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DEVELOPMENTAL FEATURES OF PORCINE HAEMAL NODES: A HISTOLOGICAL PERSPECTIVE

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ABSTRACT

Histological techniques were employed to provide detailed information on the histological features of haemal nodes in piglets and adult pig. Ten pigs were used for this study. The result demonstrated progressive changes in the structure of porcine haemal nodes. The capsule and trabeculae of piglet haemal nodes exhibited dense irregular connective tissues with reticular cells and smooth muscle cells. The cortex was more central while the medulla was peripheral with poorly defined boundaries. However, the sinusoids contained less densely packed erythrocytes. In adult pigs, haemal nodes demonstrated capsules composed of loose irregular connective tissue, fat cells, and mostly reticular cells while the trabeculae showed dense irregular connective tissues with reticular cells and smooth muscle cells. The atypical cortex and medulla of adult pig haemal nodes were distinct. The paracortex of adult pig haemal node showed high endothelial venules and the sinusoids contained densely packed erythrocytes. Afferent lymphatics, efferent lymphatics, blood vessels and veins were observed in the haemal nodes of piglets and adult pigs. Finally, the present study has provided essential information on the structural features of haemal nodes in piglets and adult pigs, revealing its atypical nature, and probable roles of blood storage, erythrophagocytosis and immune functions.

Keywords: Piglets, Adult pigs, Haemal nodes, High endothelial venules, Histological features

INTRODUCTION

The immune system comprises specific cells and organs that are distributed throughout the mammalian body. The primary function of these organs and cells is to protect the organism against invasion and damages by microorganisms and foreign bodies. The functional morphology of the organs of the immune system has been largely elucidated, but one entity that has not received adequate attention is the haemal node. Initially, its existence and nature was in doubt hence the emergence of the nomenclature: haemal and haemolymph nodes, with the implication that haemal node, like the spleen filters blood while

haemolymph node filters both blood and lymph. Pathologists initially regarded them as haemorrhagic lymph nodes (Meyer, 1917). There has been a recent upsurge of interest in their histological characterization (Casteleyn *et al.*, 2008; Zidan and Pabst, 2010; Ozaydin *et al.*, 2012).

The haemal node, an organ recently considered as a haematopoietic and secondary lymphoid structure was initially believed to be peculiar to ruminants (Casteleyn *et al.*, 2008; Singh, 1959; Garguilo *et al.*, 1987; Ezeasor and Singh, 1988). However some reports have shown that haemal nodes are present in non-ruminant species (Oláh and Törö, 1970; Zidan and Pabst, 2004) including man (Jordan, 1934).

There has not been consensus among researchers on the occurrence of haemal nodes in pigs. Meyer (1918) reported the absence of haemal nodes in domestic pigs. Moreover, the histological features of haemal nodes reported so far in ruminants and some non-ruminant species have not established the exact structural and functional significance of the node. Rather, available information have raised questions on the structural features that make haemal node a peculiar organ.

Therefore, the present study is designed to investigate the developmental features of piglet and adult pig haemal nodes with emphasis on their structural and functional significance.

MATERIALS AND METHODS

Animals: Ten pigs comprising of 10 weeks old piglets and 2 – 3 years old adult pigs were used for this study. They were obtained from local markets in Nsukka local government area, Enugu State, Nigeria and sacrificed for human consumption. Apparently healthy animals were used. Following slaughter, haemal node samples were collected for histological investigations. The handling and welfare of the experimental animals was in accordance with the ethics and regulations prepared by INSA, Animal Welfare Divisions of the Ministry of Environment and Forest, Council of International Organization of Medical Sciences (WHO/UNESCO), NIH and PHS. The research protocol was approved by the University of Nigeria, Nsukka.

Histological Procedures: Segments of haemal nodes were fixed by immersion in Bouin's fluid for 48 hrs. These were later dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. Following mounting on wooden blocks, 5µm thick sections were obtained with a rotary microtome. These were mounted on glass slides and stained with haematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam® digital camera (Motic China Group Co., Ltd, Xiamen, China).

RESULTS

Histology of Piglet Haemal Node: The haemal nodes of piglets were composed of the capsule, cortex, medulla and hilum. Each haemal node was surrounded by a thin capsule of dense irregular connective tissue with evenly distributed smooth cells and reticular cells (Figure 1). Circumferential lymphatic vessels which were observed within the capsule converged and exited the capsule as a large lymphatic vessel. The trabeculae extended from the capsule through the subcapsular region and terminated in the periphery of the cortex. Similar to the capsule, the trabeculae was composed of dense irregular connective tissue, reticular cells and smooth muscle cells. The trabeculae contained radial lymphatic vessels and arterioles, and their lateral boundaries showed erythrocyte-filled sinusoids (Figures 1, 2 and 3).

Furthermore, the cortex of the piglet haemal node was more central and the medulla more peripheral (Figure 1). The cortex and medulla showed sinusoids which were lined by endothelium. These sinusoids contained numerous erythrocytes, isolated lymphocytes and macrophages that lined the sinusoids. Although the cortex and medulla of piglet haemal nodes were observed, the boundary between the cortex and medulla were not distinct (Figure 2). The cortex was less compact and contained diffuse infiltration of small lymphocytes with 3 to 15 primary lymphoid follicles. The primary lymphoid follicles were randomly distributed within the cortex while the paracortex was characterized by diffuse lymphocytic infiltration. The medulla of piglet haemal node was composed of medullary cords and medullary sinusoids. The medullary sinusoids showed dilated erythrocytes-filled spaces with isolated lymphocytes interspersed between the erythrocytes. The subcapsular, trabeculae and medullary sinusoids showed dark-brown pigment of haemosiderin.

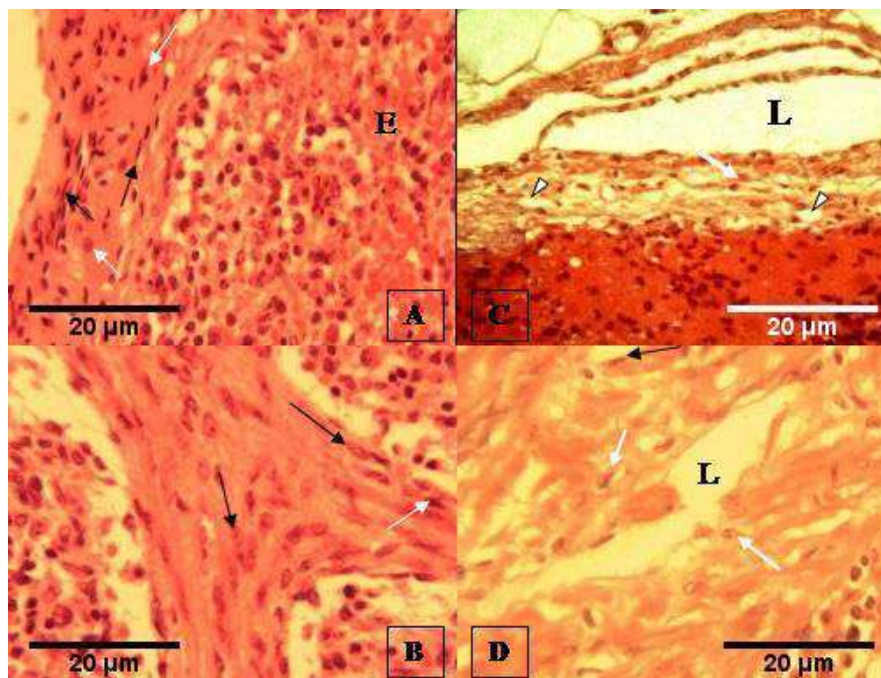


Figure 1: The capsules of piglet (A) and adult (C) pig haemal nodes; and the trabeculae of piglets (B) and adult pig (D) haemal nodes showing smooth muscle cells (black arrow), reticular cells (white arrow), fat cells (arrow head) and lymphatic vessels (L), H&E stain, X400

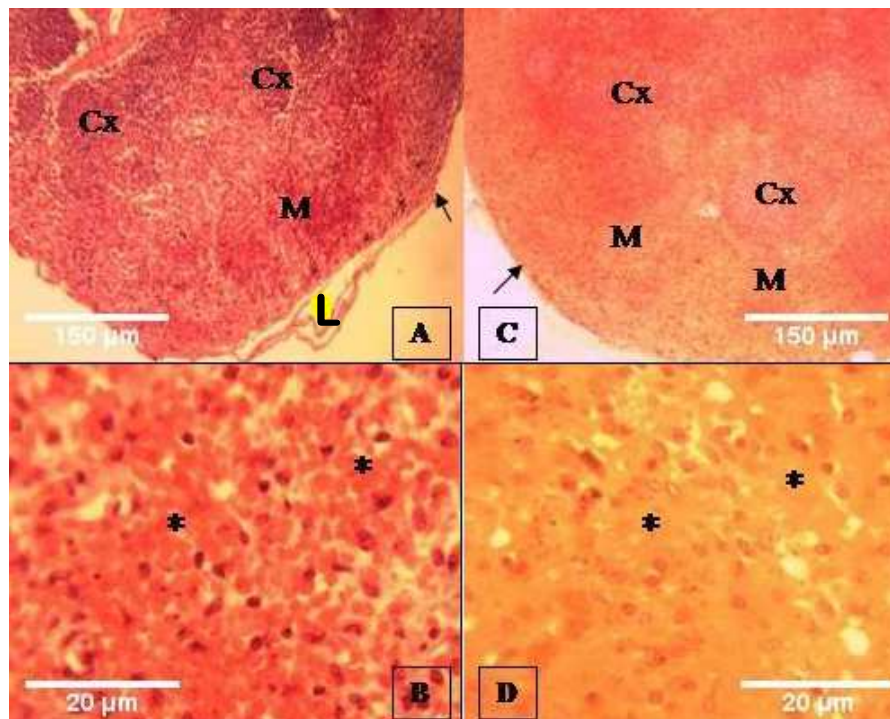


Figure 2: Light micrograph of piglet (A) and adult pig (C) haemal nodes demonstrating the capsule (arrows), lymphatic vessel (L), cortex (Cx) and medulla (M). Note the concentration of erythrocytes in the medullary sinusoids (asterisks) of piglets (B) and adult pig (D) haemal nodes, H&E stain, X400

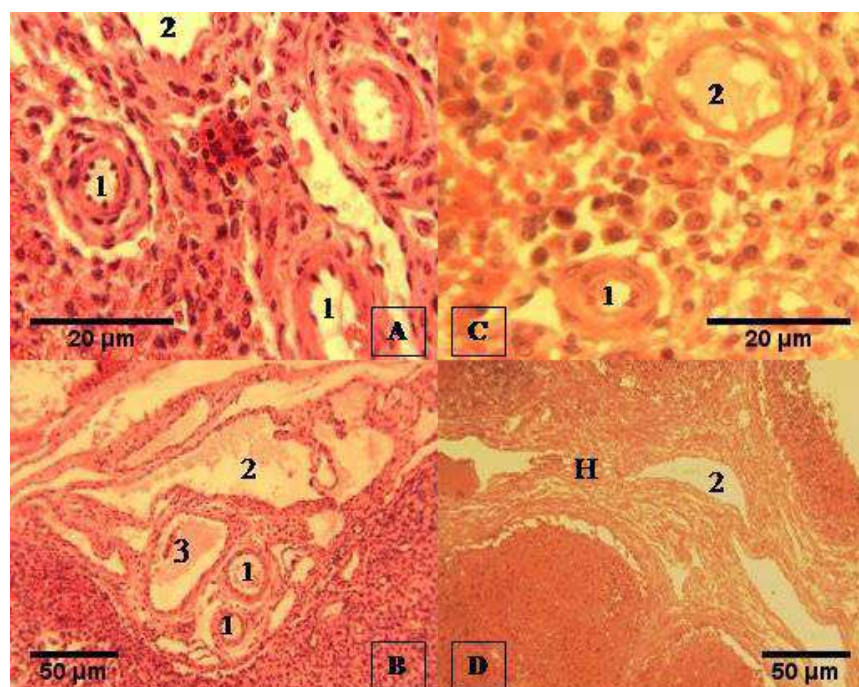


Figure 3: The trabeculae of piglet (A) and adult pig (C) haemal nodes containing blood vessels (1), and lymphatic vessels (2). Note the hilum of piglet (B) and adult pig (D) haemal nodes demonstrating blood vessels (1), lymphatics (2), vein (3) and a connective tissue framework (H). H & E stain, X400

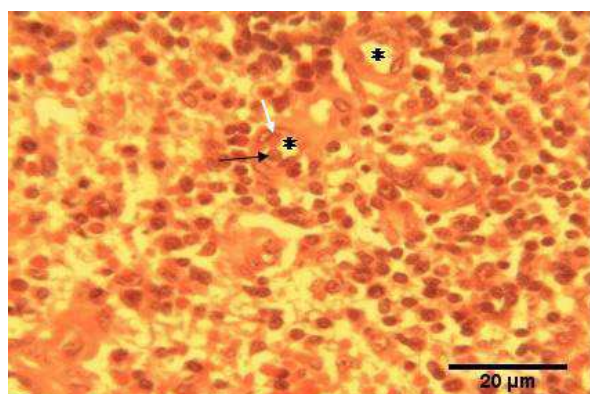


Figure 4: The paracortex of adult pig haemal node exhibiting high endothelial venules (asterisks). Note the lymphocyte (black arrow) and erythrocyte (white arrow) within the lumen of high endothelial venules. H&E stain, X400

The piglet haemal nodes exhibited a single hilum which was located in a thickened and indented area on the capsule. The hilum contained an artery, a vein and a large lymphatic vessel (Figure 3).

Histology of Adult Pigs Haemal Nodes: The haemal nodes of adult pigs exhibited a connective tissue capsule made up of a loose connective tissue, reticular cells and few smooth muscle cells which were distributed along points

of the circumferential lymphatic vessels. The loose connective tissues of the capsules were infiltrated by adipose cells which gave the capsule of adult pig a unique appearance (Figure 1). The capsule extended as the trabeculae deep into the haemal node tissues. The trabecula was composed of dense irregular connective tissue, evenly distributed reticular cells and smooth muscle cells (Figure 1). Radial lymphatic vessels, arterioles and sinusoids were observed within the trabeculae. In addition, adult pig haemal node showed the subcapsular sinusoid which was delimited by endothelial lining cells and was continuous with the medullary sinusoid (Figure 2). Blood capillaries were observed within the subcapsular sinusoid.

Furthermore, the haemal node parenchyma of adult pigs was characterized by a central cortex and a more peripheral medulla (Figure 1). These two regions were well defined (Figure 2). The cortex was compact and exhibited outer and inner cortical regions. The outer cortex was composed of randomly distributed primary and secondary lymphoid follicles while the inner cortex showed diffuse infiltration of lymphocytes. Inter-follicular areas of the outer cortex and inner cortical regions

contained diffuse infiltration of small lymphocytes and blood-filled sinusoids. Adult pig haemal node also exhibited a paracortex characterized by intense infiltration of lymphocytes, isolated plasma cells and reticular cells. It also exhibited numerous high endothelial venules (HEVs). These HEVs were lined by simple squamous to simple cuboidal endothelium and each lumen contained occasional isolated red blood cell (Figure 4).

The medulla of adult pig haemal nodes showed extensive areas of blood sinusoids, the medullary sinusoid. It was characterized by medullary cords and medullary sinusoids. Each cord was composed of connective tissue, reticular cells, lymphocytes, plasma cells and also exhibited arterioles and capillaries. The medullary sinusoids exhibited numerous red blood cells and isolated lymphocytes. These sinusoids appeared as spaces which coalesced establishing continuity with the subcapsular and trabeculae sinusoids. The sinusoids of adult pig haemal node exhibited dark brown haemosiderin pigments (Figures 1 and 2).

A single hilum was observed in the core of the organ. It contained a lymphatic vessel whose lumen was distended and the wall reinforced by the increased deposition of connective tissue fibres. The connective tissue framework was composed of dense irregular connective tissue, and reticular cells (Figure 3).

DISCUSSION

In the present study, changes in the connective tissue profile of the capsules of piglets and adult pig haemal nodes from dense irregular connective tissue to loose irregular connective tissue type were age related. It is most probable that at pre-pubertal age, both the capsule and trabeculae were composed of dense irregular connective tissue, reticular cells and smooth muscle cells, typical of the capsules and trabeculae of most lymphoid organs. However, as the animal attained adulthood, the tissues of the capsule may have been replaced by adipose tissues. This was evidence in the present study as the capsule of adult pig haemal node was composed of loose connective tissue type, adipose tissues, reticular cells and scanty

smooth muscle cells (present only at points of the capsular lymphatic vessels) while the trabeculae exhibited similar histology as the trabeculae of the piglet haemal node. Previous studies have shown that the histology of organ could be influenced by age, nutritional and physiologic states of the animal (Palmer, 2011; Zidan *et al.*, 2012). The smooth muscle cells together with the intrinsic nerves could modulate the contractile activity of the capsule and trabeculae, thus, concentrating the red blood cells within the blood sinusoids. Moreover, recent findings have shown that dense irregular connective tissue fibres together with reticular cells and fibroblast may contract and relax in a smooth muscle-like manner and may influence biomechanical behavior of the capsule and trabeculae (Tomasek *et al.*, 2002; Schleip, 2003). Therefore, apart from the role of providing a framework for the lymphatics and blood vessels (Ezeasor and Singh, 1988; Zidan and Pabst, 2004), the dense irregular connective tissue of the capsules and trabeculae together with their cells could play a role in the contraction of the capsule and trabecula.

Furthermore, the poorly defined cortico-medullary boundary of the parenchyma of piglet haemal node and the well defined boundaries of the cortex and medulla of adult pig haemal node is a function of age. It proves that the partitioning of the parenchyma of organs may be progressive, becoming clearly defined as the animal progresses in age. Moreover, the atypical parenchyma which was similar to that reported in the lymph nodes of pigs (Bacha and Bacha, 2000) showed that both the lymph nodes and haemal nodes of pigs may be of similar embryonic origin and development. The parenchyma of the cortex of piglet haemal nodes had more of lymphocytic infiltration than lymphoid follicles. The presence of lymphoid follicles often suggests the animal's exposure to certain degrees of pathogenic challenges (Allen and Cyster, 2008; Zidan and Pabst, 2012). According to Casteleyn *et al.* (2008) the number of lymphoid follicles in lymphoid tissues may increase with increasing age. Thus, the presence of lymphoid tissues in the cortex of porcine haemal nodes reveals the role of the organs in lymphocyte and antibody production.

One characteristic feature of adult pig haemal node in the present study which was absent in the piglet haemal node was the present of HEVs in the paracortex. The presence of high endothelial venules in the paracortex of adult pig haemal node together with the luminal content of isolated lymphocyte is very significant, as it may play a role in lymphocyte recirculation (Zidan and Pabst, 2004; Cupedo *et al.*, 2004). HEVs have been reported in the paracortex of dromedary camel haemal nodes and in lymph node paracortex (Zidan and Pabst, 2012; Cupedo *et al.*, 2004).

According to the classification of Weller (1938) that there are two distinct types of nodes: haemal nodes and haemolymph nodes, the nodes observed in this study represents haemal node. This is true because the parenchyma of the studied haemal nodes showed erythrocyte-filled sinusoids. Whereas, the sinusoids of adult pig haemal node contained densely packed erythrocytes, the nascent sinusoids of piglet haemal nodes showed less densely packed erythrocytes. More so, the population of red blood cells in the sinusoids of porcine haemal nodes was similar to the concentration of red blood cells demonstrated in the splenic sinusoids (Suttie, 2006), strongly suggesting that porcine haemal nodes may serve to store red blood cells and may also play a compensatory role of blood storage after splenectomy. The report of erythrocytes in the sinusoids of porcine haemal node is collaborated by the reports of erythrocyte-filled sinusoids of haemal nodes of ruminants by several previous authors (Bassan *et al.*, 1999; Casteleyn *et al.*, 2008; Ozaydin *et al.*, 2012), and dromedary camel (Zidan and Pabst, 2004). In addition, there has been controversy among researchers regarding the mechanism of by which these erythrocytes entered the sinusoids. Different authors hypothesized that erythrocytes entered haemal node sinusoids through afferent lymphatics (Job, 1918), reflux via lymphaticovenous communications (Andreasen and Gottlieb, 1946), communication between cortical capillaries and sinusoids (Zhang *et al.*, 2013) and erythropoiesis within the haemal node tissues (Cerutti and Guerrero, 2008). The

capillaries, arterioles, small blood vessels, and the HEVs observed in the present study may be the source of the red blood cells.

The occurrence of lymphatic vessels in haemal node is another subject of controversy among authors. In the present study, the lymphatic vessel which entered through the hilum and the one which exited through the capsule may be afferent and efferent lymphatic vessels respectively. The report of afferent and efferent lymphatics in piglet and adult pig haemal nodes is not strange, as the afferent lymphatic is necessary for selective conveyance of lymphocytes and antigen presenting cells into the cortical tissues of the haemal nodes, and the efferent lymphatic is also required to carry lymphocytes and plasma out of the haemal nodes (Haig *et al.*, 1999). This observation is supported by the reports of both afferent and efferent lymphatic vessels in the haemal nodes of sheep (Dellman and Brown, 1987), dromedary camel (Zidan and Pabst, 2004) and Buffalo (Zidan and Pabst, 2010). However other authors reported the absence of both afferent and efferent lymphatic vessels in the haemal nodes of sheep (Thorp *et al.*, 1991; Dassanayake *et al.*, 2013), hair goat (Ozaydin *et al.*, 2012), while Ezeasor and Singh (1988) observed only efferent lymphatics in West African Dwarf goat haemal nodes.

In conclusion, the present study has provided essential information on the structural features of haemal nodes in piglets and adult pigs, revealing its atypical nature, and probable roles of blood storage, erythrophagocytosis and immune functions.

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ZOOPLANKTON COMMUNITIES OF THE RIVER OSSIOMO, OLOGBO, NIGER DELTA, NIGERIA

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ABSTRACT

Zooplankton communities of the River Ossiomo at Ologbo, Niger Delta, Nigeria were investigated from April 2012 to December 2012. Three stations were studied from upstream to downstream with a distance of about 2 kilometres between each station. A total of 42 taxa were identified; 11 species of cladocerans, 6 copepods and 5 rotifers in the following order of dominance: copepoda > cladocera > rotifera. A total zooplankton population of 1330 individuals was recorded during the study period. Copepods and cladocera represented the predominant species (51.1% and 43.6% of the total zooplankton community respectively) followed by rotifers (5.3%). Copepods and cladocerans were dominated by both cyclopoid (51.1%) and chydorids (27.8%), respectively. The dominant copepod and cladocera species were Thermocyclops neglectus and Alona eximia representing 33.1% and 15.8% of the total zooplankton, respectively. The calculated diversity indices indicated that station 1 was more diverse followed by station 3, while zooplankton species in station 2 were least diverse. Community composition was similar at both stations 2 and 3, but varies seasonally across the three stations. Higher species number and density was found during the wet season with a trend of declining proportion towards the dry months.

Keywords: Zooplankton, Species, Population, Diversity, Abundance, River Ossiomo

INTRODUCTION

Zooplanktons are heterotrophic planktonic animals floating in water which constitute an important food source for many species of aquatic organisms (Guy, 1992). Cyclopoida, Ostracoda, and Cladocera are very important in the food chain of freshwater fish (Egborge, 1981). Their characteristics, coupled with high sensitivity to changes in environmental factors have drawn the attention of several hydrobiologists worldwide, who had investigated their occurrence composition, distribution and their significant roles in the study of aquatic pollution. Zooplankton studies are of necessity in fisheries, aquaculture and paleolimnological research as they have been known to leave an impression record of geological past (Stout,

1974; Aoyagui and Bonecker, 2004). They are globally recognized as pollution indicator organisms in the aquatic environment (Rutherford *et al.*, 1999; Yakubu *et al.*, 2000; Abowei and Sikoki, 2005). Zooplankton plays an important role in the biological cycling of carbon and other elements in the ocean. Seasonal zooplankton dynamics and the mechanisms driving their variability are highly susceptible to changes in environmental variables, especially in shallow, semi-enclosed bays with heavily populated shores where increased anthropogenic nutrient input severely affects marine communities (Marcus, 2004). An increase in nutrient loading can cause an increase in phytoplankton productivity and standing stocks, especially in large-sized phytoplankton (Breitburg *et al.*, 1999).

Less attention has been given to the study of zooplankton community of smaller rivers such as Ossiomo River, which are all over the country and contain significant proportion of nation's aquatic biodiversity. This study reports a survey of zooplankton communities of the River Ossiomo in Ologbo, Edo State, Niger Delta, Nigeria.

MATERIALS AND METHODS

Study Area: The study was carried out on a stretch of River Ossiomo (Latitudes 6°30' – 6°32'0"N; Longitude 5°39'– 5°40'30"E) (Figure 1), which is a tributary of Benin River, South-South, Nigeria.

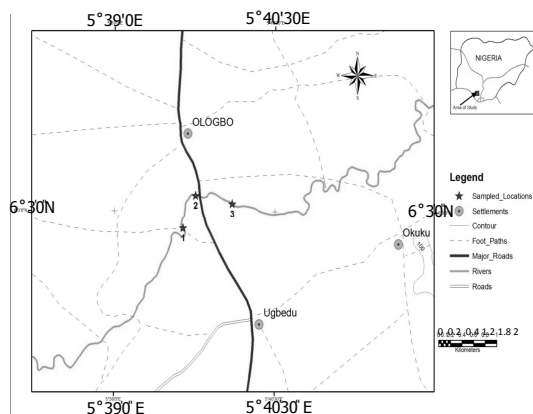


Figure 1: Map of Ologbo showing sampling locations along River Ossiomo

River Ossiomo stretches over a 250 km distance within Edo State and Delta State, South-South, Nigeria. It is supplied by rivers Ikpoba, Okhuaihe and Akhaiwan. Ossiomo River drains into the Benin River at Koko, a coastal community in Delta State, Nigeria and where Benin River empties into the Atlantic Ocean (Tawari-Fufeyin *et al.*, 2008).

This study area falls within the well-known rainforest belt of Nigeria, with a wet season ranging from March to October and a dry season from November to March. The Ologbo community, an adjacent settlement to River Ossiomo is essentially rural and it is situated in Ethiope West, Delta State, Nigeria, its geographical coordinate are 6°3'0" North and 5°40'0" East (Tawari-Fufeyin *et al.*, 2008). The River Ossiomo thus provides a source of water for domestic use especially for many rural

settlers and communities. The river is fairly wide and flanked by secondary vegetation of rubber trees *Hevea brasiliensis*, palm trees *Elaeis guinensis*, Bamboo trees *Bambusa* sp. and shrubs. On the river are floating vegetation such as *Salvinia* sp., *Lemna* sp. and *Eichornia crassipes* (Tawari-Fufeyin *et al.*, 2008). No major industry, except few logging merchants and few extractive industries is sited in this area. Farming is the major occupation of the inhabitants, while fishing is secondary (Tawari-Fufeyin *et al.*, 2008).

Three sampling stations were chosen: Station 1 (Upstream), Station 2 (Midstream) and Station 3 (Downstream). The upstream station (Station 1) is about 2 km away from station 2 at Ologbo community. Apart from boating and fishing activities, the marginal vegetation here is mainly grasses and macrophytes like water hyacinth (*E. crassipes*). Station 2 is the wharf side at Ologbo community; the river has marginal vegetation encroaching into the river waterways consisting of shrubs and grasses. This station has highest level of human activities/disturbance, these include; bathing, swimming, and washing of clothes and household utensils. Station 3 (downstream) is also about 2 km downstream from station 2. The human activities here include fishing, dredging, occasional oil spillage and lumbering.

Sampling: Samples were collected at monthly interval for 9 months from April, 2012 to December, 2012. Samplings were done between 10.00 hours and 14.00 hours local time (GMT +01) on each sampling day in three sampling stations. Zooplankton samples meant for identification was collected at each station. Composite zooplankton samples were collected in each sampling location, using both qualitative and quantitative methods of sampling. Qualitative plankton samples were collected by towing 55µm mesh students plankton net just below the water surface for 5 minutes at each sampling station. In quantitative sampling, 100 liters of water was filtered through 55µm students' plankton net with the aid of a 10 liters bucket sampled randomly 10 times at each station.

The sampled zooplanktons were preserved in 4% formalin solution in a 250 ml plankton bottles (UNESCO, 1974).

In the laboratory, zooplanktons were sorted into their various taxa under a binocular dissecting microscope (American Optical Corporation, Model 570), and slides were prepared using polyvinyl lactophenol as mountant, while drawing, counting and identifications were done with an Olympus Vanox Research Microscope (Model 230485) at x60 magnifications with an attached drawing tube (Model MKH 240-790). Identification of specimens was carried out at the University of Benin, Animal and Environmental Biology Laboratory using the relevant taxonomic keys (Onabamiro, 1952; Green, 1962; Smirnov, 1974; Dumont, 1981; Van de Velde, 1984; Jeje and Fernando, 1986; Gabriel *et al.*, 1987; Jeje, 1988; Boxshall and Braide, 1991).

Data Analysis: The percentage relative abundance of the specimens was estimated by direct count. Each quantitative sample was concentrated to 10 ml and from this 1 ml of sample was taken and all individual taxa present were counted. Relative abundance was calculated as the number of individuals per litre of water filtered through the net. Species diversity indices (Margalef's index, Evenness index and Shannon-Wiener index) were used in analysing the zooplankton community structure. The BASIC programme SPDIVERS.BAS for diversity index was used for diversity, while Kruskal-Wallis non-parametric test was used to test for significant differences between stations. All statistical methods used to analyze the zooplankton community structure including inter station comparisons carried out to test for significance differences in the abundance of zooplankton using one-way ANOVA. The Pearson correlation coefficient at a confidence limit of 95% was applied using SPSS 16.0 to study the relation between zooplankton distribution and the environmental variables (Zar, 1984; Ogbeibu, 2005). The Bray-Curtis similarity index was computed using the software packages PRIMIER program V 5.1.

RESULTS

Environmental Parameters: Most of the physico-chemical conditions of the water investigated namely pH, depth, transparency, turbidity, suspended solids, conductivity, hydrogen carbonate, sodium, potassium, calcium, magnesium, chloride, sulphate, nitrate, phosphate, dissolved oxygen and BOD all showed no significant variations ($p > 0.05$) among the three sampled stations (Table 1). However, the flow rate, air temperature, water temperature, differed significantly ($p < 0.05$) among the stations. The flow rate at station 3 was significantly faster than those of the other two stations which were not significantly different ($p > 0.05$) from each other, while temperature (air and water) at station 1 were significantly higher ($p < 0.05$) than the other two stations which were also not significantly different ($p > 0.05$) from each other (Table 1). The water was generally fresh with conductivity values ranging from $70.11 \mu\text{Scm} - 62.03 \mu\text{Scm}$.

The water was slightly acidic in nature with the mean hydrogen ion concentration ranged from 6.01, 5.86 to 5.76, respectively in stations 1, 2 and 3 (Table 1). The concentration of calcium and magnesium salts with carbonates constitutes the total hardness of water; this was also generally low indicating that the river was soft water. The range in dissolved oxygen concentration ($7.54 - 7.12 \text{ mg l}^{-1}$) was high. The range values for the essential primary productivity nutrients; nitrate ($0.20 - 0.16 \text{ mg l}^{-1}$), sulphate ($0.94 - 0.88 \text{ mg l}^{-1}$) and phosphate ($0.33 - 0.25 \text{ mg l}^{-1}$) were low.

Species Composition and Population Density:

A total of 22 species of zooplankton were identified from River Ossiomo during the period of study. Most of them were cladocerans (11 species), copepods (6 species) and rotifers (5 species) (Table 2). The lowest number of species was recorded in station 2 during all seasons. On the other hand, the upstream sustained the highest number of species (21 taxa) at station 1 (Figure 2).

Zooplankton was represented by holoplanktonic groups with a total of 1330 individuals.

Table 1: Physical and chemical conditions of the studied stations in River Ossiomo, April 2012 – Dec. 2012

Parameters	Station 1			Station 2			Station 3			P-Value	FMEnv. Permissible Limits	WHO Standard/ Guideline Value
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max			
Ambient Temperature	29.57 ^a \pm 1.63	26.60	32.30	27.01 ^b \pm 1.48	25.0	29.50	28.32 ^b \pm 1.44	26.50	30.40	**P<0.05	N/A	-
Water Temperature	27.56 ^a \pm 1.48	25.30	29.50	25.87 ^b \pm 0.85	24.8	27.40	26.46 ^b \pm 0.84	25.0	27.80	**P<0.05	35 ⁰ C	-
Depth(m)	1.75 \pm 0.85	0.63	2.70	1.47 \pm 0.19	1.10	1.70	1.32 \pm 0.25	1	1.75	P>0.05	-	-
Flow Rate(m/s)	0.09 ^b \pm 0.05	0.04	0.18	0.08 ^b \pm 0.04	0.01	0.15	0.13 ^a \pm 0.30	0.1	0.19	*P<0.05	-	-
Transparency (m)	1.26 \pm 0.60	0.50	2.30	1.30 \pm 0.22	1.00	1.70	1.01 \pm 0.14	0.70	1.20	P>0.05	-	-
pH	6.01 \pm 0.43	5.42	6.8	5.76 \pm 0.29	5.40	6.13	5.86 \pm 0.39	5.19	6.38	P>0.05	6.5-8.5	6.5-8.5
EC(μS/cm)	70.11 \pm 22.85	40	99	62.03 \pm 21.64	28	90	64.1 \pm 24.15	30	94	P>0.05	N/A	1000
Turbidity (NTU)	4.37 \pm 0.82	3.5	5.7	3.97 \pm 0.74	3.1	5.2	4.19 \pm 0.82	3.3	5.4	P>0.05	5.0NTU	-
TSS (mg/l)	5.8 \pm 1.14	4.5	7.5	5.52 \pm 0.91	4.1	7	5.01 \pm 1.62	2.3	7	P>0.05	<10	-
TS (mg/l)	42.11 \pm 10.00	24.1	56.6	38.82 \pm 15.30	22.7	76.3	40.73 \pm 19.39	22.5	87.3	P>0.05	-	-
TDS (mg/l)	33.84 \pm 11.39	17.6	49.1	29.6 \pm 9.07	18.6	45.7	30.57 \pm 9.75	18.1	47.2	P>0.05	500	1000
DO(mg/l)	7.48 \pm 2.06	5.6	12.1	7.12 \pm 1.20	5.8	8.9	7.54 \pm 1.66	5.4	10	P>0.05	5.0	-
HCO₃⁻ (mg/l)	53.28 \pm 11.66	30.5	62	48.63 \pm 19.54	24.4	91.5	50.53 \pm 21.64	24.4	91.5	P>0.05	-	-
Na(mg/l)	1.37 \pm 1.67	0.33	4.99	1.35 \pm 1.87	0.32	5.75	1.43 \pm 1.87	0.32	4.89	P>0.05	200.00	200
K(mg/l)	0.26 \pm 0.37	0.03	1.23	0.25 \pm 0.36	0.05	1.2	0.25 \pm 0.35	0.03	1.17	P>0.05	-	-
Ca(mg/l)	1.00 \pm 0.50	0.01	1.55	0.92 \pm 0.46	0.04	1.43	0.97 \pm 0.51	0.06	1.89	P>0.05	-	200
Mg (mg/l)	0.52 \pm 0.32	0.05	0.94	0.44 \pm 0.27	0.05	0.8	0.48 \pm 0.28	0.06	0.85	P>0.05	-	200
Cl (mg/l)	36.39 \pm 24.55	9.3	88.8	31.12 \pm 20.78	7.75	74.4	26.44 \pm 24.46	5.2	88.8	P>0.05	200	250
P (mg/l)	0.33 \pm 0.21	0.1	0.76	0.25 \pm 0.11	0.12	0.39	0.31 \pm 0.28	0.07	0.94	P>0.05	-	-
NO₃⁻ (mg/l)	0.19 \pm 0.22	0.04	0.69	0.16 \pm 0.23	0.02	0.71	0.20 \pm 0.22	0.02	0.63	P>0.05	10.0	10
SO₄⁻ (mg/l)	0.94 \pm 0.77	0.12	2.56	0.88 \pm 0.66	0.2	2.48	0.91 \pm 0.85	0.22	2.97	P>0.05	500.0	250

Note: P>0.05 - Not Significant, *P<0.05 – Significant, **P<0.05- Highly Significant; Similar superscript across the row shows that there is no significant difference between the mean of the stations

Table 2: Species composition and population density in River Ossiomo, April 2012 – Dec. 2012

Species Composition (taxonomy)	Station 1	Station 2	Station 3	% Relative Abundance	Total
Phylum Arthropoda	-	-	-	-	-
Class Crustacea	-	-	-	-	-
Sub Class Branchiopoda					
Order Cladocera	-	-	-	43.61	-
Bosminidae	-	-	-	-	-
<i>Bosmina longirostris</i>	60	-	-	-	60
Cercopagididae	-	-	-	-	-
<i>Bythosrephes longimanus</i>	10	-	-	-	10
Chydoridae	-	-	-	-	-
<i>Dadaya macrops</i>	50	20	30	-	100
<i>Alona eximia</i>	70	50	90	-	210
<i>Euryalona orientalis</i>	10	-	-	-	10
<i>Pseudochydorus globosus</i>	20	10	20	-	50
Daphnidae	-	-	-	-	-
<i>Daphnia hyaline</i>	20	-	10	-	30
<i>Simocephalus expinous</i>	10	-	-	-	10
Macrothricidae	-	-	-	-	-
<i>Grimaldima brazzai</i>	20	10	20	-	50
Moinidae	-	-	-	-	-
<i>Moina reticulata</i>	10	-	-	-	10
Sididae	-	-	-	-	-
<i>Diaphanosoma excisum</i>	20	10	10	-	40
Sub Class Copepoda	-	-	-	51.13	-
Order Cyclopoida	-	-	-	-	-
Cyclopidae	-	-	-	-	-
<i>Cryptocyclops bicolor</i>	80	-	10	-	90
<i>Eucyclops macruroides denticulatus (Lilljeborg, 1901)</i>	10	-	-	-	10
<i>Metacyclops minutus</i>	10	30	10	-	50
<i>Mesocyclops bodanicola</i>	30	10	20	-	60
<i>Microcyclops varicans</i>	20	-	10	-	30
<i>Thermocyclops neglectus</i>	210	160	70	-	440
Superclass Rotifera	-	-	-	5.26	-
Class Monogononta	-	-	-	-	-
Order Ploima	-	-	-	-	-
Lecanidae	-	-	-	-	-
<i>Monostyla hamata</i>	10	-	-	-	10
<i>Monostyla cornuta</i>	10	-	-	-	10
Lepadellidae	-	-	-	-	-
<i>Lepadella ovalis</i>	10	-	-	-	10
Proalidae	-	-	-	-	-
<i>Proales decipiens</i>	20	-	10	-	30
<i>Proales simplex</i>	-	10	-	-	10
(Total)No of Individuals	710	310	310	-	1330

Copepods were the predominant component of the holoplankton in River Ossiomo during both seasons in terms numerical abundance, while cladocerans were the predominant component in terms of species diversity. Numerically copepods made up 51.1% of the total zooplankton population with 680 individuals m⁻³ (Figure 3). Among the most dominant copepods species were *Thermocyclops neglectus* and *Cryptocyclops bicolor* (64.7%, 33.1% and 13.2%, 6.8% of the total copepods and total zooplankton, respectively).

Cladocerans form the second most important group in terms of numerical abundance, comprising about 43.6% of the total zooplankton count representing 580 individuals (Figure 3). Cladocerans were mostly represented by *Alona eximia* and *Dadaya macrops* (forming 36.2%, 15.8% and 17.2%, 7.5% of total cladocerans and total zooplankton respectively). Although rotifers were represented by 5 species, collectively they formed only 5.3% of the total zooplankton density in the river, with relatively higher densities at the upstream of the river (station 1).

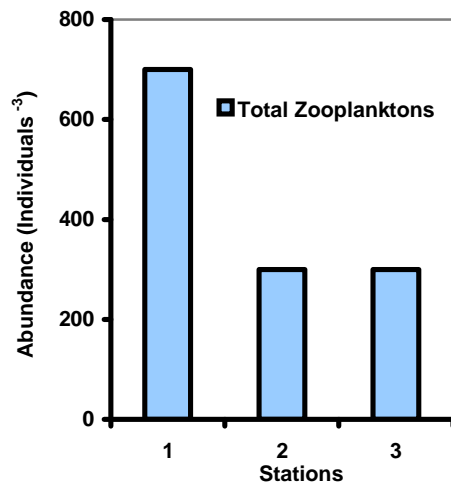


Figure 2: Spatial distribution of the total zooplankton recorded across study stations in River Ossiomo during the period of study

Spatial and Seasonal Distribution of Zooplankton: The zooplankton standing crop throughout the study area was 1330 individuals. As illustrated in Figure 2, the highest density (710) was recorded at station 1. Station 2 and 3 harboured a lower standing crop with a density of 310 at both stations. Based on numerical abundance copepods were the most dominant zooplankton group, making up half of the zooplankton population in most of the studied stations (Figure 3).

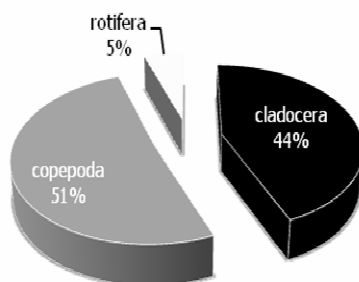


Figure 3: Percentage of occurrence of the recorded zooplankton groups in River Ossiomo during the period of study

The highest copepod densities were observed in the upstream and midstream (stations 1 and 2) decreasing gradually towards the downstream (station 3). The abundance was lowest at station at station 3. The freshwater copepod *Eucyclops macruroides* was recorded only at station 1 with 10 individuals (Figure 4).

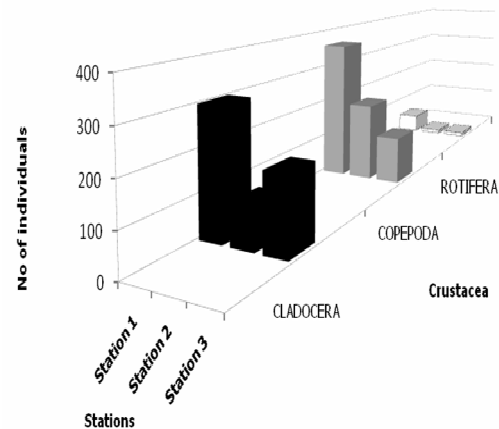


Figure 4: Spatial distribution of zooplankton groups in River Ossiomo during the period study

Cladocerans were the most dominant group in the downstream (station 3) making up 58.1% of the total zooplankton population at this station. Their abundance decreased gradually: densities were minimal in the midstream (station 2). Rotifers showed nearly the same distributional pattern as copepods. Their densities were highest at in the upstream (station 1) and decreased gradually towards the downstream (station 3). Freshwater rotifers *Proales decipiens* and *Proales simplex* were only recorded at station 3 and 2 respectively (Figure 4).

The seasonal total zooplankton standing stock throughout the study showed that the river was productive through the period of study. Abundance was lower during dry season. The zooplankton population was higher in the wet season, showing a distinct peak in the month of June and July for nearly all stations.

In wet season, the zooplankton standing crop was larger than in dry season. Copepods represented 53.6% (590 individuals) of the total zooplankton. They were represented by 6 species with the dominance of *Thermocyclops neglectus* (420 individuals, 38.2%). *Cryptocyclops bicolor* and *Mesocyclops bodanicola* were fairly frequent species. Cladocerans were the second dominant group with a density of 460 individuals, accounting for 41.8% of the total count. Regarding species composition, cladocerans were more diversified (13 species).

Rotifers contributed about 4.6% to the total community. They were represented by 5 species

During dry season, cladocerans dominated the zooplankton community (110 individuals) consisting 47.8% of the total population. Cladocerans were represented by *Bosmina longirostris*, *Dadaya macrops* and *Alona eximia*. Of these *Dadaya macrops* was the dominant at stations 1 and 3 for the month of December. Copepods were the second dominant group with a density of 100 individuals, representing 43.5% of the total zooplankton count. Copepods were represented by *Cryptocyclops bicolor*, *Thermocyclop neglectus*, *Microcyclops varican* and *Mesocyclops bodanicola*. The leading species was *Thermocyclop neglectus* in station 1 for the month of October and November. Rotifers constituted 8.7% of the total zooplankton represented only by *Proales decipiens*. It was present in stations 1 and 3 in the month of December only (Figure 5).

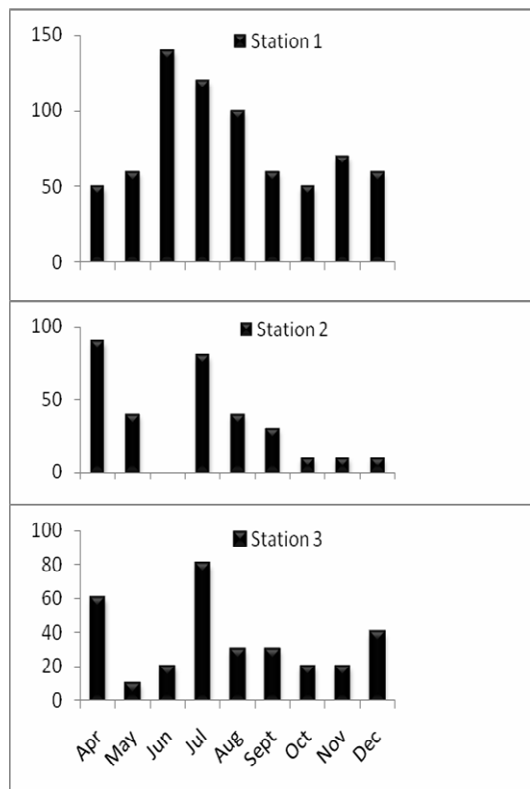


Figure 5: Spatial and seasonal distribution of the total zooplankton across study stations in River Ossiomo during the period of study

Species Diversity: The diversity indices were designed to measure species richness, the number of species in a community and the degree of evenness of the species' relative

abundance. However, spatial variations in the number of species and individuals were reflected by the species diversity (Shannon-Weiner index). River Ossiomo showed the lowest average species richness (1.592) recorded at station 2, while the highest average of 2.508 was recorded at station 1 (Figure 6). The zooplankton in station 3 recorded the highest Evenness index (0.6916); this was followed by station 1 (0.5846) then station 2 (0.5461), in their order of decreasing values (Table 2).

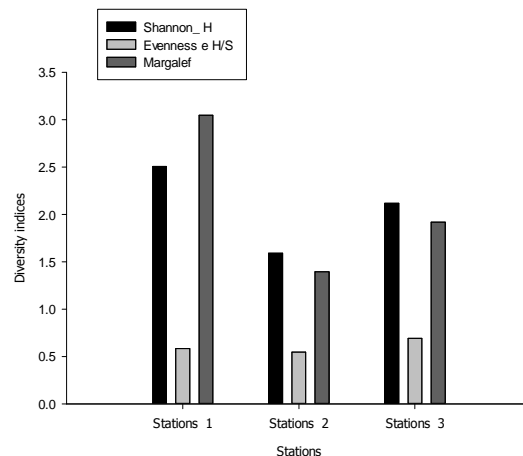


Figure 6: Diversity indices of total zooplankton recorded at different stations in River Ossiomo during the period of study

Cluster Analysis: In order to reveal the similarities and differences among the investigated stations, cluster analysis was performed based on the total abundance of the zooplankton community (Figure 7).

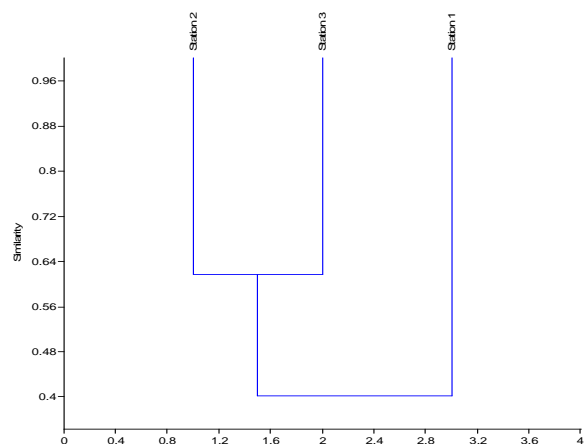


Figure 7: Dendrogram showing similarity of sampling stations on the basis of their zooplankton composition in River Ossiomo during the period of study

The results showed the presence of two main clusters with a high similarity. The first cluster contains only station 1, which is located in the upstream, where copepods are dominant. The second cluster consists of the other stations (2 and 3) located in the midstream (wharf side) and downstream of the river, were characterized by relatively low abundance.

DISCUSSION

Twenty two (22) species of crustacean zooplankton made up of 11 species of Cladocera, 6 species of Copepoda and 5 species of Rotifera were recorded in River Ossiomo during the study. The numbers of zooplankton species recorded from this study were common in several other rivers in Nigeria and elsewhere (Bidwell and Clarke, 1977; Jeje and Fernando, 1986; Egborge, 1994; Imoobe and Egborge, 1997; Tawari-Fufeyin *et al.*, 2008; Imoobe *et al.*, 2008; Imoobe and Akoma, 2009; Imoobe, 2011). The values of Margalef's index, Evenness index and Shannon-Wiener index indicated a fairly rich diversity of zooplanktons supported by the nutrient status of the water body. This suggested that the river was not under serious pollution threat at the time of the study. This was in agreement with the earlier studies by Imoobe (1997) who reported fifty-one species of crustacean zooplankton from Jamieson River located within the same locality.

The calculated diversity indices using Shannon-Wiener index revealed that station 1(2.508) was more diverse, followed by station 3(2.116), while station 2(1.592) was the least diverse. This pattern was expected because station 2 had more disturbances especially from human activities and the rate of flow of water was high at this station. Also the distribution pattern of the individuals found in this station was few, low and least even.

Zooplankton abundance and species number in Ossiomo River varied monthly. The high abundance of zooplankton recorded during the wet season (June – July) was similar to reports in previous studies elsewhere (Saint-Jean, 1983; Okogwu, 2010; Imoobe, 2011). Seasonal alteration of zooplankton abundance observed in this study may be due to physico-

chemical condition of the water. Flooding during the wet season may have contributed positively to zooplankton population growth as a result of species recruitment from other flooded water bodies and inflow of nutrients from the drainage basin that will trigger off increase in phytoplankton production and consequently zooplankton productivity. However, seasonal dynamics of zooplankton communities in the tropics has been attributed to a number of other factors such as the environmental characteristics of the water, predation, quality of edible algae and competition (Hellawell, 1986; Ovie and Adeniji, 1994).

Copepods and cladocerans were the most important groups of crustacean zooplankton in River Ossiomo, while the former was dominated by the cyclopoids, the later was dominated by the chydorids. A total of eleven species belonging to seven families and eleven genera were reported for cladocera, while a total of six species belonging to one family and six genera were recorded for copepods, the lowest zooplankton class recorded was rotifera which has total of five species belonging to three families and three genera.

Copepoda are known to occur in plankton of most water bodies and have been ranked as one of the most abundant. Raymont (1983) recorded that though ubiquitous; copepods are more in the marine environment than in the freshwater. The cyclopoids dominated in this study and this agreed with the findings of Egborge (1981) and Jeje and Fernando (1986), where the 11 cyclopoid copepods were the dominant group in Lakes Asejire and Kainji, respectively.

The five species of rotifers that were found belong to the families of Lecanidae (represented by *Monostyla hamata* and *M. cornuta*), Lepadellidae (*Lepadella ovalis*) and Proallidae (*Proales decipiens* and *P. simplex*). This result contrasted an earlier study of Tawari-Fufeyin *et al.* (2008) who recorded no representative of rotifer in the same river.

The spatial and seasonal distribution of crustacean zooplankton species (Figure 5) showed that while some species were restricted to certain stations for certain month, others were found in all the stations. Eight species of

crustacean zooplankton, namely, *Dadaya macrops*, *Alona eximia*, *Pseudochydorus globosus*, *Grimaldima brazzai*, *Diaphanosoma excisum*, *Metacyclops minutes*, *Mesocyclops bodanicola* and *Thermocyclops neglectus* occurred in all the three stations. Out of the 22 species of the zooplankton, only 1 species was absent in station 1, 13 species were absent in station 2, while 10 species were absent in station 3. The predominant human activities in station 2 must have resulted in such high depletion in the population. The high density of zooplankton in station 1 was due to 6 species that were exclusively restricted to station 1 to include *Simocephalus expinous*, *Moina reticulata*, *Eucyclops macruroides*, *Monostyla hamata*, *Monostyla cornuta*, *Lepadella ovalis*, while only *Proales simplex* was restricted to station 3. No species was restricted to station 2, this might be because it is the downstream of the study area hence the water current drift many of the zooplankton in station 1 and 2 into station 3.

In conclusion, the study revealed there was no evidence of water pollution recorded in any of the stations. The contamination from occasional oil spillage was found to be below World Health Organization (WHO) and Federal Ministry Environment (FMEEnv) acceptable limit for water pollution. The zooplankton showed a high significant positive correlation with air temperature. All parameters were found to influence the distribution and abundance of the fauna along the stretch of the river. Future work on this particular river is recommended to ascertain the extent of future composition, distribution, diversity and ecology of zooplankton and other higher taxa like the fishes in the river may be in a longer distance across the river to ascertain the trend of physico-chemical parameters and assemblage of the zooplankton and others invertebrates and vertebrates.

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KNOWLEDGE BASED ASSESSMENT OF INTESTINAL PARASITIC INFECTIONS AMONG STUDENTS ATTENDING BOARDING SCHOOLS IN EBONYI STATE, NIGERIA

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ABSTRACT

There is an apparent lack of information on the risk and clinical symptoms of Intestinal Parasitic Infections (IPIs) among students attending boarding secondary schools in Ebonyi State, Nigeria. This questionnaire-based survey attempts to assess some behavioural habits, possible risk factor(s) as well as clinical symptoms experienced by these students. 256 questionnaires were filled by (52.7% males and 47.3% females) from four boarding schools between June and July 2015. Results showed that on hand-washing practice after defecation, while there was much more students who washed their hands with soap and water (79.7%) than with water only (10.2%), only a few do not wash their hands (2.7%). Also, students wash (86.3%), do not wash (1.2%) or sometimes wash (11.3%) their hands before meals. There were students who do (16.4%), do not (53.5%) or sometimes do (22.3%) bite off on their fingernails using their teeth. Records were taken of those who walk (11.3%), do not walk (68.0%) or sometimes walk (18.0%) on bare feet. A greater number of students use water cisterns (41.0%) than pit toilets (36.7%), and pit toilets (36.7%) than bushes (19.5%). Borehole constituted the most ready source of drinking water for students (75.8%). Parents are predominantly farmers, traders, teachers and civil servants. Clinical symptoms were more occasional than frequent. The hygiene behavioural practices are commendable. Thus there is possibly a low risk of IPIs among these students. However, promotion of healthy hygienic practices should be further encouraged.

Keywords: Intestinal parasitic infections, Risk, Clinical symptoms, Boarding schools, Ebonyi State

INTRODUCTION

Intestinal parasitic infections (IPIs) are easily transmitted in an atmosphere of close human contacts. Students in boarding institutions, particularly those living under crowded conditions, are highly vulnerable to acquiring these infections (Al-Madani and Mahfouz, 1995; Gamboa *et al.*, 2011; Sagnuankiat *et al.*, 2014). The risk of infection is further compounded by the hygiene practices of some of these students. The habits of fingernail biting, walking on bare feet, eating with unwashed hands and defecating without washing the hands after toilet use are some of the bad habits that raises

the risk of infection (Dambhare *et al.*, 2010; Tamirat and Getye, 2014; Al-Delaimy *et al.*, 2014).

IPIs are common in developing countries particularly in areas where basic facilities such as pipe-borne water, proper faecal waste disposal systems and adequate healthcare services are lacking (Agi, 1995; Al-Delaimy *et al.*, 2014). Places with poor sanitary habits and improper personal and environmental hygiene are more often inhabited by low income earners and thus are worse hit by IPIs (Wordemann *et al.*, 2006; Juarez and Rajal, 2013). IPIs results in a wide-spectrum of clinical symptoms ranging from apparently

symptomless to life-threatening conditions (Ichhpujani and Bhatia, 2002; Polimeno *et al.*, 2010; Muennig *et al.*, 2015). Common manifestations include abdominal pains, diarrhoea, anal itching and weight loss (Ichhpujani and Bhatia, 2002). These may occur occasionally or frequently depending on a number of factors such as infection burden, duration and risk of repeated exposures.

A number of studies conducted in different parts of Ebonyi, southeastern Nigeria revealed a high prevalence of IPIs among school children (Anim and Akamnonu, 2009; Ugbogu and Asogu, 2013). This is largely a consequence of ignorance of the routes and risks of IPIs. We strongly believe that if school-age children are adequately informed about IPIs, habits that predispose them to acquiring IPIs will be dropped easily. Seeking information from the students as to their level of awareness on the causes and the clinical symptoms associated with IPIs is pivotal to the disease management (Workneh *et al.*, 2014) because it will expose the ignorance level as well as bring to light the amount and areas where enlightenment needs to be strengthened so as to impact positively on behavioural habits and thereby reduce the incidence of infection. Thus inline with Idowu and Rowland (2006) and Canete *et al.* (2012), questionnaires were employed as information gathering tool to achieving the aforementioned set objectives.

MATERIALS AND METHODS

Study Area: The survey was carried out in Ebonyi Central and South, two of the three senatorial zones of Ebonyi State. Ebonyi State is located in the South Eastern part of Nigeria. It lies between longitude 7⁰35'N and latitude 6⁰45'E. There are two distinct seasons; rainy season from April to October and dry season from November to March. A good number of Ebonyians are civil servants, students, drivers, manual labourers and artisans, but are predominantly traders and farmers. They produce crops such as rice, yam, potatoes, maize and cassava in large quantities. The rural dwellers especially, rely on rivers and streams for water (Nworie *et al.*, 2014). Several of the

people make use of pit toilets and bushes as means of faecal waste disposal, a practice which inadvertently promote IPIs transmission. The State has a rich presence of academic institutions which include Universities, Colleges of Education and Agriculture, and boarding secondary schools.

Study Population and Sample Estimation:

The present questionnaire based survey was conducted among students attending boarding secondary schools between June and July, 2015. Four boarding schools were selected for this survey; an exclusively boys' and an exclusively girls' boarding secondary schools from each of the two [Ebonyi Central (Ezza High School and Ezza Girls) and South (Eghugbo Technical College and Sir Francis Ibiyam Grammar School)] senatorial zones. These schools are well known and the enrolment of children by their parents is relatively encouraging. Using the Creative Research Systems Survey Software (Version 11.0) with a Confidence Limit (CL) and Confidence Interval (CI) of 95% and 5.5 respectively, a population estimate of 2,800 students resulted in a sample size of 285 students out of which only 256 respondents were processed. The other 29 were voided due to a range of errors.

Data Collection: Verbal consents were granted by the schools' heads. Data were gathered by the use of questionnaires. The questionnaires were designed to address four major aspects: environmental, socio-economic, sanitary behavioural risk factors of IPIs and some of their clinical symptoms. The socio-economic factors were age, sex and parents' (father and mother) occupations. The sanitary behavioural aspect covered questions on hand washing after defecation (yes/no, and if yes, with soap and water, or with water only), and before meals (yes/no/sometimes), walking on barefoot (no/yes/sometimes) and biting of fingernails (no/yes/sometimes). Environmental risks were on the type of faecal waste disposal system (pit toilet, water cistern, bush) and source of drinking water (borehole, stream, well, river, storage tank, any other source). The students were to give a 'frequently' or 'occasionally'

answer to each of the clinical symptoms (diarrhoea, anal itch, flatulence, abdominal pains, foul-smelling stools) presented in the questionnaires.

Statistical Analysis: The odd ratio (OR) analysis was performed using Medcalc Statistical Software (version 15.6) to assess the relative exposure of students in the use of the different faecal waste disposal systems (pit toilet, water cistern and bush). ORs were statistically significant if $p < 0.001$ at 95% confidence interval. The Social Science Statistical Software was used in chi-square analysis of sanitary behavioural data and between occasional and frequent occurrences of clinical symptoms. Chi-square analysis was significant if $p < 0.01$. Frequency distributions of the responses of subjects on various aspects of the survey were carried out. Categorical variables were tabulated as numbers and percentages in parenthesis.

RESULTS

A total of 256 students [males 135(52.7%); females 121(47.3%)] responded to the questionnaires. These respondents were within the age bracket of 11 – 19 years. Data on personal hygiene of the students, according to their age groups are presented in Tables 1 – 4. Significant differences between students (males and females) who washed their hands after defecation with water only, and with water and soap ($\chi^2 = 11.7$, $p = 0.000615$, $p < 0.01$) and students who walked, did not walk and sometimes walked on bare feet ($\chi^2 = 13.2$, $p = 0.001395$, $p < 0.01$) were observed. There were no significant differences between students who washed, failed to wash and sometimes washed their hands before meals ($\chi^2 = 4.3$, $p = 0.116745$, $p > 0.01$); and those who used, did not use and sometimes used their teeth to bite off their fingernails ($\chi^2 = 4.0$, $p = 0.136924$, $p > 0.01$).

Water cistern (WC) was used by 105(41.0%) students, pit latrine by 94(36.7%) and bush by 50(19.5%). Some other students used a combination of these faecal waste disposal methods.

The number of students using WCs were higher than those using pit latrines (OR = 3.56, CI (95%) = 1.98 to 6.39, $p < 0.001$); and pit latrines than bushes (OR = 3.44, CI (95%) = 1.68 to 7.06, $p < 0.001$) (Table 5).

Borehole constituted the most ready source of drinking water for a majority (194, 75.8%) of students. This was distantly followed by storage tanks (19, 7.4%), streams (17, 6.6%), wells (3, 1.2%), river (2, 0.8%), rainfall (1, 0.4%) and tap (1, 0.4%). There were other students who listed a combination of some of these water sources.

Parents are predominantly traders (22.7% fathers, 48.0% mothers), farmers (12.5% fathers, 8.6% mothers), teachers (9.0% fathers, 10.2% mothers), civil servants (16.0% fathers, 10.9% mothers), artisans (9.0% fathers, 3.5% mothers) and health workers (3.1% fathers, 9.4% mothers).

Data on the clinical symptoms of IPIs among students revealed that more students were occasionally than frequently down with the symptoms of IPIs ($\chi^2 = 4.5$, $p = 0.340138$, $p > 0.01$) (Table 6).

DISCUSSION

The present questionnaire survey assessed a number of risk factors and clinical symptoms of IPIs among students attending boarding secondary schools in Ebonyi State, Nigeria. The hygienic behaviour of any individual has a significant correlation with the risk of infection (Dambhare *et al.*, 2010; Tamirat and Getye, 2014; Al-Delaimy *et al.*, 2014). The route of transmission of IPIs is largely faeco-oral. The majority of students in this study wash their hands after defecation (89.9%) and before meals (86.3%). This could be a result of good education on hand washing, presence of washing facilities around points of defecation and food service. Additionally, a greater number of these students after defecation wash their hands with soap and water (79.7%) and consequently they are at much lesser risk of IPIs than those who used only water (10.2%).

Table 1: Knowledge based assessment of hand washing behaviour after defecation among students attending boarding schools in Ebonyi State, Nigeria

Age Group (Years)	Wash hands after defecation		
	Water only	Yes (%) Soap and Water	No (%) None
11 – 12	4(1.6)	21(8.2)	0(0.0)
13 – 14	7(2.7)	62(24.2)	2(0.8)
15 – 16	10(3.9)	87(34.0)	4(1.6)
17>	5(2.0)	34(13.3)	1(0.4)
Total	26(10.2)	204(79.7)	7(2.7)

Table 2: Knowledge based assessment of hand washing behaviour before meals among students attending boarding schools in Ebonyi State, Nigeria

Age Group (Years)	Wash hands before meals		
	Yes (%)	No (%)	Sometimes (%)
11 – 12	20(7.8)	1(0.4)	4(1.6)
13 – 14	71(27.7)	1(0.4)	4(1.6)
15 – 16	95(37.1)	0(0.0)	14(5.5)
17>	35(13.7)	1(0.4)	7(2.7)
Total	221(86.3)	3(1.2)	29(11.3)

Table 3: Knowledge based assessment of the behavioural practice of walking on bare feet among students attending boarding schools in Ebonyi State, Nigeria

Age Group (Years)	Walk on bare feet		
	Yes (%)	No (%)	Sometimes (%)
11 – 12	4(1.6)	17(6.6)	4(1.6)
13 – 14	11(4.3)	53(20.7)	8(3.1)
15 – 16	8(3.1)	80(31.3)	20(7.8)
17>	6(2.3)	24(9.4)	14(5.5)
Total	29(11.3)	174(68.0)	46(18.0)

Table 4: Knowledge based assessment of fingernails biting behaviour among students attending boarding schools in Ebonyi State, Nigeria

Age Group (Years)	Biting of fingernails		
	Yes (%)	No (%)	Sometimes (%)
11 – 12	3(1.2)	16(6.3)	5(2.0)
13 – 14	12(4.7)	41(16.0)	14(5.5)
15 – 16	17(6.6)	60(23.4)	25(9.8)
17>	10(3.9)	20(7.8)	13(5.1)
Total	42(16.4)	137(53.5)	57(22.3)

Table 5: Knowledge based assessment of faecal matter disposal methods among students attending boarding schools in Ebonyi State, Nigeria

Age range	Pit latrine	Water cistern	Bush
11 – 12	12	6	6
13 – 14	28	34	12
15 – 16	43	49	14
17>	11	16	18
Total	94	105	50

Table 6: Knowledge based assessment of rates of clinical symptoms among students attending boarding schools in Ebonyi State, Nigeria

Clinical symptoms	Males (%)		Females (%)		Total (%)	
	Occ	Fre	Occ	Fre	Occ	Fre
Diarrhoea	34(13.3)	9(3.5)	39(15.2)	1(0.4)	73(28.5)	10(3.9)
Anal itch	43(16.8)	3(1.2)	37(14.5)	5(2.0)	80(31.3)	8(3.1)
Flatulence	56(21.9)	16(6.3)	46(18.0)	3(1.2)	102(39.8)	19(7.4)
Abdominal pain	72(28.1)	9(3.5)	72(28.1)	5(2.0)	144(56.3)	14(5.5)
Foul-smelling stool	53(20.7)	9(3.5)	46(18.0)	8(3.1)	99(38.7)	17(6.6)

Key: Occ = occasionally, Fre = frequently

A study carried out among school children in the eastern region of Nepal reportedly showed that children who washed their hands after defecation using soap and water had a significantly lower prevalence of infection (24%) when compared to those using only water (63.2%) (Sah *et al.*, 2013). Students who failed to wash their hands are very much vulnerable to the disease. Failure to wash hands after defecation may result from an eagerness to play with friends, laziness, lack or distance of washing facilities from defecation points among other reasons.

Intestinal parasites like hookworms and *Strongyloides* are capable of penetrating intact human skin like the soles of the feet which are in constant contact with soil (Ichhpujani and Bhatia, 2002). Hence, the need to always put on foot wares. About 86.0% of students admittedly use shoes regularly. The good practice by a majority of students to always be on foot wares would help in reducing the risk of parasitic infection. The comparatively low prevalence of *Ascaris lumbricoides*, hookworm and *Schistosoma mansoni* recorded in some school children have been presumably attributed among other factors to the regular wearing of shoes (Gelaw *et al.*, 2013).

Although many of the students have formed the habit of biting (16.4%) or sometimes biting (22.3%) off their fingernail, those who do not (53.5%) were much more. The biting of infected fingernails, undoubtedly, results in auto-infection of individuals. The provision of nail clippers for these students will greatly discourage the use of their teeth thus reducing possible incidences of re-infection (Mahmud *et al.*, 2015).

The choice of faecal waste disposal method is critical in assessing the risk of IPIs (Agi, 1995; Al-Delaimy *et al.*, 2014).

This study revealed that WCs was the most used faecal waste disposal method than other methods. The steady decline in the number of students using WC (105, 41.0%), pit toilet (94, 36.7%) and the bush (50, 19.5%) suggest a reduction trend in the risk of acquiring IPIs through waste disposal systems. Communities where individuals use bushes following lack of or refusal to use toilet facilities, rank high in IPIs (Agi, 1995; Abate *et al.*, 2013). Similarly, in a cross sectional study on primary school children in Addis Ababa, children who used traditional type of toilet had the highest prevalence of IPIs as well as those whose toilets were farther from their vicinity (Ashenafi and Mohammed, 2014). To this end, while advocating for a total elimination of the practice of defecating in bushes, we recommend the constructed of good numbers of water closet toilets which are easily accessible and having adequate washing facilities in place of pit latrines.

Choice and source of drinking water has strong correlation with risk of IPIs (Agi, 1995; Al-Delaimy *et al.*, 2014; Ashenafi and Mohammed, 2014). The intermittent visits of some of the students to their homes after each day's academic activity may account for some of the various responses recorded (stream, well and river) as these may be the only source of water.

The occupational engagements of parents is an index of their economic status which may constitute a risk of acquiring these infections (Wordemann *et al.*, 2006; Juarez and Rajal, 2013; Sah *et al.*, 2013). Parents of students were mostly traders, farmers, teachers and civil servants. Some are artisans and health workers. In a study among children in Jos, Nigeria, significantly higher infection rates were recorded among children whose female parents

or care givers were petty traders, artisans and farmers compared to those whose parents were civil servants and health workers (Jombo *et al.*, 2011). In another study, infection rate was highest among children of farmers (60.94%) and was least among children of civil servants (30.17%) (Ogbuagu *et al.*, 2009).

The use of questionnaires in assessing the clinical symptoms of IPIs is a rapid assessment technique (RAT) in evaluating the prevalence, magnitude and burden of the disease in a population. Questionnaires have been used in collecting information on symptoms of IPIs in previous studies (Niyyati *et al.*, 2009; Escobedo *et al.*, 2008). There is no gainsaying that the population where IPIs are markedly present will experience frequent manifestations of the disease symptoms than the population where the infections are barely seen. For all five clinical symptoms assessed from the students' testimonial, it was shown that there was the likelihood of students going down with the symptoms of IPIs occasionally than frequently. This result thus suggests a possible low prevalence of the disease among boarding students in contrast to previous parasitological survey of the general population across age groups elsewhere (Anim and Akamnonu, 2009; Ugbogu and Asogu, 2013). We advise that a screening exercise be conducted among these students in order to ascertain the prevalence of IPIs. Also, enlightenment campaigns on personal and environmental hygiene should be a regular phenomenon.

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PARASITOLOGICAL IDENTIFICATION AND HISTOLOGICAL EXAMINATION OF *FASCIOLA GIGANTICA* SEQUEL TO OCCURRENCE OF BOVINE BILLIARY FASCIOSIS IN CATTLE SLAUGHTERED AT BODIJA ABATTOIR, IBADAN

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ABSTRACT

Fasciola gigantica is the parasitic fluke causing tropical fasciolosis leading to great economic losses in cattle production in Nigeria. One hundred and five (105) flukes were collected and identified after careful examination of the bile ducts from twenty one (21) infected slaughtered cattle out of a total of two hundred (200) cattle examined from Bodija Municipal Abattoir. Semichon's Acetic-Carmine staining technique and histological examination was carried out for parasitic identification while simple faecal floatation and sedimentation techniques was done for parasite egg examination. Postmortem examination showed enlarged and friable liver covered with fibrin tags while bile ducts were thickened, distended and packed with adult flukes when sliced open. Microscopic and histological examination revealed greyish-brown leaf-shaped adult *Fasciola gigantica* flukes, bearing a cone shaped projection and a pair of broad shoulders with the intestinal ceca branched as well as the testes and the ovary. The vitelline follicles fill the lateral fields of the worm and the common genital pore just anterior to the acetabulum. Large *Fasciola gigantica* eggs which are oval, yellowish to greenish in colour and bears a polar operculum. Public enlightenment especially among the butchers and abattoir workers should be periodical on the public health importance of the parasite and how unwholesome abattoir practices can lead to accidental human infection. This will greatly reduce the practice of selling infected organ that have been deemed unfit for human consumption.

Keywords: *Fasciola gigantica*, Cattle, Semichon's Acetic-Carmine technique, Histology, Bile duct

INTRODUCTION

Food-borne trematodiasis are a group of neglected tropical diseases (Furst *et al.*, 2012). *Fasciola gigantica* is a parasitic flatworm of the class Trematoda, which causes tropical fasciolosis. The incidence of human infection has apparently increased over the past 20 years (Tolan, 2011).

It has a complex lifecycle that includes a hepatic phase as well as a biliary phase (Yen *et al.*, 2011). Two distinct clinical phases occur during the course of this infection, the first corresponds to the hepatic migratory phase of the life cycle of the flukes and the other corresponds to the presence of the parasites in their final location in the bile ducts (Marsden, 1999; Yen *et al.*, 2011).

Esteban *et al.* (2003) noted that fasciolosis has been shown to be a re-emerging and widespread zoonoses affecting a number of human populations, apart from its veterinary and economic importance.

Sequel to the continuous occurrence of fasciolosis in Bodija abattoir which is the main recipient and distributor of cattle moved from different parts of northern states to Ibadan metropolis, thus the aims of this study are to identify the parasite using different techniques and also carry out awareness on health implications of the unwholesome practices of butchers in the abattoir as a possible risk factor in human fasciolosis.

MATERIALS AND METHODS

Collection and Preservation of Parasites:

One hundred and five (105) flukes were collected after careful examination of the bile ducts from twenty one (21) slaughtered infected cattle out of a total of two hundred (200) cattle examined for flukes infestation from Bodija Municipal Abattoir. Faecal samples voided by the animals were also collected using plastic gloves and put into clean, dry, leak-proof, transparent plastic bottles for parasitological examination.

The flukes were sorted out and placed in a beaker containing 50 ml of 0.85% cold saline as described by Bush (2009) to wash the worms, remove mucus from their body and prevent contraction due to the muscular nature of the trematodes. These samples were transported to Veterinary Parasitology Laboratory (Faecal) and Histopathology Laboratory (Flukes) of the Faculty of Veterinary Medicine, University of Ibadan for processing. The worms were then relaxed in distilled water for 30 minutes to allow the worms void most of their eggs as an egg-filled uterus will obscure most features of internal anatomy. Fixation of worms was carried out as described by Beaver *et al.* (1984). The trematodes were transferred to 70% alcohol for storage, fixation and preservation of the fluke's cellular architecture.

Staining and Mounting of the Parasites:

Preparation of Semichon's Acetic-Carmine Stain was carried out as described by Kia (2003).

Trematodes from 70% ethyl alcohol were placed into diluted solution of carmine and this was overstrained then destained as described by Ash and Orihel (1987). After staining, the worms were rinsed in 70% ethyl alcohol and destained in weak acid alcohol made of 2 drops of concentrated HCL in 100ml of 70% ethyl alcohol for about 2 hours. The colour of the stain was leached from the tissue until they were clear but internal organs remained well stained. The trematodes were then placed in solution of 70% ethyl alcohol containing 2 drops of saturated aqueous Na_2CO_3 for 30 minutes. This step neutralized the acid and also prevented continued destaining. The parasites were rinsed in 70% alcohol and dehydrated through 80%, 95% and 100% ethyl alcohol with 15 minutes intervals between each alcohol change. Finally the parasites were then cleared in xylene for 15 minute and transferred to a glass slide with few drops of the mounting medium placed on the slides then a cover slip was placed over the preparation. The slides were then placed on a slide warmer for an hour and viewed under a binocular light microscope. Investigation and identification of *Fasciola* was done according to their distinct morphological characteristics following the standard guidelines given by Urquhart *et al.* (1996).

Histopathological Examination of the Parasites:

The protocol for histological preparations was as described by Usende *et al.* (2013). Briefly, the trematodes were fixed by immersion in 10% Formalin and dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned with a microtome at 5µm thickness, and placed on slides used for Haematoxylin and Eosin stain to examine general histological pattern. Detailed microscopic study was performed using high power light Olympus microscope at x40 to x400 magnifications and photomicrographs taken with computer enabled digital camera.

Faecal Examination for Parasite Egg: Direct microscopic examination, sodium chloride floatation and sedimentation techniques (Urquhart *et al.*, 1996) were used to process the

faecal samples. Identification of the parasite eggs were made on the basis of morphology and size. Faecal smears were prepared from fresh faecal samples on glass slides using saturated salt solution and covering with cover slips. The slides were examined microscopically for helminth eggs, using 10x and 40x objectives.

RESULTS

Postmortem examination revealed sero-sanguinous fluid filled abdominal cavity. The liver was enlarged, friable and had a thickened opaque hepatic capsule. Sectioning through hepatic parenchyma produced a gritty sound and cut surface revealed distended, calcified bile ducts which contained numerous adult flukes (Figure 1).



Figure 1: Adult *Fasciola gigantica* from the engorged bile ducts of slaughtered infected cattle into 0.85% cold saline. Mag. X0.4

Microscopic and histological examination revealed grayish-brown leaf-shaped adult *Fasciola gigantica*, which are broader anteriorly than posteriorly with the anterior end bearing a cone shaped projection and a pair of broad shoulders (Figure 2).

The intestinal ceca are branched as are the testes and the ovary, vitelline follicles fill the lateral fields of the worm, the uterus extends through the mid-portion and the common genital pore is just anterior to the acetabulum (Figures 3, 4 and 5).

Large *Fasciola gigantica* eggs were also seen which are oval, yellowish to greenish in colour and bears a polar operculum (Figure 6).

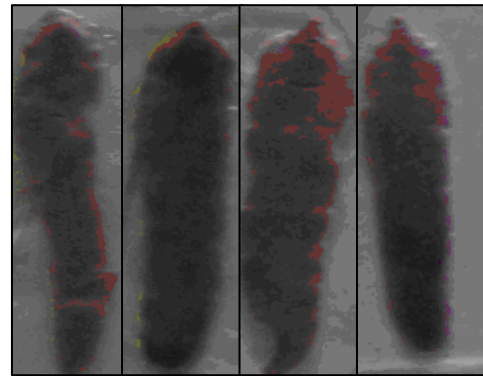


Figure 2: Mounted *Fasciola gigantica* following Semichon's Acetic-Carmine staining before viewing under microscope Mag. X1

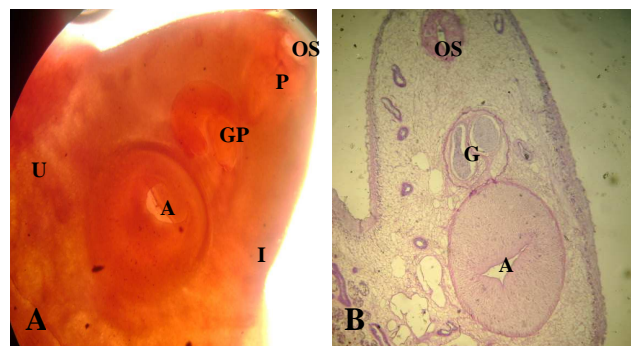


Figure 3: (A) Photomicrograph of the anterior portion of *Fasciola gigantica* mounted in carmine stain and (B) Histology of anterior portion of *Fasciola gigantica*, showing oral sucker (OS), pharynx (P), genital pore (GP), acetabulum (A), intestine (I) and uterus (U). Mag. X10

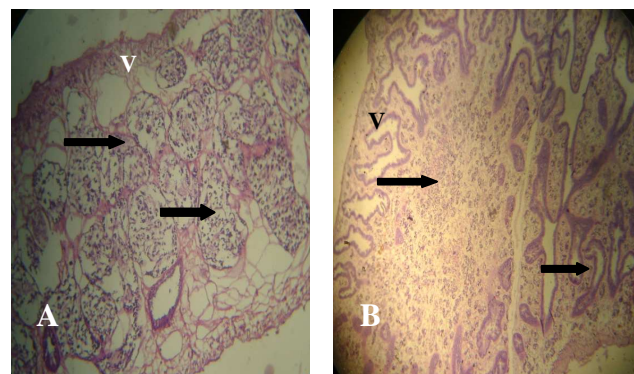


Figure 4: Histology of the ventral portion of sectioned *Fasciola gigantica* showing vitellaria (V), ovaries and testes (Arrow). Mag. X10

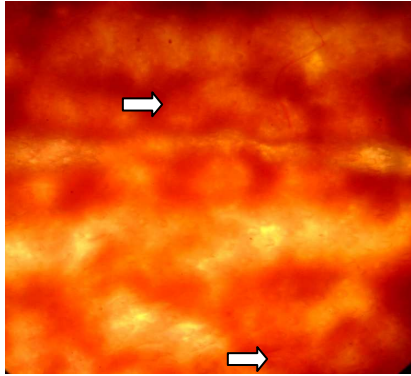


Figure 5: Photomicrograph of the ventral-mid portion of *Fasciola gigantica* mounted in carmine stain showing vitellaria and testes (Arrow). Mag. X10

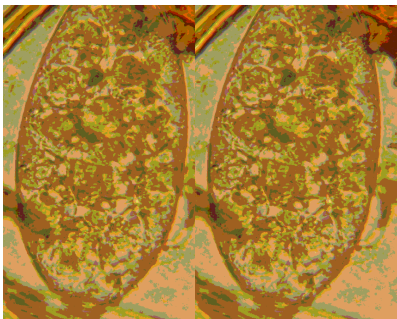


Figure 6: Showing large *Fasciola gigantica* egg which is oval, yellowish to greenish in colour and bears a distal polar lid (operculum) as seen under microscope from infected cattle. Mag. X10

DISCUSSION

A vital function of meat inspection is to aid in monitoring diseases by providing feedback information to the veterinary service to control diseases, to produce wholesome products and to protect the public from zoonotic hazards (Gracey *et al.*, 1999). In this present study, postmortem examination and parasitic identification in cattle was conducted in Bodija municipal abattoir and enlightenment awareness was made to the butchers on how unwholesome abattoir practices can lead to accidental human infection and how butchers' refusal to grant partial or total infected organ condemnation can greatly predispose meat consumers to health hazards.

Different occurrence and prevalence of fasciolosis have been reported in different abattoirs in Nigeria (Alawa *et al.*, 2011; Ardo *et*

al., 2013; Abraham and Jude, 2014; Onyeabor and Wosu, 2014; Kalu *et al.*, 2015) which implies that awareness creation about the public health importance and economic losses associated with the infection at farm level needs urgent attention.

Accurate morphological differentiation between the liver fluke species *Fasciola hepatica* and *Fasciola gigantica* is difficult. However, *Fasciola gigantica* has been reported as the parasitic specie causing tropical fasciolosis and it is regarded as one of the most important single platyhelminth infections of ruminants in West Africa (Goral *et al.*, 2011). The disease is zoonotic (Yilmaz *et al.*, 2013). Fasciolosis in Nigeria has been of major concern to the meat industry of which several researchers have proposed different control measures which might ensure a lasting solution to the occurrence and debilitating effect of the disease on farms where cattle are transported to abattoir for slaughter. It is vital to take into cognizance that *Fasciola gigantica* infection can cause serious effect on the animal host thereby leading to production losses as these debilitating effects looks seemingly harmless at onset and may be difficult to assess. However, the production losses caused by these parasites can be estimated by comparing the performance of infected cattle that receive minimal control or no control at all with naturally infected cattle, in which the parasites are well controlled as the entire metabolism of the untreated animals undergoes functional deterioration.

This study greatly beckon on the government to ensure a compensatory scheme in which butchers are rewarded appropriately when they cooperate with veterinary health workers in carrying out partial or total organ condemnation of infected animals in the abattoir during postmortem meat inspection as this gesture from the government will greatly prevent exposure of the populace to the consumption of these parasite infected organs and therefore safeguarding the health of the masses. In Ibadan metropolis, offal from butchered cattle which are sold to food vendors are a major delicacy which consumers enjoy eating with their local meals. When these organs are infected with parasites, it leads to

serious consequences to the health of consumers (Fearon *et al.*, 2014). It is paramount to note that many infected persons are asymptomatic during the larvae migratory phase, though some experience fever and pain in the right upper quadrant of the abdomen with general malaise of varying degree, including myalgia and urticaria.

Furthermore, Enlightenment trainings and mandatory hygienic abattoir practices should be enforced on butchers in slaughter slabs, as various unwholesome practices were observed in the abattoir in which butchers were seen washing their bodies with abattoir effluents and waste water inside which bovine manures have been entrained through abattoir run offs, these practices can possibly lead to accidental ingestion of parasite eggs as Bestas *et al.* (2014) rightly reported that humans are incidental hosts for *Fasciola gigantica* as these flukes cause illnesses in patients who become infected by ingesting contaminated water containing encysted larvae.

Conflict of interests: The authors declare that they have no competing interests.

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IMMUNOLOGIC AND HAEMATOLOGIC EFFECTS OF METHANOLIC STEM BARK EXTRACT OF *AZADIHIRACTA INDICA* ON CHICKENS EXPERIMENTALLY INFECTED WITH VELOGENIC NEWCASTLE DISEASE VIRUS (KUDU 113) STRAIN

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ABSTRACT

The study was aimed at evaluating the weight, haematologic and immunologic effects of crude methanolic stem bark extract of Azadihiracta indica stem bark on vaccinated chickens experimentally challenged with the velogenic Newcastle disease virus. One hundred day old cockerels were randomly divided into four equal groups (I, II, III and IV). Birds in all groups were vaccinated with La Sota strain of Newcastle disease vaccine. The birds were challenged with the velogenic strain of Newcastle disease virus (VNDV) at 42 days of age. Following challenge, groups I, II and III were given oral treatment of methanolic stem bark extract of Azadihiracta indica at 200mg/kg, 400mg/kg and 600mg/kg, respectively until day 56 of age, while group IV was not given the treatment. Thereafter, birds in all the groups were assessed for onset of clinical signs, changes in live body weight, humoral immune responses and haematologic changes. The birds in groups I, II and III showed no sign of Newcastle disease while birds in group IV exhibited mild depression and huddling. The mean body weight of the vaccinated-treated groups were significantly ($p < 0.05$) higher than that of the vaccinated-untreated group on day 56 of age. On days 63 and 70 of age, the mean body weight of group III was significantly higher than groups I and II. The mean haemagglutination inhibition titres of group III was significantly higher ($p < 0.05$) than groups I, II and IV. The mean PCV, Hb and RBC values of the vaccinated-treated group was significantly ($p > 0.05$) higher than the vaccinated-untreated group on day 49 of age. The WBC count of groups I and II were significantly higher ($p > 0.05$) than groups III and IV on days 49 and 56 of age. The mean absolute heterophil counts of vaccinated-treated groups was significantly ($p > 0.05$) higher than the vaccinated-untreated group on days 56 and 63 of age. The mean absolute lymphocyte counts of the vaccinated-treated increased significantly ($p > 0.05$) than that of vaccinated-untreated birds days 49 of age till the end of the experiment.

Keywords: *Azadihiracta indica*, Stem bark extract, Chickens, Newcastle disease, Immune response, Haematological changes

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease affecting wild and domestic avian species (Seal *et al.*, 2000, Alexander, 2003). ND

is caused by an avian paramyxovirus serotype of the genus Avulavirus belonging to the family paramyxoviridae. The disease is worldwide in distribution (Alexander *et al.*, 1997). Since its first outbreak in Ibadan, Nigeria in 1952, the

disease is still one of the most important diseases of chickens in Nigeria (Hill *et al.*, 1953; Ezeokoli *et al.*, 1984; Ezema *et al.*, 2009).

The impact of ND is most notable in domestic poultry due to high susceptibility of poultry and the severe consequences of virulent strains on the poultry industries (Alexander, 2003). ND continues to be of serious economic threat to the poultry industry resulting in increased morbidity and mortality rates and loss of eggs for both breeding and human consumption (Abdu *et al.*, 1992). ND vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virus (Alexander, 2003; Senne *et al.* 2004; Kapezynski, 2005) and in response to the threat presented by ND, several countries have put in place vaccination campaigns to prevent the epizootics. Outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied (Burridge *et al.*, 1975) therefore; there is an increasing awareness on the use of various plants in treatment and control of animal diseases (Atawodi and Spiegelhalter, 1994).

Neem (*Azadirachta indica*) a Meliaceae family tree, is a hardy evergreen tree commonly found in South Asia and parts of Africa including Nigeria. All parts of the neem plant have been found useful in the treatment of various ailments (Subapriya and Nagini, 2005). It has been demonstrated to exhibit anti-inflammatory, antipyretic, antiarthritic (Kaur *et al.*, 2004), antihyperglycaemic (Murthy *et al.*, 1978), diuretic (Binde *et al.*, 1958) and immunomodulatory properties (Arivazhagan *et al.*, 2000). Neem oil has been reported to have anti-fertility properties and immune stimulating effects (Upadhyay *et al.*, 1992).

The continuous reoccurrence of Newcastle disease even in vaccinated flocks, is of the greatest challenges facing the poultry industry in Nigeria, hence the need for an alternative remedy to prevent the devastating activity of this disease on the poultry industry. In south-east Nigeria, during the harramattan period, the stem of neem is traditionally used in prevention of Newcastle disease by soaking in the drinking water of birds and it is believed that neem bark has both protective and curative

property against Newcastle Disease hence the need to study the immunomodulatory effects of neem especially as an adjuvant to ND vaccination, with an objective to evaluate the effects of neem stem bark extract on the haematological indices and immune responses of chickens experimentally infected with the velogenic strain (Kudu 113) of NDV.

MATERIALS AND METHODS

Plant material: The barks of *Azadirachta indica* were collected during the months of March 2009, at Nsukka, in Enugu State, Nigeria. The plant was authenticated at the Bio-resources development and conservation programme, Nsukka, Enugu state, Nigeria. Extraction of the dried bark was performed by soaking into 80% methanol for 48 hours with intermittent shaking at room temperature (28° C). The resultant extract was concentrated using a rotatory evaporator. The extract was solubilized in 5% Tween 80 and acute toxicity test of the extract was done.

Acute Toxicity Test: 20 birds were randomly divided into 4 groups (A, B, C and D) of 5 birds each. The birds in each group (A, B, C and D) were given different doses of the extract (150 mg/kg, 300 mg/kg, 600 mg/kg and 1,200 mg/kg), respectively. The birds were observed for 24hrs for signs of acute toxicity which includes depression, weakness, nervous signs, excitability and death.

Experimental Birds: A flock of 100 day old cockerels were procured from CHI hatchery in Nigeria. They were housed in an isolated pen in the Poultry Disease Research unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The birds were given hitchner B1 at day old, gumboro vaccine on day 10 and 21 while La Sota vaccine was given on day 28. The birds were fed with commercial poultry feed ad libitum and provided with drinking water.

Viral Inoculum: The viral inoculum was obtained from National Veterinary Research Institute Vom, Jos, Plateau State, Nigeria. Viral

content of an ampoule was reconstituted with 0.5ml of phosphate buffered saline (PBS). This was added to 9.5ml of PBS (1/10) and later to 10ml of PBS and then double diluted to 20ml ($ELD_{50} = 10^{5.32}$).

Experimental Challenge: The birds were randomly divided into 4 equal groups (groups I, II and III) of twenty chicks each on 28 day of age. By day 42 of age, birds in all the groups were inoculated intramuscularly with 0.2ml challenge dose of VNDV strain (Kudu 113) with titre $10^{9.5}$ per milliliter of the inoculum. Following challenge with VNDV strain, birds in groups I, II and III were given oral treatment with 200, 400 and 600mg/kg body weight of the plant extract, respectively daily for two weeks, while group IV was not treated. On days 28, 42, 49, 56 and 63 of age, blood samples were collected from each group for serology and haematology. Sera from the blood samples were stored at -20°C until used.

Clinical Signs: The birds were clinically monitored twice daily for clinical signs of ND from day 42 till the end of the experiment. Ten birds in each group was randomly selected and live body weight taken on days 42, 49, 56, 63 and 70 of age.

Haemagglutination (HA) and Haemagglutination Inhibition (HI) Tests: Two milliliter of blood was collected from each of birds in a test tube containing EDTA as anticoagulant. The blood was washed in phosphate buffered saline (PBS) and centrifuged at 3000 rpm for 5 minutes. This was repeated until a clear supernatant was obtained. The packed red blood cells (RBC) were re-suspended in a measured volume of PBS solution to make 0.5% RBC suspension (Beard, 1989).

The antigen titre for running HI tests was determined by standard HA technique using La Sota as antigen (Alexander, 2003). The reciprocal of the highest dilution of La Sota ND antigen causing 100% agglutination of an equal volume of standardized RBCs was taken as the HA titre of the antigen. The HI titres were determined by the method of Beard (1989).

Haematology: Blood samples were collected were used in determining the haematological profile of the birds which includes; PCV was determined using the microhaematocrit method (Coles, 1986), HbC determined by the cyanomethamoglobin method (Kachamar, 1970), RBC count determined by the haemocytometer method (Schalm *et al.*, 1975) and total and differential WBC count determined using the improved Neubaur counting chamber (Campbell and Coles, 1986).

RESULTS

Toxicity of Methanolic Stem Bark Extract of *Azadihiracta indica*: The result of the acute toxicity tests showed that the extract is safe even at dose of 1200mg/kg. At all the dose levels tested, there was no death recorded within 24 hours post treatment.

Clinical Signs Induced by Methanolic Stem Bark Extract of *Azadihiracta indica*: They birds in all the groups did not show any typical sign of ND however, slight depression was observed in 2 birds on days 45 till 48 of age in group IV.

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Body Weight of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: There was a decrease in the mean body weight of both the treated and untreated vaccinated groups on days 45 and 56 of age, however the mean body weight of the vaccinated-treated groups were significantly ($p < 0.05$) higher than that of the vaccinated-untreated group on day 56 of age. On days 63 and 70 of age, the mean body weight of group III was significantly higher than groups I and II (Table I).

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Immune Response of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: The mean haemagglutination inhibition titres (\log^2) of all the groups increased on days 49 – 63 of age of the birds in all the experimental groups (Table 2), however on days 56 and 63 of age, the mean haemagglutination inhibition titres (\log^2) of group III was significantly higher ($p < 0.05$) than group I, II and IV.

Effect of Methanolic Stem Bark Extract of *Azadirachta indica* on PCV, Haemoglobin Concentration and RBC count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain:

There was a decrease in the mean packed cell volume, haemoglobin concentration and red blood cell count in all the groups on day 49 of age, however the mean PCV of the vaccinated-treated group was significantly ($p>0.05$) higher than the vaccinated-untreated group on day 49 of age, whereas there was no significant difference ($p<0.05$) among the vaccinated-treated group till end of the experiment (Table 3). The mean haemoglobin concentration of the vaccinated-treated group was significantly ($p>0.05$) higher than the vaccinated-untreated group, however there was no significant difference on the mean haemoglobin concentration among the vaccinated-treated groups on day 49 of age. On day 63 of age the mean haemoglobin concentration of group III was higher than group I and II as well as group IV (Table 4). The mean red blood cell count of the vaccinated-untreated group were significantly ($p>0.05$) higher than the vaccinated-untreated group on day 49 of age, whereas on days 56 and 63 of age, the mean red blood cell count of group III was significantly ($p>0.05$) higher than groups I, II and IV.

Effect of Methanolic Stem Bark Extract of *Azadirachta indica* on Total leucocyte Count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain:

There was an increase in the mean White blood cell count for both vaccinated-treated and vaccinated-untreated groups on days 49 and 56 of age, however the mean WBC count of group I and II were significantly higher ($p>0.05$) than groups III and IV on days 49 and 56 of age (Table 6).

Effect of Methanolic Stem Bark Extract of *Azadirachta indica* on Differential leucocyte count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain:

The mean absolute heterophil counts increased in all the groups on days 49 and 56 of age, however the mean absolute heterophil counts of vaccinated-treated

groups was significantly ($p>0.05$) higher than the vaccinated-untreated group on days 56 and 63 of age. The mean absolute lymphocyte counts of the vaccinated-treated increased significantly ($p>0.05$) than that of vaccinated-untreated birds days 49 of age till the end of the experiment, however the mean absolute lymphocyte count of group I of the treatment group were significantly ($p>0.05$) higher than that of group III on day 49 of age, group II and III on day 56 and II on day 63 of age (Table 8).

DISCUSSION

The significant decrease in weight observed in both the vaccinated-treated and vaccinated-untreated groups on days 49 and 56 of age are similar to reduction in weight reported by Okoye *et al.* (2000) in birds challenged with velogenic ND virus, the decrease in weight is a common occurrence in septicaemic or viraemic diseases due to reduction in feed and water intake. Decrease in weight was notably more severe in the vaccinated-untreated group than the treated vaccinated group and the group III of the vaccinated-treated group with the highest dose of the extract had the least reduction in weight, this may be due to the inhibitory property of neem on viruses observed by Waafa *et al.* (2010) in an in vitro study with neem leaf and fruit extract on ND virus, the inhibitory property of neem on the virus may have led to slight reduction in feed intake, ability of the birds to overcome the infection, and a return of feed/water intake.

The slight reduction in weight observed in the vaccinated-treated group than in the vaccinated-untreated group was also reported by Eze *et al.* (2012) in birds challenged with velogenic NDV and treated with methanolic leaf extract of *Moringa oleifera* than those challenged with the VNDV but not treated with the extract.

Following vaccination with La Sota vaccine and challenge with the VNDV strain of Kudu 113, high NDV antibody titre were observed in all the groups, this is similar to what was reported that following challenge with VNDV, that NDV HI titers are usually high (Illango and Oluho-munkini, 2005; Kakenji *et al.*, 2007). The presence of high NDV antibody titres is necessary to provide long term protection against ND (Ritchie *et al.*, 1994; Sa'idu *et al.*, 2006; Ruwaan *et al.*, 2009).

Table 1: Effect of different doses of *Azadihiracta indica* extract on body weight of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	393.80 ± 17.58	393.00 ± 11.59	369.00 ± 10.35	385.8 ± 15.65
45	347.70 ± 12.60	348.10 ± 14.69	332.00 ± 6.60	330.80 ± 11.81
49	343.50 ± 16.85	350.70 ± 16.27	340.50 ± 17.07	327.00 ± 24.38
56	480.50 ± 27.87 ^b	481.00 ± 19.13 ^b	497.00 ± 24.14 ^a	437.50 ± 29.87 ^c
63	544.00 ± 28.74 ^c	597.50 ± 19.51 ^b	620.00 ± 20.00 ^a	598.78 ± 24.18 ^b
70	607.00 ± 28.74 ^c	638.50 ± 18.64 ^b	658.00 ± 18.56 ^a	639.44 ± 17.57 ^b

*Different superscripts in a row indicate significant difference between the groups ($p < 0.05$).

Table 2: Effect of different doses of *Azadihiracta indica* extract on immune response of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Groups Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	8.00 ± 0.32	8.20 ± 0.49	8.60 ± 0.93	8.00 ± 0.32
49	8.80 ± 0.20	8.70 ± 0.32	8.90 ± 0.45	8.40 ± 0.60
56	10.00 ± 0.49 ^b	10.80 ± 0.37 ^b	11.87 ± 0.49 ^a	8.80 ± 0.32 ^c
63	11.77 ± 0.24 ^b	11.87 ± 0.37 ^b	12.60 ± 0.68 ^a	9.00 ± 0.34 ^c

The data are given as mean haemagglutination inhibition titre (\log_2) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$).

Table 3: Effect of different doses of *Azadihiracta indica* extract on PCV of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	27.88 ± 0.23	28.25 ± 0.44	28.50 ± 0.54	27.68 ± 0.54
49	27.00 ± 0.58 ^a	27.67 ± 0.88 ^a	27.33 ± 0.88 ^a	26.67 ± 0.38 ^b
56	27.67 ± 2.33	27.33 ± 0.33	27.00 ± 1.53	26.67 ± 1.45
63	27.33 ± 1.81	28.33 ± 0.33	28.33 ± 1.20	27.00 ± 1.53

The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$).

Table 4: Effect of different doses of *Azadihiracta indica* on haemoglobin concentration of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group Days	II 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	8.79 ± 0.02 ^a	8.70 ± 0.23 ^a	8.82 ± 0.15 ^a	8.92 ± 0.19 ^a
49	8.47 ± 0.23 ^a	8.51 ± 1.26 ^a	8.53 ± 1.11 ^a	8.27 ± 1.32 ^b
56	8.83 ± 0.33	8.86 ± 0.47	8.87 ± 0.55	8.97 ± 0.61
63	8.87 ^{bc} ± 1.77	8.97 ± 0.67 ^{bc}	9.23 ± 0.42 ^a	8.93 ± 0.26 ^c

The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$).

Table 5: Effect of different doses of *Azadihiracta indica* on RBC count of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	2.54 ± 0.03	2.54 ± 0.03	2.50 ± 0.02	2.52 ± 0.03
49	2.38 ± 0.27	2.48 ± 0.26	2.48 ± 0.45	2.23 ± 0.62
56	2.41 ± 0.78 ^b	2.54 ± 0.07 ^a	2.62 ± 0.25 ^a	2.40 ± 0.40 ^b
63	2.55 ± 0.78 ^b	2.55 ± 0.48 ^b	2.62 ± 0.19 ^a	2.50 ± 0.14 ^b

The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$)

Table 6: The Effect of different doses of *Azadihiracta indica* extract on total leucocyte count ($10^3/\mu\text{l}$ of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	15.23 ± 0.44	15.23 ± 0.43	15.25 ± 0.24	15.22 ± 0.22
49	17.93 ± 2.00 ^a	17.47 ± 1.74 ^b	16.67 ± 1.57 ^b	16.53 ± 1.66 ^b
56	18.47 ± 2.06 ^a	18.07 ± 2.03 ^a	17.53 ± 1.35 ^b	17.40 ± 2.21 ^b
63	16.20 ± 1.44	16.53 ± 1.95	15.07 ± 1.79	15.30 ± 0.64

The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$)

Table 7: Effect of different doses of *Azadihiracta indica* extract on heterophil count ($10^3/\mu\text{l}$ of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	2.87 ± 0.44	2.75 ± 0.13	2.72 ± 0.26	2.78 ± 0.22
49	4.68 ± 0.65	4.94 ± 0.10	4.51 ± 0.73	4.62 ± 0.90
56	5.01 ± 1.17 ^a	4.90 ± 12.74 ^{ab}	4.63 ± 0.18 ^b	4.07 ± 1.39 ^c
63	4.34 ± 0.60 ^a	4.26 ± 0.30 ^a	4.02 ± 1.53 ^b	3.57 ± 0.56 ^c

The data are given as mean haemagglutination inhibition titre (\log_2) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$)

Table 8: Effect of different doses of *Azadihiracta indica* extract on absolute lymphocyte counts ($10^3/\mu\text{l}$ of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	9.41 ± 0.23	9.44 ± 0.11	9.40 ± 0.34	9.41 ± 0.23
49	10.79 ± 1.23 ^a	10.53 ± 0.50 ^a	10.12 ± 0.39 ^b	9.23 ± 0.44 ^c
56	10.27 ± 0.99 ^a	10.05 ± 0.48 ^b	10.03 ± 0.83 ^b	9.16 ± 0.41 ^c
63	9.77 ± 1.67 ^a	9.57 ± 0.86 ^b	9.73 ± 0.41 ^{ab}	8.98 ± 1.65 ^c

The data are given as mean haemagglutination inhibition titre (\log_2) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p < 0.05$)

It is also important to note that NDV HI antibody titre was significantly higher in the vaccinated-treated group than the vaccinated-untreated group, this shows that the extract has immune stimulating effects (Upadhyay *et al.*, 1993).

The gradual increase in the NDV antibody titres observed in the vaccinated-treated groups is an indication that the birds had more protection against ND, this increase has also been reported by Ahsan *et al.* (1991), Sadekar *et al.* (1998) and Kwakwukwe *et al.* (2013) following

challenge with VNDV and treatment with *Azadirachta indica* extract.

The slight decreased in the mean PCV, Hb and RBC observed in both the vaccinated-treated groups and vaccinated/ untreated group has also been noted by other researchers who reported that decrease in blood parameters following challenge with VNDV occurs as a result of the destruction of the RBC by the ND virus (Caldron *et al.*, 2005; Ruwaan *et al.*, 2009). The decrease was more severe in the vaccinated-untreated group than in the vaccinated-treated group this shows that the vaccination to a certain degree could not prevent the destructive capabilities of the NDV virus on the RBC, that maybe why ND outbreaks have been reported in chicken flock despite vaccination (Alexander, 2003; Senne *et al.*, 2004; Ezema *et al.*, 2009), however in the treated groups *Azadirachta indica* extract at the different doses enhanced the immune system enabling the birds to overcome the destructive effects of the ND virus.

There was an observable increase in WBC count in both the untreated and treated vaccinated groups, however the mean WBC was significantly higher ($p < 0.05$) in group I and II of the vaccinated-treated group than group IV of the vaccinated-untreated on days 49 and 56 of age. The leukocytosis may be attributed to increased production of leucocyte in the haemopoietic tissues (Yongola *et al.*, 2006; Ravindraa *et al.*, 2009). Low leucocyte count in stressed chickens is a primary consequence of suppression of the immune system and increased susceptibility to disease (Wambura, 2009). This increase in WBC is a finding consistent with that of Kwawukume *et al.* (2013) in chickens challenged with NDV and treated with neem leaf extract, this is attributable to the neems ability to boost the macrophage response in the body which stimulates the lymphocytic system and boost the production of WBC (Sadekar *et al.*, 1998). Ruwaan *et al.* (2012) also observed increased WBC on VNDV challenged birds treated with the root extract of *Anorthosis nobilis*. The higher WBC count noted in low dose treatment groups (Group I, II) as against the higher dose treatment group (III) is a finding consistent with

the report of Kwawukume *et al.* (2013), who also observed a higher WBC count in birds fed with 5% neem leaf extract than those fed with 10% neem leaf extract this he attributed to probable toxicity of neem in higher concentration (Kwawukume *et al.*, 2013).

The mean absolute heterophil counts of vaccinated-treated group was significantly higher than the vaccinated-untreated group, that might have resulted from the fact that heterophils exhibit high level of apoptosis when infected by NDV (Ravindraa *et al.*, 2009). The level of the heterophils usually indicates the severity of the initial immune response; therefore their high values in the vaccinated-treated groups showed that *Azadirachta indica* possibly protected them from apoptosis.

The mean absolute lymphocyte counts increased in the vaccinated-treated group on day 49 of age and decrease subsequently till the end of the experiment this is in agreement with the report that the increase in lymphocytes might be physiologic, reactive, proliferative in disease conditions (Wambura, 2009). Birds that normally have high circulating lymphocytes in the initial response to infective pathogens might develop leucopenia due to lymphopenia. While the low level of the mean lymphocyte counts in group IV is in agreement with the report that NDV has the ability to cause agglutination and lyses of lymphocytes of affected birds thereby reducing the no of circulating lymphocyte (Bennet *et al.*, 2003; Khesorn, 2009).

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HAEMONCHOSIS AND HAEMOPARASITES OF SMALL RUMINANTS REARED IN NORTH WESTERN, NIGERIA

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ABSTRACT

Small ruminants (goats and sheep) production systems worldwide are significantly constrained by haemo and gastrointestinal parasites. The study was carried out in North-western Nigeria from November 2011 to October 2012 with the aim to identify the common haemoparasites and haemonchosis in small ruminants. Three hundred abomasum samples with corresponding blood samples were collected from 200 goats and 100 sheep, respectively at necropsy. The abomasa were examined by Hansen and Perry method for the presence of Haemonchus contortus while blood samples were examined using the thin blood smear and Haematocrit Centrifugation Techniques (HCT). The prevalence of Haemonchus contortus in small ruminants was 80.3% with goats and sheep having prevalence of 78% and 85%, respectively. The prevalence for H. contortus in small ruminants during late dry, early dry, late rain and early rain were 64.9%, 84.1%, 89.9% and 81.1%, respectively. The prevalence were statistically significant among the seasons ($p < 0.05$) with highest prevalence in the late rainy season. The total number of adult worms collected was 21862. The highest adult worm burdens were obtained during late rainy season (August to October) when a mean worm burden of 180.2 ± 51.45 and PCV of 26.63 ± 0.63 were recorded. The mean worm burdens for early dry, late dry and early rain were 42.60 ± 6.93 , 31.67 ± 5.56 and 61.10 ± 11.33 , respectively. The PCV values for the season were 27.73 ± 0.79 , 26.60 ± 0.87 and 28.40 ± 0.65 , respectively. The values of PCV for the four sub-seasons were not significant from one another but the value of PCV obtained during early rain was different from those of other sub-seasons. The PCV had a weak negative correlation with worm burdens with Pearson correlation coefficient of -0.2632 which was highly significant ($p < 0.001$). Out of 200 goats and 100 sheep examined for haemoparasites, only one goat had heavy infection with Trypanosoma vivax and a sheep had mixed infection with Theileria ovis and Anaplasma ovis. The prevalence of three protozoans encountered was 0.33% each in small ruminants. The PCV of infected goat and sheep with protozoan parasites was 25% and 20%, respectively. The results suggest that Haemonchus contortus may be the major cause of anaemia in the study area. It is therefore recommended that further research be embarked upon to determine the effect of nutrition in ameliorating the effects of helminth infections and anaemia in small ruminant.

Keywords: Haemonchosis, Haemoparasites, Prevalence, Small ruminants, Sub-seasons

INTRODUCTION

Small ruminants play an important socio-economic role within traditional animal husbandry systems in many developing countries, including Nigeria, where over 80% of the nation's livestock lies in the hand of small holders or other traditional groups (Dalhatu and Ala, 2010). However, in recent times, benefits derived from these animals were notably below expectation owing to low productivity (Jatau *et al.*, 2011). One of the most important factors responsible for the decline in productivity is disease. Gastrointestinal and blood parasitic infections seemed to be the most prominent in this regard.

Among different types of parasitic infections, gastrointestinal nematode infections (GNIs) are the most important as far as their adverse effects and prevalence are concerned (Molento, 2009; Tadesse *et al.*, 2009). They cause weight loss, reduced feed intake, impaired fertility, lowered immunity, damaged gastric function and high mortality rate, leading to enormous economic losses (Carvalho *et al.*, 2012). One exception to this is the highly pathogenic nematode parasite of small ruminants, *Haemonchus contortus*, which is considered to be the main culprit causing anaemia and hypoproteinaemia in ruminants. It has been estimated that each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Urquhart *et al.*, 2000). Surveys in countries around the world have shown that amongst domestic animals, sheep and goats suffer more frequently from haemonchosis (Nwosu *et al.*, 2007; Tariq *et al.*, 2008). *Haemonchus contortus* is capable of causing acute disease and high mortality in all classes of livestock. Death rate due to acute haemonchosis is very high and may go up 50% in small ruminants (Itty *et al.*, 1997).

Small ruminants in Sub-Saharan Africa may be infected with a wide variety of haemoparasites such as the Rickettsiae: *Anaplasma* and *Ehrlichia* (*Cowdria*), and the protozoan parasites: *Theileria*, *Babesia* and *Trypanosoma* (Bell-Sakyi *et al.*, 2004; Okaiyeto *et al.*, 2008). Some haemoparasite species are only evident when the host is undergoing a

clinical response to infection, while other members of the same genera may be seen in blood smears from apparently healthy animals. Infection with many of these haemoparasites species results in a state of pre-immunity, in which the host becomes a long term asymptomatic carrier serving as a source of infection for the tick or insect vector (Young *et al.*, 1988). The tropical environment is for various reasons eminently suitable for the development of these parasitic diseases (Payne, 1990).

This study was therefore conducted to identify the common haemoparasites and haemonchosis of small ruminants in relation to the anaemia in North-western Nigeria, with the aim to advising the livestock farmers on the control strategies of these parasites in order to minimise small ruminants' production losses. The abattoir was selected because it will represent a wide range of husbandry and environmental practices.

MATERIALS AND METHODS

Study Area: The study was carried out in Dogarawa (Trading) slaughter slab in Zaria, Savannah zone of North-western Nigeria from November 2011 to October 2012. The zone is characterised by a tropical climate with two main seasons; a rainy season (May to October) and a dry season (November to April). The minimum temperature recorded was 13.8^oC in December and maximum of 37.1^oC in April. The relative humidity was highest (83.8%) in the month of August and lowest (18.0%) in the month of March and with total annual rainfall of about 1417.3 mm. The sheep breed available at the Dogarawa slaughter slab located in the study area was mostly the Yankasa, while the goats were mostly of the red Sokoto breed. The small ruminants were bought by butchers from Zaria town, the adjoining peri-urban areas, town markets and surrounding villages. The small ruminant management system in these areas vary from free range grazing with little or no supplementation to tethering during the cropping season (April – November); while roaming freely during the dry season. Although this system of management is cheap and less

labour intensive, it is characterized by low productivity and high losses due to accidents, diseases and theft (Baah *et al.*, 2012). For the purpose of conducting the study and the subsequent analysis, the calendar months in the year were divided into four sub-seasons. These were early dry (November, December and January), late dry (February, March and April), early rain (May, June and July) and late rain (August, September and October) sub-seasons.

Sample Collection: Blood and abomasum samples were collected from 200 goats and 100 sheep slaughtered in Dogarawa slaughter slab between November 2011 to October 2012. Immediately following slaughter, 5ml of blood samples were collected from the severed jugular vein into bijou bottle containing EDTA as anticoagulant. Following evisceration, abomasum was legated with string and separated from omasum and duodenum to avoid leakage and mixing of contents. Each sample was collected into a clean labelled polythene bag within 30 minutes of evisceration and transported immediately on ice to the Department of Veterinary Parasitology and Entomology Laboratory for examination. The blood samples and corresponding abomasum samples collected from the same animal were labelled with the same number. Twenty to thirty samples for both goats and sheep were collected each month for the period of sample collection.

Haemoparasites: A thin blood smear was prepared from each blood sample using the method of Hansen and Perry (1994) and Cheesbrough (1999). A drop of blood on one end of a clean glass slide, then use a spreader to spread the blood by allowing the spreader to touch the blood, then spread gently but firmly along the surface of the horizontal slide so that the blood is dragged behind the spreader to form the film with a feathered edge, air-dry and fixed in methanol for 5 minutes. Stained in 1:10 Giemsa and Buffer dilution and stain for 25-30 minutes and rinse with distilled water then allow to dry. The smears were examined at x100 magnification (oil immersion) for presence of haemoparasites and identification.

Packed Cell Volume: The remaining blood samples were used to determine the packed cell volume (PCV). After gently mixing the blood, a 75 × 1.5 mm capillary tube was filled with blood up to $\frac{3}{4}$ of its length by capillary action and one end sealed. Then, all of the blood-filled tubes were centrifuged for 4 minutes at 16000 rpm using a microhaematocrit centrifuge. Finally, each tube was placed in a micro-haematocrit reader, to determine the percentage of packed red cell volume (PCV) for each animal (Hansen and Perry, 1994; Urquhart *et al.* 2000).

Haemonchus contortus: Each abomasum was opened on the tray with the help of a scissor. The contents were then washed several times using tap water, paying particular attention to cleaning between the folds of the mucous membranes. The parasites were recovered by passing the content through a sieve of 100 µm diameter mesh and were later back-washed into another container. The samples were examined for adult *H. contortus*. The parasites were picked with wire loop with the aid of an illuminator (Picker x-ray in Veterinary Helminthology Laboratory ABU-Zaria) (Hansen and Perry, 1994; Taylor *et al.*, 2007). The worms were preserved in 10% formalin and were then poured into Petri dishes and examined under a stereomicroscope. Identification was made using keys developed by various researchers (Hansen and Perry, 1994). Some parasites that were not cleared were cleaned with lactophenol for detailed morphological examination.

Data Analysis: The percentage prevalence of parasite species was calculated as number of individuals of a host species infected with a particular parasite species divided by the number of host examined times 100. Data obtained for adult *H. contortus* counts were expressed in tables as mean ± SEM. Data were further subjected to t-test and analysis of variance (ANOVA) followed by Turkey's post hoc test where necessary. Chi-square and odds ratio were also used to test for association between the species of the host and seasons of the year. Value of $p < 0.05$ was considered significant.

Pearson correlation was also used to test for relationship between PCV and adult *H. contortus* counts. GraphPad prism version 4.0 Windows from Graphpad Software, San Diego, California USA was used to analyze the data.

RESULTS

The results of necropsy examination in small ruminants are shown in Table 1. From the abomasa of 300 small ruminants examined for adult *H. contortus*, the prevalence was 80.3%. The prevalence in goats and sheep were 78% and 85%, respectively. The difference in the two species of small ruminants was not statistically significant ($p > 0.05$) but considering species as a risk factor, sheep were one- and-half times more at risk of infection with adult *H. contortus* than goats. The prevalence for *H. contortus* during late dry, early dry, late rain and early rain were 64.9%, 84.1%, 89.9% and 81.1%, respectively. The prevalence was statistically significant among the sub-seasons ($p < 0.05$) with highest prevalence during the late rainy sub-season.

The total number of adult *H. contortus* collected was 21862. The highest adult worm burdens were obtained during late rainy season (August to October) when a mean worm burden of 180.2 ± 51.45 was recorded. The least was obtained during late dry season (February to April) when a mean worm burden of 31.67 ± 5.56 was recorded (Table 2). The mean worm burden during the late rainy sub-season was significantly higher ($p < 0.05$) than the means for the other three sub-seasons. The mean burdens for the early dry and early rain were not significantly different from one another but were both significantly higher ($p < 0.05$) than the mean for the late dry sub-season. The PCV values for early dry, late dry, early rain and late rain were 27.73 ± 0.79 , 26.60 ± 0.87 , 28.40 ± 0.65 and 26.63 ± 0.63 , respectively. The values of PCV for four sub-seasons were not significantly different from one another but the value of PCV obtained during early rain was different from other sub-seasons. The PCV had a weak negative correlation with worm burdens at Pearson correlation coefficient of -0.2632 which was highly significant ($p < 0.001$).

The prevalence of protozoan infections among the small ruminants indicated that out of the 200 goats examined, only one goat had heavy infection with *Trypanosoma vivax* with prevalence of 0.5% in the month of March (Table 3). On the other hand, 100 sheep examined, only one had mixed infection with *Theileria ovis* and *Anaplasma ovis* in the month of September. The prevalence of protozoan infections in small ruminants was 0.6%. Three protozoans encountered were *Trypanosoma vivax*, *Theileria ovis* and *Anaplasma ovis* with 0.3% prevalence each (Table 3). Goats and sheep that were infected with protozoa parasites had 25% and 20% PCV respectively.

DISCUSSION

The result of the abomasum examination suggested that haemonchosis is present in the study area. The prevalence of 80.3% recorded in the study area is similar to the range of 77 – 100% reported from other geographical zones of Nigeria (Chiejina, 1986; Nwosu *et al.*, 1996a,b; Ajanusi and Chiezey, 2005). Though, the findings are higher than the results of other surveys in sheep and goat carried out in North-eastern Nigeria (Nwosu *et al.*, 2007). The occurrence of haemonchosis in an area is influenced by a multifactorial system, which comprises hosts, parasite and environmental effects (Muhammad *et al.*, 2009).

In this study, species and seasons play an important role in prevalence and worm burdens of haemonchosis in small ruminants. Higher prevalence was observed in sheep than in goats and this agreed with other works in Nigeria (Nwosu *et al.*, 2007; Jatau *et al.*, 2011) and elsewhere in the world (Waruiru *et al.*, 2005; Asif *et al.*, 2008). High prevalence in sheep is assumed to be due to the grazing habit where they graze closer to the ground fostering opportunity of exposure to parasites. However, it is in contrary to reports of Keyyu *et al.* (2006) and Raza *et al.* (2007) where they had higher prevalence in goats than in sheep. In this regard, it is assumed that sheep do have a considerably higher immunological response to gastrointestinal parasites when compared to goats (Urquhart *et al.*, 2000).

Table 1: Prevalence of *H. contortus* in small ruminants by species of the host and season of the year

Category	Number Examined	Number positive (%)	X ² (P- value)	Odd ratio
Species				
Goat	200	156 (78)	2.068(0.1505)	1
Sheep	100	85 (85)		1.6
Total	300	241 (80.3)		
Season				
Late dry	74	48(64.9)	16.95(0.0007)	1
Early dry	63	53(84.1)		2.9
Late rain	89	80(89.9)		4.8
Early rain	74	60(81.1)		2.3
Total	300			

For species ($P>0.05$) while season ($P<0.05$)

Table 2: Seasonal *H. contortus* counts and corresponding packed cell volume examined in small ruminants in North-western Nigeria

Season	Month	Worm count	Season	PCV	Season
Early Dry	November	72.70±14.80	42.60±6.93 ^b	34.10±0.84	27.73±0.79
	December	51.38±12.23		23.70±1.10	
	January	15.68±3.95		29.49±1.20	
Late Dry	February	23.53±6.95	31.67±5.56 ^a	26.13±1.23	26.60±0.87
	March	27.50±12.26		24.0±1.46	
	April	41.16±9.24		28.89±1.54	
Early Rain	May	100.5±29.78	61.10±11.33 ^b	27.80±1.43	28.40±0.65
	June	23.33±6.65		31.17±0.90	
	July	56.18±10.02		26.68±0.73	
Late Rain	August	74.81±8.95	180.2±51.45 ^b	26.54±0.78	26.63±0.63
	September	273.2±148.7		26.63±1.43	
	October	188.7±30.01		26.70±0.98	

Means within the same column with different superscripts are statistically significant

Table 3: Prevalence of Protozoan infections in small ruminants in North-western Nigeria

Small ruminants	Number Examined	Prevalence			PCV
		<i>Trypanosoma vivax</i>	<i>Theileria ovis</i>	<i>Anaplasma ovis</i>	
Goats	200	1 (0.5)	0 (0.0)	0 (0.0)	25
Sheep	100	0 (0.0)	1 (1.0)	1 (1.0)	20
Total	300	1 (0.3)	1 (0.3)	1 (0.3)	

Number in parenthesis = percentage, PCV = packed cell volume

Considering the seasons of the year as a predisposing factor, high prevalence and worm burdens were observed during the late rainy season. This result agreed with the findings of Hansen and Perry (1994) and Almalaik *et al.* (2008). The high prevalence and worm burdens obtained in this study could be as a result of the management system operated by most small

ruminant owners especially during the rainy season when animals are confined to avoid damage to crops. Consequently, such animals are overstocked with the pens not properly cleaned. These factors with the high humidity during the rainy season predispose them to the parasitic infections. It has been reported that the tropical climatic conditions, especially rainfall

and temperature, favour the development and survival of parasitic nematode eggs to infective stages (Josiah *et al.*, 2015). This might explain the high prevalence rate observed in this study.

The mean PCV recorded from the four sub-seasons of the year during the study period in small ruminants were lower, this indicated the onset of anaemia arising from the infections. The mean PCV of infected animals during the late rainy sub-season when the worm burdens were high and availability of good quality pasture was the same with mean PCV of infected animals during the dry season when the worm burdens were low and absent or low quality of pasture. The lower mean value of PCV during the late rainy sub-season is likely due to the blood-sucking effects of the adult worms in which each adult sucks up to 0.05ml of blood per day (Urquhart *et al.* 2000). In addition, the worms secrete anticoagulant so that the site of attachment continues to bleed even after the worm has become replete and detached (Ajanusi and Chiezey, 2005). Infection with *Haemonchus* spp may cause severe anaemia and hypoproteinemia, leading to depression, loss of condition, reduced productivity and eventually death (Al-Shaibani *et al.*, 2009).

The lower mean value of PCV during late dry sub-season might due to poor nutrition. Poor nutrition lowers the resistance of the animal, thus enhancing the establishment of worm burdens and increasing the pathogenicity of the parasites. It is well known that adequately fed animals are more able to tolerate parasitism than animals on a low plane of nutrition (Waruiru *et al.*, 2004; Knox *et al.*, 2006). Thus, small ruminants affected by blood-sucking parasites, such as *H. contortus*, may be able to maintain their haemoglobin levels as long as their iron and protein intakes are adequate. However, if the animals iron reserves and protein intake is reduced then their haemopoietic systems become exhausted, and they may die (Vatta *et al.*, 2002). The study therefore demonstrated a significantly ($p < 0.0001$) negative correlation between worm burdens and PCV. This means that as worm burdens increased, the PCV decreased and vice versa. This result agreed with the result of

Ajanusi and Chiezey (2005), Menkir *et al.* (2007) and Okaiyeto *et al.* (2008).

The results of this study also suggested that protozoan infection may not be a threat to small ruminants in the study area, as the prevalence of three protozoans encountered was 0.33% each for *Trypanosoma vivax*, *Theileria ovis* and *Anaplasma ovis*. This is lower than those of Ajanusi and Chiezey (2005) which reported prevalence of 9.2% and 3% for *Anaplasma ovis* and *Theileria ovis*, respectively and that of Okaiyeto *et al.* (2008). Though the PCV of two infected small ruminants with protozoans were lower than the mean PCV recorded in small ruminants with haemonchosis. The observed anaemia characterised by low mean PCV values of two infected animals suggested that the haemo parasitic infection may be the cause of the anaemia. Similar observation was earlier made by Okaiyeto *et al.* (2008).

In conclusion, the findings from this study indicated the infection of small ruminants with haemonchosis and environmental factors such as rain and relative humidity with optimal temperature play major role in the infection. Small ruminant farmers may not have noticed the effects of the haemonchosis on their animals because of the sub-clinical or chronic nature of the infection, however, their effects is usually manifested in productivity. It is therefore recommended that further research be embarked upon to determine the effect of nutrition in ameliorating the effects of helminth infections and anaemia in small ruminants. It is also important to note that since haemoparasitic diseases constitute a major handicap to livestock farmer where they are present, farmers should be constantly advised in the study area on routine control of ectoparasites that may transmit them.

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OPTIMUM DIETARY PROTEIN REQUIREMENT OF GENETICALLY MALE TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURED IN FLOATING HAPA SYSTEM

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ABSTRACT

The study was conducted to investigate the optimum dietary protein level needed for growing genetically male tilapia, Oreochromis niloticus. Diets containing crude protein levels 40, 42.5, 45, 47.5 and 50% were formulated and tried in triplicates. Test diets were fed to 20 fish/1m³ floating hapa at 5% of fish body weight daily for 24 weeks. Survival of fish was not affected by dietary protein levels. Growth rate varied inversely with dietary protein levels to a maximum at 40%. Protein utilization and turnover decreased with increasing protein levels in diets. Quadratic regression analysis of growth indices against protein inclusion levels indicated that the optimum dietary protein required for maximum growth was 40%. This result also paralleled the least values of protein and calorie deposited for 40% protein level whereas the other levels were significantly ($p < 0.05$) higher. The study concludes that 47.5% and 50% protein levels may depress tilapia growth and feed utilization, while 40% protein gave optimum growth.

Keywords: Genetically male tilapia, Dietary protein levels, Growth, Protein requirement

INTRODUCTION

Aquaculture is an integral component of the overall agricultural production system in Nigeria. The country with hundreds of rivers and ponds is notable for being a fish-loving nation where fish plays an important role in the diets, constituting the main and often irreplaceable animal protein source in both urban and rural households (Otubusin, 2011). The major fish species cultured in Nigeria include catfishes, tilapia and carp. Tilapia is one of the most widely cultured fish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2013), and this contribution has been exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia. Among these are: genetic improvement, ease of culture, highly adaptable to a wide range of environmental conditions (Ponzoni *et al.*, 2008). However, a major problem in tilapia culture is that females grow slower than males. Early

sexual maturation diverts energy from growth to reproduction and unwanted breeding results in overcrowding and competition. The most effective solution to this problem is to produce and grow only male fish. Researches have addressed this problem in an innovative way through the application of basic genetics, to develop a unique product in genetically male tilapia (GMT) (Mair *et al.*, 1997). The GMT so developed has proved to be excellent production fish in both extensive and intensive systems using ponds, raceways, cages and tanks (Ek Nath *et al.*, 2007). They are now in use in more than 20 countries around the world (Gupta and Acosta, 2004; Gupta *et al.*, 2004) including Nigeria. In formulating diet for fish it is important to meet all nutritional requirements since lack of quality feed for economic production adversely affects growth rate, disease manifestation and total harvest (Alatise *et al.*, 2006). Dietary protein is used by fish for growth, energy and body maintenance. Therefore, understanding protein requirement

for maximum growth of any species of fish is a step forward in developing cost-effective feed for fish farming and this has to do with determining the optimum amount required to produce maximum growth rate. Protein requirements of catfish had been widely reported (Jamabo and Alfred-Ockiya, 2008; Diyaware *et al.*, 2009). However, little information on nutritional research with respect to genetically male tilapia had been established. Therefore, the present study had been designed to determine protein requirement of rearing the YY-male tilapia for maximum production.

MATERIALS AND METHODS

Collection of Fish Sample: The GMT fingerlings were procured from Durante Fisheries Industries, Ibadan, Oyo State, Nigeria. Fish were transported in oxygen bags to Department of Zoology, University of Uyo, Uyo, Akwa Ibom State, Nigeria where the experiment was conducted.

Experimental Design: A complete randomized block design (CRBD) consisting of five treatments (blocks) replicated thrice was used for the study. Five outdoor concrete tanks (8 x 5 x 1.65m³) at Vika Farms Limited, Mbak Etoi, Uyo, Akwa Ibom State, latitude 5° 3' North and longitude 7° 56' East was used. The experimental design had fifteen 1 x 1 x 1m³ hapa placed on the concrete tanks at the rate of three hapa per tank. Each hapa was rigged and suspended to maintain a depth of 0.75m in water and a free board of 0.25m. The float lines were tied to the four corners of each compartment using kuralon rope (Number 15) as described by Otubusin (2000).

Diets Preparation: Diet compositions for the feeding trial are presented in Table 1. All ingredients were carefully weighed out, mixed, made into pellets using 2 mm meat mincer, air-dried and labelled separately according to diets. Proximate analysis was done on the dietary ingredients and the resultant experimental diet (AOAC, 2004).

Fingerling Rearing: Each hapa was randomly stocked with GMT (2.00 ± 0.01g) at 20 fish/1m³ /hapa and raised for 24 weeks. The stocked fish were fed at 5% of their body weight three times daily. The stocked fish (20%) were sampled fortnightly. Fish weights were measured using a Furi Digital Balance (Model: FEJ-6000) to the nearest 0.1g.

Growth Performance: The following variables were calculated: Mean weight gain (MWG) (g) = final weight (g) – initial weight (g), Average daily growth (ADG) = MWG (g)/length of feeding trial (days), Specific growth rate (SGR, %/day) = 100(ln W₂ – lnW₁)/T₂-T₁, Where: W₂ = Weight at time T₂; W₁ = Weight at time T₁, Feed conversion ratio (FCR) = Total dry feed fed (g)/MWG (g), Protein index (PI) = Survival (W₁-W₀)t and Percentage survival rate (%SR) = 100(number at end of feeding trial/number at start of feeding trial).

Protein Requirement Determination: The curvilinear plateau analysis between protein inclusion levels and selected growth parameters [average daily growth (ADG), specific growth rate (SGR) and protein index (PI)] was used to determine the nutritional requirement of GMT as described by Aksnes *et al.* (1996). The second degree polynomial analyses on the growth parameters were generated to give quadratic prediction equations for the best performing diet. Test of significance for the relationship between protein inclusion levels and ADG, SGR and PI was done and values of correlation coefficients (r) obtained were used to assess the relationship between protein inclusion levels and nutritional response of catfish.

Proximate Analysis of Fish Carcass: Proximate analysis of fish carcass was done according to standard AOAC method (AOAC, 2004). Moisture Content was done by oven-drying to a constant weight; Total ash by muffle furnace combustion; Crude fibre by trichloroacetic acid method; Lipid content by soxhlet extraction method; Protein by micro-kjeldahl method, Carbohydrate was calculated as difference obtained after subtracting moisture, total organic nitrogen (protein), ether

Table 1: Proportion of dietary ingredients (% dry matter), cost and proximate composition of experimental diets fed to genetically male tilapia cultured in floating hapa system

Ingredients (g)	40.0%	42.5%	45.0%	47.5%	50.0%
Fishmeal	172.10	186.00	200.00	215.30	230.60
Soyabean	172.00	186.00	200.00	215.30	230.60
Cornmeal	239.70	182.00	124.30	67.50	11.20
Groundnut cake	345.10	375.00	404.60	431.00	456.60
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30	0.30
Fish Premix*	0.50	0.50	0.50	0.50	0.50
Fish oil	70.00	70.00	70.00	70.00	70.00
Cost					
₹/kg	217.50	222.70	227.80	233.80	239.90
₹/kg	1.09	1.11	1.14	1.17	1.20
Proximate Composition					
Protein	38.83	40.05	43.43	45.01	48.25
Carbohydrate	27.84	25.98	21.54	18.99	15.39
Fat	8.38	8.40	8.64	8.75	8.87
Moisture	14.10	14.00	13.82	13.75	13.72
Ash	6.48	7.11	7.82	8.49	8.61
Fibre	4.72	4.74	4.75	4.76	4.78

*Pfizer livestock product; 1kg fish premix contains: Vitamin A: 10,000,000 I.U.D; D3: 2,000,000 I.U.D; E: 23,000mg; K3: 2,000mg; B1: 3000mg; B2: 6,000mg; niacin: 50,000mg; calcium pathonate: 10,000mg; B6: 5000mg; B12: 25.0mg; folic acid: 1,000mg; biotin: 50.0mg; choline chloride: 400,000mg; manganese: 120,000mg; iron: 100,000mg; copper: 8,500mg; iodine: 1,500mg; cobalt: 300mg; selenium: 120mg; antioxidant: 120,000mg.

extract, ash and fibre from 100%. Caloric value was estimated based on physiological fuel values (0.2364 KJ/g for protein; 0.3954 kJ/g for lipid and 0.1715 kJ/g for carbohydrate) as described by (Henken *et al.*, 1986).

Data Analysis: Data analyses were carried out using Statistical Package for Social Sciences (SPSS 19.0, 2010 version).

RESULTS

The results of growth performances and survival rates of GMT fed diets containing different protein levels for 24 weeks indicated that all the growth indices were highest in diet containing 40% protein. There were no significant differences ($p > 0.05$) in food conversion ratio and survival rate in all the treatments. Fish fed the 40% protein diet had the best growth performance ($p < 0.05$), with a mean weight gain of 190.27 ± 29 g, average daily growth of

2.26g/day, specific growth rate of $5.43 \pm 0.02\%$ /day and protein efficiency ratio of 13.33 ± 0.16 . The least weight gain (156.07 ± 1.7 g) and poorest specific growth rate (5.20 ± 0.02) was observed in fish fed 50% protein diet (Table 2). Generally, there was an inverse relationship between protein level and growth performance of fish.

The results of proximate composition of fish carcass revealed inverse relation between protein content in diet and lipid content in fish muscle. However, linear relation existed between protein in diet and protein and gross energy contents in fish muscle (Table 3).

The results of the analyses between protein inclusion levels and average daily growth (ADG: $y_{\max} = 2.26$ g/day at $x_{\max} = 40\%$ protein). Similar results were obtained for specific growth rate (SGR: $y_{\max} = 5.43\%$ /day at $x_{\max} = 40\%$ protein) and protein index (PI: $y_{\max} = 2.23$ kg/day at $x_{\max} = 40\%$ protein). Results of the second degree polynomial analyses on these

Table 2: Growth response of genetically male tilapia fed diets with varied crude protein levels for 24 weeks

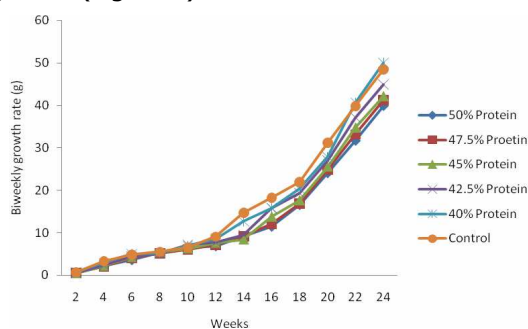
Variables	40%	42.5%	45%	47.5%	50%
MWG	190.27±2.9 ^e	171.8±0.5 ^d	166.6±0.2 ^c	160.93±1.3 ^b	156.07±1.7 ^a
ADG	2.26±0.03 ^c	2.04±0.02 ^b	1.96±0.01 ^b	1.92±0.01 ^b	1.86±0.02 ^a
SGR	5.43±0.02 ^c	5.32±0.01 ^b	5.30±0.01 ^b	5.3±0.01 ^b	5.20±0.01 ^a
FCR	0.14±0.00	0.14±0.00	0.14±0.00	0.13±0.00	0.14±0.00
PER	13.33±0.16 ^d	13.47±0.39 ^e	10.52±0.5 ^c	10.14±0.4 ^b	9.03±0.44 ^a
PI	4.71±0.03 ^b	5.05±0.17 ^c	4.32±0.30 ^a	4.13±0.19 ^a	3.97±0.18 ^a
% SR	100±0.00	100±0.00	100±0.00	93.33±1.67	100±0.00

Key: MWG = Mean weight gain (g), ADG = Average daily growth, SGR = Specific growth rate, FCR Feed conversion ratio, PER = protein efficiency ratio, PI = Protein index, %SR = Percentage survival rate

Table 3: Proximate composition of the carcass of genetically male tilapia fed diets with varied crude protein levels for 24 weeks

Variables	40%	42.5%	45%	47.5%	50%
Crude protein	15.17±0.16 ^a	15.87±0.43 ^b	16.49±0.01 ^c	16.85±0.16 ^d	17.55±0.15 ^e
Crude lipid	5.99±0.05 ^c	6.17±0.19 ^c	5.85±0.08 ^c	5.58±0.03 ^b	5.08±0.06 ^a
Ash	2.81±0.10 ^b	2.48±0.28 ^a	3.08±0.06 ^b	3.03±0.03 ^b	3.38±0.18 ^c
Gross energy	6.0±0.02 ^a	6.27±0.06 ^b	6.29±0.02 ^c	6.32±0.04 ^c	6.32±0.11 ^c

growth parameters gave quadratic prediction equations: ADG: $y = -0.8148x^2 + 1.6477x \pm 0.234$; SGR: $y = -0.1871x^2 + 1.0326x \pm 0.3412$; PI: $y = -0.8328x^2 + 1.6739x \pm 0.4023$. Test of significance for the relationship between protein inclusion levels and ADG, SGR and PI gave high correlation coefficients (ADG: $r = 0.9549$; SGR: $r = 0.9764$ and PI: $r = 0.9586$ at $p < 0.05$). This high correlation ($r > 0.9$ at $p < 0.05$) proved a strong relationship between protein inclusion level of 40% and growth performance of GMT. This showed that growth rate varied inversely with the amount of protein in diet. The biweekly growth curve indicated that fingerlings of GMT required 40% protein in the diet for optimum growth (Figure 1).

**Figure 1: Growth curve of genetically male tilapia fed diets containing different levels of protein over a 24-week growth trial**

DISCUSSION

The whole body composition of genetically male tilapia was influenced ($p < 0.05$) by dietary protein levels in diets. Fish fed 40% protein diet had lower carcass protein and higher carcass lipid than those fed 45%, 47.5% or 50% protein diets. These results were similar to those obtained by Al-Hafedh (1999). Ash content was unaffected by dietary protein levels and followed no particular trend. Similar result was presented by Khattab *et al.* (2000) who reported that ash content was unaffected by protein level in Nile tilapia collected from fish ponds. In this study, high protein level (50% protein) did not significantly enhance fish growth. These results were in agreement with Hamza and Kenawy (1997) who reported that 40% protein was more potent than other levels for Nile tilapia growth. Many authors obtained conflicting results from their studies on the effect of dietary protein level on the growth of Nile tilapia. Abdelghany (2000) reported that optimum dietary protein level for growth of Nile tilapia fry was 30% crude protein. Al-Hafedh (1999) concluded that better growth of Nile tilapia was obtained at dietary protein levels of between 40% and 45%. However, this study

revealed that GMT would grow optimally at 40% protein level. Food conversion ratio was not affected by protein levels. This trend was not in agreement with that obtained by (Khattab *et al.*, 2000). Protein efficiency ratio, productive value and index are commonly used as indicators of protein quantity, quality and amino acid balance in fish diet. These parameters were used to assess protein utilization and turnover, where they were related to dietary protein intake and its conversion into fish gain and protein gain. Results showed that PER, PPV and PI were significantly affected by protein level in diets. This indicated that protein utilization decreased with increasing dietary protein levels in diets. These results may be due to the fact that major part of weight gain is related to the deposition of protein, and the protein accretion is a balance between protein anabolism and catabolism. Furthermore, gastric emptying rate or solubility of the protein has been shown to affect the utilization of dietary protein (Epse *et al.*, 1999). The results of this study concluded that a diet containing 40% protein would be adequate and suitable for tilapia growth and optimum performance.

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CLINICAL CHEMISTRY AND HAEMATOLOGICAL ASSESSMENT OF QUAIL EGG-PRETREATED ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

This study investigated the possible hepatoprotective effect of quail egg solution on acetaminophen intoxicated rats. Thirty adult rats of mixed sexes were assigned into five groups of six per group. The rats in groups 2, 3, and 4 were pretreated with 30, 15, 7.5 mg/ml ad lib respectively of quail egg solution for 7 days before intoxication with 2000 mg/kg acetaminophen. Rats in group 5 were not pretreated but intoxicated with 2000 mg/kg acetaminophen (negative control) while the group 1 rats were neither pretreated nor intoxicated and served as positive control. Forty eight hours post induction, blood for some biochemical and haematological analysis was collected and the remaining rats treated until 14th day when the rats were humanely sacrificed and vital organs (liver and kidney) collected for histopathology. The results showed that the ALT activity of 30 mg/ml pretreated rats were significantly ($p < 0.05$) lower than those of the negative control rats. Significant ($p < 0.05$) increases were seen in the RBC, WBC, PCV and Hb levels of quail egg pretreated rats when compared with the negative control. However no significant ($p > 0.05$) changes were seen in AST activity, MCHC and MCH levels of both the test groups and the controls. Histomorphometry examination revealed less severe vacuolar degenerative changes in the liver of 30 mg/ml pretreated rats when compared to the rats of other intoxicated groups. It was concluded that quail egg at the concentration of 30 mg/ml ameliorated hepatotoxicity and improved haematologic indices of acetaminophen-induced toxicity in rats.

Keywords: Acetaminophen, Hepatotoxicity, Quail egg, Hematology, Histopathology, Liver enzymes

INTRODUCTION

Liver diseases are among the major problems in the globe today. Paracetamol[®] (acetaminophen), a widely used antipyretic and analgesic drug produces acute liver damage if frequently administered (Keffe and Friedman, 2004). Paracetamol toxicity has been reported to be the foremost cause of acute liver failure in the western world, and accounts for most drug overdoses in the United States, United Kingdom,

Australia and New Zealand (Hawkins *et al.*, 2007). The hepatotoxicity of paracetamol is as a result of formation of toxic metabolites when part of it is activated by hepatic cytochrome p-450 to a highly reactive metabolite, N-acetyl-p-benzoquinonimine (NAPQI) (Vermeulen *et al.*, 1992; Wallace, 2004)

The liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) are measures of liver homeostasis (Robert, 1999). The haematological

examination is among the methods which may contribute to the detection of some changes in health status, which may not be apparent during physical examination but which affect the fitness of the animals (Kronfeld and Medway, 1969). In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for hepatic disorder. However, there are several herbs/herbal formulation claimed to possess beneficial activity in treating hepatic disorders (Ojo *et al.*, 2006).

The quail eggs are produced from small, short-tailed game birds of the family Phasianidae which resembles partridges but are generally smaller. The birds are found in chaparral, sagebush, oakwood lands and the northern east. Quail birds are common in Europe, Asia and some parts of Africa. The egg has an average weight of 10.13g, with the albumen, yolk and shell weighing 6.12g, 3.2g and 0.7g respectively (Anca *et al.*, 2008).

Quail eggs are slowly becoming an easy to get product on the market. More and more people are beginning to show interest in their use as an active natural medicine instead of the chemical product with so many side effects. Quail egg has been shown to be effective in the treatment and management of so many diseases (Tanasom *et al.*, 2013). This study is therefore tailored to evaluate the haematological, biochemical responses and the hepatic histomorphologic changes associated with quail egg pretreatment to acetaminophen-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals: Adult Wister albino rats of mixed sexes aged between 10 and 16 weeks with weight of 120-180 g were obtained from animal house of Faculty of Veterinary Medicine, University of Nigeria Nsukka. The animals were acclimatized for about 7 days under standard environmental condition, with a 12 hour light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

Quail Egg: Quail eggs used were obtained from the Faculty of Veterinary Medicine, University of

Nigeria, Nsukka Farm. The freshly laid eggs weigh between 10 – 15 g.

Experimental Design: Thirty adult male rats were assigned to five treatment groups replicated thrice with two rats per replicate. The groups include group 1 – pretreated with distilled water and no paracetamol intoxication (positive control), group 2 – pretreated with 30 mg/kg quail egg solution and 2000 mg/ml paracetamol, group 3 – pretreated with 15 mg/kg quail egg solution and 2000 mg/kg paracetamol, group 4 – pretreated with 7.5 mg/ml quail egg solution and 2000 mg/kg paracetamol and group 5 - pretreated with distilled water and 2000 mg/kg paracetamol (negative control). The rats were pretreated with aqueous solution of quail eggs of varying concentrations for seven days. On day 7, 2000 mg/kg of acetaminophen (paracetamol) was administered orally. Forty eight hours post acetaminophen administration, blood was collected from the rats for the determination of some haematological and biochemical parameters. Thereafter, 2 rats per group were humanly sacrificed and vital organs (liver and kidney) collected for histopathology. The remaining rats in different groups were administered with the quail egg solution *ad libitum* till day 14.

Preparation of Quail Egg: An empty beaker was weighed. The shells of the quail eggs were broken with spatula and the contents emptied into the beaker. The weight of the beaker and the content was recorded. The weight of the egg yolk and albumin was obtained by subtracting the weight of the beaker from the weight of the beaker and its contents. A stock solution of quail egg (weight : volume) was prepared using the weight of the egg and distilled water. Serial dilutions of the stock solution - 7.5, 15.0 and 30 mg/ml of quail egg were made for the different treatment groups.

Induction of Liver Damage: The dose of 2000 mg/kg of paracetamol (acetaminophen) was administered to the rats orally.

Blood Collection: Blood samples were collected from the animals using orbital techniques for clinical chemistry and haematological determination. Blood samples for clinical chemistry determination were collected from the retrobulbar plexus of the median canthus of the eye of the rats (Parasuraman *et al.*, 2010). A microcapillary tube was carefully inserted into the medial canthus of the eye to puncture the retrobulbar plexus and thus enable outflow of about 2 ml of blood into a clean glass tube. The blood was kept at room temperature for 30 minutes to clot. Afterwards, the test tubes containing the clotted blood sample were centrifuged at 3000 revolution per minute using a table centrifuge. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle for the clinical chemistry determinations. Blood for haematologic determinations was collected with EDTA bottle.

Determinations of Liver Biomarkers: The serum aspartate aminotransferase and alanine aminotransferase activities were determined by the Reitman-Frankel colorimetric method for the *in-vitro* determination of AST in serum or plasma using AST test kit (QCA, Spain) (Reitman and Frankel, 1957).

Haematological Determinations

Packed cell volume: The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002).

Haemoglobin concentration: The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method (Higgins *et al.* 2008).

Erythrocyte count: The erythrocyte count was determined by the haemocytometer method (Thrall and Weiser, 2002).

Total leukocyte count: The total leukocyte count was determined by the haemocytometer method (Thrall and Weiser, 2002).

Mean corpuscular values: The mean corpuscular values – mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm *et al.*, 1975).

Histomorphometric Examination: The histological examination of the tissues of the liver and kidney of the rats were done using the method of Drury *et al.* (1967).

Statistical Analysis: The data generated was analyzed with one way analysis of variance (ANOVA) using SPSS version 20. P values less than 0.05 were accepted as significant. The results were presented in tables as mean \pm standard error of mean.

RESULTS AND DISCUSSION

There was a significant ($p < 0.05$) increase in WBC count of the rats in groups 2 and 3 when compared with the negative control (Table 1). There was concentration dependent reduction in RBC and WBC counts of acetaminophen induced hepatotoxicity in rats treated with graded concentrations of quail egg when compare to the normal group (Table 1). The effects of graded concentrations of quail egg on day 7 and 14 RBCs were similar in all treatments (Table 1). Vitamin B₂ (Riboflavin), Omega-3 and Omega-6-fatty acids contained in quail egg have been associated with boosting of erythropoiesis (Hillman *et al.*, 2005).

Packed cell volume and Hb levels of Groups 2 and 3 were statistically comparable ($p > 0.05$) to that of the positive control on days 7 and 14 but were significantly ($p < 0.05$) higher than those of the group 5 rats (Table 2). This could indicate that quail egg can improve the erythrocyte indices of paracetamol-intoxicated rats (Weiss and Goodnough, 2005).

One way analysis of variance did not reveal significant ($p > 0.05$) changes in MCV, MCH and MCHC across all the groups (Table 3).

There was no significant ($p > 0.05$) change in the activity of serum AST in the treated groups when compared with the

untreated paracetamol-intoxicated rats (Figure 1).

Table 1: Effect of graded concentration of quail egg on red blood cell (RBC) and white blood cell (WBC) counts of acetaminophen induced hepatotoxicity in rats

Groups	Day 7		Day 14	
	RBC ($\times 10^6$ cells/mm ³)			
1	6.81 \pm 0.21 ^c	7.03 \pm 0.18 ^d		
2	6.20 \pm 0.25 ^{bc}	6.60 \pm 0.25 ^{cd}		
3	5.85 \pm 0.44 ^{bc}	6.23 \pm 0.27 ^c		
4	4.29 \pm 0.35 ^b	4.96 \pm 0.33 ^b		
5	5.72 \pm 0.25 ^a	3.73 \pm 0.12 ^a		
WBC ($\times 10^3$ cell/mm ³)				
1	9.720.14 ^c	9.650.15 ^b		
2	9.270.16 ^c	9.450.18 ^b		
3	9.580.93 ^c	8.480.49 ^b		
4	6.500.17 ^b	5.270.71 ^b		
5	5.470.29 ^a	5.27071 ^a		

Similar letter superscript across each column indicates not significantly different means ($p > 0.05$). Different letter superscript across each column indicates significantly different means ($p < 0.05$)

Table 2: Effect of graded concentration of quail egg on packed cell volume (PCV) and haemoglobin concentration of acetaminophen induced hepatotoxicity in rats

Groups	Day 7		Day 14	
	PCV (%)			
1	44.67 \pm 0.88 ^b	45.00 \pm 0.58 ^b		
2	42.00 \pm 2.08 ^b	43.67 \pm 2.84 ^b		
3	40.00 \pm 0.58 ^{ab}	43.00 \pm 0.58 ^b		
4	36.00 \pm 1.73 ^a	40.00 \pm 2.52 ^b		
5	34.67 \pm 2.40 ^a	32.00 \pm 3.05 ^a		
Hb (g/dl)				
1	14.73 \pm 0.37 ^b	15.20 \pm 0.11 ^b		
2	14.23 \pm 0.43 ^b	14.40 \pm 0.70 ^b		
3	13.40 \pm 0.31 ^{ab}	14.80 \pm 0.31 ^b		
4	12.17 \pm 0.44 ^a	13.67 \pm 0.73 ^b		
5	12.13 \pm 0.88 ^a	10.50 \pm 0.76 ^a		

Similar letter superscript across each column indicates not significantly different means ($p > 0.05$). Different letter superscript across each column indicates significantly different means ($p < 0.05$)

Results showed that there was no significant ($p < 0.05$) change in the level of serum aspartate aminotransferase (AST) activity of rats in different test groups when compare to the normal group. Results showed that there was significant ($p > 0.05$) increase in serum alanine aminotransferase activity of rats in different test

groups compared with serum alanine aminotranferase activity of rats that were not pretreated (group 5 negative control).

Table 3: Effect of graded concentration of quail egg on mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin of acetaminophen induced hepatotoxicity in rats

Groups	Day 7		Day 14	
	MCV (fl)			
1	65.33 \pm 1.86	63.67 \pm 1.20		
2	68.33 \pm 5.69	64.67 \pm 6.06		
3	69.00 \pm 5.51	69.33 \pm 2.60		
4	81.33 \pm 8.41	65.67 \pm 1.45		
5	85.33 \pm 6.93	82.33 \pm 11.20		
MCHC (g/dl)				
1	32.70 \pm 0.15 ^a	33.33 \pm 0.43 ^a		
2	33.33 \pm 0.47 ^a	32.90 \pm 0.68 ^a		
3	35.97 \pm 3.08 ^a	33.30 \pm 0.46 ^a		
4	33.50 \pm 0.60 ^a	33.00 \pm 0.60 ^a		
5	33.27 \pm 0.55 ^a	32.30 \pm 0.53 ^a		
MCH (pg)				
1	21.67 \pm 0.67	21.00 \pm 0.58		
2	22.67 \pm 1.86	20.67 \pm 1.45		
3	22.67 \pm 1.86	23.00 \pm 0.58		
4	21.67 \pm 0.33	27.00 \pm 3.21		
5	27.67 \pm 3.93	27.67 \pm 2.03		

Similar letter superscript across each column indicates not significantly different means ($p > 0.05$). Different letter superscript across each column indicates significantly different means ($p < 0.05$)

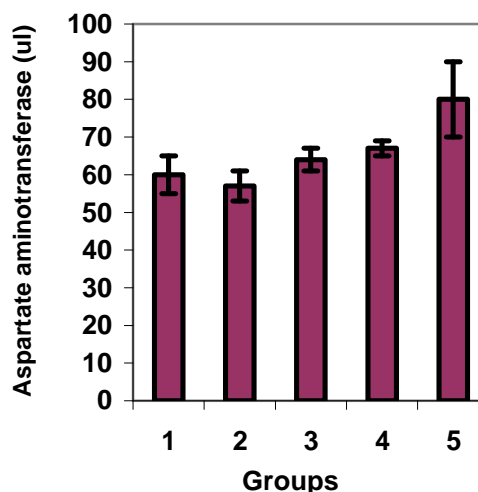


Figure 1: Effects of quail egg pretreatment on aspartate aminotransferase of acetaminophen-induced hepatotoxicity in rats

Group 2 rats showed significant ($p < 0.05$) reduction when compared to groups 3, 4, and 5 rats. Furthermore, the results for the liver

enzymes showed that there was significant ($p>0.05$) increase in serum alanine aminotransferase in rats from the different test groups compared with serum alanine aminotransferase activity of rats that were not pretreated (group 5 negative control). Group 2 rats showed significant ($p<0.05$) reduction in ALT when compared to groups 3, 4 and 5 rats. Aspartate aminotransferase unlike ALT is not known to be a specific marker for liver damage (Nyblom *et al.*, 2006). ALT activities in group 5 rats were significantly ($p<0.05$) higher when compared with those of the quail egg-pretreated rats (Figure 2).

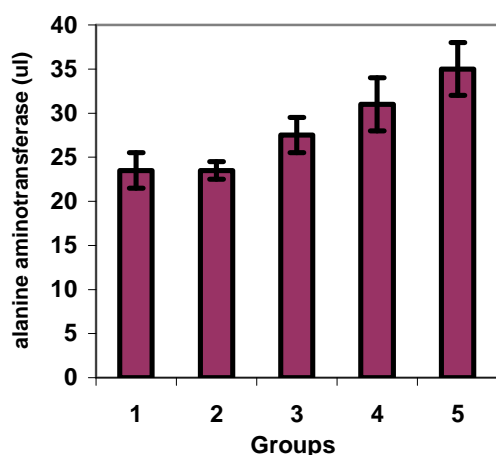


Figure 2: Effects of quail egg pretreatment on alanine aminotransferase of acetaminophen-induced hepatotoxicity in rats

This could be attributed to the effect of paracetamol on the liver (Robles-Daiz, 2014). The ALT activities of the normal control rats and that of group 2 rats were statistically comparable ($p>0.05$). This implied that the 30 mg/ml quail egg pretreatment prevented excessive leakage of ALT into the circulation. Quail egg could have achieved this by the presence of omega-3-fatty acid which according to Korever and Klasing (1997) is associated with decrease in inflammatory response, improvement in growth rate, erythropoiesis and leucopoiesis and increase specific immunity. It could be also be as a result of Vitamin C, and B₂ contained in the quail eggs which are known to be hepatoprotective (Takate *et al.*, 2010).

The liver enzymes, alanine aminotransferase (ALT), aspartate

aminotransferase (AST) are measures of liver homeostasis (Robert, 1999). Serum aminotransferase (AST and ALT) are markers of hepatocellular injury (Nyblom *et al.*, 2006). The aminotransferases are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases. In most acute hepatocellular disorder, the ALT is higher than AST (Robert, 1999). Paracetamol is a commonly and widely used analgesic and antipyretic agent, but over doses of paracetamol depletes the normal levels of hepatic glutathione. Cytochrome P450 enzyme system metabolizes paracetamol and forms a minor but significant alkylating metabolite known as NAPQI (N-acetyl-p-benzoquinone imine), which in turn is irreversibly conjugated with the sulfhydry groups of glutathione (Jollow *et al.*, 1973). Production of NAPQI (responsible for the toxic effects of paracetamol) is mainly because of two isoenzymes of cytochrome P450 (CYP2E1 and CYP1A2). Excess production of paracetamol metabolite caused the initial hepatic damage and subsequent activation of inflammatory mediator TNF- α , which in turn contributed to tissue necrosis (Jollow *et al.*, 1973).

Histopathology results indicated that the quail eggs in the pre-treated groups had lesser vacuolar degeneration of their hepatocytes compared to the hepatocytes of the negative control (Figures 3 – 7).

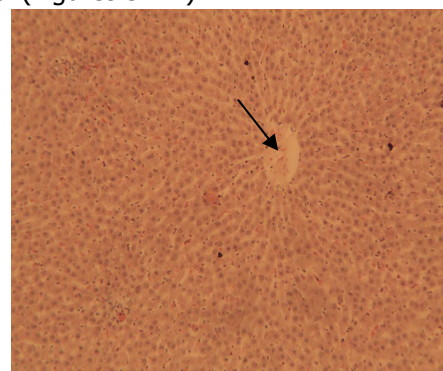


Figure 3: Section of liver from group one rats showing normal hepatocytes arranged in cords. Central vein (arrow), H&E X100

The hepatocytes of the group 2 rats (pretreated with 30 mg/ml of quail egg) had lesser damage compared to the hepatocytes of the other test groups.

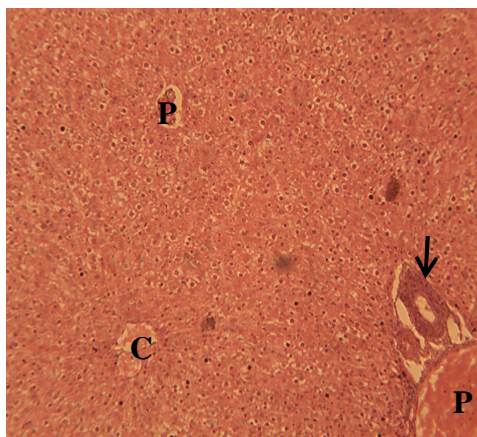


Figure 4: Section of liver from group two rats showing moderate vacuolation of the hepatocytes. Central vein (C), portal area (P), bile duct (arrow), H&E X 00



Figure 5: Section of liver from group three rats showing severe diffuse vacuolar degeneration and a moderate periportal cellular infiltration (arrow), H&E X100

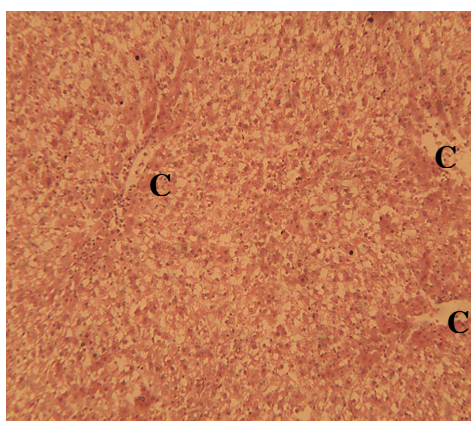


Figure 6: Section of liver from group four rats showing varying hepatocellular vacuolar degeneration. It tends to vary from moderate to severe, panlobular to midzonal and periportal, and random. Central vein (C), H&E X 100

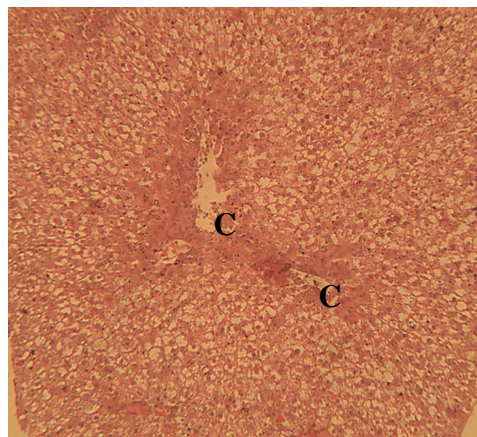


Figure 7: Section of liver from group five rats showing varying hepatocellular vacuolar degeneration. However, the degenerative change tends to involve primarily the periportal and midzonal areas of the hepatic lobules. Note; the hepatocytes around the central vein appears normal. Central vein (C), H&E X 100

This indicates that quail egg pretreatment at this concentration was able to mitigate injury to the hepatocytes. The samples collected from the kidney showed no histopathological lesion. The glomeruli, Bowman's capsule and renal tubules were normal in all groups. In conclusion, results of the study indicate that quail egg could be hepatoprotective and erythropoetic in acetaminophen-induced hepatotoxicity in rats

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CONSUMER PREFERENCE FOR SWINE OFFALS AND ITS HEALTH IMPLICATIONS IN KUMASI, GHANA

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ABSTRACT

Global meat consumption rate had doubled in most countries over the last five decades. In Ghana, total animal production has also increased over the last decade with consumption of pork and its offals becoming prominent. Therefore this study aims to evaluate factors that influence consumer preference for pig offals and the associated public health risks. One hundred (100) respondents in the Kumasi Metropolis were randomly selected through structured questionnaires and samples of offals randomly collected, sectioned, processed and examined for any histopathological indicators. Data collected were analyzed through the use of descriptive statistical tools. The results showed that edible offal preferential consumption exist within the increasing demand and value supply chain with the liver (32%) and stomach (23%) being the most preferred offal. Factors such as nutritional value, delicacy, availability, cost and level of education are noted to influence the purchasing power and preference of consumers. The consumers are less conscious regarding the risks stemming from the common zoonotic diseases and health concerns. Histological assessment of the most preferred offals (liver) showed no remarkable histopathological changes. Based on this, the preference for pig livers may not be associated with considerate health risk.

Keywords: Consumer, Histopathology, Kumasi, Pig offal preference

INTRODUCTION

Meat is a term most often used to describe the skeletal muscle tissue of an animal meant for consumption. It is mostly composed of roughly 75% water, 20% protein, and 5% fat, carbohydrates, and assorted proteins. Meat is not homogeneous as different types of meat vary in composition depending on the source and fatty acid composition (De-Smet *et al.*, 2002).

Meats have been reported to be high in nutritional value ranging from amino acids, vitamins, minerals, anti-oxidants such as ubiquinone among others. As such, reports have

showed that over the last five decades, meat consumption rate in some countries such as China have doubled, while other have shown significant increase in meat consumption per capital. Although Ghana showed a slight meat consumption per capital drop from 10.6 % to 9.9 % within the same period of review while total meat production had increased from 77,723 tonnes in 2001 to 244,742 tonnes in 2010 (Brown, 2009; Adzitey, 2013). Pork meat is known to be the fourth most consumed meat type after game, chicken and beef respectively. However, with a population growth rate estimate of 0.912% as at 2013, a population density growth of 36 persons per square

kilometer in 1970 to 78 persons per square kilometer in 2012 (Wikipedia, 2015; Ghana Embassy, 2015; World Bank, 2015), and growing global concerns for wildlife conservation, the bulk of meat type readily consumed by Ghanaians is gradually drifting away from wildlife to other meat sources (chicken beef, pork respectively) over the last decade (Adzitey, 2013).

Moreover, due to this observed increased trend in global and local meat consumption, associated health risks from both infectious and non-infectious diseases have been on the rise such as Creutzfeldt-Jakob disease, colorectal cancers, helminthosis, cardiovascular diseases, macular eye degeneration (Engelking, 2015) among others. Hence there is a gradual increase in demand for meat offal both globally and locally.

Meat offal, additionally called assortment or organ meat is a summative term used to describe meat from smooth muscles and internal organs such as guts gotten from butchered animal carcasses which includes liver, digestive tracts, lungs, heart and kidney. Offal is a vast reservoir of vital amino – acids, vitamins, minerals and of some miniaturized scale supplements, in that, they have a higher bioavailability which can barely be adjusted for by plant-derived pro vitamins (Biesalski, 2005).

However, the public health implications of pig offal could mask the nutritional benefits especially in a developing country such as Ghana where issues of meat safety and quality have not received needed attention as compared to developed countries and has such resulted in reemergence of some zoonotic infectious diseases (Adzitey, 2013). The incidence of offal-borne diseases continues to adversely affect the health and productivity of people in the country and beyond. Offal could be a source of some zoonotic diseases such as cysticercosis, brucellosis, fascioliasis, tuberculosis and others as it is often consumed under cooked (Phiri *et al.*, 2006).

To this end, this study was designed to assess factors that tend to influence consumer preference for offal across major determinants such as occupational, educational and tribal

background and to access the associated potential risk.

MATERIALS AND METHODS

Study Design: The survey is a cross-sectional study, which sought to evaluate the preferential consumption of pig offal and associated diseases in the various retail markets in the Kumasi metropolis. Structured questionnaires were administered to 100 consumers of pig offal who were randomly selected prior to sample collection. Based on data from preferential consumption survey for specific pig offal, samples of such were collected from selected butcher shops and vendors within the metropolis for histopathological assessment.

Study Area: The Kumasi metropolis has an area of approximately 254 square kilometers and is located between latitudes 6°35" and 6°4" N and longitudes 10°30" and 10°35" E (Figure 1). It was purposely selected because it is a densely populated city with non Muslim working class who have the means to afford animal protein. The city plays a major role in the food chain of Ghana as compared to the northern region of Ghana where greater percentage of its population are Muslims and often pigs produced within the northern area are sent down to Kumasi where it has a ready market. 50 – 60% of pigs in Ghana are concentrated in the Ashanti and Brong-Ahafo regions of Ghana and over 90% of this lot is made up of Ashanti black pigs (Frimpong *et al.*, 2012).



Figure 1: Map showing the various locations in Kumasi metropolis (Wikipedia, 2015)

Study Population: Adult grown-up members who demonstrated preference for pig offal were subsequently selected for this investigation, also included are sellers of the offal within Kumasi metropolis. Pig offal especially the livers used for histological assessments were obtained from randomly selected retailers or merchants in the Kumasi metropolis.

Sample Size Determination: The sample size required was determined using the formula: $n = t^2 \times p(1-p)/m^2$, where n = required sample size, t = confidence level at 95% (standard value of 1.96), p = estimated prevalence in the project area was estimated at 5% (0.05) since there was no previous report on the condition in Ghana and m = margin of error at 5% (standard value of 0.05). Therefore, $n = (1.96)^2(0.05)(0.95)/0.05^2 = 73$. The adequate sample size is 73 and 100 respondents were sampled.

Experimental technique: The sampling lasted for five (5) months and a total of one hundred (100) well-structured questionnaires adapted from previous study with offals or ruminants (Ayroe *et al.*, 2016) was modified, pre-tested and administered as a tool for evaluation of the preference of selected participants to swine offals. The respondents' data was collected and analysed for preferential consumption of such offals.

Histopathological Assessment: The most preferred pig offal samples (liver) were randomly collected from various retail points and preserved in 10% buffered formalin, routinely processed and stained with Haematoxylin and Eosin (H&E) for histopathological evaluation using light microscopy (Dellman and Brown, 1987).

Data Analysis: The data collected was analysis in percentages utilizing the Statistical Package for Social Sciences (SPSS) version 20.0 suits and Microsoft Office Excel was used in the plotting of graphs.

RESULTS

Educational Impact on Preference: The educational background of respondents had impact on preferential consumption with reference to traditions and their purchasing power. Education plays a very important role in the preference of the various pig offals which is in cognisance of the health implication of the offals to the consumer (Figure 2).

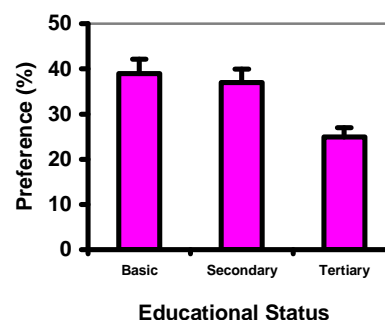


Figure 2: Effect of educational status on preference of pig offals in Kumasi, Ghana

The Source of Pig Offal: The fundamental sources of retail offal among others include open market (49.0%), butchers shop (39.0%), own animal (3.0%) and the super market (9.0%). Pig offals were comparatively cheap and the proximity of offals access point to the consumer defines the patronage of the offal (Table 1).

Table 1: Major sites for the purchased of swine offals in Kumasi, Ghana

Where do you often get your pig offals	Frequency	Percent
Open market	49	49.0
Butchers shop	39	39.0
Super market	9	9.0
Own animals	3	3.0
Total	100	100.0

Specific Preferential Consumption of Offal:

Despite the fact that buyers of pig offals were from different walks of life, with different foundations, special utilization of offal exists among the respondents in Kumasi. Many respondents showed strong preference for the liver (32%) however, the heart was the least preferred offal accounting for 3% (Figure 3).

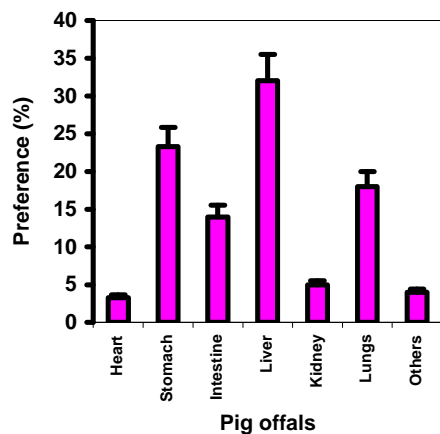


Figure 3: Consumer's preference of pig offals in Kumasi, Ghana

The reasons assigned by respondent for their preferences were nutritional value 41%, delicacy 53% and cost 5% (Figure 4). It implies that the cost factor has little influence in the purchasing of the offal. However, the nutritional value and the delicacy appear to have an influence on the offal purchase.

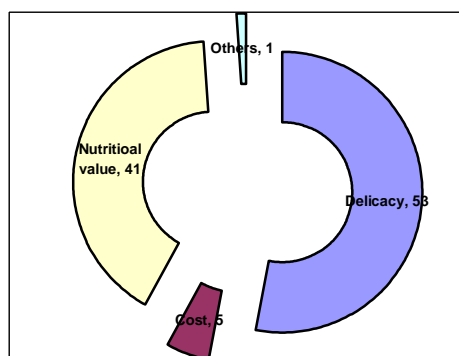


Figure 4: Consumer's reasons for the preference of pig offals in Kumasi, Ghana

The Occupation of Respondent: The results revealed that traders consume pig offals the most as they constitute (20%) of the respondents while house wife, farmer, teacher with a percentage of 15%, 13% and 11%, respectively (Figure 5). It is known from this study that delicacy, nutritional value and the traders' proximity to the pig offal sales points do influence the purchase of offals from the cold stores and only occasionally visit to the other offal sales points.

Tribe Influence: Out of the 100 respondents, Ashanti (19%), Frafra (17%), Akan (18%) tribes were the highest (Figure 6). As a result of the

choice of study area, the Ashanti including Akan were in the majority among the 100 sampled respondents chosen; therefore tribe influence may not be ascertained in this investigation.

Pathological Assessment: Majority of the pig livers examined were apparently normal with one representing 1% showing milk spots which is associated with larval migration. The photomicrograph of the normal and the gross picture of that with milk spots are presented in Figure 7.

DISCUSSIONS

This investigation evaluates consumer preference of pig offals in Kumasi. In this study it was observed that preferences for offal do not always coincide with the actual dietary and food consumption pattern of the respondents. The preferred offal was the liver which further showed that the level of education positively impacted on the preference as most respondent were influence by knowledge of the delicacy and nutritional value than cost (Ayroe *et al.*, 2016).

In this study also, it was observed that the source of the offals was more from the open market than butchers or supermarket which showed that accessibility, varieties and affordability of the offals in the open market might have accounted for the preference observed hence the consumers' proximity to the pig offal sales points and affordability of offals may influence the purchase of offal from the cold stores as described in similar study in ruminants (Ayroe *et al.*, 2016).

The preference shown in the study differ from similar studies with ruminant offals where forestomach was the most preferred (Ayroe *et al.*, 2016), the forestomach of goats or cattle gives varieties than that of pig.

The occupation of respondent also positively influenced the choice for the liver as most of the respondent are experienced and are aware of the nutritive value than other offals. In a similar study from Ghana on the preference of offals from small ruminants, occupation of the respondents had correlated positively with preference (Ayroe *et al.*, 2016).

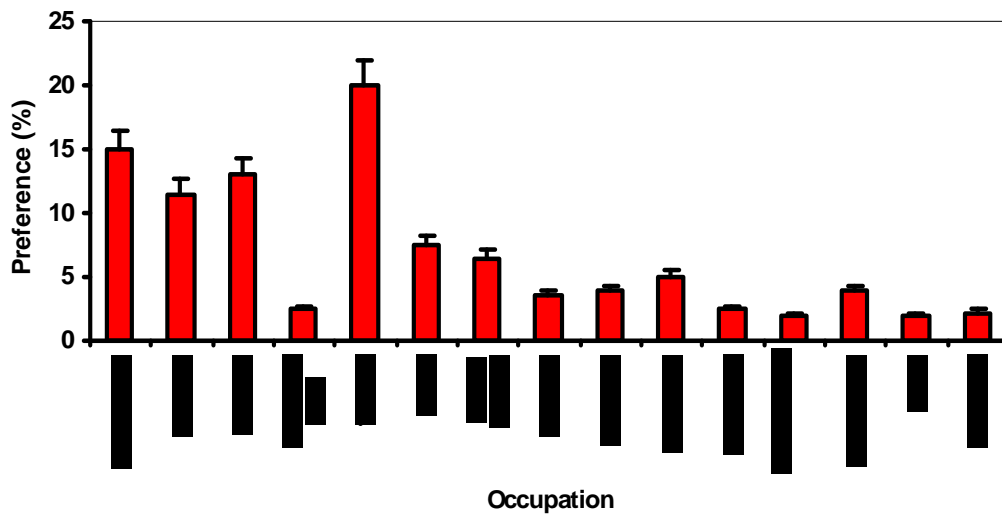


Figure 5: The influence of occupation on preference of pig offals in Kumasi, Ghana

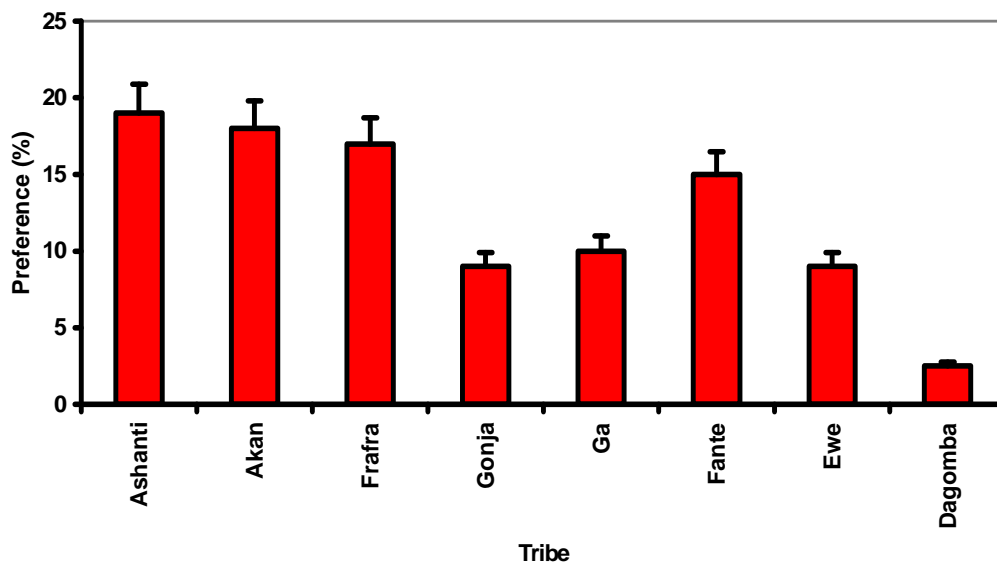


Figure 6: The influence of tribe on preference of pig offals in Kumasi, Ghana

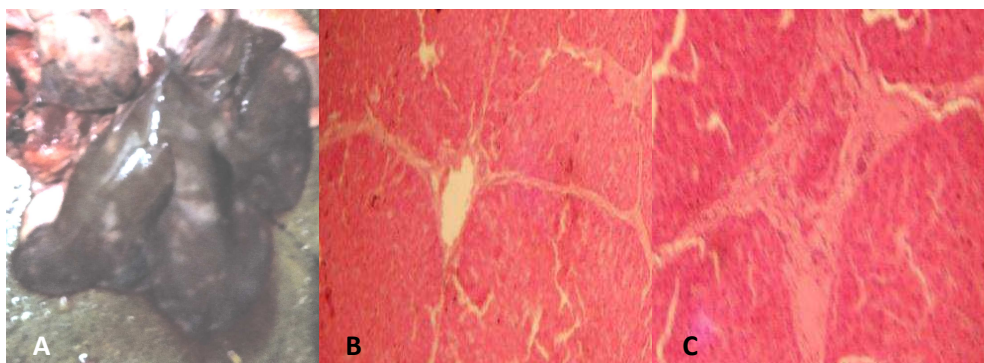


Figure 7: Gross and histopathological assessment of the most preferred offal (liver). Gross picture of the liver with milky spots (A) and photomicrograph of tissue section stained with Haematoxylin and Eosin (B and C) showing a normal liver. Mag. x 100

The tribal influence may not be easily ascertained as a result of the choice of study area where the Ashanti including Akan constituted the majority of the respondents.

The pathological assessment of the most preferred offals also revealed that hepatic abscess associated with milk spots is not commonly encountered as most of the histological screening of the tissue revealed no significant pathological change hence it could be that livers from pigs in this area of study are safe.

Conclusions: This survey revealed that preferential consumption of pig offals exists within the Kumasi Metropolis. Preference for offal does not generally concur with the genuine dietary and sustenance utilization design. Various variables informed the purchasers' inclination for the stomach (23%) and the liver (32%) which was the preferred consumable offals in this study. Delicacy, taste, nutritional value and educational background were noted to play a role in the buying force and inclination of purchasers. Histopathological assessment revealed no conceivable risk connected with the consumption of the most preferred of offals (liver). Based on this assessment, it is strongly recommended that pig offals especially liver should be patronize by consumers since its health risk are minimal.

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A SIX YEAR REVIEW OF THE TRENDS IN PREVALENCE OF MALARIA INFECTION IN CHILDREN IN SECONDARY AND TERTIARY HEALTH CARE OUTLET IN ANAMBRA STATE, NIGERIA

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ABSTRACT

This study examined the trends of malaria infection among in children in the paediatric unit of some tertiary and secondary health outlet in Anambra State, Nigeria over a six year period. A retrospective study of malaria prevalence in children aged 0 – 14.9 years between January 2005 and December 2010 was conducted to establish the trends. There was an increase in the number of children with malaria infection from 2005 to 2010. Seasonal prevalence occurred with 52.3% malaria parasite infection during rainy season and 47.7% during the dry season. Children between the ages of 0 – 3 years had the highest malaria prevalence between 2005 – 2010. There was an overall yearly increase in the number of malaria cases which may be as a result of influx of mothers/caregivers to the hospitals for further treatment after initial home management of malaria in their children.

Keywords: Trends, Malaria, Prevalence, Children, Tertiary and secondary health outlet, Anambra State, Nigeria

INTRODUCTION

Malaria continues to claim one to two million lives a year, mainly those of children in sub-Saharan Africa. An estimated 3.2 billion people are at risk of malaria, of which 1.2 billion are at high risk. In high-risk areas, more than one malaria case occurs per 1000 population. Malaria killed 437,000 children before their fifth birthday in 2013, the majority in sub-Saharan Africa. According to the latest estimates, malaria mortality rates were reduced by about 47% globally and by 54% in the WHO African Region between 2000 and 2013 (WHO, 2014). Reduction in mortality depends, in part, on improving the quality of hospital care, the training of healthcare workers and improvements in public health (Ralph and Akyea 2013).

New analysis reveals that the prevalence of malaria parasite infection (including both symptomatic and asymptomatic infections) has decreased significantly in Africa since 2000. The number of people infected fell from 173 million in 2000 to 128 million in 2013 – a reduction of 26 %. This has occurred despite a 43% increase in the African population living in malaria transmission areas. Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97 % of Nigeria's population. The remaining 3 % of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. Malaria has the least prevalence, 27.6 percent, in children age 6 to 59 months in the

South East region (United States Embassy in Nigeria, 2011).

WHO in 2011 recommended prompt parasitological confirmation by microscopy or Rapid Diagnostic Test (RDT) for all patients with suspected malaria before treatment begins. Artemisinin-based combination therapy (ACT) is the standard treatment of uncomplicated malaria. Prevention programs focus on the distribution and use of bed nets, called Long Lasting Insecticidal Nets (LLINs), including evidence-based health communication programs on the mode of malaria transmission and the importance of sleeping under ITNs. This study examined the trends of severe and uncomplicated malaria cases in General and Teaching Hospitals in Anambra State, Nigeria over a five year period.

MATERIALS AND METHODS

A retrospective study of malaria cases of children between January, 2005 and December, 2010 was conducted to establish the trends. The hospital records of the paediatric unit of the selected General Hospitals were studied and analyzed. Monthly diagnosis of malaria cases in eleven (11) General Hospitals was recorded. The hospitals purposefully selected for this study were: CHC - Comprehensive Health Centre, Atani, GHE - General Hospital, Ekwulobia, GHO - General Hospital, Osomala, GHA - General Hospital, Amanuke, GHEU - General Hospital, Enugu Ukwu, NATHU - Nnamdi Azikiwe Teaching Hospital, Umuinya, GHI - General Hospital, Ifitedunu, GHAG - General Hospital, Agulu, AUTHA - Anambra State University Teaching Hospital, Awka, GHU - General Hospital, Umueri and GHN - General Hospital, Nnobi (Figure 1).

Data Analysis: Percentages were used to determine the prevalence of infection in each year and across gender and age groups.

RESULTS AND DISCUSSION

A retrospective studies on the prevalence of malaria from 2005 to 2010 in Anambra State using the hospital records showed that 2010

had the highest prevalence (27.5%) followed by 2009 (18.2 %), 2006 (15.5 %) and 2005 had the lowest prevalence of 11.7 % (Figure 1). Also from 2005 – 2010, the months of September, October and November had the highest prevalence of malaria infection (9.5 %) followed by June and August (8.9 %), July (8.8 %) December (8.5 %), January 7.8 %, February 7.5 % May 7.4 %, March 7.3 % and April 6.5 % (Figure 2). There was 52.3 % malaria parasite infection during rainy season and 47.7 % during the dry season (Figure 3).

The retrospective prevalence of malaria infection in different secondary and tertiary hospitals in Anambra State, Nigeria, showed that Anambra State University Teaching Hospital, Awka had the highest infection (41.4 %) followed by Nnamdi Azikiwe Teaching Hospital, Umuinya (27 %) and Umueri General Hospital had the lowest prevalence of 2.3 % (Figure 4).

Combined sex monthly prevalence of childhood malaria infection in the year under review showed that there was 9.5 % malaria infection in the months of September to November, followed by June and August (8.9 %), July (8.8 %) and December (8.5 %). The lowest prevalence (6.5 %) was recorded in the month of April (Figure 5).

Also the retrospective study showed that children between the ages of 0 – 3 years had the highest malaria prevalence from 2005 – 2010. The highest prevalence was recorded in 2010 (65.7%), followed by 60.9% (2008), 60.4 %, 60.2 %, 59.4 % and 59.1 % in 2009, 2007, 2006 and 2005, respectively. Children between the ages of 3.1 – 5.0 years had the highest prevalence of infection in 2007 (27.2 %), while those between 5.1 – 12 years had the highest prevalence in 2006 (14.2 %) (Figure 6).

There was progressive increase in percentage admitted cases of malaria in children, directly related to upward movement of years under review. This was in line with the study of Ralph and Akyea (2013) who reported a similar trend in a five year review of in-patient cases of malaria at the Children's Ward of Volta River Authority (VRA) Hospital, Akosombo, Ghana. This may be attributed to the malaria advocacy and education embarked upon by Anambra

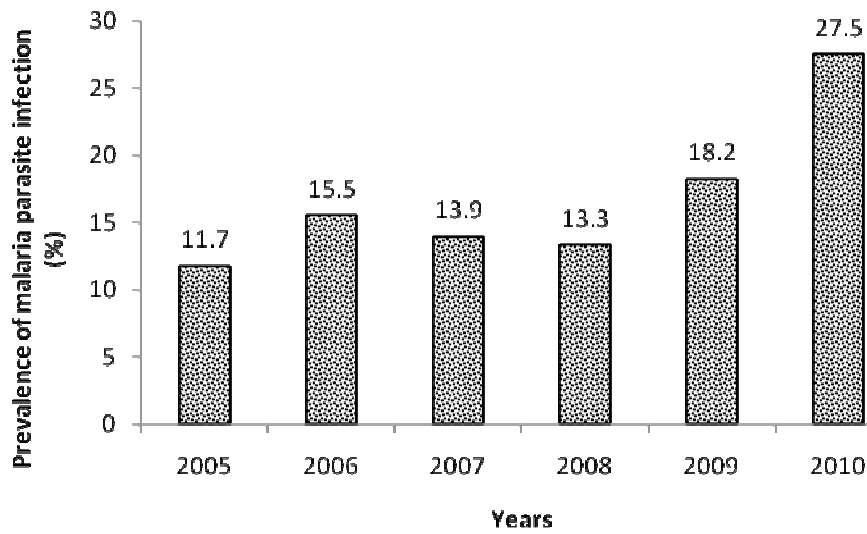


Figure 1: Prevalence of malaria parasite infection in Anambra State, Nigeria from 2005 – 2010

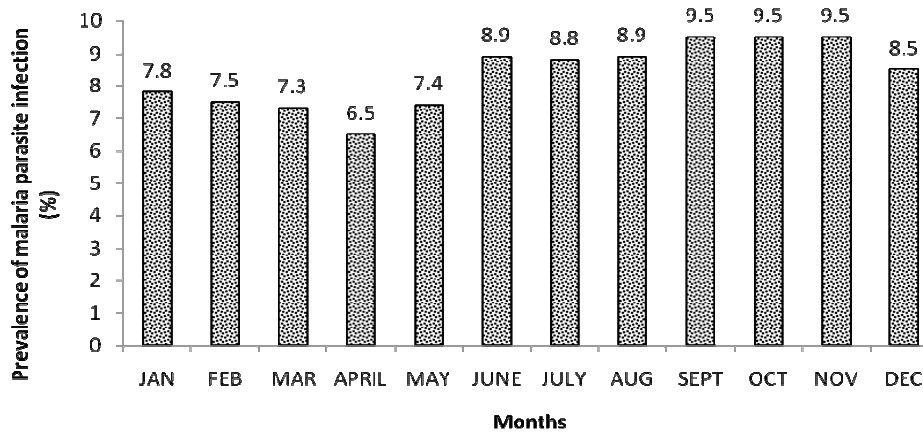


Figure 2: Monthly prevalence of malaria parasite infection from 2005 - 2010 in Anambra State, Nigeria

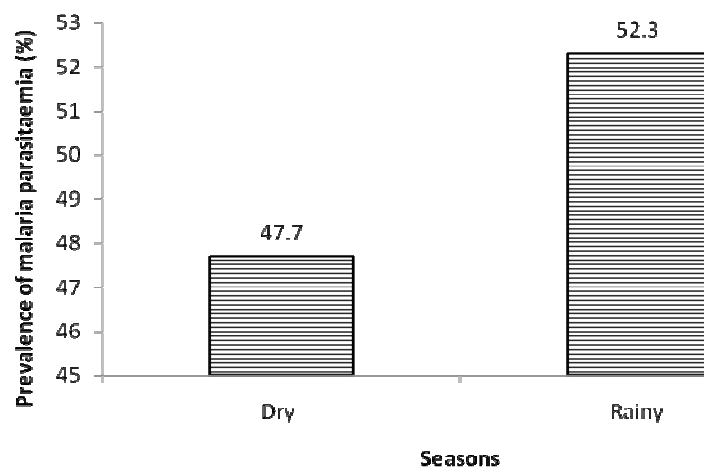


Figure 3: Seasonality of malaria parasite infection in Anambra State, Nigeria from 2005 – 2010

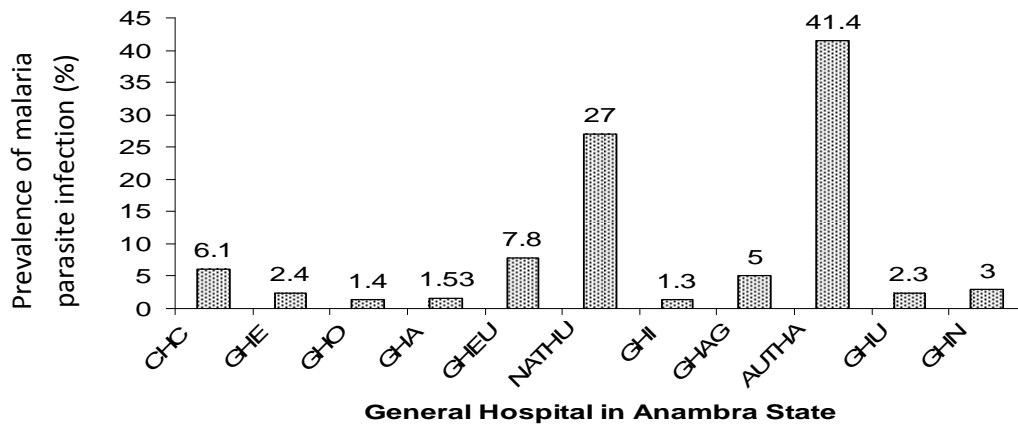


Figure 4: Prevalence of malaria parasite infection in General Hospitals in Anambra State, Nigeria from 2005 – 2010. Key: CHC - Comprehensive Health Centre, Atani, GHE - General Hospital, Ekwulobia, GHO - General Hospital, Osomala, GHA - General Hospital, Amanuke, GHEU - General Hospital, Enugu Ukwu, NATHU - Nnamdi Azikiwe Teaching Hospital, Umunya, GHI - General Hospital, Ifitedunu, GHAG - General Hospital, Agulu, AUTHA - Anambra State University Teaching Hospital, Awka, GHU - General Hospital, Umueri and GHN - General Hospital, Nnobi

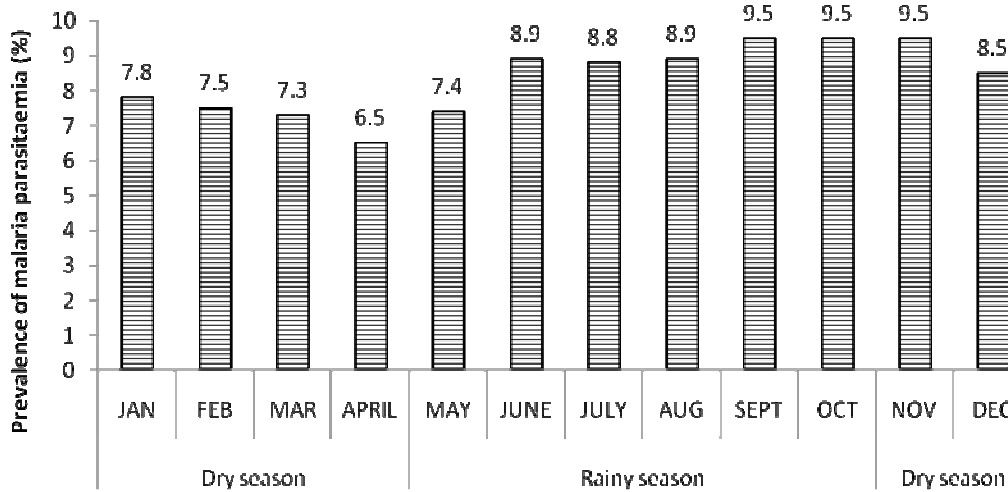


Figure 5: Combined monthly sex prevalence of childhood malaria infection from 2005 -2010 in Anambra State, Nigeria

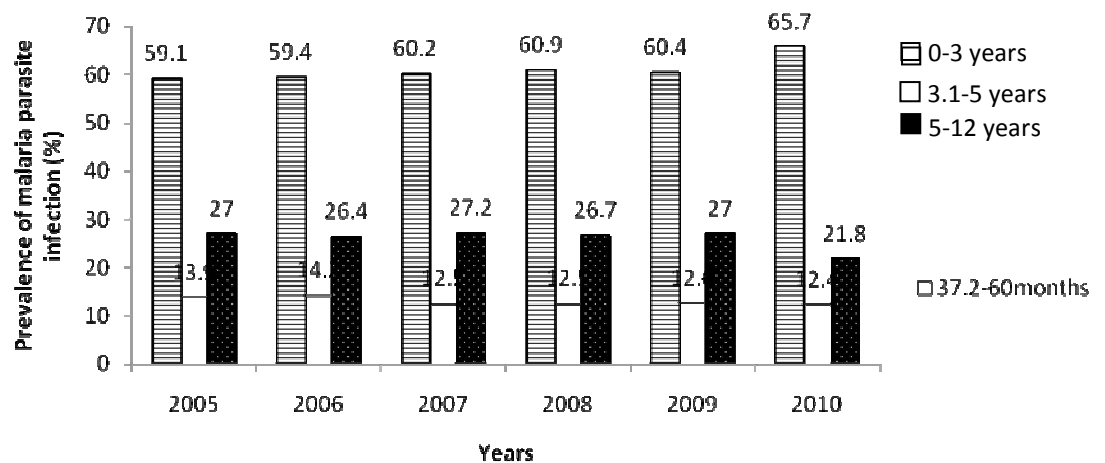


Figure 6: Age prevalence of malaria parasite infection in Anambra State, Nigeria from 2005 - 2010

State Government in line with the Federal Government policy to ensure effective control of malaria especially among the vulnerable groups (children under five years and pregnant women). Also this showed that there was significant improvement in the referral practises by the health workers at different levels of health care in the state.

The monthly prevalence of malaria infection over the years in focus showed that the months of January to December are suitable for malaria transmission in Anambra State, hence the presence of malaria infection in both wet and dry season (Ayanlade *et al.*, 2010; Iwuora, 2014). This is influenced by the availability of conditions suitable for malaria parasite transmission. The conditions that are suitable for both the development of *Plasmodium* and mosquitoes were defined as the coincidence of precipitation accumulation greater than 80 mm, mean temperature between 18^o C and 32^o C and relative humidity greater than 60% (Ayanlade *et al.*, 2010).

The result of this study showed that children between the ages of 0 – 3 years were persistently more infected than the other age groups in the year under review. This result was in line with the study of Alhaji (2012) and Austin *et al.* 2012). Further more 58.3% was reported for children aged 0 – 5 years in Awka, Anambra State (Mbanugo and Ejims, 2000) and 61 % recorded in Abuja (Matur *et al.*, 2001). This may be due to the fact that at that age, their immunity to parasitic infections has not been fully developed.

In this study, it was found that tertiary hospitals had the higher prevalence of *Plasmodium* infection than the General Hospitals and Comprehensive Health Centres. This may be due to excessive visits of malaria patients and the improvement in further treatment behaviour of mothers/caregivers in the state.

Conclusion: There was an overall yearly increase in the number of malaria cases admitted to the paediatric unit of hospitals in Anambra State from 2005 to 2010 which showed improvement in the further treatment behaviour of mothers/caregivers but calls for effort in the improvement of integrated

management and control of childhood malaria in the state.

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CONTENTS		PAGES
1.	DEVELOPMENTAL FEATURES OF PORCINE HAEMAL NODES: A HISTOLOGICAL PERSPECTIVE – UDOUMOH, Anietie Francis and EZEASOR, Daniel Nwagbo	2241 – 2248
2.	ZOOPLANKTON COMMUNITIES OF THE RIVER OSSIOMO, OLOGBO, NIGER DELTA, NIGERIA – IKHUORIAH, Suleman Ovuehor, ORONSAYE, Charity G. and ADEBANJO, Isaac Adeniyi	2249 – 2259
3.	KNOWLEDGE BASED ASSESSMENT OF INTESTINAL PARASITIC INFECTIONS AMONG STUDENTS ATTENDING BOARDING SCHOOLS IN EBONYI STATE, NIGERIA – EBHODAGHE, Faith and ISAAC, Clement	2260 – 2267
4.	PARASITOLOGICAL IDENTIFICATION AND HISTOLOGICAL EXAMINATION OF <i>FASCIOLA GIGANTICA</i> SEQUEL TO OCCURRENCE OF BOVINE BILLIARY FASCIOSIS IN CATTLE SLAUGHTERED AT BODIJA ABATTOIR, IBADAN – FALOHUN, Olufarati Oludunsin, OBEBE, Oluwasola Olaiya, ONYICHE, Emmanuel ThankGod, OKE, Philip Oladele, OMOTAINSE, Oluwadamilola Samuel and ADELABU, David Babatunde	2268 – 2273
5.	IMMUNOLOGIC AND HAEMATOLOGIC EFFECTS OF METHANOLIC STEM BARK EXTRACT OF <i>AZADIRACTA INDICA</i> ON CHICKENS EXPERIMENTALLY INFECTED WITH VELOGENIC NEWCASTLE DISEASE VIRUS (KUDU 113) STRAIN – OKOROAFOR, Obianuju, EZEMA, William, UDEGBUNAM, Rita, EZE, James, EZE, Ukamaka, ARONU, Chinwe, OMORNUWA, Augustine, OKOSI, Riona and NGENE, Augustine	2274 – 2283
6.	HAEMONCHOSIS AND HAEMOPARASITES OF SMALL RUMINANTS REARED IN NORTH WESTERN, NIGERIA – JOSIAH, Gana James, OMALU, Innocent Chukwuemeka James, MAKUN, Hussaina Jumai, CHIEZEY, Ngozi P. and ABAH, Onuche-Ojo Isaiah	2284 – 2291
7.	OPTIMUM DIETARY PROTEIN REQUIREMENT OF GENETICALLY MALE TILAPIA (<i>OREOCHROMIS NILOTICUS</i>) CULTURED IN FLOATING HAPA SYSTEM – EFFIONG, Mmandu Uwem	2292 – 2297
8.	CLINICAL CHEMISTRY AND HAEMATOLOGIC ASSESSMENT OF QUAIL EGG-PRETREATED ACETAMINOPHEN-INDUCED HEPATOTOXIC RATS – ABA, Patrick Emeka, JOSHUA, Parker Elijah, ONWUDINJO, Rex Chibuikem, OMOJA, Valentine Uneojo and EZEASOR, Chukwunonso Kenechukwu	2298 – 2304
9.	CONSUMER PREFERENCE FOR SWINE OFFALS AND ITS HEALTH IMPLICATIONS IN KUMASI, GHANA – NONTERAH, Esmard Weldam, ASENSO, Nyarko Timothy, EMIKPE, Benjamin Obukowho and ASARE, Derrick Adu	2304 – 2310
10.	A SIX YEAR REVIEW OF THE TRENDS IN PREVALENCE OF MALARIA INFECTION IN CHILDREN IN SECONDARY AND TERTIARY HEALTH CARE OUTLET IN ANAMBRA STATE, NIGERIA – OKEKE, Obiageli Panthe, IMAKWU, Cyril Ali, EYO, Joseph Effiong and OKAFOR, Fabian Chukwuemenam	2311 – 2315

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