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PRODUCTION OF SOME VIRULENCE FACTORS UNDER DIFFERENT GROWTH CONDITIONS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *Aeromonas hydrophila*

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ABSTRACT

The production of some virulence factors under different growth conditions and antibiotic susceptibility pattern of Aeromonas hydrophila were investigated in this study. The virulence factors tested on the isolates included haemolytic activity, exopolysaccharide (capsule) and toxin production. Other cell property evaluated was antibiotic resistance. Of the several chemotherapeutants tested, streptomycin had Minimal Inhibitory Concentration (MIC) and Minimal Cidal Concentration (MCC) at 25 µg/ml; flumequine, MIC 15 µg/ml and MCC 20 µg/ml; nitrofurantoin, MIC 20 µg/ml and MCC at 20 µg/ml; chloramphenicol, MIC 15 µg/ml and MCC 30 µg/ml and nalidixic acid, MIC 25 µg/ml, MCC 30 µg/ml, respectively. Virulence characteristics were apparent from the study; the properties exhibited such as ß-haemolytic activity (F= 1.32551; p < 0.001) and toxin production (F = 0.141; p > 0.05) were evidence of the pathogenic potential of A. hydrophila.

Keywords: Virulence, Haemolytic activity, Susceptibility, Antibiotics, *Aeromonas hydrophila*

INTRODUCTION

Virulence is an important property of microorganisms in relation to their pathogenicity and is defined as the capacity of the organism to invade tissues, multiply and produce toxic effects. It is estimated by the minimal lethal dose (MLD), which is the smallest dose of the organism (number or weight of cells from a culture) that will kill a particular species of animal. Virulence depends on two factors that may be largely independent of one another; namely, the invasiveness or aggressiveness, and the toxigenicity or toxin - producing property of the organism. Thus, the tetanus bacillus for instance is highly toxigenic but only weakly aggressive; in contrast, the Pneumococcus is markedly aggressive but its toxicity is minimal (Cruickshank *et al.*, 1980).

Aeromonas hydrophila has been isolated from both polluted and unpolluted water worldwide (Schubert, 1976), and its ability to produce virulence factors like enterotoxin, haemolysin, endotoxins, cytotoxins (Barney *et al.,* 1972; Berheimer and Avigad, 1974; Chopra and Houston 1999), as well as its antibiotic resistance potential (Chopra and Houston, 1999; Albert, 2000; Okpokwasili and Okpokwasili, 1994) has been documented. Chopra *et al.*, (2000), reported a cytotoxic enterotoxin production; in a study of nine isolates of the organism, 69 % were found to produce cytotoxin and haemolysin. Fatal and non-fatal infections caused by the organism have been reported. *A. hydrophila* has also been observed as a life threatening pathogen, associated with a variety of clinical manifestations, including septicemia (Seatha *et al.*, 2004; Mathewson and Dupont, 1992), meningitis (Seatha *et al.*, 2000); endocarditis (Davis *et al.,* 1978), corneal ulcers (Feaster *et al.,* 1978), wound infections (Shackelford *et al.*, 1973; Mani *et al.*, 1995), peritonitis (Salton and Schick, 1973) and acute diarrheal diseases (Albert, 2000). *A. hydrophila's resistance* to chemotherapeutants such as; ampicillin, colistin sulphate, sulphonamide, tetracycline and cotrimoxazole, has been reported (Okpokwasili and Okpokwasili, 1994). Earlier reports indicated that the environmental strains of *A. hydrophila* were capable of producing toxins and that isolates recovered from healthy and moribund fish were cytotoxic and most strains were enterotoxic in the Rabbit Ileal Loop (RIL) and suckling mouse tests. The organism was concluded to produce an *Escherichia coli* ST-like (heat stable) and heat labile toxins (Kaper *et al.,* 1980).

This study investigated the expression of some virulence characteristics and antibiotic resistance of *A. hydrophila* isolated from fish with a view of establishing its pathogenicity.

MATERIALS AND METHODS

Isolation, identification, enumeration and maintenance of *Aeromonas hydrophila:* The bacterium*, Aeromonas hydrophila* was isolated from the fish *Epiplatys bifasciatus*, of the family, Cyprodontidae, from Taylor Creek, a fresh water creek in Yenegoa, Bayelsa State of Nigeria. Reference strain, ATCC 7966 (*A. hydrophila*), made available by G. S. C Okpokwasili, served as the control.

Culturable aerobic heterotrophic bacterial counts were obtained after appropriate serial dilutions and plating in Rimler Shotts agar and Tryptone Soy agar (TSA) plates (Shotts and Rimler, 1973). The *Aeromonas hydrophila* strain was tested for production of various virulence factors, namely,

capsule or slime production, α or β haemolysis, antibiotics susceptibility as well as toxin production following cultivation in the hydrocarbons: gasoline, toluene, kerosene and diesel oil as sole carbon sources and glucose as control.

Substrate Utilization by *A. hydrophila*: Production of some virulence factors under different growth conditions was determined using the vapour phase transfer method (Mills *et al.,* 1978) as modified by Okpokwasili and Amanchukwu (1988). The components of the medium were: 0.42 g $MgSO_4.7H_20$; 0.297 g KCl; 0.85 g $KH_2 P0_4$; 0.42 g Na $N0_3$; 1.27 g $K_2H P0_4$; 20.12 g NaCl, and 20 g TSA agar powder. The methods of Harrigan and McCance (1976) were adopted for the characterization and identification of the isolates. Purity of the samples was maintained by inoculation on TSA slants at 4 °C, and sub-culturing fortnightly onto freshly prepared TSA slants and stored in a refrigerator.

Haemolysis: Alpha or Beta haemolytic activity of culture filtrates of *A. hydrophila* on Ox red blood cells was used to determine the ability of the organism to produce haemolysin (pathogenicity factor) in different hydrocarbon substrates. One hundred (100) ml of Mineral Salts Broth (MSB) distributed in 5 ml volumes into 20 test tubes was supplemented with 0.1, 0.25, 0.5 and 0.1 ml of each hydrocarbon: gasoline, toluene, kerosene, diesel and glucose, the control. These were inoculated with the test organism and incubated at 37 ^oC for 24 h, after which the culture was centrifuged at 3000 rpm for 15 min to clarify and then filtered through a Whatman No. l filter paper. Equal (1-mL) volumes of the filtrate and 1 % Ox RBC (washed thrice in saline, resuspended in same solution and diluted appropriately to obtain the working concentration) were mixed in clean test tubes and incubated in a water bath at 37 ^oC for 1 h. The mixture was then centrifuged at 3000 rpm for 5 min to remove unlysed RBC and debris. The Optical density (OD) of the supernatant at 420 nm was read in a Spectronic-20 to determine the degree of haemolysis (α or β), as a measure of haemolysin produced in the culture filtrate. Similar determination was carried out with culture filtrate of glucose-supplemented MSB as control.

Capsule (Exopolysaccharide) Formation: The method described by Cruickshank *et al.,* (1980), was adopted for the capsule formation test. Approximately 10 ml volume of *A. hydrophila* was grown in mineral salts medium (MSM), mended with 1% (w/v) glucose and maintained at pH 7.2 in a mechanical shaker (swirling flask) at 13 ^oC at 100 rpm for 5 days. Swirling flask procedures described by Pazur and Forberg (1980), were adopted for the exopolysaccharide capsule production in liquid broth as most procedures currently in use do not achieve selective release of exopolymers from the cells. This procedure was found to enhance clumping of cells as a result of the selective pressure exerted by the swirling process. Increase in the number and size of observable clumps in the suspension and the presence of collar of cells on the walls of the swirling flasks at the liquid-air interface correlated with subsequent increase in the culture biomass detected visually. Capsular material was not easily stained and remained relatively uncolored with a comparatively weak dye such as Methylene blue. Consequently, the India ink wet mount procedures for exopolysaccharide capsule examination was adopted in this study and was found most appropriate, as the black dye sharply outlined the edge of the capsule. The capsular and slime layers were observed in a wet preparation in which India ink was added for contrast (Duguid, 1959). The thickness of the capsule observed virtually was qualitatively scored as high (+++), moderate (++), or low (+).

Antibiosis: Antibiotic susceptibility testing was done using the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) procedure (Cruickshank *et al.,* 1980). The following antibiotics in their various concentrations (µg/ml), were used: kamamycin monosulphate (50 µg/ml), streptomycin sulphate (25 µg/ml), furazolidone (60 µg/ml), ampicillin (100 µg/ml), amikacin (65 µg/ml), flumequine (20 µg/ml), nalidixic acid (30 µg/ml), tetracycline (100 µg/ml), erythromycin (60 µg/ml), cephalothin (65 µg/ml), chloramphenicol (30 µg/ml), gentamycin (70 µg/ml), oxolinic acid (25 µg/ml), nitrofurantoin (20 µg/ml).

A two-fold serial dilution of each antibiotic was prepared in Tryptone soy broth (TSB), and 0.1 ml volume of a 12 h TSB culture of the test organism was added to each dilution. Two controls were included, a blank containing TSB + antibiotic but no bacterial inoculation and TSB with bacterial inoculation but no test antibiotics. Following incubation at 37 ^oC for 24-48 h, each culture was examined for bacterial growth. All dilutions with no apparent growth (indicated by lack of turbidity) was subcultured in TSA, and the highest dilution showing no growth in the TSA subculture were taken as containing the minimal bactericidal concentration.

Toxin Production: The potential for toxin production of the pre-grown isolates was tested by growth in Tryptone Soy broth (TSB) amended with 0.6% yeast extract and incubated at 37°C for 48h. The toxin was harvested from the spent broth by centrifuging at 4000 rpm for 15 mins. The supernatant liquid containing the toxin was partially purified by re-dissolving in 10 ml of distilled water and filtered through Whatman No. 1 filter paper, after which 55 ml of the extracted toxin was used for the virulence assay.

Virulence Studies: Ten 3-months old each of three fish species, *Clarias gariepinus, Clarias gariepinus* X *heterobranchus bidorsalis* hybrid, and *Oreochromis mossambicus* obtained from African Regional Aquaculture Centre (ARAC), Aluu, about 500 m northwest of the University of Port Harcourt, Choba Campus, Port Harcourt Rivers State were introduced into 3 litres of pre-sterilized pond water in clean aquaria. Each aquarium was covered with wire

gauze. To different aquaria was added 0.1, 0.25, 0.5 and 1.0 ml of supernatant of TSB culture fluid of the *A. hydrophila* supplemented with 0.6 % yeast extract and clarified by centrifugation at 4000 rpm for 15 min. After 96h of exposure the number of fish that died were counted and recorded. Following the preliminary tests, confirmatory experiment was carried out with higher doses or concentrations of the culture filtrate. Consequently, 5 ml, 10 ml, 15 and 20 ml of the filtrate were administered to the test fish by the bathe method (immersion of filtrate into the aquaria). All experiments were carried out in triplicates.

Fish Behavioural Studies and Bioassay: Significant behavioural responses of fish to the administered toxin were studied following 96 h of exposure, and the percentage death or survival recorded. The lethal dose of the toxin that could kill fifty percent of the fish samples (LD_{50}) was then determined using the graphic procedure (Litchfield and Wilcoxon, 1949) for estimating the median effective dose and the dose percent effect curve. The interpolated value at 50 % mortality ratio gave the LD_{50} of each toxin at the corresponding concentrations.

Data Analysis: The data for the total heterotrophic bacterial count and substrate utilization test were tested by 2-way analysis of variance with substrate (five levels) and volume of substrate (four levels) as fixed factors.

Haemolytic activity was measured as optical density and/or absorbance (at 420 nm) of liberated haemoglobin using the Spectronic-20. Analysis of variance was then used to test the difference between means as well as their level of significance.

Data from the bioassay and mortality ratios were analysed by 2 -way or 3-way analysis of variance as appropriate. Where data were not normally distributed, appropriate transformation was applied to the values before the analysis. The data obtained from these were used to calculate the fifty-percent lethal dose (LD_{50}) using the graphic method (Litchfield and Wilcoxon, 1949).

RESULTS

Substrate Utilization by *A. hydrophila:* The ability of *Aeromonas hydrophila* to utilize the various substrates as sole sources of carbon and energy has been outlined in Table 1. There was also a remarkable difference in the pattern of growth, and hence the optical density of each substrate at the various concentrations. A significant main effect of concentrations of substrate ($F = 5.3319$; $p < 0.01$) on bacterial counts was observed. Gasoline, toluene and kerosene were utilizable, only at low concentrations. Utilization of the substrate by *A. hydrophila* decreased with increase in hydrocarbon concentration. A significant difference was observed in the growth of *A. hydrophila* while utilizing diesel oil ($F = 12.5693$; $p < 0.001$). A gradual but appreciable increase in turbidity occurred, signifying the ease in which *A. hydrophila* utilized the substrate (Table 1).

Haemolytic Activity: β-haemolysis was observed from haemolytic activity of Ox Red Cells by *A. hydrophila* (Table 2). From analysis of variance, gasoline 0.0983, and diesel oil, 0.5178, had a significant mean difference of 0.4195. There was no significant statistical effect of concentration of substrate ($F = 0.2292$; $p > 0.05$) on the isolate. However, a significant main effect of substrate ($F= 1.32551$; $p <0.001$) was evident.

The substrate differential haemolysis of Ox red blood by *A. hydrophila* was shown in Figure 1. The peak depicted by diesel oil is an indication of the less toxic and/or sublethal or benign constituents of the diesel oil compared to those of gasoline, toluene or kerosene. A similar pattern of sublethal effect was shown by glucose, the control. The higher toxic level of gasoline was shown by the zero slope of its differential haemolysis (Figure 1).

Capsule Formation: Result of the capsule production test by *A. hydrophila* was presented in Table 3. Growth on glucose was observed to enhance minimal capsule production, while high capsule production generally occurred among the hydrocarbon grown isolates

Antibiotic Sensitivity: The result of the antibiotic sensitivity, aimed at determining the most effective chemotherapeutants against *Aeromonas hydrophila* through their Minimal Inhibitory Concentration (MIC) and the Minimal Cidal Concentration (MCC) are presented on Table 4. Chemotherapeutants with good antimicrobial activity included: streptomycin (MIC and MCC at 25 μg/m), flumequine, (MIC 15 μg/ml and MCC 20 μg/ml), Nitrofurantoin, (MIC 20 μg/ml and MCC 20 μg/ml); chloramphenicol, (MIC 15 μg/ml and MCC 20 μg/ml). Nalidixic acid, (MIC 25 μg/ml and MCC 30 μg/ml). The order of efficacy was as follows: flumequine > Nitrofurantoin > Oxolinic acid > Chloramphenicol > Streptomycin > Nalidixic acid.

Fish Behaviour: Table 5 presents the different behaviors observed in the various fish samples following exposure to the culture fluid aimed at determining the toxicity of the medium. Time taken to achieve death of each species correlated with the concentration of the culture fluid used. High concentration produced a concomitant increase in the death rate at reduced time interval. *O. mossambicus* showed least resistance to the culture fluid. Significant behavioral changes like erratic movement were observed at about 6 h after treatment. *O. mossambicus* was observed to be susceptible to high doses of the treatment, while *C. gariepinus* species took a longer time to manifest observable effects at about 9 h. The hybrid of *H. bidorsalis* X *C. gariepinus* was the most resistant even at a higher dose, manifesting observation changes at the 11 h following treatment.

Table 1: Utilization of Hydrocarbon Substrates by *Aeromonas hydrophila*

Substrate	Concentration (ml^{-})	OD $_{420\ nm}$ Over Time (in Days)							
		0	1	2	3	4	5	6	7
Gasoline	0.1	0.002	0.013	0.017	0.022	0.026	0.030	0.036	0.041
	0.25	0.001	0.015	0.022	0.028	0.033	0.039	0.044	0.048
	0.5	0.003	0.010	0.008	0.007	0.005	0.005	0.004	0.002
	1.0	0.002	0.006	0.004	0.004	0.003	0.002	0.002	0.001
Toluene	0.1	0.001	0.019	0.023	0.027	0.032	0.035	0.038	0.042
	0.25	0.003	0.022	0.028	0.031	0.037	0.041	0.046	0.049
	0.5	0.004	0.012	0.010	0.010	0.009	0.008	0.008	0.006
	1.0	0.002	0.010	0.008	0.007	0.006	0.006	0.004	0.003
Kerosene	0.1	0.003	0.021	0.028	0.033	0.039	0.045	0.049	0.055
	0.25	0.002	0.027	0.032	0.038	0.046	0.047	0.045	0.050
	0.5	0.004	0.018	0.015	0.013	0.011	0.011	0.010	0.008
	1.0	0.002	0.016	0.013	0.009	0.007	0.007	0.006	0.005
Diesel Oil	0.1	0.002	0.029	0.037	0.044	0.052	0.058	0.065	0.069
	0.25	0.001	0.033	0.041	0.048	0.057	0.064	0.072	0.078
	0.5	0.004	0.038	0.049	0.056	0.062	0.069	0.074	0.082
	1.0	0.002	0.043	0.055	0.064	0.071	0.074	0.082	0.089
Glucose	0.1	0.004	0.027	0.032	0.037	0.045	0.050	0.059	0.064
	0.25	0.002	0.031	0.035	0.043	0.049	0.057	0.065	0.069
	0.5	0.002	0.036	0.038	0.049	0.053	0.061	0.072	0.077
	1.0	0.003	0.041	0.043	0.055	0.059	0.069	0.078	0.088

Table 2: Haemolytic Activity of *A. hydrophila*

Concentration of Substrate	OD $_{420\ nm}$	Gasoline	Toluene	Kerosene	Diesel Oil	Glucose
0.1	0.165	0.201	0.206	0.345	0.329	0.2492
0.25	0.096	0.183	0.195	0.412	0.344	0.246
0.5	0.070	0.093	0.116	0.601	0.407	0.2574
1.0	0.062	0.80	0.109	0.713	0.502	0.2932
$\bar{x}$	0.0983^{b}	0.1393^{b}	0.1565^{b}	0.5178^{a}	0.3955^{a}	

Means with different superscripts are significant

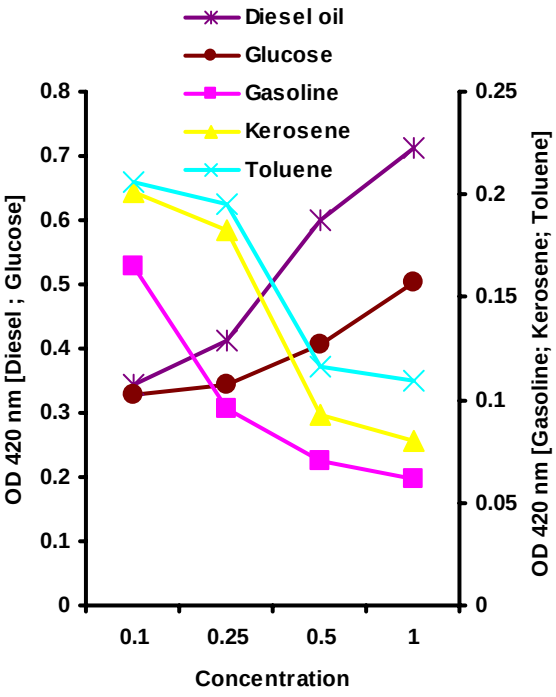

Figure 1: Substrate differential haemolysis of Ox red blood by *A. hydrophila*

Very slow death was observed after several days of the treatment: 24 h – 96 h for *O. mossambicus* and 36 – 96h for *C. gariepinus* species and *the* hybrid of *H. bidorsalis* X *C. gariepinus* respectively.

Bioassay: Response of fish to treatment with the culture fluid is presented in Table 6. The effect was more pronounced at a higher concentration of the toxin as more death occurred at 15 than at 20 ml concentrations. However, there was no statistical difference in the effect of various concentrations used for the bioassay ($F = 0.141$; $p > 0.05$). The effectiveness of the treatment expressed in the mortality rate of fish was shown in Figure 2. *O. mossambicus* showed more instantaneous response to treatment, followed by *C. gariepinus* and lastly by the hybrid of *H. bidorsalis* X *C. gariepinus* which were observed to be most resistant. The percentage survivors of samples were observed to decrease with increased concentration of toxin, with complete death recorded in the 20 ml volume. However, the hybrid of *H. bidorsalis* X *C. gariepinus* were more resistant, and so more survivors were observed even at the 20 ml lethal dose of the toxicant than the other samples. There was nevertheless, no significant statistical effect of mortality rate of fish ($F = 0.393$; $p > 0.05$). The LD_{50} is represented in (Figure 3).

DISCUSSION

This study had analysed the virulence potential of *Aeromonas hydrophila*, as well as its susceptibility pattern to a wide range of antibiotics with a view of determining its pathogenicity. Virulence was observed

Table 3: Capsule Production by *A. hydrophila*

Substrate	Concentration (ml)			
	0.1	0.25	0.5	1.0
Glucose	-	+	+	+
Gasoline	+++	++	+	-
Toluene	+++	+++	++	+
Kerosene	+++	+++	+++	++
Diesel Oil	+++	+++	+++	++

+++ High production; ++ Moderate production; + Low production, - No capsule production

Table 4: Minimal Inhibitory Concentration (MIC) and Minimal CIDAL Concentration (MCC) of Chemotherapeutants and Susceptibility Pattern of *A. hydrophila*

Chemotherapeutants	MIC (µg/ml)	MCC (µg/ml)
Kamamycin	45	50
Streptomycin	25	25
Furazolidone	40	60
Ampicillin	100	100
Amikacin	45	65
Flumequine	15	20
Nitrofurantoin	20	20
Tetracycline	80	100
Erythromycin	40	60
Cephalothin	50	65
Chloramphenicol	15	30
Gentamycin	50	70
Oxolinic acid	20	25
Nalidixic acid	25	30

Table 5: Behavioural Response of Fish Following Intoxication

Behavioural Response	Time of Response (h) after Treatment		
	O. mossambicus	***C. gariepinus***	***H. bidorsalis* X *C. gariepinus***
Fish appear active at surface, gulping air	6 h	9 h	11 h
Fish lying listlessly near water surface	6 h	9 h	11 h
Erratic swimming movements	7 h	10 h	12 h
Fish twisting onto side, exposing abdomen	8 h	11 h	14 h
Slower and irregular movements	10 h	14 h	16 h
Fish on bottom of aquaria, fins folded	12 – 24 h	18 h	20 h
Floating on the surface lifeless, with operculum, mouth open, bulging eyes exposed	24 – 96 h	24 – 96 h	24 – 96 h

TABLE 6:Effect of Toxin on Mortality of Fish Samples

Fish	Mortality Concentration of Toxin (ml)							
	0.1	0.25	0.5	1.0	5	10	15	20
O. mossambicus	0	0	0	0 .2	0 .2	0 .4	0 .7	0.9
C. gariepinus	0	0	0	0	0 .1	0.1	.3	0.8
H. bidorsalis* X *C. gariepinus	0	0	0	0	0	0.1	0.3	0.4

to be an important property of *A. hydrophila* in relation to its pathogenicity; and depended on two factors that may be largely independent of one another; namely, the invasiveness or aggressiveness, and the toxigenic or toxin - producing property of the organism. The ability of *Aeromonas hydrophila* to utilize the various substrates as sole sources of carbon and energy is shown in this study.

There was a significant main effect of concentration of substrate ($F = 5.3319$; $p < 0.01$) at lower concentrations more than higher concentrations. Comparatively, gasoline supported less growth than diesel oil. This may be due to its properties: short-chain carbon length (C_5 – C_9); specific gravity, 0.68 – 0.77; boiling point 30 – 200; flash point – 40 as well as the presence of additives such as anti-knock, mercaptans, anti-oxidants and corrosion inhibitors, which are toxic to microorganisms. Diesel oil, on the other hand, has carbon chain C > (14); boiling point, 180 – 360 and flash point 77. Diesel oil was remarkably utilized probably due to its rich mineral content such as sulphur and some heavy metals (cations) some of which are essential in the synthesis of amino acids in microorganisms (Atlas, 1995). From analysis of variance, significant main effect of 0.1925 (8.130 – 7.9375) was evident. Similarly, toluene and diesel oil had significant main effect of 0.145 (8.130 – 7.985). Toluene has carbon atoms C_{10} - $C_{14;}$ specific gravity 0.78; boiling point 160 – 285, flash point, 77 and complex inhibitory chemicals like lead anti-knock additives (Bertoni *et al.,* 1996).

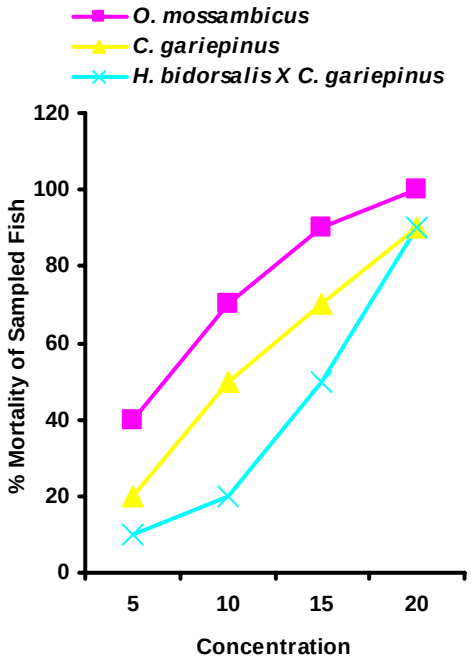

Figure 2: Percentage response of fish to *A. hydrophila*

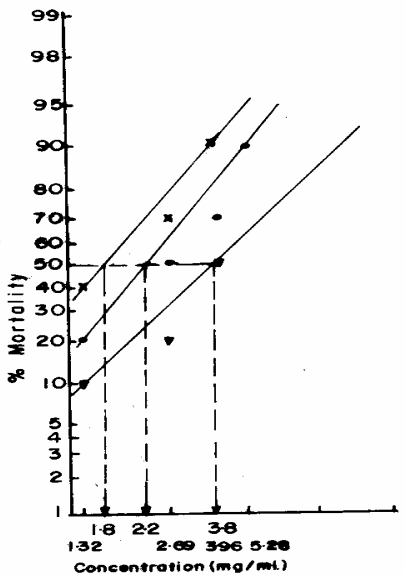

Figure 3: LC_{50} of samples in response to *A. hydrophila*
***x---x Tilapia; o---o Clarias; >---< Clarias* hybrid**

Gasoline, toluene and kerosene were utilized only at low concentrations as they are thought to be poor substrates because of their toxic effect on microorganisms. Data from analysis of variance showed no significant main effect of concentration of substrates of gasoline and toluene, 7.985 – 7.9375 (0.0475); kerosene and toluene, 8.0175 – 7.985 (0.0325). This is attributable to their close carbon range (C_5 - C14), aliphatic components as well as inhibitory additives. The toxicity is probably due to disorganization of cytoplasmic membrane resulting mainly from non-specific effect on membrane proteins including those associated with transport and oxidation (Dutta and Harayama, 2001). Utilization of substrate at sub lethal concentrations might also be genetically regulated, in consonance with the findings of Kozlovsky *et al*., (1993) who reported that genetic information for synthesizing enzymes responsible for degrading these hydrocarbons is enclosed in the extra-chromosomal genetic elements (plasmid) and that the enzymes of a degradative pathway are plasmid – specific. Glucose was maximally utilized as a carbon source, and may represent growth conditions of *A. hydrophila* in its natural environment (Rom-ling *et al.,* 1994) at uncontaminated sites.

Haemolysins are substances that bring about the haemolysis or dissolution of red blood cells. Haemolysin was produced by all the *Aeromonas hydrophila* isolates. This was indicated by the β - haemolysin (ability to lyse red cells completely) exhibited by the organism, in consonance with the reports of Barney *et al*., (1972); Beiheimer and Avigad, (1974), Chopra and Houston (1999). This phenomenon suggests the production of two types of haemolysin responsible for such phenomenon: a heat stable glycolipid, and a heat-labile phospholipase C (PLC), which can both lyse and agglutinate human red cells and platelets respectively (Shortridge *et al.,* 1990*)*. Capsule production was also demonstrated in the study, conforming to the reports of Kenne and Lindeberg, (1983); Sutherland, (1983) who showed that microorganisms produce a large number of structurally diverse extracellular polysaccharides (EPS) with resultant unique rheological properties. The phenomenon of capsule formation is of prime importance among pathogenic bacteria as it enhances the virulence of the organisms as it acts as a defense for the organism against bactericidal factors in body fluids. In capsulate bacteria, the slime is generally similar in chemical composition and antigenic character to the capsular substances. Microbial exopolymers were observed to occur as slime fibers loosely associated with or dissociated from the cells.

Generally, bacterial sensitivity to antibiotics increases with increase in antibiotic concentration. It is noteworthy that the efficacy of antibiotics varies. While some are able to exhibit high bacteriostatic and bactericidal effect on a wide range of organisms at low concentrations, some can only do so at a very high dosage. Of the several chemotherapeutants (in μg/ml) used during this study, streptomycin, flumequine, nitrofurantoin, chloramphenicol and nalidixic acid (in order of potency) were drugs of choice, as resistance of the bacterium was very low, and the drugs had both bacteriostatic and bactericidal effect on the organism. In general, *Aeromonas* strains show a drug sensitivity (susceptibility) to the quinolones (flumequine and oxolinic acid) and the nitrofurance; nitrofurantoin according to the reports of Okpokwasili and Okpokwasili, (1994). However, high resistance to such drugs as: ampicillin, cephalothin, erythromycin, tetracycline, furazolidine and amikamicin observed in the study correlates with the findings of Okpokwasili and Okpokwasili (1994). Drug resistance may be natural or acquired characteristic of microorganisms. It is inferred from

the study that the resistance of *A. hydrophila* to used drugs could have resulted from impaired cell wall or an envelope penetration, enzyme inactivation or altered binding sites. Similarly, acquired drug resistance could have been as a result of mutation, adaptation or gene transfer; and spontaneous mutation, which could have occurred at low frequency. However, Aoki *et al.*, (1981), Toranzo *et al.*, (1985) reported that genetic resistance may be chromosomally or plasmid – mediated, and that plasmid - mediated resistance is typical of the Gram-negative enteric pathogens (such as *A. hydrophila)*. By the process of conjugation, resistant plasmids might have been transferred both between the bacterial strains. Such resistance factors could have coded for multiple antibiotic resistance due to possession of resistant factors (R – plasmids) thereby rendering the drugs impenetrable to the bacterial cell as well as causing conversion of an active drug to an inactive product by enzymes produced by the organism (inactivating enzymes), which correlates with the findings of Röling *et al,* (2002). Nevertheless, it is possible that the observed high resistance pattern of *A. hydrophila* to antibiotics used in the study could have resulted from drug abuse, especially since the drugs in question are cheaper, and more readily available, a scenario about which Toranzo *et al.*, (1985), had warned against in a bid to check drug misuse and the prevalence of bacterial resistance and the associated risk of transfer of resistance to pathogens especially of the aquatic organisms which may induce gastroenteritis. It is further inferred that antibiotic resistance to *A. hydrophila* might also have displayed an intrinsic resistance to the inhibitory or lethal effects of the drugs. Such resistance might depend, for example, on the absence or inaccessibility of those structural and/or functional features against which the antibiotic is effective.

Toxin production evident in the study conforms to the findings of Xu *et al.,* (1998) who reported a heat-labile enterotoxin produced by *A. hydrophila* that produced fluid accumulation in Rabbit Ileal Loop (RIP), and an enterotoxic response in Y – adrenal cells. In a study of 9 isolates of *A. hydrophila*, 69 % were found to produce cytotoxin, and haemolysin Chopra *et al.*, (2000). Kaper *et al.*, (1980) and Sanyal *et al.,* (1978) reported that all strains of *A. hydrophila* isolated from diarrheic and healthy individuals, animals and drinking water, river water and sewage were enterotoxigenic.

From results of previous studies, Barney *et al.*, (1972); Beiheimer and Avigad, (1974); Chopra and Houston (1999), Aeromonas, (*A. hydrophila)* was shown to produce endotoxins, cytotoxin and haemolysin. Xu *et al.*, (1998) and Albert, (2000) on the other hand reported a heat-labile enterotoxin produced by *A. hydrophila* similar to that described in this study, which produced fluid accumulation in RIL and an enterotoxic response in Y-1 adrenal cells. Similarly, Chopra *et al.*, (2000), reported that *A. hydrophila* can produce an enterotoxin, but it is different from that of *Vibrio cholerae* and enterotoxigenic *E. coli*

Exposure of fish to the *A. hydrophila* toxins extracted during the study resulted in some behavioural responses in the tested fish. However, the toxic effect was more pronounced at high, rather than sublethal or low doses. However, *H. bidorsalis* X *C. gariepinus* proved more resistant to the toxic than the other species. This might be attributed to the improved vigor and immuno-competence exhibited by the hybrid. The toxic effect was more pronounced at a higher concentration of the toxicant as more death occurred at 15 than at 20 ml concentrations. The effectiveness of the toxins is therefore expressed in the mortality rate of test samples. The LD_{50} of fish samples shown in Fig., indicated that *O. mossambicus* had LD_{50} at 1.32; *C. gariepinus* at 2.69 and *H. bidorsalis* X *C. gariepinus* at 3.96. The vulnerability of O. *mossambicus* is thus shown by its LD_{50}, 1.32, attributed to such factors as trauma or stress that might have been encountered by the fish while the ability of the other two species to withstand the toxic effect at least a longer period than the *O. mossambicus* is shown by their respective LD_{50}. However, the LD_{50} of the various fish samples was achieved at higher concentrations of the toxins.

In conclusion, results of this study indicated that exopolysaccharide capsule formation, antibiotic resistance, haemolysin production, endo and exo-toxin production are pathogenic potentials of *A. hydrophila.* These features are evidence that the organism is an outstanding life threatening pathogen worthy of further investigation.

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Fasciola gigantica IN ONITSHA AND ENVIRONS

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ABSTRACT

The presence of Fasciola gigantica in cattle slaughtered in Onitsha abattoir and three other abattoirs in Onitsha area of Anambra State, Nigeria was investigated from November to December 2004. The study involved actual postmortem inspection on the slaughtered cattle. The liver were examined for Fasciola by making length wise incision on the ventral side of the liver in such a way that the bile duct and gall bladder are cut open. All cases of Fasciola were detected from the liver. Afor-Igwe abattoir recorded the prevalence rate of 10.8% while the prevalence rates of 7.0%, 7.7% and 13.4% were recorded at Nkwor-Ogidi abattoir, Oye Olisa abattoir and Onitsha main market abattoir respectively. Out of a total of 1580 cattle examined, 166(10.51%) were infected with F. gigantica. Of the 166 diseased liver, 26(15.7%) had light worm load, 77(46.4%) medium worm load and 63(38%) had heavy worm load. The lowest number of worm recovered per liver was 3 while the highest was 88. This study has established the presence of F. gigantica in Onitsha Area. It was also observed that most diseased liver were not condemned. This situation calls for serious attention of the veterinary workers in the state. In view of the fact that these cattle which were brought from the Northern part of Nigeria were made to trek to places of pasture (near streams and rivers) within Onitsha area where the snail intermediate host of the parasite thrives, it is suggested that grazing of cattle should be highly restricted to lesser snail infected areas. The range land system (Artificial pasture land) seems to be the panacea to fascioliasis in cattle.

Keywords – *Fasciola gigantica,* Cattle, Liver, Onitsha

INTRODUCTION

Meat derived from cattle, sheep and goats provides major sources of animal protein for the populace of Eastern Nigeria. These ruminants incidentally serve as definitive host to the parasitic helminthes trematode of the family, Fasciolidae, commonly known as liver flukes. There are various species of these but the economically important ones are *Fasciola gigantica* in the tropics and *F. hepatica* in the temperate region (Ikeme and Obioha, 1973).

F. gigantica is a parasite of the liver and bile ducts of cattle, sheep, goats and wild ruminants in Africa and Asia. It is of great veterinary importance, causing the disease fascioliasis in cattle, accounting for considerable economic loss annually (Ukoli, 1990). The negative impact of helminth infections on livestock productivity in tropical countries has long been established. Reports by Ndarathi *et al.* (1989) and Olusi (1997) contained recent appraisals of this problem.

The primary objective of this research is to investigate the presence and intensity of *F. gigantica* in cattle slaughtered in Onitsha Urban and environs. This investigation hopefully would not only show the necessity for the routine monitoring and surveillance of this parasite infection on cattle, goat and sheep, but also should make it possible to assess the potential public health and economic importance.

MATERIALS AND METHODS

Study Area and Cattle: The study area is Onitsha urban and its environs. The sites are Onitsha main market abattoir, Afor-Igwe abattoir, Nkwor-Ogidi abattoir and Oye Olisa abattoir all within 10 km radius of Onitsha in Anambra State of Nigeria. Onitsha is a big city with many traders and businessmen and thus large numbers of cattle were slaughtered daily. The cattle slaughtered in this area were brought off the Hausa and Fulani herds men from the Northern part of Nigeria. The breed of cattle studied were trade cattle, white Fulani (Bunaji), Sokoto Zebu/guddi, Fulani zebu and Nigerian Fulani (Abore). The herdsmen or their agent brought them down to Onitsha and environs in lorries. For the fact that the cattle were not slaughtered as soon as they arrived, they were made to trek to places of pasture within Onitsha area.

Organ and Meat Inspection: The slaughter houses were visited for 2 months from November 2nd to December 3rd, 2004. The slaughter houses were visited 3 times every week. This was done between 5 am and 7 am, the period when cattle are slaughtered in the area. On the whole, one thousand five hundred and eighty (1580) cattle were inspected. The inspection of the meat was made possible through the co-operation of the veterinary staff on duty at the abattoir. In most abattoirs, meat inspection facilities are inadequate and procedures are not uniform or standardized.

Table 1: Infection rate of *Fasciola gigantica* in cattle slaughtered in Onitsha abattoir and environs

Month	Onitsha main abattoir		Afor-Igwe abattoir		Nkwor-Ogidi abattoir		Oye Olisa abattoir		Total	
	No. Ex.	No. Inf.%	No. Ex.	No Inf. %	No Ex.	No Inf.%	No. Ex.	No. Inf.%	No. Ex.	No Inf.%
Nov.	300	45(15.00)	202	21(10.40)	125	8(6.402)	180	13(7.22)	807	87(10.78)
Dec.	303	36(11.88)	170	19(11.18)	105	8(7.62)	195	16(8.21)	773	79(10.22)
Total	603	81(13.4)	372	40(10.8)	230	16(7.0)	375	29(7.7)	1580	166(10.5)

Table 2: *Fasciola gigantica* intensity in diseased cattle

Abattoirs	Light (0-10 worms)	Medium (11 -50 worms)	Heavy (>50 worms)	Total
Onitsha	9	44	28	81
Afor-Igwe	8	17	15	40
Nkwor-Ogidi	2	6	8	16
Oye Olisa	7	10	12	29
Total	26 (15.7%)	77(46.4%)	63(38%)	166
	Lowest No. per liver=3		Highest No. per liver =88	

The work involved actual postmortem inspection on the cattle. The livers were examined for *Fasciola* by making length wise incisions of the ventral side of the liver in such a way that the bile duct is cut open. Then forceps was used to pick the exposed worms in the bile duct and gall bladder. The flukes recovered from each cattle were placed in labelled containers and taken to the laboratory for identification, counting and preservation. Infected liver were classified according to the total number of worms recovered per liver into light (1-10), medium (11-50) and heavy (>50).

RESULT

The infection rate is shown in table 1. Onitsha main market abattoir recorded the infection rate of 13.4 % while the infection rates of 10.8 %, 7.0% and 7.7 % were recorded by Afor-Igwe abattoir, Nkwor-Ogidi abattoir and Oye Olisa abattoir respectively. On the whole there were 116 cases of *Fasciola gigantica* infections out of the 1580 cattle inspected representing 10.51%. Eighty-seven (10.78 %) of the infections were detected in November while seventy-nine (10.22 %) were detected in December. The intensity of infection is shown in table 2. Sixty-three (38 %) of the diseased liver had heavy worm loads of 50 and above.

DISCUSSION

The result obtained in this study is an indication that *F. gigantica* exist in the study area. The infection rate of *F. gigantica* in cattle slaughtered in Onitsha area found to be 10.51 % was moderately low. Although no similar study was known to have been carried out in the same area. A comparison with related study within the geographical south east Nigeria though in 1973 revealed that *F. gigantica* prevalence was 39 % in Nsukka urban abattoir (Ikeme and Obioha 1973). In Zaria, northern part of Nigeria, a high prevalence rate of 65.4 % was reported by Schillhorn *et al* (1980). However recently, low prevalence of 10.00% was recorded in same Nsukka urban abattoir by Ngwu *et al* (2004). The low rate observed in this study which was similar to that observed at Nsukka (Ngwu *et al.,* 2004) recently could be attributed to many factors which include better management of cattle. This could be due to the fact that healthier animals now reach the southern market where the study was conducted. Mode of transportation of the slaughtered cattle from the northern to the eastern part of the country would have as well influenced the result. Probably, with modernized means of transportation (trailers and lorries) the cattle are restricted to the shepherd's choice of pasture coupled with their awareness of the economic consequences of leading the cattle to infected grazing grounds.

The period of this study was another factor that could have influenced the rate of infection. This is because the prevalence rates of 41.3% was reported in rainy season while that of 32.7 % was reported during the post rainy season periods in Borno State of Nigeria (Egbe-Nwiyi and Ohaudrai, 1996). This could be due to the fact that snail which serves as the intermediate host abounds in rainy season.

A reasonable number of the diseased liver with heavy worm load were hard, small with rough and uneven surfaces with a lot of fibrous tissues and unfit for human consumption. This report recorded many cattle without infection and few with light infection. This could be attributed to the fact that the slaughtered cattle were adult animal that might have been previously infected which resulted in cirrhosis of the liver that opposed penetration of young flukes contracted later in the season.

This study has clearly demonstrated the presence of *F. gigantica* in cattle slaughtered in Onitsha area abattoir. Although the rate of infection is moderately low, the economic implications should not be overlooked. This is because some infected liver were very bad while some of them were not condemned. This situation calls for serious attention of both the veterinary workers and the public health planners in the state. Since fascioliasis constitute a major intestinal problem and liver condemnation in cattle. The grazing of cattle should be highly restricted to areas of lesser snail infected site. The range land systems (Artificial pasture land) seem to be the panacea to fascioliasis in cattle. If cattle are

fed with hays, the rate of fasciola *gigantica* will be at its low ebb.

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EFFECT OF ECOSYSTEM CHANGES ON AIR-BORNE AND VEGETATION-DWELLING ARTHROPODS IN AGU-AWKA AREA OF AWKA

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ABSTRACT

The study on the impact of ecosystem changes on air-borne and vegetation-dwelling arthropods was carried out in the Agu-Awka area of Awka, Anambra State capital. Areas investigated were roadsides, cultivated agricultural, built-up, uncultivated agricultural and forest sites using the sweep net for arthropods on vegetation and the sticky trap for air-borne flying arthropods. The forest site acted as control. Ecosystem changes from close forest to open environments reduced species richness for vegetation-dwelling arthropods but increased the species richness of air-borne arthropods. For the vegetation-dwelling fauna, the forest site recorded 14 species while the disturbed built-up sites had only 4 species. The differences between the sites were significant (P < 0.05). For the air-borne arthropods, there were no species in the forest while the highest number of species (7) was recorded in the uncultivated agricultural sites. The differences over the study sites for air-borne species were not significant (P > 0.05). The ecosystem change decreased significantly the species abundance of vegetation-dwelling arthropods from 42 in the forest to 14 in the built-up sites (P < 0.05), while the species abundance of air-borne fauna was significantly increased from 0 in the forest to 43 in the uncultivated agricultural sites (P > 0.05). The species diversity for the vegetation-dwelling arthropods decreased significantly from 0.856 in the forest to 0.384 in the built-up sites (P < 0.05), while it increased significantly from 0.000 in the forest to 0.611 in the uncultivated agricultural sites for the air-borne arthropods (P < 0.05). For the vegetation-dwelling arthropods, 6 insect species and 6 spider species were dominant in the sites that had undergone environmental changes while 1 insect species and 3 spider species were dominant in the forest. For the air-borne fauna, no species was found in the forest while 7 insect species were dominant in the sites which had experienced ecosystem changes.

Keywords: Ecosystem change, Flying insects, Vegetation-dwelling arthropods, Agu-Awka

INTRODUCTION

Man often causes ecosystem changes due to activities such as agricultural clearing, rangeland grazing, urbanisation, road construction and mining (Majer and Beeston, 1996). Such activities can affect genetic diversity, species diversity and ecosystem diversity (WRI, IUCN and UNEP, 1992). Loss of biodiversity is a great problem of environmental and ecological consequences and humanity depends on biodiversity for fuel, food medicine and raw materials. The continuous removal of forests for various agricultural and industrial purposes has caused the loss and degradation of the primary tropical forests, leaving only man-made ones. This destruction causes extinction or loss of richness for those species whose habitats have been altered by man (Adebayo, 1995).

Studies of arthropod responses to ecological change can enhance man's understanding of the effects of human disturbance and landscape modification on the terrestrial ecosystem. In addition, species diversity can be measured using the number of species present and their relative abundance (Watt *et al*, 2002). Agu-Awka, located at the edge of Awka, the state capital, is about 7.6 km^2. The area has been witnessing ecosystem changes, such as agricultural clearings, road construction and urbanisation. This study was carried out to determine the impact of man-made ecosystems on the species richness, abundance and diversity of vegetation-dwelling and air-borne arthropods.

MATERIALS AND METHODS

Five major sites were chosen at Agu-Awka to cover roadsides, cultivated agricultural, built-up, uncultivated agricultural and forest sites. The forest site was used as the control. The study was carried out during the rainy season (May – July).

Study Site: Roadsides had sub-units of two tarred and two untarred roads giving a total area of 1000 m^2. The cultivated agricultural sites comprised two cassava farms and two oil-palm plantations as sub-units with a total area of 1800 m^2. The built-up sites had two residential and two industrial sub-unit giving a total area of 1875 m^2, while the uncultivated agricultural sites were made up of two primary succession and two secondary succession sub-units of a total area of 1800 m^2. The slight variations in the total areas of sites studies were not significant.

Sampling Techniques: The sweep net was used to sample arthropods on vegetation while the sticky tap was used to catch air-borne flying arthropods (Sutherland, 1997). Ten sweeps were taken along each roadside, 5 sweeps for each side of the road, 0.5 m from the road edge at alternating intervals of 100 metre between 8 am and 11 am on sampling days. For each of the other sites (cultivated and uncultivated agricultural sites, built-up and the forest sites), perpendicular lines were marked out in each site and 5 sweeps taken along each of the perpendicular lines at designated intervals of not less than 7 m and not more than 10 m apart.

The sticky traps were green-coloured beer bottles, smeared with venoline jelly to act as an adhesive substance and were hoisted upside-down on 1.5 m wooden poles. The traps were set up between 8 am and 11 am in the morning and examined after 24 hours. Ten (10) traps were used for each roadside, five for the left side and five at alternate intervals of 100 m on the right side each placed 0.5 m from the road edge. In each of the cultivated and uncultivated agricultural and the forest sites, traps were positioned along the marked out perpendicular lines: five (5) along the horizontal and five (5) along the vertical lines at intervals not less than 6 m and not more than 8 m. In the building sites, due to the stratification of the vegetation, 8 traps were placed in a straight line across the sites in areas with low vegetation at 4 m intervals, while 2 traps were set along the sides with high vegetation cover at 10 m intervals. Trapping was conducted only once per site during the study period. The Shannon-Weaver index of diversity (Shannon-Weaver, 1963) was used to assess species diversity in the study sites ($H' = nlogn - \sum filogfi/n$, where i = the categories, f = the number of observations in category i, and n = the sample size). The total number of species in each site was used to assess species richness while the average faunal abundance/average number of individuals per site was computed from the total number of individuals of the various species encountered in the sub-units. The percentage dominance of the various species was used to determine the dominant species. A one-way analysis of variance was used to compare the biodiversity indices between the study sites. Insect identification was done with the help of the *Check-list of Insects of Nigeria* (Medler, 1980).

RESULTS

Vegetation-Dwelling Arthropods

Species richness: The species richness of vegetation-dwelling arthropods is displayed in Table 1. The highest number of arthropod species on vegetation was recorded in the forest site (14), while the industrial sites had the least number of 3 species (Table 2). The differences in species richness over the study sites were significant ($P < 0.05$).

Average faunal abundance: The forest site recorded the highest average faunal abundance (42), while the lowest was in the built-up sites (14) Table 2. The differences in average faunal abundance for the species over the study sites were significant ($P < 0.05$).

Faunal diversity: The index of diversity (Table 2) was highest in the uncultivated agricultural sites ($H = 0.863$), followed by the forest site ($H = 0.856$) and lowest, in the built-up sites ($H = 0.384$). The differences between the sites were significant ($P < 0.05$).

Dominant species: For the vegetation-dwelling arthropods, there were 6 dominant species. On the roadsides were *Lepisiota capensis* (52%), *Camponotus acvapimensis* (26%) and *Balelutha hospes* (22%). In the cultivated agricultural sites, the dominant species were *Camponotus acvapimensis* (44%), *Lepisiota capensis* (38%) and *Bemisia tabaci* (17%), while in the built-up sites, the dominants were *Camponotus acvapimensis (73%) and Nabi's balckburni* (27%). In the uncultivated agricultural sites, the dominants were *Leptopterna dolobrata* (58%), and *Camponotus acvapimensis* (42%). The forest site was dominated by only *Camponotus acvapimensis* (Table 3). For the spiders, 7 species were dominant over the study sites. On the roadsides were *Amaurobius spirilis* (40%), *Peucetia viridan* (30%), *Heteropoda venatoria* (20%) and *Sceliform coementarium* (10%). In the cultivated agricultural sites, the dominants were *Sceliform coementarium* (60%) and *Amaurobius spirilis* (40%). In the built-up sites, the only dominant species was *Sceliform coementarium* while in the uncultivated agricultural sites, the dominant species were *Sceliform coementarium, Heteropoda venatoria, Gasteracantha areuata and Peucetia viridan* each showed 25% dominance. In the forest site, the dominant species were *Amaurobius spirilis, Salticus sp.,* and *Misumena vatia*, each was approximately 30% dominant.

Air-borne Flying Insects

Species richness: The species richness of air-borne flying insects is shown in Table 4. The highest number of species (7) was recorded in the primary succession sub-unit and the least (0) in the forest site where no flying insect was recorded (Table 5). The differences in species richness over the study sites were not significant ($P > 0.05$).

Average insect abundance: The uncultivated agricultural sites had the highest average insect abundance of 43 while the built-up sites and the forest recorded 12 and 0 respectively (Table 5). The differences in average abundance for air-borne flying insects over the study sites were significant ($P < 0.05$).

Insect diversity: The uncultivated agricultural sites recorded the highest insect diversity of $H = 0.611$, while the forest site recorded the lowest insect diversity of $H = 0.000$ (Table 5). The differences between the sites were significant ($P < 0.05$).

Dominant species: There were 7 dominant insect species. In the roadsides, the dominant insect species were *Drosophila melanogaster* (86%) and *Hippodamia convergens* (14%). In the cultivated agricultural sites, the dominant species were *Drosophila melanogaster* (49%), *Tenthredinidae* (19%), *Aedes sp.* (16%), *Camponotus acvapimensis* (8%) and *Sitophilus sp.* (8%). In the built-up sites, the dominant species were *Drosophila melanogaster* (62%), *Hippodamia convergnes* (19%) and *Camponotus acvapimensis* (19%). In the uncultivated agricultural sites, the dominant species were *Drosophila melanogaster* (74%), *Epilachna varivestis* (15%) and *Aedes sp.* (11%). In the forest site, no air-borne insects were recorded (Table 6).

DISCUSSION

Ecosystem changes in the Agu-Awka area of Awka were brought about by urbanization, agricultural clearings, road constructions, fuel wood gathering and infrastructure. Arthropods are important in ecological studies as they contribute significantly to the biodiversity of the biosphere and are important to the overall health of the terrestrial ecosystem.

Table 1: Species richness/composition of vegetation-dwelling arthropods in Agu-Awka area, Awka

	Major Sites	Sub-Units	Insect Species	Spiders	Ticks
1	Roadsides	: Untarred Roads	*Camponotus acvapimensis, Balelutha hospes, Leptopterna dolabrata, Lepisiota capensis*	*Amaurobius spirilis, Sceliform coementarium Amaurobius spirilis,*	*Demodex canis*
		: Tarred Roads	*Lepisiota capensis, Camponotus acvapimensis, Hippodamia convergens, Nabis blockburni, Zonocerus variegatus, Mantis religiosa*	*Heteropoda venatoria, Peucetia viridian*	---
2	Cultivated Agricultural	: Cassava Farms	*Lepisiota capensis, Bemisia tabaci, Camponotus acvapimensis, Leptopterna dolabrata*	*Sceliform coementarium, Peucetia viridian*	---
		: Oil Palm Plantation	*Camponotus acvapimensis, Bemisia tabaci, Lepisiota capensis, Sitophilus sp., Crematogaster sp.*	*Sceliform coementarium, Peucetia viridian*	---
3	Built-up	: Residential	*Nabis blackburni, Camponotus acvapimensis, hippodamia convergens, Bemisia tabaci*	*Sceliform coementarium*	---
		: Industrial	*Camponotus acvapimensis. Harparus pennsylvanicus Bemisia tabaci*	---	---
4	Uncultivated Agricultural	: Primary Succession	*Leptopterna dolabrata, Camponotus acvapimensis, Labidura riparia, Nezara viridula, Zonocerus variegatus, Nabis blackburni, Tenthredinidae, Drosophila melanogaster*	*Gasteracantha sp., Salticus sp.*	---
		: Secondary Succession	*Camponotus acvapimensis, Lepisiota capensis, Hippodamia convergens, Nezara viridula, Leptopterna dolabrata, Zonocerus variegatus, Tenthredinidae.*	*Sceliform coementarium, Heteropoda venatoria*	---
5	Forest	: Control	*Camponotus acvapimensis, Hippodamia convergens, Lepisiota capensis, Aedes sp., Periplaneta brunnea, Bemisia tabaci, Copa occidentalis, Krausara angulifera, Fannia canicularis, Bourletiella hortensis, Neotennes connexus*	*Amaurobius spirilis, Salticus sp., Misumena vatia.*	---

Table 2: Biodiversity Indices of vegetation-dwelling arthropods in Agu-Awka area, Awka

Major Study Sites	Sub-Units of the Study Sites	Species Richness (Approx. No. of Species Present)			Average Faunal Abundance			Shannon-Weaver Diversity Index (H)	
			Insects	Spiders		Insects	Spiders	Sub-Units	Major sites
Roadsides	Untarred roads	6	4	2	20	18	2	0.675	0.721
	Tarred roads	9	6	3	32	24	8	0.766	
Cultivated	Cassava farms	6	4	2	27	24	3	0.523	
Agricultural	Oil-palm Plantations	6	5	1	28	26	2	0.551	0.537
Built-up	Residential	5	4	1	14	11	3	0.529	0.384
	Industrial	3	3	=	13	13	=	0.239	
Uncultivated	Primary Succession	10	8	2	22	19	3	0.885	
Agricultural	Secondary Succession	9	7	2	20	18	2	0.840	0.863
Forest (Control)	-	14	11	3	42	39	3	0.856	

Table 3: Dominant vegetation-dwelling insect species in Agu-Awka area, Awka

Dominant Species	Average Number of Individuals Per Site								
	Roadsides		Cultivated Agricultural		Built-Up		Uncultivated Agricultural		Forest
	1	2	3	4	5	6	7	8	9
1. ***Camponotus acvapimensis*** (Hymenoptera)	7	--	--	15	--	11	--	5	6
2. ***Lepisiota capensis*** (Hymenoptera)	--	14	13	--	--	--	--	--	--
3. ***Leptopterna***									

dolabrata **(Hemiptera)**	--	--	--	--	--	--	7	--	--
4. *Nabis blackburni* (Homoptera)	--	--	--	--	4	--	--	--	--
5. *Balelutha hospes* (Homoptera)	6	--	--	--	--	--	--	--	--
6. *Bermisia tabaci* (Homoptera)	--	--	6	9	--	--	--	--	--

Key: 1 tarred roads, 2 Tarred roads, 3 Cassava farms, 4 Oil palm Plantations, 5 Residential, 6 Industrial, 7 Primary Succession, 8 Secondary succession, 9 Control

Table 4: Species Richness/Composition of Air-Borne Flying Insects In Agu-Awka Area, Awka

	Major Sites	Sub-Units	Insect Species
1	**Roadsides**	**: Untarred Roads**	*Drosophila melanogaster, Hippodamia convergens, Componotus acvapimensis*
		: Tarred Roads	*Drosophila melanogaster, Hippodamia convergens, Tenthredinidae*
2	**Cultivated Agricultural**	**: Cassava Farms**	*Drosophila melanogaster, Aedes sp., Tenthredinidae, Sitophilus sp.*
		: Oil Palm Plantations	*Drosophila melangaster, Camponotus acvapimensis Sitophilus sp.*
3	**Built-up**	**: Residential**	*Drosophila melonogaster, Hippodamia convergens, Musca domestica*
		: Industrial	*Camponotus acvapimensis. Drosophila melanogaster*
4	**Uncultivated Agricultural**	**: Primary Succession**	*Drosophila melanogaster, Epilachna varivestis, Camponotus acvapimensis, Lepisiota capensis, Aedes sp., Aphis sp., Nezara viridula*
		: Secondary Succession	*Drosophila melanogaster, Epilachna varivestis, Camponotus acvapimensis, Aedes sp., Hippodamia convergens*
5	**Forest**		NIL

Table 5: Biodiversity Indices Of Air-Borne Flying Insects In Agu-Awka Area, Awka

Major Study Sites	Sub-Units of the Study Sites	Species Richness (Approx. No. of Species Present)	Average Faunal Abundance		Shannon-Weaver Diversity Index (H)	
			Sub-Units	Major sites	Sub-Units	Major sites
Roadsides	**Untarred roads**	3	26	26	0.174	0.209
	Tarred roads	3	26		0.244	
Cultivated Agricultural	**Cassava farms**	4	29		0.567	0.500
	Oil-palm Plantations	3	13	21	0.433	
Built-up	**Residential**	3	12	12	0.431	0.344
	Industrial	2	12		0.257	
Uncultivated Agricultural	**Primary Succession**	7	40	43	0.680	0.611
	Secondary Succession	5	45		0.542	
Forest (Control)		0	0			0.000

Table 6: Dominant Air-Borne Flying, Arthropod Species In Agu-Awka Area, Awka

Dominant Species	Average Number of Individuals Per Site								
	Roadsides		Cultivated Agricultural		Built-Up		Uncultivated Agricultural		Forest
	Untarred roads	Tarred roads	Cassava farms	Oil palm Plantations	Residential	Industrial	Primary Succession	Secondary succession	Control
1. *Drosophila melanogaster* (Diptera)	23	21	11	7	6	7	17	23	--
2. *Hippodamia convergens* (Coleoptera)	3	4	--	--	4	--	--	--	--
3. *Epilachna varivestis* (Coleoptera)	--	--	--	--	--	--	--	8	--
4. *Sitophilus sp.* (Coleoptera)	--	--	--	3	--	--	--	--	--
5. *Aedes sp.* (Diptera)	--	--	6	--	--	--	6	--	--
6. *Tenthredinidae* (Hymenoptera)	--	--	7	--	--	--	--	--	--
7. *Camponotus acvapimensis* (Hymenoptera)	--	--	--	3	--	4	--	--	--

At the base of many food chains, arthropods are important components of the diet of invertebrates and vertebrates. They also form an integral part of the nutrient and energy-processing ability of the soil and demonstrate rapid responses to ecosystem change (Coleman and Crossley, 1996).

Morris 2000 cited in Hannay (2001), stated that by studying arthropod responses to ecological changes, one can better understand the effects of human disturbance and landscape modification on terrestrial systems. In the study sites of Agu-Awka, arthropods on vegetation responded to ecosystem changes by decreases in species richness in the roadsides (8) cultivated agricultural (6), built-up (4) and uncultivated agricultural (10) sites when compared to the forest site (14). The decrease in species richness was likely due to destruction of the habitat on which the fauna lived during the course of urbanisation (Adebayo, 1995; Pielou, 1996; Kozlov and Zvereva, 1997 and Watt *et al*, 2002). The greatest reduction in species richness was recorded in the built-up sites and Blair and Launer (1997) had observed that the greater the degree of urbanisation, the greater the decline in species richness of vegetation-dwelling arthropods. The number of species recorded in the uncultivated agricultural sites (10) appeared to indicate that long fallow periods could restore species richness in agriculturally disturbed lands. Eggleton *et al* (1996) cited in Watts *et* al, (2002), reported that complete forest clearance reduced the number of termite species in the Mblamayo Forest Reserve, Cameroon, but partial manual forest clearance and establishment of a forest plantation was not detrimental to termite species richness.

For the air-borne insects at Agu-Awka, there was an increase in species richness in the other study sites compared to the forest site which recorded no air-borne species. Hetrick *et al* (1998) remarked that there was increase in species composition of aerial insects as the ecosystem changed from close forest to open environment. This could be the case in the primary succession sub-unit sites with open environment where an average of 7 species was recorded. The low species richness recorded on the roadsides (3) and built-up sites (3) could be due to severe habitat destruction and fragmentation during urbanisation (Ofomata, 1981 and Hannay, 2001).

Faunal abundance of vegetation-dwelling arthropods showed a significant decrease from 42 in the forest site to 13 in the built-up sites. This could be attributed to the devastating impact of road construction, urbanisation and agricultural practices on biodiversity (Ofomata, 1981; Coyle 1981 and Wells *et al,* 1983). Blair and Launer (1997) also remarked that there was a decrease in population of arthropods on vegetation from natural to urban areas due to a shift in habitat structure. The fairly high average abundance in the cultivated agricultural (28) and roadsides (26) sites may be due to the spread of exotic and invasive species as was reported by Hannay (2001) in his study on the impact of roads on arthropods. In the industrial sub-unit of the built-up sites, no spider was recorded. Rypstra *et al*.(1999) reported that structural complexity of the environment enhanced spider abundance. The industrial infrastructure in Agu-Awka had limited areas for spiders to attach their webs. The tarred road sub-unit recorded 8 spiders as opposed to 3 in the forest site. This situation may be due to fact that open environments with vegetation, as seen on roadsides, cultivated and uncultivated agricultural sites, with their abundant insects, fauna, provided much food and cover to the spiders that preyed on insects. Faunal abundance for air-borne or free-flying insects increased in the other sites compared to the forest. The increase could be due to a shift from close forest to open environment during urbanization which attracted invasive pioneer species (Haskell, 2000 and Hannay, 2001).

Species diversity of fauna on vegetation decreased from 0.856 in the forest site to 0.384 in the built-up sites. This decline in species diversity was probably caused by habitat destruction during urbanization and subsequent migration to new habitats. This resulted in great faunal concentration in a few habitats and reduced evenness in distribution (Pielou, 1996; Kozlov and Zvereva, 1997 and Ofomata, 1981). The high faunal diversity in the uncultivated agricultural sites could be attributed to the fallow length and apparent recovery of the site (0.863) from the initial impact of agricultural practice and invasion by fauna from adjacent sites to occupy vacant ecological niches (Mader, 1984 and Hannay, 2001). The fairly low faunal diversity of the built up sites (0.384) may be attributed to these areas experiencing greatest loss of vegetation and faunal species through destruction and construction activities (Ofomata, 1981; Kozlov, 1997 and Hannay, 2001). Faunal diversity of the air-borne or free-flying insects increased in other study sites compared to the forest (0.000 to 0.611). The increase could be due to change from close forest to open environment (Hetrick *et al,* 1998). The differences in the faunal diversity were significant showing that faunal diversity increased unevenly in the different sites. The uncultivated agricultural sites had the highest diversity of 0.611 due to these areas having relatively low vegetation and open environment from where the air-borne insects could fly freely. The low diversity of the roadsides (0.209) was probably due to high wind movement from passing vehicles that does not permit easy insect flights despite the low vegetation and open environment (Hannay, 2001).

In the forest, site with close vegetation and no ecosystem change, only one insect species (*Camponotus acvapimensis*) and three (3) spider species were dominant while in the other sites which had open environment and experienced varying ecosystem changes, five other insect species apart from *Camponotus acvapimensis* and six (6) spider species were dominant. For the air-borne insects, the forest close environment did not favour the flying insects which accounted for their absence. In the other sites which had open environment due to ecosystem changes, 7 dominant insects were encountered. Whitney and Forster (1988) and Motzkin *et al*; (1996) had observed that land use strongly influenced the upsurge of more arthropod species at the local as well as landscape scale during their study in New England and through competition, those favoured by the disturbed environments become dominant. For vegetation-dwelling species, *Camponotus acvapimensis* was dominant both in the forest and disturbed environments, showing that the species could be endowed with broad tolerances or adaptations to close forest and open environments. *Drosophila melano gaster* was more abundant than every other air-borne insect species in all the disturbed sites indicating that it might have a wider ecological tolerance range and adaptability to the disturbed habitats than other aerial species.

In conclusion, urbanization should be drastically scaled down in the Agu-Awka area of Awka because of its negative impact on plant and animal biodiversity. Certain areas should be designated as forest reserves where stable natural ecosystems can be maintained in the interest of promoting biodiversity. Finally, crop lands should be subjected to long fallows of 4 – 5 years in order to restore lost species richness and diversity.

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HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS OF SULPHADIMIDINE IN NIGERIAN MONGREL DOG

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ABSTRACT

Haematological and biochemical effects of sulphadimidine were studied in Nigerian mongrel dogs. Five Nigerian mongrel dogs of either sex weighing between 7 and 12 kg were used for the study. The pretreatment blood and serum samples were collected and the weight of animals taken before the administration of 100 mg/kg body weight for a period of 7 days. The animals were weighed daily. The results showed that there was no significant difference between preadministration and post administration weights (P>0.05) of dogs. Packed cell volume decreased significantly (P < 0.05) with duration sampled dogs. Liver function test revealed significant decrease (P < 0.05) of total bilirubin and alkaline phosphatase. Other indices of liver function and electrolytes indices were normal (P > 0.05). The mean weight gain (8.8 ± 2.04 kg^a) of the animals before sulphadimidine administration was comparable with the weight gain (8.77 ± 0.89 kg^b) of animals after the sulphadimidine administration. Sulphadimidine caused anaemia of moderate value (26.4 ±3.36%^{a}) in the treated samples as compared to pretreated samples (46.4±6.27^b). Total bilirubin (12.32 ±1.4 µmol^a) in pretreatment samples was decreased in comparison with treated (18.5 ±2.0 µmol/l^b) samples. Alkaline phosphatase was decreased in preadministration samples (114.2 ±5.7µg/l^b) as compared to post administration samples (130 ±9.61 µmol/l^a). Therefore longtime administration of sulphadimidine in anaemic mongrel dogs may aggravate anaemic condition. Sulphadimidine may increase renal excretion of bilirubin and decrease bone mineralization in mongrel dogs during bone formation.

Keywords: Haematology, Biochemical effect, Sulphadimidine, Nigerian Mongrel, Dog

INTRODUCTION

The systemic availability of a drug is the amount of administered drug which reaches the systemic circulation intact (Graham-Smith and Aronson, 1992). Measurement of drug concentration in the blood and urine are performed to determine the need for adjustment of the dosage or of the schedule of administration (Saganuwan *et al.*, 2003). Sulphadimidine, a systemic sulphonamide, has maintained an active place in the armamentary of antimicrobial drugs used in veterinary medicine (Saganuwan *et al.*, 2003). It has been proven clinically to be useful for wide range of microbial diseases caused by gram negative and positive bacteria, Nocardia, Actinomyces, Chlamydia, Toxoplasma and Coccidia (Bevil, 1982). Sulphadimidine is 79 % plasma protein bound with half-life of 3.88 to 15.4 hours and has particularly large percentage (60 – 90 %) excreted as acetylated derivatives (Saganuwan *et al.*, 2003). The estimation of bioavailability of sulphadimidine is usually based on the cumulative urinary excretion of the drug (Baggot, 2001).

The protein fractions in the blood are commonly estimated in the serum and do not include fibrinogen that will be precipitated when the blood clots. The main serum proteins are albumin and globulin (Kombo-Owiye and Reid, 1991). The extent of drug binding to plasma proteins varies with the concentrations of drug and plasma protein, the affinity being between drug-binding protein and drug and the number of binding sites per molecule. Within the range of therapeutic concentrations, the extent of drug binding in healthy animals is concentration dependent for some drugs and animal models (Baggot, 2001).

Albumin largely accounts for the binding of acidic drugs such as sulphonamides in plasma. The range of total plasma/serum protein concentration (6.0 - 8.5 g/dl) is similar in domestic animals and humans (Baggot, 2001). Species variation in the binding of acidic drugs may be attributed to differences in the configuration of the plasma albumin that would affect the binding capacity of protein (Baggot, 2001). The aim of the present study was not to establish only normal haematological and biochemical parameters in the healthy dogs but also, to investigate the effects of sulphadimidine on these parameters. The study may serve as a guide to avoiding adverse effects that may be caused by sulphadimidine in Nigerian mongrel dogs as species variation, sex, age, disease condition, environment and nutritional factors sometimes play great role in disposition kinetics of a particular drug.

MATERIAL AND METHODS

Experimental Animals: Five Nigerian mongrel dogs of either sex weighing between 7 and 12 kg were used for this study. The dogs were purchased in Makurdi, Benue State, Nigeria from a dog owner. The dogs were borne the same day and from the same

mother. But they were 6 - 7 months old and fed daily with boiled rice, beans and meat, water was provided adlibitum.

Drug Administrations and Sample Collection: Sulphadimidine was intramuscularly administered at the dose rate of 100 mg/kg body weight into thigh muscles of the 5 dogs daily for a period of 7 days. Prior to administration of sulphadimidine, control blood samples were collected from the dogs: 2 mls of blood was collected from the cephalic vein of each dog into test tubes containing ethylenediamminetetraacetate (EDTA) as anticoagulant for haematological parameters. Another 4 - 5 mls of whole blood was collected from each dog but allowed to coagulate and serum collected for quantitative in vitro determination of biochemical parameters: liver function test and electrolytes determination.

After that, the animals were weighed before sulphadimidine administration and after sulphadimidine administration for 7 days. At the end of 7 days trial, another 1 – 2 mls of blood sample was collected from the cephalic vein of each dog into EDTA bottle and 4 – 5 mls of whole blood was collected from each dog and allowed to coagulate in order to obtain serum for determination of haematological and biochemical parameters respectively. All dogs were weighed.

Determination of Haematological and Biochemical Parameters: Total blood cells count was done using the method of Baker (1985). Total protein was determined using biuret method (Tietz, 1995). Albumin was determined using bromocresol green method (Doumas, 1971). But conjugated bilirubin and total bilirubin were determined using the method of Jendrassik and Grof (1938) whereas Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruric Transaminase (SGPT) were determined using the method of Reitman and Frankel (1957). Sodium ion (Na^+) and potassium ion (K^+) were determined using flame photometric method (Fawcett and Scott, 1960). Both bicarbonate (HCO^-_3) and chloride (CL^-) ions were determined using titration method (Chaney and Marbach, 1962).

Statistical Analysis - The data on weight gain or loss, haematological and biochemical parameters were expressed as mean ± S.D. Tests for significance between mean parameters in respect of preadministration and post administration values were performed using student 't' test (Petrie and Watson, 2002).

RESULTS

The mean weight of the animals before administration of sulphadimidine was 8.8 ±2.04 kg[a] whereas the mean weight of the animals post administration of sulphadimidine was 8.77 ± 0.89 kg[b] (P > 0.05) i.e. there was no significant difference between the weight of the animals before and after the treatment with sulphadimidine (Table 1).

Table 1: Effect of intramuscular sulphadimidine on weight gain in Nigerian mongrel dogs

S/No	Control Pre Administration	Experimental Post Administration
1	12.00	10.00
2	7.00	7.86
3	9.00	8.71
4	9.00	9.29
5	7.00	8.0
Mean (kg)	8.80	8.77
Mean ± S.D	8.80 ±2.04	8.77 ± 0.89

Haematology revealed the significant decrease level of packed cell volume (P<0.05). Whereas white blood cells (WBC) neutrophils, lymphocytes, monocytes, eosinophils and basophils levels were not significantly increased (P>0.05) (Table 2).

Table 2: Effects of intramuscular sulphadimidine on haematological parameters of Nigerian mongrel dogs

Indices	Control Pre Administration	Experimental Post Administration
PCV %	46.4 ±6.27[b]	26.4 ±3.36[a]
WBC x 10%	7.54 ±1.45[a]	6.54 ±1.72[b]
Neutrophils %	52 ±7.78[a]	45.40 ±15.96[b]
Lymphocytes%	38.2 ±10.69[a]	44.8 ±8.99[b]
Monocytes%	5.6 ±5.37[a]	4.60 ±2.60[b]
Eosinophils%	4.2 ±2.95[a]	5.20 ±5.63[b]
Basolphils%	0.0 ±0.0[a]	0.0 ±0.0[b]

Keys: *T-test level of significance = 5%, a = Statistically significant, b= Statistically not significant, PCV= Packed cell volume, WBC = White blood cells, N = Neutrophils, L = Lymphocytes, M = Monocytes, E = Eosinophils, B = Basophils*

Liver function test revealed the increase level of total bilirubin and alkaline phosphatase (P<0.05). However, total protein, albumin, conjugated bilirubin, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) did not increase significantly P>0.05) (Table 3). Electrolytes titration has shown that sodium ion (Na^+) potassium ion (K^+), chloride ion (Cl^-) and bicarbonate ion ($HC0^-_3$) did not increase significantly (P>0.05) (Table 4).

DISCUSSION

The mean weight gain (8.8 ± 2.04 kg[a]) of the animals before sulphadimidine administration is comparable with the weight gain (8.77 ± 0.89 kg[b]) of animals after the sulphadimidine treatment. This shows that sulphadimidine has no effect on weight gain or loss. But the decrease in packed cell volume (P > 0.05) is a clear demonstration of report of Willard *et al* (1989) that in the dog, the severity of the anaemia is arbitrarily indicated by PCV range and that PCV value of 20 – 29 % was moderate. Hence sulphadimidine cause anaemia of moderate value (26.4 ± 3.36 %[a]) in dogs. Although anaemia is the most common erythrocyte disorder that can cause a variety of clinical signs (e.g. weakness, lethargy, heart murmur, pica) or may be sub-clinical and detected only as part of a diagnostic work up (Willard

et al., 1988), the above mentioned signs of anaemia were not noticed before the experiment.

Table 3: Effects of intramuscular sulphadimidine on liver function parameters of Nigerian mongrel dogs

Indices	Control Pre Administration	Experimental Post Administration
TP (g/l)	68.32 ±1.69[a]	68.82 ±2.70[b]
A (g/l)	40.3 ±3.77[a]	38.56 ±3.15[b]
TB (µmol/l)	18.5 ±2.01[b]	12.32 ±1.41[a]
CB (µmol/l)	3.08 ±1.48[a]	2.96 ±0.72[b]
AL (µg/l)	130 ±9.61[b]	114.2 ±5.12[a]
SGOT (µg/l)	20.4 ±11.39[a]	11.0 ±1.41[b]
SGPT (µg/l)	12.0 ±9.4[a]	5.2 ±1.64[b]

Keys: *T-test level of significance = 5%, a = Statistically significant, b = Statistically not significant, TP = Total protein, A = Albumin, TB = Total bilirubin, CB = Conjugated bilirubin, AL = Alkaline phosphatase, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamic pyruvic transaminase*

Table 4: Effects of intramuscular sulphadimidine on electrolytes concentration in Nigerian mongrel dogs

Indices	Control Pre Administration	Experimental Post Administration
Na^+(mmol/l)	135.0 ±1.87[a]	135.2 ±1.92[b]
K^+(mmol/l)	3.58 ±0.19[a]	3.82 ±0.29[b]
CL^-(mmol/l)	100 ±1.87[a]	100.4 ±2.07[b]
HCO^-_3(mmoll)	24.6 ±1.82[a]	25 ±1.58[b]

Keys: *Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Chloride ion, HCO^-_3 = Bicarbonate ion.*

The results of liver function test have shown total protein value of 68.32 ± 1.69g/l[a] in Nigerian mongrel dogs. This agrees with the report of Baggot (2001) that the range (60-86 g/l) of total plasma/serum protein concentration is similar in domestic animals and human, but this range was not affected by sulphadimidine administration (P>0.05). However, the total bilirubin decrease (P<0.05) is a clear demonstration of report of Willard *et al* (1989) that decreased bilirubin (12.32 ±1.41 µmol/l[a]) in comparison with (18.5 ±2.01 µmol/l[b]) may be due to drugs that displace bilirubin from albumin. This is further confirmed by Prescott *et al* (2000) that sulphonamides are bound to plasma proteins to an extent varying from 15% to 90%. But there is variation among species in binding of individual sulphonamides.

Moreso, significant difference between preadministration value (130 ± 9.61 µg/l[a]) and post administration value (114.2 ±5.72 µg/l[b]) of alkaline phosphatase may be associated with the injected sulphadimidine which might have inhibited hepatic enzyme. This is supported by Willard *et al* (1979) that bone-origin of serum alkaline phosphatase is commonly increased in animals less than 6 to 8 months old. But in this study sulphadimidine has decreased alkaline phosphatase (P < 0.05).

The decreased level of alkaline phosphatase may affect bone mineralization during bone formation. This is supported by Murray *et al* (2000) that alkaline phosphatase contributes to mineralization but in itself is not sufficient.

Lack of statistical significant difference between preadministration and post administration values (P>0.05) of electrolytes may suggest inability of sulphadimidine to cause sodium (Na^+) potassium (K^+), chloride (Cl^-) and bicarbonate (HCO^{-3}) ions imbalance.

However, the results have shown the normal values of Na^+ (135.0 ± 1.87 mmol/l[a]), K^+(3.58 ± 0.19 mmol/l[a]) and Cl^-(100 ± 1.87 mmol/l[a]) in Nigerian dogs to be lower than those reported: Na^+(141-154 mmol/l), K^+(3.8 - 5.8 mmol/l) and Cl^-(105 – 115 mmol/l) by Willard *et al* (1989) in foreign breed of dogs. Bicarbonate level remains the same in both Nigerian local (24.6 ±1.82 mmol/l[a]) and foreign (17-25 mmol/l) breeds of dogs.

Conclusion: Sulphadimidine did not cause increase weight gain or loss but significantly caused decreased packed cell volume (PCV) as total bilirubin and serum alkaline phosphatase were also significantly decreased. However Na^+, K^+, Cl^- and HCO^-_3 ions were not significantly affected. But the normal values of Na^+, K^+ and Cl^- ions were lower in mongrel dogs as compared to the foreign breed of dogs except that HCO^-_3 level remain the same in both mongrel and foreign breeds of dog.

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LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF DISTICHODUS SPECIES OF ANAMBRA RIVER

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ABSTRACT

The length-weight relationships (LWRs) and condition factor of Distichodus 169 Distichodus rostratus, 167 D. brevipinnis and 163 D. engycephalus from Anambra river were investigated from November 2004 to October 2005. LWRs showed that the b-values for the combined sexes were 3.051, 3.114 and 3.040 for D. rostratus, D. brevipinnis and D. engycephalus respectively. Thus, all the Distichodus species exhibited isometric growth with high, positive and significant correlations. The mean condition factor for the combined sexes was 1.12 ± 0.48, 1.06 ± 0.22 and 0.94 ± 0.33 for D. Rostratus, D. brevipinnis and D. engycephalus respectively. Except for D. brevipinnis, there was no significant difference (P > 0.05) in the condition factor (K) between the males and females of other species. The condition factor also demonstrated interseasonal variability in all the species. The importance of condition factor in the breeding activities of Distichodus species is discussed.

Keywords: *Distichodus*, Length-weight relationships, Condition factor

INTRODUCTION

Distichodus species are among the major exploitable fish species and are widely distributed in Nigeria, Nilo-Sudan, Niger, Volta, Chad and Nile basins (Teugels *et al.* 1992). They are used extensively in aquaculture on account of the ready availability of their seeds in the wild for stocking, good adaptation to climate, ability to support high population densities and to feed on grasses and weeds in ponds (Satia, 1990). Although a sizeable amount of literature exists on their biology, especially their length-weight relationships and condition factor (Imevbore and Okpo 1972; Arawomo, 1982; Francisco, 1992; Entsua-Mensah *et al.,* 1995; King, 1996, 1998; Nwani, 1998; Ezenwaji, 2004 among others) in some inland water bodies there is still paucity of information on the biology of *Distichodus* species in Anambra river, their importance and potentials not withstanding. The present study thus examines the length-weight relationship and condition factor to fill a gap in the current knowledge of the species.

MATERIALS AND METHODS

Study Area: The Anambra river has its source in Ankpa highlands of Kogi State of Nigeria about 100 km North of Nsukka (Azugo, 1978). It lies between latitudes $6^{0}10^{-}$ and $7^{0}40^{-}$ East of the Niger (Awachie, 1975). Essentially the river has a southward course crossing the Kogi / Enugu State boundary, then meanders through Ogurugu to Otuocha from where it flows down to its confluence with the Niger at Onitsha. The main river channel, which has a total length of about 207.40 km (Azugo 1978), has its bank covered by such plants like *Echinoclae species, Salvinia nymnellula, Ludiwigia decurrens, Imperita cylindirica, Andropogon spp, Jussiaea spp, Pennisetum spp and Cynodon spp*. There is a rainy season (April – September / October) and a dry season (October / November - March). From December to January / February, the basin is influenced by the harmattan but their effect is not well marked. Agricultural activities are very high and crops such as yam, cassava, rice, millet, vegetables, groundnut, potatoes, banana, and plantain are produced in large quantities. Fishing methods in the river basin include bailing out of water or pumping out water from ponds with water pumps, construction of fish fences, the use of "atalla", hooks and line, set lines, lift nets, dragnets, beach seines, cast nets, among others (Awachie and Ezenwaji, 1981; Eyo and Akpati 1995). Species of fishes found in the river include *Distichodus, Alestes, Mormyrids, Clarias, Labeo* and *Heterobranchus* among others.

Fish Sampling: Fish samples were collected monthly around Otoucha and Ogurugu river ports along the Anambra river between November 2004 and October 2005, using gill nets of mesh sizes ranging from 38.1 mm to 177.8 mm. Baskets, traps and hook and lines were also used. Fish collected were preserved in ice and transported to the laboratory for measurements. Each fish was weighed to the nearest 0.1 g and total and standard lengths were determined to the nearest centimeter.

Biometrics: The length -weight relationships of the Distichodus species were determined employing the power curve: $W = aL^{b}$, where W = wet weight of fish in grams, L = standard length in centimeters, and a and b are regression constants. The logarithm-transformed data gave the straight line relationship thus: Log W = log a + bLog L. The condition factor for each specimen was calculated using the method of Bagenal and Tesch (1978) thus: $K= W/L^{3} X 100/1$;

where K = condition factor, W = weight of fish in grams and L = length of fish in centimeters. The coefficient of variation (CV) was determined as: CV = [S/X x 100/1] % (King and Udo, 1996); where S = standard deviation and x = population mean.

Data Analysis: T-test was used to verify if the 'b' values obtained were significantly different from 3. The sexual and seasonal variations in condition factor were also determined using t-test, while the coefficient of variation (CV) was tested by the F-test.

RESULT

Length-Weight Relationship: The length-weight relationship and related parameters of the male, female and combined sexes of *Distichodus* species in Anambra river are presented in Table 1. In all the *Distichodus* species studied, the b values were not significantly different from 3 ($P > 0.05$) thus indicating isometric growth patterns for all the species. The correlation coefficients were all positive and highly significant. The intercept 'a' showed high heterogeneity among the populations (CV = 1003.5 %) and varied from 5.6×10^{-3} in *D. brevipinnis* combined sex to 4.5×10^{-2} in *D engycephalus* combined sex. Conversely, the exponent 'b' showed low variation among the populations (CV = 8.937 %) and ranged from 2.845 in male *D. brevipinnis to* 3.051 in the combined sex of *D. rostratus*

Condition Factor (K): The monthly variations in the mean condition factors for the three species of *Distichodus* are presented in Table 2. The mean K-value of male *D. rostatus* varied from 0.36 ± 0.04 in April to 1.79 ± 0.11 in September with an annual mean of 1.08 ± 0.45. The mean K for the wet season (1.35 ± 0.51) was significantly higher than the dry season (0.82 ± 0.14) ($t = 2.40$, df_{10}, $P < 0.05$). The coefficient of variation (CV) (Table 3) for the wet season (CV = 58.48 %) was significantly different from the dry season (CV = 43.45 %) ($F = 2.30$, $P < 0.05$). The female K-value ranged from 0.57 ± 0.10 in February to 1.98 ± 0.17 in August with a mean of 1.17 ± 0.50. Mean K-value for the wet season (1.53 ± 0.49) was significantly differed from that of the dry season (0.82 ± 0.15) ($t = 3.38$, df_{10}, $P < 0.05$). The coefficient of variation (CV) for the wet season (51.13 %) was not significantly different from the dry season (48.43 %) ($F=1.08$, $P > 0.05$). There was -no significant difference in the average condition factor between male and female *D. rostratus* ($t = 0.464$, df_{22} $P > 0.05$). The K-value for the combined sex was 1.12 ± 0.48. The wet season value of 1.67 ± 0.28 was significantly different from the dry season value of 0.58 ± 0.18 ($t = 2.10$ df_{10} $P < 0.05$). However, the coefficient of variation for the wet (55.75 %) was not significantly different from the dry season (44.29 %) ($F = 1.94$, $P > 0.05$).

The mean K-value for male *D. brevipinnis* was 1.02 ± 0.20. The factor varied from 0.70 ± 0.35 in November during the onset of dry season to 1.32 ± 0.12 in September during the peak of the rains. The mean K-value for the dry season (0.91 ± 0.19) and wet season (1.14 ± 0.14) were not significantly different ($t = 0.30$, df_{10}, $P>0.05$). The coefficient of variation (CV) for the dry season (81.73 %) was significantly different from that of the wet season (40.98 %) ($F = 2.67$, $P < 0.05$). The average female condition was 1.12 ± 0.25 and varied from 0.79 ± 0.26 in November to 1.63 ± 0.35 in May. The K-value of 0.93 ± 0.11 for the dry season differed significantly from that of the wet season (1.30 ± 0.22), ($t= 3.90$, df_{10}, $P < 0.05$). In contrast, the coefficient of variation (CV) for the wet (37.52 %) was not significantly different from the dry season (37.06 %) value ($F = 1.07$, $P > 0.05$). The mean male K-value of *D. brevipinnis* was significantly different from that of the female ($t= 1.08$, df_{22}, $P < 0.05$). The mean K-value for the combined sex was 1.06 ± 0.22 and was not significantly different between the seasons, 1.20 ± 0.40 for the wet and 0.86 ± 0.14 for the dry seasons, ($t = 1.12$, df_{10}, $p > 0.05$). Similarly the coefficient of variation for the wet season (CV = 40.33 %) and dry season (41.51 %) were not significantly different ($F = 1.63$, $P > 0.05$).

Male condition factor for *D. engycephalus* ranged from 0.63 ± 0.07 in December to 1.35 ± 0.23 in August with a mean value of 0.90 ± 0.30. Average condition factor for the dry season (0.67 ± 0.03) and wet season (1.12 ± 0.27) were significantly different ($t = 4.10$, df_{10}, $P< 0.05$). Similarly the coefficient of variation (CV) for the dry (26.50 %) and wet (49.29 %) seasons were significantly different ($F = 2.64$, $P < 0.05$). The mean condition factor for the female *D. engycephalus* was 0.98 ± 0.37 and ranged between 0.67 ± 0.07 to 1.74 ± 0.90. The average condition factor for the dry (0.72 ± 0.06) and wet (1.23 ± 0.38) seasons were significantly different ($t = 3.27$, df_{10} $P<0.05$). Similarly, the coefficient of variation for the wet season (CV = 52.86 %) significantly differed from the dry season (CV = 31.11 %) ($F= 2.17$, $P< 0.05$). There was, however, no significant difference between the condition factors of males and females D. *engycephalus* ($t = 0.58$, df_{22}, $p>0.05$). Considering the combined sex, the average condition factor was 0.94 ± 0.33. The wet season value of 1.38 ± 0.45 was significantly different from the dry season value of 0.69 ± 0.08 ($t= 2.04$, df_{10}, $p < 0.05$). The coefficient of variation (CV) for the dry (50.58 %) was also significantly different from the dry season value of 27.63 % ($F = 2.04$, $p < 0.05$)

DISCUSSION

The isometric growth pattern exhibited by *Distichodus* species of Anambra river is consistent with the b-values reported for other African *Distichodus* species (Entsua-Mensah *et al,* 1995., Palomeres *et al*. 1996., Francisco, 1992). The result however differed from the allometric growth of some distichodontides in Nigeria (King 1996a, Ezenwaji, 2004) with b values ranging from 2.158 to 3.354. The variation especially in the case of Ezenwaji (2004) may be attributed to the number of specimens used (< 60).

Table 1: Length-weight relationship of *Distichodus* species of Anambra river basin

Species	Sex	a	b	r	Number N	Length Max	Range (cm) Min
D. rostratus	M	0.0071	2.993	0.854	84	32.06	11.00
D. rostratus	F	0.0082	3.012	0.6894	85	34.04	13.06
D. rostratus	M and F	0.0064	3.051	0.8809	169	35.08	13.60
D. brevipinnis	M	0.0066	2.845	0.7102	81	38.80	12.04
D. brevipinnis	F	0.0064	3.040	0.6640	86	38.80	13.60
D. brevipinnis	M and F	0.0056	3.014	0.7460	167	39.60	13.62
D. engycephalus	M	0.0074	3.011	0.6024	76	9.00	27.50
D. engycephalus	F	0.0088	2.996	0.5832	84	10.06	31.40
D. engycephalus	M and F	0.0455	3.040	0.6194	163	30.20	11.50

a = regression intercept, b = slope and r = correlation coefficient

Table 2: Monthly variations in the condition factor (cf = w. 1000/L^3) of *Distochodus* species in Anambra River Basin

Month	*D. Rostratus*			*D. brevipinnis*			*D. engycephalus*		
	Male	Female	Male and Female	Male	Female	Male and Female	Male	Female	Male and Female
Nov 2004	0.78±0.7	0.78±0.16	0.78±0.15	0.70±0.35	0.75±0.26	0.75±0.31	0.68±0.07	0.77±0.11	0.72±0.09
Dec 2004	0.08 ± 0.14	1.93±0.13	0.87±0.14	1.08±0.22	1.02±0.18	1.05±0.20	0.63±0.07	0.67±0.08	0.64±0.08
Jan 2005	1.05±0.23	0.97±0.24	1.01±0.24	0.03±0.22	0.89±0.30	0.89±0.26	0.64±0.09	0.66±0.08	0.66±0.99
Feb 05	0.90±0.10	0.89±0.12	0.90±0.11	1.12±0.08	1.06±0.30	0.09±0.19	0.07±0.18	0.74±0.12	0.72±0.15
March 05	0.36±0.04	0.59±0.28	0.48±0.1	0.90±0.26	1.43±0.43	1.18±0.35	1.34±0.18	1.42±0.19	1.43±0.19
April 05	1.63±0.47	1.74±0.56	1.69±0.52	1.18±0.20	1.63±0.35	1.41±0.28	0.74±0.05	0.73±0.16	0.74±0.11
May 05	1.44±0.17	1.43±0.23	1.44±0.20	1.23±0.15	1.09±0.21	1.16±0.18	16±0.15	1.74±0.90	1.45±0.53
June 05	1.28±0.46	1.65±0.20	1.47±0.30	1.04±0.21	1.11±0.16	1.08±0.19	1.17±0.09	1..20±0.15	1.19±0.12
JULY 05	1.58±0.21	1.98±0.17	1.78±0.19	1.14±0.18	1.23±0.10	1.19±0.19	1.35±0.23	1.45±0.17	1.40±0.20
AUG 05	1.79±0.11	1.76±0.14	1.77±0.13	1.32±0.12	1.32±0.12	1.32±0.12	0.90±0.9	0.81±0.54	0.76±0.54
SEPT 05	0.67±0.21	0.76±0.18	0.63±0.20	0.76±0.37	1.00±0.35	0.73±0.36	0.71±0.36	0.81±0.54	0.76±0.54
Mean	$\bar{x}$ =1.08±0.45	$\bar{x}$ =1.17±0.50	$\bar{x}$ =1.12±0.48	$\bar{x}$ =1.02±0.20	$\bar{x}$ =1.12±0.25	$\bar{x}$ =1.06±22	$\bar{x}$ =0.90±0.30	$\bar{x}$ =0.98±0.37	$\bar{x}$ =0.94±0.33

Table 3: seasonal variation in condition factor and coefficient of variation (CV) among three *Distichodus* species of Anambra river

Species	Condition factor			Coefficient of variation (%)		
	Dry season	Wet season	T-Value	Dry season	Wet season	T-Value
			D. rostratus			
M	0.82±0.14^a	1.35±0.51^b	2.40	43.45^a	58.48^b	2.30
F	0.82 ±0.15^a	1.53±0.49^b	3.38	48.43^b	51.13^a	1.08
M and F	0.58±0.18^a	1.67±0.28^b	2.01	44.29^a	55.75^a	1.94
			D. brevipinnis			
M	0.91±0.19^a	*1.14±0.14^a*	0.39	81.73^a	40.98^b	2.67
F	0.93±0.11^a	*1.30±0.22^b*	1.30	37.06^a	37.52^a	1.07
M and F	**0.86**±0.14^a	*1.20±0.40^a*	1.12	41.51^a	40.43^a	1.63
			D. engyphalus			
M	*0.67±0.03^a*	*1.12±0.27^b*	4.10	26.50^a	49.29^b	2.64
F	0.72±0.06^a	*1.23±0.38^b*	3.27	31.11^a	52.86^b	2.17
M and F	*0.69±0.08^a*	1.38±0.45^b	1.05	50.58^a	27.63^b	2.04

a and b indicate significant corresponding means at P=0.05

The non-significant difference in the average condition factor in male and female *Distichodus* species excluding *D. brevipinnis* is in line with the findings of Arawomo, 1982; Francisco 1992., Ahmed and Saha, 1996; King, 1996; Nwani, 1998., Ezenwaji 200 among others.

The present study also revealed that except for *D. brevipinnis* the mean condition factor for the combined sexes in the wet season were significantly higher than that of dry season. This agrees with the mean K of female *Periophthalmus barbarus* in Imo river (King and Udo, 1996) and *Heterobranchus bidorsalis* in Idodo river (Anibeze 1995). The high condition factor noted in the wet season could be attributed to increased food availability occasioned by flooding, favourable environmental condition and gonad development. Conversely, the low condition factor observed during the dry season may be attributed to physiological stress due to changes in physical and chemical conditions of the habitant. Earlier workers (Olatunde, 1983; Nwadiaro and Okorie, 1985; Mgbenka and Eyo, 1992; Ikomi, 1996; Ekanem, 2000) made similar observation.

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TOXICITY, GROWTH AND SURVIVAL OF *Clarias gariepinus* JUVENILES EXPOSED TO DIFFERENT CONCENTRATIONS OF CRUDE OIL FRACTIONS-POLLUTED WATER

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ABSTRACT

Studies were carried out on the toxicity, growth and survival of Clarias gariepinus juveniles exposed to different concentrations of oil-polluted water. Thirty-nine aerated aquaria (60 × 30 × 30 cm^3), arranged in a 4 × 3 Complete Randomized Block Design were used for the study. Three oil types: the Bonny light crude oil (BLCO), the premium motor spirit (PMS) and kerosene (DPK) at oil concentrations of 1.00, 1.50, 2.00 and 2.50 ml L^{-1} were used in triplicates of 5 ml to contaminate 15 L of dechlorinated tap water and 20 fingerlings of Clarias gariepinus (22 ± 0.24 g) exposed to it. A control treatment (0.00 ml L^{-1}) of non-oil contamination was also used in triplicates. A 96-hour toxicity phase in the oil-polluted water preceded a 42 days recovery phase. 38% crude protein diet was fed to fish during exposure and recovery phases at 3% and 5% body weight per day, respectively. Water temperature, pH, fish mortality and normalized biomass index (NBI) of each aquarium were monitored. The total organic nitrogen, soluble organic nitrogen and colloidal organic nitrogen in addition to soluble and adsorbed ammonia in the aquaria water and sediments were analyzed using standard methods. Results showed that the water temperature was 26 ± 2.04° C, pH was 6.50 ± 0.30 and fortnightly feed intake of fish increased between days 14 and 42. This increase, which corresponded with the increase in the fortnightly weight gain, could be attributed to the reduction of stress caused during the 96-h toxicity phase. The increase in the soluble ammonium and the exchangeable ammonium concentrations of water correlated with the increase in the concentrations (1.50 – 2.50 ml L^{-1}) of BLCO, PMS and DPK. Percent mortality of fish reduced between days 14 and 42 irrespective of oil treatment while fish exposed to the control treatment had lower percent mortality than those exposed to the oil treatments. This trend was corroborated by the relatively higher NBI for the control during the exposure (-0.02) and recovery {0.08 (14 days), 0.08 (38 days) and 0.21 (42 days)} periods than those of oil treatments (-49.64 to -0.10).

Keywords: *Clarias gariepinus,* Toxicity, Soluble ammonium, Feed intake, Weight gain

INTRODUCTION

Akingbade (1991) recorded varying levels of petroleum hydrocarbons in the body organs of fishes, frog and snails resulting from over 3000 cases of oil spillages and release of about 2.4 million barrels of crude oil that had taken place in the Nigerian coastal environment. Concentrations of crude oil and fuel (0.05 - 10 ml L^{-1}) toxic to fish eggs and fingerlings have been studied (Lonning, 1977).

Freshwater fish are used as 96-hour bioassay organisms (Kopperdaul, 1976) for the determination of crude oil toxicity. Many workers (Stobber *et al*., 1978; Cardwell, 1979) have reported on the toxicity resulting from oil spills that occurred in aquatic environments near big oil industries and stated that fish larvae, fingerlings and eggs are quite sensitive bioassay test organisms. *Clarias gariepinus* Burchell, 1822 in the Nigerian waters is a highly esteemed hardy fish due to possession of accessory air-breathing organs which enable it to tolerate diverse aquatic conditions (Reed *et al*., 1967). *C. gariepinus* fry and fingerlings may be nonetheless very delicate and sensitive to aquatic pollutants including crude oil and its products.

Ammonia toxicity is reported to be one of the common causes of death of fish during rearing (Hampson, 1976). He also stated that nitrogen is generally excreted directly as ammonia without detoxicative metabolism, though some may be excreted as trimethylamine oxide, urea, uric acid or creatine. Ammonia concentrations in water depend on equilibrium between rate of production, exchange in water by flow-through in open or partially open systems and oxidative conversion to nitrite and nitrate by bacterial activity (Schulze-Weinhenbrauck, 1974). The mechanism of ammonia toxicity is by high ammonia concentrations in the blood resulting from failure of ammonia excretion or its uptake from the water at the surface membranes particularly at the gills (Hampson, 1975). The unionized ammonia (NH_3) is the form which can pass readily through cell

membranes (Hampson, 1976). This form is readily soluble in the lipid segments of the membrane and apparently needs no active transport, while the ionic forms occur as large hydrated and charged entities which cannot readily pass through charged micropores of the hydrophobic membrane components. The toxicity of ammonia is thus extremely dependent on conditions in the water which affect the equilibrium: $NH_4 + H_2O \leftrightarrow NH_3 + H_3O^+$. This equilibrium is drastically changed by variations in hydrogen ion concentration, temperature and ionic concentration (salinity) of the water.

Anderson *et al*. (1974) noted that an understanding of the effects of crude oil on ammonia toxicity, growth, feeding energetics and swimming activity of fingerlings is needed to assess the impact of oil pollution on fish production. Although the uptake of crude oil fractions and its components from water is very rapid and bioaccumulations if they are not metabolized do occur, much is not known about what happens to these compounds within the fish (Stageman and Sabo, 1976). Although fish has oxidative enzymes for metabolic detoxification of xenobiotics including aromatic petroleum hydrocarbons (Payne and Penrose, 1975), little is known about the metabolism of crude oil compounds in juveniles of *C. gariepinus*. In Nigeria, work has been done on the effect of different concentrations of Bonny-light crude oil on the mortality rate of *Heterobranchus bidorsalis* (Nwamba *et al*., 2001). Also, working with *Oreochromis niloticus*, exposed to diesel, Dede and Kaglo (2001) suggested that death of the tilapia fingerlings might be related to decrease dissolved oxygen content in water due to presence of diesel.

Against this background, the toxicity, growth and survival of *C. gariepinus* juveniles in oil product-polluted water were studied. Criteria for assessment included: total organic nitrogen, soluble organic nitrogen, colloidal organic nitrogen, soluble ammonium, exchangeable ammonium, concentrations and their effects on feed intake, weight gain and normalized biomass index.

MATERIALS AND METHODS

Seven hundred and eighty (780) juveniles of *C. gariepinus* (22 ± 0.24 g) were purchased from a private fish hatchery at Otor-Oweh in Isoko North L. G. A., Delta State, Nigeria and conveyed to Ebonyi State University, Abakaliki. The movement of fish was done with five 25-litre plastic containers, while the water in the containers were aerated with a New Generation Bell portable aerator (Model PAT-NO49-83537), energized by a 6.0 volt motorcycle battery. To avoid undue stress arising from high temperatures, ice cubes were added at regular intervals to water containing fish during transportation. At the Fisheries Research Laboratory in Abakaliki, the fish were acclimatized for 14 days on a maintenance ration of chick starter diets fed at 3% body weight per day (bw d^{-1}). A 38% crude protein diet was formulated from locally available ingredients (Table 1) and pelleted with a locally fabricated pelletizer. The feed was oven-dried at 60° C for 3 hours and preserved in a pest-free cupboard within the laboratory.

Table 1: Gross and Proximate Composition of Diets Fed to the African catfish (*Clarias gariepinus*) juveniles

Feed ingredient	Percent Composition
Yellow maize	9.81
Soyabean meal	54.76
Fish meal	16.43
Blood meal	10.95
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	0.40
Total	***100.00***
Nutrient	
Crude protein (CP)	34.88
Ether extract (EE)	4.44
Ash (AS)	11.08
Dry matter (DM)	8.23
Nitrogen-free extract (NFE)	41.87

[1]Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): Thiamin, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3000; niacin, 150; vitamin B_{12}, 0.06; retinyl acetate (500,000IU/g), 6; menadione-Na-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alpha tocopherol, 50; cholecalcipherol (1,000,000 IU/g); ethoxyquin, 2.0. [2]Contained as g/kg of premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; Na_2Cl_2, 45; Na_2SO_4, 44.88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 5; $CuSO_4.5H_2O$, 5; $NaSeO_3$, 0.11; $MnSo_4.H_2O$, 0.7, and cellulose, 380.97.

Thirty-nine aerator-equipped, transparent plastic aquaria (60 × 30 × 30 cm^3) were arranged to accommodate 4 treatments (T_1, T_2, T_3 and T_4) each of Bonny-light crude oil (BLCO), premium motor spirit (PMS) and kerosene (DPK) at oil concentrations of 1.00, 1.50, 2.00 and 2.50 ml L^{-1} per treatment in a 4 × 3 Complete Randomized Block Design (CRBD). A control experiment (T_5) had no oil treatment (0.00 ml L^{-1}). 5 ml of each of the oil concentrations (1.00 - 2.50 ml L^{-1}) were introduced in triplicates to 15 litres of water contained in each of the 36 aquaria while 3 aquaria (controls) had no oil treatments. Each of the 36 aquaria was randomly stocked with 20 juveniles of *C. gariepinus*. This fish were fed the formulated diet at 3 % body weight per day (bw d^{-1}) for 96 hours during the toxicity phase of the study and later 5% bw d^{-1} for 42 days during the recovery phase. Fish mortality/survival was monitored during the toxicity phase of 96 hours at intervals of ½, 1, 2, 4, 8, 16, 32, 48 and 96 hours. The oil-treated water in all the 36 aquaria were discarded after 96 hours and the surviving fish rinsed several times in clean dechlorinated tap water. The recovery phase commenced immediately after the aquaria had been refilled with 15 litres of fresh water. Mortality/survival of fish was also monitored at fortnightly intervals within 42 days (i.e. 14, 28 and 42 days). The water temperature and pH were monitored with the aid of a maximum-minimum

thermometer and a pH meter (Model PH-1-201), respectively.

Laboratory analyses of nitrogen and ammonia in the water and sediments of each aquarium were carried out after 4 days and on a fortnightly basis. The soluble organic, colloidal organic and total organic nitrogen were determined by the method of Avnimelech and Lacher (1979). Soluble and absorbed ammonia extracted from water with potassium sulphate (K_2SO_4) solution were analyzed colorimetrically (Solorzano, 1969).

The four days and fortnightly fish survivals were estimated via the normalized biomass index (NBI) thus: NBI = $\{(W_F \times N_F)\}$ $\{W_I \times N_I)\} \times 1/100$ (Beck, 1979), where, W_F = final weight of fish, N_F = final number of fish, W_I = initial weight of fish, N_I = initial number of fish. The fortnightly feed intake (FFI) was determined from the equation: FFI = feed intake per day × 14, while the fortnightly weight gain (FWG) was calculated thus: FWG = final fortnightly weight gain – initial fortnightly weight gain. The analysis of variance (ANOVA) was used to establish statistical differences between treatment means and Duncan's Multiple Range Test to partition the means (Steel and Torrie, 1990).

RESULTS

The water temperature was 26 ± 2.04° C and pH was 6.50 ± 0.30 throughout the study period. The results of the feed intake of *C. gariepinus* juveniles exposed to the crude oil and its products for 4 days and during recovery for 42 days are shown in Table 2. The fish consumed less feed as the concentrations of the three oil treatments increased from T_1 (1.00 ml L^{-1}) to T_4 (2.50 ml L^{-1}). Fish under the control treatment, T_5 (0.00 ml L^{-1}) consumed higher quantity of feed (1.66 ± 0.18 g) than those exposed to the crude oil fractions. The pattern of feed intake within the first 14 days of recovery indicated that less but not significantly different ($P > 0.05$) feed was consumed by fish exposed to BLCO concentrations of 1.00 ml L^{-1} (T_1), 2.00 ml L^{-1} (T_3) and 2.50 ml L^{-1} (T_4) than those exposed to the corresponding PMS and DPK concentrations (Table 2). Fish under the control treatment, 0.00 ml L^{-1} (T_5) consumed more diet than those exposed to the various concentrations of the three oil types (BLCO, PMS and DPK).

At day 28, the fish exposed to 2.00 ml L^{-1} and 2.50 ml L^{-1} BLCO concentrations still consumed comparatively less feed (3.84 ± 0.12 and 3.79 ± 0.22 g respectively) than those exposed to PMS (4.76 ± 0.06 and 4.79 ± 0.05 g respectively) and DPK (4.86 ± 0.13 and 5.60 ± 0.174 g respectively). The control fish at days 28 and 42 still consumed comparatively higher quantity of feed than those under oil treatments. At day 42, however, the fish exposed to DPK fed less (5.99 ± 0.21 g) (1.00 ml L^{-1}), (4.87 ± 0.20 g) (1.50 ml L^{-1}) and (6.97 ± 0.21 g) (2.00 ml L^{-1}) than those exposed to BLCO and PMS. Generally, the *C. gariepinus* juveniles exhibited significant differences ($P < 0.05$) in their responses to feed intake when recovering from BLCO, PMS and DPK exposures within day 14 and 28 recovery periods and highly significant different ($P < 0.01$) responses within 42 days recovery period (Table 2).

Table 3 shows the weight gain of fish within 4 days oil exposure period and the fortnightly weight gain (FWG) within 42 days recovery period. At day 14, fishes exposed to 1.00 mlL^{-1} concentration of BLCO, PMS or DPK showed better weight gain (0.02 ± 0.001, 0.03 ± 0.001 and 0.03 ± 0.002 g, respectively) than those exposed to higher concentrations (1.50-2.50 mlL^{-1}) of the oil types. However, fishes under the control treatment (0.00 ml L^{-1}) had better weight (0.04 ± 0.001 g) than those exposed to the oil treatments. The FWG of fish within the first 14 days increased in accordance with the increasing concentrations of oil (1.00 – 2.50 ml L^{-1}) exposed to the fish. Hence, FWG decreased from T_1 (1.00 ml L^{-1}) i.e. {1.48 ± 0.02 g (BLCO), 1.45 ± 0.02 g (PMS) and 1.48 ± 0.03 g (DPK)}. The weight gain recorded with the control (1.74 ± 0.03g) was also higher than those recorded with fish subjected to the oil treatments (Table 3). In addition, the fish had better weight while recovering from the DPK-treated water than from the PMS- or the BLCO-treated water (Table 3). The same trend in weight gain was demonstrated by the fish at 28 and 42 days recovery periods. There were significant differences ($P < 0.05$) in the FWG of *C. gariepinus* juveniles as they recovered from exposures to BLCO, PMS and DPK at days 14, 28 and 42 (Table 3).

Tables 4 how the nitrogen and ammonium concentrations of water in which *C. gariepinus* juveniles were exposed (4 days) and recovered from their exposures (42 days) to concentrations of BLCO, PMS and DPK. The total organic nitrogen (TON), the soluble organic nitrogen (SON), the colloidal organic nitrogen (CON), the soluble ammonium (SA) and the exchangeable ammonium (EA) in water (Table 4) increased in accordance with the increasing concentrations of crude oil fractions to which the fishes were exposed.

The control experiment had lower concentrations of TON, SON, CON, SA and EA than those of the oil-treated water (Tables 4a and 4b). The DPK-treated water had comparatively lower values TON, SON, CON, SA and EA irrespective of the exposed concentrations (Table 4).

Table 5 shows the percent mortality and the normalized biomass index (NBI) of fish within the toxicity (4 days) and the recovery (42 days) phases of the study. The NBI estimated the magnitude of growth and survival of fish within the study period. At day 4, fish percent mortality was least in the DPK-treated water (25.00%) than in the PMS-treated (27.00%) and the BLCO-treated (30.00%) waters. These results were corroborated by the lower NBI values recorded with fish exposed to BLCO (-49.64) and PMS (-49.40): relative to DPK (-13.31) (Table 5). Fish of the control experiment had a better NBI value (-0.02) than those subjected to the oil treatments. The trend exhibited by fish during the toxicity phase (4 days), with regard to percent mortality and NBI values, was reflected during the fortnightly recovery phase (42 days). At days 14, 28 and 42, fish recovering from exposure to the DPK-treated water

Table 2: Mean feed intake (g) of *Clarias gariepinus* juveniles within 4 days exposure to oil fractions and within 42 days recovery period recorded at fortnightly intervals[1]

Experimental phase	Period (days)	Oil type	Treatment (ml L^{-1} crude oil fraction)				
			T_1 (1.00)	T_2 (1.50)	T_3 (2.00)	T_4 (2.50)	T_5 (0.00)
Exposure	4	BLCO	1.56 ± 0.10^{a}	1.54 ± 0.10^{a}	1.50 ± 0.10^{a}	1.47 ± 0.10^{a}	
		PMS	1.58 ± 0.12^{a}	1.55 ± 0.08^{a}	1.52 ± 0.11^{a}	1.49 ± 0.13^{a}	
		DPK	1.61 ± 0.11^{a}	1.58 ± 0.10^{a}	1.56 ± 0.10^{a}	1.53 ± 0.11^{a}	4.42 ± 0.12^{b}
Recovery	14	BLCO	3.40 ± 0.12^{a}	3.74 ± 0.10^{b}	3.27 ± 0.11^{a}	3.31 ± 0.11^{a}	
		PMS	3.44 ± 0.13^{a}	3.10 ± 0.06^{b}	3.78 ± 0.10^{c}	3.51 ± 0.12^{a}	
		DPK	3.64 ± 0.14^{a}	3.06 ± 0.08^{b}	3.34 ± 0.11^{ab}	3.61 ± 0.12^{d}	4.42 ± 0.20^{d}
	28	BLCO	4.88 ± 0.21^{a}	4.03 ± 0.14^{b}	3.84 ± 0.12^{b}	3.79 ± 0.05^{b}	
		PMS	4.37 ± 0.04^{a}	4.44 ± 0.03^{a}	4.76 ± 0.06^{b}	4.79 ± 0.22^{a}	
		DPK	4.50 ± 0.14^{a}	3.62 ± 0.16^{b}	4.86 ± 0.13^{c}	5.60 ± 0.17^{d}	5.38 ± 0.11^{e}
	42	BLCO	8.35 ± 0.21^{a}	10.00 ± 0.13^{b}	5.21 ± 0.32^{c}	6.14 ± 0.22^{d}	
		PMS	6.77 ± 0.20^{a}	7.03 ± 0.14^{b}	19.44 ± 0.30^{c}	5.92 ± 0.32^{d}	
		DPK	5.99 ± 0.21^{a}	4.87 ± 0.20^{b}	6.97 ± 0.21^{c}	8.68 ± 0.30^{d}	12.14 ± 0.22^{e}

[1]BLCO = Bonny light crude oil, PMS = Premium motor spirit, DPK = Kerosene, T_1 – T_5 = Treatments, T_5 = Control treatment. [2]Means follow by the same superscript in the same row are not significantly different (P > 0.05).

Table 3: Mean weight gain (g) of *Clarias gariepinus* juveniles within 4 days exposure to oil fractions and within 42 days recovery period recorded at fortnightly intervals[1, 2]

Phase	Period (days)	Oil type	Treatment (ml L^{-1} crude oil or crude oil product)				
			T_1 (1.00)	T_2 (1.50)	T_3 (2.00)	T_4 (2.50)	T_5 (0.00)
Exposure	4	BLCO	0.02 ± 0.001^{a}	0.01 ± 0.002^{b}	0.02 ± 0.002^{a}	0.01 ± 0.001^{b}	
		PMS	0.03 ± 0.001^{a}	0.02 ± 0.001^{b}	0.02 ± 0.001^{b}	0.02 ± 0.002^{b}	
		DPK	0.03 ± 0.002^{a}	0.01 ± 0.001^{b}	0.02 ± 0.00^{c}	0.02 ± 0.001^{c}	0.04 ± 0.001^{d}
Recovery	14	BLCO	1.48 ± 0.01^{a}	1.45 ± 0.02^{a}	1.41 ± 0.01^{b}	1.32 ± 0.02^{c}	
		PMS	1.60 ± 0.04^{a}	1.57 ± 0.03^{a}	1.50 ± 0.02^{b}	1.45 ± 0.02^{b}	
		DPK	1.64 ± 0.04^{a}	1.59 ± 0.03^{ab}	1.53 ± 0.04^{bc}	1.48 ± 0.03^{c}	1.74 ± 0.03^{d}
	28	BLCO	1.72 ± 0.02^{a}	1.67 ± 0.01^{b}	1.63 ± 0.03^{b}	1.56 ± 0.02^{c}	
		PMS	1.80 ± 0.04^{a}	1.76 ± 0.04^{ab}	1.71 ± 0.03^{b}	1.63 ± 0.02^{c}	
		DPK	1.81 ± 0.03^{a}	1.76 ± 0.04^{b}	1.69 ± 0.04^{b}	1.61 ± 0.03^{c}	1.96 ± 0.03^{d}
	42	BLCO	1.71 ± 0.03^{a}	1.67 ± 0.02^{a}	1.62 ± 0.03^{ab}	1.57 ± 0.02^{b}	
		PMS	1.76 ± 0.02^{a}	1.73 ± 0.03^{a}	1.67 ± 0.03^{b}	1.59± .02^{c}	
		DPK	1.48 ± 0.14^{a}	1.41 ± 0.04^{ab}	1.35 ± 0.04^{b}	1.28 ± 0.00^{c}	1.86 ± 0.03^{d}

[1]BLCO = Bonny light crude oil, PMS = Premium motor spirit, DPK = Kerosene, T_1 – T_5 = Treatments, T_5 = Control treatment. [2]Means followed by different superscript in the same row are significantly different (P < 0.05).

Table 4: Nitrogen and ammonium concentration of crude oil- and crude oil product-treated water stocked with *Clarias gariepinus* juveniles within 4 days exposure and 42 recovery period[1, 2, 3]

Water parameter[2]	Oil type	Treatment (ml L^{-1} crude oil or crude oil product)				
		T_1 (1.00)	T_2 (1.50)	T_3 (2.00)	T_4 (2.50)	T_5 (0.00)
		4 days exposure period				
Total organic nitrogen (TON) mgN L^{-1}	**BLCO**	0.42 ± 0.03^{a}	0.46 ± 0.02^{ab}	0.52 ± 0.03^{b}	0.61 ± 0.05^{ac}	
	PMS	0.44 ± 0.04^{a}	0.51 ± 0.03^{ab}	0.58 ± 0.04^{b}	0.66 ± 0.06^{c}	0.20 ± 0.03^{d}
	DPK	0.20 ± 0.01^{d}	0.24 ± 0.01^{a}	0.35 ± 0.01^{b}	0.43 ± 0.0^{c}	
Soluble organic nitrogen (SON) µgN L^{-1}	**BLCO**	5.53 ± 0.21^{a}	5.62 ± 0.21^{a}	5.83 ± 0.22^{a}	5.92 ± 0.31^{a}	
	PMS	6.62 ± 0.63^{b}	6.71 ± 0.56^{b}	8.75 ± 0.63^{b}	9.12 ± 0.72^{b}	0.26 ± 0.02^{b}
	DPK	0.26 ± 0.03^{b}	0.31 ± 0.02^{a}	0.37 ± 0.04^{a}	0.46 ± 0.01^{a}	
Colloidal organic nitrogen (CON) µgN L^{-1}	**BLCO**	83.52 ± 4.10^{a}	91.60 ± 4.02^{b}	94.21 ± 4.17^{b}	96.74 ± 2.10^{b}	
	PMS	76.13 ± 3.22^{a}	78.54 ± 3.33^{a}	70.71 ± 3.13^{a}	74.61 ± 4.32^{a}	8.77 ± 4.11^{c}
	DPK	8.42 ± 0.72^{c}	9.62 ± 0.03^{a}	10.12 ± 0.14^{b}	11.27 ± 0.52^{c}	
Soluble ammonia (SA) µgNH_4 L^{-1}	**BLCO**	15.08 ± 1.15^{a}	15.06 ± 1.44^{a}	16.06 ± 1.21^{a}	16.36 ± 1.13^{a}	
	PMS	17.03 ± 2.21^{a}	17.08 ± 2.10^{a}	17.23 ± 2.20^{a}	17.28 ± 1.24^{b}	3.09 ± 0.22^{c}
	DPK	4.06 ± 0.03^{a}	4.02 ± 0.01^{a}	4.06 ± 0.31^{a}	5.23 ± 0.10^{b}	
Exchangeable ammonia (EA) µgNH_4 L^{-1}	**BLCO**	44.62 ± 1.40^{a}	45.61 ± 2.41^{a}	46.72 ± 2.22^{a}	48.46 ± 3.12^{a}	
	PMS	50.43 ± 1.32^{a}	53.47 ± 2.62^{ab}	55.24 ± 2.36^{bc}	58.13 ± 2.29^{c}	4.61 ± 0.06^{d}
	DPK	5.03 ± 0.35^{a}	5.69 ± 0.24^{ab}	6.77 ± 0.42^{bc}	6.27 ± 0.32^{c}	
		42 days recovery period				
Total organic nitrogen (TON) mgN L^{-1}	**BLCO**	3.10 ± 0.63^{a}	3.70 ± 0.31^{ab}	4.40 ± 0.30^{b}	5.10 ± 0.41^{bc}	
	PMS	3.40 ± 0.62^{a}	4.01 ± 0.53^{ab}	4.43 ± 0.40^{b}	5.24 ± 0.42^{c}	1.01 ± 0.22^{d}
	DPK	1.11 ± 0.20^{d}	1.71 ± 0.15^{a}	2.43 ± 0.25^{b}	3.13 ± 0.20^{c}	
Soluble organic nitrogen (SON) µgN L^{-1}	**BLCO**	46.20 ± 2.01^{a}	46.80 ± 0.21^{ab}	47.50 ± 1.12^{b}	48.22 ± 1.12^{bc}	
	PMS	77.15 ± 8.10^{a}	77.70 ± 6.20^{a}	78.40 ± 7.02^{a}	79.11 ± 6.11^{a}	
	DPK	1.35 ± 0.20^{d}	2.05 ± 0.22^{a}	2.80 ± 0.13^{b}	3.52 ± 0.21^{c}	1.06 ± 0.11^{d}
Colloidal organic nitrogen (CON) µgN L^{-1}	**BLCO**	925.42 ± 40.04^{a}	926.02 ± 41.03^{a}	926.72 ± 30.16^{a}	927.42 ± 21.04^{a}	
	PMS	671.31 ± 22.02^{a}	673.01 ± 23.03^{b}	673.81 ± 20.02^{a}	674.53 ± 11.14^{b}	
	DPK	94.72 ± 1.10^{a}	95.32 ± 0.81^{a}	96.21 ± 0.51^{a}	97.11 ± 1.23^{a}	75.57 ± 31.01^{c}
Soluble ammonia (SA) µgNH_4 L^{-1}	**BLCO**	149.18 ± 40.16^{a}	149.88 ± 44.05^{a}	150.58 ± 42.22^{a}	151.28 ± 43.02^{a}	
	PMS	163.22 ± 10.20^{a}	163.90 ± 11.12^{a}	164.63 ± 10.30^{a}	163.34 ± 11.14^{a}	
	DPK	34.53 ± 12.01^{b}	35.13 ± 9.11^{b}	35.83 ± 11.01^{b}	36.53 ± 10.11^{b}	29.14 ± 11.10^{b}
Exchangeable ammonia (EA) µgNH_4 L^{-1}	**BLCO**	345.23 ± 13.00^{a}	346.14 ± 11.01^{a}	346.94 ± 10.20^{a}	347.64 ± 12.12^{a}	
	PMS	441.11 ± 14.07^{b}	441.73 ± 15.06^{b}	443.43 ± 16.08^{b}	444.13 ± 13.08^{b}	32.50 ± 14.07^{b}
	DPK	48.57 ± 0.70^{a}	49.17 ± 0.52^{a}	49.87 ± 0.61^{a}	50.57 ± 0.60^{a}	

Table 5: Percent mortality and normalized biomass index of *Clarias gariepinus* juveniles during 4 days of exposure to crude oil fractions and 42 days of recovery

Experimental period	Duration (days)	Oil type[1, 2]	Initial number of juveniles[3, 4]	Number of dead juveniles	Number of survivors	Percent mortality	Normalized biomass index
Exposure period[5]	4.00	BLCO	240.00	72.00	168.00	30.00	-49.64
		PMS	240.00	65.00	175.00	27.00	-49.40
		DPK	240.00	60.00	180.00	25.00	-13.31
		Control	60.00	1.00	59.00	2.00	-0.02
Recovery period of 42 days[6]	14.00	BLCO	168.00	31.00	137.00	18.11	-0.52
		PMS	175.0	26.00	149.00	15.00	-0.33
		DPK	180.00	23.00	157.00	13.00	-0.27
		Control	59.00	1.00	58.00	1.00	0.08
	28.00	BLCO	137.00	22.00	115.00	16.00	-0.35
		PMS	149.00	18.00	121.00	12.00	-0.57
		DPK	157.00	14.00	143.00	9.00	-0.27
		Control	58.00	1.00	57.00	1.00	0.08
	42.00	BLCO	115.00	14.00	101.00	12.00	-0.17
		PMS	121.00	10.00	111.00	8.00	-0.10
		DPK	143.00	9.00	134.00	6.00	-0.04
		Control	57.00	0.00	57.00	0.00	0.21

[1]BLCO = Bony light crude oil, PMS = Premium motor spirit, DPK = Kerosene. [2]Concentrations of oil types used = 1.00, 1.50, 2.00 and 2.50 ml L^{-1}; [3]Initial number of juveniles derived from four treatments x 3 replicates x 20 juveniles = 240; [4]Initial number of juveniles in the control is derived from 3 replicates x 20 juveniles = 60 juveniles; [5]Four-day summations of fish mortality/survivals in the oil types and Control were adopted; [6]Fortnightly summations of fish mortality/survivals were adopted.

had lower percent mortality and higher NBI values than those recovering from BLCO and PMS exposures (Table 5). Fish of the control experiment expectedly died less and survived better than those exposed to the oil treatments.

DISCUSSION

The decrease in the feed consumption by fish in the first 4 days (toxicity phase) with increasing concentrations of BLCO, PMS and DPK (Table 2), indicated that feed intake was affected by oil concentrations in water. Irrespective of the BLCO concentrations (1.00 - 2.50 ml L^{-1}), a similar trend of increase in the fortnightly feed intake (FFI) of fish (Table 2) existed between days 14 and 42. This result indicated that fish response to feed intake improved with time. This improvement was also exemplified by the fish exposed to the various concentrations of PMS and DPK (Table 2). The quantity of feed consumed by the fish during the recovery phase (42 days) was generally higher than that consumed during the toxicity phase (4 days) (Table 2). This implied that the presence of crude oil fractions in water affected the quantity of food consumed by the fish. In addition, there were consistent increases in the quantity of feed consumed by the fish as the recovery period progressed from day 14 to day 42. Lagler *et al.* (1977) reported that the increased food consumption relative to increasing size and age may be due to the interaction of factors affecting internal motivation or drive for feeding, such as: season, temperature, time and nature of last feeding, food stimuli perceived by the senses, lateral line system, hunger, curiosity and gluttony. The increase in the sizes of fish (mean weight gain) between days 14 and 28 of the recovery period (Table 3) might have been accompanied by the development of more senses to perceive food stimuli in accordance with the report of Lagler *et al.* (1977).

Fish under the control treatment, T_5 (0.00 ml L^{-1}) apparently fed better on forth nightly basis than those subjected to oil pollution (Table 2). This result further indicated that the contamination of water with crude oil fractions affected food consumption by fish. This result was consistent with the report of Hunt and Linn (1990) who reported a reduced feed intake by *Clarias* sp. exposed to 1.50 ml L^{-1} of an agro-chemical, carbynl (I-naphthyl methyl carbonate) solution.

The values of the weight gain for fish treated with the various concentrations of BLCO, PMS and DPK (Table 3) followed the pattern exhibited by the feed intake of fish. This result implied that there was a concomitant increase in weight with time (days 14 to 42) as the fish recovered. Jauncey (1982) reported a similar weight increase with time for juvenile *Sarotherodon mossambicus* fed eight isoenergetic diets with protein levels ranging from 0% to 56%.

The increases in the values of the soluble ammonium (SA) and the exchangeable ammonium (EA) of water with increasing concentrations of BLCO, PMS and DPK during the toxicity phase (Table 4), corresponded with the increases in SA and EA values recorded during the recovery phase (Table 4). The comparatively higher values of SA and EA recorded during the recovery phase (42 days) than those recorded during the toxicity phase (4 days) may be due to copious excretion of SA and EA into water by *C. gariepinus* juveniles. The values of SA and EA in the control treatment were comparatively lower than those of the oil-polluted treatment (Table 4).

This result was in agreement with Avnimelech and Lacher (1979).

The higher percent mortality (30.00%) and lower NBI (-49.64) values of fish when exposed to BLCO at day 4 than when recovering from this exposure at day 14 (18.00%; -0.52), day 28 (16.00%; -0.35) and day 42 (12.00%; -0.17) (Table 5) indicated that fish mortality and the propensity to survive increased with the period the fish was allowed to recover from oil pollution. The range values of SA (149.18 ± 40.16 – 151.28 ± 43.02 µg NH_4 L^{-1}) and EA (345.23 ± 13.00 – 347.64 ± 12.12 µg NH_4 L^{-1}) for BLCO treated water alone (Table 4) implied that these range values of SA and EA could cause fish mortality of between 12.00% and 18.00% within 42 days (Table 5). The range values of the growth and survival index (NBI) of fish recovering from the BLCO exposure (-0.52 to - 0.17) (Table 5) corroborated the range values of fish mortality (12.00-18.00%) already indicated. Hampson (1976) had earlier reported that ammonia toxicity was one of the common causes of fish mortality during rearing. The stress factor occasioned by oil pollution must have retarded the incorporation of nitrogen in the fish into fish flesh and facilitated its conversion to ammonia. In his study, ammonia must have entered the water through the metabolic and excretory mechanism of fish or by bacterial action in the water (Hampson, 1976).

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MANAGEMENT TECHNIQUES FOR REVITALIZATION AND EFFECTIVE UTILIZATION OF YINAGU RIVER IN MADAGALI LOCAL GOVERNMENT AREA OF ADAMAWA STATE

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ABSTRACT

The study examined the management techniques towards the revitalization and effective utilization of the resources of Yinagu river in Madagali LGA of Adamawa State. A total of 200 fishermen aged between 45 years and above were sampled using semi-structured interviews and closed ended questionnaires from January 1998 to December 2003. Factors affecting fish production in Yinagu river were identified in their order of perceived importance as the use of nets of small mesh size (73.5%), poaching (60.0%), flooding (40.0%), rainfall (34.0) and blockage of the river tributaries (18.0%). The management techniques employed to effectively utilize the resources of Yinagu river include the specification of fishing sites, use of two seasonal fishing, use of rituals, local administration, creation of buffer zones between the water body and sites of farming activities among others.

Keywords: Management, Revitalization, Effective utilization, Yinagu river, Productivity, Exploitation

INTRODUCTION

The increasing demand for fish protein and farmland, and irrigational practices have tremendously affected the sustenance of Yinagu river The combine forces of these factors had subjected the river to its present deplorable state, because it has gone beyond the river's resistance, there by leading to a cumulative effect of over-utilization of the river, which now is at the verge of extinction.

Brown (1987), reported that revitalization of a given resource can only be achieved through the co-operation of the users and the government. According to Brown, the future economic stand of a particular resource depends on the level of its utilization and management. In support of Brown's report, Hepher (1990) suggested that because of the disappearing water bodies in the world there is need to explore more method of water resources management if the present world increasing human population must be sustained.

It is widely accepted across the globe that fish is among the most common resource of the river on which human lives depend, the common man who cannot afford the purchase of beef, mutton, goat meat, poultry etc is left with fish as the only source of animal protein. According to ADADP (1995), man is led to exploit this resource beyond the river's resistance resulting in its drastic reduction in terms of fish productivity. ADADP further reported that about 80 – 95% of the rural populace depend on fish as the main source of animal protein. Consequently, many people are involved in buying and selling of fish and fish products thereby making a fishery sector a source of employment.

The inhabitants of the study area mostly Margi constitute one of the leading consumers of fish protein in the North – East sub-region, because of the surplus of fish usually obtained from the river and its tributaries. As a result of the preference of fish protein by Margi people, a standard fish dish referred to as "Margi special" is widely accepted across the state. Gabon (1993), reported that most rivers in Adamawa State have been converted to mere standing ponds due to human activities such as farming practices, uncontrolled draining of water during fishing etc. usually enhanced by government negligence. According to Gabon, people catch the fishes, destroy the river banks and pollute the waters with agrochemicals used in farming thereby destroying both the physical and biological components of the river. ADADP (1995) reported that although the revenue generated from fishery sector was enormous, natural riverian environments endowed with considerable resources and attractive features were sometimes hardly appreciated by humans and thus abused. Amos (2002), stressed that effective management of our local rivers can increase fish productivity across sub-Saharan Africa. Hence, concerted effort should be geared toward stopping further deterioration of our riverian ecosystem.

This paper examines the traditional and modern management techniques and how they can be effectively utilized for the revitalization of Yinagu river that use to provide most of the fish protein required by the people of Adamawa-North and Borno-South before its present state of non-productivity (ADADP, 1995).

1. The specific objectives to be investigated include:
2. Assessment of the fish productivity level of Yinagu river from 1998 to 2003.
3. Identification of the factors affecting fish production in the study area.
4. Identification of the traditional and modern management techniques employed in the sustenance of Yinagu river.

5. To ascertain whether both management techniques were effective and
6. To suggest other traditional and modern management techniques for effective utilization and sustenance of Yinagu river.

MATERIALS AND METHODS

Study Area: The research was conducted in Yinagu and its neighbouring villages in Madagali local government area of Adamawa State, located in Adamawa North senatorial zone, which shares a common boundary with Izege in Gwoza local government area of Borno State. The Yinagu river lies between longitude 14^0 48 E and latitude 12^0 32 E and the tributaries are located as follows: Yedzaram (13^0 42N, 11^0 24 E), Dir-Uwal (14^0 33 N, 12^0 17 E), Birishishiwa (12^0 23 N, 10^0 21 E and Tsugadi (13^0 16 N, 9^0 18 E) as reported by Satumari (2004)

The climatic condition is typical that of tropical regions of the world, with mean daily temperature ranging between 28 – 34 ^{0}C. During harsh periods, usually from March to May, the temperature may rise up to 38-39^{0}C. The relative humidity is variable, with the peak of it during rainy season especially from late July to September (Toyo, 1996). The mean annual rainfall ranging from 700-900mm and the rainy season last for about 3-4 months, usually June to September (Akosim *et al,* 1996). The vegetation is constituted by the guinea savannah and consists of abundant woody plant species (Akosim *et al,* 1996). The primary occupation of the inhabitants is farming, which at times is being complimented by petty trading and fishing.

A total of 26.2 km^2 length of the river and its tributaries were covered. The sample area include; Kwappa, Yaffa, Kirchinga, Mbitiku, Uddah, Birishishiwa and Yinagu-Via villages, through which the river's tributaries pass. Research was conducted on the following rivers; Yadzaram, Dir-Uwal, Biri-Shishiwa and Tsugadi as they constitute the Yinagu river.

Data collection: A total of 200 professional and non-professional fishers aged 45 years and above residing along the river and its tributaries were randomly sampled using semi-structured interviews and closed ended questionnaires from January 1998 to December 2003. Other information through personal observation and literature search were equally utilized. For easy administration of the questionnaire, participatory rural appraisal method was employed because it allows free interaction and understanding between the researcher and the respondent (Dunn, 1994). The age preference was to ensure that such person or persons have witnessed the changes that took place within the period of 6 years of fishing activity and its management. The knowledge of sampled villages helped to prevent the concentration of respondents in a given village, thus avoiding bias. The assessment of fish productivity level, identification of traditional and modern management techniques and ascertaining of the effectiveness of both management techniques were based on analysis of data collected from respondents and personal observations. The contents of questionnaire include; Name of village, age, years of fishing experience, educational qualification, fishing gears used types of fish captured, type of craft use, possible factors affecting fish productivity, traditional and modern management techniques in use in the management and sustenance of Yinagu river, and the effectiveness of both management techniques.

RESULTS AND DISCUSSION

Fishing Pattern: Awi (2002) reported that the fishing patterns include: the dry season fishing which takes place between March and May and rainy season fishing between September and October. Fishing in two seasons is only by obtaining a tariff from the local government authority. The tariff on fishing in Yinagu river also specifies that the upper part of the river is prohibited from being fished except seldom poaching done by some indiscreet fishers. Fishing in this portion is only done during Yinagu Fishing Festival which is usually between the months of March and May, organized jointly by the local and the state governments.

Fishing Equipment: The fishing equipment are mostly the locally constructed fishing nets e.g. cast nets, bag nets, hook nets, drag nets and also the use of free hand fishing. Generally, variable numbers of fishing gears are used during any fishing event. Adamawa State Agricultural Development Programme, ADADP (1995), reported that the use of a particular fishing gear depend on individual fisher (Table 1)

Table 1: Fishing Gears used in Yinagu river fisheries

S/No	Fishing Gears	Mean Quantity of Fishing Gears Per Fishing Festival
1	**Cast nets**	760
2	**Bag nets**	500
3	**Hook nets**	200
4	**Drag nets**	460
5	**Free hand fishing**	variable

Use of Gears: The use of a given gear depends on the season of fishing. The cast and hook nets are used during rainy season fishing, (when the water volume is high) while the bag nets, drag nets and free hand fishing are used during the dry season fishing (when the volume of water in the river is low).

Specification of Fishing Gears: The local administration of Yinagu river in conjunction with the government specifies that fishing nets with smaller mesh sizes should not be used to avoid catching of fingerlings, but lack of enforcement of management

techniques, the fishes are caught indiscriminately. The most common fishes caught are C*larias* species, *Synodontis* species, *Tilapia* species*, Microlestes species, Protopterus* species, *Mormyrus* species, *Alestes* species etc, although *Clarias* and *Tilapia species* are normally caught in large quantities (Awi, 2002).

Types of Craft Use: The fishing gear nets are made up of locally processed A*donsonia digitata* fibres. The nets only differ in shape and sizes. In some cases, hooks are attached e.g. hook nets popularly called "Taru" in Hausa and "Cadra" in Margi language.

Years of Fishing Experience: Every member of the sampled fishers should have being fishing for at least 6 years of un-interruption.

Fish Productivity Level of Yinagu river from 1998 to 2003: Adamawa State Agricultural Development Programme ADADP (2004) reported that the fish productivity level of Yinagu river had drastically reduced over the years from 1998 to 2003 (Table 2). According to Adamawa State Agricultural Development Programme, the reduced productivity was attributed mainly due to poor management of the resource.

Table 2: Responses on Estimate of Fish Productivity Level of Yinagu river

S/no	Year	Estimated quantity of fish in kg
1.	1998	5,786
2.	1999	3,924
3.	2000	3,566
4.	2001	3,022
5.	2002	2,978
6.	2003	2,224

Factors Affecting Fish Productivity

The factors affecting fish production in Yinagu river were as tabulated in table 3 thus:

Rainfall: 34% of the respondents believed that the yearly quantity of water in the river was directly influenced by the amount of the annual rainfall, which had direct influence on fish production. They believed that the more the quantity of water in the river the higher the chances of increased fish production.

Flood: Forty percent (40%) of the respondents revealed that the rate and extent of flood affects fish production in the river. Flood exposes most of the fingerlings to danger and in addition carries them along to distant water bodies. Odihi (1992), reports that the bank of the river may suffer erosion and this can lead to destruction of spawning sites. Generally, high fishing activity is recorded during low waters of the dry season (Welcome, 1979) in tropical flood plain rivers.

Poaching: Sixty percent of the respondent attributed decline in fish production of Yinagu river to be as a result of poaching.

Use of nets of smaller mesh size: 73.5% of the respondents consider that the inability of managers of Yinagu river to determine the type of gear net mesh size for fishing, made the fishers to use any type of gear net during their illegal activities. This result to indiscriminate catching of both bigger fishes and fingerlings.

Blockage of tributaries using pegs: 18% of the fishers had fished through the blockage of the river's water source. These acts results in death of most fishes especially the fingerlings, and also prevent them from entering the main water body.

Table 3: Observed Factors Affecting Fish Productivity in Yinagu River by Respondents

S/NO	Factor	No. of Respondents	Percentages (%)
1	**Rainfall**	68	34.0
2	**Flood**	80	40.0
3	**Poaching**	120	60.0
4	**Use of nets of smaller mesh size**	147	73.5
5	**Blockage of tributaries**	36	18.0

Management Techniques

The following management techniques are being practiced for the sustenance of Yinagu river. Details of the respondents' reactions are given in Table 4.

Local Administration: The management of the River is headed by the Village head, who gives directive to "ptilmi" (sarkin ruwa) and from Ptilmi to fishers' group leaders and from group leaders to individual fisher. Also the reports on fishing or poaching activities are passed back through the same route in a reverse order. Details shown by Information flow channel below (Figure 1).

Village head

↕

Ptilmi (Sarkin ruwa)

↕

Fishers' group leaders

↕

Individual fisher

Figure 1: Information flow channel in rural resources management. Source: Environmental study, 2005.

Use of rituals: To enable continuous fish production by the river, "Annual rituals" are performed using black goat at the beginning of rainy season, usually after the first rainfall and then a day before the fishing festival. Sixty-eight percent (68 %) of the respondents (Table 4) reported that such practice helps to appease the gods of the land and the river. According to the respondents, the ritual performance is done by Ptilmi. The ritual perform is an obligation and dues paid to the gods of the land and water (Ntasimda, 1985).

Use of two seasonal fishing: 62% of the respondents (Table 4) reported that the used of seasonal fishing helps to monitor the activities of poachers and determine the size of fish that should be caught, because the nets used are usually inspected by a committee headed by Ptilmi before usage. The long period between the fishing activities allow the fingerlings to grow and be recruited into the fishery. This management technique when strictly adhere to as reported by Food and Agricultural Organization, FAO (1999) will guarantee the increased fish productivity of a given water body. The two seasons are dry season (March to May) and rainy season (September to October). The fishing is usually done within these months.

Creation of buffer zones between the water body and sites of farming activities: 85% of the respondents (Table 4) reported that farming activities was only allowed at 80 metres away from the water body. This helps to prevent unnecessary blockage of water ways and excessive deposit of sand in the river. Continuous sand deposition may result to river extinction. Amos (2002), reported that creation of buffer zones permits the development of the river and its surrounding close to its natural form. This will lead to increase fish productivity of the river.

Table 4: Operational and Effectiveness of Management Techniques in Yinagu river Fishery

S/NO	Operational Management Techniques	No. of Respondents	(%)	Effectiveness of responses	%
1	Local administration	182	91.0	144	72
2	Use of rituals	136	68.0	62	31
3	Use of two season fishing	124	62.0	116	58
4	Creation of buffer zones between the water body and sites of farming activities	170	85.0	130	65
5	Specification of fishing sites	63	31.5	42	21

Specification of fishing sites: 21 % of fishers reported that partial fishing is allowed at the lower part of the river. This constitute only one-tenth of the river and in addition is clearly demarcated from the main water body by off-shore sand deposits as reported by Awi (2002).

Effectiveness of management techniques: Although the fishers reported that management techniques such as: local administration, creation of buffer zones and the use of two season fishing were adequate (table 4), but with the present trend of things such as: increasing demand for fish protein, farmland, irrigation practices and other resources of the river made the techniques less effective (Awi, 2002). The effectiveness of particular management technique was assessed based on the number of respondents in agreement with a given operation, out of the total sampled population of 200 fishers.

Conclusion: The decreasing fish productivity of Yinagu river is due to poor approaches to the management techniques because of increasing human population, that led to increased exploitation coupled with environmental factors and poor fishing practices. Results have shown that if the management techniques in place are observed and sustained, then Yinagu river can regain its productivity level

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EFFECT OF SMOKE-DRYING ON THE PROXIMATE COMPOSITION OF *Tilapia zillii, Parachanna obscura* AND *Clarias gariepinus* OBTAINED FROM AKURE, ONDO-STATE, NIGERIA

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ABSTRACT

The proximate composition of fresh, smoked and deteriorated fish samples (Tilapia zilli, Parachanna obscura and Clarias gariepinus) were determined using standard methods of analyses. It was revealed that Tilapia zilli contained; moisture 4.11 - 67.33 %, protein 20.10 – 65.90 %, ash 3.41 – 14.64 %, fat 4.44 – 7.73 % and carbohydrate 4.72 – 11.89 %, Parachanna obscura had moisture 6.47 - 68.61%, protein 18.23 – 64.67%, ash 2.68 – 13.20 %, fat 3.55 – 8.87 % and carbohydrate 6.79 – 10.25 %, while Clarias gariepinus produced moisture 4.61- 56.99%, protein 17.21 – 68.05%, ash 4.82 – 15.32 %, fat 4.79 – 8.19% and carbohydrate 1.92 – 17.35%. It was observed that smoke drying methods increased the protein, ash and fat contents of the samples. The low fat content observed for the deteriorating sample might be due to rancidity with the resultant rancid odour.

Keywords: *Tilapia zillii, Parachanna obscura, Clarias gariepinus,* Smoke-drying, Proximate composition

INTRODUCTION

Among the good quality animal protein sources, fish is the most perishable. An estimated 50 % of the fish produced in the remote coastal settlements and hinterland perish before reaching the consumers, as a result of poor handling, preservation and processing practices adopted by the artisanal fishers, commercial fish farmers and fisheries entrepreneurs (Eyo, 1997). Spoilage set in because fish is susceptible to microbial and enzymatic deterioration and quality reduction occur, if proper steps are not applied to process the fish, (Emokpae, 1985). The fish loses its organoleptic characteristics and becomes progressively more unacceptable for human consumption. There are several ways of accessing quality of fish product, whether smoked, dried, frozen or canned, and these are physical examination, biochemical, microbiological, entomological and sensory methods (Clucas and Sutcliffe, 1981).

The process of fish drying involves the removal of moisture from fish flesh; this could be achieved through sun-drying, smoke-drying, application of pressure and use of absorbent pads. Sun drying is presumably the oldest method of fish preservation employing hot heat from sun and atmospheric air (Awoyemi and Eyo, 1998). The demerit of sun drying being the length of time it takes for drying. For better product, sun drying is supplemented with smoke drying. Smoke dried fish, if stored under good conditions can be kept for several months (Tobor, 1984). Dvorak and Vognarova (1965) reported that smoking caused some decrease in available lysine. The loss in available lysine may vary from 6 – 33 % at 25 ^{o}C to 53 – 56 % at 40 ^{o}C during hot smoking. Lysine reduction is directly proportional to the temperature and duration of smoking (Akande *et al.,* 1998). Clifford et al., (1980) reported a 25% loss of available lysine on the surface and a 12% loss at the center of hot smoked fish fillet. Other basic amino acids were reduced by 6.6% on the surface but remained unchanged at the center of hot smoked fish fillet.

MATERIALS AND METHODS

The fish samples (*Tilapia zillii, Parachanna obscura* and *Clarias gariepinus*) were procured from the Ondo State Agricultural Development Project, (ADP) Akure. Processing of the fishes involved gutting, washing, hanging to drain the moisture, smoking and packaging.

Fresh Sample: A fresh flesh sample each from the three species was taken to the laboratory for the proximate analysis.

Smoke Dried Sample: All fish species were smoked whole in a kiln for between 4 – 10 days (depending on the species) using smoke from charcoal heated cooking pot. The fish samples were exposed to the same drying conditions. After drying, the fish samples were packed into different cellophane bags and stored on a shelf at room temperature and were observed for deterioration.

Table 1: Proximate composition of fresh, dried and deteriorating samples of *Tilapia zillii, Parachanna obscura* and *Clarias gariepinus* from ADP ponds, Ondo State

Proximate parameters	Fresh	Dried	Deteriorating
	Tilapia zillii		
Moisture content	67.33 ± 0.60	4.11 ± 0.06	6.13 ± 0.03
Crude protein	20.10 ± 0.37	65.90 ± 0.96	63.12 ± 0.68
Ash	3.41 ± 0.04	14.64 ± 0.03	12.28 ± 0.06
Fat	4.44 ± 0.17	7.73 ± 0.08	6.58 ± 0.06
Carbohydrate	4.72 ± 0.08	7.62 ± 0.36	11.89 ± 0.32
	Parachanna obscura		
Moisture content	68.61 ± 0.37	6.47 ± 0.04	9.54 ± 0.08
Crude protein	18.23 ± 0.47	64.67 ± 0.00	63.78 ± 0.01
Ash	2.68 ± 0.03	13.20 ± 0.04	11.25 ± 0.03
Fat	3.55 ± 0.19	8.87 ± 0.03	5.18 ± 0.08
Carbohydrate	6.93 ± 0.20	6.79 ± 0.01	10.25 ± 0.21
	Clarias gariepinus		
Moisture content	56.99 ± 0.80	6.52 ± 0.09	4.61 ± 0.03
Crude protein	17.21 ± 0.41	68.05 ± 0.90	61.28 ± 0.01
Ash	4.82 ± 0.09	15.32 ± 0.02	11.59 ± 0.03
Fat	4.79 ± 0.08	8.19 ± 0.01	5.17 ± 0.02
Carbohydrate	16.19 ± 0.01	1.92 ± 0.08	17.35 ± 0.05

Deteriorated Sample: On week five, after deterioration has set in, another sample was taken from each of the three fish species for proximate analysis.

Proximate Analysis: Proximate analysis of the sample for moisture, fibre and fat were determined using the methods described by AOAC (1990). Nitrogen was carried out by the micro-kjeldahl method described by Pearson (1981) and the percentage Nitrogen was converted to Crude protein by multiplying by 6.25.

Statistical Analysis: All determinations were carried out in triplicate and data obtained analyzed for their central tendencies and variances using Statistical Package for Social Sciences (SPSS for Windows version 10).

RESULTS AND DISCUSSION

It was observed that spoilage of fish flesh resulted from the action of enzymes and bacteria; this can be slowed down through the application of salt and removal of moisture to increase the shelf life of fish, (Connel, 1980).

The result of proximate composition of smoked fish samples showed that the crude protein level was higher than those of fresh sample and the deteriorating fish sample (Table 1). Doe and Olley (1983) reported that smoking resulted in the concentration of nutrients due to low residual moisture level. The gross difference in crude protein level of the fresh sample and dried sample across the species was substantial, *Tilapia:* 20.10 % and 65.90 %, *Channa:* 18.23 % and 64.67 % and *Clarias:* 17.21 % and 68.05 % respectively (Table 1).

At the deterioration, the crude protein of the fish samples was relatively lower when compared to the dried fish samples. Mould growth was observed on the fish samples during storage, part of the protein may have been used up by the mould for growth (Proctor, 1977). The decrease in crude protein at deterioration might be due partly to microbial and enzymatic breakdown and assimilation by mould. This was supported by Eyo (1983), who observed the same trend in the protein content of some fish species kept for longer period of time. The fat content of the three species when dried were higher than the fresh and deteriorating stages, which may be disadvantageous especially with regards to rancidity development during storage. The lower level of fat content observed for the deteriorating stage was due to loss to rancidity with the resultant rancid odour and offensive flavour.

The result showed that bacteria multiply when the fish samples were kept for longer period of time. Spoilage and development of bacteria in smoked fish is always due to improper handling of fish product either prior to smoking or after smoking.

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PHYTOCHEMICAL CHARACTERIZATION AND BIOCHEMICAL STUDIES OF *Cissus multistriata EXTRACT* ADMINISTERED TO *Rattus novergicus*

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ABSTRACT

The leaves of cissus multistriata were collected, air-dried for two weeks and pulverized into powder; this was followed by extraction with either chloroform or water. The phytochemical screening of the extracts revealed the presence of carbohydrates, proteins, vitamin C, saponins, steroids, cardiac glycosides, lipids and vitamin E; whereas balsam, anthraquinone, tannins, alkaloids, cardenolides and phlobactannin were completely absent. The aqueous extract was administered to the experimental rats at doses of 100, 200, 800, 1600, 3200, and 6400 mg/kg body weight for three weeks. The control group were injected 5 ml physiological saline 3 times daily for 21 days. The test animals showed appreciable body weight increase when compared with the control group. The body weight increase was dose dependent. Analysis of the blood samples for enzymes activities, (indicators for the possible damage to the liver and kidney) showed that the leaf extract was slightly toxic to these organs. Measurement of enzymes activities revealed that lactate dehydrogenase, alkaline phosphatase, acid phosphatase, alanine and aspartate amino transferases activities were observed to have increased throughout with increase in dosage of the extract down the group. A part from the enzymes, other renal and hepatic profiles were monitored which included serum urea, creatinine, albumin and bilirubin. There was increase in the renal and hepatic profiles monitored and the increase was dose dependent. The result of this investigation indicated that prolonged use and at a high dosage of the extract could be deleterious to the liver and kidney.

Keywords: *Cissus multistriata, Vitaceae,* liver, kidney, albino rats, enzymes, analytes

INTRODUCTION

The truth about natural products with medicinal efficacy must not be allowed to die (Anslem, 2002). Selection of such natural products for use in orthodox medicine was not based on prior knowledge of its constituents but on factors like seasonal, availability, astronomical, mystical, religious and signatures of nature that the Native Doctor accepted as influencing his life (Anslem, 2002).

Cissus is a genius plant with over 350 species of tropical and subtropical which are chiefly woody vines of the grape Family (Vitaceae). The leaves are often fleshy and somewhat succulent and often used for medicinal purposes. They contain some bioactive compounds. These compounds are found in the leaves, roots, stem and bark (Burkill, 1985). It is a slightly fleshy climber flowers; ultimate branching of inflorescence cymose, unexpanded corolla, sub-globose leaflet; three oblong-eliptic rounded at base, sharply apiculate at apex; 4 - 7 cm long, 2 - 3 cm broad, petiolules fruits, 3-4 seeded. It is found in places like Togo, and Nigeria around river basins. Its distribution extends to Sudan, East Africa, Belgian-Congo and Angola (Burkill, 1985).

It is a well known plant to the traditional medicine practioners in Nigeria. It is called *Ojekere* or *Okanigbo* by the Igalas, *Ewebiomo* in Yoruba, *Ochekihiozeowehi* by the Ebira's in Kogi State, and *Mukala* by the Ibos in Nigeria.

It is used as medicinal plant for the treatment of diverse ailments in different locations. The Ebira's use the stem prepared in form of decoction as internal cleanser for new born babies while the Yoruba's use the leaves for the treatment of infertility in women and stomach ailment in children. It is commonly used by the Ibaji's in Kogi State for the treatment of malnutrition diseases such as kwashiorkor and marasmus in children. Other uses of this plant include its use as cough remedy, fracture healing etc.

Rattus novergicus were used in this study because they have close physiology to that of humans cheap to obtain and give a more reliable response. The objective of this study was to test the toxic effect of *cissus multistriata* leaf extract on organs of the body. This plant is used by many herbal doctors for the treatment of different ailments without proper dosage. Elevated blood levels of liver and kidney enzymes are indications of organ toxicity or tissue damage, hence their measurement in this work. This research also aims to screen the plant for its bioactive components that might be responsible for the healing properties claimed by the users.

MATERIALS AND METHODS

Preparation of leaf extract: The leaves of *cissus multistriata* were collected from Ega in Idah Local Government Area, Kogi State, Nigeria during rainy season when the plant thrives very well. The leaves

were washed to remove dirts; air dried for two weeks and then pulverized using mortar and pestle. The percolation method of extraction was employed. The powdered or pulverized plant sample was soaked in a solution of chloroform and water (3:5 v/v) for 92 hours, filtered and the filtrate evaporated to give off the chloroform and water. The solid concentrate of the extract was stored in vials.

Experimental Animal Care and Management: The experimental animals used were albino rats (*Rattus novergicus)* weighing between 210 – 274 g obtained from ABU Agricultural farm, Zaria, Kaduna State, Nigeria. The animals were grouped into six test groups of four rats each labeled 1 to 6. The seventh group served as control. All rats were fed and watered *ad libitum* using 20 % CP Guinea feed.

Crude Extract Administration: 100, 200, 800, 1600, 3200 and 6400 mg/kg body weight of rat was injected three times daily for 21 days to rats in treatment groups 1, 2, 3, 4, 5 and 6 respectively. All injectibles were dissolved in 5 ml physiological saline. Rats in treatment group 7 were injected 5 ml physiological saline, 3 times daily for 21 days.

Blood Sampling and Enzyme Activity Assay: At the end of the treatment period, rats in all groups were anaesthetized, dissected and bleed via cardiac puncture. Blood samples collected per group were centrifuged at 3000 rpm. The resultant supernatant rich serum was used for enzyme activity assay thus:
Serum acid phosphatase activity was determined based on the method of Friedman and Young (1997). Alkaline phosphatase activity was determined using the method of Rosalki *et al,* (1993). The method of Tietz (1994) was followed in the determination of serum aspartate amino transferase. The determination of alanine amino transferase was carried out following the methods of Friedman and Young (1997). Lactate dehydrogenase activity was also measured according to the method of Friedman and Young (1997). Serum urea determination was carried out using the method described by Talke and Schuber (1965). The method of Teitz (1991) was followed in the determination of serum creatinine. Similarly, serum albumin determination was carried out using the methods of Tietz (1991) and Doumas *et al.* (1971). The methods of Jendrassik and Grof (1938) and Sherlock (1951) was used in the determination of serum bilirubin.

Phytochemical Screening: The plant sample was screened for the presence of bioactive components following the methods of Sofowora (1982), Trease and Evans (1993), Brian and Anthony (1989), WHO (1998), and Finar (1974)

Data Analysis: The results are expressed as mean $\pm$ S.D. Analysis of variance (ANOVA) was used to test for the differences among all the groups at $P < 0.05$. To find out where the significant difference lies among the groups, the Duncan's multiple range test was used in comparing the means (Dixon and Massey, 1957; Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The result of the phytochemical screening showed the presence of some bioactive compounds such as carbohydrate, cardiac glycosides, flavonoids, lipids, proteins, sponnins, steroids and vitamin C while compounds like alkaloids, anthraquinones, balsam, cardenolides, phlobactannin, tannin and vitamin E were absent in the aqueous extract. The screening of the chloroform extract revealed the presence of lipids and vitamin E (Table 1).

Table 1: Phytochemical Screening of *Cissus multistriata*

S/N	Compound	Aqueous Extract	Chloroform Extract
1	**Alkaloid**	-	-
2	**Anthraquinone**	-	-
3	**Balsam**	-	-
4	**Carbohydrate**	++	-
5	**Cardenolide**	-	-
6	**Cardiacglycosides**	++	-
7	**Flavonoids**	+	-
8	**Lipids**	+	++
9	**Protein**	++	-
10	**Phlobactanin**	-	-
11	**Saponins**	++	-
12	**Steroids**	+	-
13	**Tannins**	-	-
14	**Vitamin C**	+	-
15	**Vitamin E**	-	++

***Key:* ++ =** *presence of bioactive compounds in high concentration, + = Presence of bioactive compounds in low concentration. - = Absence of bioactive compounds.*

The aqueous extract contained more of the bioactive compounds than the chloroform extract. The chloroform extract had only the fractions that are soluble in non-polar solvents. The bioactive compound contents of this plant support its uses, in providing energy; build up of worn-out tissues and regulation of internal temperature of the body. The presence of protein, vitamins and carbohydrate justifies the use of this plant in management of malnutrition. The plant also contains vitamin E and C known as tocopherol and ascorbate respectively. Both vitamins are antioxidants and as such could be useful in free radical scavenging in living system and are essential dietary constituents for humans. Vitamin C is necessary for connective tissue and promotes the healing of wounds and fracture (Barbara, 1996). This justifies the use of the plant extract for bone fracture healing.

The phytochemical screening of the aqueous extract of this plant also revealed the presence of flavonoids. The effectiveness of this plant as an anti-oedema and anti-inflamation may be due to the presence of this bioactive ingredient. Further more, is steroid which backs up the use of this plant by indigenous people of Igala land for the treatment of infertility in both males and females.

Table 2: Mean Body Weights of Rats before and After Exposure to Dosage of *Cissus multistriata Extract*

Group	Dose (mg/kg)	Before administration	One week (g)	Two weeks (g)	Three weeks (g)
1	100	210.84 ± 2.25[a]	214.74 ± 1.37[b]	216.15 ± 1.48[b]	219.98 ± 1.69[b]
2	200	225.73 ± 2.85[b]	220.16 ± 3.86[a]	230.17 ± 4.67[a]	231.96 ± 7.42[a]
3	800	231.16 ± 0.66[c]	236.30 ± 5.60[c]	237.33 ± 4.77[a]	240.17 ± 6.46[a]
4	1600	233.13 ± 0.98[d]	237.61 ± 3.12[d]	240.83 ± 5.65[a]	242.91 ± 7.18[a]
5	3200	264.11 ± 0.99[e]	265.27 ± 1.22[e]	267.07 ± 2.16[a]	270.12 ± 1.46[a]
6	6400	274.18± 4.11[f]	284.09 ± 0.05[a]	295.48 ± 1.50[a]	301.69 ± 3.33[a]
Control	—	220.03 ± 2.70[g]	220.65 ± 2.30[a]	220.95 ± 2.22[a]	221.31 ± 2.42[a]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).

Table 3: Mean Values of Serum Enzyme Activities in Albino Rats

	Dose in mg/kg body	Enzymes (U/L)				
Group	weight	Serum ACP	ALP	AST	ALP	LDH
1	100	10.3± 0.50[b]	22 ± 0.82[b]	94 ± 1.41[b]	199 ± 1.63[a]	256 ± 2.50[a]
2	200	17.8 ± 0.36[c]	24 ± 0.82[c]	96 ± 0.82[a]	219 ± 1.41[a]	294± 2.16[a]
3	800	20.8 ± 0.23[a]	26 ± 0.82[a]	98 ± 0.82[a]	250 ± 0.82[a]	303 ± 2.16[a]
4	1600	24.4 ± 0.36[a]	28 ± 0.82[a]	101 ±1.41[a]	251 ± 1.41[a]	306 ±2.16[a]
5	3200	30.2 ±0.24[a]	29 ± 0.82[a]	105 ± 0.82[a]	284 ± 1.63[a]	314 ± 2.16[a]
6	6400	41.0 ± 0.46[a]	32 ± 0.81[a]	105 ± 2.16[a]	383 ± 1.50[a]	325 ± 6.16[a]
Control	—	12.4 ± 0.47[a]	20 ± 0.82[a]	91 ± 0.82[a]	149 ± 1.41[a]	322 ± 1.41[a]
Pre-treated rats -		12.39 ± 0.47[e]	20 ± 0.82[d]	89 ± 0.79[f]	148.5 ± 1.39[g]	240 ± 2.51[g]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).; ACP = Acid phosphatase, ALT= alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase

Table 4: Analyte Determination

Group	Dose mg/kg	Serum urea (mg/dl)	Serum Creatinine (mg/dl)	Serum albumin (mg/dl)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)
1.	100	21 ± 0.82[b]	0.9 ± 0.08[b]	1.68 ± 0.04[a]	0.25 ± 3.03[b]	0.01 ± 0.00[a]
2.	200	22 ± 0.41[c]	1.1 ± 0.08[a]	1.68 ± 0.19[a]	0.29 ± 2.58[a]	1.01 ± 0.00[a]
3.	800	25 ± 0.82[d]	1.0 ± 0.08[c]	1.70 ± 0.12[a]	0.34 ± 2.58[c]	0.02 ± 2.58[b]
4.	1600	27 ± 0.82[a]	1.1 ± 0.26[a]	1.90 ± 0.08[a]	0.46 ± 2.28[d]	0.04 ± 2.58[g]
5.	3200	30 ± 0.96[a]	1.0 ± 0.08[d]	1.80 ± 0.08[a]	0.52 ± 0.03[a]	0.09 ± 2.58[a]
6.	6400	40 ± 0.82[a]	1.2 ± 0.08[a]	1.60 ± 0.08[b]	0.58 ± 2.58[a]	0.12 ± 2.58[a]
Control	-	25 ± 0.82[a]	0.9 ± 0.08[a]	1.60 ± 0.14[a]	0.30 ± 2.58[a]	0.03 ± 2.58[a]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).

Steroid according to Barbara (1996) is one of the group of hormones chemically related to cholesterol, they include estrogen, androgen, progesterone, and the corticosteroids. Appreciable increase in the body weight of the animals was observed and the increase was dose dependent (Table 2). All the test groups showed appreciable increase in body weight compared to the control. This increase was statistically significant ($P < 0.05$).

The results of the serum enzyme activities are presented on table 3. The activity of acid phosphatase increased with increase in dosage of the extract. There was a significant difference ($P < 0.05$) in the increase in activity when compared with the control. This similar trend was observed for all the enzymes assayed. The effect caused by the extract on the liver could be minimal in groups 1 and 2 whose mean values when compared with the control showed no significant difference ($P > 0.05$) and more toxic to this organ of the animals in group 3 to 6 as their mean values when compared with the control showed a significant difference ($P < 0.05$). This observation correlates with that of Gupta and Verma (1990) that *cissus quardrangularis* was not toxic at lower doses but at higher ones.

Marcus and Milton (1980), observed high acid phosphatase activity in the serum to correlate with hepatobiliary disease and disease of the reticulo-endothelial system as a result of damaged liver. The extract had highest effect on animals in group 6 that was administered the highest dosage of extract and damage to the liver might have occurred in most of the treated rats. An elevated level of these enzymes, AST and ALT in acute infection, toxic hepatitis, cirrhosis of the liver and liver neoplasm has been described (Rej and Horder, 1993; Marcus and Milton, 1980). Animal in groups 5 and 6 had the highest serum enzyme activity and hence more damage must have been done to them compared with other groups and the control.

Similarly, alkaline phosphatase and lactate dehydrogenase activities were observed to have increased with increase in dosage of extract.

These enzymes activity signifies more damage to these organs and vice versa as the presence of these enzymes in the serum is an indication that these organs were affected. The result of the serum urea

concentrations showed that there was gradual increase in the serum urea concentration as the dosage increased (Table 4). There was no significant difference (P> 0.05) when group 1 was compared with the control. Comparing group 6 of dose 6400 mg/kg body weight with the control, a statistically significant difference (P < 0.05) increase was observed. An increase in serum urea in conjunction with a concomitant increase in serum creatinine levels may be an indication of kidney malfunction (Teitz, 1991).

There was increase in serum creatinine concentration with increase in dosage of extract (Table 4). Creatinine levels depend on the glomerular filtration rate (GFR). Serum creatinine is doubled when GFR is considered to be halved. The increase in serum creatinine level in this study may not have significant consequences to kidney. The result of serum bilirubin concentration as shown on table 4 reflects that increase was dose dependent. Bilirubin concentrations are effective sources of measurement of liver function. The increase in the bilirubin concentration showed that the plant extract had a degree of toxicity to the liver. This is in support of the view of Weiss *et al* (1983).

The low serum concentration of the renal and hepatic profiles monitored is an indication that the plant may be safe at lower doses and could be deleterious at higher doses. From the overall results, it may be inferred that the plant extract was slightly toxic. Despite the numerous benefits derived from this plant in terms of its medicinal values, prolonged use should be disallowed. The presence of bioactive compounds is contributory to its medicinal value. The effect of this extract on the two organs showed that the plant extract toxicity was dose dependent as indicated by the levels of enzyme activities, which were used as indicators of renal and hepatic diseases.

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SERO-EPIDEMIC SURVEY OF HEPATITIS B IN A POPULATION OF NORTHERN NIGERIA

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ABSTRACT

The rates of infection of various hepatitis B virus serological markers were measured on the basis of age, sex and socio-economic activities amongst the community population of Mubi, a known border community in North-Eastern Nigeria. Sera of 992 subjects consisting of 613 males and 379 females were analysed by radioimmunoassay. The overall HBV exposure among the subjects surveyed was 40.3 %. The rate of HBsAg infection was 9.0 %; 19.0 % for anti-HBs and 12.2 % for anti-HBc. The occurrence of HBV markers by age of the subjects showed that infants less than 1 year old had the highest HBV exposure rate of 43.9%; the rate declined at the 1-10 years age group and increased steadily thereafter with age until the ≥ 51 years age bracket. The incidence of the HBV markers by sex of subjects showed that infection rates were higher in males (43.4%) than in females (35.4%). The rate of HBs infection rose progressively with age and significantly higher (p<0.01) in males (20.1%) than in females (17.2 %). The infection rate of HBc did not correlate with increase in age and significantly higher (P < 0.01) in males (13.2 %) than in females (10.8%). The distribution of the HBV markers was associated with differences in socio-cultural environment and practices (Fig. 2); thus, prison inmates who constituted the bulk of commercial blood donors had the highest rate of infection (28.5 %), followed by traders/artisans (21.0%) and students/pupils (18.0%). This study suggests vertical (maternal to infant) and horizontal transmission early in life in the spread of HBV markers in Mubi area and recommends passive active HB immunization (anti-HB vaccine), personal and urban hygiene and that testing for HBsAg by the most sensitive methods should be required for all blood donors. HBsAg-carriers and People who are known to have the infection or to be at high risk e.g. prostitutes, prisoners, etc should be discouraged from donating blood.

Keywords: Hepatitis, Radio-immunoassay, Immunization, Cirrhosis, Serological-markers, Morbidity

INTRODUCTION

Viral hepatitis causes of considerable mortality both from acute infection and chronic disease conditions and ranks among the ten top killer diseases (Blumberg, 2002). Infection with hepatitis B virus (HBV) is a worldwide problem. It has been reported that hepatitis B related illnesses causes an estimated 1 – 2 million deaths per year world wide and 5,000 – 6,000 deaths per year in America (Blumberg, 2002; HBF, 2005). The World Health Organization (WHO) estimates that 400 million out of the about 2 billion subjects infected worldwide are at risk of developing hepatological and non-hepatological manifestations. Between a third to a quarter of these people are expected to develop digestive haemorrhage and progressive liver diseases including cirrhosis and hepatocellular cancer (Poynad, 2001; HBF 2005). The HBV infection varies widely worldwide from high (≥ 8 %) e.g., in Africa, Asia and the Western Pacific to intermediate (2 - 7.9 %) e.g., in Southern and Eastern Europe and low (≤ 2 %) e.g., in Western Europe, North America and Australia (Poynad, 2001).

In Nigeria, Hepatitis B virus (HBV) infection is a major health problem due to its associated mortality. Apart from the asymptomatic nature of the disease in most cases, the documentation of mortality is very poor. Furthermore, many people especially in the poor rural communities do not seek medical assistance early except for major health problems. Another factor is that most of the available health institutions lack the requisite manpower, equipment and reagents for virologic diagnosis. However, recent surveys have incriminated hepatitis B as a major aetiological agent of chronic disease in Nigeria (HBF, 2005).

Symptoms of hepatitis B virus infection are few, hardly noticeable When symptoms are present, they vary significantly depending on the overall health of the infected person and generally include extreme tiredness, loss of appetite, nausea and vomiting, fever, headache, muscle aches, abdominal disturbances and jaundice.

Acute hepatitis, liver cirrhosis and hepatocellular carcinoma are presently important causes of hospitalization and death due to HBV. Hepatitis B virus is one of the major diseases of mankind and at high risk of developing cirrhosis and primary hepatocellula carcinoma and subsequent death. Hepatitis B virus related to hepatoma is the most common malignancy accounting for 20 – 50 % of all cancer-related deaths among males in Asia and African (Ojo, 1997). As a consequence of the chronic complications of Hepatitis B, there is a great demand on the health care system leading to considerable economic implications.

The basic reason for screening of blood before transfusion is to avoid the occurrence of complications in the recipient due to the blood received, particularly to avoid the transfusion of pathogenic micro-organisms such as *Mycobacterium tuberculosis*, *Treponema palladium* (causative agent of syphilis), *Plasmodium* (malaria parasite), the Human Immuno Deficiency Virus (HIV) and hepatitis B virus, etc. The screening of blood of donors for HBV is not routinely done in most rural health facilities in Africa, consequently, majority of the blood transfusion are undertaken without screening for hepatitis B.

Symptoms of acute hepatitis often subside without treatment within a few weeks or months. A few cases develop into a chronic and incurable form of the disease, eventually resulting in liver cirrhosis or cancer. Currently, there are 5 agents licensed in the United States for the treatment of chronic hepatitis B viz. interferon alfa-2b; pegylated-interferon alfa-2a; and the oral agent's lamivudine, adefovir dipivoxil, and entecavir. The oral antiviral agents against hepatitis B virus (HBV) are usually used for long-term periods in order to increase the probability of hepatitis B e antigen (HBeAg) seroconversion in HBeAg-positive patients and/or to maintain remission in both HBeAg-positive and HBeAg-negative CHB. Liver transplants may be beneficial to infected patients, but the virus remains in the body after transplantation surgery and may eventually attack the new liver (Papatheodoridis, 2006). There is effective vaccine that can prevent hepatitis B.

This study determined the infection rate of HBV serological markers in a population in North East Nigeria on the basis of age, sex and socio-economic activities. This would partly help to re-focus attention to other killer-diseases apart from the HIV/AIDS scourge. Based purely on economic point of view; hepatitis B is more significant than HIV/AID. Infact, the Hepatitis B virus is said to be about 100 times more infectious than HIV, the virus that causes AIDS (HBF, 2005).

MATERIALS AND METHODS

Study Location: The study location is the Mubi General Hospital, Mubi, in Mubi North LGA of Adamawa State. Mubi is an ancient urban settlement in the defunct North Eastern region and a notable border community, being bounded by Boukoula District of the Republic of Cameroun. The Mubi General Hospital is the largest health facility in the area and heavily patronized by residents of 6 adjourning LGAs - Mubi North and South, Gombi, Hong, Maiha and Askira-uba in neighboring Borno state. Also, Boukoula District of Cameroun Republic. The Hospital therefore serves a population of more than 250,000 people including serving as a referral for the state university, a Federal Polytechnic, a state College of Agriculture, School of Health Technology and several primary and post-primary schools all located in Mubi metropolis. Also serviced are the Police Mobile Training school Limankara, Prison and Custom formations and traders from within and outside Nigeria who patronized the thriving Mubi cattle market.

Sampling Frame: A total of 992 subjects who were either patients or blood donors at the Mubi General Hospital were enlisted for this survey and composed as shown (Fig. 1).

Serological Markers: The Hepatitis B virus (HBV) Markers studied were:

i HBsAg (Hepatitis B surface antigen) using the Austria II-125 radio-immunoassay method.
ii Anti-HBs (Anti-hepatitis B surface antibody) using the AUSAB® radio-immunoassay method.
iii Anti HBc (Anti-hepatitis B core antibody) using the CORAB radio-immunoassay method.

7 mls of blood was obtained from each subject using sterile, disposable syringes and needles. For pending tests, sera were extracted and stored, frozen at -20^{o} c. A close-ended questionnaire was used to obtain information on the age, sex and occupation of subjects.

Statistical Analysis: The chi-square (χ^2) test with Yates correction for small numbers (Swincow, 1983) was used to analyze the data.

RESULTS

The overall incidence of HBV markers among the subjects surveyed was 9.2 % for HBsAg, 19.0 % for anti-HBs and 12.3 % for anti-HBc. The overall HBV exposure was 40.3 %.

The distribution of HBV markers by age of the subjects is shown on Table 1. Infants less than 1 year old had the highest HBV exposure rate of 43.9 %. That is, at birth and within the first 12 months of life, this percentage of the infant population was positive for at least one HBV marker. The rate declined at the 1-10 years age group and increased steadily thereafter with age until the $\geq$51 years age bracket.

The incidence of the HBV markers by sex of subjects showed that infection rates were higher in males (43.4 %) than in females (35.4 %). In males, the infection rate was highest amongst 41-50 years age group (46.5 %) and least in the 1 – 10 years (38.3 %) age group (Table 2). In females, the highest and least rates were in infants <1year old (42.9 %) and 1 – 10 years (30.0 %) age group respectively (Table 3). Values in the other age groups were intermediate.

The infection rate for anti-HBs was significantly higher ($P < 0.05$) in males (20.1 %) than in females (17.2 %). The highest rate of infection with HBs was recorded among the $\geq$ 50 years age group (22.5 %) and the lowest rate among subjects of the < 1 year (14.6 %) age bracket. The rate of HBs infection rose progressively with age. The infection rate for anti-HBc was significantly higher in males ($P < 0.001$) than in females. The anti-HBc infection rate was highest among the infants <1 year of age (19.5 %) and the least rate (9.3 %) was in the 21 - 30 years age bracket. Infection did not correlate with increase in age grouping.

Table 1: Hepatitis B Virus Markers by Age Grouping in a Population of Northern Nigeria

Age Group	No Examined	HBsAg No. +ve (%)	Anti-HBs No. +ve (%)	Anti-HBc No. +ve (%)	Total HBV Exposure No. +ve (%)
<1	41	3(7.3)	6(14.6)	9(22.0)	18(43.9)
1 – 10	180	14(7.7)	27(15.0)	23(12.8)	64(35.6)
11 – 20	160	15(9.4)	28(17.5)	22(13.6)	65(40.6)
21 – 30	289	32(11.1)	57(19.7)	27(9.3)	116(40.1)
31 – 41	156	13(8.3)	33(21.2)	20(12.8)	66(42.3)
41 – 50	126	10(7.9)	28(22.2)	16(12.7)	54(42.9)
≥ 51	40	3(7.5)	9(22.5)	5(12.5)	17(42.5)
Total	992	91(9.2)	188(19.0)	122(12.3)	400(40.3)

Table 2: Hepatitis B Virus Markers amongst Males in a Population of Northern Nigeria

Age Group	No Examined	HBsAg No. +ve (%)	Anti-HBs No. +ve (%)	Anti-HBc No. +ve (%)	Total HBV Exposure No. +ve (%)
<1	13	1(7.7)	2(15.4)	3(23.1)	6(46.2)
1 – 10	120	10(8.3)	19(15.8)	17(14.2)	46(38.3)
11 – 20	96	11(11.5)	18(18.8)	15(15.6)	44(45.8)
21 – 30	177	22(12.4)	36(20.3)	17(9.6)	75(42.4)
31 – 41	99	9(9.1)	22(22.2)	14(14.1)	45(45.5)
41 – 50	86	7(8.1)	21(24.4)	12(14.0)	40(46.5)
≥ 51	22	2(9.1)	5(22.7)	3(13.6)	10(45.5)
Total	613	62(10.1)	123(20.1)	81(13.2)	266(43.4)

Table 3: Hepatitis B Virus Markers amongst Females in a Population of Northern Nigeria

Age Group	No Examined	HBsAg No. +ve (%)	Anti-HBs No. +ve (%)	Anti-HBc No. +ve (%)	Total HBV Exposure No. +ve (%)
<1	28	2(7.1)	4(14.3)	6(21.4)	12(42.9)
1 – 10	60	4(6.7)	8(13.3)	6(10.0)	18(30.0)
11 – 20	64	4(6.3)	10(15.6)	7(10.9)	21(32.8)
21 – 30	112	10(8.9)	21(18.8)	10(8.9)	41(36.6)
31 – 41	57	4(7.0)	11(19.3)	6(10.5)	21(36.8)
41 – 50	40	3(7.5)	7(17.5)	4(10.0)	14(35.0)
≥ 51	18	1(5.6)	4(22.2)	2(11.1)	7(38.9)
Total	379	28(7.4)	65(17.2)	41(10.8)	134(35.4)

The distribution of the HBV markers on the basis of socio-economic groupings (Fig. 2) showed that prisoners had the highest rate of infection (28.5 %), followed by traders/artisans (21.0 %) and students/pupils (18.0 %).

DISCUSSION

The overall infection rate observed in this study for HBsAg (9.2%) and some HBV markers (40.3%) meets he number needed to treat (NNT) criteria for the disease (Craxi *et al.*, 2005) With a HBsAg carrier rate of 9.2%, Mubi area in Adamawa state Nigeria is classifiable as hyper-endemic HBV focus using the standard of Ponad (2001).

The age distribution of antigenemia in this study indicates early transmission. Incidence of HBV markers is high in infants <1 year (43.9%) and children 1-10 years (35.6%) indicating possible vertical (maternal infant) transmission utero or parentally and very early in life (horizontal) leading to the acquisition of HbsAg chronic carrier status.

Further investigations may be required to demonstrate whether the infected children in this study were infected utero (carriers) or contaminated with maternal blood during delivery (transient HBV antigenemia). Their low HBsAg blood levels appear to support the later suggestion. It has been observed that the risk of infection is about five time mothers higher in children of HBsAg positive mothers than those of HBsAg negative mothers. Transmission of HBV from mother to neonate can be activated through contact with material blood and other infectious fluid during labour, colostrium, and breast milk (Bornino, 1992). There is substantial risk of perinatal infection if the mother has acute HBV in the second or third trimester of pregnancy or within two months after delivering. Most children infected during perinatal period become persistent carriers. It is estimated that 5-10% of adults, 30-50% of children and 90% of babies will not get rid of the virus, develop chronic infection and can pass the virus on to others and are at increased risk for liver problems later in life (HBF 2005).

The infection rates for anti-HBc were significantly higher in males than females. The high prevalence among males agrees with the report of WHO (1983) that certain sex-specific pattern or occupational activities may expose males more often to Hepatitis B virus or that some immunological deficiency or genetic predisposition may mean that a larger percentage of infected males than females develop a chronic infection. The WHO report emphasized that the underlying mechanism of the sex difference in response to Hepatitis B virus remain obscured.

The prevalence of infection is associated with differences in socio-cultural environment and practices (Fig 2). The prevalence of infection with hepatitis B virus varies from country to country and depends on a complex mix of behavioural, environmental, and host factors (WHO, 1983). Factors likely to affect the occurrence of HBV markers are age, level of literacy, immunization records, skin scarification, human bite, sexual behavior of sexually active adults and teenagers, sharing of contaminated ear-rings, toothbrushes, razor, syringe or tattoo needles, level of drug-use, residential pattern, congregation of susceptible with infective, level of hygiene (personal and urban), general low level of living, etc. Unfortunately, prison inmates constituted the bulk of commercial blood donors encountered during this study.

It is obvious from this and similar report in tropical Africa that unlike parts of western Europe and North America with sporadic HBV infection which occur mostly in adults, the disease is endemic and present very early in life in most areas and subjects as young as 1-10 years old already have infection rates similar to the adult population (Boxall *et al.,* 2006). Carrier rates in the tropics are generally higher among children and also among those of lower economic class (Ojo, 1997). The World Bank in the 1993 world development report stated that the addition of Hepatitis B vaccine to the Expanded Programme on Immunization (EPI) was among the cost effective health interventions in most developing countries (Cooksley, 1997). The objective of the World Health Organization (WHO) included the introduction of Hepatitis B immunization to EPI of all countries by 1997 and reduction in the incidence of new carriers among children by 80% by the year 2001 (Cooksley, 1997). The Centre for Disease Control (CDC) and the American Academy of Pediatrics recommend that all infants, children and adolescents up to 18 years of age and all adults at risk of infection should receive the HBV vaccine.

This study suggests vertical (maternal to infant) and horizontal transmission early in life in the spread of HBV markers in Mubi area.

Recommendations: The study recommends immunization (mass vaccination of young children against HBV), good personal hygiene and urban sanitation, health communication (education) and formal education generally as potent tools in the fight against HBV markers in this area.

Women, who acquire Hepatitis B before or while pregnant, can transmit the disease to their children. Diagnosis for HBV markers should be incorporated into the ante-natal schedule. Perinatal transmission can be prevented with the identification of HBsAg positive women and administration of immunoprophylaxis to their newborns. A national prevention programme for HBV with universal screening of pregnant women and vaccination of infants has been found effective in some countries such as Greece (Vassiliki *et al.,* 2006).

Testing for HBsAg by the sensitive methods should be required for all blood donors. People who are known to have the infection or to be at high risk e.g. prostitutes, prisoners, etc should be discouraged from donating blood. Blood transfusion should be undertaken only when absolutely necessary for life threatening conditions.

Persons identified in the course of sero-epidemiologic investigations as transient or persistent carriers of HBsAg such as those diagnosed positive in the present study should be treated and educated regarding the mechanism of HBV spread so that the rate of transmission might be minimized.

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PREVALENCE OF GASTRO-INTESTINAL PARASITES IN RELATION TO AVAILABILITY OF SANITARY FACILITIES AMONG SCHOOLING CHILDREN IN MAKURDI, NIGERIA

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ABSTRACT

The prevalence of gastro-intestinal parasites in school children in relation to availability of sanitary facilities was investigated. Stool samples from 580 pupils from nine schools in Makurdi were examined for intestinal parasites. Sanitary facilities available within the schools were also noted. The overall prevalence rate of parasitic infection was 54.13%. Pupils in schools that had lower ratio of number of pupils per toilet had lower infection rates than those from schools with high ratio of number of pupils per toilet. This was however not statistically significant (X^2 2.272, df = 2, P > 0.05). The following parasites were encountered, namely Ascaris lumbricoides (11.89%), Ancylostoma duodenale (18.62 %), Strongyloides steroralis (1.89%), Trichuris trichura (4.65%), Tapeworm (3.79 %), Entamoeba histolytica (7.06 %), Schistosoma mansoni (1.55 %) and Entemoeba coli (2.41 %). The implications of these results were discussed highlighting the need for provision of sanitary facilities: like children friendly toilets, portable water and fencing the school premises from trespassers as long-term intervention strategies. Occasional activities like mass school based chemotherapy and health education are recommended as immediate intervention strategies to prevent and control intestinal parasites.

Keywords: Intestinal parasites, School children, Sanitary facilities

INTRODUCTION

Several environmental and socio-economic factors have been identified to be responsible for the continued persistence of intestinal parasites in children; some of these include poor sanitary conditions, unhygienic practices, absence of portable water, poor housing and poverty (WHO 1991, Edungbola and Obi 1992; Crompton and Savioli, 1993; Nwoke 2004, Amuta *et. al.,* 2004). Recent global estimate indicated that more than a quarter of the world's populations are infected with one or more of the most common parasites; *Ascaris lumbricoides,* hookworm and *Trichuris tichura* (Manen *et. al.,* 1997; Chan *et al.,* 1994).

School age children (3 – 16 years) are particularly at risk. Infants growing up in an endemic community where sanitation and waste disposal facilities are inadequate are usually infected soon after weaning. About 20 % of disability adjusted life years (DALYS) lost due to communicable diseases among children are a direct result of intestinal nematodes (Hanson 1999). Clinical manifestation among children habouring these parasites include abdominal pain, nausea, reduced appetite, iron-deficiency anaemia, retarded growth and impaired cognitive performance (Edungbola and Obi, 1992; Ogbe *et. al.,* 2002).

The presence or absence of sanitary facilities at home has been established as a strong determinant of the prevalence of gastro-intestinal parasites (Feachem *et. al.,* 1983; Manen *et al.,* 1997; Omudu, 2003). However, contemporary education polices has tremendously increased the time children have to spend in school. The school environment has therefore emerged as epidemiological foci in childhood parasitism.

Amuta *et. al.* (2004), reported a positive correction between contamination of school compounds with faecal pathogens and the availability of sanitary facilities in schools in Makurdi, Nigeria. This study further investigates possible relationship between the presence of sanitary facilities in school and prevalence of gastro-intestinal parasites in school children.

MATERIALS AND METHODS

This study was conducted in Makurdi, the Benue State capital. Nine primary schools participated. Permission was sought and received from the respective authorities in charge of the schools. A total of 580 pupils aged 5 – 18 years were randomly selected for parasitological investigation and the school was physically inspected for availability of sanitary facilities.

Additional bio-data information sought from the pupils included name age, sex, place of residence, type of toilets used at home and source of drinking water. Afterwards, the randomly selected children were each given a clean, dry, well-labeled specimen bottle with which their faecal samples were to be deposited. The procedure of introducing faecal material into the bottles was explained and

demonstrated to pupils with the assistance of their class teachers.

Faecal samples collected were transported to the laboratory for analysis. They were examined for ova; cyst and/or larvae of gastro-intestinal tract parasites using the direct wet mount microscopic examination and the formal -ether concentration technique (Wentworth, 1988; Ukaga *et al.,* 2002).

Inspection of Sanitary Facilities: A structured questionnaire was designed, discussed with school authorities and pre-tested was administered to participating school Head teachers to take inventory of available sanitary facilities within the school premises. Sanitary and demographic issues addressed in the questionnaire are shown in Table 1. Physical inspection of the school premises was also conducted to ascertain state of cleanliness.

Table 1: Questionnaire to assess sanitary and demographic conditions among schooling children in Makurdi, Nigeria

School identification code----------
Population of pupils----------
Please tick the appropriate answers

1.	Type of toilet facility Pit latrine------- Flush toilet -------None-------
1a.	Number of toilet facility ----------
1b.	Number of functional toilet ----------
2.	School's source(s) of water, Tap water ------ Well ------ Storage tank------None
2a.	Number of water sources available----------
3.	Availability of fence around the school Yes ------ No------
4.	Location of refuse dump within premise outside premise
4a.	Status of refuse dumps Approved------ Unapproved------
5.	General cleanliness of school compound very neat ------ Neat------ dirty ------ Very dirty------

Data Analysis: Chi-square test will be used to test association between the presence of sanitary facilities and prevalence of infection. Prevalence of infection and questionnaire will be analysed using simple percentage.

RESULTS

An overall prevalence of 54.13 % (314) infection rate was recorded in this study. Eight parasite namely *Ascaris lumbriciodes* (11.89 %), Hookworm, *Ancylostoma duodenale* (18.62 %), *Trichuris trichiura* (4.65 %) *Taenia* species (3.79 %), *Stronglyloides stercorais* (1.89 %), *Entomoeba histolytica* (7.06 %) *Schistosoma mansoni* (1.55 %) and *Entamoebi coli* (2.41 %) were isolated from the stool samples (Table 2). The infection rate was higher among female pupils (51.53 %) than their male counterpart. Chi-square test was utilized to test association between the presence of sanitary facilities and prevalence of infection; the result at ($P > 0.05$) showed that there was no significant association between availability of sanitary and level of infection.

While the percentage infection rates in schools with better sanitary facilities were lower (Table 3), this also was however not statistically significant when compared with other schools ($P > 0.05$).

The sanitary and demographic appraisal revealed that a high ratio of number of pupils shared toilet in most of the schools (Table 4). The absence of portable water within school premises was observed in all but two of the schools. The general cleanliness of school premises was poor but for only school H which was assessed very neat. In most of the schools, refuse were dumped in unapproved location thereby making it difficult for evacuation by concerned agencies and contributing to the build up of pathogens.

DISCUSSION

This study revealed a high prevalence of intestinal parasites in school pupils, this findings was in line with similar studies in Nigeria (Luka *et al.,* 2000; Ndifon, 1991; Adeyeba and Akinlabi, 2002; Ukpai and Ugwu, 2003) and else where (Menan *et al.,* 1997; Silva *et al*., 1997). The reasons for the high prevalence may be attributed to poor environmental conditions and personal hygiene, inadequate supply of portable water, poor excreta and waste disposal system. The difference in infection rate between male and female pupils was not statistically significant. Luka *et al., (*2000), Ukpai and Ugwu (2003) and Akogun and Badaki (1998) recorded higher infection rates in male and reasoned that this was as a result of gender differences in recreational activities.

The study observed that sanitary facilities were inadequate in schools and this is of epidemiological significance considering the number of hours pupils spend in school. The ratio of the number of pupils per toilet far exceeds that recommended (Feachem *et al.,* 1983). Furthermore the unavailability of water within school premises combines with the above factor to exacerbate the risk of infection. Amuta *et al* (2003) reported faecal contamination of soil samples collected from school compounds in Makurdi, as a result of indiscriminate stooling by pupils. Mizgajska, (1993), Etim and Akpan (1999), Nocks and Tanko, (2000) reported same for schools in India, Calabar and Zaria respectively.

This widespread contamination of the school environment with pathogenic organisms underscores the importance of proper disposal of waste in the protection and promotion of sustainable health. The provision of adequate sanitary facilities in school interrupts transmission of faecal –oral pathogens. Epidemiological evidence suggests that improvement of sanitation and community hygiene have considerable impact in reducing communicable diseases as do improved water supply. The absence of drinking water in schools may drive pupils to other unhygienic sources thereby increasing risk. Feachem *et al,* (1983) reported 20 % reduction in prevalence and intensity of intestinal parasitic infection through

provision of water, sanitation and improvement of personal hygiene in communities.

Table 2: Sex related distribution of gastrointestinal parasites in pupils

Gastro-intestinal Parasites	Male			Female			Total		
	a	b	c	a	b	c	a	b	c
Ascaris lumbriodes	287	32	11.15	293	37	12.62	580	69	11.89
Ancylostoma duodenale	287	52	18.11	293	56	19.11	580	108	18.62
Trichuris trichuira	287	12	4.18	293	15	5.12	580	27	4.65
Taenia species	287	12	4.18	293	10	3.41	580	22	3.79
Strongyliodes stercolaris	287	4	1.39	293	7	2.39	580	11	1.89
Entamoeba histolytica	287	19	6.62	293	21	7.16	580	40	7.06
Schistosoma mansoni	287	4	1.39	293	5	1.70	580	9	1.55
Entamoeba coli	287	14	4.87	293	14	4.77	580	28	2.41
Total	**287**	**149**	**47.03**	**293**	**165**	**51.53**	**580**	**314**	**54.13**

a = No. Examined, b = No. Infected c = Percentage (%)

Table 3: Prevalence of gastrointestinal parasites in pupils in relation to sanitary facilities

Schools	Sanitary facilities	Number examined	Number infected (%)
A	**Toilet/No water/No Fence**	60	33 (55.00)
B	**Toilet/No water/Partially fenced**	60	30 (50.00)
C	**Toilet/No water/Partially fenced**	60	41(68.33)
D	**Toilet/No water/No fenced**	60	35 (58.33)
E	**Toilet/No water/Fenced**	60	20 (33.33)
F	**Toilet/water/Fenced**	60	27(45.00)
G	**Toilet/No water/No Fenced**	60	40 (66.67)
H	**Toilet/water/Fenced**	80	28 (35.00)
I	**Toilet/No water/Partially Fenced**	80	60(75.00)
Total		**580**	**314 (54.13)**

Table 4: Some sanitary and demographic information on schools

Schools	Type/number of toilets	Available water for pupil	Available refuse dump	Population of pupils	Ratio of pupil per toilet
A	**Pit latrines 8 (17.39 %)**	STK- 1 WLL- 0 PIP- 0	Approved-0 Unapproved-2	1308	163:1
B	**Pit Latrines 11(23.91 %)**	STK- 1 WLL- 0 PIP- 0	Approved-0 Unapproved-1	2210	276:1
C	**Pit Latrines 5 (10.86 %)**	STK-0 WLL- 0 PIP- 0	Approved-1 Unapproved-1	1364	682:1
D	**Pit Latrines 14 (30.43 %)**	STK- 1 WLL- 1 PIP- 0	Approved-0 Unapproved-3	1966	140:1
E	**Water cistern 6 (30.00 %)**	STK- 1 WLL- 1 PIP- 1	Approved-1 Unapproved-2	500	83:1
F	**Pit Latrines 4(8.69 %)**	STK- 1 WLL- 1 PIP- 0	Approved-1 Unpproved-0	755	188:1
G	**Pit Latrines 2 (4.34 %)**	STK- 1 WLL- 0 PIP- 0	Approved-1 Unapproved-0	613	306:1
H	**Water cistern 14 (70.00 %)**	STK- 1 WLL- 1 PIP- 1	Approved-2 Unapproved-0	1225	87:1
I	**Pit Latrines 2 (4.34 %)**	STK- 1 WLL- 1 PIP- 0	Approved-0 Unapproved-2	230	115:1
TOTAL	**Pit latrine 46(69.69 %)** **Flush toilet 20(30.31 %)**	**STK**-8(57.14%) **WLL**4(33.33%) **PIP**-2(14.28 %)	**Approved**-6(35.29 %) **Unapproved-**11(64.71 %)	10,171	154:1

Keys: STK= Storage tank, WLL= Well, PIP= Tap water

The school environment offers an ideal terrain for intervention activities aimed at controlling parasitic diseases and elimination of potential risks. Provision of adequate toilet facilities that children are trained in using and are happy to use will certainly discourage indiscriminate defaecation elsewhere. Provision of portable water within school premises and fencing

the school compound to ward-off trespassers and stray animals that defeaecate inside or round classrooms. This will go a long way in reducing parasitic disease transmission within the school environment. Schools without fence are vulnerable to trespassers who often defecate inside classes or within school premises. Traner (1985) and Edungbola and Obi (1992) highlighted further intervention initiatives that can be practicably undertaken by respective school authorities

School-based parasitic disease intervention though mass chemotherapy and interactive health education has already commenced in some privilege communities in Nigeria (Etim *et al.,* 2002; Ogbe *et al.,* 2002).

The outcome of this study underscores the urgent need for provision and improvement of sanitary facilities in schools. Consistent intervention strategies targeted at the parasites by way of deworming campaigns, environmental sanitation through provision of sanitary facilities and adherence to personal hygiene ethics through health education. These will go a long way to reducing the scourge of gastro-intestinal parasites in children. The involvement of parents and other stakeholders in designing and implementing these interventions are fundamental to their success.

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SOCIO-ECONOMIC IMPACT OF ONCHOCERCIASIS WITH PARTICULAR REFERENCE TO FEMALES AND CHILDREN: *A REVIEW*

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ABSTRACT

The socio-economic impact of onchocerciasis (river blindness) on humans is reviewed with special reference to females and children. The results of many studies reveal that onchocerciasis is usually a serious threat to public health and an impediment to socio-economic development in areas with high intensity and high endemicity of the disease. In such places, blindness and serious visual impairment are common, and mortality among the blind may be four times as high as among non-blind persons of the same age in the same community. As a result of debilitation and blindness, the infected person is unable to maintain for long any type of productive activity. Inhabitants of fertile river valleys move to the less fertile upland country. Many young men migrate to urban areas, reducing the productivity of the community and disrupting family life. Employees classified as having a severe Onchocercal Skin Disease (OSD) earned 15 % less in daily wages than those not infected. People with Onchocercal Skin Disease are stigmatized in their communities. OSD limits the range of social involvement and can affect sexual life of affected individuals. With reference to women and children, young females with OSD suffer stigmatization more than young men. This affects their age of marriage and the kind of partners they marry, limiting them to already married men, divorced men, elderly men, childless men, etc. Severe itching that often accompanies OSD may reduce the period lactating mothers breastfeed their babies. Children, particularly females, from households headed by individuals with onchocerciasis, especially blindness and OSD are more at risk of being school dropouts. Academic performance of school children with visual impairment is adversely affected. To reduce these effects, there is need for intense public enlightenment to augment the efforts of World Health Organization (WHO) in combating the disease using mass treatment with ivermectin (Mectizan).

Keywords: Onchocerciasis, Onchocercal skin disease, Stigmatization, Visual impairment

INTRODUCTION

Onchocerciasis is a parasitic disease caused by infection with the filarial nematode, *Onchocerca volvulus*. The adult worms (macrofilariae) lodge in palpable nodules under the skin of infected humans, although they can also be found free in subcutaneous tissue (Nnochiri, 1964; Samba, 1994). The microfilariae are found in the intercellular fluid, including that of the eye, and their death and subsequent disintegration result in inflammatory reactions. If microfilarial load is high following a prolonged period of exposure to massive infection, this may lead to serious visual impairment including blindness. In addition, the microfilariae give rise to intensely itching rashes, to wrinkling, thickening and depigmentation of the skin, to lymphadenitis resulting in hanging groins and elephantiasis of the genitals and to general debilitation, including loss of weight (Samba, 1994).

Onchocerciasis is transmitted by different species of *Simulium* (blackfly) in different parts of the world where the disease is endemic. In West Africa, the disease is transmitted by *Simulium damnosum* complex, which is made up of about 26 cytospecies some of which are S. *damnosum s. s., S. sirbanum* found in Sudan and Guinea savannas, *S. squamosum* and *S. sanctipauli* in the forest zone (Dunbar and Vajime, 1972; Dunbar, 1976). These flies breed mainly in fast flowing streams and rivers. Species of *Simulium neavei* complex are the main vectors of onchocerciasis in East and Central Africa and include *S. neavei s.s., S. woodi, S. nyasalandicum, S. hightoni, S. goinyi* and *S. ovazzae.* These flies breed mainly in rivers and streams in highland areas of East and Central Africa and live in obligate phoresy with freshwater crabs of the genus *Potomonautes,* prawns of the families Atyidae and Palaemonidae and nymphs of mayflies (Crosskey, 1990). In Central and South America, the main vector is *Simulium ochraceum.* Others include *S. simplicicolor, S. metallicum, S. callidum, S. sanguineum* and *S. guianense* (Lacey and Charlwood, 1980).

Onchocerciasis is a disease of the warm tropical environment in which the flies that carry it live under conditions favourable for their development all year round (Crosskey, 1990). In Africa, the disease has been described as a disease of the future because as the development of the hinterlands proceed, particularly as dams and water projects increase, it will cease to be a disease affecting only small, isolated, poverty stricken and primitive communities in the bush and will become more and more a threat to sophisticated development personnel and other such workers (Duke, 1972).

The disease is endemic in much of tropical Africa and parts of Central and South America and Yemen. Almost all (96%) of the estimated 122.9 million at risk of the disease globally live in sub-Saharan Africa and 17.5 million of the estimated 17.7 million who are infected live in Africa (WHO, 1995a; Gemade *et al.*, 1998). The worst affected area is the

savanna zone of West Africa especially in the Volta River basin comprising parts of Benin, Ghana, Mali, Niger and Togo and the whole of Burkina Faso, where there may be up to 15% blindness rate in some endemic villages. At least 70,000 people are blind in these areas (WHO, 1980; Nwoke, 1990).

In Nigeria, onchocerciasis is widespread and a cause of blindness in most rural communities. Of all the countries of the world, Nigeria has the greatest number of persons with onchocerciasis (Edungbola, 1991). Visual impairment due to onchocercal eye disease can be demonstrated in about 30% of children aged 5years who live in hyper-endemic communities in Nigeria; 35% of males and 27% of females in such communities are visually impaired at the age of 30years (Gemade and Utsalo, 1990; Gemade *et al.*, 1998). The number of Nigerians living in high-risk areas (demarcated by river systems with villages that had ≥ 19% prevalence) and who therefore require urgent Mectizan treatment was estimated at about 13 million (Gemade *et al.*, 1998).

Blindness and impaired vision are the most dangerous disabilities associated with the disease and are seen more among endemic communities living around the foci of transmission. Onchocercal blindness is more common in the savanna bio-climatic zone than in the rain forest zone with sclerosing keratitis standing out as the ocular lesion with the highest prevalence. Males are more affected than their female counterparts, with sex differentials observed to be most marked in the savanna (Nwoke and Ikonne, 1993).

Onchocerciasis is often associated with changes in the skin. Itching and scratching are the most important early manifestations of onchocercal dermatitis and may affect any part of the body. Alteration in skin pigmentation also occurs early in the disease and may affect any part of the body. Papular rash may develop at any time on any part of the body and is usually associated with severe itching, which leads to scratching, bleeding and ulceration with secondary infection. Sowda is a severe form of onchocercal dermatitis first described in Yemen. Those affected have intensely itchy, dark and thickened skin, with papular rash and enlarged, soft, non-tender, regional lymph nodes. Sowda is usually localized and typically involves one leg but more generalized form may involve both legs or any part of the body. Other forms of onchocercal dermatitis are known as lizard skin and leopard skin. In long-standing onchocercal dermatitis, the skin generally becomes atrophic, fragile, wrinkled and inelastic and areas of it, often the shins, develop the classical spotting depigmentation of leopard skin (Hagan, 1998).

Presence of palpable nodules is another evidence of onchocerciasis in a person. Nodules tend to be more numerous and widely distributed in the rainforest than in the savanna but numbers of microfilariae in the skin are higher in the savanna. Skin depigmentation, lymphadenopathy and hanging groin are more frequent in the rainforest than in the savanna but severe skin atrophy is more common in the savanna (Anderson *et al.*, 1974; Hagan, 1998).

The classical method of determining the prevalence and intensity of onchocerciasis is by the demonstration and counting of microfilariae in biopsies obtained by skin snipping. Although very specific, this technique is inadequate for detecting early, light or prepatent infections and is becoming increasingly unacceptable to the populations investigated due to different reasons, one of which is the awareness of the potential risk of secondary infections especially with HIV (Boatin *et al.*, 1998).

The future challenges for diagnostics in onchocerciasis lie in developing an optimal test, which is highly sensitive, highly specific, easy to carry out, cheap and acceptable to the populations studied. In the present era of widespread use of Mectizan, such a test should be capable of predicting the return of microfilariae in treated individuals. Serological test 0-150 PCR and DEC patch test appear to have prospects of meeting most of these challenges (Boatin *et al.*, 1998).

In view of the limitations of skin snip method and immunodiagnosis, there has been a search for an alternative rapid assessment method. Edungbola *et al.*, (1993) reported that the rate of leopard skin (LS) and palpable nodules showed significant variation with the microfilarial rates. The prevalence rates of these clinical features increase with increase in the community microfilarial rate.

The Rapid Assessment Method (RAM), based on nodule palpation and leopard skin, is a standardized epidemiological procedure with proven reliability. The RAM is simple, rapid, non-invasive, cheap, applicable and practicable over a wide range of ecological conditions, reliable regardless of the severity and duration of the infection, non-technical, acceptable to villagers, with absence of risk of other infections, and good for impact monitoring and evaluation. It is useful in preliminary screening for detailed prospecting of onchocerciasis endemicity (Edungbola *et al.*, 1993; Withworth and Gemade, 1999).

Control of onchocerciasis involves the control of the vector by means of insecticides used against *Simulium* larvae in the watercourses where they breed, and use of drug against the parasite in man. The drug of choice for treatment of onchocerciasis is ivermectin (Mectizan), which is effective against microfilariae and drastically reduces the microfilarial loads and the risk of developing ocular lesion (Awadzi *et al.*, 1989; Remme *et al.*, 1989) and Onchocercal Skin Disease (Brieger *et al.,* 1998). In West Africa, the World Health Organization's Onchocerciasis Control Programme (OCP) has been so successful in reducing the prevalence of onchocercal blindness in the savanna areas within its region of operation that this condition is no longer of public health significance in such areas (WHO, 1991).

Onchocerciasis is a widespread filarial disease that produces grave socio-economic consequences. The disease affects the productivity, social and sexual lives of sufferers due to blindness

and other debilitating effects (Nwoke, 1990). WHO (1980) reported that onchocerciasis is a major cause of blindness in parts of Africa and is a serious obstacle to socio-economic development.

According to Kale (1998), the greatest burdens related to human onchocerciasis are the result of the eye and skin lesions and severe itching produced by the microfilariae. He also said that the skin lesions are a major socio-economic burden in terms of disability-adjusted life-years (DALY).

The present paper reviews the effects of onchocerciasis on different aspects of socio-economic lives of those suffering from the disease with particular reference to effects on females and children. This will give a general picture of the severity of the socio-economic consequences of onchocerciasis. It will also form baseline information that will help those involved in the fight against onchocerciasis to have a better focus and a greater determination.

MATERIALS AND METHODS

A comprehensive search was made from the Internet, various journal articles and textbooks of reports on the socio-economic effects of onchocerciasis in various parts of the world. Such articles were assembled and studied.

RESULTS

Symptoms of Onchocerciasis Related to Socio-Economic Effects: From the materials reviewed, many symptoms of onchocerciasis relating to the socio-economic consequences of the disease were highlighted by the various authors. Some of these symptoms are given in Table 1.

Using WHO 1995b report on the importance of Onchocercal Skin Disease (which is a multi-centre study carried out in 8 centres in 5 countries of Africa: Nigeria, Cameroon, Ghana, Tanzania and Uganda), among the reported symptoms, itching was rated as the most troubling symptom by persons affected by Reactive Onchocercal Skin Disease and Depigmentation, both of which constitute Onchocercal Skin Disease. This is shown in Tables 2 and 3a.

The non-affected also rated itching as the most troubling symptom of Onchocercal Skin Disease followed by appearance as shown in Table 3b.

Some Psychosocial Effects of Onchocerciasis on Infected Persons: Some psychological and social effects of onchocerciasis on infected persons are given in Table 4.

Economic Effects of Onchocerciasis: Onchocerciasis has been shown to have serious economic consequences. Some of the economic effects are shown in Table 5.

Specific Socio-economic Effects of Onchocerciasis on Females and Children: Some of the socio-economic effects of onchocerciasis are particularly cruel against children and females. Table 6 shows these effects.

In considering the overall marital status in relation to onchocercal infection, Ukpai and Ezeji, (2003), reported that among a population of examined females in Okigwe, Imo State, singles that were up to marriageable age had the highest percentage of infection indicating that such girls are avoided by males thus reducing their marriage prospects. This is shown in Table 7.

DISCUSSION

Symptoms related to Psychosocial Effects of Onchocerciasis: Various symptoms of onchocerciasis were reported to lead to psychosocial and economic consequences in infected persons. Some of these symptoms include itching, Onchocercal Skin Disease, palpable nodules, insomnia, fatigue, Musculo-Skeletal pain, headache, visual impairment and blindness, hanging groins and elephantiasis of the genitals among others. Among all these, itching was reported to be the most troublesome symptom with the most profound socio-economic consequences. Appearance (OSD) was also rated very high. Visual impairment and blindness are particularly important in affecting farming (Workneh *et al*., 1993; Kim *et al*., 1997, Ubachukwu and Anya, 2001) and academic performance (Ubachukwu and Anya, 2003).

Socio-economic Effects of Onchocerciasis: The socio-economic liabilities as a result of onchocerciasis are enormous. The blackfly vectors of *Onchocerca volvulus* are a serious nuisance in the endemic communities because of the resultant skin lesions from their bites. Susceptible persons may be uncomfortable for weeks with an almost unbearable pruritus and scratching. In many individuals, this persists throughout the whole course of the infection. Sometimes, the itching and scratching may be so severe as to cause insomnia (Nwoke *et al.,* 1987).

The various skin changes associated with onchocerciasis such as rashes, hypopigmentation and scaling, oedema and depigmentation have distressing effects on the lifestyle of the infected individuals (Nwoke, 1986; Nwoke *et al*., 1987), sometimes constituting destitutes (Nwoke, 1990). The presence of hanging groin and elephantiasis of the genitalia commonly seen in adult males and genital distortion seen in females nearly always results in the infected individual's unwillingness towards a free interaction within his or her locality. In infected individuals with the pendulous sacs, sexual life is greatly affected if not completely hindered (Nwoke, 1986; 1990; Nwoke *et al*., 1987).

A study on perception and social implication of onchocerciasis in Edo State, Nigeria, showed that attitude of non-affected towards the affected is partially discriminatory and suspicious. The affected are socially withdrawn due to frustration of their health condition (Wagbatsoma and Okojie, 2004). Similar results have also been obtained in other parts of the country (e. g. Amazigo and Obikeze, 1991; Ubachukwu, 2001a and b).

Table 1: Symptoms of onchocerciasis related to socio-economic effects

Symptom	Study Area	Reference
Itching	Jos, Nigeria	Nwoke, 1986; Nwoke *et al.*, 1987;
	Nigeria, Cameroon, Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
	Ethiopa	Kim *et al.,* 1997
	Enugu State, Nigeria	Ubachukwu, 2001a; 2001b
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001; Ukpai and Ezeji, 2003
Onchocercal Skin Disease (OSD)	Jos, Nigeria	Nwoke, 1986; Nwoke *et al.*, 1987;
	Nigeria, Cameroon, Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
	Enugu State, Nigeria	Ubachukwu, 2001b
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001; Ukpai and Ezeji, 2003
	Imo State, Nigeria	Ukpai and Ezeji, 2003
Palpable nodules	Jos, Nigeria	Nwoke, 1986; Nwoke *et al.,* 1987;
	Nigeria, Cameroon, Ghana, Tanzania, and Uganda	WHO, 1995; Hagan, 1998
	Enugu State, Nigeria	Ubachukwu and Anya, 2001
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001; Ukpai and Ezeji, 2003
	Imo State, Nigeria	Ukpai and Ezeji, 2003
Insomnia	Review work	Nwoke, 1990
	Nig., Cam., Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
	Africa	APOC, 2006
Fatigue	Nig., Cam., Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
Musculo- Skeletal Pain	Imo State, Nigeria	Ukpai and Ezeji, 2003
Headache	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001
Visual impairment and blindness	Review work	Nwoke, 1990
	OCP area of West Africa	Samba, 1994
	Ethiopia	Kim *et al.,* 1997
	Enugu State, Nigeria	Ubachukwu and Anya 2003
	Africa	APOC, 2006
Hanging groins and elephantiasis of the genitals	Jos, Nigeria	Nwoke, 1986; Nwoke *et al.,* 1987
	Review work	Nwoke, 1990
	OCP area of West Africa	Samba, 1994.
Reduced fertility/ Complete infertility	West Africa	Okungu, 2000

Table 2: Frequency of symptoms rated most troubling by persons affected by Reactive Skin Disease (%)

Symptom	Awka N=60	Calabar N=53	Cameroon N=55	Enugu N=33	Ghana N=65	Ibadan N=17	Tanzania N=37	Uganda N=83
Itching	80.0	71.7	65.5	40.6	53.8	64.7	48.6	45.8
Appearance	11.7	9.4	12.7	3.1	16.9	17.6	5.4	9.6
Insomnia	0.0	1.9	7.5	0.0	3.1	0.0	0.0	0.0
Backache	0.0	0.0	1.8	6.3	0.0	5.9	33.3	6.0
Joint Pain	3.3	3.8	3.6	12.5	1.5	0.0	5.4	2.4
Fatigue	0.0	5.7	0.0	0.0	1.5	0.0	2.7	1.2
Headache	0.0	1.9	0.0	0.0	3.1	0.0	0.0	2.4
Others	5.0	5.7	5.5	31.3	16.9	11.8	24.3	31.3

(After WHO, 1995b)

Table 3a: Frequency of symptoms rated most troubling by persons affected by Depigmentation (%)

Symptom	Awka N=40	Calabar N=33	Cameroon N=42	Enugu N=31	Ghana N=28	Ibadan N=49	Tanzania N=15	Uganda N=15
Itching	45.0	51.5	57.1	12.9	46.4	36.7	33.3	40.0
Appearance	2.5	9.1	4.8	0.0	25.0	14.3	6.7	0.0
Insomnia	2.5	0.0	2.4	0.0	0.0	0.0	0.0	0.0
Backache	0.0	9.1	0.0	12.9	0.0	24.5	13.3	6.7
Joint Pain	30.0	15.2	4.8	25.8	0.0	4.1	6.7	20.0

Fatigue	0.0	3.0	7.1	0.0	0.0	2.0	6.7	0.0
Headache	0.0	0.0	4.8	6.5	0.0	2.0	13.3	0.0
Others	20.0	6.1	16.7	38.7	7.1	16.3	13.3	33.3

(After WHO, 1995b)

Table 3b: Most troubling symptom according to non-affected (% of respondents)

Symptom	Reactive Skin		Depigmentation	
	Male	Female	Male	Female
Itching	50.7	53.2	33.0	37.2
Appearance	24.0	25.4	32.1	24.0
Can't say	6.0	8.1	12.8	20.9

(After WHO, 1995b)

Table 4: Psychological Effects of Onchocerciasis

Effect	Study Area	Reference
Low effect of self esteem	Nigeria, Cameron, Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001
Stigmatization of infected persons and their families	Ette, Enugu State, Nigeria	Amazigo and Obikeze, 1991
	Nigeria, Cameroon, Ghana, Tanzania and Uganda	WHO, 1995; Hagan, 1998
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001
	Enugu State, Nigeria	Ubachukwu, 2001a and b
	Imo State, Nigeria	Ukpai and Ezeji, 2003
	Edo State, Nigeria	Wagbatsoma and Okojie, 2004
	Africa	APOC, 2006
Dampens marriage prospects	West Africa	Okungu, 2000
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001
	Enugu State, Nigeria	Ubachukwu, 2001a and b
	Imo State, Nigeria	Ukpai and Ezeji, 2003
	Africa	APOC, 2006
Hinders breastfeeding	Review paper	Amazigo, 1994
	Anambra State, Nigeria	Eneanya and Nwaorgu, 2001

Table 5: Economic effects of onchocerciasis

Effects	Nature of Effects	Study Area	Reference
Effects on productivity	Itching, musculo-skeletal pain and severe fatigue interfere with productive ventures e.g.	Nig, Cam, Ghana, Tanzania and Uganda	WHO, 1995,
		Nig, Ethiopia, Sudan	WHO, 1997,
	a. Farm work	Ethiopia	Workneh *et al.,* 1993,
		Ethiopia	Kim *et al*., 1997
		Review work	Benton, 1998
		Enugu state, Nig,	Ubachukwu and Anya, 2001
	b. Academic performance	Enugu state, Nig,	Ubachukwu and Anya, 2003
Effects on education	Children especially females of infected parents drop from school	Nig, Ethiopia, Sudan	WHO, 1997
		Review work	Benton, 1998
Direct costs	More money spent on health issues	Nig, Ethiopia, Sudan	WHO, 1997
		Review work	Benton, 1998
Indirect costs	More time spent on seeking health-care and less time spent on household activities	Nig, Ethiopia, Sudan	WHO, 1997
		Review work	Benton, 1998
Reduced labour supply	Through death	Review work	Nwoke, 1990
	Thru blindness	Review work	Nwoke, 1990
	Reduced efficiency	Review work	Nwoke, 1990
	Loss of potential working days	Review work	Nwoke, 1990
Depopulation	Emigration leading to depopulation of infected areas and over population of less fertile uninfected areas disruption of family life.	Review work	Hamon and Kartman 1973
		Review work	Vajime, 1982
		Review work	Nwoke, 1990
		Review work	Kale, 1998
		Hawal valley, Nigeria	Bradley, 1976
Demographic	Uneven distribution	Hawal valley, Nigeria	Bradley, 1976

imbalance	of the population by age and sex	Review work	Vajime, 1982
Hinder effective sully of land	Fertile lands are abandoned	Review work	Nwoke, 1990

Table 6: Socio-economic effects of onchocerciasis on children and females

Effects	Study Area	Reference
Children, especially girls, drop out of school to care for the blind.	Nigeria, Ethiopia and Sudan Review work	WHO, 1997 Benton, 1998.
Academic performance of children is hindered	Enugu State, Nigeria Africa	Ubachukwu and Anya 2003 APOC, 2006.
Social discrimination against adolescent girls with rashes diminishing their marriage prospects	Nigeria, Cameroon, Ghana, Tanzania and Uganda Ette, Enugu State Review paper West Africa Enugu State, Nigeria Imo State, Nigeria Africa	WHO, 1995, Hagan, 1998 Amazigo and Obikeze, 1991 Amazigo, 1994 Okungu, 2000 Ubachukwu, 2001a, 2001b Ukpai and Ezeji, 2003 APOC, 2006
Hinders stability of marriage	West Africa	Okungu, 2000
Social discrimination against families of girls with rashes	Ette, Enugu State Nigeria, Cameroon, Ghana, Tanzania and Uganda	Amazigo and Obikeze, 1991 WHO, 1995; Hagan, 1998
Reduces duration of breastfeeding	Review work	Amazigo, 1994

Table 7: Overall marital status related infection

Marital Status	No. examined	No. infected	% of infection
Not up to the age of marriage	22	9	40.90
Single but up to marriageable age	120	73	60.83
Married	110	54	49.10
Separated	44	17	38.60
Divorced	43	12	27.90
Widowed	61	42	60.80

(After Ukpai and Ezeji, 2003)

Onchocercal Skin Disease can have major adverse psychological and socio-economic effects (WHO, 1995b; Murdoch *et al.*, 2002). Results from a multi-country study by the Pan-African Group on Onchocercal Skin Disease (WHO, 1995b) showed that OSD limits the range of social involvement of affected persons. Affected individuals reported that manifestation of onchocerciasis impair their ability to work and interact socially, and also affect other facets of their lives resulting in loss of sleep, inadequate sexual performance, weakness, worry, pains and embarrassment. Affected individuals feel ashamed of themselves, worry a lot over their skin condition, fear that the disease might kill them and experience low morale.

While society feels sorry for and pities those who suffer from the skin condition, they also avoid, despise and make fun of them. Affected people are stereotyped as weak, emotionally dull and cold and as unable to perform their duties, let alone, feed themselves. They are considered dangerous and dirty, are avoided for fear that they might pass on their disease to others. People would not elect them to positions of leadership. Oncho-affected individuals have poor self-image, suffer lack of confidence and are not willing to accept positions of leadership, thinking they might embarrass the people they would represent (WHO, 1995b).

Onchocerciasis rarely leads to death but when it does, it cuts off the individual's supply of labour years in the future. As a result of debilitation and blindness, the infected person is unable to maintain for long any type of productive activity. Because onchocercal blindness is mostly found among the working age groups, such permanent disability withdraws the affected individual's supply of labour years requiring vision (Nwoke, 1990). The blind people are usually poverty-stricken and have a lower life expectancy than normal people (Vajime, 1982). Mortality among the blind may be four times as high as among non-blind persons of the same age in the same community (Samba, 1994). Blindness in 20 % of the adult males reduces farming capacity below survival level (Hamon and Kartman, 1973).

Nwoke (1990) reported that onchocerciasis affects the effective supply of labour in three ways:

i) as a cause of death, it removes the individual's supply of labour years in the future

ii) as a result of permanent disability through blindness and serious visual impairment, onchocerciasis withdraws

the individual's supply of labour years to activities requiring vision and

iii) partial visual impairment and/or non-disabling manifestation may also reduce the efficiency of labour days worked. Also as a result of so many people suffering from onchocercal blindness, there is loss of a huge number of potential working days.

Kim *et al.* (1997) reported that the human toll of the disease is devastating due to high numbers of blind people and constant itching which affects productivity. Infected persons have difficulty attending to their jobs. Infected persons with OSD were reported to be 15% less productive than those not infected. This was because they earned 15% less in daily wages than those not infected. Workneh *et al*. (1993) and WHO (1995b) also reported that non-ocular onchocerciasis has a negative impact on work productivity.

According to Ubachukwu and Anya (2001), the most disturbing aspects of onchocerciasis are impaired vision and blindness. Blindness hinders a person permanently from agricultural activities and makes such a person an economic liability. Blindness leads to loss of income for the family because most of the time, the blind person is the breadwinner of the family. In addition, the blind becomes a socio-economic burden to the other members of the family, as he/she needs to be cared for instead of caring for others. Serious visual impairment, on the other hand, does not lead to complete disability (morbidity) from farm work as blindness but reduces the working efficiency of such a person (Ubachukwu, 2001a; Ubachukwu and Anya, 2001). In addition, they reported that the presence of a large number of nodules, especially around the hip, to a large extent, hinders the farmer from farm work. Nwoke (1986) and Nwoke *et al*. (1987) also reported that the itching and rashes associated with onchocerciasis cause serious scratching, which can be so severe as cause loss of sleep and even necessitate complete absenteeism from work.

Onchocerciasis, therefore, leads to both morbidity and debility resulting in complete loss of productive years and reduction in labour input (work time) and labour output or efficiency (amount of work per unit time) respectively. The bites of *Simulium* in the farms also reduce the time a farmer effectively puts into farm work (Ubachukwu and Anya, 2001).

The skin lesions of onchocerciasis have recently been shown to be a major socio-economic burden in terms of disability-adjusted life-years (DALY) (Kale, 1998). Computation of disability-adjusted life-years lost because of onchocerciasis shows that the total burden of human onchocerciasis in Africa is about 884,000 DALY lost annually. The estimate of DALY lost per year because of itching is greater than that lost from ocular manifestations of the disease. DALY is computed as the sum of years of life lost because of early mortality and years lived with disability because of a given disease (Benton, 1998).

There are reports that low population densities and desertion of many fertile river valleys in the savanna zone of West Africa are mainly due to onchocerciasis (Budden, 1956; Bradley, 1976, Nwoke, 1990). These reports were also supported by Hamon and Kartman (1973) who reported that fertile lands become deserted while less fertile uplands become overcrowded. Highlighting this observation, Kale (1998) reported that although the major manifestations of the disease show geographical variation, they are often sufficiently severe to prevent human use of the often very fertile land close to the rivers in which the vectors breed.

Serious economic and social setbacks result from distorted distribution of population due to depopulation. If emigration is not checked, onchocerciasis free lands can become increasingly overused and possibly ruined beyond recovery. Demographic imbalance also results, marked by uneven distribution of the population by age and sex because men afflicted by onchocercal blindness desert the villages while women and children stay back. This jeopardizes family life and the division of labour (Bradley, 1976). Emigrants impose demands upon other territories that are often agriculturally less fertile, consequently resulting in constant population maladjustment (Nwoke, 1990). Due to the habitual migration of disabled people from endemic areas to urban centres to beg for alms, it is often a common social trend to see chains of blind adults being led to markets or around cities by children with good sight (Nwoke, 1990).

Hamon and Kartman (1973) summarized the socio-economic effects of onchocerciasis as follows:

1. blindness lowers farming capacity seriously
2. fertile lands become deserted while less fertile uplands become overcrowded
3. fishing in infested water is reduced
4. labour forces engaged in development activities e.g. building of dams, are protected at great cost.

Some studies have shown that onchocerciasis imposes both direct and indirect costs on people suffering from the disease. Preliminary results of multi-country study (WHO, 1997; Benton, 1998) show that on the average, an individual who has a severe manifestation of OSD spends almost $20 more per annum on health-related expenditures than an uninfected individual. Given the low level of income in the study countries (Nigeria, Ethiopia and Sudan), these extra costs can represent as much as 15 % of the annual income of an infected individual. The study also shows that there are substantial 'time costs' of infection, those with severe OSD spending more time seeking health care and spending less time in household activities.

Other reported effects of onchocerciasis include male sterility (Hughes, 1954 cited by Budden, 1956) and habitual abortion (Ikejiani, 1954). Ikejiani (1954) reported two cases of habitual abortion involving onchocerciasis patients in Nigeria. According to him, after treatment with hetrazan, these women produced children without difficulty.

Socioeconomic Impact of Onchocerciasis on Females and Children: According to Amazigo (2004) in a paper titled 'Women's Health and Tropical Diseases: A focus on Africa', women constitute a significant percentage of the total population in Africa and to achieve better global health condition, a focus on African women is thus necessary. Infections are confined to the world's poverty belt of the tropics and subtropics largely in sub-Saharan Africa. Low-income levels are associated with debilitating disease patterns. According to this report, of all geographical regions, Africa has the highest tropical diseases morbidity and mortality ratios. Historical changes in economic and agricultural roles of men and women leave women with the major responsibility for subsistence farming and family welfare. Consequently, adolescent and adult females in Africa now make the greatest contribution to agricultural production. These changes in roles have increased exposure of females to infective bites of flies, which transmit tropical diseases and increase their role in the transmission of diseases (Amazigo, 2004). It is, therefore, not surprising that females suffer a lot of socio-economic consequences of one of the tropical diseases with severe public health and socio-economic importance- human onchocerciasis.

A review of some of the ways onchocerciasis affects females and children socially, psychologically and economically is hereby documented. In a recent review paper, Amazigo (2004) reported that certain health conditions and problems associated with the highly prevalent tropical infectious diseases are shared by males and females at almost equal prevalent rates but they have each particularly serious consequences for females because of their reproductive functions. These problems result in increased risk during pregnancy and childbirth. Some tropical infections (e.g. onchocerciasis) that cause gross disfigurement are particularly cruel for adolescent females and women because of their effects on marriage prospects, education and self-esteem (e.g. Amazigo and Obikeze, 1991; WHO, 1995b; 1997; Amazigo, 2004).

Children especially girls are forced to drop out of school in order to care for the blind. The result of a multi-country study (WHO, 1997; Benton, 1998) indicates that (i) the risk of children becoming non-attendees (school dropouts) is twice as high if the head of their household has OSD than if the head is uninfected, (ii) severe OSD in heads of households is more likely to have a detrimental impact on the attendance in school of female children than of male.

Studies on the effects of onchocerciasis on school academic performance in a standard examination (Junior Secondary School Certificate Examination, JSSCE) in Nigeria (Ubachukwu and Anya, 2003) show that visual impairment has profound negative effect on school performance. The result of the regression analysis between performance and various manifestations of onchocerciasis shows a strong inverse correlation ($r \sim -0.72$) between performance and visual impairment. In school, constant distraction caused by unrelenting itching impairs any educational achievements, especially among girls who already suffer from gender-based inequalities of opportunity (APOC, 2006).

There are reports of social discrimination against people with onchocerciasis especially rashes (Amazigo and Obikeze, 1991; Amazigo, 1994; WHO, 1995b; Okungu, 2000; Ubachukwu, 2001a and b; APOC, 2006). The results of these studies show that there is serious social discrimination against people with Onchocercal Skin Disease. This discrimination is more serious against adolescent girls and young women than men, diminishing their marriage prospects. This is reflected in the fact that the infected adolescent girls do not get married as early as their uninfected counterparts because men tend to avoid them. These infected girls are also limited in their choice of marriage partners to men that are elderly, divorced, widowed, childless, disabled etc. Even if they are married, their skin condition affects the stability of their marriage and therefore jeopardizes their future happiness (Okungu, 2000). They are therefore subjected to perpetual unhappiness and frustration in life. According to Okungu's (2000) report, "many mothers agreed that onchodermatitis affects the marriage chances of a young woman because a man wants a pretty girl for marriage. Moreover, it is commonly believed in West Africa that whatever has happened to a mother can happen to a child, so the disease can be transmitted to offspring at birth". The belief that the disease can be transmitted from one person to the other including from mother to child during pregnancy/birth is widespread and underlies most of the discriminatory attitudes observed in most communities (Amazigo, 1993; Okungu, 2000; Ubachukwu, 2001a; 2004a) These infected girls also rarely appear in public gatherings due to shame and whenever they do, they try to cover the rashes on their bodies as much as possible with their wears, although this is very inconveniencing. The families of such girls are also discriminated against. They are usually looked down upon (Amazigo and Obikeze, 1991; WHO, 1995b).

In women with onchocercal itching, the duration of breastfeeding was reported to be reduced by more than 9 months for 25% of the infected women who breastfed infants after the onset of disease condition (Amazigo, 1994).

Summary and Recommendations: The greatest burdens related to human onchocerciasis are the results of the eye and skin lesions and severe itching produced by the microfilariae (Kale, 1998). Onchocerciasis affects the productivity, social and sexual lives of infected persons due to blindness and other debilitating effects (Nwoke, 1990), and is a major obstacle to socio-economic development (WHO, 1980). The skin lesions of onchocerciasis have recently been shown to be a major socio-economic burden in terms of disability-adjusted life years (Kale, 1998).

Onchocerciasis rarely leads to death (Nwoke, 1990), although it has been reported that mortality among the blind may be four times as high as among non-blind persons of the same age in the

same community (Samba, 1994). Blindness lowers productivity of infected persons and makes them both social and economic burdens (Ubachukwu and Anya, 2001). Even the academic performance of children is seriously affected by visual impairment (Ubachukwu and Anya, 2003). Children, especially girls, are forced to drop out of school in order to care for the blind (WHO, 1997; Benton, 1998). People with onchocercal dermatitis are socially discriminated against. This discrimination is worse for adolescent girls than for young men, to the extent that the marriage prospects of these girls are adversely affected (Amazigo and Obikeze, 1991; WHO, 1995b; Ubachukwu, 2001a, 2001b). This greater discrimination against girls is based on the belief in the communities that the "wealth of a man is his beauty", so if a man has rashes but has wealth, it does not matter much (Ubachukwu, 2001a; 2001b) and also that "whatever happens to a mother will happen to a child so the disease can be transmitted to the offspring at birth" (Okungu, 2000; APOC, 2006).

To combat onchocerciasis, there is need to improve people's attitude towards the disease and improve disease awareness through appropriate health education, which will encourage the acceptance of ivermectin as adequate treatment and compliance to the treatment regimen to reduce morbidity and promote self-esteem (Wagbatsoma and Okojie, 2004; Ubachukwu, 2004a, 2004b). Stigma and other findings show that suffering arising from non-blinding onchocerciasis also requires efforts of control programmes (WHO, 1995b). Different control programmes have been launched in different parts of the world e.g. the World Health's Onchocerciasis Control Programme in West Africa (OCP), (Samba, 1994), the African Programme for Onchocerciasis Control (APOC), (WHO, 1996; Benton, 1998) and the Onchocerciasis Eradication Programme of the Americas (OEPA), (WHO, 1996; Etya'ale, 1998). These control programmes are supported by world bodies such as World Health Organization, World Bank, United Nations Development Programme, Food and Agricultural Organization, Pan American Health Organization and different non-governmental development organizations (NGDO). However, the need for elimination of onchocerciasis is far from being met. There is still need for more commitment on the part of the world bodies and the NGDO and for more NGDO and public-spirited individuals to get involved in efforts to eliminate onchocerciasis as a public health and socio-economic burden and save the lives of infected individuals especially children and adolescent girls from the psychological and socio-economic consequences of onchocerciasis.

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