*
6-8	Active Decay	*Chrysosporium tropicum, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus and Rhizopus* species,

The checklist of fungi associated with *Gallus gallus domesticus* carrion in the study area is presented in Table 4.9. The following fungi at various depths of the study were isolated: *Aspergillus niger, Aspergillus flavus, Penicillium species, Aspergillus fumigatus, Rhizopus, Trichosporon asahii, Scedosporium aurantiacum, Chrysosporium tropicum* and *Lecytophora Hoffmanii.*

Discussion

A total of four hundred and seventeen (417) necrophagous arthropods were collected consisting of three (3) taxonomic orders: Diptera, Coleoptera, and Hymenoptera, nine (9) families and sixteen (16) species were encountered in this study. Similar taxonomic orders were recorded by (Kyerematen *et al.*, 2012) in their study of insect diversity and succession pattern on different carrion types in Ghana.

Comparatively, 217 insect species recorded in Tennesse are lower than the abundance in the present study (Cobaugh *et al*., 2015).

In this study, the Saprinus species were common in all decomposing stages (Fresh, bloated, and Active) of *G. g. domesticus*. Families, Muscidae, Scarabaeidae, Hycopcidae

were only observed in the bloated stage of decay. Also, similar information has been reported in most arthropod succession studies on carrion (Reed, 2011; Tantawi *et al.*, 1996, Sebastião and Prado e Castro, 2018). The *Musca domestica* and the blow flies were the first to arrive on the specimens in both dry and wet months just within hours of depositing the various carcasses and this was in agreement with the data of Gullan and Cranston (2010). This is because the *Musca domestica* and the blow flies feed on the keratin and help start the process of succession.

The decomposition occurred over a period of 8 days. Comparatively, it appears lower than the report by (Silahuddin *et al*., 2015) in Malaysia where they reported 14 days decomposition days to form complete decomposition of rabbit carrion weighing. These differences can be attributed to the size and weight of the carrion. Also, other factors such as differences in climate conditions mainly temperature, relative humidity and rainfall unique to each geographic region for each predefined stage of decomposition outlined by the researchers (Day *et al*., 2002 and Shi *et al*.,2009)

An important observation was the relative higher abundance of organisms isolated in dry months of the study. This can be attributed to higher ambient temperature and lower humidity as reported in other studies elsewhere (Tomberlin *et al.*, 2017; Singh *et al.*, 2018; Somavilla *et al.*, 2019).

Four parasites namely, *Hymenolepis nana, Capilaria contorta, Eimeria tenella and Ascaris gallus* were encountered in the study. The presence of these parasites in *Gallus domesticus* carrion may denote their significance as forensic tool in our locality. However, the only parasite, *Schistosoma* documented to have forensic importance was not found in this present study (Cardoso *et al*.,2017).

Bacteria isolated from the carrion includes (*Klebsiella pneumoniae, Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus* and the fungi are (*Aspergillus niger, Aspergillus flavus, Penicillium species, Aspergillus fumigatus, Rhizopus, Trichosporon asahii Scedosporium aurantiacum, Chrysosporium tropicum,* and *Lecytophora* species).

Clostridium perfringens and Clostridium frigidicanes (Vass 2001; Widya et al. 2012)

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According to Vass (2001); Widya *et al*. (2012), the species of bacteria involve in the formation of adipocere in carrion include Clostridium perfringens and Clostridium frigidicanes. The bacteria and fungi isolated in this study can be used in forensic activities involving carrion in this locality. This is similar to studies in the temperate and continental regions (Sharanowski *et al.*, 2008). Of note is the presence of *Lecytophora* species isolated from fresh stage only. This is of high forensic value considering the fact that its presence means that the death of the carrion is recent and within 2 days.

More importantly the dominance of Histeridea and Cleridea as from the 3 day onwards could be as a result of their ability to feed on the keratin and the maggots of other insects and also implies that the organism could have died after 3 days Scarabaeidae became abundant from the 5th day of decomposition and this agrees with the study of Braack (1987) who reported that as ammonia smell from the carcasses, it minimizes coleopteran adults and larvae. The dominance of Calliphoridae family as pioneer colonizers may be due to high perspicacity of decay odors by the insect from long distance of the carcass (Perrault *et al.*, 2015; Liu *et al.*, 2016).

Conclusion

Data obtained in this present study will contribute to our knowledge on succession and colonization of necrophagous organisms on carrion decay, which will be invaluable information on postmortem interval determination and forensic investigation in our locality.

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