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HUMAN INTESTINAL PARASITE INFECTIONS IN ISHIAGU, A LEAD MINING AREA OF ABIA STATE

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ABSTRACT

A survey of intestinal parasite infections in a heavy metal (Pb) mining area of Abia State (Ishiagu) was carried out using both direct wet preparation and formal/ether concentration methods. A total 512 individuals ranging from primary and secondary school children to adults were screened. Of the number sampled, 177 (34.67 %) had various intestinal parasites. The parasite prevalence were Ascaris lumbricoides (17.80 %), Hookworms (14.80%) Entamoeba histolytica (3.70 %) and Trichuris trichiura (2.3 %). Prevalence for males (35.55 %) and females (33.47%) were not significantly different (P < 0.05). Age distribution of the infections showed a gradual increase from < 10 years (14.0%) to 11-20 years group (36.67%) and peaked at 21-30 years with 57.00 % before decreasing to the least in the > 51 years (27.02 %). This gave a significant age related infection (P < 0.05). The findings were discussed in relation to the rural nature of the community and the activities at the head mining sites.

Keywords: Parasites, Heavy metal mining, Stool specimens

INTRODUCTION

General intestinal parasite infections are the most common infection in a community, with over 400 million people infected all over the world (WHO, 1987, Adeyeba and Akinlabi, 2002). The most implicated parasites are *Ascaris lumbricoides,* Hookworms (*Necator americanus* and *Acylostoma deodenale*I); *Entamoeba histolytica* and *Entamoeba coli*. Others include *Trichiuris trichiura, strongyloides stercoralis, Giardia lamblia* and *G. intestinalis* (Agi, 1997, Nikolic *et al*., 1998; Mbanugo and Onyebuchi, 2002). Several workers attribute the high prevalence of these infections to poverty, poor personal hygiene, poor environmental care, poor health services, and lack of adequate and proper awareness of the transmission mechanisms and life-cycle patterns of these parasites (Montressor *et al*., 1998; Adeyeba and Akinlabi, 2002; Mbanugo and Abazie, 2002).

The effects of these worm infections have been enormous. The migratory stages (larvae) and the obstructions caused by some adults create growth and mental problems especially in children. Skin rashes and discolourations are not excluded, while lack of certain essential vitamins remains a consistent effect (Ozeretskovskaya, 1982; Chan, 1994; Stolsfus *et al.,* 1996).

Much work has been done on intestinal parasite infections in Nigeria. However, the situation in Ishiagu remains unknown. In addition, the infection spectrum in a heavy metal mining and stone quarrying community has not been documented. This work therefore provides information in this direction.

MATERIALS AND METHODS

The study area is Ishiagu, a rural community in the Guinea Savannah belt of Abia State, Nigeria. The inhabitants are predominantly farmers. Some are engaged in lead (Pb) and Zinc (Zn) mining and stone quarrying (rock blasting) activities with various companies operating in the area. Other people are involved in various forms of trading and schooling. Modern housing systems and basic infrastructures have only recently been seen in a few spots as over 80-90% of the inhabitants still live in typical rural setting.

Specimen Collection: Wide mouthed plastic containers with press-down covers were used to collect stool specimens from subjects of various ages, ranging from primary school children to adults in the study area. The containers were distributed a day before the collection, after educating them on the collection method. All collected containers were properly labeled. A questionnaire was used to collect information relating to age, sex, occupation etc of the subjects (Table 1).

Examination of Specimens: The stool specimens were collected and examined in batches. The specimens were examined using the formal/ether concentration techniques and the direct smear method with normal saline and iodine solution (Garcia and Ash, 1979; WHO, 1991). Results obtained were subjected to chi-square analysis to further test their validity statistically.

Table 1: Research questionnaire used to study human intestinal parasite infections in Ishiagu, a lead mining area of Abia State

This questionnaire is intended for use only for this research purpose. You are therefore to answer all the questions to the best of your knowledge and ability. The information obtained will be treated with absolute confidentiality.

Please tick the appropriate answer where necessary
Name: .....
Sex: Male Female
Age in years: <10 11-20 21-30
1 – 40 41-50 >50
Level of Education:
Primary School Secondary School
Tertiary School None of all
Occupation:
Lead and Zinc Mining Stone quarrying
Schooling Farming
Trading Civil Servant
Any other
What do you know about parasitic diseases
None Very Little Average
Above Average Very much

RESULTS

Out of the 512 subjects screened for intestinal parasite infections, 177 (34.6 %) were positive with various parasites. Out of 270 males examined, 96 (35.6 %) were positive while 81 (33.47 %) of the 242 females were positive (Table 2). There was no significant difference in the sex-related prevalence ($P < 0.05$).

Table 2: Prevalence of intestinal parasites among the inhabitants of Ishiagu, Abia State

Species	No examined	% infected
	177	**34.60%**
Ascais lumbricodes	91	17.8%
Hookworms	76	14.8%
Trichuris trichiura	12	2.3%
Entaemoeba histolytica	19	3.71%
Total	512	100

Table 3, indicates that the most infected age group was the 21-30 years (57.00%), followed by 11-20 years (36.67 %). The least infected was 0 < 10 years (14.00 %) followed by > 50 years (27.02 %). Age significantly influenced the prevalence ($P < 0.05$) (Table 3).

Four intestinal parasites were observed in his study. These were *Ascaris lumbricoides, Hookworm, Entamoeba histolytica* and *Trichuris trichiura*. Table 2 shows these parasites and their prevalence among the 512 subjects screened.

Occupation did not influence the prevalence of infection except for the civil servants (16.90 %) who had significantly lower prevalence of infection than all the others group E.

DISCUSSION

This study has revealed that intestinal parasites observed in other areas are the same found in both children and adults in the lead, zinc and stone mining area – Ishiagu (Agi 1995, Nikolic *et al, 1998,* Adeyeba and Akirilabi, 2002, Mbanugo and Abazie, 2002). The parasites encountered in this work were *Ascaris lumbricoides,* Hookworms, *Entamoeba histolytica* and *Trichuris trichiura*.

Table 3: Prevalence of Human intestinal parasites according to age, sex and occupation of the inhabitants of Ishiagu

		Number Examined	Number infected
Sex	Male	270	96(35.55)
	Female	242	81(33.47)
Age in	<10	100	14(14.0)
Years	11-20	120	44(36.677)
	21-30	100	57(57.00)
	31-40	95	32(33.68)
	41-50	60	20(33.33)
	>50	37	10(27.02)
Occupation	Mining	72	23(31.94)
	Stone quarrying	102	34(33.33)
	Schooling	134	62(46.27)
	Farming	87	30((34.48)
	Trading	4	1(25.00)
	Civil service	71	12(16.90)

The observations of these intestinal parasites in subjects in Ishiagu community were in line with happenings in other areas. However, more parasites than the four reported in this work have been observed in most areas other than heavy metal mining and rock blastering areas (Amogun, 1990; Chan, 1997; Mbanugo and Onyebuchi 2002, Adeyeba, and Akinlabi, 2002). The fewer number of parasite species reported in this work could be attributed to the fact that the wastes from these mining sites in the area could have adversely affected the survival of these parasites in the soil. The inhabitants make use of the abandoned quarry and mining pits for various purposes, which could equally, affected the parasites spectrum.

The most prevalent intestinal parasite in the area was *Ascaris lumbricoides* (17.8 %), followed by Hookworms (14.8%) while the least was *Trichuris trichiura* (2.3%). The results of this study agree with previous reports elsewhere that Ascaris *lumbricoides* and Hookworm species are the most prevalent intestinal parasites in South Eastern Nigeria (Agi, 1995; Mbanugo and Abazie, 2002; Adeyeba and Akinlabi, 2002).

A total prevalence of 34.6% intestinal parasite infections observed in the study area is similar to the 33.6% (Agi, 1995) and 30.8% (Mbanugo and Abazie 2002) but lower than the 50.4% (Adeyeba and Akinlabi, 2002) and 70.80% (Awogun, 1984) reported elsewhere in the country. These variations explain that no community is the yardstick for measurement as the prevalence of intestinal parasites is a function of many interacting factors. These factors vary in different communities and even in the same community at different periods of development. There was no significant different in the sex – related prevalence as both sexes were exposed to the same or similar sources of infection at

similar or some rate. Both sexes take part in similar activities in the same areas that could predispose them to the intestinal parasites infections.

Age-related prevalence showed that infection rate increased with age and reached the peak in 21-30 yeas subjects (57.00%) before decreasing with advancing age. The work force in Ishiagu, a typical rural community, constitutes of people from 21-30 years of age. These are those engaged in the farming, lead and zinc mining and stone quarrying activities outside their homes which predispose them to infections with these parasites. On the other hand, subjects of 10-20 years are mainly school children who are generally highly infected as a result of age and lack of acquired immunity (Mbanugo and Onyebuchi, 2002, Adeyeba and Akinlabi, 2002). In this 10-20 years group, those not engaged in schooling were observed scavenging at the mining and quarrying sites, which equally exposed them to infections.

Occupational prevalence of intestinal parasitic infection showed that the only significantly different group ($P < 0.05$) was the civil servant which had the lowest prevalence of 16.90%, probably as a result of their being generally more educated than the rest of the community.

In conclusion, there was low prevalence of intestinal parasite infections with only few parasite species in the area, but the rural nature of the community, coupled with unhygienic practices at the various work sites expose the people to infections. There is therefore the need for provision of portable drinking water in addition to mass deworming treatment. The need for mass awareness campaign should also be emphasized in order to drastically reduce all the infections.

REFERENCES

ADEYEBA, O. A. and AKINLABI, A. M. (2002). Intestinal Parasitic infections among school children in rural community, South Nigerian. *Nigerian Journal of Parasitology, 23*: 11 – 18.

ADEDOYIN, M. A., AWOGUN, I. A. and TIM, J. (1990). Prevalence of intestinal parasites in relationship in diarrhoea among school children in Ilorin, Nigeria. *West African Journal of Medicine, 9(2):* 83 – 89.

AGI, P. I. (1995). Pattern of Infection of Intestinal Parasites in Sagbama Community in Niger Delta, Nigeria. *West African Journal of Medicine, 14(1):* 39 – 42.

AGI, P. I. (1997). Comparative Infections in two rural communities in then Niger Delta, Nigeria. *West African Journal of Medicine, 16(4);* 232 – 236.

AWOGUN, I. A. (1984). The Prevalence of Intestinal parasitic infections in children living in Ilorin, Kwara State, Nigeria. *West African Journal of Medicine, 4(1):* 16 – 21.

CHAN, M. S. (1994). The evaluation potential global morbidity attributable to intestinal infections. *Parasitology, 109*; 373 – 387.

MBANUGO, J. I. and ABAZIE, O. C. (2002). A Comparative Study of Intestinal parasitic Infections of Pregnant and Non Pregnant Women in Nkpor, Anambra State. *Nigerian Journal of Parasitology, 23:* 19 – 26.

MBANUGO, J. I. and ONYEBUCHI, C. J. (2002). Prevalence of Intestinal Parasites in Ezinifite Community in Aguata LGA of Anambra State. *Nigerian Journal of Parasitology, 23:* 27 – 34.

MONSTRESSOR, A., CRUMPTON, D. W. T., DUNDY, D. A., HALL, P. and SAVIOLI, C. (1998). *Guidelines for the evaluation of soil-transmitted Hemithiasis and Schistosiamiasis at Community Level.* A guide for managers of control programmes, World Health Organization, Geneva.

NDUKA F. O., NWAUGO, V. O. and NJOKU, A. J. (2001). Prevalence of Paragonomiasis in school children in some areas of South Eastern Nigeria. *Journal of Health and Veterinary. Science. 2(2): 120 – 125..*

NIKOLIC, A., DJURKOVIC, O and BOBIC, B. (1998). Intestinal Parasitic infections in Serbia. *Srpski Arhizua Celukumpo Lekarsivo, 126(1-2): 1 – 5.*

NWAUGO, V. O., NDUKA .F. O. and NWOKE, B. E. B. (2002). Aspect of Urinary Schistosomiasis among quarry pit water users in Umuchieze Abia State. *Nigerian Society of Microbiology Annual Conference. Uyo, 14 - 17th July, Nigeria.*

OZERESTSKOVSKAYA, N. N. (1982). Intestinal Parasitic infection. *Scandinavian Journal of Infectious Diseases, Supplementary 36:* 46-51.

STOLZFUS, R. J., ALBONICO, M., CHWAYA, H. M., SARVIOLI, L., TIELSH, J., SCHULZE, K., YIP, R. and HEMOQUANT, H. H. (1996). Determination of hookworm related drug loss an its role in iron deficiency in African Children. *American Journal of Tropical Medicine and Hygiene, 55(4).* 399 – 404.

WHO (1987). Prevention and Control of Intestinal Parasitic Infections. Report of WHO Expert Committee. *Technical Report Series. 749*. World Health Organization, Geneva.

WHO (1991). *Basic Laboratory Method in Medical Parasitology.* World Health Organization, Geneva.

PREVALENCE OF MALARIA PARASITES AND ANAEMIA IN PREGNANT AND NON PREGNANT WOMEN IN ABA AND OKIGWE TOWNS OF SOUTHEAST NIGERIA

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ABSTRACT

A study of the prevalence of Malaria parasites in pregnant women attending pre - natal care in Government hospitals in two major towns (Aba and Okigwe) in Southeast Nigeria was carried out. Blood was collected by vein puncture from 500 pregnant women in different trimesters (300 from Aba and 200 from Okigwe) and 200 non - pregnant women, 100 from each town. Presence of Malaria parasite was observed microscopically on thin and thick blood smears prepared from each sample. Personal data were collected both orally and from maternity records of the women. The results were analysed statistically using the Chi - square test. Only the ring trophozoite and gametocyte forms of Plasmodium falciparum were observed in the infected samples. A total of 270 (54 %) pregnant women out of the 500 examined were infected with P. falciparum while 66(33 %) of the non - pregnant women sampled were infected. This represents a significant difference. Aba had 158 (52.6 %) out of the 300 pregnant women examined infected while Okigwe had 112(56 %) of the 200 pregnant women examined infected. There was no significant difference between the results obtained in the two towns. (P > 0.05). Peak prevalence was observed in the first trimester 64.1 % (100 out of 156) while 3rd trimester showed the lowest 45 % (68 of 150). Prevalence was also highest in primgraviidae and women in second pregnancy (67.96 %). Multiparous women (3rd pregnancy and above) had 39.31 % . Age was significant. Anaemia (Hb. < 11g/dl) was observed in 385 (77 %) of the 500 pregnant women examined. Of the 270 infected women 254(94.07 %) were anaemic. Anaemia was significantly higher in women with higher parastemia (Z.cal. = 9.06). The implications of this result on the epidemiology of malaria are discussed.

Keywords: Prevalence, Malaria, Pregnancy, Women, Anaemia, *Plasmodium*

INTRODUCTION

Malaria is a major public health problem in developing countries causing considerable morbidity and mortality especially in sub Saharan Africa. It is endemic in 103 countries with about 2000 million people exposed to infection (Menendez, 1995). An estimated 1 - 2 million deaths result each year from about 300 - 500 million clinical cases in highly endemic areas (Snow *et al.*, 1999, 2001). Mostly affected are children less than 5 years and followed closely by pregnant women. An increased risk of Malaria during pregnancy was observed over 60 years ago by Wickramasariya (Steketee and Mutabingwa, 1999). In all endemic areas it has been observed that the frequency and severity of malaria increases with pregnancy (Gilles *et al.*, 1984).

Several reasons have been adduced for this increase such as relative impairment of the immune system (Ibeziako *et al.,* 1980; Mutabingwa 1994), cytoadherence to chondroitin Sulphate A in the placenta (Fried and Duffy 1996) and an increased attractiveness of pregnant women to Malaria vectors (Lindsay *et al.*, 2000). Also in areas of high transmission, primigraviidae are more susceptible to infection than multiparous women (Okoko *et al.*, 2003). Furthermore it has been observed that increased risk of malaria varies during the course of pregnancy with the first trimester showing highest prevalence and parasite density than the 2nd and 3rd trimesters (Menendez, 1995).

Malaria in pregnancy holds severe consequences which range from anaemia to severe complications such as cerebral malaria, pulmonary oedema and renal failure in the mother (Steketee *et al.*, 2001; Saute *et al.*, 2002; Bouyou - Akotet *et al.*, 2003), increased stillbirth, intra - uterine growth retardation and low birth weights in the foetus (Kasumba *et al.*, 2000; Verhoeff *et al.*, 2001; Steketee *et al.*, 2001).

With the Government of Nigeria interested in the Roll Back Malaria Programme, this study was undertaken to provide part of the much needed baseline data for effective planning and control of Malaria especially among the population at risk, the pregnant women.

MATERIALS AND METHODS

The study area consists of two towns, Aba and Okigwe in South East Nigeria. Aba is one of the commercial nerve centers of the country and therefore more cosmopolitan in nature than Okigwe. They have tropical climate with mean daily maximum air temperature range from 28 °C – 35 °C and mean daily minimum air temperature range from 19 °C – 24 °C (Nwoke and Uwazie, 1991).

The highest temperature occurs between March and April and the lowest in January. Wet and dry seasons are distinct in the area. Wet season spans from March to October giving an annual rainfall of between 1,700 mm and 20,000 mm.

Okigwe has varied vegetation being equatorial around the banks of natural water bodies and Guinea Savannah further inland. It is rocky and hilly with springs scattered all over. Aba's vegetation is typically rain forest. It is an urban town spotting a lot of open drains, bushes puddles and inefficient waste disposal system with huge refuse litters found in strategic areas of the town.

The two hospitals from where samples were collected (Abia State University Teaching Hospital, Aba and General Hospital Okigwe) serve as the most accessible and more focal for pregnant women in these areas during pre - natal care.

Sampling: 500 pregnant women were randomly selected and their blood sampled for Malaria parasitaemia from the two hospitals. 300 pregnant women were sampled in Aba while 200 were sampled in Okigwe. Also 200 non - pregnant women, 100 from each town were sampled. Peripheral venous blood from each woman was used in preparing thick and thin blood films, which were stained with Giemsa and examined for Malaria parasites using standard quality and controlled procedures (Alonso *et al.*, 1994). The presence and level of parasitemia was observed and recorded. A sample was recorded as positive, on the microscopic detection of any *Plasmodium* stage on the slide. The sampled females were grouped into age groups 11 - 20, 21 - 30, 31 - 40 and 41 - 50 years old. All other information needed on the pregnant women was gotten from their maternity records and also orally from the women as presented in (Table 1.)

Table 1: Information obtained from women on the prevalence of malaria parasites in pregnant and non-pregnant women in Aba and Okigwe towns of Southeastern Nigeria

Information obtained orally	Information obtained from maternity records
Age	Age
Marital status	Trimester
Parity	Parity
Chemoprophylaxis	Prescribed Chemoprophylaxis

Haemoglobin Estimation: Haemoglobin was estimated using Haemiglobincyanide (HICN) technique. Haemoglobin level of >11g/dl was considered normal while low anaemia was 11 - 9g/dl, moderate anaemia 8.9 – 7 g/dl and severe anaemia <7g/dl. (Bouyou - Akotet *et al.*, 2003).

Data Analysis: The data were analysed statistically using Chi - square and Normal distribution (Z test).

RESULTS

Plasmodium falciparum was the only malaria parasite observed in the blood samples of the pregnant women sampled in the study. Trophozoites and gametocytes were the erythrocytic stages observed during the study. Out of the 500 pregnant women examined for malaria parasites in both towns, 270 (54 %) had positive infections while 66(33 %) out of the 200 non - pregnant women were equally infected. There was significant difference in malaria prevalence among the women ($P > 0.05$). In Aba 158 (52.6 %) out of the 300 pregnant women examined were positive for malaria parasitemia while 112 (56 %) out of the 200 examined in Okigwe were positive. There was no significant difference in malaria prevalence among the towns. ($P < 0.05$) (Table 2).

Table 2: Prevalence of malaria parasites in pregnant and non pregnant women in Aba and Okigwe towns

Town	Pregnant		Non-Pregnant	
	NE	NI	NE	NI
Aba	300	158 (52.7%)	100	30 (30%)
Okigwe	200	112 (56%)	100	36 (36%)
Total	500	270 (54%)	200	66 (33%)

NE means No. Examined: NI means No. infected

Highest prevalence of 64.1 % (100 out of 156) was observed in women in the first trimester of pregnancy followed by 52.5 % (102 of 194) in the 2nd trimester, with the least seen in the 3rd trimester 45 % (68 out of 150). Statistically significant differences in malaria prevalence among women in different trimesters was observed ($P > 0.05$) (Table 3).

Table 3: Effect of gestation and parity on the prevalence of malaria parasites in pregnant women in the two towns

Gestation Period	NE	NI (%)
1st Trimester	156	100 (64.1%)
2nd Trimester	194	102 (52.5%)
3rd Trimester	150	68 (45%)
Parity	NE	NI (%)
1st Pregnancy	124	86 (69.35%)
2nd Pregnancy	132	88 (66.67%)
3rd and above (Multiparous)	244	96 (39.31%)

NE means No. examined; NI means No. infected

Parity was statistically significant ($P > 0.05$) as primgravidae (69.35 %) and secungravidae (66.67 %) were more infected than multi - parous women (3rd pregnancies and above) that had 39.31 % infection. Age was significant ($P > 0.05$). The age prevalence (Table 4), followed a concave pattern, being more prevalent in younger females 11 - 20 years, 68 %) and older (41 - 50 years, 67 %) age groups than in mid age groups of (21 - 30, 50 %) and (31 - 40, 53 %). In the non - pregnant women, those of age group 11 - 20 were more infected (62.5 %).

Anaemia was observed in 385 of the 500 pregnant women examined giving an overall prevalence of 77 %. Out of the 270 infected pregnant women 254 (94.07 %) were anaemic, while 160 out of the 230 (69.56 %) uninfected pregnant women were anaemic (Table 5). There was a significant difference between results of the two groups.

Table 4: Effect of age on prevalence of malaria parasites in pregnant and non-pregnant women in the two towns

Age	Pregnant		Non-pregnant	
	NE	NI	NE	NI
11-20	44	30 (68%)	40	25 (62.5%)
21-30	288	146 (50%)	98	27 (27.55%)
31-40	138	74 (53%)	37	10 (27.03%)
41-50	30	20 (67%)	25	4 (16.00%)

Table 5: Prevalence of anaemia in the pregnant and non-pregnant women examined in Aba and Okigwe towns of Southeastern Nigeria

	Pregnant		Non-pregnant	
	NE	NA	NE	NA
Infected	270	254 (94.07%)	66	30 (45.45%)
Un-infected	230	160 (69.5%)	134	28 (20.90%)
Total	500	385 (77%)	200	58 (29%)

NE means No. Examined: NA means No. Anaemic

Anaemia was also found to be higher in pregnant women with higher parasitaemia (mean = 7.712 ± 0.8750 g/dl) as against those with lower parasitaemia (Mean = 9.8230g/dl ± 2.0725). The difference was statistically significant (Z Cal = 9.06). Also (45.45 %) of the infected non - pregnant women were anaemic.

Chloroquine was observed to be the chemoprophylaxis of choice with 198 (39.6 %), followed by Daraprim (33.6 %) and Fansidar 12 %. However only 14.8 % of the women were on no chemoprophylaxis. Those on Chloroquine also had a low infection of 46.6 % as against 66.7 % by those on Fansidar and 72 % infection by those on no drugs at all. (Table 6).

Table 6: Drugs used by the pregnant and non pregnant women examined in Aba and Okigwe towns of Southeastern Nigeria

Drug	Pregnant		Non-pregnant	
	ND	NI	ND	NI
Chloroquine	198(39.6%)	92(46.6%)	96	28 (29.17%)
Daraprim	168(33.6%)	92(55%)	12	3 (25.00%)
Fansidar	60(12%)	40(66.7%)	62	22 (33.33%)
No Drugs	74(14.8%)	53(72%)	30	23 (76.67%)

ND means No. on Drugs: NI means No. Infected

DISCUSSION

The prevalence *of Plasmodium falciparum* infection in pregnant women in Aba and Okigwe, South Eastern towns of Nigeria, were 52.6 % and 56.0 % respectively. These rates were comparable to the 47.5 % reported in Onitsha (Nwokedi, 1992), 42 % reported in Ghana (Mockenhaupt et al.,(2000), 57.5 % reported in Gabon (Bouyou - Akotet *et al*., 2003) and 41 % observed in Uyo Nigeria (Opara *et al*., 2004). However they are higher than 7.3 % reported in Port Harcourt (Ibeziako *et al*., 1980), 18.5 % reported in Keneba Gambia (Watkinson and Rushton 1983), 23 % reported in Mozambique (Saute *et al*., 2002) and 26.75 % reported in Malawi (Rogerson *et al*., 2003),. These rates were however much lower than 97.2 % observed in India (Meintra *et al*., (1993)

The high rate of prevalence observed could be due to the environmental conditions inherent in Aba and Okigwe, which favours *P. falciparum* transmission. It has been recognized that a temperature range of 16^0 C – 38^o C and relative humidity of 60 % were suitable for malaria parasite transmission (WHO, 2000).

The attitude of the women of not starting pre - natal care early in pregnancy may also have contributed to the prevalence. Some of the women began pre - natal care either towards the end of 1st trimester or mid second trimester. Also some avoided antimalaria chemoprophylaxis for fear that the foetus may be affected. Some did not take the standard dosage of the drugs as reported in Dakar, Senegal, where 11.9 % of pregnant women took Chloroquine in inappropriate dosages (Faye *et al*., 1998).

The prevalence obtained within the first and second trimesters agreed with those of Bernard (1991), Nair and Nair (1993,) Rayanal (1998), Zhou *et al*. (2002) and Anosike *et al*. (2004) who observed peak prevalence in weeks 10 - 20 of pregnancy. This may be attributed to the expression of adherent proteins on the surface of infected red blood cells (IRBCs), enabling the IRBCs to adhere to micro vascular capillaries of vital organs causing severe pathological conditions (Menendez, 1995; Miller *et al*. 2002). Achur *et al*. (2000) have shown that Chondroitin Sulfate Proteoglycans (CSPGs) present in the intervillous spaces mediate the adherence of IRBCs in the placenta. Agbor- Enoh *et al*. (2003) had suggested that the high prevalence was due to the rapid expansion of the placenta corresponding to the concomitant expression of significant levels of extra cellular CSPGs providing binding sites for IRBCs. This coupled with the absence of Chondroitin - 4 - sulfate ((C4s) - IRBC) adhesion - inhibitory antibodies prior to 12 - 20 weeks of gestation enhanced the prevalence.

Also parity played a role in the prevalence rates. Primgravidae and Secungravidae accounted for 67.96 % of the infection as against 39.31 % in multiparous women. These results were similar to the 65 % reported in Malawi (Mattelli *et al*., (1994), 65 % reported in Senegal (Diagne *et al*., 1997), 62 % in Tanzania (Wakibara *et al*., 1997) and 64 % in Gabon (Bouyou - Akotet *et al*., 2003). The results were definitely higher than 26.2 % observed among the Primigravidae in Malawi (Rogerson *et al*., 2003) but disagrees with Saute *et al*. (2002) in Mozambique that observed no significance in prevalence levels with parity. Duffy and Fried (1999), Ricke *et al*. (2000), O'Neil-Dunne *et al*. (2001), and Okoko *et al*. 2003 had suggested that the early onset of efficient antibody response in Multigravidae and the delayed production of antibodies in Primigravidae appeared to account for the gravity dependent and differential prevalence of Plasmodium Malaria in pregnant women. Duffy and Fried (1999) showed that plasma from Kenyan multigravid women inhibited adhesion of placental parasites to Chondrotin sulfate A (CSA).

Furthermore Okoko *et al*. (2003) reported that malaria infection of the placenta causes a shift from Th2 to Th1 cytokine profile that may be detrimental to pregnancy. The prevalence of anaemia in this study was in agreement with mean percentage for Africa put at 61 %. It also agreed with those of Nair and Nair (1993) in Tanzania, Van Den Broek *et al*. (2000) in Southern Malawi and Bouyou - Akotet *et al*. (2003) in Gabon.

Different drugs including Daraprim, Chloroquine and Fansidar were used by sampled pregnant women. Chloroquine was found to be the most widely used and most effective with a low prevalence of 46.6 % amongst its users. This agreed with the findings of Bouyou - Akotet *et al*. (2003) in Gabon. Our finding may not be unconnected with its recommended safety during pregnancy and its cheap cost. In conclusion, the study agrees that pregnancy was among other factors affecting the prevalence of malaria. Efforts should be geared towards control among the population at risk especially the Primgravidae.

REFERENCES

ACHUR, R. N., VALIYAVEETTIL, M., ALKHALIL, A., OCKENHOUSE, C. F. and GOWDA D. C. (2000). Characterization of proteoglycans of human placenta and identification of unique chondroitin sulfate proteoglycans of the intervillous spaces that mediate the adherence of *Plasmodium falciparum* - infected erythrocytes to the placenta. *Journal of Biological Chemistry, 275:* 40344 - 40356.

AGBOR-ENOH, S.T., ACHUR, R.N., VALIYAVEETTIL, M. LEKE, R. TAYLOR, D. W. and GOWDA, D. C. (2003). Chondroitin Sulfate Proteoglycan Expression and binding of *Plasmodium falciparum* - infected erythrocytes in the human placenta during pregnancy. *Infection and Immunity, 71*: 2455 - 2461.

ALONSO, P. L., SMITH, T., SCHELLENBERG, J. R. M., ASANA, H., MWANKUSYE, S., UKASE, H., BASTOS DE AZEVEDO, I., CHONGELA, I., KOBERA, S. and MENENDEZ, C. (1994). Randomized trial of efficacy of *Spf 66* vaccine against *Plasmodium falciparum* Malaria in children in Southern Tanzania. *Lancet, 344*: 1177 - 1181.

ANOSIKE, J.C., NWOKE, B. E. B., NJOKU, A. J, UKAGA, C. N. and ONYEKWERE, O. C. (2004). A study on the prevalence of Malaria in pregnant women visiting antenatal clinic in Federal Medical Centre, Owerri. A paper presented at the 28th annual conference of The Nigerian Society for Parasitology, September 22nd - 25th 2004, held at Imo State University Owerri.

BERNARD, J. B. (1991); Malaria: Risks during Pregnancy. *Transactions Of Royal Society of Tropical Medicine* a*nd Hygiene 13,* 21 - 27.

BOUYOU-AKOTET, M. K., IONETE-COLLARD, D. E., MABIKA-MANFOUMBI, M., KENDJO, E., MATSIEGUI, P., MAVOUNGOU, E. and KOMBILA M. (2003). Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon, *Malaria Journal, 2*: 18 - 24.

DIAGNE, N., CISSE, B. ROGIER, C. and TRAPE J. F. (1997). Incidence of Clinical Malaria in Pregnant women exposed to intense perennial transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 91:* 166 – 170.

DUFFY, P. E. and FRIED, M. (1999). Malaria during pregnancy, parasites, antibodies and Chondrotin Sulfate A. *Biochemical Society Transactions, 27(4)*:478 - 482.

FAYE, O., GAYE, O. and MOLEZ, J. F. (1998). Impact of the use of permethin pre-impregnated mosquito nets on Malaria transmission in a hyper endemic village of Senegal. *Medicine Tropicale Mersale, 58(4):* 335 - 360.

FRIED, M. and DUFFY, P. E. (1996). Adherence of *Plasmodium falciparum* to Chondroitin Sulfate A in the human Placenta. *Science, 272:* 1502 - 1504.

GILLES, H. M., LAWSON, J. B., SIBELAS, M., VOLLER, A. and ALAN, N. (1984). Malaria and Pregnancy, *American Journal of Tropical Medicine and Hygiene, 33(4): 517 - 525.*

IBEZIAKO, P. A., OKERENGWO, A. A. and WILLIAM, A. I. O. (1980). Malaria Immunity in Pregnant Nigerian Women and their baby. *Journal Gynaecology and Obstetrics,* 18: 147 - 149.

KASUMBA, I. N., NALUNKUMA, A. J. and MUJUZI, G. (2000). Low birth weight associated with maternal Anaemia and Plasmodium falciparum infection during pregnancy in a peri-urban/urban area of low Endemicity in Uganda. *Annals of Tropical Medicine and Parasitology, 94*: 7 - 13.

LINDSAY, S., ANSELL, J., SELMAN, C., COX, V., HAMILTON, K., and WALRAVEN, G. (2000). Effect of pregnancy on exposure to Malaria mosquitoes. *Lancet*, *355:* 1972 - 1973.

MEINTRA, N., JOSHI, M. and HAZRA, M. (1993). Maternal manifestations of Malaria in pregnancy: a review. *Indian Journal of Maternal and Child Health, 4(4):* 98 - 101.

MATTELLI, A., DONATO, F., SHEIN, A., MUCH, J. A., LEOPARDI, O., ASTORI, L. and CAROSI, G. (1994). Malaria and Anaemia in pregnant women in Urban Zanzibar Tanzania. *Annals of Tropical Medicine and Parasitology, 88:* 475 - 483.

MENENDEZ, C. (1995). Malaria during Pregnancy: A Priority area of Malaria research and control. *Reviews of Parasitology Today, 5*: 178 - 183.

MILLER, L. H., BARUCH, D. I., MARSH, K. and DOUMBO, O. K. (2002). The pathogenic basis of Malaria. *Science, 45*: 673 - 679.

MOCKENHAUPT, F. P., RONG, B. TILL, H., EGGETTE, T. A., BECK, S., GYASI-SARPONG, C., THOMPSON, W. N. and BIENZLE, U. (2000). Sub-microscopic *Plasmodium falciparum*

infections in pregnancy in Ghana. *Tropical Medicine and International Health, 5:* 167 - 173.

MUTABINGWA, T. K. (1994). Malaria and pregnancy: Epidemiology, Pathophysiology and control options. *Acta Tropica, 57:* 241 - 242.

NAIR, L. S. and NAIR A. S. (1993). Effects of Malaria infection on Pregnancy. *Indian Journal of Malariology, 30(4):* 204 - 214.

NWOKE, B. E. B. and UWAZIE, O. U. (1991). Studies on the blackflies *Simulium* of Imo State Nigeria. The distribution of Immature stages in Isuikwuato Okigwe area. *Nigerian Journal of Parasitology, 12*: 29 - 37

NWOKEDI, M. C. (1992). *Prevalence of Malaria in Pregnant women in Onitsha, South East Nigeria.* B.Sc. Thesis. Abia State University, 36 pp

O'NEIL-DUNNE, I., ACHUR, R.N. and AGBOR-ENOH, S. T. (2001). Gravidity dependent production of antibodies that inhibit binding of *Plasmodium falciparum* infected erythrocytes to placental Chondroitin Sulfate Proteoglycan during pregnancy. *Infection and Immunology, 69:* 7487 - 7492.

OKOKO, B. J., ENWERE, G. and OTA, M. O. C. (2003). The epidemiology and consequences of Maternal Malaria: A review of immunological basis. *Acta Tropica, 7:* 193 - 205.

OPARA, K. N., IBANGA, E. S., WALI, N. B. and USIP, L. P. (2004). Falciparum Malaria and Susceptibility to Genetic Markers of Pregnant Women in Uyo, Southeast Nigeria. *Abstract of the 28th Annual Conference of the Nigeria Society of Parasitology* held at Imo State University Owerri. pp. 76.

RAYANAL, P., PENEAU, C., CARLES, G., BOUSQUET, F., MIGNOT, V., and ARBULLE, P. (1998). Pregnancy and Malaria, Study of 143 cases in French Guyana. *Journal of Gynaecology, Obstetrics, Biology and Reproduction, 27(8):* 798 - 805.

RICKE, C. H., STAALSOE, T., KORAM, K., AKANMORI, B. D., RILEY, E. M., THEANDER, T. G. and HVIID, L. (2000). Plasma anti - bodies from malaria - exposed pregnant women recognize variant surface antigens on *Plasmodium falciparum* infected erythrocytes in a parity - dependent manner and block parasite adhesion to chondroitin sulfate. *Journal of Immunology, 165*: 3309 - 3316.

ROGERSON, S. J., MKUNDIKA, P. and KANJALA, M. K. (2003). Diagnosis of *Plasmodium falciparum* Malaria at Delivery: Comparison of Blood Film Preparation Methods and of Blood Films with Histology. *Journal* of *Clinical Microbiology, 41*: 1370 - 1374.

SAUTE, F., MENENDEZ, C. MAYOR, A., APONTE, J., GOMEZ - OLIVE, X, DGEDGE, M. and ALONSO, P. (2002). Malaria in Pregnancy in Rural Mozambique: The role of Parity Submicroscopic and Multiple *Plasmodium falciparum* infection*. Tropical Medicine and International Health, 7:* 19 - 28.

SNOW, R. W, CRAIG, M. H., DEICHMANN, U. and LE-SOEUR D. (1999). A preliminary continental risk map for Malaria mortality among African Children*. Parasitology Today, 15:* 99 – 104.

SNOW, R. W., TRAPE, J. F. and MARSH, K. (2001). The past, present and future of childhood mortality in Africa. *Trends in Parasitology, 17:* 593 - 597.

STEKETEE, R. W. and MUTABINGWA, T. K. (1999). Malaria in Pregnant Women: Research, Epidemiology, policy and practice. *Annals of Tropical Medicine and Parasitology, 93*: S7 - S9.

STEKETEE, R. W., NAHLEN, B. L., PARISE, M. E and MENENDEZ, C. (2001). The burden of Malaria in Pregnancy in Malaria endemic areas. *America Journal of Tropical Medicine and Hygiene, 64(supplementary)*: 28 - 35.

VAN DEN BROEK, N. R., ROGERSON, S. J., MHANGO, C. G, KAMBALA, B., WHITE, S. A. and MOLYNEUX, M. E. (2000). Anaemia in Pregnancy in Southern Malawi: Prevalence and risk factors. *British Journal of Obstetrics and Gynaecology, 107:* 445 - 451.

VERHOEFF, F. H., BRABIN, B. J., VAN BUREN, S., CHIMSUKU, L., KAZEMBE, P., WIT, J. M and BROADHEAD, R. L. (2001). An analysis of inter - uterine growth retardation in rural Malawi. *European Journal of Clinical Nutrition, 55*: 682 - 689.

WAKIBARA, J. V., MBOERA, L. E. and NDAWI, B. T. (1997). Malaria in Mvumi, Central Tanzania and the *in vivo* response of *Plasmodium falciparum* to chloroquine and sulphadoxine pyrimethamine. *East African Medical Journal, 74*: 69 – 71.

WATKINSON, M. and RUSHTON, D. I. (1983). Plasmodial Pigmentation of Placenta and outcome of Pregnancy in a West African Mother. *British Medical Journal, 287*: 251 - 254.

WHO (2000). WHO Expert Committee on Malaria. Twentieth Report, World Health Organization, Geneva.

ZHOU, A., MEGNEKOU, R., LEKE, R., FOGAKO, J., METENOU, S., TROCK, B., TAYLOR, D. W. and LEKE, R. F. (2002): Prevalence of *Plasmodium falciparum* infection in Pregnant Cameroonian women. *American Journal of Tropical Medicine and Hygiene, 67(6):* 566 - 570.

EFFECT OF FEED RESTRICTION ON GROWTH PERFORMANCE AND ECONOMY OF PRODUCTION OF BROILER CHICKS

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ABSTRACT

An experiment was conducted to determine the effect of feed restriction on growth performance and economy of production using One Hundred and Twenty (120) ANAK 2000 broiler chicks. The dietary treatments consisted of providing feed ad libitum (full fed) and two feed restriction treatments: restricting feeding 80 % of ad libitum between 28 – 70 days of age (DOA); and for 28 – 47 DOA with re-alimentation to full fed 48 - 70 DOA. The three treatments were identified as D_1, D_2, and D_3 respectively. A one-way analysis of variance (ANOVA) in Completely Randomized Design (CRD) was used to analyze data collected on growth performance variables. A cost – benefit analysis was utilized for the economy of production. Analysis of results obtained revealed that final body weight and weekly weight gain of broilers on D_1 and D_3 were similar ($P < 0.05$) but differed from D_2 ($P < 0.05$). No significant difference ($P < 0.05$) was found between D_2 and D_3 and between D_2 and D_1 in weekly feed intake and feed efficiency respectively. Feed efficiency was improved by restriction followed with re-alimentation. A reduced feed cost (N)/Kg weight gain, highest revenue and least cost-benefit ratio were obtained from reduced from birds on D_3.

Keywords: Broiler chicks, feed restriction, Growth performance, Economy of production

INTRODUCTION

The production performance of the broiler chicks is greatest when free access to feed and water is given Feed, incidentally, is the most expensive factor in growing broiler birds (Obioha, 1992). Inadequacy and inconsistency of feed supply is a major bottleneck to efficient animal production in tropical farming system (Melaku and Peters, 2000). Nji *et al.*, (2002) attributed these short – fall in feed supply to two major factors viz: (1) scarcity and high cost of conventional protein and energy feedstuff, and (2) competition for these products by man, livestock and agro – industrial sectors. Quantitative feed restriction programme has been successfully applied in managing these scares feedstuff. However, improper use of this approach can lead to considerable weight loss and poor production (Bowes *et al.,* 1988). Thus, the application of the knowledge of feed management in nutrition must interact with economic consideration that influences the amount of feed supplied as ration. Plavink and Hurwitz (1988) observed that the timing, severity and duration of restriction had significant effect on the subsequent ability of broilers to recover from a growth defect. Several studies have shown that early nutrition and hydration has long – term benefits in growth rates than early deprivation (Noy and Sklan, 1999, 2000). This is, primarily, because the development of the digestive tract in poultry is rapid and more susceptible to variations with different nutrients and their availability to the body system (Dibner *et al.,* 1996). Nwachukwu and Ibe (1990) provided broilers 95, 90 or 85 % of the daily feed consumption of birds fed *ad libitum* from 2 –6 weeks of age before re-feeding them on *ad libitum* from 7 – 9 weeks of age. They reported a depressed body weight by all levels of feed restriction; furthermore, economic parameters considered did not show feed restriction as having advantage over full – feeding. Their findings could have been influenced by the time at which feed restriction was commenced and the duration. This study examined responses of broilers subjected to three different feeding regimes from 28 day of age. Measurements included growth performance variables and economic parameters.

MATERIALS AND METHODS

Experimental Site: The study was carried out in the Poultry Research Unit, Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki.

Animal Management: a total of 120 day - old ANAK 2000 strains of broiler chicks obtained from S and D Farm Limited, Abeokuta were used for the study. The 120 chicks were brooded together in the brooding unit (deep litter system) for 28 days using 100 watts electric bulb. At 28 day of age (DOA), the chicks were randomly allotted to three dietary treatments consisting of 60 birds per treatment. Each treatment was replicated four times thus they were 10 birds per replicate. The feeding trial lasted for 6 weeks. The chicks were fed finisher diet (Guinea Feed).

Dietary Treatments: three dietary treatments were used for the study. These were identified as D_1 = Chicks fed *ad libitum* from 28 –70 DOA; D_2 = Chicks

fed 80% *ad libitum* 28 - 70 DOA; and D_3 = Chicks fed 80 % *ad libitum* 28 – 47 DOA and then re-alimented to *ad libitum* 48 - 70 DOA. The percentage feed restriction was based on previous 24 – hour feed consumption values of *ad libitum* control group (D_1).

Parameters Measured: The chicks were weighed as individual replicate groups at the beginning of the experiment (28 DOA). Taking the average weekly body weight of the birds and calculating the amounts of weight gained per week measured growth rates. From the feeder weights, the amount of feed consumed was calculated for the six weeks of experimentation. By dividing the average weekly weight gain by the average weekly feed consumed for individual bird/treatment, feed efficiency was established for the experiment. Multiplying total feed consumed by cost/kg feed got the total cost of feed. The quotient of total cost of feed and total weight gain gave the feed cost/ kg gain. Revenue referred to the product of final body weight and cost/kg live weight. Gross margin was obtained by subtracting the total cost of feeding from revenue whereas the cost – benefit ratio was obtained by dividing total cost of feeding by gross margin.

Statistical Analysis: Data obtained on all parameters, expect those on economics of production were subjected to a one – way Analysis of Variance in a Completely Randomized Design (Obi 2001). Significant means ($P < 0.05$) were separated using Duncan's New Multiple Range Test (Obi 2001).

RESULTS AND DISCUSSION

Table 1 shows the results of the growth performance variables of the birds fed the dietary treatments. There was no significant difference ($P < 0.05$) in final body weight of birds on D_1 and D_3. Such similarities did not exist between these two treatments and D_2. This observation could be traced to the fact that following re-alimentation, restricted chicks consumed feed voraciously, which translated to a good gain for the chicks on D_3 (Plavnik et al., 1986). The slight numerical difference in final body weight of D_1 and D_3 ($D_1 = 0.07 > D_3$) supports the submission of Mollison *et al.*, (1984) that although the compensatory growth of the restricted group at certain periods may equal that of the unrestricted group, the final body weight of the restricted group never catches up with that of the unrestricted group. The mean weekly weight gain, feed intake and feed efficiency of birds were significant ($P < 0.05$) improved by re-alimentation. Beane *et al.*, (1979) reported that re-alimentation following the restriction of feed intake of broilers fed 85% of full fed control birds resulted in greater weight gains and a better feed efficiency. Feed restriction often results in apparent decrease in maintenance requirement due to depressed metabolic rate, suggesting that birds become more and more efficient in utilizing reduced food intake. This is based on the concept of a reduced maintenance requirement in animals recovering from periods of growth/feed restriction – where the carry over effects of lowered metabolic rates allows more food to be available for growth purposes (Lawrence and Fowler, 1998).

Table 1: Effect of Dietary Treatment on Performance Characteristics of Broiler Chicks

Parameters	D_1	D_2	D_3	SEM
Mean Initial body at 28 DOA (kg/chick)	0.57^a	0.56^a	0.58^a	0.01
Mean final body weight (kg/chick)	2.45^a	2.15^b	2.38^a	0.04
Mean weekly weight gain (kg/chick)	0.31^a	0.27^b	0.30^a	0.01
Mean weekly feed intake (kg/chick)	0.93^a	0.79^b	0.81^b	002
Feed efficiency	0.34^b	0.34^b	0.37^a	0.01

^{ab} Means differently superscripted are significantly different from one another ($P < 0.05$); ± SEM = Standard Error of the Mean.

The results of the economics of production are summarized in table 2. Quantitative feed restriction proved a benefit of this procedure. Feed cost was highest in D_1 and least in D_2 (a difference of N40.32). Feed cost (₦)/kg weight gain decreased in this order D_3, D_1 and D_2 (₦129.60, ₦142.47 and ₦143.09 respectively). Revenue, a factor determined by final body weight and ruling market price was highest for D_1 and D_3. The result on gross margin (₦)/ bird showed a contrary trend with that of feed cost (₦)/kg weight gain ($D_3 > D_1 > D_2$). D_3, thus had a better cost-benefit ratio than the other treatments. These results were in agreement with results of Pasternak and Shalev (1983). They reported significant positive monetary returns due to feed restriction. Proudfoot and Hulan (1982) also indicated that bird subjected to initial feed restriction and later returned to *ad libitum* made higher profit than the control birds.

Table 2: Economics of Production of Feed Restriction on Broiler Chicks[1]

Parameters	D_1	D_2	D_3
Total feed consumed (kg/chick)	5.58	4.74	4.86
Cost (₦) kg feed	48	48	48
Total cost of feeding (₦/chick)	268.64	227.52	233.28
Final Body Weight (kg/chick)	2.45	2.15	2.38
Total weight gain (6 weeks) kg/chick	1.88	1.59	1.80
Feed cost (₦)/ kg Weight gain	142.47	143.09	129.60
Cost of production (₦)[2]	267.84	227.52	233.28
Revenue (₦)	857.50	752.50	833.00
Gross Margin (₦)	589.66	524.98	599.72
Cost – benefit Ratio	0.45	0.43	0.39

[1] Cost/kg live weight chicken = ₦350; [2] Cost of production based on feed cost only (other costs remain constant)

Conclusion: There were signs of improved growth performance detected in birds fed D_3, resulting in a concomitant improvement in cost – benefit ratio of the dietary treatment. The results of this trial, thus, help in emphasizing the importance of feed restriction (80% *ad libitum*) of broiler chicks from 28 – 47 DOA, followed by re-alimentation to *ad libitum* (48 – 70

DOA). With such approach, our results indicate that the farmer would certainly achieve least cost of production and at the same time maximize profit.

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REFERENCES

BEANE, W. L, CHERRY, J. A and WEANER Jr. W. D. (1979). Intermittent Light And Restricted Feeding of Broiler Chickens. *Poultry science, 58:* 567 – 571.

BOWES, V. A., JILLIAN, R. J., LEESON, S. and STRITZINGER, T. (1988). Effect of Feed Restriction on Feeding Efficiency and Incidence of Sudden Death Syndrome in Broiler Chickens. *Poultry Science, 67:* 1102 – 1104.

DIBNER, J. J, KITCHELL, M., ATWELL and C., IVERY, F. (1996). The Effect of Dietary Ingredients and Age on the Microscopic Structure of the Gastrointestinal Tract in Poultry. *Journal of Applied Poultry Research, 5(1):* 16 – 21.

LAWRENCE, T. L. J. and FOWLER, V. R. (1998). *Growth of Farm Animals.* CAB International, Wallingford, Oxon, United Kingdom.

MELAKU, S. and PETER, J. K. (2002). Effects of Supplementation of Sole or Mixtures of Selected Multipurpose Trees (MPT) on Feed Intake, Live weight and Scrotum circumference Changes in Menz Sheep Fed a Basal diet of Tef (*Eragrostis tef*) straw. *Deutsher Tropentag Book of Abstracts and Proceedings:* 231 – 233.

MOLLISON, B., GUENTER, W. and BOYCOTT, B. R. (1984). Abdominal Fat Deposition and Sudden Death Syndrome in Broilers: The Effect of Restricted Intake, Early Life Calorie (Fat) Restriction and Caloric: Protein Ratio. *Poultry Science, 63:* 1190 – 1200.

NJI, F. R., NIESS, E, and PFEFFER, E. (1999). Performance of Growing Broiler Chicks Fed Bambara Groundnuts (*Vigna Subterranean*). *Deutsher Trogentag, Book of Abstracts and Proceedings:* 156.

NOY, Y. and SKLAN, D. (1999). Different Types of Early Feeding and Performance in Chicks and Poultry. *Journal of Applied Poultry Research, 8 (1):* 16 – 24.

NOY, Y and SKLAN, D. (2000). Hydrolysis and Absorption in the Small Intestines of Posthatch Chicks. *Poultry Science*, *79(9):* 1306 – 1310.

OBI I. U. (2001). *Statistical Method of Detecting Differences between Treatment Means.* SNAAP Press Nigeria Limited, Enugu, Nigeria.

OBIOHA, F. C. (1992). *A Guide to Poultry Production in the Tropics.* Acena Publications, Onitsha, Nigeria.

NWACHUKWU, E. N. and IBE, S. N. (1990). Effects of Quantitative Feed Restriction on Broiler Growth and Monetary returns. *Nigerian Journal of Animal Production, 17:* 10 – 13.

PROUDFOOT, F. G. and HULAN, H. W. (1982). Effect of Reduced Feeding Time using all Mash or Crumble Pellet Dietary Regimes on Chicken Broiler Performance Including the Incidence of Death Syndrome. *Poultry Science, 61:* 750 – 754.

PASTERNAK, H. and SHALEV, B. A. (1983). Genetic Economic Evaluation of Traits in a Broiler Enterprise: Reduction of Food intake. *British Poultry Science, 24:* 531 – 536.

PLAVNIK, L., MCMURTY, J. P., and ROSE BROUGHT, R. N. (1986). Effects of Early Feed Restriction in Broilers: Growth Performance and Carcass Composition. *Poultry science, 50:* 68 – 69.

PLANVIK, L. and HURWITZ, S. (1988). Effect of feed Restriction on Chicks: Effects of Age, Duration and Sex. *Poultry Science,* 67: 384 – 390.

THE EXPOSURE OF *Heterobranchus bidorsalis* JUVENILES TO DIFFERENT CONCENTRATIONS OF BONNY-LIGHT CRUDE OIL AND THEIR EFFECTS ON AMYLASE AND CRETININE KINASE ACTIVITIES

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ABSTRACT

The effects of exposing Heterobranchus bidorsalis juveniles (14.08 ± 0.12 g) to different concentrations of Bonny-light crude oil (BLCO) on amylase and cretinine kinase activities were studied. The exposure of the fish to 1.00, 2.00, 4.00, 8.00 ml L^{-1} BLCO and a control for 4 days toxicity period indicated that the significant increases (P < 0.01) in the serum amylase (SRA) and the hepatic cytosolic amylase (HCA) activities in the fish were BLCO concentrations dependent. Reduced SRA and HCA activities noticed within the first 14 days of the recovery period implied that the removal of the oil pollutant from the ambient water chemistry probably lowered the pressure on the blood serum and liver amylase enzyme to catalyse the metabolism of the ingested carbohydrates. Significant increases (P < 0.05) in the serum cretinine kinase (SRCK) and the hepatic cytosolic cretinine kinase (HCCK) activities in the fish also followed the pattern shown by the SRA and the HCA activities. The increased SRA, HCA, SRCK and HCCK activities in the blood serum and liver of the fish were indications of a shift in the carbohydrate metabolism due to crude oil exposure.

Keywords: *Heterobranchus bidorsalis*, Bonny-light crude oil, Serum, Cytosolic, Amylase, Cretinine kinase.

INTRODUCTION

Increasing awareness of the adverse effects of anthropogenic activities and pollution on aquatic environment has focused interest on health of fish populations and possibilities to utilize these health parameters for assessment of the quality of aquatic environment (Henry *et al*., 2004). The potential of using biomarkers for monitoring both environmental quality and the health of organisms inhabiting polluted ecosystems has received increasing attention in recent years (Lopes *et al*., 2001; Samecka-Cymerman and Kempers, 2003; Gauthier *et al*. (2004).

The effect of xenobiotic contamination (including crude oil) in an ecosystem can be estimated through analysis of biochemical changes in organisms inhabiting that polluted environment (Tuvikene *et al*., 1996; Norris *et al*., 2000; Brewer *et al*., 2001). The biochemical response of aquatic organism to pollution is given by changes several key enzymes, especially those of biotransformation systems. The value of tissue enzyme activities in the diagnosis of the effects of pollutant is an emerging area in aquatic toxicology and remediation programmes (Oluah *et al*., 2005). Thirugnanam and Forgash (1977) studied the anticholinesterase effect of chlorpyrifos to *Fundulus heteroclistis* and reported that increased concentration of the mosquito pesticides in salt water mash habitat resulted in maximum anticholinesterase inhibition in the fish. Increased glucose-6-phosphatase and glycogen phosphorylase activities were observed in *Cyprinus carpio* exposed to paraquat (Simon *et al*., 1983). A herbicide (Basalin) in contact with a freshwater fish, *Nemachelinus* sp. affected the activities of lactate dehydrogenase, alkaline phosphatase and glutamic pyruvate transaminase in the fish (Verma et al. 1981). Oluah and Amalu (1998) reported increased activities of alanine and aspartate aminotransferases in *Clarias albopuntatus* exposed to copper. Certain pesticides were observed to inhibit alkaline phosphatase and glucose-6-phosphatase activities in the fish *Mytulus vittatus* (Werma, *et al.,* 1981).

Omoregie *et al.* (1997), however, reported that the exposure of fish to crude oil fractions caused changes in the oxygen consumption, tissue glycogen and glucose levels of the fish. This study presents the results of the exposure of *Heterobranchus bidorsalis* juveniles to different concentrations of Bonny-light crude oil and their effects on amylase and cretinine kinase activities. The essence was to determine the impact of the various concentrations of this crude oil pollutant on the energy metabolism of this highly priced food fish in Nigeria.

MATERIALS AND METHODS

Six hundred (600) juveniles of *Heterobranchus bidorsalis* (Geoffroy St. Hilaire, 1809) (14.08 ± 0.12g} were transported from a private fish hatchery at Otor-Oweh, Delta State to the Fisheries Laboratory of Enugu State University of Science and Technology, Enugu. At the Fisheries Laboratory in Enugu the fishes were acclimatized for 14 days and fed 38% crude protein diet at 3% body weight per day (bw d^{-1}).

Batches of twenty (20) juveniles of *H. bidorsalis* were randomly stocked in triplicates in 15 plastic containers with 24 litre dechlorinated tap water and which were previously contaminated with 5 ml of Bonny-light crude oil (BLCO) at 1.00, 2.00, 4.00 and 8.00 ml L^{-1} concentrations. Three (3) plastic containers not contaminated with BLCO were left as the controls. Mosquito-mesh nets were used to cover the containers to prevent fish escape.

Two experimental phases were adopted for the study. The toxicity phase lasted for 4 days (96h), while the recovery phase lasted for 42 days and was monitored at fortnightly (14 days) intervals. Fish were monitored each day in both phases for mortality and the surviving fish recorded. At the end of the toxicity period, the surviving fishes and plastic containers were washed and replenished with dechlorinated tap water. A 38% crude protein diet (Tables 1) was fed to fish at 3% bw d^{-1} during the toxicity period (4 days) and 5% bw d^{-1} during the recovery period (42 days). Records of the water temperature (26 ± 0.50° C) and pH (6.80 ± 0.02) were taken with the aid of a maximum and minimum mercury-in-glass Celsius thermometer and a pH meter (Model Ph-I-20-L) respectively.

The blood and liver tissues were sampled at day 4 (for the toxicity period) and at days 14, 28 and 42 (for the recovery period). The blood samples were collected by both the cardiac puncture method and the severance of the caudal peduncle using disposable hypodermic syringe (Oluah, 1999). The liver was excised and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized as described by Devi *et al.* (1993) and then placed in ice-cold 0.25M sucrose (Oluah et al., 2005). The liver homogenate was centrifuged at 5000 rpm for 15 minutes at 4° C and the supernatant was transferred into clean microfuge tubes. The samples were stored at -80° C until enzymatic assays were carried out (Ozmen *et al*., 2005). The blood was similarly centrifuged for 15 minutes at 1000 rpm to obtain the serum. The serum was also stored at -80° C in clean microfuge tubes.

Total protein concentrations of the liver supernatants and blood serum were determined according to the method of Lowry *et al.* (1951) using BSA as the standard at 695 nm. Blood serum (Serum amylase (SRA) and Serum cretinine kinase (SRCK)), hepatic cytosolic amylase (HCA) and hepatic cytosolic cretinine kinase (HCCK) concentrations were determined. All enzymatic assays were conducted spectrophotometrically at appropriate wavelengths using a microplate reader system (VersaMax, Moleclar Devices Corp., USA) at 25° C. Samples were assayed in triplicates. Data collected were analysed using descriptive statistics and analysis of variance (ANOVA) to indicate statistical significance ($P < 0.05$). Differences were partitioned with the least significant difference.

RESULTS

The values of the serum amylase (SRA) and the hepatic cytosolic amylase (HCA) were lower in the control fish than in those exposed to BLCO concentrations (1.00 – 8.00 ml L^{-1}) (Table 2) for both of the toxicity and recovery periods of the study. Amylase enzyme concentration in the fish blood serum increased significantly ($P < 0.01$) during the toxicity period as the concentration of oil in the water increased from 1.00 ml L^{-1} BLCO (103.30 ± 1.01 µ L^{-1}) to 8.00 ml L^{-1} BLCO (283.35 ± 1.1 bµ L^{-1}). There was a corresponding increase in the HCA concentration as the BLCO concentrations increased from 1.00 to 8.00 ml L^{-1} (Table 2).

Table 1: Gross Composition of the Experimental Diet Fed to *Heterobranchus bidorsalis* Fingerlings Stocked in Crude Oil Polluted Water

Feed ingredient	*% Composition*
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free extract	34.46
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): thiamine, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3,000; niacin, 150; vitamin B_{12}, 0.06; retinyl acetate (500,000 IU/g), 6; menadione-Na-bisulphate 80; inositol, 400; biotin, 2; vitamin C, 200; alphatocopherol, 200; cholecalcipherol, 1,000,000 IU/g.

[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; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 0.50; $CuSO_4.5H_2O$, 0.50; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and Cellulose, 380.97.

When the oil pollutant was removed during the 14 days recovery period, both the SRA and the HCA concentrations in the fish were reduced by a measure of 20 % (Table 2) irrespective of the BLCO concentration to which the fishes were exposed. In addition, there were obvious increases in the SRA and HCA concentrations in the fish as the recovery period extended from day 14 to day 42. Significant variations ($P < 0.01$) in the SRA and HCA.

Table 2: Serum and Hepatic Cytosolic Amylase Concentration in *Heterobranchus bidorsalis* Juveniles Exposed to Different Concentrations of Bonny-light Crude Oil (BLCO) for 4 Days (Toxicity) and 42 Days (Recovery) Periods[1]

Study Period	Duration (days)	BLCO concentration (ml L^1)									
		0.00 (Control)		1.00		2.00		4.00		8.00	
		SRA	HCA	SRA[2]	HCA[3]	SRA	HCA	SRA	HCA	SRA	HCA
Toxicity		75.36	113.04	103	154.45	144.62	216.93	202.47	303.71	283.35	425.03
Phase	4	±0.29^{h}	±1.03^{a}	±1.01^{a}	±1.08^{b}	±1.12^{c}	±1.51^{d}	±1.43^{d}	±1.56^{e}	±1.16^{f}	±1.82^{g}
Recovery	14	76.14	116.08	86.64	123.96	115.70	173.54	161.98	242.97	226.68	340.02
Phase		±0.44^{i}	±1.15^{c}	±0.62^{a}	±1.07^{b}	±1.12^{c}	±1.08^{d}	±1.10^{e}	±1.23^{f}	±1.24^{g}	±1.31^{h}
	28	78.04	119.12	86.77	130.16	121.49	182.22	170.82	255.12	238.01	357.02
		±0.56^{h}	±1.13^{i}	±0.71^{a}	±1.06^{b}	±1.04^{c}	±1.12^{d}	±1.11^{e}	±1.32^{f}	±1.16^{g}	±1.23^{h}
	42	80.11	123.34	99.79	149.64	139.71	209.55	195.59	293.39	272.71	410.57
		±0.63^{i}	±1.04^{j}	±0.16^{a}	±1.13^{b}	±1.14^{c}	±1.16^{d}	±1.17^{e}	±1.46^{f}	±1.13^{g}	±1.38^{h}

[1]Means in the same row followed by the same superscript differ significantly (P < 0.05). [2]Serum amylase concentration (μ L^{-1}), [3]Hepatic cytosolic amylase (μ g^{-1})

Table 3: Serum and Hepatic Cytosolic Cretinine Kinase Concentration in *Heterobranchus bidorsalis* Juveniles Exposed to Different Concentrations of Bonny-light Crude Oil (BLCO) for 4 Days (Toxicity) and 42 Days (Recovery) Periods[1]

Study Period	Duration (days)	BLCO concentration (ml L^1)									
		0.00 (Control)		1.00		2.00		4.00		8.00	
		SRCK	HCCK	SRCK[2]	HCCK[3]	SRCK	HCCK	SRCK	HCCK	SRCK	HCCK
Toxicity		0.28	0.42	0.33	0.50	0.45	0.68	0.63	0.95	0.88	1.32
Phase	4	±0.03^{a}	±0.06^{c}	±0.01^{a}	±0.03^{b}	±0.02^{c}	±0.04^{d}	±0.06^{d}	±0.05^{e}	±0.04^{f}	±0.01^{g}
Recovery	14	0.30	0.45	0.26	0.42	0.36	0.54	0.50	0.76	0.71	1.06
Phase		±0.02^{c}	±0.02^{b}	±0.02^{a}	±0.04^{b}	±0.02^{c}	±0.03^{d}	±0.02^{d}	±0.05^{e}	±0.04^{e}	±0.08^{f}
	28	0.36	0.51	0.27	0.42	0.38	0.57	0.53	0.80	0.75	1.11
		±0.02^{c}	±0.03^{d}	±0.02^{a}	±0.04^{b}	±0.01^{c}	±0.04^{d}	±0.03^{d}	±0.06^{e}	±0.05^{f}	±0.09^{g}
	42	0.42	0.57	0.31	0.48	0.44	0.59	0.61	0.92	0.86	1.28
		±0.02^{b}	±0.03^{c}	±0.02^{a}	±0.04^{b}	±0.03^{b}	±0.04^{c}	±0.05^{d}	±0.07^{e}	±0.06^{f}	±0.10^{g}

[1]Means in the same row followed by the same superscript differ significantly (P < 0.05). [2]Serum cretinine kinase concentration (μ L^{-1}), [3]Hepatic cytosolic cretinine kinase (μ mg^{-1})

Table 4: Percent mortality and Survival of *Heterobranchus bidorsalis* juveniles during exposure to Different Concentrations of Bonny-light Crude Oil (BLCO) (4 days) and recovery (42 days)

Study Period	Duration (days)	% Mortality BLCO concentration (ml L^1)					% Survival BLCO concentration (ml L^1)				
		0.00 (Control)	1.00	2.00	4.00	8.00	0.00 (Control)	1.00	2.00	4.00	8.00
Toxicity Phase	4	0.00	10.00	0.00	40.00	50.00	100.00	90.00	100.00	60.00	50.00
Recovery Phase	14	0.00	8.00	6.00	32.00	40.00	100.00	92.00	92.00	68.00	60.00
	28	0.00	2.00	1.00	24.00	36.00	100.00	98.00	99.00	76.00	64.00
	42	0.00	1.00	0.00	16.00	26.00	100.00	99.00	100.00	84.00	74.00

concentrations in the fish were also recorded as the fishes recuperated, from their exposures to the various BLCO concentrations and control (Table 2). As was the case with the amylase concentration, both the serum cretinine kinase (SRCK) concentration and the hepatic cytosolic cretinine kinase concentration were least in the control fish than in those exposed to BLCO concentrations (Table 3). The cretinine kinase concentration in fish blood serum also increased significantly ($P < 0.05$) during the toxicity period (Table 3) as the concentrations of BLCO increased from 1.00 ml L^{-1} (SRCK = 0.33 ± 0.01 µ L^{-1}) to 8.00 ml L^{-1} (SRCK = 0.88 ± 0.04 µ L^{-1}). The corresponding values of the HCCK concentration at this period were 1.00 ml L^{-1} (HCCK = 0.50 ± 0.03 µmg^{-1}) to 8.00 ml L^{-1} (HCCK = 1.32 ± 0.10 µmg^{-1}).

Twenty percent (20 %) reductions in the values of SRCK and HCCK concentrations in the fish were also recorded within the first fortnight (14 days) of the recovery period, irrespective of the BLCO concentrations applied (Table 3). The concentrations of the cretinine kinase enzyme, however, increased as the recovery period extended from day 28 to day 42. Generally, there were significant variations ($P < 0.05$) in the SRCK and the HCCK concentrations in the fish as they recovered from their exposures to the various concentrations of BLCO.

The percent mortality (PM) and survival (PS) of the fish during the toxicity and recovery periods of the study (Table 4) indicated that the fish exposed to 4.00 and 8.00 ml L^{-1} BLCO died more and survived less. The control fish, however, recorded zero percent (0.00 %) mortality and a hundred percent (100.00 %) survival during both study periods.

DISCUSSION

Fish viscera are known to be a rich source of enzymes, including amylase and cretinine kinase, many of which present high activity at low concentrations. Fish digestive enzymes exhibit optimal activity at temperatures much higher than the ambient temperature of fish (Fereidoon and Janaka-Kamil, 2001). Changes in the activity of tissue glycogen and glucose modulating enzymes have been reported in common carp exposed to paraquet (Simon *et al.*, 1983). Omoregie *et al*. (1997) reported that the exposure of fish to crude oil fractions caused changes in the oxygen consumption, tissue glycogen and glucose levels of the fish.

The result of our study indicated that the increases in SRA and HCA activity of *H. bidorsalis* juveniles were dependent on the BLCO concentrations to which the fishes were exposed (Table 2). This result is consistent with the report of Oluah *et al*. (2005) who obtained increases in the serum and liver lactate dehydrogenase (LDH) activity in *Clarias albopunctatus* exposed to increasing concentrations of sublethal Gammalin 20 and Acetellic 25EC. Although Oluah *et al*. (2005) recorded increases in LDH activity with the duration of exposure of *C. albopunctatus* to the agro-chemical pollutants, this study recorded reduced SRA and HCA concentrations within 14 days (Table 2), as the fishes recuperated from the stress of exposing them to 1.00 - 8.00 ml L^{-1} BLCO concentrations. The present result implies that the removal of the oil pollutant from the ambient water chemistry must have reduced the pressure on the serum and hepatic (liver) amylase activity to metabolize the ingested carbohydrate (Table 1a) and release energy required for fish to respond to the infiltrating oil pollutant in the blood stream. The haemotological effects of starvation (Norman *et al*., 1980), stress (Scott and Rogers, 1981), and health condition of the fish (Munkittrick and Leatherhead, 1983) consequent upon altered water chemistry have been studied. Oluah (2001) stated that the alterations of water quality usually predispose the fish to stress and disease which as a result, provoke quick responses in the physiology of the fish, especially haematological parameters.

The increases in the SRA and HCA (Table 2) and the SRCK and HCCK (Table 3) concentrations in the fish between days 14 and 42 of this study were consistent with the report of Oluah *et al*. (2005) mentioned above. Other workers who had earlier recorded similar results include: Christensen *et al*. (1977) and Devi *et al*. (1993) who reported increased muscular LDH activity in brook trout (*Salvelinus fontinalis*) and fiddler crab (*Uca pugilator*) exposed to cadmium respectively. Parathion was also found to elicit increased LDH activity in rat (Gallo and Lawryk, 1991); while lindane caused a 2-fold increase in liver myeloperoxidase activity in rat (Junge *et al.,* 2001).

While the amylase enzyme catalyses the biochemical conversion of the ingested carbohydrate in the fish intestinal tract to glucose, cretinine kinase is involved in the glycolytic pathway to energy metabolism of glucose/glycogen via the blood and the liver of the fish. Therefore, the increased activities of SRA, HCA, SRCK, HCCK in both the serum and the liver of *H. bidorsalis* juveniles of this study are indications of a shift in the carbohydrate metabolism arising from glucose and glycogen catabolism which eventually culminate in the release of energy needed for metabolic activities in the fish. Neff and Anderson (1987) enunciated some deleterious effects of exposing fish to crude oil contamination to include: alteration of the immune response metabolism, changes in liver metabolism and haemorrhage. The present results are in consonance with the report of these workers since the highest percent mortality and the lowest percent survival of *H. bidorsalis* juveniles (Table 4) were recorded during the 4 days toxicity period.

REFERENCES

BREWER, S. K., LITTLE, E. E., DELONEAY, A. J., BEAUVAIS, S. L., JONES, S. B. and ELLERSLECK, M. R. (2001). Behavioural dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykis*) exposed to cholinesterase-inhibiting chemicals. *Archives of Environmental Contamination and Toxicology*, *40:* 70 – 76.

CHRISTENSEN, G., MUNT, E. and FIANDT, I. (1977). The effect of methyl mercuric chloride, cadmium chloride and lead nitrate in six biochemical factors of brook trout (*Salvelinus fontinalis*). *Toxicology and Pharmacology*, *42:* 523 – 530.

DEVI, M., REDDY, P. S. and FINGERMAN, M. (1993). Effect of exposure on lactate dehydrogenase activity in the hepatopancreas and abdominal muscles of Fiddler crab *Uca pugilator. Comparative Biochemistry and Physiology C, 106:* 739 – 742.

FEREIDOON, S. and JANKA-KAMIL, Y. V. A. (2001). Enzyme from fish and aquatic invertebrates and their application in the food industry. *Trends in Food Science and Technology*, *12:* 435 – 464.

GALLO, M. A. and LAWRYK, V. J. (1991). *Organic phosphorus pesticides studies.* Williams and Wilkins, Baltimore. 1123 pp.

GAUTHIER, L., TARDY, E., MOUCHET, F. and MARTY, J. (2004). Biomonitoring of the genotoxic potential (micronucleus assay) and detoxifying activity (EROD induction) in the River Dadou (France), using the amphibian *Xenopus laevis. Scientific Total Environment, 323:* 47 - 61.

HENRY, F., AMARA, R, COURCOT, L., LACOUTURE, D. and BERTHO, M. L (2004). Heavy metals in four fish species from the French coast of the Eastern English Channel and Southern Bight of the North Sea. *Environmental International*, *30:* 675 – 683.

JUNGE, B., CARRION, Y., BASIO, C., GALLANO, M. PRINTARNIO, S., TAPIA, G. and VIDELA, L. A. (2001). Effect of iron over load and lindane in toxication in relation to oxidation stress, Kuffer cell function and liver injury in rat. *Toxicology and Applied Pharmacology, 170:* 23 – 28.

LOPES, P. A., PINHEIRO, T., SANTOS, M. C., MATHIAS, M. L., COLLARES-PEREIRA, M. J. and VIEGAS-CRESPO, A. M. (2001). Response of antioxidant enzyme in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. *Scientific Total Environment, 286:* 153 – 163.

LOWRY, O. H., ROSEMBROUGH, N. J. and FARR, A. L. (1951). Protein measurement with Pholin Phenol reagent. *Journal of Biology and Chemistry*, *193:* 265 – 275.

MUNKITTRICK, K. R. and LEATHERHEAD, J. F. (1983). Haematocrit values in feral goldfish, *Carassius auratus* L. as indicators of the health of the population. *Journal of Fish Biology*, *6:* 153 – 161.

NEFF, J. M. AND ANDERSON, J. W. (1981) Response of marine animals to petroleum and specific petroleum hydrocarbons. New York: John Wiley and Sons, 177 pp.

NORMAN, Y, WOO, S. and CHENG, S. (1980). Metabolic effects of starvation in the snakehead (*Ophiocephalus meculatus*). *Comparative Biochemistry and Physiology*, 67: 623 – 627.

NORRIS, D. O., CAMP, J. M., MALDONADO, J. A. and WOODLING, J. D. (2000). Some aspects of hepatic function in feral brown trout *Salmo trutta*, living in maul contaminated water. *Comparative Biochemistry and Physiology, 127:* 71 – 78.

OLUAH, N. S. (1999). Plasma aspartate amino transferase activity in the catfish *Clarias albopunctatus* exposed to sublethal zinc and mercury. *Bulletin Environmental Contamination Toxicology*, *63*: 343 - 349.

OLUAH, N. S. (2001). The effect of sublethal cadmium on the haematology of the freshwater catfish, *Clarias gariepinus* (Pisces: Clariidae). *Journal of the Science of Agriculture, Food Technology and the Environment*, *1*: 9 - 14.

OLUAH, N. S. and AMALU, C. C. (1998). The effect of heavy metals on the aminotransferase activity in the freshwater catfish *Clarias albopunctatus. Journal of Aquatic Sciences, 5:* 33 – 36.

OLUAH, N. S., EZIGBO, J. C. and ANYA, N. C. (2005). Effect of exposure to sublethal concentrations of Gammalin 20 and Actellic 25 EC on the liver and serum lactate dehydrogenase activity in the fish *Clarias albopunctatus. Animal Research International, 2(1):* 231 – 234.

OMOREGIE, E., UFODIKE, E. B. C. and ONWULIRI, C. O. (1997). Effect of water soluble fractions of crude oil on carbohydrate reserves of *Oreochromis niloticus. Journal of Aquatic Sciences,* 12: 1 – 8.

OZMEN, M., GUNGORDO, A., KUCUKBAY, F. Z. and GULER, R. E. (2005). Monitoring the effect of water pollution on *Cyprinus carpio* in Karakaya Dan Lake, Turkey. *Ecotoxicology*, *15:* 157 – 169.

SAMECKA-CYMERMAN, A. and KEMPERS, A. J. (2003). Biomonitoring of water pollution with *Elodea Canadensis*, A case study of three small Polish rivers with different levels of pollution. *Water, Air and Soil Pollution*, *145:* 139 – 601.

SCOTT, A. L. and ROGERS, W. A. (1981). Haematological effects of prolonged sublethal hypoxia on channel catfish, *Ictalurus punctatus. Journal of Fish Biology, 18:* 591 – 601.

SIMON, L. M., NEMOSOK, J. and BOROSS, L. (1983). Studies on the effect of paraquat on glycogen mobilization in the liver of common carps (*Cyprinus carpio*). *Comparative Biochemistry and Physiology C*, *75:* 167 – 169.

THIRUGNANAM, M. and FORGASH, A. T. (1977). Environmental impact of mosquito pesticide toxicity and anticholinesterase activity of choryophyrifos to fish in a salt marsh habitat. *Archives of Environmental Contamination and Toxicology*, *5:* 415 – 425.

TUVIKENE, A., HUUSKONEN, S., ROY, S. and LINDSTRÖM-SEPPÄ, P. (1996). Biomonitoring of South Estonian waters by means of xenobiotic metabolism in rainbow trout (*Oncorhynchus mykiss*) liver. *Comparative Biochemistry Physiology C, 114:* 171 – 177.

VERMA, S. R., RANI, S. and DAULA, R. C. (1981). Pesticides induced physiological alteration in certain tissues of a fish *Mytulus vittalus. Toxicological Letters, 9:* 327 – 332.

WERMA, S. R., RAMI, S. and DANOLA, R. E. (1091). Pesticide-induced physiological alteration in certain tissues of fish, *(Mylitus vitattus). Toxicological Letters, 9:* 327 – 332.

HUMAN ONCHOCERCIASIS: CURRENT EPIDEMIOLOGICAL AND DERMATOLOGICAL ASSESSMENT OF THE DISEASE IN UFUMA, NIGERIA

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ABSTRACT

Epidemiological and dermatological assessment of Onchocerciasis was carried out in Ufuma. Anambra State, southeastern Nigeria, between October and December 2005. Total of 404 consenting individuals from the nine villages in Ufuma, were systematically examined by the nodular palpation method. Community nodular prevalence of 30.9 % was recorded, indicating that Ufuma was mesoendemic for Onchocerciasis. 208 (51.5 %) individuals had various degrees of Onchocerciasis-induced skin diseases (OSD). Concomitant infections (nodules with OSD) affected 105 (26.0 %) of all OSD+ve individuals, indicating that certain subjects presented with palpable nodules did not develop any OSD. Age, gender and inter-village specific prevalence of onchocercal nodules, OSD and concomitant infections (nodules with OSD) were also reported in this study. The practice of lay nodulectomy observed in the area is a strong indication of the people's attempt to control the disease. Awareness created by this study, in Ufuma, that Onchocerciasis is a controllable medical condition, may enhance compliance with Community Directed Treatment with Ivermectin (CDTI) in the study area.

Keywords: Onchocerciasis, Onchocercoma, Onchodermatitis, Endemicity, Ufuma, Nigeria

INTRODUCTION

Onchocerciasis (river blindness) is caused by the parasitic filarial worm, *Onchocerca volvulus*, and transmitted by *Simulium damnosum* complex in different parts of the world. The disease is acclaimed to be one of the major public health problems that afflict populations in endemic areas of the world where it may cause disfiguration of the skin and blindness. About 125 million people worldwide are at risk of Onchocerciasis, of which 96 % live in Africa (WHO, 1995). The disease thrives in fertile, arable lands around rapidly flowing rivers that provide breeding sites for the insect vector (Nwoke *et. al*., 1998). Onchocerciasis was also responsible for poor academic performance and a higher drop out rate among infested children while low productivity, low income, higher health related costs and gender differences in the stigma associated with OSD were found among infested adults (Nwoke, 1990; Vlassoff *et. al*., 2000; Okolo *et. al*., 2004).

Onchocerca volvulus infection areas in Africa have been defined in terms of the savannah and forest vegetation zones. However, there are areas of Africa, sometimes called 'forest savannah mosaic' (Okonkwo *et. al*., 1991), where the savannah and forest zones merge. Ufuma in Orumba-North Local Government Area of Anambra State, southeastern Nigeria, is a typical example of such an area. Much work had been done on the prevalence and importance of OSD in Anambra and adjoining states in Eastern Nigeria (Nwaorgu *et. al*., 1994; Ahmed *et. al*, 1995; Nwoke, *et. al*., 1998; Dozie and Onwuliri, 2004; Ivoke, 2004) but the epidemio-dermatological picture of the disease in Ufuma has not been fully documented. We considered Ufuma, which was selected for this study, a potential Onchocerciasis infection focus because of the observable levels of OSD in the town. The study was therefore designed to investigate the prevalence of Onchocerciasis in Ufuma, where no published data exist. Results from this study will be useful in the surveillance of the disease in Anambra State. It may also stimulate further studies on the gender differences in the stigma associated with OSD as well as on some non-clinical signs, which have been reported in infected individuals in other areas of the country (Vlassoff *et al*., 2000; Dozie and Onwuliri, 2004).

MATERIALS AND METHODS

Study Area: This study was carried out in the nine villages in Ufuma, which is located between Latitude 6°28´– 6°32´N and Longitude 7°31´– 7°32´E in Orumba North Local Government Area of Anambra State, southeast Nigeria. The major bodies of water in the area are the perennial, well-aerated fast-flowing Mmam and Aghomiri rivers, confirmed by the natives to be the breeding sites of *Kpu-kpu* – a descriptive name for the hump backed *Simulium* fly – in the area. Luxuriant growth of bamboo (*Bambusa sp*.) along the Mmam and Aghomiri riverbanks and several geological formations on the riverbeds caused resistant rapids, providing suitable breeding sites for the *Simulium damnosum* complex, the vector of *Onchocerca volvulus*, in the area. The rivers provide water for drinking and other domestic purposes for the people. The area has two distinct climatic seasons in the year. The seven months (April-October) of wet season, with a break in July/August, is followed by

five months (November-March) of dry season when the harmattan occasionally occurred. Average annual rainfall (1620.4 m), mean daily maximum air temperature (32.2±2.2˚C), mean daily minimum air temperatures (23.3±1.1˚C), mean soil temperature (29.7±2.4˚C) and average relative humidity (84 %) have been recorded at the Mamu Forest Reserve in the area. The vegetation is typical rainforest but forest savannah mosaic had appeared around Umueji, Umuonyiba and Umuonyibauka villages, perhaps due to extensive human intervention. Farming, fishing and palm-wine tapping, are the main occupation of the people who are of Ibo ethnic origin. However, few petty traders and daily-paid job seekers shuttle to nearby commercial towns of Ekwulobia and Umunze for daily businesses. Ufuma has an important religious center where devotees travel to from different parts of the country. These devotees and non-resident indigenes of the town were not part of the study population.

Community Mobilization: The researchers, accompanied by two community-based facilitators (male and female) visited the area of study in mid-October 2005. Oral interviews with opinion leaders and some indigenes revealed the people's awareness of the existence of *Kpu-kpu* - the vernacular descriptive name for the hump-backed *Simulium* fly - in the area. The bites of *Kpu-kpu* sometimes resulted in the development of several *Kputu-kpu* or *Akpu* (nodules) under the skin of victims. Varying degrees of *Oko* (intense itching), *Agho-oko* (scratches from Aghomiri) and *Mmuma-miri* (skin rashes from Mmam water) experienced by the people were associated with the bite of *Kpu-kpu* and contact with water from Aghomiri and Mmam rivers. Some indigenes also believed that these conditions were hereditary. Incision marks observed on the body of some Onchocerciasis victims evidenced the practice of lay nodulectomy in the area. Most of the villagers interviewed were willing to accept Ivermectin treatment for the disease since they had in the past patronized medicine vendors for Banocide®. The community was therefore adequately sensitized and mobilized for the eventual study.

Dermatological Examination: The study was carried out between October and December 2005 on four hundred and four (404) consenting individuals from the nine villages of the town. Each subject supplied information on his/her experiences with Onchocerciasis. They were then systematically examined for the presence of palpable nodules, and visually for Onchocercal Skin Diseases (OSD) characterized by itching, leopard skin, hanging groin and skin atrophy. Blindness was determined by the subject's inability to count fingers at a distance of less than three meters (WHO, 1996). The findings were recorded in a standard structured format.

Data Collation and Analysis: Data were collated and stratified by age, gender and village. Prevalence of Onchocerciasis was determined on the basis of the results of the nodule palpation and observed OSD. Endemic levels were according to the classification adopted by Tada *et. al.* (1973) as follows: hyperendemic (nodular prevalence, NP ≥ 40 %), mesoendemic (NP = 20-39 %), and hypoendemic (NP ≤ 19 %). Chi-square analysis and Student's *t*-test were respectively used to compare categorical variables (e.g., gender) and continuous variables (e.g., age between groups. *P*-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The study population (n = 404) was stratified into 9 villages, gender and 6 age-groups (see Table 1). Each village provided about 45 (11.1 %) of the study population, which comprised 165 (40.2 %) males and 239 (59.2 %) females. 10-19 years age-group had the highest number of subjects 130 (32.2 %) examined, followed by 30-39 years age-group with 78 (19.3 %). Age groups ≤9 and ≥ 40 years however contributed 64 (15.8) and 82 (20.3 %) respectively. The short-fall in the number 50 (12.4 %) provided by 20-29 years age-group was thought to be due to the high rate of rural to urban drift observed in that group. Generally, there were more females than males in all age groups studied.

Table 2 shows the prevalence of onchocercal skin nodules in the study population. 125 (30.9 %) subjects were positive (+ve) for onchocercoma, indicating that at the community level Ufuma was mesoendemic for Onchocerciasis. Similar level of endemicity was reported from Oji-River areas in Enugu State (Nwaorgu *et. al.*, 1994), which has common boundaries with the study area. Inter-village nodular prevalence, however, ranged from 8.7 % in Umuonyibauka to 53.3 % in Enugwu-Abo. Going by the classification of endemicity adopted by Tada *et. al.*, (1973), only Umuonyibauka (Nodular Prevalence, NP = 8.7 %) was hypoendemic while Umuagu (NP = 42.5 %) and Enugwu-Abo (NP = 53.3 %) were hyperendemic for the disease. Umunebo (NP = 24.4 %), Umuogem (NP = 25.0 %), Umuonyiba (NP = 25.0 %), Umueji (NP = 27.2 %), Umuebu (NP = 35.3 %) and Umuinem (NP = 35.7 %) were mesoendemic for Onchocerciasis. The nearness of Enugwu-Abo and Umuagu to Oji River and their location within the rain forest vegetation which are breeding sites for *Simulium damnosum* complex, vectors of *Onchocerca volvulus*, was thought to be responsible for the hyperendemic levels recorded for the two villages. But Umuonyibauka village (in savannah forest mosaic), which was hypoendemic, is farthest from these rivers, and its inhabitants least came in contact with the blackfly. This is in line with the findings of Akogun *et al.* (1999) and Ivoke (2004).

Nodular prevalence of 30.3 % (females) and 31.4 % (males) suggested that both gender were equally exposed to the blackfly. It could be observed that 20-29 years age group had the highest nodular prevalence (50 %), flowed by 30-39 years age group with 37.2 % then 40-49 years age group with 28.1 %. These age groups 20-49 were found to be the most active segment of the community.

Table 1: Stratification of the study population

Village	Study population			≤ 9 yrs.			10-19 yrs.			20-29 yrs.			30-39 yrs.			40-49 yrs			≥ 50 yrs.		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
Enugwu-Abo	14	31	45	3	5	8	7	9	16	2	4	6	3	4	7	2	3	5	1	2	3
Umuagu	17	30	47	3	4	7	5	10	15	3	4	7	2	4	6	3	3	6	2	4	6
Umunebu	21	21	42	3	3	6	5	11	16	3	3	6	3	4	7	1	3	4	1	2	3
Umuinem	18	33	51	3	6	9	8	9	17	1	3	4	4	6	10	2	3	5	2	4	6
Umueji	20	24	44	2	4	6	5	7	12	2	3	5	3	7	10	4	2	6	2	3	5
Umuogem	19	29	48	4	4	8	7	11	13	3	2	5	4	5	9	1	3	4	3	1	4
Umuonyiba	21	19	40	3	2	5	7	3	10	3	4	7	4	7	11	2	2	4	1	2	3
Umunebo	15	26	41	3	5	8	5	7	12	2	3	5	4	4	8	2	2	4	1	3	4
Umuonyibauka	20	26	46	2	5	7	6	8	14	2	3	5	3	7	10	1	3	4	2	4	6
Total	**165**	**239**	**404**	**26**	**38**	**64**	**55**	**75**	**130**	**21**	**29**	**50**	**30**	**48**	**78**	**18**	**24**	**42**	**15**	**25**	**40**

M = Male. F = Female, T = Total (M + F).

Table 2: Community specific prevalence of onchocercal nodules in the study population

Village	Study population						Onchocerca nodules +ve					
	Total examined		***Male examined***		***Female examined***		***Total +ve***		***Male +ve***		***Female +ve***	
	no.	**%**	**no.**	**%**	**no.**	**%**	**no.**	**%**	**no.**	**%**	**no.**	**%**
Enugwu-Abo	45	11.1	14	31.1	31	68.9	24	53.3	9	64.3	15	48.4
Umuagu	47	11.6	17	36.2	30	63.6	20	42.5	7	41.2	13	43.0
Umunebu	51	10.9	18	35.3	33	64.7	18	35.3	8	44.4	10	30.3
Umuinem	42	12.7	21	50.0	21	50.0	15	35.7	6	28.6	9	42.9
Umueji	44	10.1	20	45.5	24	54.5	12	27.2	5	25.0	7	29.2
Umuogem	48	10.4	19	39.6	29	60.4	12	25.0	6	66.7	6	20.7
Umuonyiba	40	11.9	21	52.5	19	47.5	10	25.0	3	14.3	7	36.9
Umunebo	41	9.9	15	36.6	26	63.4	10	24.4	4	26.7	6	30.1
Umuonyibauka	46	11.4	20	43.5	26	56.5	4	8.7	2	10.0	2	7.7
Total	**404**	**100.0**	**165**	**40.8**	**239**	**59.2**	**125**	**30.9**	**50**	**30.3**	**75**	**31.4**
Age *(Years*												
≤ 9	64	15.8	26	40.6	38	59.4	9	14.1	3	11.5	6	15.7
10-19	130	32.2	55	42.3	75	57.7	33	25.4	13	23.6	20	28.6
20-29	50	12.4	21	42.0	29	58.0	25	50.0	10	47.6	15	51.7
30-39	78	19.3	30	38.5	48	61.5	29	37.2	12	40.0	17	35.4
40-49	42	10.4	18	42.9	24	57.1	16	28.1	7	38.8	9	32.5
≥ 50	40	9.9	15	37.5	25	62.5	13	32.5	5	33.3	8	32.0
Total	**404**	**100.0**	**165**	**40.8**	**239**	**59.2**	**125**	**30.9**	**50**	**30.3**	**75**	**31.4**

Females engaged more with farming, fetching of water from the streams and wood gathering while the males were engaged in fishing, palm-wine tapping and extensive land preparation for cultivation. These activities ensure prolonged exposure and therefore the rate of vector-contact in the area. Nodular prevalence of 14.1 % recorded in infants less than 9 years old was not un-expected because, in the absence of house-helps, they frequently accompanied their mothers to the streams and farms, making vector-contact in the process. Subjects over 50 years old also had 32.5 % nodular prevalence, an indication that Onchocerciasis is a cumulative disease.

Prevalence of onchocercal skin diseases (OSDs) in the study population, as well as in individuals with concomitant infections (onchocercal nodules with OSDs) is shown in Table 3. Total of 208 subjects were affected by OSDs, giving a prevalence of 51.5 % at the community level, but the endemicity of OSDs appeared to follow the same pattern as that obtained for ochocercal nodules by the nodular palpation technique. Inter-village OSD +ve prevalence ranged from 23.9 % in Umuonyibauka to 91.1 % Enugwu-Abo village, indicating that 6 villages in Ufuma were hyperendemic while 3 were mesoendemic for OSDs. There was no significant difference between 52.7 % prevalence of OSDs in males and 50.6 % observed in females ($P > 0.05$). However, the highest prevalence of OSDs was recorded in subjects between 20 to 40 years old, in line with the findings of Ahmed (1995). 105 (50.5 %) of all 208 OSD+ve subjects had concomitant infections of onchocercal nodules with OSDs, which was widespread in the community. Ranging from 36.4 % in Umuonyibauka to 64.7 % in Umuebu. Community nodule with OSD+ve prevalence was 26.0 %, indicating that certain individuals presenting with onchocercal nodules did not develop any OSD. Hence the community prevalence of OSDs (51.1 %) > Nodular prevalence (30.9 %) > Nodule with OSD (26.0 %).

The prevalence of OSDs among gender, age-groups in the villages are shown in Table 4. At the community level, the study population presented varying degrees of Onchocerciasis-induced dermatological changes and blindness. The prevalence of OSDs at the community level was 37.4 % for Itching with rashes, 10.9 % (Leopard skin), 1.7 % (Skin atrophy), 0.5 % (Hanging groin) and 1.0 % (Blindness).

Itching with rashes was observed in all age groups but appeared to increase with age, peaking at 20 to 29 years age-group.

Table 3: Prevalence of concomitant infections (Nodules with OSD*) in the study population

Village	Study population	Nodule +ve		Community nodule prevalence *(n=404)*	OSD +ve		Community OSD +ve prevalence *(n=404)*	Nodule with OSD +ve		Community Nodule with OSD +ve prevalence *(n=404)*
	no.	no.	%	%	no.	%	%	no.	%	%
Enugwu-Abo	45	24	53.3	5.9	41	91.1	10.2	23	56.1	5.7
Umuagu	47	20	42.5	4.9	34	72.3	8.4	16	47.1	4.0
Umunebu	51	18	35.3	2.9	17	38.6	4.2	11	64.7	2.7
Umuinem	42	15	35.7	4.5	26	51.0	6.4	13	50.0	3.2
Umueji	44	12	27.2	3.7	25	59.5	6.2	12	48.0	3.0
Umuogem	48	12	25.0	2.5	18	43.9	4.5	9	50.0	2.2
Umuonyiba	40	10	25.0	2.9	21	43.6	5.2	10	47.6	2.5
Umunebo	41	10	24.4	2.5	15	37.5	3.7	7	46.7	1.7
Umuonyibauka	46	4	8.7	0.9	11	23.9	2.7	4	36.4	1.0
Total	**404**	**125**	**30.9**	**30.9**	**208**	**51.5**	**51.5**	**105**	**50.5**	**26.0**
Gender										
Male	165	50	30.3	12.4	87	52.7	21.5	42	48.3	10.4
Female	239	75	31.4	18.5	121	50.6	30.0	63	52.1	15.6
Total	**404**	**125**	**30.9**	**30.9**	**208**	**51.5**	**51.5**	**105**	**50.5**	**26.0**
Age years)										
≤ 9	64	9	14.1	2.2	17	26.6	4.2	11	64.7	2.7
10-19	130	33	25.4	8.2	62	47.7	15.3	27	43.5	6.7
20-29	50	25	50.0	6.2	41	82.0	10.2	22	53.6	5.4
30-39	78	29	37.2	7.2	50	64.1	12.4	27	54.0	6.7
40-49	42	16	28.1	3.9	22	52.4	5.4	10	43.5	2.5
≥ 50	40	13	32.5	3.2	16	40.0	4.0	8	50.0	2.0
Total	**404**	**125**	**30.9**	**30.9**	**208**	**51.5**	**51.5**	**105**	**50.5**	**26.0**

**Certain subjects presented with more than one OSD*

Table 4: Community prevalence of Onchocercal Skin Diseases (OSDs*) in the study population

Village	Study population	Itching with rashes		Leopard skin		Skin atrophy		Hanging groin		Blindness	
	no.	no.	%	no.	%	no.	%	no.	%	no.	%
Enugwu-Abo	45	25	55.6	10	22.2	3	6.6	1	2.2	2	4.4
Umuagu	47	22	46.8	7	14.9	2	4.2	1	2.1	2	4.2
Umunebu	51	19	37.3	5	11.9	1	2.4	0	0.0	0	0.0
Umuinem	42	20	47.6	6	11.7	0	0.0	0	0.0	0	0.0
Umueji	44	14	31.8	3	6.8	0	0.0	0	0.0	0	0.0
Umuogem	48	16	33.3	4	8.4	1	0.0	0	0.0	0	0.0
Umuonyiba	40	11	27.5	4	10.0	0	0.0	0	0.0	0	0.0
Umunebo	41	15	36.6	3	7.3	0	0.0	0	0.0	0	0.0
Umuonyibauka	46	9	19.6	2	4.3	0	0.0	0	0.0	0	0.0
Total	**404**	**151**	**37.4**	**44**	**10.9**	**7**	**1.7**	**2**	**0.5**	**4**	**1.0**
Gender											
Male	165	63	38.2	18	10.9	3	1.8	1	0.6	2	1.2
Female	239	88	36.8	26	10.9	4	1.7	1	0.4	2	0.8
Total	**404**	**151**	**37.4**	**44**	**10.9**	**7**	**1.7**	**2**	**0.5**	**4**	**1.0**
Age years											
≤ 9	64	17	26.6	0	0	0	0.0	0	0.0	0	0.0
10-19	130	53	40.7	9	6.9	0	0.0	0	0.0	0	0.0
20-29	50	27	54	10	20	4	8.0	0	0.0	0	0.0
30-39	78	31	39.7	17	21.8	2	2.6	0	0.0	0	0.0
40-49	42	13	30.9	5	11.9	1	2.4	1	2.4	2	4.8
≥ 50	40	10	25.0	3	7.5	0	0	1	2.5	2	5.0
Total	**404**	**151**	**37.4**	**44**	**10.9**	**7**	**1.7**	**2**	**0.5**	**4**	**1.0**

**Certain subjects presented with more than one OSD*

This observation confirms the report (Ivoke, 2004) that onchocercal cutaneous changes were more prevalent in the younger age groups. There was no significant difference between the prevalence of itching with rashes, 36.8 % in females and 38.1 % in males ($P > 0.05$). The use of Banocide® (Di-ethyl-carbamazine, DEC) in the area contributed to the development of itches in affected individuals. The drug usually killed onchocercal microfilariae (mf), whose antigenic substances were suspected to trigger

off hypersensitive reactions that manifested in itching and development of skin rashes. Leopard skin was not reported in subjects under 9 years old. 30-39 years age-group, with 21.8 % prevalence, was mostly affected, followed by 20-29 years age-group with 20.0 %. This result was in line with the findings of Edungbola (1987) who reported the leopard skin only in patients over the age of 20 years. Generally, more females 26 (10.9 %) than males (18 (10.9 %) were affected by leopard skin lesion but this difference was not significant ($P > 0.05$). Skin atrophy was not investigated in subjects over the age of 50 years in order not to confuse it with senile atrophy, which may occur in subjects that were advanced in age. Hanging groins and blindness showed no gender preference in the study population but occurred in ≥ 50 years old individuals. Low prevalence of blindness in the area may be as a result of the use of Banocide® by the people. The drug, which caused the death of onchocercal micofilariae, prevented them from migrating to the eye balls where their eventual death produced sclerosing keratitis – a hardening inflammation of the cornea – the cause of blindness (Pearlman, 1996). The prevalence of senile atrophy, hanging groins and blindness were too low to be considered of diagnostic value but they are important because if Onchocerciasis is not treated in time, it proves to be a long-term, disfiguring and blinding disease.

The practice of lay nodulectomy (also reported from an endemic area in Abia State by Abanobi *et al.*, 1999) and the use of Banocide® observed in the study area were evidences of the people's attempt at treating Onchocerciasis. The awareness created by this study in the community, that Onchocerciasis is a controllable medical condition, may enhance compliance with any Community Directed Treatment with Ivermectin (CDTI) in the study area. This study has established the endemicity of Onchocerciasis in Ufuma and confirms earlier reports that nodular palpation method was of great value in Community Assessment of human onchocercal endemicity (Nwoke, 1998; Nock *et. al.*, 1998; Uzoegwu *et al.*, 2004).

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REFERENCES

ABANOBI, O. C., EDUNGBOLA, L. D., OBIRI, G. and NWOKE, B. E. B. (1999). Onchocercal nodules Prevalence and the practice of lay nodulectomy in Mballa, Isuochi, Abia State, Nigeria. *Nigerian Journal of Parasitology, 20:* 45 – 50.

AHMED, K., ENEANYA, C. I., AKPA, A. U. C., AMAZIGO, U. and REMME, J. H. F. (1995). The Importance of Onchocercal Skin Diseases: The report of a Multi-country study by the Pan-African Study Group on Onchocercal Skin Disease. *UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. TDR/AFR/RP/95.1 Applied Field Research Reports No. 1.*

AKOGUN, L. B and ONWULIRI, C. O. (1991). Hyperendemic Onchocerciasis in the Taraba River Valley of Gongola State (old Adamawa Province), Nigeria. *Annals of Parasitology and Human Comparative, 6(1):* 22 – 26.

EDUNGBOLA, L. D., WATTS, S. J. and KAYOLE, O. O. (1987). Endemicity of striking manifestation of Onchocerciasis in Shao, Kwara State, Nigeria. *African Journal of Medical Sciences, 16:* 147 – 156.

DOZIE, I. N. S and ONWULIRI, C. O. E. (2004). Onchocerciasis in Imo State, Nigeria. Prevalence of some Non-classical Signs and Symptoms. *Bio-Research*, *2(2):* 42 – 45.

IVOKE, N. (2004). An Epidemio-Dermatological Assessment of Onchocercal Skin Diseases in Awhum, Enugu State, Nigeria. *Bio-Research*. *2(2):* 46 – 53.

NOCK, H., RIPIYE, P. and GALADIMA, M. (1998). Diagnostic Value of Nodules and Leopard Skin in Community Assessment of Human Onchocercal Endemicity. *Nigerian Journal of Parasitology*, *19:* 19 – 24.

NWAORGU, O. C., OHAEGBULA, A., ONWELUZO, I. E., ALO, E. T., NWEKE, L. N., AGU, V. L. and EMEH, E. (1994). Results of a Large-scale Onchocerciasis Survey in Enugu State, Nigeria. *Journal of Helminthology*, *68(2):* 155 – 159.

NWOKE, B. E. B. (1990). The Socio-economic Aspects of Human Onchocerciasis in Africa: The present appraisal. *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, *34(1):* 37 – 44.

NWOKE, B. E. B., DOZIE, I. N. S., GEMADE, E. I. I. and JIYA, J. Y. (1998). The present Status of Human Onchocerciasis in Southern Nigeria using Rapid Epidemiological Mapping of Onchocerciasis (REMO). *Nigerian Journal of Parasitology, 19:* 11 – 18.

OKOLO, C. G., DALLAH, C. N. and OKONKWO, P. O. (2004). Clinical Manifestations of Onchocerciasis and Some Aspects of its Control in Achi Oji River Local Government Area. Enugu State, Nigeria. *Nigerian Journal of Parasitology*, *25:* 101 – 106.

OKONKWO, P., AKPA, A., IHEKWABA, A., NWAGBO, D., UMEH, R., ADIBUA, S., EZIKE, V. and OGBUOKIRI, I. (1991). Studies on Onchocerciasis in forest-savannah mosaic areas of Nigeria, 1: Investigations in Gbaraga, Oji River. *Annals of Tropical Medicine and Parasitology*, 85: 617 – 623.

PEARLMAN, E. (1996). Experimental Onchocercal Keratitis. *Parasitology Today,* 12: 261 – 267.

TADA, I., IWAMOTO, T. and WONDE, T. (1973). Quantitative Studies on the Emergency of *Onchocerca volvulus* microfilariae from the skin. *Japan Journal of Tropical Medicine and Hygiene*, *1:* 13 – 24.

UZOEGWU, P. N. and ALOH, G. (2004). Comparison of Clinical, Parasitological and Serological Diagnostic Methods for the definitive diagnosis of Onchocerciasis in Nsukka Senatorial Zone. *Animal Research International*, *1(3):* 181 – 189.

VLASSOF, C., WEISS, M., OVUGA, E. D. L., ENEANYA, C. I., NWEL, P. T., BABALOLA, S. S., AWEDOBA, A. K., THEOPHILUS, B., COFIE, P. and SHETAB, P. (2000). Gender differences in the Stigma Associated with Onchocercal Skin Disease (OSD) in five African sites. *Social Science and Medicine, 50:* 1353 – 1368.

WHO (1995). Onchocerciasis and its Control. Word Health Organization Expert Committee on Onchocerciasis Control Geneva: *World Health Technical Report Series*. No. 852.

WHO (1996). Word Health Organization Expert committee on Onchocerciasis Second Report. *World Health Organization Lecture Report Series*. No. 334.

A NEW AND SIMPLE METHOD OF CONFIRMATORY DETECTION OF MATING IN ALBINO RATS (*Rattus norvegicus*)

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ABSTRACT

A new and simple method of detecting mated female albino rats was developed and tested for precision and accuracy. The method involved the gross observation of grey to yellowish protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides. Results of test of the new method showed that the mean length of time between observation of the protein coagulates on vaginal smears and delivery of the mated pregnant females was 21.39 ± 0.11 days (range = 20 – 23 days, n = 58), which concurred with the normal 21-day gestation length of rats. The coefficient of variation (CV) of imprecision of the new method was found to be 3.74 %. This new method is simple, easy to apply and does not interfere with fertilization and pregnancy, and also does not involve either the use of specially designed rat cages or microscopy of vaginal smears, which were the constraints of the former methods of confirming mating in rats.

Keywords: Albino rats, Detection of mating, New method, Vaginal smears

INTRODUCTION

Laboratory animal experimentation is an important tool for the investigation and understanding of various biological principles and study of human and animal disease mechanisms. It forms the backbone of biomedical, bio-agricultural and bio-industrial researches that enable the development of new medical and veterinary pharmaceuticals, vaccines, surgical materials and diagnostic techniques, and also the investigation of experimental diseases as models of most human and animal diseases (CCAC, 1984; NIH, 1999a; Gallagher, 2003). The rat is the most widely studied experimental animal because the rat model possesses enormous strengths and versatility of application that have made it the most appropriate and almost indispensable animal model for the study of human biological mechanisms and diseases (NIH, 1999b; Gallagher, 2003). Presently rats comprise more than 28 % of laboratory animals and provide important animal models for almost every aspect of biomedical and behavioural research, including reproductive physiology and behaviour, repro-toxicology and reproductive diseases (CCAC, 1984; NIH, 1999b).

Rats are the most preferred experimental animals for reproductive studies because of numerous reasons, which include their short gestation length (20 – 22 days), short oestrous cycle (4 – 5 days), litter size of about 7 – 9, weaning age of about 21 days and a relative short period/age (7 – 8 weeks) of sexual maturity (Hafez, 1970; Baker *et al.,* 1980). In addition, rat pregnancies are more size consistent (compared to mouse), rat cycling is relatively non-pheromonal (similar to humans), rats can be bred quickly after parturition, and rat brains show early sexual dimorphism (NIH, 1999b).

In reproductive studies, it is usually necessary to precisely and accurately date and time the sex act in order to estimate various pregnancy and birth expectations and also to know the number of matings that occur before pregnancy results. Mating is not always easy to judge, and it is not always practical to observe the copulatory act, which usually may be nocturnal (Hafez, 1970; Inglis, 1980). Routine confirmation of mating in the rat is therefore usually made by checking for the presence of spermatozoa in a vaginal lavage or by visualization of the copulatory plug (Bennett and Vickery, 1970; Berthelot, 1981). Checking of spermatozoa in vaginal lavage is a technical and time consuming procedure involving microscopy, while visualization of the copulatory plug can only be carried out using specially designed single-rat cages that permit the copulatory plug to fall through the floor mesh on to a tray beneath – in some cases the plug may for a variety of reasons not be found (Bennett and Vickery, 1970; Mathews and Adler, 1978; Inglis, 1980; Berthelot, 1981). In the present study, we describe a method of confirming mating in rats by grossly visualizing remnants of the copulatory plug on vaginal smears made on glass slides, without using specially designed cages or microscopy procedures. The development of this new method was based on the

fact that the copulatory plug is a coagulated mass of proteins (Mathews and Adler, 1978; Voss, 1979; Seitz and Aumuller, 1990; Carballada and Esponda, 1993), and that even the smallest remains can be picked up by a vaginal swab and be grossly visualised on clean glass slides.

MATERIALS AND METHODS

The experimental rats used for the study were the out-bred strain of the Sprague-Dawley (SD) albino rats. Eighty two (82) matured SD rats comprising of 70 females of 12 - 14 weeks of age and 12 males of 14 - 16 weeks of age, procured from and maintained in the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The rats weighed 150 – 170 g at the time of commencement of the study. They were kept in standard clean rat cages, fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited, Sapele) containing 16 % crude protein, and supplied with clean drinking water all through the study.

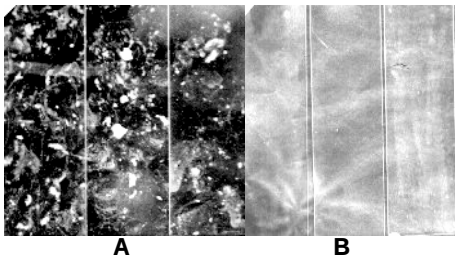
The seventy (70) female rats were randomly distributed into 14 cages such that each cage contained 5 female rats. Each of the rats was identified with an indelible marker. After the distribution and identification of the female rats, a vaginal smear of each of them was made on a labelled clean glass slide. The smear was collected by carefully inserting a cotton-tipped swab moistened with normal saline into the vaginal cavity of the rats. The swabs used measured about 7.5 cm in length with the cotton-tipped end of 1.5 cm circumference, almost the same dimensions as the typical “cotton buds’ used in cleaning the inside of the ear in humans. The swabs were applied gently against the vaginal wall and rolled around carefully before being withdrawn. Immediately after withdrawal from the vaginal cavity, the moist swab was rolled / smeared onto a labelled clean glass slide. The smear was observed grossly to check for the presence of protein coagulates (remnants of the copulatory plug).

After the initial smears were collected from the female rats, the 12 male rats were randomly distributed into 12 out of the 14 cages, such that the male:female ratio in these 12 cages was 1:5. No males were introduced into the two remaining cages that contained 10 females that served as control. After the introduction of males into the 12 mating cages, vaginal smears were made as described above for each of the females in all the 14 cages twice a day (6.30 am in the morning and 6.30 pm in the evening), and the smeared slides were observed grossly for protein coagulates. The observation of grossly visible protein coagulates on the vaginal smear of each female was recorded as evidence of mating. Pregnancy in the mated females was followed up individually in the cages such that already mated females could be re-mated if the initial mating did not result in pregnancy. Once the protein coagulates were observed on the vaginal smear of each rat, the rat was thereafter weighed at four-day intervals to check the progress of the pregnancy. Obviously pregnant ones were removed from the mating cages at the last trimester of pregnancy when their abdomen was found to be conspicuously enlarged; they were kept in single-rat nursing cages for delivery. Pregnant females kept in nursing cages were observed twice daily to record the date of delivery. For each rat, the day that the protein coagulates were found on their vaginal smear (day of mating) was regarded as day 1 of pregnancy and the day of delivery was taken as the last day of gestation. The number of matings that resulted in pregnancy was also recorded for each female rat.

Data generated from the study were presented as frequency tables along with means with standard deviations. The mean body weight of the mated-pregnant rats was compared with that of the unmated-controls using students t - test. The precision and accuracy of the procedures used was determined by computing the coefficient of variation (CV) of imprecision around the mean gestation period.

RESULTS

Gross observation of the initial vaginal smears made before introducing the males into the mating cages showed no protein coagulates, but smears made from females in the mating cages showed the presence of grey to yellowish protein coagulates (Figure 1) in about 18 out of the 60 females exposed to males within the first 24 hours of introducing males into the mating cages.



A **B**

Figure 1: Protein coagulates observed on slides on which vaginal smears of mated rats were made (3 slides on the left labelled A); compare with the clear slides with no protein coagulates on the right (3 slides labelled B) on which vaginal smears of unmated control rats that were not exposed to males were made

By the second day of exposure to males, 15 more females showed protein coagulates on their vaginal smears. The observation of protein coagulates on the vaginal smears of females exposed to males continued, and by day 5 of exposure to males, protein coagulates had been observed in the vaginal smear of all the 60 females that were exposed to males. All through the study period, the observation of these protein coagulates in vaginal smears made from females exposed to males was found to be consistent, and this was never observed in the

vaginal smears of the 10 control rats which were not exposed to males. The number of times these protein coagulates were observed on the vaginal smears of individual female rats exposed to males (number of mating before pregnancy) ranged from once to three times with a mean of 1.21 ± 0.45 times (Table 1).

Table 1: Frequency table showing the number of times protein coagulates were observed on the vaginal smear of female rats exposed to males (number of mating before pregnancy resulted)

No. of times that protein coagulates were observed on vaginal smears	Number of females
1	47
2	10
3	1

Mean = 1.21; Standard deviation = 0.45; Mode = 1; Median = 1; n = 58

Out of the 60 female rats exposed to males, 58 (96.7 %) became pregnant and delivered, while 2 (3.3 %) had pseudo-pregnancy. None of the control rats that were not exposed to males showed any signs of pregnancy, pseudo-pregnancy or delivery. The time period between the last observation of protein coagulates on the vaginal smears of those that became pregnant and the day of delivery (gestation period) ranged from 20 – 23 days with a mean of 21.39 ± 0.80 days (Table 2). The mode and median of the gestation lengths were both 21 days respectively.

Table 2: Frequency table showing the period of time between grossly observing protein coagulates on vaginal smears and the day of delivery (gestation period) of the female rats exposed to males

Time period (days)	No. of females
20	6
21	29
22	18
23	5

Mean = 21.39 days; Standard deviation = 0.80; Mode = 21 days; Median = 21; n = 58

In assessing the precision and accuracy of this method of detecting mating, the CV of imprecision computed around the mean gestation period was calculated to be 3.74 % for the 58 females that got pregnant and delivered.

The mean body weights of the mated-pregnant female rats were found to be significantly higher ($P < 0.01$) than that of the unmated controls from day 8 post-observation of protein coagulates on vaginal smear (post-mating) and was consistently significantly higher ($P < 0.01$) until delivery (Figure 2). No significant difference ($P > 0.05$) was observed between the mean body weights of the mated and unmated controls during the first four days post-observation of protein coagulates.

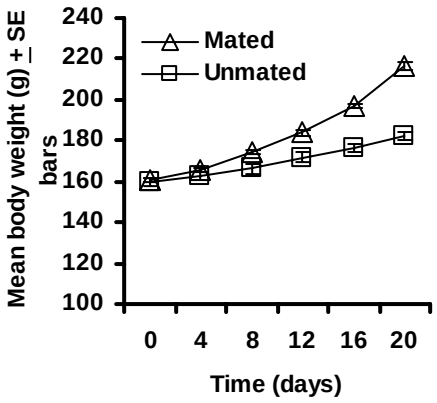


Figure 2: Comparison of the body weights of the mated and unnmated rats

DISCUSSION

The copulatory plug, also known as the vaginal plug, is a white or grey to yellowish waxy coagulated mass of proteins which is usually deposited by males in the female reproductive tract at mating in some mammalian species including rats; it is formed by the secretions of the male accessory sex gland (Bennett and Vickery, 1970; Seitz and Aumuller, 1990; Carballada and Esponda, 1993; Ramm *et al.,* 2005). The plug had been reported to function in the "enforcement of chastity" (Voss, 1979) and stimulation of sperm transport to the uterus (Matthew and Adler, 1978; Carballada and Esponda, 1992). The plug usually fills the vagina from the vulva to the cervix, but soon after deposition shrinks and falls out and can be observed when rats are kept in specially designed cages that allow the plug to fall off without being soiled by faeces (Bennett and Vickery, 1970; Inglis, 1980; Berthelot, 1981). The present study had shown that remnants of this copulatory plug could be grossly observed on smears made from vaginal swabs of mated female rats. The mean time period of 21.39 days (range of 20 - 23 days) between the observation of the protein coagulates on vaginal smears and delivery of the pregnant rats concurred with the normal 21-day gestation period (range of 20 – 22 days) of albino rats (Hafez 1970; Inglis, 1980). The observation of the protein coagulates on vaginal smears of mated female rats in this study is a confirmatory indicator of mating. This method of detecting mating could also be said to be precise and accurate with the CV of imprecision being 3.74 % (less than 5 %).

The collection of vaginal swabs for making the smears did not interfere with fertilization and pregnancy as indicated by the fact that 96.7 % (58 out of 60) of the mated females from whom swabs were collected became pregnant and delivered their offspring.

Two out of the 60 mated females (3.3 %) exhibited pseudo-pregnancy – a post-mating state in which female rats exhibit signs and endocrine

changes associated with pregnancy when in the actual sense they are not pregnant (Kovacic, 1970; Inglis, 1980). Pseudo-pregnancy in rats is caused by infertile mating following insufficient intromissions that prolong the life / action of the corpus luteum and thus the diestrus period up to 13 days (Wilson *et al.,* 1965; Bennett and Vickery, 1970; Renfree, 1994).

The finding in this study of a significant increase in the body weight of the mated-pregnant rats when compared to the unmated controls from day 8 post-mating validates earlier claims that monitoring body weight changes could be used to detect pregnancy in these species (Hendricks and Houston, 1970; Inglis, 1980; Grant, 2006).

In conclusion, it can be stated that this new method of detecting mating by observing protein coagulates on vaginal smears made on glass slides is simple, precise and accurate. It overcomes the constraints of the former methods of confirming mating as it neither involves the use of specially designed cages nor does it require microscopy of vaginal smears.

REFERENCES

BAKER, H. J., LINDSEY, T. R. and WEISBROTH, S. H. (1980). *The Laboratory Rat.* Volume 2, Research Applications. Academic Press, New York.

BENNETT, J. P. and VICKERY, B. H. (1970). Rats and mice – copulation and mating behaviour. Pages 302 –303. *In:* HAFEZ, E. S. E. (Ed.). *Reproduction and Breeding Techniques for Laboratory Animals.* Lea and Febiger, Philadelphia.

BERTHELOT, J. Y. (1981). Mating methods to produce accurate timed pregnancy in rats. *Laboratory Animal Science, 31:* 180 –186.

CARBALLADA, R. and ESPONDA, P. (1992). Role of fluid from seminal vesicles and coagulating glands in sperm transport into the uterus and fertility in rats. *Journal of Reproduction and Fertility, 95:* 639 – 648.

CARBALLADA, R. and ESPONDA, P (1993). Structure of the vaginal plugs generated by normal rats and rats with partially removed vesicles. *Journal of Experimental Zoology, 265:* 61 – 68.

CCAC (1984). Research Animals in Canada – Laboratory Rats. Volume 2. Canadian Council for Animal Care (CCAC), Ottawa, Ontario.

GALLAGHER, R. (2003). Animal research is for human welfare. *The Scientist, 17:* 1- 3.

GRANT, K. (2006). Rat Breeding Guide – The Layman's Guide to Breeding Pet Rats. Rat and Mouse Club of America (RMCA), Florida. http://ratguide.com/breeding/pregnancy/determiningpregnanacy.php. Accessed on April 6, 2006.

HAFEZ, E. S. E. (1970). *Reproduction and Breeding Techniques for Laboratory Animals*. Lea and Febiger, Philadelphia.

HENDRICKS, A. G. and HOUSTON, M L. (1970). Gestation and prenatal development. Pages 157 – 176. *In:* HAFEZ, E. S. E. (Ed.). *Reproduction and Breeding Techniques for Laboratory Animals*. Lea and Febiger, Philadelphia.

INGLIS, J. K. (1980). Reproduction, breeding and heredity. Pages 156 – 234. In: INGLIS, J. K. (Ed.) *Introduction to Laboratory Animal Science and Technology.* Pergamon Press, Oxford.

KOVACIC, N. M. I. (1970). Endocrinology of reproduction. Pages 5 – 27. *In:* HAFEZ, E. S. E. (Ed.). *Reproduction and Breeding Techniques for Laboratory Animals.* Lea and Febiger, Philadelphia.

MATTHEWS, M. K. and ADLER, N. T. (1978). Systematic inter-relationship of mating, vaginal plug position, and sperm transport in rats. *Physiology of Behaviour, 20:* 303 – 309.

NIH (1999a). *Report of the National Institutes of Health Model Organism Database Workshop.* Dec. 7 – 8, 1998. National Institutes of Health (NIH), USA.

NIH (1999b). *Report of the National Institutes of Health Rat Model Priority Meeting*. May 3, 1999. National Institutes of Health (NIH), U.S.A.

RAMM, S. A., PARKER, G. A. and STOCKLEY, P. (2005). Sperm competition and the evolution of male reproductive anatomy of rodents. Proceedings of the Royal Society B, 272: 949 – 955.

RENFREE, M. (1994). Endocrinology of pregnancy, parturition and lactation. Pages 677 – 766. In: LAMMING, G. E. (Ed), Marshall's Physiology of Reproduction, 4th ed., Volume 3. Longmans, London.

SEITZ, J. and AUMULLER, G. (1990). Biochemical properties of secretory proteins from rat seminal vesicles. *Andrologia, 22(Supplement 1):* 25 – 32.

VOSS R. S. (1979). Male accessory glands and the evolution of copulatory plug in rodents. *Occasional Papers of the Museums of Zoology, University of Michigan, 689:* 1 – 27.

WILSON, J. R., ADLER, N. and LEBOEUF, B (1965). The effects of intromission frequency on successful pregnancy in the female rat. *Proceedings of the National Academy of Sciences, 35:* 1392 – 1395.

EFFECT OF CRUDE OIL AND ITS PRODUCTS ON BILIRUBIN OF AFRICAN CATFISH *Clarias gariepinus*

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ABSTRACT

The study was aimed at determining the effect of Crude oil and its product on bilirubin of a Catfish. Fishes with an average weight of 21.08 ± 0.12g were subjected to the toxic and recovery phases of different concentrations (0.2 ml/L, 0.4 ml/L, 2.0 ml/L. and 4 ml/L) of Crude oil, diesel, kerosene and petrol for 4 days and 26 days respectively. The bilirubin level of fishes subjected to different concentrations of toxicants was higher than that of the control. The biochemical parameters, investigated showed significant (P < O.05) difference when compared to the control. The bilirubin level increased with increasing concentrations of toxicants. Increased bilirubin level suggests liver cell damage or a metabolic disturbance in the liver involving defective conjugation and / or excretion of bilirubin.

Keywords: Crude oil concentration, *Clarias gariepinus*, Bilirubin, Toxicity, Recovery

INTRODUCTION

Fish kills caused by spills of light oils and petroleum products was observed in contaminated areas such as lakes lagoons and some shallow water (Baker, 1969). *Clarias gariepinus*, an omnivore fresh water fish is a popular delicacy relished throughout tropical Africa. Their hardy nature and the possession of accessory air-breathing organs enable them tolerate adverse aquatic conditional (Reed *et al.,* 1967)

Crude oil and its products can cause damage to aquatic ecosystem in a number of ways. Oxygen is not soluble in oil and therefore does not easily pass through even when a thin of oil is present on water. Oil can therefore limit the amount of oxygen that enters into a body of water from the atmosphere. Crude oil and its products may also contain water soluble fractions that are toxic either directly or through the metabolic pathways to aquatic organisms and fish. Light oil are known to be more toxic than heavy oil (Tatem *et al.,* 1979). Clerk (1987) proposed that freshly spilled oil was more toxic than weathered oil, which must have lost volatile fractions. This is because lighter and freshly spilled oil contain low boiling fractions which are the major constituents of crude oil.

Bilirubin is a by product of haemoglobin degradation. Crude oil exposures of adult marine fish species have been reported to increase the mortality rate and changes in the haemoglobin content of blood (Tatem et al, 1979). Rise in serum level of total bilirubin can be due to liver cell damage or a metabolic disturbance in the liver and increased destruction of red blood cells (Arthur et al, 1986). This study, therefore, considered the problem arising from exposing *Clarias gariepinus* to crude oil and its product and its concomitant effect on bilirubin metabolism.

MATERIALS AND METHODS

Experimental Design: The experiment was conducted at the Enugu State University of Science and Technology (ESUT) Research Laboratory Enugu, Nigeria. 150 Juvenile fishes of Clarias gariepinus (Mean weight 21.08 ± 0.12g) were transported from a private fish hatchery at Otor-oweh in Delta State, Nigeria, in two plastic containers (90L) to fisheries Laboratory of ESUT. The fishes were acclimatized in the fisheries Laboratory for 14 days and were maintained on 38 % crude protein diet at 3 % body weight daily. 102 juvenile fishes were subjected to different concentrations (0.2 ml/L, 0.4 ml/L 2.00 ml/L, and 4.00 ml/L) of crude oil, petrol, kerosene and diesel. Each of these four oil samples were introduced in triplicates to 48 plastic containers (90L) at the concentrations stated above. The control experiment (0.00 ml/L) consisted of three (3) plastics containers (90L) without any oil treatment.

The fishes were randomly stocked in a Completely Randomized Block Design (CRBD) in 51 plastic containers (90 L) at two fish per container. Each container was filled to 10L mark with declorinated tap water. The fishes in each set up were left for 4 days in different concentrations of crude oil and products respectively for the toxic phase while in the recovery phase, the respective fishes exposed to the toxicants for 4 days were put in fresh water and left for the next 26 days. Blood samples were collected from the respective fishes in the toxic phase and recovery phase for the analysis of bilirubin (Powel, 1994).

Statistical Analysis: Data collected were subjected to analysis of variance (ANOVA). Differences in the means of treatment at recovery and toxicity phases were compared. The means were separated using New Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

In the toxic phase, fishes treated with the toxicants had higher total bilirubin concentration than the control (Table 1).

Table 1: Effects of exposure of *C. gariepinus* juveniles to crude oil and its products on bilirubin level (mg/100ml)

Concentration of toxicant (ml/l)	Toxicity	Recovery
	Control	
0.00	$\pm$ 0.01^{a}	0.01 $\pm$ 0.01^{a}
	Kerosene	
4.00	4.25 $\pm$ 0.56^{f}	1.19 $\pm$ 0.08^{ed}
2.00	0.43 $\pm$ 0.01^{b}	0.34 $\pm$ 0.01^{ab}
0.40	2.45 $\pm$ 0.02^{d}	-
0.20	0.68 $\pm$ 0.10bc	-
	Diesel	
4.00	0.17 $\pm$ 0.05^{ab}	0.51 $\pm$ 0.3^{b}
2.00	0.85 $\pm$ 0.01^{c}	0.68 $\pm$ 0.51^{bc}
0.40	1.45 $\pm$ 0.01^{cd}	0.17 $\pm$ 0.01^{a}
0.20	0.77 $\pm$ 0.02^{bc}	0.34 $\pm$ 0.03^{ab}
	Crude Oil	
4.00	0.85 $\pm$ 0.01^{c}	-
2.00	2.55 $\pm$ 0.12^{e}	2.55 $\pm$ 0.14^{d}
0.40	0.68 $\pm$ 0.03^{bc}	0.85 $\pm$ 0.09^{bc}
0.2	5.27 $\pm$ 0.14^{g}	1.19 $\pm$ 0.07^{ed}
	Petrol	
4.00	-	-
2.00	2.64 $\pm$ 0.15^{ef}	1.02 $\pm$ 0.05^{c}
0.40	0.51 $\pm$ 0.02^{b}	0.60 $\pm$ 0.03^{bc}
0.20	2.04 $\pm$ 0.01^{d}	-

The total bilirubin level of fishes treated with kerosene ranged from 0.43 – 4.15 mg/ 100ml; diesel ranged from 0.77 – 1.45 mg/100ml; crud oil ranged from 0.68 – 5.27 mg/100ml and petrol ranged from 0.51 – 2.64 mg/100ml. Therefore the highest level of total bilirubin concentration was found in crude oil (5.27 mg/100ml) and the lowest in diesel (0.17 mg/100ml). There was no pattern of increase or decrease in the level of total bilirubin with respect to the concentration of toxicants. Higher concentration of total bilirubin was recorded in the fishes recovering from exposure to toxicant than in the control (Table 1).The concentration of the fishes recovering from diesel ranged from 0.17 – 0.68 mg/100ml; crude oil ranged from 0.85 – 2.55 mg/100ml; petrol ranged from 0.60 – 1.02 mg/100ml and kerosene ranged from 0.34 – 1.19 mg/100ml. The highest concentration of total bilirubin was recorded in crude oil (2.55 mg/100ml) and the lowest in diesel (0.17 mg/100ml). The concentration of total bilirubin increased with increasing concentration of toxicants. The biochemical parameter investigated showed significant ($P < 0.05$) difference when compared to the control. Fishes that were exposed to the toxicants have higher average concentration of conjugated bilirubin than the control (Table 2).

Table 2: Effects of exposure of *C. gariepinus* juveniles to crude oil and its products on conjugated bilirubin level (mg/100ml)

Concentration of toxicant (ml/l)	Toxicity	Recovery
	Control	
0.00	0,02 $\pm$ 0.01^{a}	0.02 $\pm$ 0.01^{a}
	Kerosene	
4.00	1.62 $\pm$ 0.02^{h}	1.19 $\pm$ 0.07^{de}
2.00	1.36 $\pm$ 0.07^{a}	1.02 $\pm$ 0.05^{d}
0.40	0.68 $\pm$ 0.05^{de}	1.02 $\pm$ 0.02^{d}
0.20	0.68 $\pm$ 0.51^{de}	-
	Diesel	
4.00	1.19 $\pm$ 0.12^{fg}	0.94 $\pm$ 0.02^{d}
2.00	0.68 $\pm$ 0.04^{de}	0.68 $\pm$ 0.01^{c}
0.40	0.34 $\pm$ 0.02^{ed}	0.43 $\pm$ 0.03^{b}
0.20	0.26 $\pm$ 0.01^{c}	0.34 $\pm$ 0.01^{ab}
	Crude Oil	
4.00	1.11 $\pm$ 0.05^{fg}	-
2.00	1.02 $\pm$ 0.08^{f}	0.85 $\pm$ 0.01^{c}
0.40	1.02 $\pm$ 0.01a	0.43 $\pm$ 0.02^{b}
0.2	0.09 $\pm$ 0.02^{cd}	0.20 $\pm$ 0.01^{ab}
	Petrol	
4.00	-	-
2.00	0.77 $\pm$ 0.01^{c}	2.30 $\pm$ 0.09^{e}
0.40	0.60 $\pm$ 0.06^{de}	0.03 $\pm$ 0.01^{a}
0.20	0.51 $\pm$ 0.02^{d}	-

The conjugated bilirubin concentration of fishes exposed to kerosene ranged from 0.68 – 1.62 mg/100ml, diesel ranged from 0.26 – 1.19 mg/100ml; crude oil ranged from 0.09 – 1.11 mg/100ml and petrol ranged from 0.51 – 0.77 mg/100ml. The highest concentration of conjugated bilirubin was observed in kerosene (1.62 mg/100ml) and the lowest in crude oil (0.09 mg/100ml). The concentration of conjugated bilirubin increased with increasing concentration of toxicants. Fishes recovering from exposure to toxicants have higher conjugated bilirubin concentration than the control (Table 2). The conjugated bilirubin level of fishes recovering from exposure to diesel ranged from 0.34 –0.94 mg/100ml; crude oil ranged from 0.26 –0.85 mg/100ml; petrol ranged from 0.03 –2.30 mg/100ml and kerosene ranged from 1.02 – 1.19 mg/100ml. The highest concentration of conjugated bilirubin was observed in petrol (2.30 mg/100ml). Conjugated bilirubin concentration increased with increasing concentration of toxicants. The average concentration of conjugated bilirubin showed significant ($P < 0.05$) difference when compared to the control.

Higher level of bilirubin was recorded in the fishes exposed to toxicant than the control and the level of total and conjugated bilirubin increased with increasing concentration of toxicants. Increase in total bilirubin can be due to damage to the liver cells or increased destruction of red blood cells and increase in conjugated bilirubin level can be due to obstruction of the bile duct (Arthur *et al.,* 1996). Highest level of total bilirubin was found in crude oil and the lowest in diesel in both the toxic and recovery phases. This suggests that crude oil increase the level of total bilirubin more than petrol and kerosene while diesel do not have much effect.

REFERENCES

ARTHUR, C. G. and JOHN, E. H. (1986). Metabolism and Temperature Regulation. Pages 886 – 888. *In: Medical Physiology,* 9th edition. W. B. Sanders Company, London.

BAKER, E. G. (1969). Origin and Migration of oil. *Science, 129:* 871 – 878.

CLERK, R. B. (1987). Oil Pollution in Sea Water. Marine Pollution Bulletin, (8) 5: 204 – 205.

POWEL, W. N. (1994). Determination of bilirubin in vertebrates. *Annual Journal of Clinical Pathology, 14:* 55 – 57.

REED, W., BURCHAR, J., HOPSON, A. J., JONATHAN, J., and IBRAHIM, Y. (1967). *Fish and fisheries of Northern Nigeria.* Government Press, London. 226 pp.

TATEM, H. E., COX, B. A., and ANDERSON, J. W. (1979). The toxicity of oils and Petroleum hydrocarbons on Marine Fishes. *East Coastal Marine Science, 6:* 365 – 374.

DENTAL DISORDERS AMONG RESIDENTS OF UGBO-ODOGWU ESCARPMENT, UDI HILLS, EASTERN NIGERIA

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ABSTRACT

Oral epidemiological assessment of dental disorders was carried out between April and July 2006 among the residents of Ugbo-Odogwu escarpment on Udi Hill near Enugu, Eastern Nigeria. Dental abrasions with prevalence rate of 37.3 %, attrition (31.3 %), calculus (87.5 %), caries (78.8 %), gingivitis (62.6 %), gum recession (53.8 %), halitosis (82.7 %), periodontitis (52.6 %), stains (78.6 %) and teeth erosions (24.8 %) were the specific dental disorders observed in the area. Every subject had one form or the other of these oral conditions occurring concomitantly. Gender and age specific prevalence of dental disorders as well as nutritional habits, suspected to play major roles in the initiation and development of dental disorders in the study population were discussed. The result of this study could be used to develop a Management Information System (MIS) for Dental Health Care in Nigeria. It may also stimulate further research interests in the relationships between dental disorders and the nutritional habits of other communities in the developing world.

Keywords: Oral Epidemiology, Dental caries, Oral disorders, Eastern Nigeria

INTRODUCTION

Coal mining had attracted immigrants who encamped at several sites on Udi Hill escarpments near Enugu. Ikpeze (2005) reported that the encampments later metamorphosed into unregulated villages namely, Ugbo-Odogwu (≈1.12 km^2), Agu-Abor (≈0.76 km^2), Ugwu Aaron (≈0.64 km^2) and Ugwu-Alfred (≈0.7 km^2), which are generally regarded as semi-urban slums with inadequate social amenities and security. This author opined that parasitic infections and unsanitary conditions largely contributed to different health problems in the areas. Experience, from the Federal School of Dental Technology and Therapy Clinics Enugu, indicated that outpatients from these villages had almost entirely preventable oral problems, which sometimes necessitated expensive dental therapies.

Healthy teeth and gums are part of the general good health. The teeth are used for the mastication of foods and are essential for good nutrition, understandable speech and attractive facial appearance of the individual. There is a dearth of published information on dental diseases from this part of the country. The major aim of this study is to carry out an epidemiological assessment of dental disorders in Ugbo-Odogwu, where no previous data exist. The result of the study will provide useful epidemiological data for the development of a Management Information System (MIS) on Public Health; create dental health awareness and help to enhance compliance with community directed dental health education and counseling in the study area and elsewhere in the country. The result may also stimulate further research interests on the relationship between dental disorders and nutritional habits of many communities in Nigeria.

MATERIALS AND METHODS

Study Area: The study area, Ugbo-Odogwu, situates on the Udi Escarpment via the Enugu-end of the Onitsha – Enugu Expressway (Figure 1).

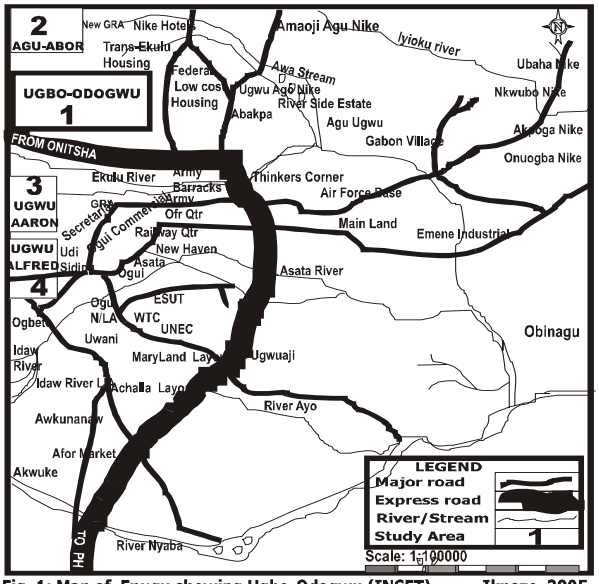

Figure 1: Map of Enugu town showing Ugbo-Odogwu (Ikpeze, 2005)

It has recently been integrated into Enugu-East Local Government Area. Ogbanokwute Spring, which serves as its only source of water, influenced the early migrant settlement in the area. However, Ekulu River, which traverses the Expressway, is a few kilometers from Ugbo-Odogwu. The population (n≈10000) is composed mainly of retired coal miners, railway artisans, peasant farmers and their wives. The active population included petty traders, commuter-bus operators, school children, trades apprentices, junior civil servants and touts who shuttle to Enugu for their daily businesses. There is the potential for rapid urbanization of Ugbo-Odogwu due to its proximity to the Expressway. Two Missionary Centres provide Primary school education. The nearest Secondary School is about 3 km away from the express road. The Federal School of Dental Technology and Therapy, Trans-Ekulu, which is a tertiary institution, is about 2 km away from the study area.

Awareness Mobilization: Information dissemination on the impending study was achieved through church announcements. The support of opinion leaders and landlords in the area was solicited. A female facilitator, recruited from the Ugbo-Odogwu accompanied the research team during the sensitization visits and throughout the study period. The community was thus adequately mobilized for the eventual study, which took place between April and July 2006.

Sample Population: Stratified random sampling technique was used to select the sample population of 80 individuals. It was primarily intended that equal numbers of each gender be used for the study. The researchers carried out personal interviews and clinical examinations of the oral cavities of the subjects to ascertain their dental health. The clinical signs studied included halitosis (bad breath), dental caries, tooth erosion, tooth stains, bleeding gums, calculus formation, gingivitis, gum recession, teeth abrasions, periodontitis and mobile tooth.

Data Collation and Analysis: Well-structured formats were used to record the subject's bio-data, dental disorders and nutritional habits. The results of analyses of epidemiological parameters were presented in the form of tables, histograms and descriptive assays. For tabular presentations, data were sorted, arranged, condensed and set out in such a way as to bring out the essential points. For histograms, the variables of interest were shown on the axis while the adjoining bars were drawn so that their areas represented the relative frequency of events studied.

RESULTS AND DISCUSSION

The study population was stratified under, gender, age groups, occupation and literacy levels (Table 1). Each age group provided 10.0 % subjects to the sample population of 80, comprising 39 (48.75 %) males and 41 (51.25 %) females.

Table 1: Stratification of the sample population of Ugbo-Odogwu (April-July 2006)

Age (years)	Male no.	Female no.	Total no. (%)
≤10	5	5	10 (10.0)
11-20	4	6	10 (10.0)
21-30	6	4	10 (10.0)
31-40	4	6	10 (10.0)
41-50	5	5	10 (10.0)
51-60	6	4	10 (10.0)
61-70	4	6	10 (10.0)
≥71	5	5	10 (10.0)
Total	**31**	**41**	**80 (100.0)**
Occupation			
Students	8	12	20 (25.0)
Civil servants	3	9	12 (15.0)
Petty traders	12	18	30 (37.5)
Farmers	8	2	10 (12.5)
Pensioners	8	0	8 (10.0)
Total	**31**	**41**	**80 (100.0)**
Educational level			
Primary	27	23	50 (62.5)
Secondary	6	14	20 (25.0)
Tertiary	5	2	7 (8.75)
Informal	1	2	3 (3.75)
Total	**31**	**41**	**80 (100.0)**

School children contributed 25.0 %, junior civil servants (15.0 %), petty traders (37.5 %), peasant farmers (12.5 %) and retired coal miners and railway artisans (10.0 %). Only 62.5 % of the study population obtained primary school education, 25.0 % (secondary education), 8.75 % (tertiary education), while the remaining 3.75 % did not have formal education. This result indicates that Ugbo-Odogwu residents are lower class citizens but literate enough to benefit from any coordinated community directed dental health education and counseling, to be complimented with the provision of appropriate social amenities and improved environmental sanitation.

10 specific dental disorders were observed in the area (Table 2). All the people examined were found to have one form of dental disorder or the other. Concomitant infections with 2 to 3 dental disorders were common in the community. The overall frequency of single dental disorders was 473 or 59.1 % prevalence rate, with a male: female ratio of 53.5: 46.5 (i.e. 1.15:1). The relative frequency of specific dental disorders was dental calculus 14.8 %, followed by halitosis (14.0 %), caries (13.5 %), and stains (13.3 %). Others were gingivitis (10.6 %), gum recession (9.1 %), periodontitis (8.9 %), abrasions (6.3 %), attrition (5.3 %), and teeth erosion (4.2 %). Males had higher prevalence of attrition (21.3 %), gum recession (31.2 %) periodontitis (31.2 %, stains (42.2 %) and teeth erosion (31.2 %) than females, with 9.9 %, 22.5 %, 21.2 %, 32.4 % and 9.9 % respectively (Figure 2). However, females had a higher prevalence of caries (43.7 %) than males (36.2 %). Generally, there were no marked gender differences in the prevalence of abrasions (18.7 – 18.8 %), calculus (43.7 – 43.8 %), gingivitis (29.9 – 32.5 %) and halitosis (39.9 – 42.5 %).

Table 2: Gender distribution and prevalence of dental disorders in the study population (n = 80) of Ugbo-Odogwu (April-July 2006)

Dental disorders	Population with dental disorders								
		Gender distribution		Relative frequency			*Prevalence		
Specific dental disorders	Total no.	Male (M) no. (%)	Female (F) no. (%)	T (%)	M (%)	F (%)	T (%)	M (%)	F (%)
1. Abrasions	30	15 (50.0)	15 (50.0)	6.3	3.17	3.17	37.3	18.7	18.6
2. Attrition	25	17 (68.0)	8 (32.0)	5.3	3.59	1.69	31.3	21.3	9.9
3. Calculus	70	35 (50.0)	35 (50.0)	14.8	7.39	7.39	87.5	43.7	43.8
4. Caries	64	29 (45.3)	35 (54.7)	13.5	6.13	7.39	79.9	36.2	43.7
5. Gingivitis	50	24 (48.0)	26 (52.0)	10.6	5.07	5.48	62.4	29.9	32.5
6. Gum recession	43	25 (58.1)	18 (41.9)	9.1	5.28	3.80	53.7	31.2	22.5
7. Halitosis	66	34 (51.5)	32 (48.5)	14.0	7.18	6.76	82.4	42.5	39.9
8. Periodontitis	42	25 (59.2)	17 (40.8)	8.9	5.28	3.59	52.4	31.2	21.2
9. Stains	63	37 (58.7)	26 (41.3)	13.3	7.82	5.49	78.6	46.2	32.4
10. Teeth erosion	20	12 (60.0)	8 (40.0)	4.2	2.53	1.69	24.8	14.9	9.9
Cumulative Frequency	**473**	**253 (53.5)**	**220 (46.5)**	**100**	**53.5**	**46.5**	**59.1**	**31.6**	**27.5**
Multiple (concomitant) dental disorders									
1) Abrasions with Halitosis	12	7 (58.3)	5 (41.7)	3.2	1.85	1.35	14.9	8.7	6.2
2) Abrasions with Periodontitis and Halitosis	10	6 (60.0)	4 (40.0)	2.6	1.59	1.06	12.4	7.5	4.9
3) Calculus with Attrition and Halitosis	8	5 (62.5)	3 (37.5)	2.1	1.32	0.79	9.9	6.2	3.7
4) Calculus with Erosions and Stains	5	4 (80.0)	1 (20.0)	1.3	1.06	0.26	6.2	4.9	1.3
5) Calculus with Halitosis	40	25 (62.5)	15 (37.5)	10.6	6.63	3.97	49.9	31.2	18.7
6) Calculus with Stains	50	23 (46.0)	27 (54.0)	13.3	6.10	7.16	62.4	28.7	33.7
7) Calculus with Stains, Gingivitis and Halitosis	12	5 (41.7)	7 (53.8)	3.2	1.33	1.86	14.9	6.2	8.7
8) Caries with Calculus	60	28 (46.7)	32 (53.3)	15.9	7.42	8.48	74.9	34.9	40.0
9) Caries with Calculus and Gum recession	30	19 (63.3)	11 (36.7)	8.0	5.04	2.91	37.4	23.7	13.7
10) Caries with Calculus and Halitosis	45	20 (44.4)	25 (55.6)	11.9	5.30	6.63	56.2	24.9	31.3
11) Caries with Calculus, Stains and Halitosis	30	14 (46.7)	16 (53.3)	8.0	3.71	4.24	37.4	17.5	19.9
12) Caries with Gingivitis	30	5 (16.7)	25 (83.3)	8.0	1.33	6.63	37.4	6.2	31.2
13) Caries with Periodontitis	25	15 (60.0)	10 (40.0)	6.6	3.97	2.65	31.2	18.7	12.5
14) Caries with Stains	20	15 (75.0)	5 (25.0)	5.3	3.97	1.23	24.9	18.7	6.2
Cumulative Frequency	**377**	**191 (50.7)**	**186 (49.3)**	**100**	**50.7**	**49.3**	**47.1**	**23.8**	**23.3**

Prevalence = Relative frequency (%) x MF, where MF is the multiplying factor. [MF for specific dental disorders = (473/80) = 5.9125. MF for multiple dental disorders = (377/80) = 4.7

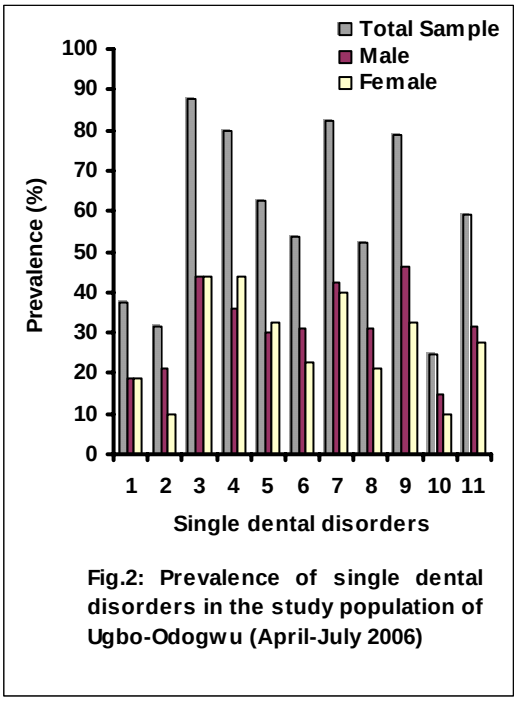

Fig.2: Prevalence of single dental disorders in the study population of Ugbo-Odogwu (April-July 2006)

Barker and Less (1996) opined that tooth-tooth contact or input of foreign objects such as nails, pens, and pins and chewing of bones etc., in the mouth caused tooth abrasion. It is worth knowing that most young people in the study area took delight in opening bottles of beer and soft drinks with their teeth, and not realizing the inherent harmful effects. Heymsfiled *et al*. (1994) reported high prevalence of dental caries from Western Europe, North and South America, Japan and Australia. Shills *et al*. (1994) noted that while 65 % of North American population required dental treatment and about one out of every 20 persons needed to have at least one tooth extracted as a result of caries.

Overall frequency of multiple dental disorders was 377 or 47.1 % prevalence rate, with a male: female ratio of 50.7: 49.3 (i.e. 1.03:1). Caries with calculus was of most frequent occurrence (15.9 %), followed by calculus with stains (13.3 %), caries with calculus and halitosis (11.9 %), and calculus with halitosis (10.6 %). The staple foods in Nigeria, including Ugbo-Odogwu, contain high carbohydrate, and also encourage tooth-tooth contact. People need to keep their mouths clean after such meals to avoid infection that may lead to oral diseases.

There was higher prevalence of calculus with halitosis (31.2 %), caries with calculus and gum recession (23.7 %), and caries with stains (18.7 %) in males than in females with 18.7 %, 13.7 %, and 6.2 % respectively (Figure 3). However, females had higher prevalence of calculus with stains (33.7 %), caries with calculus (40.6 %), caries with calculus and halitosis (31.3 %) and caries with gingivitis (31.2 %) than males with 28.7 %, 34.9 %, 24.9 % and 6.2 % respectively.

Age-specific distribution of dental disorders is shown in Table 3. Abrasions, attrition, periodontitis and teeth erosions appeared to be rare in subjects

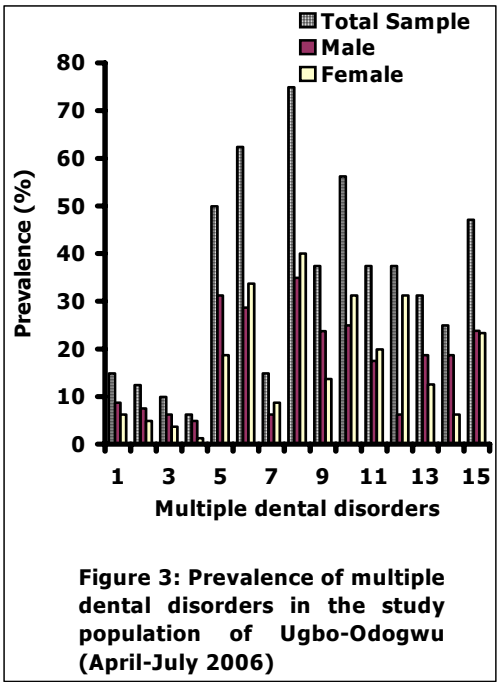

Figure 3: Prevalence of multiple dental disorders in the study population of Ugbo-Odogwu (April-July 2006)

halitosis, abrasion with periodontitis and halitosis, calculus with attrition and halitosis, calculus with erosions and stains were rare and not observed in age groups less than 30 years old. Calculus with halitosis, calculus with stains, caries with halitosis, and caries with calculus and halitosis appeared to follow almost the same pattern and were widespread in the study population. Cumulative frequencies of specific and concomitant dental disorders (Figure 4) indicated that the conditions were generally age dependent. This was in line with the findings of Adegbembo (1995) who reported that the prevalence of caries increased from childhood to adulthood in Nigeria.

Gender and age distribution of subjects with peculiar nutritional habits is presented in Table 4. The consumption of biscuits, cake, candies and soft drinks were rampant in the area, especially among the adolescents and young adults. Cigarette smoking, sniffing of ground tobacco (snuff), drinking of wine were not observed in subjects under 20 years of age, but the bibbing of alcohol (palm wine, spirits, brandy, Locally-brewed Gin etc) was a common spectacle to behold in the study area.

Table 3: Age-frequency distribution of dental disorders in the study population (n = 80) of Ugbo-Odogwu (April-July 2006)

Dental disorders	Age-frequency distribution								Cumulative frequency
Specific dental disorders	**≤10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**60-70**	**≥71**	**no.**
1. Abrasions	0	0	0	1	5	7	8	9	30
2. Attrition	0	0	0	2	4	5	6	8	25
3. Calculus	7	8	8	9	9	9	10	10	70
4. Caries	8	9	9	8	7	7	8	8	64
5. Gingivitis	1	1	6	7	8	8	9	10	50
6. Gum recession	1	3	5	6	6	7	7	8	43
7. Halitosis	4	6	9	9	9	9	10	10	66
8. Periodontitis	0	0	0	6	8	9	9	10	42
9. Stains	6	7	7	8	8	8	9	10	63
10. Teeth erosion	0	0	0	0	3	3	6	8	20
Cumulative frequency	**27**	**34**	**44**	**56**	**67**	**72**	**82**	**91**	**473**
Multiple (concomitant) dental disorders									
1) Abrasions with Halitosis	0	0	0	1	1	3	3	4	12
2) Abrasions with Periodontitis and Halitosis	0	0	0	1	1	2	2	4	10
3) Calculus with Attrition and Halitosis	0	0	0	0	0	1	2	5	8
4) Calculus with Erosions and Stains	0	0	0	0	0	1	1	3	5
5) Calculus with Halitosis	4	6	2	5	6	4	7	6	40
6) Calculus with Stains	4	6	7	6	5	6	6	10	50
7) Calculus with Stains, Gingivitis and Halitosis	0	1	0	2	1	1	4	3	12
8) Caries with Calculus	5	6	7	7	8	8	9	10	60
9) Caries with Calculus and Gum recession	0	0	2	3	4	6	7	8	30
10) Caries with Calculus and Halitosis	4	7	6	5	6	4	7	6	45
11) Caries with Calculus, Stains and Halitosis	2	4	3	4	5	4	3	5	30
12) Caries with Gingivitis	1	3	2	4	3	5	6	6	30
13) Caries with Periodontitis	0	0	1	2	4	5	7	6	25
14) Caries with Stains	2	3	1	3	3	2	3	3	20
Cumulative frequency	**22**	**36**	**31**	**43**	**47**	**52**	**67**	**79**	**377**

under 30 years old, but caries, halitosis and stains were widespread in the study population. Increase in dental caries was reported in 12 year-old school children in all social classes in Ondo State, Nigeria, but the increase was only noticed in 5 year-olds who came from the upper class (Olujugba and Lennon, 1986). Halitosis, gum recession and gingivitis were however more pronounced in the older age groups. Concomitant disorders, such as abrasion with

An adult respondent from Ugbo-Odogwu explained that they indulge in alcohol because of the chilly weather of the escarpment, especially during the harmattan season. Males took kolanuts, fruit juice, liquor, snuff and wine more than females (Figure 5). Apparently, the people were unawares of the dental health implications of their nutritional habits. Wilkins (1976) posited that some indulge in such habits for the fun of it; others out of necessity,

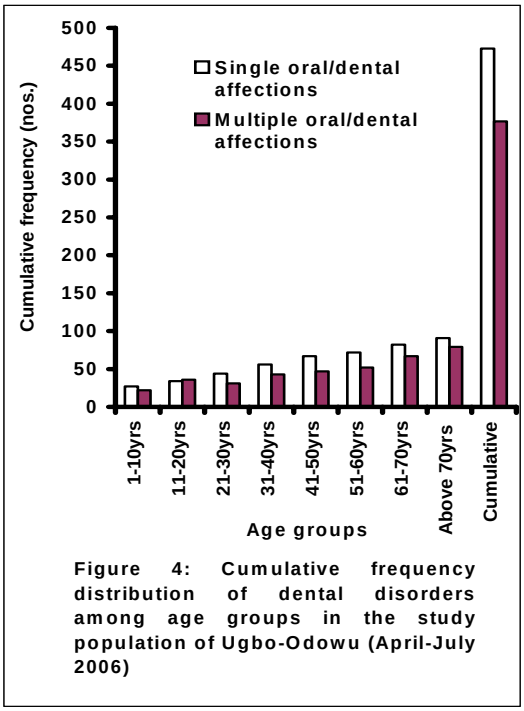

Figure 4: Cumulative frequency distribution of dental disorders among age groups in the study population of Ugbo-Odowu (April-July 2006)

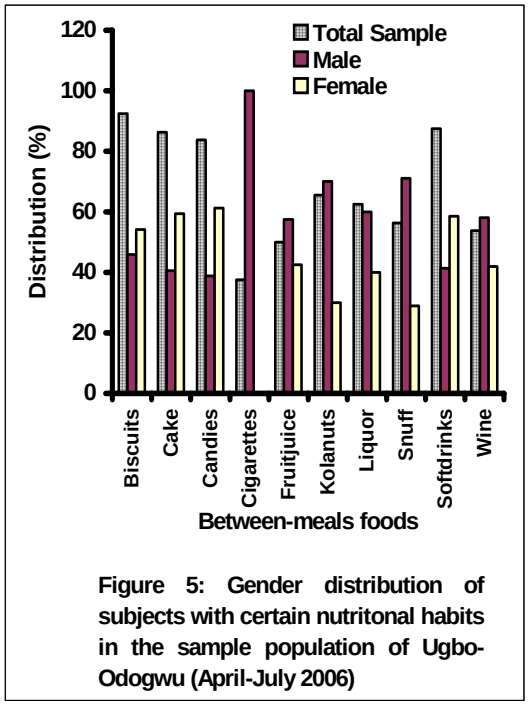

Figure 5: Gender distribution of subjects with certain nutritonal habits in the sample population of Ugbo-Odogwu (April-July 2006)

Table 4: Gender and age distribution of subjects with certain nutritional habits in the study population (n = 80) of Ugbo-Odogwu (April-July 2006)

Nutritional habits	Gender			Age (years)							
	Total no.(%)	Male no.(%)	Female no.(%)	≤10 no.	11-20 no.	21-30 no.	31-40 no.	41-50 no.	51-60 no.	61-70 no.	≥71 no.
Biscuits	74 (92.5)	34 (45.9)	40 (54.1)	10	10	10	10	10	9	8	7
Cake	69 (86.3)	28 (40.6)	41 (59.4)	10	10	10	10	9	9	6	5
Candies	67 (83.8)	26 (38.8)	41 (61.2)	10	10	10	9	9	8	6	5
Cigarettes	30 (37.5)	30 (100.0)	0 (0.0)	0	0	0	8	9	5	4	4
Fruit juice	40 (50.0)	23 (57.5)	17 (42.5)	2	2	5	8	9	7	5	2
Kolanuts	50 (65.5)	35 (70.0)	15 (30.0)	0	0	5	8	9	8	10	10
Liquor	50 (62.5)	30 (60.0)	20 (40.0)	3	4	4	5	8	9	8	9
Snuff	45 (56.3)	32 (71.1)	13 (28.9)	0	0	1	6	8	10	10	10

while many as a result of influence of peer groups, age, gender, location, economic status and availability of such foods and beverages. These factors were observed to be in operation in the study area. Kolanuts and hot drinks were usually presented to visitors at every home in the study area. Children were always given biscuits and snacks to be taken at break time in the school, while various brands of cigarettes, kolanuts, candies, soft drinks and snacks were hawked and openly patronized in the area. Brown nicotine stains from tobacco, red caffeine stains from kolanuts and stains of different colours were found on the teeth as reported. Caries activity became higher when sucrose was the major dietary component of soft drinks, candies and snacks. Unrefined sugar (carbohydrate) did not cause caries, calculus or any other dental disorder per-se, but most dental disorders resulted from plaque and calculus formation due to poor oral hygiene (Shills *et al*., 1994; Barker and Less, 1996). Carbohydrate foods usually stuck to teeth and oral tissues, and if not cleaned, formed the substrate for plaque. Widespread consumption of carbohydrate foods, biscuits, cakes and soft drinks may have influenced the formation of dental calculus, caries and stains in the study area. We observed that the rough way most people in the study area cracked palm-nuts and kernel with their teeth, as well as the improper used chewing sticks and brushes may not be unconnected with the levels of abrasions, gum recession and attrition reported in this study.

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REFERENCES

ADEGBEMBO, A. O. (1995). National Survey of Dental Caries Status and Treatment Needs in Nigeria. *Journal of WHO Oral Health Country/Area Profile Programme, 45*: 35 – 44.

BARKER, H. M and LESS, R. (1996). *Nutrition and Dietetics for Health Care*. 9th Edition. Churchill Livingstone, USA.

HEYMSFILED, S. B., TIGBE, A. and WARY, Z. (1994). Nutritional Assessment by Anthropometric and Biochemical Methods. Pages 812 – 838. *In:* SHILLS, M. E., OLSON, J. A. and SHIRES, M. (Editors). *Modern Nutrition in Health and Diseases,* 8th Edition. Williams and Wilkins, USA.

IKPEZE, O. O (2005). Stratification and Livestock Population Census for Enugu Urban, Nigeria: A Pilot Survey. *Animal Research International, 2(2)*: 332 – 335.

OLUJUGBA, O. O. and LENNON, M. A. (1986). Oral Epidemiology: Dental Caries Experience in 5 and 12 year–old School Children in Ondo State, Nigeria. *Journal of the Inter-Country Centre for Oral Health for Africa*. *1:* 1 – 45.

SHILLS, M. E., OLSON, J. A. and SHIRES, M. (1994). Assessment of Nutritional Status. Pages 112 – 308. *In*: SHILLS, M. E., OLSON, J. A. and SHIRES, M. (Editors). *Modern Nutrition in Health and Diseases,* 8th Edition. Williams and Wilkins, USA.

WILKINS, E. M. (1976). *Clinical Practices of the Dental Hygienist.* 6th Edition. Lea and Febiger. Philadelphia.

COMPARATIVE EFFICACY OF ANCYLOL, IVOMEC, MEBENDAZOLE AND PIPERAZINE AGAINST *Ancylostoma caninum* IN EXPERIMENTALLY INFECTED PUPS

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ABSTRACT

The efficacy and side effects of single dose treatment at different dose regimen of four anthelmintics against Ancylostoma caninum in experimentally infected pups were evaluated and compared based on total worm count and egg per gram (epg) count. Ancylol at both normal (1 mg/kg/BW) and elevated dose level (1.5 ml/kg/BW) showed 93.15 % and 93.87% (based on worm count) and 93.13 and 93.75 % (based on epg count) respectively. Whereas Ivomec® (a brand of Ivermectin) at normal dose level (1 ml/50 kg) and elevated dose level (1.5 ml/50 kg) was found to be effective. The results were 79.48 % and 86.81% based on worm count and 89.44 % and 92.50 % based on epg count. Mebendazole and Piperazine even at elevated dose level was observed ineffective. Pups treated at normal and elevated dose level revealed acute toxicosis whereas those treated with Mebendazole showed cough and vomiting tendencies which later subsided and also, there was no risk involved in the administration of the drugs. Statistical analysis showed that there was statistical difference (P < 0.05) in the efficacy of the drugs. However there was significant difference (P < 0.05) between % efficacy and dosage. There was also significant difference between (P < 0.5) between epg count and drugs. The use of various compounds against ancylostomiasis in dogs has been discussed.

Keywords: Anthelmintics, Efficacy, *Ancylostoma caninum*

INTRODUCTION

Ancylostomiasis (hookworm disease) is a disease of worldwide distribution. The most widespread of all hookworm species is *A. caninum* and it parasitizes dog throughout the tropics and subtropics. Due to its high prevalence and its zoonotic significance, *Ancylostoma caninum* has gained major importance in the field of veterinary as well as public health research. In recent years the realization that *A. caninum* can cause human gut disease has sparked off renewed interest in its study.

In the normal canine host, infection with *A. caninum* usually follows skin contact with L3 larvae that have developed in the soil. Invasive L3 larvae undergo either tracheal migration to reach the gut (leading to obvious infection) or somatic migration to invade and stay as reservoirs mainly in the skeletal muscle fibres. Heterogenous infections in animals and amphixenous anthropozoonosis are common causing apthous ulceration, eosinophilic enteritis (Prociv, 1998)

Many highly effective anthelmintics are available but such drugs must be used correctly to obtain favourable clinical response. The ideal anthelmintics should have a wide margin of safety, considerable activity against immature stages, easy to administer, inhibit re-infection and be compatible with other compound not require long withholding periods because of residue and be cost effective. To overcome the problem of hookworm infestation, the anthelmintics with good efficacy and least toxic effects is desired.

The present study deals with the anthelmintics efficacy of Ancylol, Ivomec, Mebendazole and Piperzine against *A. caninum* infection in pups.

MATERIALS AND METHODS

100 g of stool from each of 3 infected dogs selected was mixed with 300 g of sterilized sand (hot air oven sterilized). This was kept in a moist chamber at 25 ^{O}C (Sen *et al.,* 1965). The culture solution with which the filter paper was kept wet and protected consisted of 0.01 % HCl and 0.2 % NaCl (Bhai and Pande, 1981). The culture was sprayed with mycostatin (Bhai and Pande, 1981) ten days later, the dish was tilted and about 0.5 ml of water which percolated was aspirated into test tubes.

Bearnan's apparatus (Cheesbrough, 1987) was used to isolate larvae that may remain in the filter paper. Larvae collected were further cleaned in sucrose gradient (400g sucrose in a litre of distilled water). The larvae were kept at 4 ^{o}C for few days before use (Banerjee *et al*., 1970; Ikeme, 1976). Finally active larvae were collected by using muslin impregnated with 1.5 % agar (Warren, 1965).

Ninety six pups (mongrel breed) of about 4 weeks age were dewormed with Ivomec at 1 ml/50 kg BW for 2 days and divided into four groups each subdivided into 3 subgroups with each subgroup containing 6 pups.

For each experiment a separate infected control group without treatment was maintained. All the pups were infected orally with 300 larvae of *A. caninum* On day 24 post infection, each was treated with different anthelmintics. Each of the subgroup was treated with different dosage of the anthemintics. The treatment schedule followed in this experiment is shown in Table 1.

Two methods were adopted for therapeutic evaluation of the drugs. One of the methods was based on total egg count by Stools dilution method (Soulsby, 1982) and the other was based on total worm count (Georgi, 1969). The eggs per gram values of 96 pups were determined at pre-treatment and day 7, 14, 28 and 30 days post treatment. For the total worm count, the animals were sacrificed. Necropsy was performed according to Sen *et al* (1965). The gastrointestinal system of each pup was removed. The stomach, small intestine and large intestine were cut open lengthwise with a fine scissors and the content scrapped and washed into the separated containers and later strained through sieves. The sieves were spread out with the aid of needles and searched for worms visually and with handlens.

Drug efficacy was evaluated using the method described by USDVM thus: Efficacy = (Mean of the control - mean treated) x 100/ Mean control (Robinson *et al*., 1976).

Data collected were analysed using descriptive statistics and analysis of variance (ANOVA) and F-LSD to indicate statistical significance ($P < 0.05$).

RESULTS

Efficacy of the drugs tested based on worm count and epg count were shown on Tables 1 and 2. Table 3 revealed that Ancylol had the highest efficacy followed by Ivomec super especially at elevated and normal levels. Mebendazole and Piperazine were least efficacious even at elevated dose level. Ancylol has the highest efficacy of 93.87 % and 93.15 % at elevated and normal does levels respectively while at subdose level, efficacy of 84.31 % was obtained. Using Ivomec at elevated and normal dose levels, efficacy of 86.81 % and 79.48 % efficacy were obtained. At elevated and normal dose levels of Mebendazole efficacy of 67.39 % and 61.28 % were obtained. While at subdose level 44.44 % efficacy was obtained. Using piperazine, at elevated and normal levels 58.88 % and 52.58% were obtained. But at subdose level 42.51 % efficacy was obtained. Statistical analysis using ANOVA and F-LSD showed that there was statistical difference in the efficacy of the drugs ($P < 0.05$)

The result in Table 2, revealed efficacy of the based on epg count. From the result it could be seen that the highest efficacy were obtained at elevated dose level in the entire anthelmintic group as the case may be. Statistical analysis using ANOVA showed that there was significant difference ($P < 0.05$) between epg counts in Ancylol treated group and control and also between Ivomec treated group and control group. F-LSD for epg count and dose also showed significant difference ($P < 0.05$) between the treated groups and the control group; moreover, there was also a significant difference between epg count and days (periods) of the experiment.

DISCUSSION

From the result of the study, pups treated with Ancylol had very few worms in their intestinal lumen showing efficacy of 93.2 % and 93.8 % for normal and elevated dose treatments respectively. Efficacy based on epg count was also high. However, faster respiration and depression were noticed immediately after drug was injected into the body. This lasted for about 30 minutes before normalcy returned. Tiwari and Bandopadhayay (1995) had earlier reported similar reactions in dogs treated with disophenol. Furthermore, in another study, Pneumarthy *et al.* (1995) reported acute toxicosis in pups injected disopenol subcutaneously at 0.8 ml/kg. Misraulia *et al.* (1989) reported Ancylol poisoning in dog at a higher dose level. In their study, the symptoms of toxicity developed few minutes of post injection. Signs of acute toxicosis were not reported in an Irish wolf Hound breed given recommended dose of Ancylol (Legendre, 1973). This may be attributed to the genetic difference among the different breeds of pups. Therefore, further research is needed in this area. Tiwari and Bandopadhayay (1995) reported a case of sub-acute/chronic toxicosis in a pedigreed Doberman Picher dog aged 15 months suffering from acute hookworm infection and anaemia. According to their report, Ancylol at 1 ml/kg subcutaneously produced after dew days oedematous swelling at the site of injection anorexia, vomiting, faster respiration and depression. The toxicosis observed in this study later subsided when a teaspoonful vitamin B12 was given orally.

Result from our study indicated that the group treated Ivomec at subdose level had efficacy of 53.5 % based on worm count and 88.3 % based on epg count. At a normal dose level, efficacy of 79.5 % based on worm count was obtained while at elevated dose level, 86.8 % efficacy was obtained. This implies that even at elevated dose level, efficacy with the normal dose are almost the same, therefore increasing the dose is merely a waste of resources as the difference was not statistically significant. In an earlier study, similar result in respect to efficacy of Ivomec at different dose levels has been reported by (Anderson and Robinson, 1982) for *Toxocara canis* infected Indian dogs. On the contrary, Ramizs (1984) obtained a lesser efficacy in Labrado breed infected with Ascarid worm. This really showed that efficacy of drugs can also depend on the breed of the animals and the brand of the drug used. From the result it could be seen that even Ivomec was not an effective drug against hookworm disease because a reasonable number of worms persist in the intestine after treatment. Incidentally it is sad to report that it is the common drug used in most Veterinary Clinics in Anambra State. No wonder why the disease persist despite the efforts made to eradicate it.

Table 1: **Efficacy of Ancylol, Ivomec, Mebendazole and Piperazine against *A. caninum* infections in pups**

Sub group	No of animals	Dose of drug used	Av. Worm recovered	% Worm established	% Efficacy
		Ancylol treatments (ml/kg BW)			
1	6	0.5	32.33	10.77	84.31 a
2	6	1	15.00	3.00	93.15 a
3	6	1.5	7.67	2.55	93.87 a
Control	6	Water	90	30.00	
		Ivomec treatments (ml/50 kg BW)			
1	6	0.5	43.33	16.55	44.44 c
2	6	1	18.67	11.44	61.28 b
3	6	1.5	12.00	9.55	67.39 b
Control	6	Water	90	30.00	0
		Mebendazole treatments (mg/10 kg BW)			
1	6	0.5	51.33	17.11	42.51 c
2	6	1	42.67	14.22	52.58 c
3	6	1.5	37.00	12.33	58.58 c
Control	6	Water	90	30.00	0 d
		Piperazine treatments (ml/kg BW)			
1	6	125	51.33	17.11	42.51 c
2	6	150	42.67	14.22	52.58 c
3	6	175	37.00	12.33	58.58 c
Control	6	Water	90	30.00	0 d

Unsimilar letters on the same column= significantly different means.

Table 2: Efficacy of anthelmintics against *A. caninum* infections in pups based on epg count

Sub group	No of animals	Pre-treatment period	7 dpt	14 dpt	21 dpt	30 dpt	% Efficacy
			Ancylol treatments (ml/kg BW)				
1	6	340	250	130	79	31	91.83 a
2	6	338	185	92	51	18	95.13 a
3	6	350	150	88	48	12	96.75 a
Control	6	350	374	405	396	363	1.89 e
			Ivomec treatments (ml/50 kg BW)				
1	6	320	280	121	88	42	88.33 b
2	6	342	205	96	66	38	89.44 b
3	6	356	186	92	45	27	92.50 a
Control	6	350	372	375	362	356	1.11 e
			Mebendazole treatments (mg/10 kg BW)				
1	6	345	310	290	155	142	61.07 c
2	6	340	226	181	152	137	59.65 c
3	6	348	280	210	140	118	66.47 c
Control	6	350	354	356	350	345	1.10 e
			Piperazine treatments (ml/kg BW)				
1	6	340	333	321	305	202	42.28 d
2	6	346	321	309	303	191	45.42 d
3	6	352	312	301	290	182	48.00 d
Control	6	350	352	354	350	348	0.60 e

Unsimilar letters on the same column= significantly different means.

On the group treated with Mebendazole, it was observed that few minutes after the administration of the drug all the pups showed signs of cough and vomiting tendency for about 30 minutes and later subsided. At normal dose level and even at an elevated dose level, the efficacy was low. However, some studies have shown that this may be due to strain variation, as different strains of *A. caninum* have variable degree of infectivity (Zenkov, 1971). Melhorn (1998) had earlier reported that Mebendazole was effective against *Echinococcus granulosus* with survival of few worms in the intestinal lumen of dog. Anderson (1975) reported 98.8 % efficacy of Mebendazole against nematodes of dogs and ruminants. Gemmel *et al* (1975) has also reported effectiveness of Mebendazole against *Moniezia expansa* in dogs. Sangeeva and Suryanaraya (1995) reported that two dose levels of Mebendazole given 24 hrs apart at the dose rate of 50 mg/kg was 100 % effective in treating dog infected with *Dipylidium caninum*.

Kates *et al.* (1972) recorded an outstanding efficacy of Mebendazole against most intestinal nematodes and lung worms of sheep. This was confirmed by Armour *et al.* (1981) who recorded 99 % reduction in worm and burdens over a grazing season with a mean improvement in weight gain.

On the group treated with Piperazine adipate, there was 52.5 % efficacy at normal dose level. Those that receive sub dose treatment showed efficacy of 42.5 % while those of elevated dose level showed 58.6 %.

On necropsy, many worms were seen in the intestinal lumen. It was also observed that the liver of the pups were grossly damaged and adversely inflamed especially those treated at sub dose level. Pulmonary damage in the form of haemorrhagic pneunonitis was also observed.

Signs and symptoms such as diarrhoea, rough skin coat, falling of furs, paleness of mucous membrane and weakness persisted after treatment. Similar result in an earlier study of Piperazine efficacy has been reported for *Toxocara* infection in Australian pups (English and Sprent, 1965). Robinson *et al* (1976) had reported 68 % efficacy against immature *Toxocara canis* in Australian pups dogs when given 150 mg/kg, whereas Shearer and Genmelt (1969) suggested higher dose of 200 mg/kg for complete removal of mature worms. It has also been found to be highly effective against *Moniezia expansa* and *Thysaniezia giardi* in sheep, goat and cattle at 65 mg/kg b. wt (English and Sprent, 1965). However, it has been reported to be infective against *Stilesia hepatica* in calve (Genealor and Reinackle, 1970, Medherkar *et al.,* 1987; Gruss *et al.,* 1988). The ineffectiveness reported in Piperazine might be due to the drug selectivity to helminth species because from result of this study, Piperazine even at elevated dose level did not give encouraging result.

REFERENCES

ANDERSON, D. L. (1975) Efficacy of Mebendazole against nematodes of dogs and ruminants. *Parasitology Today, 12*: 43 – 45.

ANDERSON, D. L. and ROBINSON, E. L. (1982). Efficacy of some anthelmintic against experimentally induced *Toxocara canis* infection in pups. *American Journal of Veterinary Resources, 43*: 1681 – 1684.

ARMOUR, J., BRUCE, R. G. and MITRA, S. K. (1981). Comparative efficacy of Mebendazole and Piperazine against intestinal nematodes of ruminants. *Journal of Veterinary Medicine, 18:*121 – 126.

BANERJEE, D., PRAKASH, O. and DEO, M. G. (1970). Studies on *Ancylostoma caninum* infection in mice following percutaneous and intraperitoneal routes of infection. *Indian Journal of Medical Research, 58*: 1313 - 1320.

BHAI, I. and PANDE, A. K. (1981). Experimental Ancylostomiasis in Swiss albino mice adrenal and thyroid histopathology. *Journal of Helminthology, 55*: 257 – 305.

CHEESBROUGH, M. (1987). *Medical Laboratory Manual for Tropical Countries.* Volume 1. Butterworth and Company Limited, Kent. 186 pp.

ENGLISH, P. B. and SPRENT, F. J. A. (1965). Efficacy of Piperazine and Ivermectin against *Toxocara canis* infection in pups. *Australian Veterinary Journal, 41*: 50.

GEMMEL, M. A., JOHNSTONE, P. D. and GUDEMANS, G. (1975). Efficacy of drugs against nematodes in dog. *Research in Veterinary Sciences, 19*: 229.

GENEALOR, J. and REINACKLE, R. K. (1970) Therapeutic studies in ruminants. *Journal of South Africa Veterinary Medical Association, 41*: 211 – 215.

GEORGI, J. R. (1969). *Parasitology for Veterinarians*. W. B. Saunders Company, Philadelphia. 237 pp.

GRUSS, B., MALAN, F. S., ROPER, N. A., PLESSIS, C. A. and ASHBURNER, A. J. (1988). Efficacy of drugs in dogs. *Journal of South African Veterinary Association, 59:* 204 – 210.

IKEME, M. M. (1976). Human hookworm infections: a diagnostic dilemma. *Journal of Medical and Pharmaceutical Marketing, 4(5*): 295 – 298.

KATES, K. C., ALLEN, R. W. and WILSON, G. I. (1972). Anthelmintic Studies with Mebendazole. A comparison of the efficacy of pyrantel and Mebendazole in dogs. Pages 38 – 39. *In: Prognosis and Abstracts of 43rd Annual Meeting of American Society of Parasitology.*

LEGENDRE, A. B. (1973). Anthelmintic studies in dogs. *Australian Veterinary Practice, 6*: 27 – 29.

MEDHERKAR, D. R., RAMANATHAN, S., KHAN, M. A. and ROY, R. M. (1987). Chemotherapeutic studies. *Livestock Advances, 12*: 5 – 7.

MELHORN, H. (1998). *Parasitology in focus: Facts and trends.* Springer Verlag, Berlin, Germany. 924 pp.

MISRAULIA, K. S., SISODIA, R. S. and MEHTA, H. K. (1989). Disophenol toxicosis in dog. *Indian Journal of Veterinary Medicine, 9:* 24 – 26.

PNEUMARTHY, L. P., DEHME, W. and MENHUSEN, J. J. (1995). Disophenol toxicosis in a dog. *American Journal of Veterinary Research, 36*: 1259 – 1260.

PROCIV, P. (1998). Indigestion, not scorched earth beats alien hookworm? *Parasitology Today, 14(2*): 51 – 53.

RAMIZS, A. (1984). Comparative efficacy of anthelmintic against experimentally induced Ascaridia infection in pups. Pages 25 – 27. *In: Proceedings of 3rd International Scientific Conference on Animal Prospects.*

ROBINSON, M., HOOKE, F. G. and IVERSON, K. E. (1976). Comparative efficacy of Piperazine Ivermectin against experimentally induced *Toxocara canis* infection in pups. *Australian Veterinary Practice, 6:* 104 – 106.

SANJEEVA R. S. and SURYANARAYANA, C. (1995) Clinico-Biochemical and therapeutic studies on toxocarasis in dogs. *Indian Veterinary Journal, 72:* 1076 – 1079.

SEN, H. G., JOSHI, U. N. and SETH, D. (1965). Effect of cortisone upon *Ancylostoma caninum* infection in Albino Mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 59(6):* 684 – 689.

SHEARER, G. C. and GENMELT, M. A. (1969). Anthelmintic studies in dogs. *Research in Veterinary Science, 10:* 296 – 297.

SOULSBY, E. J. L. (1982). *Helminths, Arthropods and Protozoa of domesticated animals* 7th Edition, Bailliere, Tindal and Casell Limited, London, 766 pp.

TIWARI, S. P. and BANDOPADHYAY. A. C. (1995). Sub-acute Disophenol toxicosis in pedigreed dog. *Indian Veterinary Journal,* 72*:* 645 – 646.

WARREN, L. G. (1965). Biochemistry of the dog hookworm. 1. Oxidative metabolism. *Experimental Parasitology, 17:* 1 – 9.

ZENKOV, A. V. (1971). Chemotherapeutics in dog nematode Infection. *Helminthology Abstract, 44*: 94 – 98.

EFFECT OF NUTRITION ON THE BIRTH WEIGHT AND MULTIPLE BIRTHS OF TRYPANOSOME INFECTED FEMALE *Rattus norevegicus*

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ABSTRACT

Trypanosomiasis is a disease of agricultural interest in livestock. The research was therefore aimed at finding out if adequate nutrition would ameliorate reproductive disorder in trypanosome-infected pregnant rats. Twenty female rats of 120 days old were used. They were kept 5 rats in each cage replicated three times. Four treatments diet each containing trypanotolerant; 80 mg of Vitamin E and 0.3 mg of selenium (additives) were used. In Treatment 1, trypanosome infected reproducing female rats were fed Diet 1 (Control) comprising chick mash mixed with the additives. In Treatment 2, the rats were fed Diet 2 comprising dietary protein and carbohydrate mixed with the additives. In Treatment 3, the rats were fed Diet 3 made up of dietary protein and the additives. In Treatment 4, the rats were fed Diet 4 made of dietary carbohydrate and the additives. The birth weight was measured and number of ratlets from each treatment was also counted to determine the effect of the diets on the birth. At the end of the experiment, it was observed that trypanosome-infected pregnant rats fed Diet 2 (with adequate concentrations of carbohydrate and protein) significantly (P < 0.05) had higher birth weight of offspring and multiple births than the rats fed with other treatments diets indicating that adequate nutrition promoted reproduction in trypanosome-infected rats.

Keywords: Nutrition, Birth-weight, Multiple births, Trypanosome-infection

INTRODUCTION

Nutritional status of female reproducing animals before and during gestation affects the offspring. Carbohydrate, protein and micronutrients deficiencies in female either before conception or in early pregnancy have been implicated in causing low birth weight in offspring (Galloway et al., 1994). Birth weight is crucial to the survival of an individual animal (Wynn *et al.,* 1991). Modest restrictions or under-nutrition in maternal nutrition around the gestation period can lead to premature births and long term adverse health effects for the offspring (Wong, 2003). It was noted that genotype interacts with nutrients intake in pregnant females and their effect on birth can be modulated nutritionally (Harggarty, 2002). The nutrient, Vitamin E enhances fertility in animals (Bieri et al., 1983; Mino *et al*., 1985). Also, Vitamin E enhances multiple births (Levine *et al.,* 1976).

Trypanosomiasis is one of the most important livestock diseases in Sub-Saharan Africa that retards livestock production (Morrison *et al*., 1981). Trypanosome infection is associated with reproductive disorders including abortion in pregnant animals among others (Ogwu *et al*., 1980). Improvement on host's nutrition is important in modulating the severity of patho-physiological effect of trypanosomiasis and also, it influences the rate of recovery (Katungka-Rwakishaya, 1996).

Against the background, the objective of this study therefore was to find out the effect of adequate nutrition on the birth weight and multiple births of trypanosome-infected female rats. We were also interested in finding out if adequate nutrition will ameliorate the adverse effects of trypanosomiasis on low birth weight and abortions in trypanosome-infected pregnant rats.

MATERIALS AND METHODS

Induction of Rodent Trypanosomiasis: Twenty 120-days old female albino rats (*Rattus norvegicus*) were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house. The cages were labeled A to D corresponding to four treatments while each experimental set up was replicated three times. The rats had unlimited supply of clean water.

Twenty female rats were paired with male rats so as to ensure mating. The female rats were monitored for pregnancy. Pregnancy was detected by presence of pan plug which was released when pregnancy occurred (Cukierski *et al*., 1991). These rats were infected within 10^{th} to 14^{th} day of pregnancy with NITR type of trypanosomes from Faculty of Veterinary Medicine, University of Nigeria, Nsukka by injecting the rats with 0.1 ml of trypanosome-infected rat's blood in normal saline.

Table 1: Ingredient and proximate composition and of diets fed to trypanosome-infected rats

Ingredient	Composition			
	Diet 1 (control)	Diet 2	Diet 3	Diet 4
Chick mash (g)	1000	500	300	300
Corn meal (g)	-	250	-	300
Dextrose (g)	-	-	-	400
Caseinogen (g)	-	-	400	-
Soyabean meal (g)	-	240	300	-
Vitamin E (mg)	80	80	80	80
Selenium (mg)	0.3	0.3	0.3	0.3
Crayfish meal (g)	-	10	-	-
Nutrients				
Moisture	10.9	16.25	14.65	12.25
Protein	18.39	20.01	29.95	10.39
Ash	8.4	13.40	10.75	11.05
Fibre	10.25	13.70	15.20	10.25
Fat	10.25	3.95	11.50	5.55
Carbohydrate	41.81	32.69	17.95	50.51

The blood of the infected rats was examined under the microscope to detect level of parasitemia using a matching chart (Herbert and Lumbsden, 1976).

Diets: The rats were fed with diets 1 - 4 containing different levels of protein and carbohydrate (Table 1). Each diet additionally had a constant level of 80 mg of vitamin E and 0.3 mg of selenium thoroughly mixed with the other ingredients to enhance trypanotolerance (Mgbenka and Ufele, 2004). The rats were fed in cages whose labels were identical to the diets (A – D). The proximate composition of the diets was determined by the method of Windham (1996) (Table 1).

Birth: At the end of gestation, the number of ratlets delivered by each rat was recorded and the weights of the ratlets were taken using triple beam balance.

Data Analysis: The data were analysed for significant differences by analysis of variance (ANOVA) using SPSS version 11.0 for windows. Specific differences in treatment means were determined using Least Significant Difference (LSD) and the Duncan's New Multiple Range Test (DNMRT) (Steel and Torrie, 1990).

RESULTS

The ingredient and proximate compositions of the diets are shown on Table 1. Figure 1 showed the mean birth weight (g) of ratlets of the groups of rats and the corresponding diets. From Figure 1, it is seen that ratlets of rats fed with Diet 2 had the highest birth weight (29.40 ± 3.54 g) compared to ratlets from rats fed other diets 10.53 ± 3.30, 11.93 ± 3.60, 12.80 ± 3.43 g for Diets A, C and D respectively. The birth weights of the ratlets from rats fed other diets in reducing sequence were Diet 4 > Diet 3 > Diet 1, but were not significantly different ($P < 0.05$) from one another. Figure 2 showed the mean multiple births of groups of rats and corresponding diets.

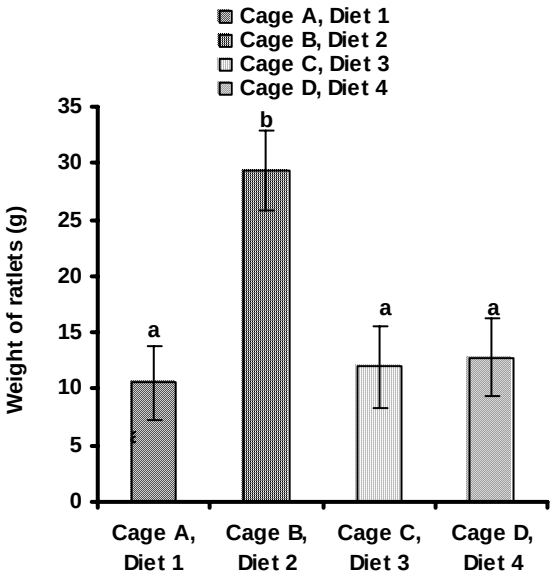

Figure 1: Mean birth weight (g) of ratlets of the groups of rats and the corresponding diets. Bars with different letters on top are significantly different ($P < 0.05$).

From Figure 2, it was observed that rats fed with Diet 2 also had the highest multiple births (4.80 ± 0.59) when compared to rats fed other diets 2.10 ± 0.65, 2.33 ± 0.71, 2.53 ± 0.68 ratlets for rats fed Diets A, C and D respectively. It was observed that rats fed with diets 1, 3 and 4 aborted some of the pregnancy. Rats fed Diet 2 had a very minimal abortion rate (6.6 %) compared to the rats fed other diets (46.7, 53.3 and 46.7 % for Diets A, C and D respectively). The number of ratlets rats fed other diets in reducing sequence were Diet 4 > Diet 3 > Diet 1, but not significantly different ($P < 0.05$) from one another.

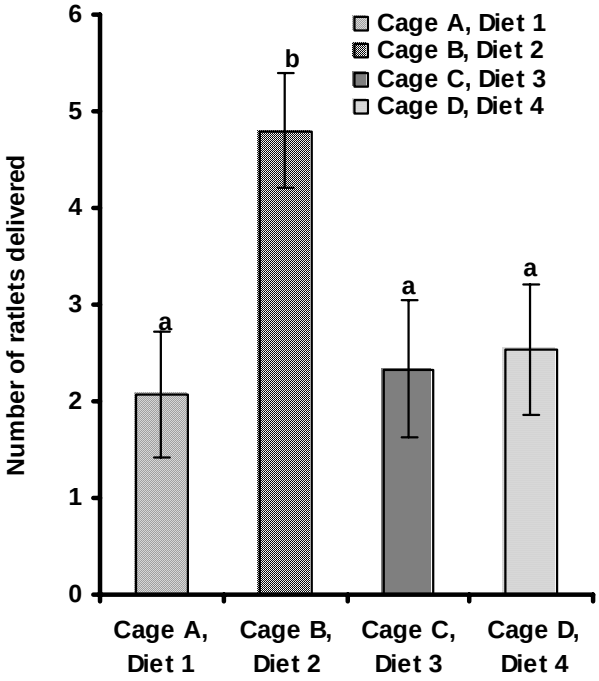

Figure 2: Mean multiple births of groups of rats and corresponding diets. Bars with different letters on top are significantly different (P < 0.05)

DISCUSSION

Diet 2 which contained 20.01 % protein and 32.69 % carbohydrate provide satisfactory nutrient to alleviate the effect of trypanosomes on trypanosome-infected pregnant rats. The abortion rates exhibited by the rats on different diets is corroborated by total the number of ratlets delivered (Figure 2). Langley-Evans and Nwagwu (1998) had earlier reported that protein range above 18 % was adequate for pregnant rats. Adequate nutrition therefore mitigated abortion in the trypanosome infected rats contrary to Ogwu *et al*. (1980), report that trypanosomiasis causes abortion in pregnant animals. From the results of the experiments it was observed that adequate nutrition enhances trypanotolerance. Our study was in agreement with the statement of Katungka-Rwakishaya (1996) that improvement on host's nutrition was important in modulating the severity of patho-physiological effect of trypanosomiasis and also influences the rate of recovery from the infection. The rats fed Diet 2 also had high birth weight. Wynn *et al*. (1991) had noted that birth weight was crucial for the survival of offspring. Our results indicated that adequate concentrations of carbohydrate and protein produced ratlets with higher birth weights. Furthermore, from our results, it was observed that malnutrition in pregnant rats can lead to physiological stresses and low birth weight of offspring. Our finding was similar to those of Galloway *et al*. (1994) who reported low birth weight in malnourished pregnant rats. The observed high multiple births in most of the rats may be attributed to the vitamin E contents of the diets. Vitamin E has been reported to enhance multiple births in pregnant rats (Bieri, *et al*., 1983; Mino *et al*., 1985).

Conclusively, adequate nutrition of trypanosome-infected expectant mother rats promoted trypanotolerance. It also promoted multiple births and enhanced high birth weight of the ratlets which in turn promoted survival of the offspring.

REFERENCES

BIERI, J. G., CORASH, L. and HUBBARD, V. S. (1983). Medical uses of vitamin E. *New English Journal of Medicine, 308:* 1063 -1071.

CUKIERSKI, M. A., SINA, J. L., PRAHALADA, S. and ROBERTSON, R. T. (1991). Effects of seminal vesicle and coagulating gland ablation on fertility in rats. *Reproduction and Toxicology, 5:* 347 - 352.

GALLOWAY, R., JOHN, S. and ANDERSON, M. (1994). Pregnancy nutritional status and its impact on birth-weight. *Sub-Committee on Nutrition (SCN) News,* 11: 6 - 10.

HAGGARTY, P. (2002). Gene-nutrient interactions in pregnancy. *Expression of Interest,* 1: 1 – 6.

HERBERT, W. J. and LUMBSDEN, W. H. R.(1976). *Trypanosoma brucei*. A rapid matching method for estimating the host's parasitemia. *Experimental Parasitology*, 40: 427 - 431.

KATUNGKA-RWAKISHAYA, E. (1996). Interaction between animal nutrition and parasites, studies with experimental trypanosomiasis in sheep. Pages 1 – 9. *In:* LEBBIE, S. H. B. and KAGWINI, E.(Eds). *Small Ruminant Research and Development in Africa*. International Livestock Research Institute (ILRI) Nairobi Kenya.

LANGLEY-EVANS, S. C. and NWAGWU, M. (1998). Impaired growth and increased glucocorticoid-sensitive enzyme activities in tissues of rats fetuses exposed to maternal low protein diets. *Life Science*, *63:* 605 - 615.

LEVINE, S. L., ADAMS, A. J., MURPHY, M. D. and FARRELL, P.M. (1976). Survey of vitamin E status in children. *Pediatrics Research*, 10: 35 6 - 358.

MGBENKA, B. O. and UFELE, A. N. (2004). Effect of vitamin E and selenium dietary supplementation on blood parameters in trypanosome infected rats *(Rattus rattus). Bioresearch,* 2(1): 8 - 17.

MINO, K., KITAGAWA, M. and NAKAGAWA, S. (1985). Red blood cell tocopherol concentrations in a normal population of Japanese children and premature infants in relation to assessment of vitamin E status. *American Journal of Clinical Nutrition*, 41: 631 - 638.

MORRISON, W. I., MURRAY, M. and MCINTYRE, W. I. M. (1981). Bovine trypanosomiasis. Pages 469 – 497. *In:* RISSTIC, M. and MCINTYRE, W. I. M. (Eds), *Disease of Cattle in Tropics.* Martinis Nijhoff Publishers, The Hague.

OGWU, D., NJOKU, C. A. and OSORI, D. I. K. (1980). Effects of experimental *T. vivax* infection on first, second and third trimesters of pregnancy in heifers. *Therioenology,* 25(3): 383 - 398.

STEEL, R. G. D. and TORRIE, J. H. (1990). *Principles and Procedures of Statistics.* McGraw-Hill, New York, 451 pp.

WINDHAM, W. R. (1996). Animal Feed. Pages 1 – 38, (Chapter 4). *In:* Cuniff, P. (Ed). *Official Methods of Analysis of Association of Analytical Chemists (AOAC) International*. 16th Edition, Volume 1. Gaithersburg, Maryland, USA.

WONG, J. (2003). Premature birth link to lack of nutrition before pregnancy. *University of Toronto News Bulletin*. Pages 1 - 3.

WYNN, A., CRAWFORD, M., DOYLE, W. and WYNN, S. (1991). Nutrition of women in anticipation of pregnancy. *Nutrition and Health*, 7: 69 - 88.

IN-VITRO ANTHELMINTIC EFFICACY OF CRUDE AQUEOUS EXTRACTS OF NEEM (*Azadirachta indica*) LEAF, STEM AND ROOT ON NEMATODE

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ABSTRACT

The anthelmintic efficacy of the aqueous extracts of neem (Azadirachta indica) leaf and stem and root barks against the hatching of eggs and the survival of larvae of nematode parasites of small ruminants were studied. The results of the in vitro egg hatch assay showed that the aqueous extracts of the leaf and stem bark produced significant anthelmintic effect through reduction in nematode egg hatch. The reduction in egg hatch was concentration dependent being highest (51 % and 50 % for the leaf and stem bark extracts respectively) at the highest concentration (100 mg/ml) of the extracts but inferior to those produced by albendazole (100 % at 40 mg/ml). Aqueous extracts of the leaf and root bark produced significant reduction in larval survival within 60 minutes at ambient temperature (30 – 35 ºC). Larval death was similar in both extracts and concentration dependent, increasing with increasing concentration of the leaf and root bark extracts. The reduction in larval survival due to the extracts was similar to that produced by albendazole. In general, the aqueous extract of neem leaf was more efficacious in limiting nematode larvae survival and in-vitro egg hatch. The results confirm the folkloric claims that neem has anthelmintic effect and thus suggest its possible usefulness as an anthelmintic.

Keywords: Anthelmintic efficacy, Aqueous extract, Leaf, Stem, Root, Bark, *Azadirachta indica*

INTRODUCTION

Gastrointestinal helminthiasis, especially parasitic gastroenteritis (PGE) constitutes a major set back in the productivity of small ruminant livestock in Nigeria and other tropical countries (Schillhorn van Veen, 1973; Akerejola *et al.,* 1979; Chiejina, 1987; Nwosu *et al.,* 1996 ab). The control of PGE is usually achieved through anthelmintic medication and grazing management in cattle (Chiejina and Emehelu, 1986; Chiejina, 1987) and goats (Nwosu *et al.,* 1996b). However, extensive use of anthelmintics has resulted in drug resistance for many nematodes of sheep, goats and cattle (Jackson, 1993; Pritchard, 1994). Since newer anthelmintics are not being brought into the market, there has been increasing search for novel, environmentally friendly and more sustainable drugs for control of helminthosis. Consequently, several plants traditionally said to have medicinal properties are being investigated for their potency. The plant, Azadirachta indica commonly known as neem is a good candidate for such investigation.

All parts of the plant including the leaves, bark, fruits, seed, oil and sap have been shown to have medicinal properties and contain over ten different active components with azadirachtin as the most potent and widely studied (Nwosu, 2001). Neem is very popular in traditional medicine and neem-derived medicinal preparations have been shown to be efficacious against a wide range of animal diseases including bacterial (ITDG and IIRR, 1996), protozoal and other parasitic conditions (Ekanem, 1978; Ivbijaro, 1987; Khalid *et al.,* 1989; Dwivedi, 1999), promote cutaneous wound healing following mange infestation as well as act as fly-repellent against haematophagous insects (ITDG and IIRR, 1996; Nwosu, 2001). However, it is evident that only a small proportion of the possible medicinal usefulness of the plant in veterinary practice has been exploited (Nwosu, 2001). In this paper, we evaluated the anthelmintic efficacy of aqueous extracts of neem leaf, root and stem barks against in-vitro hatching of eggs and the survival of the larvae of nematode parasites of small ruminants.

MATERIALS AND METHODS

Neem: Fresh leaves, stem and root barks of neem were collected from adult plant within the University of Maiduguri, Nigeria. A botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria, where voucher specimen of the plant was deposited, confirmed the identity of the plant. The stem and root barks were pealed off the plant using a sharp knife while the leaves were hand-cut. The samples were collected into polythene bags and transported to the laboratory for processing.

The neem leaves, stem and root barks were sun-fried for 10 days at 8 hours per day. They were separately ground into powder using a pestle and mortar. The active components were then exhaustively soxhlet extracted using the aqueous method (Mittal *et al.,* 1981; WHO, 1992). The extracts were concentrated in a conical flask maintained overnight at 60˚C.

Table 1: *In-vitro* anthelmintic efficacy of crude aqueous stem bark and leaf extracts of neem (*Azadiracta indica*) against strongylid nematodes of small ruminants

Extract/Drug concentration	No. of samples with egg hatch*	No. of larvae hatched Mean ± S.D.	Range	Reduction in egg hatch (%)
		Water control		
Stem bark extract	50	98 ± 47	23 - 283	0**
100 mg/ml	50	49 ± 28	9 - 133	50.0b
50 mg/ml	50	56 ± 30	11 - 146	42.9b
25 mg/ml	50	67 ± 38	17 - 156	31.6c
		Leaf extract		
100 mg/ml	50	48 ± 21	23 - 102	51.0b
50 mg/ml	50	60 ± 32	18 - 167	38.8b
25 mg/ml	50	74 ± 40	36 - 198	24.5c
		Albendazole		
40 mg/ml	0	0	0	100a
20 mg/ml	25	2 ± 1	1 - 4	98.0a
15 mg/ml	43	3 ± 2	1 - 8	96.9a

**Total number of samples tested = 50; **Larval recovery from water control cultures was used as standard (i.e. 0 % reduction in egg hatch); abc Figures in same column with different superscripts are significantly different (P < 0.05).*

Table 2: Survival of infective nematode larvae following incubation in albendazole or neem leaf and root bark extracts for 60 minutes at room temperature

Extract/Drug concentration	Number of surviving larvae Mean ± S.D.	Range	Percent larval death after 60 minutes
Water control	246 ± 101	117 - 608	0*
	Leaf extract		
100 mg/ml	7 ± 7	1 - 34	97.2a
50 mg/ml	12 ± 8	2 - 38	95.1a
25 mg/ml	16 ± 8	4 - 45	93.5a
	Root bark extract		
100 mg/ml	10 ± 7	2 - 30	95.9a
50 mg/ml	14 ± 8	2 - 38	94.8a
25 mg/ml	18 ± 9	2 - 48	92.7a
	Albendazole		
25 mg/ml	10 ± 7	2 – 40	95.9a
12.5 mg/ml	15 ± 7	6 - 47	93.9a
- 6.25 mg/ml	19 ± 8	10 - 54	92.9a

**Larval survival in water (control) was used as standard (i.e. 0% larval death; abc Figures in same column with different superscripts are significantly different (P<0.05)*

Each extract was diluted in three concentrations (25, 50 and 100 mg/ml). The in-vitro anthelmintic efficacy of the various concentrations of the leaf and stem bark extracts was evaluated against the hatching of nematode eggs using the egg hatch assay (Kelly et al., 1981) while the survival of infective larvae in various concentrations of the leaf and root bark was evaluated by culturing a known number of larvae in the solutions for 60 minutes. In all cases, the proportion of unhatched eggs or dead larvae, at each concentration of the extracts was calculated by relating the number of hatched eggs or surviving larvae to the total number of eggs or larvae cultured (Chiejina, 1984).

Faecal Samples: Faecal samples were collected directly from the rectum of trade sheep and goats during slaughter at the Maiduguri Metropolitan Abattoir. Faecal egg counts were determined by the modified McMaster technique using saturated sodium chloride solution as the floating medium (MAFF, 1977). Only samples with counts of at least 500 eggs per gram of faeces were used in the test. Faecal culture and larval recovery were done using the test tube filter paper method described by Harada and Mori (1955). Nematode eggs and larvae were identified using standard parasitological criteria (MAFF, 1977; Soulsby, 1982).

Albendazole: Albendazole, containing 250 mg of Albendazole B. P. (Sam Pharmaceutical, Nigeria Limited) was used for the study. Three dilutions (25, 12.5 and 6.25 mg/ml) of the drug were used for the study based on previous studies (Onyeyili *et al.,* 2001 ab; Nwosu *et al.,* 2001, 2004).

Data Analysis: The results were summarized as means + Standard Error while differences between the means were analysed at the 5 % level of significance using the one way analysis of variance (ANOVA) (GraphPad Instat, 2000).

RESULTS

The results of the egg hatch assay using the aqueous extract of neem leaf and stem bark are presented in Table 1. Compared to the control (water cultures), both the leaf and stem bark extracts showed significant reduction in nematode egg hatch. In both cases, the reduction in egg hatch was concentration dependent with the greatest reduction in egg hatch at the highest concentrations (100 mg/ml) of the

extracts used in the study. The reduction in egg hatch was similar ($P > 0.05$) with the leaf and stem bark extracts at the various concentrations tested but these were significantly less ($P < 0.05$) effective than albendazole in limiting egg hatch.

The survival of strongyle larvae cultured for 60 minutes in water or various concentrations of albendazole and the aqueous extracts of the leaf and root bark of neem are presented in Table 2. The results showed that both the leaf and root bark extracts caused larval death in a similar manner ($P > 0.05$). Larval survival in both extracts was also concentration dependent, decreasing with increasing concentration of the extracts. The reduction in larval survival caused by the leaf and root bark extracts was similar ($P > 0.05$) to that produced by albendazole at the concentrations used in the study.

DISCUSSION

The results of the study revealed that neem leaf, root and stem bark extracts have some anthelmintic properties against strongylid nematodes of sheep and goats since they significantly limited the hatching of nematode eggs and the survival of nematode larvae cultured in them. These findings confirm the folkloric claims regarding the anthelmintic efficacy of neem against intestinal helminths. Previous studies have shown that neem extracts similarly affected the survival of salmonella (ITDG and IIRR, 1996) Plasmodium and *Trypanosoma* species (Ekanem, 1978; Ivbijaro, 1987; Khalid *et al.,* 1989). That the leaf, stem or root bark extract showed similar effects in reducing egg hatch or larval survival suggest that the extracts contain similar active components possibly in similar concentrations and probably possess the same mechanisms of action. These observations suggest the possible usefulness of the aqueous extracts of neem leaf, stem and root barks in the treatment of nematode infections in both man and domesticated livestock.

At the various concentrations used in this study, the efficacy of the extracts was concentration-dependent as they showed graded effect that was highest at the 100 mg/ml concentration. However, at this concentration (100 mg/ml), the effects of the extracts were significantly ($P < 0.05$) inferior to albendazole in preventing nematode egg hatch but similar in preventing nematode larval survival. The efficacy of the extracts or drug in limiting egg hatch has a direct bearing on the effective penetration of their active components through the eggshell to reach the larvae. This may explain the greater efficacy of the extracts and the drug in limiting larval survival than egg hatch at the various concentrations tested during the study.

Previous studies suggested that the anti-trypanosomal activity of neem extracts was associated with the alkaloids or other ingredients and had effect in-vitro but became degraded or metabolised and thus ineffective when introduced into the host animal (Dwivedi, 1999). Consequently, there is need for further studies, especially in-vivo studies, in order to confirm the present observations as well as purify the extract, determine the active components, their lethal dose, appropriate route of administration as well as ascertain which particular parasite species and/or developmental stage are most susceptible to the effect of the extracts so as to further enhance their anthelmintic usefulness. When these studies have been carried out, a new anthelmintic that will be readily available and acceptable to the rural farmers may be produced. Meanwhile, this study highlights the possible anthelmintic usefulness of neem extracts in the control of nematode infections of small ruminants.

REFERENCES

AKEREJOLA, O. O., SCHILLHORN VAN VEEN, T. W. and NJOKU. C. O. (1979). Ovine and caprine diseases in Nigeria: a review of economic losses. *Bulletin of Animal Health and Production in Africa, 27:* 65 - 70.

CHIEJINA, S. N. (1984). Bovine parasitic gastroenteritis in Nsukka. Worm egg output and herbage infestation following dry season calving and the performance of calves during their first grazing season. *Tropical Veterinarian, 2:* 128 - 132.

CHIEJINA, S. N. (1987). Parasitic gastroenteritis in cattle and small ruminants: pathogenesis, diagnosis and treatment. *Zariya Veterinarian, 2:* 45 - 64.

CHIEJINA, S. N. and EMEHELU, C. O. (1986). Evaluation of three strategic anthelmintic programmes for prophylaxix of parasitic gastroenteritis in cattle in eastern Nigeria. *Tropical Animal Health and Production, 18*: 55 – 63.

DWIVEDI, S. K. (1999). Evaluation of indigenous herbs as anti-trypanosomal agents. Pages 10 – 15. *In:* MATHIAS, E., RANNEKAR, D. V. and MCCORKLE, C. M. (Eds.). *Ethnoveterinary medicine: alternatives for livestock development.* BAIF Development Research Foundation, Pune, India, November 4 – 6, 1997.

EKANEM, J. (1978). Dongo-Yaro, does it work? *Nigerian Medical Journal, 8:* 8 - 10.

GRAPHPAD INSTAT (2000). GraphPad InStat version 3.00 for Windows, GraphPad Software, San Diego, California, USA www.graphpad.com

HARADA, Y. and MORI, O. (1955). A new method for culturing hookworm. *Yonago Acta Medica, 1:* 177 - 179.

IBRAHIM, M. A., NWUDE, N. J., ALIU, V. O. and OGUNSUSI, R. A. (1983). Traditional concept of animal disease and treatment among Fulani herdsmen in Kaduna State Nigeria. *Overseas development Institute Pastoral Development Network Paper*

ITDG and IIRR, (1996). *Ethnoveterinary medicine in Kenya: a field manual of traditional health care practices.* Intermediate Technology Development Group and International Institute of Rural Reconstruction, Nairobi, Kenya, 226 pp.

IVBIJARO, M. F. (1987). Prospects for neem. Pages 525 – 533. *In:* SCHMUTTERE. H. and ASCHER, K. R. S. (Eds.). *Natural pesticides from the neem tree (Azadirachta indica A Juss) and other tropical plants.* German Agency for Technical Co-operation (GTZ), Nairobi, Kenya, July 10 – 15, 1986.

JACKSON, F. (1993). Anthelmintic resistance – the state of play. *British Veterinary Journal, 149:* 123 – 138.

KELLY, J. D., WEBSTER, J. L. and GRIFFIN, D. L. (1981). Resistance to benzimidazole anthelmintics in equine strongyle. *Australian Veterinary Journal, 57:* 163 - 171.

KHALID, S. A., DUDDECK, H. and GOUZALEZ-SIERRA, M. (1989). Isolation and characterization of antimalarial agent of the neem tree, Azadirachta indica. *Journal of Natural Products, 52:* 922 – 926.

MAFF. (1977). *Manual of Veterinary Parasitological Laboratory Techniques.* Technical Bulletin No. 18, Ministry of Agriculture, Fisheries and Food, London. 129 pp.

MITTAL, G. C., AGUWA, C. N., EZEIRU, V. U. and AKUBUE, P. I. (1981). Preliminary pharmacological studies on antivenom action of *Diodia scandens* leaves. *Nigerian Journal of Pharmacy, 12:* 432 - 436.

NWOSU, C. O. (2001). Formulation of neem-derived biopesticides as alternative to persistent organic pollutants (POPS) for the control of pests of livestock. *Paper presented at the Expert Group Meeting on Sustatinable Utilization of Neem Tree in Northern Nigeria*, Dutse Jigawa State, 18 – 19th September, 2001. 8 pp.

NWOSU, C. O., OGUNRINADE, A. F. and FAGBEMI, B. O. (1996a). The seasonal prevalence of *Haemonchus* species in Red Sokoto (maradi) goats in Nigeria. *Veterinary Research Communications, 20:* 367 - 370.

NWOSU, C. O., OGUNRINADE, A. F. and FAGBEMI, B. O. (1996b). Prevalence and seasonal changes in the gastro-intestinal helminths of Nigerian goats. *Journal of Helminthology, 70:* 329 - 333.

NWOSU, C. O., HARUNA, N. K., OGUGBUAJA, V. O., AKINNIYI, A. O. and ONYEYILI, P. A. (2001). Anthelmintic efficacy of ethanolic stem bark extract of *Sacoglottis gabonensis* (Baill) against strongyline nematodes of small ruminants. *Journal of Life and Environmental Sciences, 3:* 127 - 130.

NWOSU, C. O., MOBEE, K. M., GULANI, I. G., IGBOKWE, I. O. and OGUGBUAJA, V. O. (2004). Anthelmintic efficacy of crude aqueous extracts of Garcina kola seed and stem bark against strongylid nematodes of small ruminants in Nigeria. *Nigerian Journal of Parasitology, 25:* 1 - 5.

ONYEYILI, P. A., NWOSU, C. O., AMIN, J. D. and JIBIKE, G. I. (2001a). Anthelmintic activity of crude aqueous extract of Nauclea latifolia stem bark against ovine nematodes. *Fitoterapia, 72:* 12 - 21.

ONYEYILI, P. A., AMIN, J. D., H. I. GAMBO, NWOSU, C. O. and JIBIKE, G. I. (2001b). Toxicity and anthelmintic efficacy of ethanolic stem bark extract of *Nauclea latifolia* sm. *Nigerian Veterinary Journal, 22:* 74 - 82.

PRICHARD, R. K. (1994). Anthelmintic resistance. *Veterinary Parasitology, 54:* 259 – 268.

SCHILLHORN VAN VEEN, T. W. (1973). Small ruminant health problems in Northern Nigeria with emphasis on the helminthiasis. *Nigerian Veterinary Journal, 2:* 26 - 31.

SOULSBY, E. J. L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th edition, Baillere Tindall, London. 809 pp.

WHO (1992). *Quality Control Methods for Medicinal Plants Materials*. World Health Organization, Geneva, 26 pp.

MACROINVERTEBRATE FAUNA OF A TROPICAL FRESHWATER STREAM IN NIGERIA

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ABSTRACT

Macroinvertebrate fauna of Ogbei stream in Anambra state, Nigeria was studied from monthly samples taken from six stations or sites with a benthic scoop net and a serrated cylindrical sampler (SCS) for 12 months (May, 2002 – April, 2003). A total of 11420 macroinvertebrates were collected belonging to 4 classes, 13 orders, 28 families and 50 species. The fauna was dominated numerically by Insecta (98.29 %), Arachnida (0.81%) and Oligochaeta (0.66%). Diptera was the most abundant taxon (42.62%), followed by Odonata (36.89%), Coleoptera (9.76 %) and Hemiptera (8.22 %). Station 3 had the highest percentage of abundance of the macrofauna (28.56 %) followed by station 2 (19.54 %). The highest faunal diversity was recorded in station 6. The macroinvertebrate composition, distribution abundance and diversity were influenced by substrate composition, good water quality and availability of food.

Keywords: Tropical freshwater stream, Macroinvertebrate composition, Abundance, Distribution, Diversity

INTRODUCTION

Aquatic macroinvertebrates are an assemblage of aquatic community represented by members of almost all the invertebrate taxa. Most macroinvertebrate are benthic (benthos), others are planktonic or nektonic or surface water dwellers. Macroinvertebrates have attracted a lot of interest among biologists and environmentalists in view of their importance in food chain of fishes and as long term indicators of water quality. Macroinvertebrates play a crucial role in the transfer of energy from primary producers and detritus to fish (Turcotte and Harper, 1982; McQueen *et al*., 1986; Hanson, 1990). They are also involved in nutrient recycling in aquatic system (Gladden and Smock, 1990) and are used as biological indicators in the assessment of water quality (Rosenberg and Resh, 1993; Crown *et al.*, 1995; Ajao and Fagade, 2002).

Substantial literature on Nigerian aquatic macroinvertebrates are available. Apart from Egborge's (1993) attempt to put together available information on the diversity of aquatic faunal resources of Nigeria, information on the biology and ecology of aquatic macroinvertebrates fauna in different bodies of water in Nigeria are scattered. Egborge (1993) compiled a check list of over 1620 species of invertebrates so far identified in Nigeria, out of which 67.3 % would be considered as macroinvertebrates.

Studies on aquatic macroinvertebrates in some Nigerian water bodies (Victor and Ogbeibu, 1985 and 1991; Ogbeibu and Victor 1989; Ogbeibu and Egborge, 1995; Eyo and Ekwonye, 1995; Ogbeibu and Oribhabor, 2001; Idowu *et al.*, 2004; Odo, 2004) indicate variations in the faunal composition of the water bodies studied. Hence the need to study the macroinvertebrates of a particular water body if the resources of the system are to be managed properly.

Ogbei stream is a major source of water in Nkpologwu community, as a number of human activities take place in and around the stream. Secondly the stream has been proposed for impoundment for domestic and agricultural purposes. Impoundments have been known to create conditions that affect the stability of aquatic life in the system. As there has been no previous scientific study on the stream, this pre-impoundment study was deemed necessary.

A comprehensive scientific study of Ogbei stream aimed at documenting its physico-chemical and biological characteristics is on. The present paper only reports the species composition, abundance, distribution and diversity of the macroinvertebrate fauna of the stream. It is hoped that the comprehensive report on the stream will provide useful pre-impoundment data on which future development and management of the stream resources will be based.

MATERIALS AND METHODS

Study Area: Ogbei stream arises from Umuezeagwu highlands, flows eastwards through Isioji village in Nkpologwu town (5^0 58^1 and 6^0 01^1 N and 7^0 06^1 and 7^0 08^1 E) and stretches through Akpo (in the south) before joining Otalu river to empty into Anambra river (Figure 1). There are two distinct seasons in the area. The rainy/ wet season (April – September) which is sometimes punctuated with a short break of no rain for about two weeks in August. The dry season lasts from October to March and may be punctuated by harmatan (dry and cold north – South wind) between December and January. The temperature of the area ranges between 24 ^{0}C and 32 ^{0}C in the rainy and dry seasons respectively (Emejulu *et al.,* 1992).

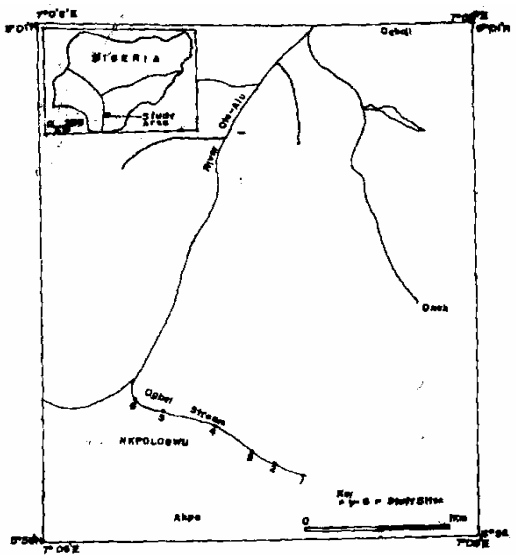

Figure 1: Map of the study area showing the sampling stations

The stream is fringed with riparine vegetation among which are *Pandamus tectorius, Costus afar, Cyathea medullaris*, *Marantochola leucantha*, *Acioa barteri* and *Raffia hookeri.*

A number of activities like bathing, fermenting and sieving of cassava, soaking and washing of tapioca; collection of sand and fishing take place in the stream. A few agricultural activities (farming and gardening) also take place around the stream.

Six sampling stations were selected along the stream based on accessibility, human influence and type of substrate/habitat. The features of which are summarized in Table 1. Each station was about 10 - 12 m apart.

Sample Collections: The macroinvertebrates were sampled with a scoop net and serrated cylindrical sampler. The scoop net was used in the shallow areas along the shore by dragging it with the open end against the water current. It was also used around the aquatic vegetation, in which case the vegetation was disturbed by kicking to dislodge the invertebrates. Sampled specimens were sieved using 0.2 mm and 0.6 mm mesh size sieves and sorted into taxa. The specimens collected were preserved in 10 % formalin and labelled according to stations. Submerged branches of trees, logs and stems were also examined for attached macroinvertebrates.

The SCS was used for quantitative macroinvertebrate sampling. The sampler was pushed into the substratum as fast as possible and the contents of the sampler scooped out into a bucket for washing. The invertebrates were sieved, sorted and preserved in labelled specimen bottles.

All specimens were identified in the laboratory under a dissecting microscope using appropriate taxonomic keys, manuals and texts books (Hutchinson, 1970; Bidwell and Clarke, 1977; Gladden and Smock, 1990; Ajao and Fagade, 2002).

Faunal Diversity and Dominance: Faunal diversity index for taxa richness was analyzed using Shannon-Wiener index (H) (Shannon and Wiener, 1963). General diversity, evenness of distribution (E) were determined according to Krebs (1978). Hutchinson's t-test was used to detect significant differences between general diversity indices (Hutchinson, 1970).

RESULTS

Species Composition and Abundance: A total of 11420 macroinvertebrates collected during the sampling period were identified into 4 classes, 13 orders and 50 species (Table 2). The fauna was dominated numerically by Insecta (98.29%) with 44 species followed by Arachnida (0.81 %) and Oligochaeta (0.66 %) with three species each. The major taxa of the Insecta were Diptera (42.62 %), Odonata (36.89 %) and Coleoptera (9.76 %). *Chironomus transvalensis* (23.58 %), *Coenagrion scitulum* (12.69 %), *Polypedium* sp (11.96 %) and *Libellula* sp (11.15 %) were the prominent species encountered in the collections.

Distribution in Relation to Stations: Oligochaetes represented by *Dero obtusa* and *Dugesia polychroa* were more at station 4 (1.33 %) than in other stations. *D. obtusa* was recorded in all the stations (Table 2).

Decapod crustaceans represented by *Sudanonautes* sp. were found in all the stations but mostly at station 4 (0.44 %). Most of the arachnids were more in station 5 (1.59 %) followed by station 1 (1.53 %). Though *Agronecta aquatica* and *Arrenurus* sp were found in all the stations, *A. aquatica* were most abundant at station 5, (0.84 %) while *Arrenurus* sp were mostly recorded at station 1 (1.02 %).

The major taxa of Insecta were variously distributed in all the stations. Plecopterans were most abundant in station 6 (1.29 %) and Odonata in station 3 (46.26 %). *Coenagrion scitulum, Libellula* sp, *Cordulia* sp were the most important odonatan species present in all the stations. *Hemipteran* spp (Order: Hemiptera) were most abundant at station 6(23.04 %) while *Hydropsyche* sp (Order: Tricoptera) was the most abundant species found particularly in station 4 (1.27 %).

Coleopterans (Order: Coleoptera) were prominent in stations 1 (20.45 %) and 2 (17.11 %). *Gyrinus* sp was the most abundant species contributing 75.74 % of all the coleopterans recorded, followed by *Hydrophilus* sp (20.0 %). Both species were recorded in all the stations. The order Diptera dominated the samples at all the stations and the number of individuals (4867) was significantly higher than those of other orders ($P < 0.05$). The most important diptera recorded in all the stations were *Chironomus transvalensis, Polypedilum* sp and *Strictochiromus* sp. *C. transvalensis* contributed 55.3 % of all the dipterans and were most abundant at station 1 (39.11 %). Generally, most of the macroinvertebrates were recorded in station 3, followed by station 2 (Figure 2).

Table 1: Characteristic features of selected stations

Station	Substrate	Human activities	Vegetation canopy	Light penetration
1	Swampy bank, sand, mud.	Soaking /sieving of cassava, bread fruit, tapioca; washing of clothes, bathing	No	Little
2	Sand, detritus	None	Yes	Little
3	Organic debris/ detritus, sand	Fishing, Lumbering, Palm wine tapping	Yes	Little
4	Sand, mud, stones	Palm wine tapping, lumbering	Yes	Little
5	Sand, mud, organic debris	Palm wine tapping, collection of *Pandamus tectorius* leaves	No	Much
6	Rocky swampy bank, sand and mud	Palm wine tapping	No	Much

Table 2: Abundance of macro-invertebrates in relation to the study stations

TAXA		STATIONS					
	Total No. (%)	**1**	**2**	**3**	**4**	**5**	**6**
OLIGOCHAETA	75(0.66)	3(0.19)	19(0.85)	14(0.43)	24(1.33)	13(1.21)	2(0.14)
Plesiopora	75(0.66)	3(0.19)	19(0.85)	14(0.43)	24(1.33)	13(1.21)	2(0.14)
Naididae	41(0.36)	2(0.13)		8(0.25)	12(0.66)	7(0.65)	2(0.14)
Dero obtusa	41(0.36)	2(0.13)	10(0.45)	8(0.25)	12(0.66)	7(0.65)	2(0.14)
Lumbricidae	31(0.27)	1(0.06)	8(0.36)	6(0.18)	10(0.55)	6(0.56)	-
***Lumbricus* (unidentified)**	31(0.27)	1(0.06)	8(0.36)	6(0.18)	10(0.55)	6(0.56)	-
Dugesiidae	3(0.03)	-	1(0.04)	-	2(0.11)	-	-
Dugesia polychroa	3(0.03)	-	1(0.04)	-	2(0.11)	-	-
CRUSTACEA	28(0.25)	2(0.13)	8(0.36)	8(0.04)	8(0.44)	1(0.09)	1(0.07)
Decapoda	28(0.25)	2(0.13)	8(0.36)	8(0.04)	8(0.44)	1(0.09)	1(0.07)
Sudanonidae	28(0.25)	2(0.13)	8(0.36)	8(0.04)	8(0.44)	1(0.09)	1(0.07)
Sudanonautes sp.	28(0.25)	2(0.13)	8(0.36)	8(0.25)	8(0.44)	1(0.09)	1(0.07)
ARACHNIDA	92(0.81)	24(1.53)	7(0.31)	15(0.46)	7(0.39)	17(1.59)	22(1.49)
Araneae	40(0.35)	8(0.51)	6(0.27)	7(0.21)	3(0.17)	9(0.84)	7(0.47)
Dolomedidae	2(0.02)	-	1(0.04)	1(0.03)	-	-	-
Dolomedes fimbriatus	2(0.02)	-	1(0.04)	1(0.03)	-	-	-
Agronectidae	38(0.33)	8(0.51)	5(0.22)	6(0.18)	3(0.17)	9(0.84)	7(0.47)
Agronecta aquatica	38(0.33)	8(0.51)	5(0.22)	6(0.18)	3(0.17)	9(0.84)	7(0.47)
Hydrachnella	52(0.46)	16(1.02)	1(0.04)	8(0.25)	4(0.22)	8(0.75)	15(1.02)
Arrenuridae	52(0.46)	16(1.02)	1(0.04)	8(0.25)	4(0.22)	8(0.75)	15(1.02)
Arrenurus sp.	52(0.46)	16(1.02)	1(0.04)	8(0.25)	4(0.22)	8(0.75)	15(1.02)
INSECTA	11225(98.29)	154(198.15)	2198(98.48)	3225(98.87)	1769(97.84)	1041(97.11)	1451(98.31)
Plecoptera	47(0.41)	4(0.25)	4(0.18)	7(0.21)	12(0.66)	1(0.09)	19(1.29)
Perlidae	47(0.41)	4(0.25)	4(0.18)	7(0.21)	12(0.66)	1(0.09)	19(1.29)
Dinocras sp	39(0.34)	4(0.25)	-	6(0.18)	11(0.61)	1(0.09)	17(1.15)
Neoperla sp	8(0.07)	-	4(0.18)	1(0.03)	1(0.06)	-	2(0.14)
Odonata	4213(36.89)	382(24.33)	797(35.71)	1509(46.260)	703(38.88)	401(37.41)	421(28.52)
Aeshnidae	158(1.38)	13(0.83)	22(0.99)	50(1.53)	8(0.44)	20(1.87)	45(3.05)

Table 2: Abundance of macroinvertebrates in relation to the study stations (continues)

Aeshna sp. Fabricus	158(1.38)	13(0.83)	22(0.99)	50(1.53)	8(0.44)	20(1.87)	45(3.05)
Corduliidae	437(3.8)	64(4.08)	74(3.32)	124(3.80)	66(3.65)	53(4.94)	56(3.79)
Cordulia sp	437(3.8)	64(4.08)	74(3.32)	124(3.8)	66(3.65)	53(4.94)	56(3.79)
Macromiidae	27(0.24)	18(1.25)	4(0.18)	-	3(0.17)	1(0.09)	1(0.07)
Macromia Africana	27(0.24)	18(1.25)	4(0.18)	-	3(0.17)	1(0.09)	1(0.07)
Gomphidae	159(1.39)	11(0.07)	8(0.36)	34(1.04)	32(1.77)	28(2.61)	46(3.12)
Gomphus sp.	50(0.44)	-	2(0.09)	16(0.49)	10(0.55)	11(1.03)	11(0.75)
Haginus sp.	109(0.95)	11(0.07)	6(0.27)	18(0.85)	22(1.22)	17(1.59)	35(2.37)
Libellulidae	1862(16.30)	199(12.68)	433(19.40)	603(18.49)	270(14.93)	172(16.04)	185(12.53)
Libellula sp.	1273(11.15)	167(10.64)	312(13.998)	452(13..86)	155(8.57)	100(9.33)	87(5.89)
Sympetrum sp.	510(4.47)	30(1.91)	116(5.2)	143(43.8)	95(5.25)	47(4.38)	79(5.35)
Tetragoneuria sp.	79(0.69)	2(0.13)	5(0.22)	8(0.25)	20(1.11)	25(2.33)	19(1.29)
Coenagrionidae	1570(13.75)	77(4.90)	256(11.49)	698(21.40)	324(17.92)	127(0	88(5.96)
Coenagrion scitulum	1449(12.69)	42(2.68)	234(10.48)	680(20.85)	301(16.65)	114(10.63)	78(5.28)
Ischnura sp.	121(1.06)	35(2.23)	22(0.99)	18(0.55)	23(1.27)	13(1.21)	10(0.68(
Hemiptera	939(8.22)	157(10)	140(6.27)	131(4.02)	74(4.09)	97(9.05)	340(23.04
Belostomatidae	1(0.01)	-	-	-	-	1(0.09)	-
Poissonia sp.	1(0.01)	-	-	-	-	1(0.09)	-
Gerridae	310(2.71)	59(3.76)	52(2.33)	40(1.23)	12(0.66)	46(4.29)	101(6.84)
Geris lacustris	233(2.04)	38(2.42)	51(2.58)	33(1.01)	12(0.66)	24(2.24)	75(5.08)
Naboandelus sp.	77(0.67)	21(1.34)	1(0.04)	7(0.21)	-	22(2.24)	26(1.76)
Hydrometridae	56(0.49)	29(1.85)	2(0.09)	2(0.06)	9(0.50)	4(0.37)	10(0.68)
Hydrometra sp.	56(0.49)	29(1.85)	2(0.09)	2(0.06)	9(0.50)	4(0.37)	10(0.68)
Mesoveliidae	251(2.20)	28(1.78)	49(2.20)	5(0.15)	4(0.22)	8(0.75)	157(10.64)
Microvelia sp.	251(2.20)	28(1.78)	49(2.20)	5(0.15)	4(0.22)	8(0.75)	157(10.64)
Naucoridae	25(0.22)	4(0.25)	2(0.09)	5(0.15)	8(0.44)	2(0.19)	4(0.27)
Naucoris cimicoides	25(0.22)	4(0.25)	2(0.09)	5(0.15)	8(0.44)	2(0.19)	4(0.27)
Nepidae	280(2.45)	31(1.97)	33(1.48)	78(2.39)	35(1.94)	36(3.36)	67(4.54)
Nepa apiculata	107(0.94)	1(0.06)	13(1.58)	37(1.13)	15(0.83)	15(1.40)	26(1.76)
Lacotrephes sp.	164(1.44)	27(1.72)	20(0.90)	39(1.20)	20(1.11)	20(1.87)	38(2.57)
Ranatra fusca	9(0.08)	3(0.19)	-	2(0.06)	-	1(0.09)	3(0.20)
Notonectidae	16(0.14)	6(0.38)	2(0. 09)	1(0.03)	6(0.03)	-	1(0.07)
Notonecta sp.	16(0.14)	6(0.38)	2(0. 09)	1(0.03)	6(0.03)	-	1(0.07)
Neuroptera	1(0.01)	-	1(0.04)	-	-	-	-
Sialidae	1(0.01)	-	1(0.04)	-	-	-	-
Sialis sp.	1(0.01)	-	1(0.04)	-	-	-	-
Tricoptera	40(0.35)	-	6(0.27)	4(0.12)	24(1.33)	2(0.19)	4(0.27)
Hydropsychidae	30(0.26)	-	3(0.13)	1(0.03)	23(1.27)	1(0.09)	2(0.14)
Hydropsyche sp.	30(0.26)	-	3(0.13)	1(0.03)	23(1.27)	1(0.09)	2(0.14)
Hydroptilidae	7(0.06)	-	2(0.09)	2(0.06)	-	1(0.09)	2(0.14)
Ochrotrichia sp.	7(0.06)	-	2(0.09)	2(0.06)	-	1(0.09)	2(0.14)
Philopotamidae	3(0.03)	-	1(0.04)	1(0.03)	1(0.06)	-	-
Philopotamus sp.	3(0.03)	-	1(0.04)	1(0.03)	1(0.06)	-	-
Orthoptera	2(0.02)	-	-	1(0.03)	1(0.06)	-	-
Gryllotalpidae	2(0.02)	-	-	1(0.03)	1(0.06)	-	-
Gryllotalpa robusta	2(0.02)	-	-	1(0.03)	1(0.06)	-	-

Table 2: Abundance of macroinvertebrates in relation to the study stations (continues)

Coleoptera	1115(9.76)	321(20.45)	382(17.11)	150(4.60)	102(5.64)	43(4.01)	117(7.93)
Chrysomelidae	11(0.10)	-	-	-	-	2(0.19)	9(0.61)
Donacia sp.	11(0.10)	-	-	-	-	2(0.19)	9(0.61)
Dytiscus sp.	3(0.03)	-	1(0.04)	2(0.06)	-	-	-
Dytiscidae	3(0.03)	-	1(0.04)	2(0.06)	-	-	-
Hydrophilidae	223 (1.95)	56(3.57)	41(1.84)	23(0.71)	41(2.27)	25(2.33)	37(2.51)
Hydrophilus sp.	223(1.95)	56(3.57)	41(1.84)	23(0.71)	41(2.27)	25(2.33)	37(2.51)
Gyrinidae	878(767)	265(16.88)	340(15.23)	125(3.83)	61(3.37)	16(1.49)	71(4.81)
Gyrinus sp.	878(7.67)	265(16.88)	340(15.23)	125(3.83)	61(3.37)	16(1.49)	71(4.81)
Diptera	4867(42.62)	677(43.12)	868(38.98)	1423(43.62)	853(47.18)	497(46.36)	549(37.2)
Thaumalaidae	5(0.04)	3(0.19)	-	-	2(0.11)	-	-
Thaumalia sp.	5(0.04)	3(0.19)	-	-	2(0.11)	-	-
Tabanidae	3(0.03)	-	1(0.04)	-	1(0.06)	1(0.09)	-
Tabanus sp.	3(0.03)	-	1(0.04)	-	1(0.06)	1(0.09)	-
Ceraptogonidae	1(0.01)	-	-	-	-	1(0.09)	-
Culicoides sp.	1(0.01)	-	-	-	-	1(0.09)	-
Stratiomidae	11(0.10)	1(0.06)	3(0.03)	1(0.03)	-	2(0.19)	4(0.27)
Stratiomyia sp.	11(0.10)	1(0.06)	3(0.03)	1(0.03)	-	2(0.19)	4(0.27)
Tipulidae	32(0.28)	-	-	4(0.12)	8(0.44)	-	20(1.36)
Tipula sp.	20(0.18)	-	-	3(0.09)	5(0.28)	-	12(0.81)
Megistocera longipinnis	12 (0.11)	-	-	1(0.03)	3(0.17)	-	8(0.54)
Simulidae	1(0.01)	-	-	-	-	-	1(0.07)
Simulium sp.	1(0.01)	-	-	-	-	-	8(0.54)
Syrphidae	13(0.11)	13(0.83)	-	-	-	-	-
Eristalis	13(0.11)	13(0.83)	-	-	-	-	-
Chironomidae	4801(42.04)	660(42.04)	864(38.71)	1418(43.47)	842(46.76)	493(45.99)	524(35.5)
Chironomous transvalensis	2693(23.58)	614(39.11)	520(23.30)	477(14.62)	416(23.01)	385(35.91)	281(19.04)
Polypedilium sp.	1366(11.96)	26(1.66)	286(12.81)	470(14.41)	311(17.20)	86(8.02)	187(12.67)
Strictochiromous sp.	720(6.30)	12(0.76)	54(2.42)	470(14.41)	110(6.08)	18(1.68)	56(3.79)
Tarnytarsus sp.	22(0.19)	8(0.51)	4(0.18)	10.03)	5(0.28)	4(0.37)	-
Hymenoptera	1(0.01)	-	-	-	-	-	1(0.07)
Mymaridae	1(0.01)	-	-	-	-	-	1(0.07)
Caraphractus sp.	1(0.01)	-	-	-	-	-	1(0.07)
Total	**11420(100)**	**1570(100)**	**2232(100)**	**3262(100)**	**1808(100)**	**1072(100)**	**1476(100)**

- *The figures in parenthesis show percentage relative abundance of the species*

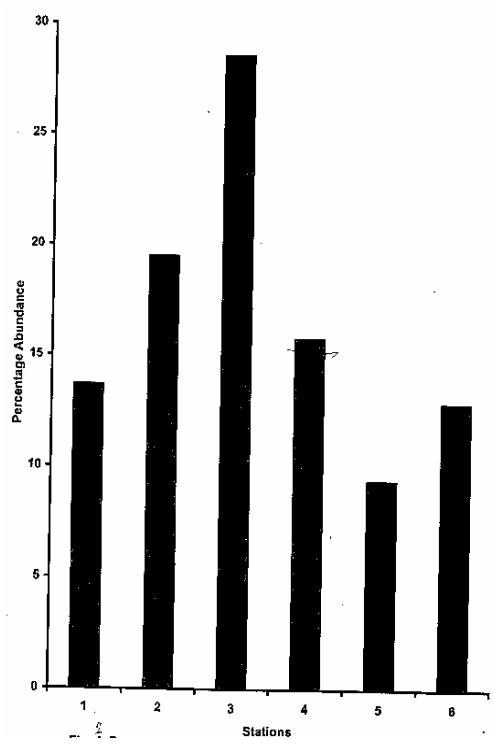

Figure 2: percentage abundance of macroinvertebrate in the stations

Monthly and Seasonal Variations of Macroinvertebrates: Table 3 shows the monthly and seasonal variations of the major taxa of the macroinvertebrates collected. Most invertebrates (13.9 %) were collected in the month of March; 11.0 % was recorded in May, while the least number of specimens was collected in August (3.3%). Crustaceans, odonatans and dipterans were most abundant in March, while hemipterans and coleopterans occurred mostly in December. The tricopterans occurred sporadically in September, December, January and March.

Generally, more macroinvertebrates were collected during the dry season (6321; 55.35 %) than during the rainy season (5099; 44.65 %). Apart from Neuroptera and Plecoptera all other taxa were recorded more during the dry season than in the rainy season (Table 3).

Faunal Diversity and Dominance: Table 4 shows the faunal diversity and dominance indices for the six stations. Species richness (Margalefs' index) was highest at station 5 (11.88) followed by station 6 (11.67).

Station I had the least species richness (10.013). Shannon-Wiener diversity index (H) was highest at station 6 (1.24) and was significantly different from other stations ($P < 0.05$). The diversity indices were statistically similar for stations 1 and 2 (1.038 and 1.035) respectively. The maximum species diversity (Hmax) was highest in station 3 (1.602), while stations 4 and 6 had the same diversity index of 1.580 respectively.

The equitability index (E) indicated that station 6 had highest evenness of distribution (0.78) while station 3 had the least evenness of distribution (0.632). Simpson's dominance index (D) was highest in station 6(11.813), followed by station 4 (7.804) and Station I had the lowest value (4.088).

DISCUSSION

Species Composition and Abundance: Species composition, abundance and distribution of aquatic macroinvertebrates are influenced by a number of factors including the physico-chemical, geomorphic and biotic factors of the aquatic ecosystem. Bishop (1973), Dance and Hynes (1980) asserted that water quality and food supply were the major factors governing the abundance and distribution of macroinvertebrate fauna in aquatic environment. Wildish (1977) considers food supply, supply of colonizing larvae and interspecific competition as the major biotic factors that determine the community composition, biomass and productivity of macrofauna in marine and estuarine environment. Ogbei stream supports a diverse assemblage of macroinvertebrate fauna. The number of taxa recorded (50 species) far exceeds what have been reported from similar biogeographical zones (Victor and Ogbeibu, 1985, 1991; Ogbeibu and Oribhabor, 2001; Odo, 2004) probably because of the favourable conditions in the stream. The variable substrate composition (sand, mud, silt, debris/organic detritus, stones etc) provided different microhabitats for the diverse groups of the fauna. Organisms cannot survive in an environment without adequate food for the organisms' survival and growth.

Besides, it is possible that the cassava, bread fruits and tapioca soaked and washed in the stream could contribute to the food resource for some macroinvertebrates.

Ibemenuga (2005) reported on the good water quality of the stream, a factor which definitely contributes to the survival, growth and abundance of the macroinvertebrates in the stream. In terms of relative abundance, dipterans were the most dominant fauna. The dominance of the dipterans with respect to number of individuals and species is in agreement with the reports of Bidwell and Clarke (1977), Townsend (1983), Sharma *et al.* (1993), Ogbeibu and Oribhabor (2001) and Ogbeibu (2001). The dominance of dipterans in the system as in other aquatic ecosystems may be attributed to their morphological and physiological adaptations to the various habitats, availability of food and sustained reproduction (Mbah and Vijime, 1989; Umeham, 1989). Chironomids which were the most abundant dipterans are known to colonize all kinds of environments including polluted waters. This is due to their ability to extract oxygen from water of very low oxygen concentration.

Distribution: Water velocity, immediate substratum of occupation and animals are very closely related. Variations in water velocity can occasion variations in stream habitats.

Table 3: Monthly /seasonal distribution of major macroinvertebrates taxa of Ogbei stream, Nigeria (May 2002 – April 2003)

Taxa	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	Range in monthly samples	Season	
														Rainy	Dry
Oligochaeta	10	7	8	1	9	5	4	5	7	9	9	3	1 - 10	36	39
Crustacea	5	3	4	1	-	2	2	-	-	5	6	-	1- 6	13	15
Arachnida	9	4	1	-	11	3	10	18	4	4	9	3	1-19	35	57
Insecta	1227	910	869	374	505	745	704	1144	1121	935	1561	1130	130 -1561	5051	6210
Plecoptera	1	5	8	2	12	2	7	3	3	1	1	2	1-8	30	17
Odonata	598	314	297	177	180	285	178	217	500	302	722	443	122 - 722	2009	2204
Hemiptera	68	41	71	30	47	55	80	336	41	46	80	44	30-336	301	638
Neuroptera	-	-	1	-	-	-	-	-	-	-	-	-	0-6	1	-
Orthoptera	-	-	-	-	-	-	-	1	-	-	1	-	0 - 1	-	2
Coleoptera	74	121	108	32	25	67	120	198	119	163	49	39	32 - 198	399	716
Diptera	486	429	384	133	238	336	319	382	433	423	703	601	133 - 703	2271	2596
Hymenoptera	-	-	-	-	-	-	-	-	-	-	1	-	0 - 1	-	1
Tricoptera	-	-	-	-	3	-	-	7	25	-	4	-		4	36
Total	**1251**	**924**	**888**	**377**	**523**	**752**	**730**	**1169**	**1132**	**953**	**1585**	**1136**	**377 – 1585**	**5099**	**6321**
	(11.0)	**(8.1)**	**(7.8)**	**(3.3)**	**(4.6)**	**(6.6)**	**(6.4)**	**(10.2)**	**(9.9)**	**(3.3)**	**(13.9)**	**(9.9)**		**(44.6)**	**(55.4)**

Numbers in parenthesis represent the percentages

Table 4: Diversity of macroinvertebrate in the study stations of Ogbei stream (May 2002 – April 2003)

	STATIONS					
	1	**2**	**3**	**4**	**5**	**6**
Number of taxa	33	39	40	38	37	
Number of individuals	1570	2232	3262	1808	1072	1476
Margalef's index (d)	10.013	11.348	11.100	11.359	11.88	11.67
Shannon Wiener index(H)	1.038	1.035	1.013	1.084	1.073	1.24
Maximum species Diversity (H max)	1.519	1.591	1.602	1.580	1.568	1.58
Equitability (E)	0.684	0.655	0.632	0.687	0.685	0.78
Dominance (D)	4.088	7.660	7.623	7.804	6.097	11.81

According to Connell (1975) distribution of animals among available habitats are generally mediated by food availability, predation intensity and tolerance of physico-chemical conditions of the system.

Oligochaetes which are often associated with silt and muddy substrata rich in organic matter were mostly encountered in station 4 rich in organic matter upon which they feed. This agrees with Petr (1972), Carter (1978), Ogbeibu and Egborge (1995) who reported the dominance of oligochaetes in muddy substratum rich in organic matter. Milbrink (1973, 1975), Janasson and Thorhauge (1976) and Mbagwu (1990) all reported that oligochaetes hid their cocoons in the deeper sediment strata for protection against predation and bacterial attack. The presence of aquatic insects is characteristic of most temperate and tropical freshwaters. They usually form a major part of the fauna in a natural stream. Aquatic insects were represented by various taxa. Hemiptera occurred mostly in station 6 where the water velocity was slow. Although Ogbeibu and Akinya (2001) reported that stones were usually devoid of insects, *Hydrophilus* sp. (Coleoptera) was found more in station 4 attached to stones/hard substrates. This is an adaptation to avoid being swept off by the current.

Diptera was largely represented by chironomids. *Chironomus transvalensis* requires a substratum with high organic matter content. According to Petr (1972) *C. transvalensis* prefers muddy bottom to sandy substrate, hence they occurred in large numbers in all the stations with muddy bottom.

The presence of sand and macrophytes in almost all the stations, debris/detritus in station 2, 3 and 5, stones in stations 4 and 6; mud in all the stations except station 3 provide adequate habitat conditions for the high faunal abundance in stations 1, 2, 4, 5 and 6.

Seasonal Variation: Among the factors that influence the distribution and abundance of macroinvertebrate fauna in the stream is depth. Depth is a prime factor in aquatic environment. Apart from plecoptera and Neuroptera, the populations of the other taxa were consistently higher in the dry season than in the rainy season months when the level of water was high. The report of Odo (2004) that coleoptera and hemiptera were more abundant in Anambra River during the dry season than in the rainy season agrees with our observations in Ogbei stream. Faunal reduction in the rainy season may be due to spate. Sudden torrential rains cause rapid and sudden rise in the rate of flow beyond the extent animals can maintain their foothold may explain the low numbers of macroinvertebrates recorded in the rainy season months. Petr (1970), Turcotte and Harper (1982) report that the densities of benthos in Black Volta River (Ghana) and Andean stream (Ecuador) respectively were greater at the end of dry season than in the rainy season. They also attributed this to spate during the rainy season. Reduced invertebrate abundance in tropical streams has also been attributed to scourging discharge (Stout, 1982).

In principle primary productivity in aquatic system tends to increase during the dry season as a result of increase in light availability. Such a situation may favour many benthic macroinvertebrates that rely on algae for food. Food availability during the dry season may bring about increase in abundance of the fauna. However, Algermeir and Karr (1983) reported low population of benthic invertebrates in some streams in central panama during the dry season. This may be due to the short duration of dry season (3 - 4 months) (Algermeir and Karr, 1983) in central panama as against 5 – 6 months in our study area.

The developmental rate of small macroinvertebrates can generally cause faunal fluctuations. Most aquatic invertebrates are benthic only at the larval stages. Their adult lives are spent outside of water. This is true of all the macroinvertebrates collected except the crustaceans and water scorpions. The life cycles of some of the fauna could also account for seasonal difference in the population size.

Diversity: The general diversity (Shannon-Wiener index, H) differed slightly among the stations and was highest in station 6 as a result of the high value of Equitability index (E). According to Victor and Ogbeibu (1985) and Ogbeibu (2001), the higher the equitability, the higher the diversity. An assessment of community and ecosystem stability using overall diversity showed station 6 as the most complex and stable station. The overall diversity may be the product of all spatial and temporal changes affecting the community (Ogbeibu an Oribhabor, 2001).

REFERENCES

AJAO, E. A. and FAGADE, S. O. (2002). The benthic macro-fauna of Lagos Lagoon. Pages 1 – 27. *Paper presented at the international Conference in Honour of Prof. F. M. A. UKOLI.* 14 – 17th January 2002 at the Department of Zoology, University of Ibadan, Ibadan.

ALGERMEIER, P. L and KARR, J. R. (1983). Fish communities along environmental gradients in a system of tropical streams. *Environmental Biology of Fish, 9 (2):* 117 – 135.

BIDWELL, A. and CLARKE, N. V. (1977). The invertebrate fauna of lake Kainji, Nigeria. *The Nigeria Field, 42(3):* 104 – 109.

BISHOP, O. N. (1973). *Limnology of a small Malayan River.* Sungai Gomback W. J. Junk. The Hague. 485 pp.

CARTER, J. J. (1978). Diversity in tropical forest and coral reefs. *Science, 199:* 1302 – 1310.

CONNELL, J. H. (1975). Some mechanisms producing structure in natural communities: a model and evidence from field experiments. Pages 460 – 490. *In:* CODY, M. L. and DIAMOND, J. M. (eds.). *Geology and Evolution of Communities.* Belknap Press, Cambridge.

CROWN, J. E., CHESSMAN, E. C., MCEVOY, P. K. and WRIGHT, I. A. (1995). Rapid assessment in the Nepean river and Blue Mountains. *Australian Journal of Geology, 20(1):* 130 – 141.

DANCE, K. W. and HYNES, H. B. B. (1980). Some effects of Agricultural land use on stream insect communities. *Environmental Pollution Series A, 22:* 19 – 28.

EGBORGE, A. B. M. (1993). *Biodiversity of aquatic fauna of Nigeria.* National Resources Conservation Council, Abuja. 173 pp.

EMEJULU, C. A., OKAFOR, F. C. and EZIGBO, J. C. (1992). Gastropod fauna of Agulu lake and adjoining freshwater system in Anambra State. *Journal of Aquatic Sciences, 7:* 35 – 38.

EYO, J. E. and EKWONYE, U. C. (1995). The macroinvertebrate fauna of pools in the flood plain (fadama) of the Anambra river, Nigeria. *Freshwater Forum, 5(3):* 160 – 162.

GLADDEN, J. E. and SMOCK, L. A. (1990). Macroinvertebrate and production in the flood plains of two lowland headwater streams. *Freshwater Biology, 24:* 533 – 545.

HANSON, J. M. (1990). Macroinvertebrate size distribution of two contrasting macrophyte communities. *Freshwater Biology, 24:* 481 – 491.

HUTCHINSON, K. (1970). A test for comparing diversities based on Shannon formula. *Journal of Theoretical Biology, 29:* 151 – 154.

IBEMENUGA, K. N. (2005). The Ecology of Macro-invertebrate fauna of Ogbei stream in Nkpologwu, Aquata Local Government Area of Anambra State, Nigeria. MSc Project Report, University of Nigeria, Nsukka. 105 pp.

IDOWU, R. T., INYANG, N. M. and MGBENKA, B. O. (2004). Macroinvertebrate studies of the littoral region in an arid zone lake (Lake: Alau), Maiduguri, Boronu State, Nigeria. *Pecop Resources Nigeria Limited, 1(1):* 1 - 12.

JANASSON, O. and THORHAUGE, E. (1976). Population dynamics of *Poramothrix harmmoniensis* in the profundal of lake Esrom with special reference to environmental and competitive factors. *Oikos, 27:* 193 – 203.

KREBS, C. J. (1978). *Ecology: The Experimental Analysis of Distribution and Abundance.* 2nd Edition. Harper and Raw Publishers, New York, 678 pp.

MBAH, C. E. and VIJIME, C. G. (1989). Preliminary Taxonomic survey of fresh water insects from northern Nigeria. *Journal of Aquatic Sciences, 4:* 27 – 39.

MBAGWU, I. G. (1990). Vertical distribution of macrobenthic invertebrates in the profundal sediment of the impoundment of the dammed river Danube (Alterworth, Lower Australia). *Journal of Aquatic Sciences, 5:* 19 – 25.

McQUEEN, D. T., POST, J. R. and MILLS, E. I. (1986). Trophic relationships in freshwater pelagic ecosystems, *Journal of Fisheries and Aquatic Sciences, 43:* 1571 – 1581.

MILBRINK, G. (1973). On the vertical distribution of oligochaetes in lake sediment. *Reports of Institute of Freshwater Research (Drottingholm), 53:* 34 – 50.

MILBRINK, G. (1975). Population biology of the cestode *Caryophyllaceous laticep* (Pellas) in Bream, Alabanis *brama* (L) and the feeding of fish on oligochaetes. *Institute of Freshwater Research Report, 54:* 36 – 51.

ODO, G. E. (2004). *Studies on the ecology of macroinvertebrate fauna as fish food in Anambra River Basin, Nigeria.* PhD Thesis, University of Nigeria Nsukka, 172 pp.

OGBEIBU, A. E. and VICTOR, R. (1989). The effects of road and bridge construction on the bank root macrobenthic invertebrates of a southern Nigerian stream. *Environmental Pollution, 56:* 85 – 100.

OGBEIBU, A. E. and EGBORGE, A. B. M. (1995). Hydrobiological studies of water bodies in the Okomo forest Reserve (sanctuary) in southern Nigeria. 1. Distribution and density of the invertebrate fauna. *Tropical Freshwater Biology, 4:* 1 – 27.

OGBEIBU, A. E. and ORIBHABOR, B. J. (2001).The ecological impact of stream regulation using benthic macro invertebrates as indicators. *Journal of Aquatic Sciences, 16(2):*132 – 138.

OGBEIBU, A. E. (2001). Distribution, density and diversity of dipterans in a temporary pond in Okomo forest reserve, southern Nigeria. *Journal of Aquatic Sciences, 16:* 43 – 52.

OGBOGU, S. S. and AKINYA, T. O. (2007). Distribution and abundance of insect orders in relation to habitat types in Opa stream reservoir system, Nigeria. *Journal of Aquatic Sciences, 17(1):* 27 – 30.

PETR, T. (1970). The bottom fauna of the rapids of the Black Volta River in Ghana. *Hydrobiolgia, 36:* 399 - 418.

PETR, T. (1972). Benthic fauna of a tropical man – made lake (Volta lake, Ghana, 1965 - 1968). *Archives fur Hydrobiologia,* 70*:* 484 – 533.

ROSENBERG, D. M. and RESH, V. H. (1993). *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman and Hall, New York, 488 pp.

SHANNON, C. E. and WIENER, W. (1963). *The Mathematical Theory of Communication.* University of Illinois press, Urban, 117 pp.

SHARMA, S., MARTHURM, R., SAXENA, M. N. and KAUSHIUK, S. (1993). Geological studies of insect communication in the Saonk, Asum and Kunuwari River of Madhya Pradesh, India. *Tropical Freshwater Biology,* 3: 287 – 294.

STUNT, R. J. (1982). Effect of harsh environment on the life history patterns of two species of tropical aquatic Hemiptera (Family, Neuioridae). *Ecology, 63:* 75 – 83.

TOWNSEND, C.R., HILDREW, A. G. and FRANCIS, J. (1983). Community structure in some South English streams. The influence of physico-chemical factors. *Freshwater Biology, 13:* 521 - 544.

TURCOTTE, P. and HAMPER, P. P. (1982). The macro invertebrate fauna of a small Andream stream. *Freshwater Biology, 12:* 411 – 419.

UMEHAM, S. N. (1989). Some aspects of the physico-chemical limnology of Lake Chad (southern sector). *Journal of Aquatic Sciences, 4:* 23 – 26.

VICTOR, R. and OGBEIBU, A. E. (1985). Macrobenthic invertebrates of a stream flowing through farmlands in southern Nigeria. *Environmental Pollution, 39:* 337 – 349.

VICTOR, R. and OGBEIBU, A. B. (1991). macroinvertebrate communities on the emotional biotope of and urban stream in Nigeria. *Tropical Zoology, 4:* 1 – 12.

WILDISH, D. J. (1977).Factors controlling marine and estuarine sublittoral macrofauna. *Helgolander Wiss Meeresunters, 30:* 445 – 454.

EFFECTS OF CEFTRIAXONE ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF TURKEY

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ABSTRACT

Short-term effects of ceftriaxone on haematological and biochemical parameters of Nigerian local turkey poults were studied. The pre-treatment blood and serum samples were collected and the weight of animals taken before the administration of body weight for a period of 4 days. The animals were weighed daily. The results showed that eosinophilia was significantly increased (P < 0.05) and total bilirubin deceased significantly (P < 0.05). Furthermore, there was significant decrease in chloride ion (P < 0.05) and increase in bicarbonate ion (P < 0.05). Other indices of haematology, liver function test and electrolyte titration were normal (P < 0.05). Ceftriaxone caused eosinophilia in treated samples (2.2 ± 0.45^a) as compared to pre-treated samples (1.6 ± 0.89^b). Total bilirubin in the post-administration samples (13.5 ± 1.05^a) was decreased in comparison with pre-administration samples (14.82 ± 0.72^b). Chloride ion decreased in the treated samples (86.6 ± 8.11^a) when compared with untreated samples (98.4 ± 2.88^b). Bicarbonate ion increased (24.8 ± 1.79^a) in the experimental samples when compared to control (24.4 ± 1.34^b). Conclusively, the short term administration of ceftriaxone may cause eosinophilia, hypobilirubinaemia, hypochloraemia and increased bicarbonate ion which may be positive response to hypochloraemia.

Keywords: Haematology, Biochemical Parameters, Ceftriaxone, Turkey

INTRODUCTION

The cephalosporins (β-lactam antibiotics) are weak organic acids, due to their low pka values, and are predominantly ionized in the blood plasma (Baggot, 2001). Protein binding of individual cephalosporins ranges from 15 % to over 80 %. The presence of food in the stomach decreases the systemic availability of oral cephalosporins (Baggot, 2001).

Ceftriaxone is a parenteral third generation cephalosporin, which has stronger activity against enterobacteriaceae, including penicillinase producing strains (Hubber, 1988). Ceftriaxone has longer duration of action because of extensive protein binding permitting once or at most twice daily dosing (Prescott, 2000) and is eliminated equally in urine and bile (Tripathi, 2003). It penetrates poorly into transcellular fluid e.g. cerebrospinal fluid (CSF), synovial fluid and aqueous humor but has half-life of 0.8 h in dog (Baggot, 2001). The overall effectiveness of therapy with cephalosporins is largely influenced by the aggregate time, though not necessarily continuous during which effective plasma concentrations are maintained (Baggot, 2001). The protein binding of individual drugs with the same class of chemical e.g. cephalosporins can differ widely in disease conditions like hepatic cirrhosis, liver abscess, acute pancreatitis, gastrointestinal disease, nephrotic syndrome and chronic renal failure (Baggot, 2001). Gastrointestinal disturbances including severe colitis were noted with ceftriaxone administration in mare (Gardner and Aucoin, 1994), probably because of its billiary excretion (Prescott, 2000). Saganuwan (2006) also reported hypoproteinaemia, and hyperkalaemia in Nigerian mongrel dog. Intravenous or intramuscular, 25 mg/kg body weight of ceftriaxone, 12-24 hourly is sufficient in dog (Prescott, 2000).

Since species variation, environmental and nutritional factors sometimes play great role in kinetic of drugs, this study was aimed at investigating the haematological and biochemical parameters of Nigerian local turkey poults given 50 mg/kg body weight of ceftriaxone. The safety of the singe dose of 50 mg/kg body weight was investigated.

MATERIALS AND METHODS

Experimental Animals: Five turkey poults of either sex each weighing 1 kg were used for the study. The turkeys were purchased from one Mr. S. Ogalue's farm at Federal Staff Quarters, Makurdi, Benue State, Nigeria. They were 5 months old and fed grower's marsh daily, water was provided *adlibitum.* The turkeys were housed in a fairly large metal cage during the experiment.

Drug Administration and Sample Collection: Ceftriaxone was administered daily into the wing vein of the 5 turkeys at the dose rate of 50 mg/kg for a period of 4 days. Prior to administration of ceftriaxone, control blood samples were collected from the turkeys; 1 ml of blood was collected from the wing vein of each turkey into test tubes

containing ethylenediamminetetraacetate (EDTA) as anticoagulant for determination of haematological parameters. Another 2 mls of whole blood was collected from each turkey but allowed to coagulate and serum collected for quantitative in vitro determination of biochemical parameters; liver function test and electrolytes determination.

At the end of 4-day trial, another 1 ml of blood sample was collected from the wing vein of each turkey into EDTA bottle and 2 mls of whole blood was collected from each turkey and allowed to coagulate in order to obtain serum for determination of haematological and biochemical parameters respectively.

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). Conjugated bilirubin and total bilirubin were determined using the method of Jendrassik and Grof (1938). Whereas serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic 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 ion (HCO^-_3) and chloride ion (Cl^-) were determined using titration method (Chaney and Marbach, 1962). All the parameters were determined before administration and 4 days after drug administration.

Statistical Analysis: The data on haematological and biochemical parameters were expressed as mean $\pm$ S.D. Tests for significance between mean parameters in respect of pre-administration and post-administration values were performed using students'-test (Petrie and Watson, 2002).

RESULTS

Haematology revealed significant increased level of eosinophils ($P < 0.05$). But white blood cells (WBC), packed cell volume (PCV), neutrophils, lymphocytes, monocytes and basophils were not significantly affected ($P > 0.05$) (Table 1). However, the animals were pale with patches of light blue colouration beneath their skins. The patches disappeared a week after the experiment.

Liver function test revealed decreased level of total bilirubin ($P < 0.05$). But total protein, Albumin, conjugated bilirubin, Alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT) and serum pyruvic transaminase (SGPT) were not affected significantly ($P < 0.05$) by the intravenous administration of ceftriaxone (Table 2).

Electrolyte concentration has shown that sodium ion (Na^+), potassium ion (k^+) and calcium ion (Ca^{++}) were not affected significantly ($P > 0.05$) as chloride ion (Cl^-) decreased significantly ($P < 0.05$). However, bicarbonate ion (HCO^-_3) increased significantly ($P < 0.05$) as a result of intravenous administration of ceftriaxone (Table 3).

DISCUSSION

The significant increase in eosinophils in the post-administration samples (2.2 ± 0.45^a) as compared to the pre-administration samples (1.4 ± 0.55^b) may be attributable to either antigen-antibody reaction or parasitism. This is supported by the report of Aka (2004) that eosinophils appear much in the following areas in the body e.g. the sites of antigen-antibody reaction, inflammation, blood clotting and in condition of heavy parasitism. Eosinophils kill parasites, and regulate the intensity of hypersensitivity reactions mediated by immunoglobulin (lgE). For a strong eosinophilic response to occur, the parasite must be in the animal's tissue (Willard et al., 1989).

Table 1: Effects of intravenous ceftriaxone on haematological parameters of turkey

Indices	Control (Pre administration)	Experimental (Post administration)
Packed cell volume %	33.2 ± 3.56^a	31.8 ± 2.28^b
White blood cells x 10 %	4.68 ± 1.45^a	5.08 ± 0.91^b
Neutrophils %	53.4 ± 16.06^a	50 ± 7.90^b
Lymphocytes %	44.6 ± 15.52^a	45.4 ± 8.23^b
Monocytes %	1.4 ± 0.55^a	2.4 ± 0.55^b
Eosinophils %	1.6 ± 0.89^b	2.2 ± 0.45^a
Basophils %	0 ± 0.0^a	0 ± 0.0^b

Key: Similar letters on the same row = statistically not significant

Table 2: Effects of intravenous ceftriaxone on liver function parameters of turkey

Indices	Control (Pre administration)	Experimental (Post administration)
Total protein (g/l)	35.18 ± 1.45^a	43.1 ± 15.33^b
Albumin (g/l)	33.02 ± 2.09^a	33.12 ± 1.90^b
Total bilirubin (μmol/l)	14.82 ± 0.72^b	13.5 ± 1.05^a
Conjugated bilirubin (μmol/l)	2.92 ± 0.63^a	2.86 ± 0.67^b
Alkaline phosphatase (μg/l)	119.6 ± 7.13^a	110.4 ± 6.10^b
Serum Glutamic Oxaloacetic transaminase (μg/l)	114.3 ± 18.79^a	114.3 ± 18.79^b
Serum Glutamic Pyruvic transaminase (μg/l)	4.0 ± 0.0^a	5.2 ± 1.64^b

Table 3: Effects of intravenous ceftriaxone on electrolyte concentration in turkey

Indices	Control (Pre administration)	Experimental (Post administration)
Sodium ion (mmol/l)	134 ± 2.45[a]	133.4 ± 2.19[b]
Potassium ion (mmol/l)	3.8 ± 0.37[a]	3.68 ± 0.46[b]
Chloride ion (mmol/l)	98.4 ± 2.88[b]	86.6 ± 8.11[a]
Bicarbonate ion (mmol/l)	24.4 ± 1.34[b]	24.8 ± 1.79[a]
Calcium ion (mmol/l)	3.26 ± 0.43[a]	3.26 ± 0.63[b]

Key: Similar letters on a row = statistically not significant

Nevertheless, the decrease in total bilirubin of experimental samples (13.5 ± 1.05[a]) in comparison with the control (14.82 ± 0.72[b]) may be due to displacement of plasma bilirubin by ceftriaxone. This finding agrees with the earlier reports of Willard et al (1989) that decrease bilirubin may be due to drugs that displace bilirubin from albumin, and Baggot (2001) reported that the binding of individual cephalosporins range from 15 to over 80 % as hyperbilirubinaemia could further decrease the albumin binding capacity of acidic drugs. Although patches of blue colouration were seen beneath the skins of the experimental turkeys, the blue colouration may be green bilverdin ix since turkeys do not produce bilirubin (Aka, 2004). This may be responsible for decreased total bilirubin observed in the experimental post-administration samples. So since ceftriaxone binds extensively to plasma proteins, invariably it may decrease renal excretion or hinder or facilitate drug elimination.

The decrease in the chloride ion (Cl^-) and increased bicarbonate ion (HCO^-_3) in the post-administration samples are a clear indication of the ability of ceftriaxone to cause metabolic alkalosis in turkey poults. Since ceftriaxone causes hypochloraemia, that may be characteristic of extensive binding acidic drugs. However, the increased bicarbonate ion observed could be a positive response to plasma hypochloraemia. This mechanism is important in maintenance of red blood cells integrity.

Conclusion: Ceftriaxone (50 mg/kg) body weight caused eosinophilia, decreased total bilirubin, hypochloraemia and increased bicarbonate ion. Hence, ceftriaxone should be administered to turkey with caution.

REFERENCES

AKA, L. O. (2004). Blood and cardiovascular system. Pages 143 – 187. *In:* AKA, L. O. (Ed.) *Foundation of Veterinary Physiology*, John Publishers, Nsukka, Nigeria.

BAGGOT, J. D. (2001). Interpretation of changes in drug disposition and interspecies scaling. Pages 93 – 185. *In:* BAGGOT, J. D. (Ed), *The Physiological Basis of Veterinary Clinical Pharmacology*, Blackwell Science Limited, United Kingdom.

BAKER, F. J. (1985). The full blood count. Pages 320 – 330. *In:* BAKER, F. J., SILVERTON, R. E., KILSHAW, D., SHANNON, R., EGGLESTONE, S., GUTHINE, D. I. and MACKENZIE, J. D. Eds.) *Introduction to Medical laboratory Technology.* 6th Edition, Butterworth and Company Limited, London.

CHANEY, A. L. and MARBACH, A. L. (1962). HCO_3- Cl: Titration Method. *Clinical Chemistry, 8:* 130 - 133.

DOUMAS, B. T., WATSON, W. A. and BIGGS, H. B. (1971). Albumin-Bromocresol green method. *Clinical Chemistry, 23:* 31 – 87.

FAWCETT, J. K. and SCOTT, J. E. (1960). Na-k: Flame photometric method. *Journal of Clinical Pathology, 13:* 156 – 158.

GARDNER, S. Y. and AUCOIN, D. P. (1994). Pharmacokinetics of ceftriaxone in mare. *Journal of Veterinary Pharmacology and Therapeutics, 17:* 155 - 157.

HUBBER, W. G. (1988). Aminoglycosides, Macrolides, Lincosamides, polymyxins, chloramphenicol and other antibacterial drugs. Pages 822 – 847. *In:* PRESCOTT, J. F., BAGGOT, J. D. and WALKER, R. D. (eds.) *Veterinary Pharmacology and Therapeutics* 13th Edition, Iowa State University Press, USA.

JENDRASSIK, L. and GROF, P. (1938). In-vitro determination of total and direct bilirubin in serum. *Journal of Biochemistry, 299:* 81 – 83.

PRESCOTT, J. F. (2000). Beta-lactam antibiotics: Cephalosporins and Cephamycins. Pages 134 – 176. *In:* PRESCOTT, J. F., BAGGOT, J. D. AND WALKER, R.D. () *Antimicrobial Therapy in Veterinary Medicine* 3rd Edition, Blackwell Scientific Publications, Iowa State University Press, USA.

PETRIE, A. and WATSON, P. (2002). Hypothesis test 1 – the test: comparing one or two means. Pages 78 – 88. *Statistics for Veterinary and Animal Science*, Blackwell Science Limited, United Kingdom.

REITMAN, S. and FRANKEL, S. (1957). Quantitative in-vitro determination of glutamic-pyruvic transaminase in serum. *American Journal of Clinical Pathology, 28:* 56 – 59.

SAGANUWAN, S. A. (2006). Haematological and biochemical effects of single intravenous bolus of ceftriaxone in Nigerian mongrel dog. Pages 28 – 31. *In: Proceedings of an International Conference on Research and Development,* Volume 1 No. 2, June 28 – 29, 2006, University of Calabar, Nigeria.

TIETZ, N. W. (1995). Protein determination. Pages 518 - 519. *In:* TIETZ, N. W. (Ed.) *Clinical Guide to Laboratory Tests*, 3rd ed., WB Saunders, Philadelphia.

TRIPATHI, K. D. (2003). Beta-lactam antibiotics. Pages 627 – 671. *In:* TRIPATHI, K. D. (Ed.) *Essentials of Medical Pharmacology*, 5th ed. Jaypee Brothers, New Delhi.

WILLARD, M. D. (1989). Gastrointestinal, pancreatic and hepatic disorders Pages 189 – 228. *In:* WILLARD, M., D., TVEDTEN, H. and TURNWALD, G. H. (Eds.) *Small Animal Clinical Diagnosis by Laboratory Methods,* WB Saunders, Philadelphia.

EVALUATION OF *IN-VITRO* ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL CONSTITUENTS OF *Cassia occidentalis*

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ABSTRACT

The research was carried out to evaluate the in-vitro antimicrobial activity and phytochemical constituents of Cassia occidentialis. Cassia leaves were collected from Kacha town in Niger State and extracted using methanol, hexane, chloroform and water extraction methods. Serial concentrations: 50, 60, 70, 80, 90 and 100 % methanol, hexane, chloroform and aqueous extracts were prepared and sterilized. The bacterial isolates used; E. coli, P. multocida, S. typhi, S. typhimurium, S. pyogenes, S. pneumoniae and K. pneumoniae were authenticated using biochemical and serological methods. The suspension (0.5) of each bacterial isolate was prepared in isotonic sodium chloride. The disc agar diffusion method was performed on 70 Mueller-Hinton agar plates, 10 per microorganism , using serial diffusion concentration: 500, 600, 700, 800, 900 and 1000 mg of hexane, methanol, chloroform and water. The results showed that all the extracts of Cassia occidentalis have antimicrobial activity on E. coli at concentrations between 900 – 1000 mg. E. coli was most susceptible to hexane extract at concentration ranges between 500 – 1000 mg, there was no antimicrobial activity exhibited against the other tested microorganisms. Phytochemical analyses showed the presence of alkaloid, tannin, saponin, glycoside and flavonoid, steroid was absent.

Keywords: Evaluation, *In-vitro,* Antimicrobial activity, Phytochemical properties, *Cassia occidentalis*

INTRODUCTION

Cassia occidentalis (Caesalpiniaceae) is a small tree growing 5 – 8 metres in height (Blumgarten, 1937). The leaves are compound, composite, paripinnate with 5 – 8 pairs of leaflets usually oval. Inflorescence occurs as auxiliary or terminal, yellow, short cluster of flower. Fruit is a pod, narrow, flat, slightly curved about 15 cm long with 10 – 12 seeds, brownish at maturity (Mann, 2003).

Traditionally, its roots, leaves, flowers and seeds are used as laxative and purgative (Todd, 1967). It is a vermifuge, anticonvulsant and used against chicken pox (Mann, 2003). Other uses include febrifuge, extrusion of guinea worm (Iwu, 1993) and black quarter (Ndi *et al.,* 2000). Previous studies have shown that its leaves exhibited *in-vitro* antibacterial, antimalarial and antihepatotoxic properties (Gasquet, 1993; Percez, 1994; Saraf, 1994). Seeds are brewed into a coffee like beverage for asthma and the flower infusion is used for bronchitis in the Peruvian Amazon (Akinloye *et al.,* 2003).

Phytochemically, the aqueous extract of *Cassia occidentalis* contained tannins, anthraquinones, sterol, cardiac glycosides, saponin and alkaloids (Muyibi *et al.,* 2000). The changing pattern of bacterial aetiology of infection and their altered sensitivities to antimicrobial agents employed in their treatment call for intensive regular exploration of indigenous plants. This will help us identify plants with antimicrobial value that will not only serve as resource for our indigenous pharmaceutical industries but will also serve as an alternative complementary medicine (Saganuwan and Gulumbe, 2006). Orji *et al*. (2003) reported that a particular characteristic of a plant is that different chemical substances are obtained in members of even the same species in different areas.

The objectives of this study was to evaluate the in-vitro antimicrobial activity and photochemical constituents of *Cassia occidentalis* of Nupe land as soil nature and environmental factors like climate, weather and humidity may affect phytochemical properties of plant grown different soil textures and environments.

MATERIALS AND METHODS

Plant: The fresh leaves of *Cassia occidentalis* were collected from Katcha, the headquarter of Katcha Local Government Area of Niger State, but identified and authenticated in Herbarium of Biological Science Department of Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Extraction: The leaves of *Cassia occidentalis* were collected fresh, pounded with mortar and pestle then dried in the sun for 30 minutes to 1 hour and grounded into powder using mortar and pestle.

The extraction was carried out as described by Saganuwan and Gulumbe (2006). Serial concentrations; 50, 60, 70, 80, 90 and 100% of the hexane, methanol, chloroform and water extracts were prepared and sterilized through 0.45 µm membrane filter paper (Cheesebrough, 1985).

Collection of Bacterial Isolates: Bacterial isolates of *S. pyogenes* and *S. pneumonia*e were obtained from Microbiology Laboratory of Sokoto specialist hospital. *K. pneumoniae* and *E. coli* were supplied by Microbiology Laboratory, College of Health Sciences, Usmanu Danfodiyo University all in Sokoto. But *P. multocida* was isolated in Veterinary Public Health Laboratory, Usmanu Danfodiyo University Sokoto, whereas *S. typhi* and *S. typhimurium* were donated by Department of Veterinary Public Health and Preventive Medicine, University of Nigeria Nsukka, Nigeria. The isolates were authenticated by chemical and serological tests as described by Cheesebrough (1985), preserved on blood agar slant and stored at 4 ^{0}C until ready to use.

Disc Diffusion Method: The isolates of *K. multocida*, *S. typhi*, *S. typhimurium*, *K. pneumoniae*, *E. coli*, *S. pyogenes* and *S. pneumoniae* were subcultured overnight at 37 ^{o}C on nutrient agar plates, ten plates per microorganism. The suspensions of each bacterial isolate were prepared as described by John *et al.* (1999) in isotonic sodium chloride solution. Dried Petri dish, ten per each microorganism of Mueller-Hinton agar were flooded with the appropriate suspension of the bacterial isolates.

Sterile 6 mm diameter absorbent filter papers (punched out from No. 1 whatman paper) were impregnated with the appropriate concentrations; 500, 600, 700, 800, 900 and 1000 mg of the hexane, chloroform, methanol and water extracts and placed on the corresponding inoculated 70 plates. Ten each of *S. typhi*, *S. pyogenes*, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, *P. multocida* and *S. typhimurium*. After the incubation at 37 ^{0}C for 24 hours, all the plates were observed for zones of growth inhibition and the diameters of the zones measured in millimeter (mm) using calibrated ruler.

RESULTS

E. coli showed diametric zones of inhibition at concentrations between 900 – 1000 mg of 10.0 and 17.0 mm with average mean of 4.5 mm as there was no any zone of inhibition shown on other microorganisms by the hexane extract of *Cassia occidentalis*. Diametric zones of inhibition were shown on *E.* coli by the chloroform extract at concentration ranges between 900 – 1000 mg of 10.0 and 16.0 mm with average mean of 4.3 mm. No zone of inhibition shown on other microorganisms by the chloroform extracts (Table 1).

E. coli showed diametric zones of inhibition at concentration ranges between 900 – 1000 mg of 10.0 and 15.0 mm with average mean of 4.16 mm as there was no any zone of inhibition shown on other microorganisms by the methanol extract. Diametric zones of inhibition of 8 mm and 10 mm were shown on *E. coli* at concentration ranges between 900 – 1000 mg with average mean of 3.67 mm as there was

Table 1: Serial concentrations of the hexane, chloroform, methanol and water extracts, and their corresponding diametric zones of inhibition

Conc. of the extracts (mg)	Diametric zone of inhibition (mm)													
	Hexane							Chloroform						
	S. ty	S. typhim	E. col	S. pneu	K. pneu	P. mul	S. pyo	S. ty	S. typhim	E. col	S. pneu	K. pneu	P. mul	S. pyo
500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
900	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
1000	0.0	0.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0
Mean: 750	0.0	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0	0.0	0.0
	Methanol							**Water**						
500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
900	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0
1000	0.0	0.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	14.0	0.0	0.0	0.0	0.0
Mean: 750	0.0	0.0	4.16	0.0	0.0	0.0	0.0	0.0	0.0	3.67	0.0	0.0	0.0	0.0

Keys: S. ty = Salmonella typhi, S. typhim = Salmonella typhimurium, E. col = E. coli, S. pneu = Streptococcus pneumoniae, K. pneu = Klebsiella pneumoniae, P. mul = Pasteurella multocida, S. Pyo = Streptococcus pyogenes

Table 2: Phytochemical constituents of *Cassia occidentalis* methanol, hexane, chloroform and aqueous leaf extracts

Extract	Alkaloid	Tannin	Glycoside	Flavonoid	Steroid	Saponin
Methanol	+	+	+	+	-	+
Hexane	+	+	+	+	-	+
Chloroform	+	+	+	+	-	+
Water	+	+	+	+	-	+

no zone of inhibition shown on other microorganisms by the aqueous extract of *Cassia occidentalis* (Table 1).

Phytochemical analyses revealed the presence of alkaloid, tannin, glycoside, flavonoid and saponin in *Cassia occidentalis* methanol, hexane, chloroform and aqueous leaf extracts as there was no steroid present in all the four extracts (Table 2).

DISCUSSION

The antimicrobial activity exhibited by hexane, chloroform, methanol and aqueous leaf extracts of *Cassia occidentalis* on *E. coli* at concentrations between 900 – 1000 mg agrees with the findings of (Gasquet, 1993; Percez, 1994; Saraf, 1994) that the leaves exhibited in-vitro antibacterial, antimalarial and antihepatotoxic properties. The plant may be used for the treatment of colibacillosis caused by *E. coli* which occurs in all species of newborn farm animals as major cause of death and economic loss in this age group (Radostits *et al.,* 2000). Leeflang (1993) had earlier observed that indigenous knowledge and practices will be useful in the promotion of animal health and meat production in the near future in Nigeria. Furthermore, Tamboura *et al.* (2000) had also reported that ethno veterinary medical health care will be the only alternative to western veterinary therapy. These ethnoveterinary remedies which rely on local plants or easily available materials are practical, effective and cheap (Tamboura *et al.,* 2000). However, lack of antimicrobial activities exhibited by all the extracts of *Cassia occidentalis* and generally at concentrations between 500 – 1000 mg on *P. multocida*, *S. typhi*, *S. typhimurium*, *S. pyogenes*, *S. pneumoniae* and *K. pneumoniae* is suggestive of limited antimicrobial activity of the plant. This was not pointed out by (Gasquet, 1993; Percez, 1994; Saraf, 1994). More so, uniformity of antimicrobial activity exhibited by hexane, chloroform, methanol and aqueous extracts of *Cassia occidentalis* leaf on only *E. coli* may confirm the limited antibacterial activity of the plant even *in-vivo.* Nonetheless, there is need to separate the chemical constituents of the plant leaf and then test each component on the microorganisms.

The results of qualitative analyses of all the four extracts confirmed the presence of alkaloid, tannin, glycoside and saponin as reported by Muyibi *et al.* (2000) that the aqueous extract of *Cassia occidentalis* contained tannins, anthraquinone, sterol, cardiac glycoside, saponin and alkaloid. But there was no steroid as it also contained flavonoid.

REFERENCES

AKINLOYE, O. A., AHMED, A. S., AJAGBONA, O. P. and OLOREDE, B. R. (2003). Some biochemical effects of various doses of aqueous seed extracts of *Cassia occidentalis* in rabbits. *Bioscience Research Communication, 15(1):* 85 – 90.

BLUMGARTEN, A. S. (1937). *Senna.* Pages 225 – 226. *In:* BLUMGARTEN, A. S. (Ed.) *Textbook of Material, Medical, pharmacology and Therapeutics*, 7th Edition, John Wily and Sons. New York.

CHEESEBROUGH, M. (1985). *Medical Laboratory Manual for Tropical Countries*, volume 17, Pages 203 – 305.

GASQUET, M. (1993). Evaluation in-vitro and in-vivo of traditional antimalarial plants. *Fitaterapia, 645:* 423 – 424.

IWU, M. M. (1993). *Catalogue of major medicinal plants. Handbook of African Medicinal plants* CRC Press, London.

JOHN, C., KEREN, K. and BRADFORD, K. (1999). Generic protocol to estimate the burden of *shigella* diarrhea and dysenteric mortality. *WHO Bulletin, 23:* 28 – 30.

LEEFLANG, P. (1993). Some observations on ethnoveterinary medicine in Northern Nigeria. *Veterinary Quarterly, 15(2):* 72 – 73.

MANN, A., GBATE, M. and UMAR, A. N. (2003). *Senna occidentalis* (Linn.). *Medicinal and Economic Plants of Nupe land*, Jube Evan Books and Publication, Kaduna, 41 pp.

MUYIBI, S. A., OLOREDE, B. R., AJAGBONNA, O. P. ONYEYILI, P. A., OSUNKWO, V. A. and MUHAMMED, B. Y. (2000). Acute toxicity and phytochemical studies of *cassia occidentalis* Linn extract in rats. S*okoto Journal of Veterinary Sciences, 2(2):* 32 – 35.

NDI, C., MUMAH, E. T., NDOKWO, K. J., MFI, A. N. NBENKUM, T., ASAH, H., EKUE, F. N. and TOYONG, J. N. (2000). Ethno veterinary plants used in the North West province of Cameroon. Pages 154 – 161. *In: Ethnoveterinary practices research and development. Proceeding of an International Workshop on Ethnoveterinary Practices*, held 14 – 18 August 2000, Kaduna Nigeria.

ORJI, E. C., OGBWZUE, D. C. and ORJI, M. U. (2003). Evaluation of antimicrobial activities of leaf extracts of *Morinda lucida* and *Erythrina senegalensis* for medical uses. *International Journal of Medical and Health Studies,* 1(1): 79 – 83.

PERCEZ, C. (1994). In-vitro anti-bacterial activity of argentine folk medicinal plants against *salmonella typhi*. *Journal of Ethnopharmacology, 44(1):* 41 – 46.

RADOSTITS, M. O., GRAY, C. C., BLOOD, D. C. and HINCHLIFF, K. W. (2000). Diseases caused by bacteria. Pages 779 – 780. *In: Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. W. B. Saunders, London.

SAGANUWAN, S. A. and GULUMBE, M. L. (2006). Evaluation of *Sida acuta Subspecies acuta* leaf/flower combination for antimicrobial activity and phytochemical constituents. *African Journal of Clinical and Experimental Microbiology, 7(2):* 83 – 88.

SARAF, S. (1994). Anti-hepatotoxic activity of *cassia occidentalis*. *International Journal of Pharmacology, 322:* 178 – 183.

TAMBOURA, H. H. M., SAWADAGO, L. L., KABORE, H. and YAMEOGO, S. M. (2000). Ethnoveterinary medicine and indigenous pharmacopoeia of Pasore Province in Burkina Faso. *Annals of New York Academy of Science, 916:* 259 – 64.

TODD, R. G. (1967). *Senna* and other purgatives. Pages 1266 – 1267. *In: Extra pharmacopoeia Martindale*, 20th Edition, W. B. Saunders, London.

EFFECTS OF ALCOHOL ON OXIDATIVE PARAMETERS OF ALLOXAN INDUCED DIABETIC ALBINO RAT

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ABSTRACT

The effects of alcohol consumption on lipid peroxidation and antioxidant status were investigated in the alloxan induced diabetic rats. Plasma from the diabetic rats not treated with alcohol (DNT); diabetic rats treated with alcohol (DT) and non diabetic rats (ND) were analysed for their malondialdelyde (MDA) and vitamin C levels. Both the glucose level and the body weight were also studied. The mean weights of the rats in the different groups were the same until the onset of diabetes and alcohol ingestion when the weight decreased. After nine (9) days of alcohol supplementation, the DT rats weighed 114.00 ± 0.41 g, and the DNT rats weighed 121.00 ± 1.22 g while the rats in the controlled group weighed 146.33 0.14 g. The glucose levels for DT, DNT and ND were 29.56, ± 0.56, 28.81 ± 0.87 and 5.42 ± 0.19 nmol/l respectively. Analysis of the lipid peroxidation product (MDA) obtained showed a significant (P < 0.05) increase in MDA values from – DT rate (38.63 ± 3.88) nmol/ml to DNT rats (28.63 ± 1.38 nmol/lml), while MDA value for ND rats was 7.88 ± 1.38 nmol/l. Plasma vitamin C values of 0.62 ± 0.05 mg/100ml, 1.107 ± 0.13 mg/100ml and 1.79 ± 0.15 mg/100ml for DT, DNT and ND respectively were obtained.

Keywords: Alcohol, Antioxidant, Lipid peroxidation, Diabetes, Rat

INTRODUCTION

Diabetes mellitus has been considered an important health hazard because of the morbidity and mortality associated with it. The fact that it cannot be cured but only managed calls for a serious concern by patients and health workers.

Diabetes mellitus may be caused and or exacerbated by certain chemicals or compounds which elicit oxidative stress (Traverso *et al.,* 1999, Ogugua, 2000) in the exposed individual. On the other hand, antioxidants have been involved in the amelioration of oxidative stress – mediated pathologies (Halliwell *et al.,* 1992; Stern, 1993). Hence oxidative stress and antioxidants have been weighed side by side in diseases states including diabetes mellitus.

The natural quest for alcohol consumption has made it "a free for all drink" despite the obvious consequences of its acute and chronic intoxication (Nwodo, 1999). The morbidity and mortality of the diseases associated with alcohol intake is both a social and health problem and the complication of diabetes mellitus may be a double tragedy for alcoholic diabetics. Fatty liver, cirrhosis and hepatitis have been associated with high intake of alcohol (Ewa and Arthur, 1996; Nwodo, 1999). This suggests that liver damage may be as a consequence of alcohol ingestion. Presence of iron in beer has been implicated in the generation of reactive oxygen species and amplification of disease conditions associated with consumption of alcoholic beverages.

Cardiac arrhythmiasis has been associated with alcohol ingestion (Finch and Huebers, 1982; Ruskin, 1989). Thus alcohol ingestion can suppress the hearts pace and thus endanger lives. Alcohol consumption can increase pulse rate and blood pressure and hence decreases the strength of the pumping action of the heart (January and Fozzard, 1988). Some unexplained heart diseases could be due to chronic heavy drinking of alcohol. According to Belotsky *et al.* (1990) alcohol irritates the interior lining – mucosa – of the oesphagus, and induces stomach erosion causing inflammation and bleeding. Also alcohol causes diarrhoea through changes in intestinal motility and rate of propulsion of materials through the small intestine (Okeagu, 1999).

The inference from the above stipulates suggests that while normal persons may suffer complications of acute and chronic alcohol, ingestion diabetics could suffer more. The thirst for alcohol has made it such that even diabetics could not resist the taste and urge and in the rural settings diabetics and people prove to it consume alcohol without reservations. Hence the thrust of the research is to follow up diabetic animals ingesting alcohol by monitoring indices of oxidative stress – glucose level, lipid peroxidation product and antioxidant vitamin C. The outcome of the result may help in the management of diabetes mellitus.

MATERIALS AND METHODS

Eighteen albino rats obtained from animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the experiment. These rats with average weight of 150 g were divided into three groups of six rats each and housed in stainless steel cages. They were fed with normal commercial chow and were allowed free access to water.

Table 1: Weight, Glucose, malondialdehyde and Vitamin C levels of diabetic and non-diabetic rats exposed to alcohol treatments

Group*	Weight (g) mean ± SD	Glucose level (mmol/l)	MDA nmol/ml Plasma	Vitamin C mg/100 ml
Non diabetic rats (ND)	146.33 ± 0.14	5.42 ± 0.19	7.88 ± 1.38	2.79 ± 0.15
Diabetic rats not treated with alcohol (DNT)	121.0 ± 1.22g	28.81 ± 0.87	28.63 ± 1.38	1.11 ± 0.52
Diabetic rats treated with alcohol (DT)	114.00 ± 0.41g	29.56 ± 0.56	38.63 ± 3.88	0.62 ± 0.52

**Results are mean ± SD; n = 6.*

The group A were the control rats while groups B and C rats were induced with diabetes mellitus. Group C rats were further treated with ethanol.

Diabetes was induced by intraperitonial injection of alloxan (200 mg/kg). The body weights of all the animals were determined before the induction of diabetes and during the experiment. Five millilitres volume of alcoholic beverage was given to each rat in group C daily for nine (9) days.

Blood glucose levels were determined daily using One Touch Blood Glucose Kit (Glucometer). At the end of the nine days the animals were sacrificed and other parameters determined as follows: Serum Malonyldialdelyde level was determined using the method of Albro *et al.* (1986) and Das *et al.* (1990) and while ascorbic acid (Vitamin C) level was determined using Tietz (1970).

RESULTS AND DISCUSSION

Table 1 showed that the normal rats had the highest weight while the diabetic rats had lower weight. Treatment with alcohol resulted to further loss in weight compared with the diabetic non-alcohol treated.. This suggests that diabetic condition could cause a reduction in weight and ethanol (beer) ingestion by diabetics compounded the problem. Decrease in weight of diabetic subjects had earlier been reported (Traverso *et al.,* 1999, Ogugua 2000). Generally, oxidative stress could lead to loss in weight which may be severed in the ethanol treated rats. Alcohol in this study increased oxidative stress which might have led to loss in body weight of the animals stressed.

Table 1 showed high levels of glucose and malondialdehyde in diabetic not treated rats (DNT) which further increased in alcohol treated diabetic rats (DT). There was above 2.6 % increased in blood glucose level of diabetic alcohol treated rats when compared with other treatments. Earlier reports proposed an overall reduction of blood glucose by alcohol (Nwodo, 1999). Prolonged ingestion of alcohol could trigger off excess production of reactive oxygen species leading to increased blood glucose level. Increased malondialdelyde level has been associated with increased glucose level (Reaven, 1995). The high MDA level in this work (Fig. 1) lay credence to this speculation. However, ethanol in low quantity may be antioxidative (hence may lower glucose level and oxidative stress index). Glucose autoxidation and increased oxidative stress has been reported (Hunt & Stocker 1990; Tukuncu *et al* 1998). The magnitude of reactive oxygen species production in the presence of ethanol may therefore modulate the level of glucose in such system. Generally, copious generation of reactive oxygen species could trigger off normal mechanism, in this case a reduction mechanism may be effected and hence elevation of blood glucose (Ogugua, 2000).

The vitamin C level was low in DNT rats compared with ND rats while DT rats had the lowest vitamin C levels (Table 1). In this system, vitamin C acted as an antioxidant and so was depleted in the process. Frei (1991) reported that vitamin C was the first antioxidant to be encountered during lipid peroxidation and so diminished in organic system. These findings were also corroborated by the report of Ogugua (2000) that Vitamin C diminished in diabetic rabbits monitored over time. For more information on the role of antioxidants in oxidative stress, both Ogugua (1994) and Fakoya *et al.* (1998) laid credence to the present findings.

Our results showed that oxidative stress became amplified in ethanol treated diabetics. Ethanol has been reported to induce oxidative stress and mediated lipid peroxidation; (Diluzo and Stefe, 1977; Bosch *et al.,* 1998; Ren *et al.,* 2000). The very low level of vitamin C in the alcoholic diabetics rats suggests aggravated depletion of vitamin C as it encounters free radicals. Thus the antioxidant status of the system is compromised in diabetics ingesting alcohol.

One may therefore re-iterate this point like a town crier that diabetics should avoid ingesting alcoholic drinks and at the same time suggest that supplementation with vitamin C or any antioxidant vitamin may help in the management of subjects with diabetes mellitus consuming alcohol.

People who are prone to diabetes (latent diabetes) may have it triggered off with alcohol ingestion while those who have developed diabetes would tend to severe complications. Hence medically challenged individual – diabetics should completely avoid alcoholic beverages.

REFERENCES

ALBRO, P. W., CORBETT, J. T. and SCHROEDER, J. L. (1986). Application of the thiobarbituric assay to the measurement of lipid peroxidation product in microsomes. *Journal*

of Biochemical and Biophysical Methods, 13: 185 – 194.

BELOTSKY, S. M., GUZU, E., KARLOV, V. and SNASTINA, T. I. (1990). Wound tissue, Respiratory burst, and local microbial inflammation. *Inflammation, 14:* 663 – 668.

BOSCH, M. F., MARTNEZ, S. and COLLEL, A. (1998). Chronical ethanol feeding induces cellular, antioxidants decrease and oxidative stress in rats peripheral nerves. *Free Radical in Biological Science and Medicine, 25:* 365 – 368.

DAS, B. S., THURNHAN, D. I., PATNACK, J. K., DAS, D. B. SATPATHY, R. and BASE, T. K. (1990). Increased Plasma lipid peroxidation in riboflavin deficient malaria infected children. *American Journal of Clinical Nutrition, 51:* 859 – 863.

DILUZO, N. R. and SLEFE, T. E. (1977). The role of metabolites in hepatic lipid peroxidation. Pages 45 – 62. *In:* FISHER, M. N. and RANKIN, J. G. (Eds). *Alcohol and Liver,* Plenum Press, New York.

EWA, K. and ARTHUR, I. (1996). Ferritin stimulation of lipid peroxidation by miicrosomes after chronic ethanol treatment. Role of Cytocrome P 450E. *Archives Biochemical Biophysics, 332:* 121 – 127.

FAKOYA, E. A. O., OSILESI, O. OGUNLEDUN, A. FAKOYA, T. A. and ODUSOGA O. (1998). Antioxidant nutrients and disease interaction. *Nigerian Journal of Nutritional Science.*

FINCH, C.A. and HUEBERS, H. (1982). Perspectives in Iron metabolism. *New England Journal of Medicine, 306:* 1520 – 1528.

FREI, B. (1991). Ascorbic acid protects lipid in human plasma and low density Lipoprotein against oxidative damage. *American Journal of Clinical Nutrition, 54:* 1113 – 1118.

HALLIWELL, B., GUTHERIDGE, J. M. C. and CROSS, C. E. (1992). Free radicals antioxidants and human diseases where are we now? *Journal of Laboratory Clinical Medicine, 119(6):* 598 – 620.

HUNT, N. H. and STOCKER, R. (1990). Oxidative stress and redox status of malaria-infected erythrocytes. Blood Cells, *16:* 499 – 526.

JANUARY, C. T. and FOZZARD, H. A. (1998). Delayed after depolarizations in heart muscle: Mechanisms and relevance. *Pharmacological Review, 40:* 219 – 227.

NWODO, O. F. C. (1999). *Alcohol,* Atlanto Press, Nsukka, Nigeria, 54 pp.

OGUGUA, V. N. (2000). *The parameters of oxidative stress in alloxan induced diabetic rabbits.* PhD Thesis University of Nigeria, Nsukka, 211 pp.

OKEAGU, F. I. (1999). *Metal ions contents in beer and oxidative stress: Implications to health.* B.Sc Project Report, Department of Biochemistry, University of Nigeria, Nsukka, 54 pp.

REAVEN, P. (1995). Dietary and Pharmacologic regimens to reduce lipid peroxidation in non-insulin dependent diabetes mellitus. *American Journal of Clinical Nutrition. 62 (6):* 14835 – 14895.

REN, J., WOLD, I. E. and EPSTIEN, P. N. (2000). Diabetes enhances acetaldehyde induced depression of cardiac myocyte contraction. *Biochemical and Biophysical Research Communication. 269(3):* 697 – 703.

RUSKIN, J. N. (1989). The cardiac arrhythmias suppression trials (CAST). *New England Journal of Medicine, 321:* 386 – 388.

STERN, A. (1993). Sickle cell anaemia. Pages 35 – 52. *In:* ARUOMA, O. I. (ed.) *Free Radical in Tropical Diseases.* Harwood Academic Press, USA.

TIETZ, N. N. (1970). Carbohydrate. Pages 174 – 176. *In: Fundamental of Clinical Chemistry.* W. B. Sunder Company, London.

TRAVERSO, N., MENINI, S., ODETTI, P. PRONZATO, M. and CATTALASSO, D. (1999). Lipid Peroxidation in hepatic subcelluar compartments of diabetic rats. *Free Radical Biological and Medicine. 26 5/6,* 538 – 547.

TUKUNCU, N. B., BAYRAKTAR, M. and VARLI, K. (1998). Reversal of defective nerve conduction with vitamin E supplementation in type 2 diabetes. *Diabetes Care, 21:* 1915 – 1918.

PLASMODIUM INFECTION IN MAN: A REVIEW

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ABSTRACT

Plasmodium infection in man is caused by the bite of an infected female Anopheles mosquito. This results in the disease, malaria. Malaria has serious debilitating effects on man. It adversely affects man's health, strength and productivity. Here, a review of Plasmodium infection in man including the life cycle, transmission, immunity, symptoms, diagnosis, pathology, prevention, control and treatment is given. Only by knowing about Plasmodium infection, the burden of infection on man and the prevention and control options can we understand the disease better and so be better prepare for the future management of this disease.

Keywords: *Plasmodium* infection, Malaria, Epidemiology, Symptoms, Treatment, Control, Man

INTRODUCTION

Plasmodium causes malaria in humans. Malaria parasites (*Plasmodium*) are parasitic protozoan belonging to the sub-class, coccidia, and family, Plasmodiidae (Smyth, 1996). The female *Anopheles gambiae* mosquito is responsible for transmitting *Plasmodium* parasites. Malaria is a major public health problem with an estimated two million children worldwide dying of malaria yearly, primarily because of *Plasmodium falciparum* and its complications (Krogstad, 1996). Malaria is reported to be responsible for 500 million clinical cases and 2.7 million deaths each year (WHO, 1996b). Sub-Saharan African region has the greatest number of people exposed to malaria transmission and the greatest number of morbidity and mortality in the world (WHO, 1996a). The high-risk groups include young children, pregnant women, non-immune travelers, refugees, displaced persons and labourers entering endemic areas (Russel and Howson, 1996). It is estimated that in Africa, malaria is responsible for over one million deaths yearly of infants and young children (Angyo *et al.,* 1996). According to David (2000), every thirty seconds, a child somewhere dies of malaria. The loss of the daily labour cost coupled with cost of treatment and high mortality associated with the disease make malaria one of the main factors retarding development in Africa (Mutero *et al.*, 1998). By adversely affecting people's health, strength and productivity, malaria further marginalizes and impoverishes them (David, 2000). In Nigeria, malaria is hyper-endemic with stable transmission (Ofovwe and Eregie, 2001).

The global effects of the disease threaten public health and productivity on a broad scale and impede the progress of many countries toward democracy and prosperity (Oaks *et al.*, 1991). In spite of control programmes in many countries, malaria continues to be one of the world's greatest killers (WHO, 1989).

Malaria in humans is caused by four species of parasitic protozoan namely: *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale*. *P. malariae* has lost whatever predominance it may once have had and *P. vivax* and *P. falciparum* are the most commonly encountered malaria parasites worldwide (Carter and Mendis, 2002). However, *P. falciparum* is the most dangerous malaria parasite and causes most deaths (Oaks *et al.*, 1991). Because of the temperature preference on its transmission, *P. falciparum* is normally present in the tropical, sub-tropical and warm temperate regions. In the tropics, *P. falciparum* account for over 80 % of malaria cases (Carter and Mendis, 2002). The four species of *Plasmodium* that infect man result in four kinds of malarial fever. *P. falciparum* results in tropical malaria, *P. vivax* causes tertian malaria, and *P. malariae* causes quartan malaria, while *P. ovale* results in ovale tertian malaria (Smyth, 1996). The four species of *Plasmodium* differ morphologically and this can be used as a criterion for identification and diagnosis of malarial type.

MATERIALS AND METHODS

A comprehensive literature search was made from the Internet and serial materials of Nnamdi Azikiwe Library, University of Nigeria, Nsukka. Various journal articles, proceedings of learned societies of parasitology, WHO documents and textbooks were consulted vis-à-vis of the biomedical and socio-economic impact of plasmodium infection in man.

RESULTS

Life Cycle of Malaria Parasite: Although the life cycles of the four species of human malaria parasites are not identical, they are sufficiently alike to permit a general description. The life cycle of *Plasmodium* parasites can be divided into three stages; the exo-erythrocytic or pre-erythrocytic stage which usually occurs in the liver, the erythrocytic stage which occurs in the erythrocytes, and the sexual stage which occurs in the mosquito (Figure 1). In exo-erythrocytic stage, an infected female anopheline

mosquito introduces sporozoites into man during feeding. These sporozoites are taken up by the blood stream and within thirty minutes, they disappear from the blood stream. The sporozoites are elongate bodies measuring about 11μm in length, with a central nucleus. The sporozoites enter the liver cells (hepatocytes) where they develop to form cryptozoites. These give rise to metacryptozoites. This rapid multiplication by schizogony is referred to as pre-erythrocytic schizogony.

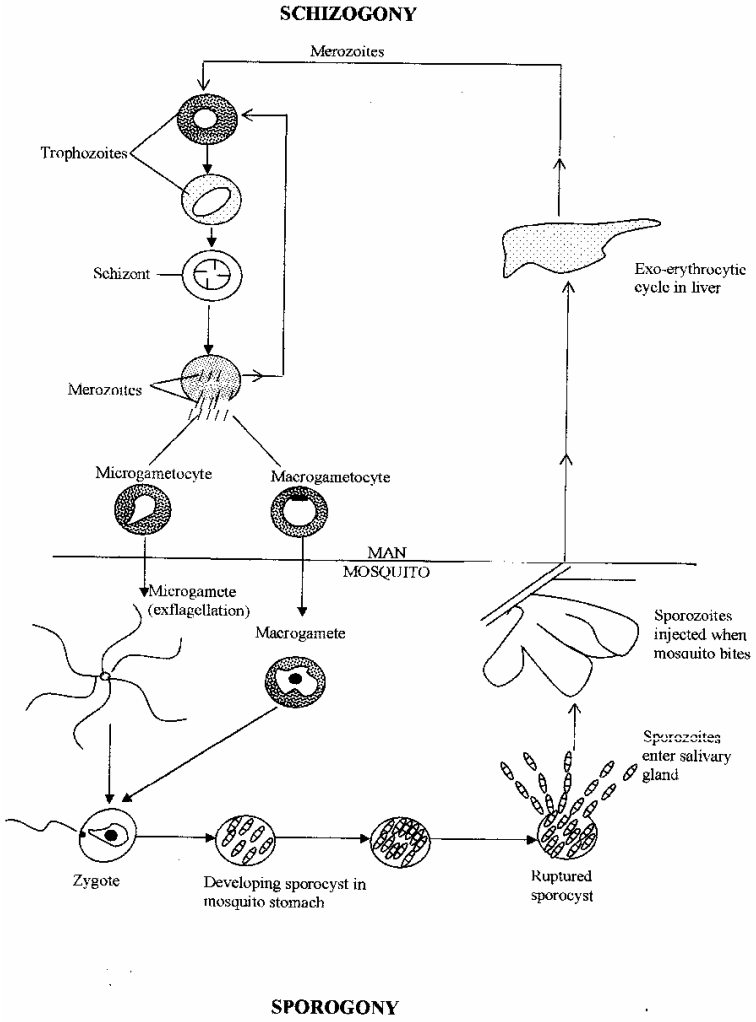

Figure 1: Generalized life cycle of malarial parasite (Mehlhorn and Walldorf, 1988)

By repeated divisions, over a period of six to nine days, metacryptozoites produce thousands of merozoites which are discharged into the blood circulation. In *P. vivax* and *P. ovale,* some injected sporozoites may differentiate into stages called hypnozoites which may remain dormant in the liver cells for sometime only to undergo schizogony causing relapse of disease when the red cells are invaded (Smyth, 1996). The life cycles of the species of *Plasmodium* affecting man are shown in Figure 2.

During the erythrocytic stage, merozoites enter into the erythrocytes. According to Aikawa (1980), entry of merozoites into a new erythrocyte is by a process called endocytosis. Endocytosis involves recognition and attachment of the merozoite to the erythrocyte membrane. On entry into an erythrocyte, a merozoite assumes the appearance of a small chromatin mass situated at the periphery of a larger mass of cytoplasm, in which a vacuole appears. Because of its characteristic appearance, this early trophozoite stage is referred to as a "signet ring". As it grows into maturity, it assumes an amoeboid shape as its nucleus divides to form up to 20 or more merozoites, depending on the *Plasmodium* species. The dividing stage is called a schizont. The mature schizont often called a segmenter causes the infected erythrocyte to rupture, thus releasing its merozoites into the blood stream. The merozoites within the blood attack new red blood cells thus repeating the erythrocytic schizogony cycle. The schizont also releases pigments and waste products which along with the merozoites are responsible for the feverish condition depicted by high temperature. Merozoites of some *Plasmodium* species show a distinct preference for erythrocytes of certain age. For instance, merozoites of *P. vivax* attack young immature red blood corpuscles called reticulocytes, those of *P. malariae* attack the older erythrocytes while those of *P. falciparum* indiscriminately enter into any available erythrocyte (Aikawa, 1980). After several generations of erythrocytic schizogony, some merozoites re-enter into red blood cells and develop into the sexual stages (sexual gametocytes) instead of schizogony stages (asexual schizonts). The male cells (microgametocytes) and the female cells (macrogametocytes) circulate in the blood until they either perish or are ingested by a female anopheline mosquito. The stage in the mosquito begins when a female anopheline mosquito feeds on infected blood, ingesting all the *Plasmodium* stages present in the blood stream. However, only the gametocytes survive to establish the sporogony cycle in the mosquito. In the midgut of the mosquito, the macrogametocyte develops a small amount of chromatin, and is thus transformed into a macrogamete. Equally in the mosquito midgut, the microgametocyte develops four to eight hair-like flagella (exflagellation) and is transformed into microgamete. The microgametes detach themselves and swim freely about in the fluid filled lumen of the midgut until they contact a macrogamete. Penetration is quickly accomplished. The fertilized macrogamete called a zygote develops into a mobile ookinete.

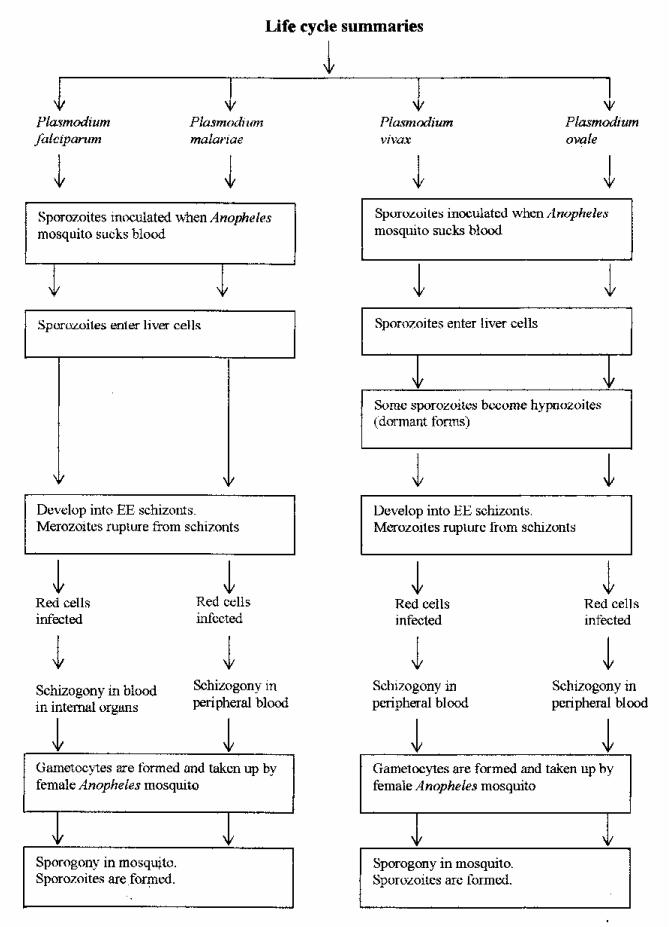

Figure 2: Life cycle of plasmodium species affecting man

The ookinete penetrates the stomach wall of the mosquito between the cells and develops as an oocyst. The oocyst gradually matures producing a spherical mass within which sporozoites develop mitotically. The oocyst usually matures in 10 – 20 days depending on temperature, *Plasmodium* species and physiological characteristics of the anopheline mosquito, attending a body size of 50 – 60μm. On rupture of the mature sporocyst, the sporozoites are released into the body cavity where they make their way to the salivary gland. On feeding, the sporozoites are injected into the tissues or directly into the blood stream of the new host (man) to initiate a new schizogony cycle.

Transmission of Malaria: Malaria is transmitted in various ways; by mosquito injection of sporozoites, by the transfer of erythrocytic stages other than gametocytes, and in a blood transfusion. Furthermore, blood donation from semi-immune persons without clinical symptoms may contain malarial parasites. In congenital malaria, infected mothers transmit parasites to their children before or during birth (Hoffman, 1996).

Malaria transmission in an area may be stable or unstable (WHO, 1996a). Stable malaria occurs when a population is continuously exposed to a fairly constant rate of malarial inoculation, while unstable malaria occurs seasonally with marked changes in transmission from one season to another and from one year to the other (Carter and Mendis, 2002). According to Carter and Mendis (2002), the differences in stability of malaria transmission, notably between tropical Africa and most other malarious regions are due largely to the behaviour and other biological characteristics of the regional species and sub-species of *Anopheles* vectors, and to their environment. The strong human - biting preferences and highly domestic habits of the tropical African vectors lead to very uniform contact between them and the human blood source in sub-Saharan Africa (Bruce-Chwatt *et al.*, 1966; Coluzzi, 1999). The climatic conditions are also highly conducive to malaria transmission, being warm and humid with relatively few fluctuations. This supports longevity of the vector mosquitoes and rapid development of the parasites within them (Oaks *et al.*, 1991). All of these features enable stable and indeed, generally intense malaria transmission in the tropics, notably Africa (Carter and Mendis, 2002).

Immunity to Malaria: Man's present understanding of immune mechanisms comes from observations made in infected people, from *in vitro* studies, and from experimental work carried out in laboratory systems (WHO, 1990). Immunity is usually established in stable malaria while immunity is unable to reach a high level in unstable malaria (Coluzzi, 1999). There are two types of clinical immunity, one, which reduces the risk of death from malaria, and another, which reduces the intensity of clinical symptoms. A third type is antiparasitic immunity, which directly reduces the numbers of parasites in an infected individual (Carter and Mendis, 2002). The number of malarial inoculations experienced and the intervals between them are all important to the malaria immune status of an individual. In the case of acute attacks of *P. falciparum* malaria, it is possible that a degree of immunity to some aspects of severe life-threatening disease may be achieved after only one or two infections (Gupta *et al.*, 1999). However, clinical immunity to other non life-threatening clinical effects of malaria requires more and frequent inoculations of malaria (Trape and Rogier, 1996). Because of the time taken to achieve effective immunity to malaria under conditions of endemic infection, antimalarial immunity is often said to be "age dependent". Very young children appear to have a poor capacity to acquire effective protective antimalarial immunity of any sort, while older children and adults may do so more readily (Baird *et al.*, 1991; Baird, 1995).

Symptoms: Symptoms of *P. falciparum* infection may include fever, chills, sweats, cough, diarrhea, respiratory distress and headache (UACHPPM, 2004). Symptoms of infection with *P. vivax, P. malariae* or *P. ovale* may begin with indefinite malaise and a slow rising fever several days in duration, followed by shaking chills and rapidly rising temperature, usually

accompanied with headache and nausea, and ending with profuse sweating. After a period free of fever, the cycle of chills, fever and sweating is repeated every one to three days (Cheesbrough, 1987).

Diagnosis: Diagnosis of malaria is accomplished through the demonstration of the malaria parasites in blood films, which could be either thick or thin. Other supportive techniques include sophisticated indirect fluorescent antibody (IFA) test, immunoglobulin values and haemagglutination tests (UACHPPM, 2004). Over the last few years, several malaria rapid tests have been developed which make a rapid diagnosis possible. They require a drop of blood from a finger prick, and involve a paper test strip that is dipped into the blood and other solution(s). After a few minutes, during which the liquids are absorbed into the strip, a reading is obtained by the presence or absence of a coloured test line on a white background (MRC, 2001).

Pathology: *Plasmodium* infection has several effects on man. It can cause anaemia and this can be severe particularly in young children. Severe anaemia exerts a heavy toll on African children in malaria endemic countries. A recent estimate suggests that approximately 1.4 - 5.7 million cases occur each year, killing 190,000 – 974,000 children less than 5 years of age, with the highest mortality occurring in infants less than 12 months old (Murphy and Breman, 2001). Blackwater fever and cerebral malaria also results from *Plasmodium* infection. Other effects are diarrhoea and vomiting, especially in children, pulmonary oedema which is rare but often fatal, and hypoglycaemia which is being increasingly reported in patients with severe malaria, especially children and pregnant women (WHO, 1986a; Murphy and Breman, 2001).

DISCUSSION

Prevention and Control: It is recommended by World Health Organization (WHO) that malaria control should be based on an epidemiological approach and that it should be planned and coordinated within primary health care with the active participation of the community (WHO, 1986b). There are two goals in the management of malaria; treating the sick and reducing the risk of malaria (WHO, 1993). Treating the sick is entirely dependent on the effective use of antimalarial drugs delivered to malaria patients in a timely manner. To achieve this, health delivery systems will have to be vastly improved, especially in most of tropical Africa (Gilson and Mills, 1995).

In reducing the risk of malaria, several methods are used. These methods include, especially in Africa, the expanded deployment and use of insecticide-treated materials, bed-nets, and curtains for those at highest risk namely, infants, young children and pregnant women (Guillet *et al*., 2001; N'Guessan *et al*., 2001). In controlled experimental trials, increased survival rates among African children sleeping under insecticide-impregnated bed-nets, (IBNs) have been consistently reported (Alonso *et al*., 1991; Binka *et al*., 1996; Curtis, 1996; Nevill *et al*., 1996). This is probably because in very young children in whom significant levels of immunity have not yet developed, protection against the sheer numbers of malaria attacks that IBNs would afford reduces the risk of a fatal infection at this very vulnerable age (Alles *et al*., 1998).

Other strategies aimed at reducing malaria transmission are the genetic manipulation of mosquito vectors (Clarke, 2002; CNN, 2002; Holt *et al*., 2002; Hoffman *et al*., 2002a; NS, 2004). Researchers at Case Western Reserve University School of Medicine created a gene called SM1, which encodes for a protein that interferes with the development of the parasite in the mosquito (SABIC, 2002). These mosquitoes have not yet been released in an attempt to replace infectious populations, as more research is required. The use of genetically modified insect vectors in the field will require considerations in terms of biosafety, ecology, ethical, legal and social issues. The genome sequence of *Anopheles gambiae* provides an architectural scaffold for mapping, identifying, selecting and exploiting desirable insect vector genes (SABIC, 2002).

The development of a transmission – blocking vaccine is another strategy aimed at reducing malaria transmission (Meuwissen, 1989; Russel and Howson, 1996; Arnot *et al*., 1998). In their work, Florens *et al*. (2002) applied a high-throughput proteomics approach to identify new potential drug and vaccine targets and to better understand the biology of *P. falciparum*. They detected chromosomal clusters encoding co-expressed proteins, which suggested a potential mechanism for controlling gene expression. DNA vaccines and recombinant viral vector vaccines are now at the pre-clinical and clinical testing stages at various centers (Webster and Hill, 2003). DNA vaccines are effective at priming cellular immune responses, which are likely to be important in liver-stage malaria. Pre-erythrocytic vaccines are an attractive prospect, as they would prevent the invasion of hepatocytes by sporozoites or destroy parasites in infected hepatocytes and would thus prevent both clinical disease and the transmission of malaria. There is evidence that vaccination with irradiated sporozoites, which abort their development at the liver stage, can lead to protection of up to 90 % of immunized human volunteers following a regime involving the bites of more than 1000 irradiated infected mosquitoes over a period of time (Hoffman *et al*., 2002b). Probably the best characterized pre-erythrocytic antigen is the circumsporozoite protein (CSP) which is expressed on the extracellular sporozoite and the intracellular hepatic stages of the parasite (Malik *et al.,* 1991). The development of blood-stage vaccines has been focused on targeting the antigens responsible for parasite entry into cells. The best characterized antigen is MSP1, a major surface merozoite protein (Cheng *et al*., 1997). Transmission-blocking vaccines (TBVs) against malaria are intended to induce immunity against the stages of the parasite that

infect mosquitoes so that individuals immunized with TBVs cannot transmit malaria. These vaccines target the sexual stage of the malaria parasite with the aim of generating antibody responses that inhibit exflagellation and fertilization of the parasites in the mosquito vector. As a result, such vaccines would not have any effect on the clinical manifestations of malaria in an individual, but could have major impact through reducing malaria transmission and thus malaria mortality and morbidity at the population or community level (Russel and Howson, 1996). TBVs against the two major species of human malaria parasite, *P. falciparum* and *P. vivax* are under development (Carter, 2001).

Appropriate construction and siting of housing and local environmental improvement which includes destruction of breeding places of mosquitoes by draining gutters properly and keeping grasses around the home low can also help in reducing human-mosquito contact. Monitoring, including by satellite, of all aspects and features of a malarious situation will be important to the timing and targeting of antimalarial interventions (Thomson and Connor, 2001). According to Kishore (2002), Remote Sensing Technologies through satellites is likely to become a rapid epidemiological tool for surveillance of vector borne diseases and malaria in particular.

The Roll Back Malaria (RBM) initiative is another measure aimed at the control of malaria. RBM is unique in that, unlike previous global campaigns against malaria, it focuses on building sustainable community capacity and also, it raises the level of political commitment and advocacy at the country level (David, 2000; Saiprasad and Benerjee, 2003). Priority areas of RBM include the improvement of health systems, disease management, provision of anti-malarial drugs and malaria related control materials, disease prevention, disease surveillance and epidemic detection and control, sustainable control, human resources development and research including inter-disciplinary operational research (WHO, 2005). The goal of RBM is to provide reliable information on progress in controlling malaria that can be used at local and national levels and can inform regional and global efforts. RBM aims to control malaria to a level where it is no longer one of the major contributors to mortality and morbidity (David, 2000).

Treatment: Treatment of malaria could be supportive or specific. Supportive treatment include measures designed to combat the anaemia, reduce fever and maintain proper hydration and nutrition while specific treatment depends on accurate diagnosis and a thorough knowledge of the actions of the antimalarial drugs. Two types of drugs are used to treat malaria; blood schizonticides and tissue schizonticides (UACHPPM, 2004). Blood schizonticides attack the parasites within red blood cells. They are used in acute infection to prevent or terminate the clinical attack. Examples include amodiaquin hydrochloride, chloroquine phosphate, chlorguanide and quinine. However, the resistance of *P. falciparum* malaria to chloroquine has been widely reported (Neequaye *et al*., 1986; Rathod *et al*., 1997; Peters, 1998; Warhurst, 2001). Here in Nigeria, resistance to chloroquine has also been reported (Umotong *et al*., 1991; Sowunmi and Salako, 1992; Molta, 1995; Erah *et al*., 2003). Hence, scientists are still carrying out research on new and more effective antimalarials. Tissue schizonticides on the other hand act on the exo-erythrocytic parasitic stages in liver cells to prevent a relapse. They include drugs like primaquine phosphate, pamaquine, and pyrimethamine. Recently, aertemisinin and its derivatives from a Chinese herb have held out a great promise in the fight against malaria (Ezigbo, 1990).

Conclusion: The knowledge about *Plasmodium* infection will go a long way in reducing the prevalence of infection in man. Overall, it can be said that the impact of malaria on man has been very great. In one way or the other, the burden of malaria continues to this day at an unacceptable level.

In the future management of malaria, the tools available, drugs, insecticides, insecticide-treated materials, etc. will be of great importance. They are, individually and collectively, very effective instruments, although they are under constant threat from drug-resistant parasites and insecticide-resistant vectors. To maintain these tools, sustained investment of effort and resources is required on a much greater scale than is taking place at present. This could happen only in the presence of the necessary economic, political and social development in all of the affected countries.

REFERENCES

AIKAWA, M. (1980). Host cell invasion by malarial parasites. Pages 31 – 46. *In:* COOK, C. B., PAPPAS, P. W. and RUDOLPH, E. D. (Eds.). *Cellular Interactions in Symbiosis and Parasitism*. Ohio State University Press, Columbus, USA.

ALLES, H. K., MENDIS, K. N. and CARTER, R. (1998). Malaria mortality rates in South Asia and in Africa: implications for malaria control. *Parasitology Today, 14 (9)*: 369 – 375.

ALONSO, P. L., LINDSAY, S. W. and ARMSTRONG, J. R. (1991). The effect of insecticide treated bed nets on mortality of Gambian children. *Lancet, 337:* 1499 – 1502.

ANGYO, L. A., PAM, C. D. and SZLACHETBA, R. (1996). Clinical patterns and outcome in children with acute severe *Plasmodium falciparum* malaria at Jos University Teaching Hospital, Nigeria. *East African Medical Journal, 73 (12):* 823 – 826.

ARNOT, D. E., KORAM, K. and KILAMA, W. (1998). Malaria vaccine research and testing in Africa. *Parasitology Today*, *14(7):* 254 - 255.

BAIRD, J. K., JONES, T. R., DANUDIRGO, E. W., ANNIS, B. A., BANGS, M. J., BASRI, H., PURNOMO and MASBAR, S. (1991). Age-dependent acquired protection against *Plasmodium falciparum* in people having two years exposure to hyper endemic malaria.

American Journal of Tropical Medicine and Hygiene, 45: 65 - 76.

BAIRD, J. K. (1995). Host age as a determinant of naturally acquired immunity to *Plasmodium falciparum*. *Parasitology Today*, *11*: 105 - 111.

BINKA, F. N., KUBAJE, A. and ADJVIK, M. (1996). Impact of permethrin impregnated bed nets on child mortality in Kassena-Kankana district, Ghana: a randomized control trial. *Tropical Medicine and Health, 1*: 147 - 154.

BRUCE-CHWATT, L. J., GARRET-JONES, C. and WEITZ, B. (1966). Ten year study (1955 – 64) of host selection by *Anopheline* mosquitoes. *Bulletin of the World Heath Organization, 35*: 405 - 439.

CARTER, R. (2001). Transmission blocking malaria vaccines. *Vaccines, 19*: 209 – 2314.

CARTER, R. and MENDIS, K. N. (2002). Evolutionary and historical aspects of the burden of malaria. *Clinical Microbiology Reviews, 15(4)*: 564 - 594.

CHEESBROUGH, M. (1987). *Medical laboratory manual for tropical countries.* Vol. 1, Second edition. Cambridge University Press, Cambridge, Great Britain. 605 pp.

CHENG, Q., LAWRENCE, G., REED, C., STOWERS, A., RANFORD-CARTWRIGHT, L. and CREASEY, A. (1997). Measurement of *Plasmodium falciparum* growth rates in vivo: a test of malaria vaccines. *American Journal of Tropical Medicine and Hygiene, 57*: 495 – 500.

CLARKE, T. (2002). Mosquitoes minus malaria. *Nature, 419*: 429 - 430.

CNN (2002). Scientists crack malaria's genetic code. Cable News Network, USA. 3 pp. >http://www.cnn.com/2002/HEALTH/conditions/10/02/malaria.genetics/ reut/index.html < Accessed July 10, 2004.

COLUZZI, M. (1999). The clay feet of the malaria giant and its African roots: Hypothesis and inferences about origin, spread and control of *Plasmodium falciparum*. *Parassitologia*, 41: 277 - 283.

CURTIS, C. F. (1996). Impregnated bed nets, malaria control and child mortality in Africa. *Tropical Medicine and International Health, 1:* 137 - 138.

DAVID, A. (2000). Roll Back Malaria: what are the prospects? *Bulletin of the World Health Organization, 78 (12)*: 1377 - 1385.

ERAH, P. O., ARENMUGHARE, G. and OKHAMEFE, A. O. (2003). *Plasmodium falciparum* malaria resistance to chloroquine in five communities in Southern Nigeria. *African Journal of Biotechnology*, *2 (10)*: 384 – 389.

EZIGBO, J. C. (1990). Parasitic protozoa. Pages 4 – 18. *In:* EZIGBO, J. C. (Ed.). *Parasitology for medical students*. New Frontiers Publishers Limited, Lagos, Nigeria.

FLORENS, L., MUSTER, N., SACCI, J. B., TABB, D. L., WITNEY, A. A., WOLTERS, D., WU, Y., GARDNER, M. J., HOLDER, A. A., SINDEN, R. E., YATES, J. R. and CARRUCCI D. J. (2002). A proteomic view of the *Plasmodium falciparum* life cycle*. Nature, 419*: 520 – 526.

GILSON, L. and MILLS, A. (1995). Health Sector reforms in sub-Saharan Africa: lessons of the last 10 years. *Health Policy, 32:* 215 – 243.

GUILLET, P., ALNWICK, D., CHAM, M. K. NEIRA, M., MU-KELABAI, K., ZAIM, M. and HEYMANN, D. (2001). Long-lasting treated mosquito nets: a breakthrough in malaria prevention. *Bulletin of the World Health Organization, 79*: 998 - 999.

GUPTA, S., SNOW, R. W., DONNELLY, C. A., MARSH, K. and NEWBOLD, C. (1999). Immunity to non-cerebral severe malaria is acquired after one or two infections. *Nature Medicine, 5*: 340 - 343.

HOFFMAN, S. L. (1996). *Malaria vaccine development: a multi-immune response approach*. American Society of Microbiology, Washington D.C., USA, 325 pp.

HOFFMAN, S. L., SUBRAMANIAN, G. M., COLLINS, F. H. and VENTER, C. (2002a). *Plasmodium,* human and *Anopheles* genomics and malaria. *Nature, 415*: 702 - 709.

HOFFMAN, S. L., GOH, L. M., LUKE, T. C., SCHNEIDER, I., LE, T. P. and DOOLAN, D. L. (2002b). Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. *Journal of Infectious Diseases, 185:* 1155 – 1164.

HOLT, R. A., SUBRAMANIAN, G. M., HALPERN, A., SUTTON, G. G., CHARLAB, R., NUSSKERN, D. R., WINCKER, P., CLARK, A. G., RIBEIRO, J. M., WIDES, R., SALZBERG, S. L., BRENDAN, L., YANDELL, M., MAJOROS, W. H., RUSCH, D. B., LAI, Z., KRAFT, C. L., ABRIL, P. W., BADEN, H., BERARDINIS, V., BALDWIN, D., BENES, V., BIEDLER, J., BLASS, C., BOLANOS, R., BOSCUS, D., BARASTEAD, M., CAI, S., CENTER, A., CHATURVEDI, K., CHRISTOPHIDES, G. K., CHRYSTAL, M. A., CLAMP, M., CRAVCHIK, A., CURWEN, V., DANA, A., DELCHER, A., DEW, I., EVANS, C. A., FLANIGAN, M., GRUNDSCHOBER-FREIMOSER, A., FRIEDLI, L., GU, Z., GUAN, P., GUIGO, R., HILLENMEYER, M. E., HLADUN, S. L., HOGAN, J. R., HONG, Y. S., HOOVER, J., JAILLON, O., KE, Z., KODIRA, C., KOKOZA, E., KOUTSOS, A., LETUNIC, I., LEVITSKY, A., LIANG, Y., LIN, J., LOBO, N. F., LOPEZ, J. R., MALEK, J. A., MCLNTOSH, T. C., MEISTER, S., MILLER, J., MOBARRY, C., MONGIN, E., MURPHY, S. D., O'BROCHTA, D. A., PFANNKOCH, C., QI, R., REGIER, M. A., REMINGTON, K., SHAO, H., SHARAKHOVA, M. V., SITTER, C. D., SHETTY, J., SMITH, T. J., STRONG, R., SUN, J., THOMASOVA, D., TON, L. Q., TOPALIS, P., TU, Z., UNGER, M. F., WALENZ, B.,

WANG, A., WANG, J., WANG, M., WANG, X., WOODFORD, K. J., WORTMAN, J. R., NU, M., YAO, A., ZDOBNOV, E. M., ZHANG, H., ZHAO, Q., ZHAO, S., ZHU, S. C., ZHIMULEV, I., CLUZZI, M., TORRE, A., ROTH, C. W., LOUIS, C., KALUSH, F., MURAL, R. J., MYERS, E.W., ADAMS, M.D., SMITH, H. O., BRODER, S., GARDNER, M. J., FRASER, C. M., BIRNEY, E., BORK, P., BREY, P. T., VENTER, J. C., WEISSENBACH, J., KAFATOS, F. C., COLLINS, F. H. and HOFFMAN, S. L. (2002). The genome sequence of the malaria mosquito, *Anopheles gambiae. Science, 298*: 129 - 149.

KISHORE, J. (2002). *National health programmes of India*. 4th edition. Century Publications New Delhi. 117 pp.

KROGSTAD, D. J. (1996). Malaria as a re-emerging disease. *Epidemiology Reviews, 18*: 77 - 89.

MALIK, A., EGAN, J. E., HOUGHTEN, R. A., SADOFF, J.C. AND HOFFMAN, S. L. (1991). Human cytotoxic T lymphocytes against the *Plasmodium falciparum* circumsporozoite protein. *Proceedings of the National Academy of Sciences of the United States of America, 88*: 3300 – 3304.

MEHLHORN, H. and WALLDORF, V. (1988). Life cycles. Pages 1 – 147. *In:* MEHLHORN, H. (Ed.). *Parasitology in focus: facts and trends*. Springer-Verlag Berlin Heidelberg, Germany.

MEUWISSEN, J. H. (1989). Current studies related to the development of transmission – blocking malaria vaccines: a review. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 83 (Supplement):* 57 - 60.

MOLTA, N. B. (1995). Susceptibility of *Plasmodium falciparum* to malarial drugs in North-eastern Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 89*: 422 - 425.

MRC (2001). *Malaria advice for Southern Mozambique, Swaziland and South Africa*. Medical Research Council. South Africa. 17 pp. >http://www.malaria.org.za<. Accessed October 10, 2004.

MURPHY, S. C. and BREMAN, J. G. (2001). Gaps in the childhood malaria burden in Africa: Cerebral malaria, neurological sequelae, anaemia, respiratory distress, hypoglycaemia and complications of pregnancy. *American Journal of Tropical Medicine and Hygiene, 64(1-2 supplements):* 57 – 67.

MUTERO, C. W., OUMA, J. H., AGAK, B. K., WANDERI, J. A. and COPELAND, R. S. (1998). Malaria prevalence and use of self-protection measures against mosquitoes in Suba District, Kenya. *East African Medical Journal, 75(12)*: 11 - 15.

NEEQUAYE, J., COE-ENE, J. and TAELMAN, H. (1986). *In-vitro* chloroquine resistant falciparum malaria in West Africa. *Lancet, 1*: 153 - 154.

NEVILL, C. G., SOME, E. S. and MUNG'ALA, V. D. (1996). Insecticide-treated bed nets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health, 1*: 139 - 149.

N'GUESSAN, R., DARRIET, F., DOANNIO, J. M., CHANDRE, F. and CARNEVALE, P. (2001). Olyset net efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Cote d'Ivorie. *Medical and Veterinary Entomology, 15*: 97 – 104.

NS (2004). Genetically Modified (GM) mosquito resistant to malaria parasites. New Scientist. New York, USA. 3 pp. >http://www.newscientist.com< Accessed June 25, 2004.

OAKS, S. C., MITCHELL, V. S., PEARSON, G. W. and CARPENTER, C. C. J. (1991). *Malaria: obstacles and opportunities*. National Academy Press. Washington D.C., 328 pp.

OFOVWE, E. G. and EREGIE, C. O. (2001). Manifestations of severe *falciparum* malaria in children aged 6 months to 5 years in Benin City, Nigeria. *The Resident Doctor, 5 (1):* 16 - 20.

PETERS, W. (1998). Drug resistance in malaria parasites of animals and man. *Advances in Parasitology, 41*: 1 - 62.

RATHOD, P. K., MCERLEAN, T. and PEI-CHEIEH, L. (1997). Variations in frequencies of drug resistance in *Plasmodium falciparum. Proceedings of the National Academy of Sciences, 94*: 9349 - 9393.

RUSSEL, P. K. and HOWSON, C. P. (1996). *Vaccines against malaria: Hope in a gathering storm*. National Academy Press, Washington D.C., 30 pp.

SABIC (2002). Biotech takes on malaria. *AgBioTech InfoSource, 79*: 1 – 2. Saskatchewan Agricultural Biotechnology Information Centre. >http//:www.agwest.sk.ca<. Accessed November 8, 2005.

SAIPRASAD, G. S. and BENERJEE, A. (2003). Malaria control: current concepts. *Medical Journal of Armed Forces of India,* 59 (1): 5 - 6.

SMYTH, J. D. (1996). *Animal Parasitology*. Cambridge University Press, Great Britain, 549 pp.

SOWUNMI, A. and SALAKO, L. A. (1992). Evaluation of the relative efficacy of various antimalarial drugs in Nigerian children under 5 years of age suffering from acute uncomplicated *falciparum* malaria. *Annals of Tropical Medicine and Parasitology, 86*: 1 - 8.

THOMSON, M. C. and CONNOR, S. J. (2001). The development of malaria early warning systems for Africa. *Trends in Parasitology, 17*: 438 - 445.

TRAPE, J. F. and ROGIER, C. (1996). Combating malaria morbidity and mortality by reducing transmission. *Parasitology Today, 12*: 236 - 240.

UACHPPM (2004). *Malaria Fact sheet*. US Army Centre for Health Promotion and Preventive Medicine (UACHPPM). 2 pp. >http://www.who.int/mediacentre/factsheets <. Accessed July 10, 2004.

UMOTONG, A. B., EZEDINACHI, E. N., OKERENGWO, A. A., USANGA, E. A., UDO, J. J. and WILLIAMS, A. I. (1991). Correlation between *in vivo* and *in vitro* response of chloroquine resistant *Plasmodium falciparum* in Calabar, South-Eastern Nigeria. *Acta Tropica, 49*: 119 - 125.

WARHURST, D. C. (2001). A molecular marker for chloroquine-resistant *falciparum* malaria. *New England Journal of Medicine, 344*: 299 - 302.

WEBSTER, D. and HILL, A. V. S. (2003). Progress with new malaria vaccines. *Bulletin of the World Health Organization, 81(12)*: 902 – 909.

WHO (1986a). Malaria action programme. Severe and complicated malaria. World Health Organization. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 80 (Supplement)*: 1 - 50.

WHO (1986b). World Health Organization expert committee on malaria. *Technical Report Series, No. 735*: 1 - 10.

WHO (1989). Tropical diseases: progress in international research, 1987-88. *9th Programme Report of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (TDR)*. WHO, Geneva, 110 pp.

WHO (1990). Fogarty/World Health Organization (WHO) International conference on cellular mechanisms in malaria immunity. *Immunology Letters, 25*: 1 - 5.

WHO (1993). *A global strategy for malaria.* World Health Organization, Geneva, Switzerland. 350 pp.

WHO (1996a). World malaria situation in 1993. Part 1. World Health Organization. *Weekly Epidemiological Record*, 71: 17 - 22.

WHO (1996b). World Health Organization fact sheet Malaria. *No. 94(revised)*. World Health Organization, Geneva, Switzerland. 5 pp.

WHO (2005). World malaria report 2005. 11 pages. >http//:www.who.org<. Accessed October 5, 2005.

PREVALENCE OF SICKLE HAEMOGLOBIN AND GLUCOSE–6–PHOSPHATE DEHYDROGENASE DEFICIENCY GENES IN THE POPULATIONS OF NORTH WEST AND SOUTH WEST PROVINCES, CAMEROON

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ABSTRACT

Hereditary disorders of erythrocytes are common in many areas of the world, including Cameroon. Limited knowledge on the consequences of high incidences of sickle haemoglobin (HbS) and glucose-6-phosphate dehydrogenase (G6PD) deficiency genes in the Cameroons might have been responsible for the haemoglobin genotype mismatched marriages among the sickle heterozygotes and drug-induced anaemia among the G6PD deficient individuals ignorantly treated with oxidant drugs having high redox potential. The situation therefore, informed the random screening of the populace of the North West and South West populations of Cameroon for these genes with a view not only to reveal their current incidences and level of interaction but also to educate the people on the consequences of these genetic defects. Our results revealed the total incidences of 32.20 % sickle and 11.61 % G6PD deficiency genes. The percentage frequency of the sickle cell gene was higher in the South western (18.80 %) than in the North West (14.51 %) populations. The percentage incidence of G6PD deficiency was 9.21 % and 1.20 % for males and females respectively in the North West and 10.85 % and 1.46 % for males and females respectively in the South West. The interaction was not significant (P > 0.01) between G6PD deficiency and HbS for the North West and South West populations. These genetic defects must have reached polymorphic levels due to natural selection through survival advantage against death from malaria and consanguineous marriages.

Keywords: Sickle cell gene, G6PD Deficiency gene, Prevalence, Cameroon

INTRODUCTION

Human beings have interacted with malaria parasites for very long and thus the parasite has had ample time to adapt and evolve with the human host (Troye–Bloomberg *et al.,* 1999). Immune processes and genetic traits have contributed in reducing the profligacy of the malaria parasite and a wide range of genetic polymorphisms have been developed to modify individual response to this lethal disease. Haemoglobinopathies and glucose-6-phosphate dehydrogenase (G6PD) deficiency are among the most common single gene disorders, which affect red blood cell (RBC) stability and integrity (Kar *et al.,* 1990). More than 700 abnormal haemoglobins have been described world wide and more than 200 million people world-wide have RBC enzyme abnormality (Arya, 1995). These genetic lesions are major causes of morbidity and mortality around the world (Angastiniostis, 1995). Sickle haemoglobin and G6PD deficiency are genetically independent, their loci being located on chromosome 11 for sickle and chromosome X for G6PD deficiency genes.

Glucose-6-phosphate dehydrogenase [G6PD, EC 1:1:1:49; D–Glucose–6–phosphate: NADP Oxidoreductase (G6PD)] is a key enzyme in the pentose phosphate pathway (PPP) that is essential for adequate supply of phosphorylated nicotinamide-adenine dinucleotide (NADPH), which protects RBCs from oxidative stress. Reduced NADPH is needed to maintain glutathione (GSH), which in turn keeps the sulfhydryl groups of haemoglobin and other RBC proteins in a reduced active form. This activity enables the RBCs to withstand lysis from oxidant damage, instituted particularly during viral/bacterial or protozoa infections, or following exposure to oxidant drugs with high redox potential such as antimalarials (primaquine and pamaquine), sulfnamide, sulfamethoxazole and other drugs and chemicals and consumption of certain food stuff (fava beans) (Beutler, 1959; Lui *et al*., 1994; Cheesbrough, 2000).

Biochemical characterization has led to the identification of about 442 distinct G6PD variants, of which 299 were characterized and about 100 variants found to be polymorphic in various human populations (Beutler, 1990). Many of which have no haematological consequences. Commonly, however, intermittent episodes of haemolytic anaemia with or without chronic haemolysis may be associated with G6PD deficiency (Beutler *et al.,* 1996). Variants including the common G6PD-B (Gd^B) (wild-type), G6PD-A^+ (Gd^A) (non-deficient type) and G6PD-A^- (Gd^{A-})(deficient type) are observed in people living in tropical and sub-tropical areas (Beutler, 1994). Molecular basis of G6PD showed that both G6PD-A^+ and G6PD-A^- differ from G6PD-B by a variation at nucleotide 367 (A→G), while G6PD-A^- had an additional mutation at nucleotide 202 (G→A). G6PD A^+ is the most common variant found in 20% blacks Africans while G6PD-A^- variant is seen in 11% black Americans (Beutler, 1994).

Sickle haemoglobin (HbS) is caused by a "typographical error" in the genetic code in which thymine replaces adenine in the DNA encoding β-globin gene. Consequently, valine replaces glutamate at the sixth position in the β-globin product (Koch *et al.,* 2000). Sickle gene is widely distributed in malarial belts of Africa, but its frequency varies widely in different West African populations (Allison, 2002). The prevalence of sickle cell disease (SCD) in Africa ranges from 1 – 10 % while the sickle heterozygous state range between 15 – 40.5 % in malaria endemic areas (American Academy of Family Physicians, 1994, Uzoegwu and Onwurah, 2003). The high frequency of HbS and G6PD deficiency genes in some malaria endemic areas parallels the historical incidence of malaria (Allison, 1954; Allison and Clyde, 1961). Sickle haemoglobin and G6PD deficiency are common in Central and West Africa, probably due to their advantage over malaria. Although, there has been published report on the incidences and interaction of the sickle and G6PD deficiency genes in many West African countries, little or no such reports exist for the North and South Western populations of Cameroon. This study therefore was aimed at providing such information, considering the consequences of these genes in the population.

MATERIALS AND METHODS

Study Area: Latitudinally, the North West (NW) province metropolitan area lies between 5º56´N and 5º58´N of the equator, and longitudinally, it falls within 10°9´E and 10º1´E, of the Greenwich meridian. The South West (SW) province lies between longitude 9°75'E and 10°E and Latitude 4°4' N and 5°75' N. Their populations make up the Southern Cameroons, which in 1961 left the Federal Republic of Nigeria to join the then Eastern Cameroon after a referendum and have a history and present of malaria endemicity (Figure 1).

Subjects: A total of 12,470 volunteer indigenes of the two provinces aged one to seventy (1 – 70) years, who sought treatment in the Provincial Hospitals and Health Centers, some secondary school students and many community volunteers in the neighbourhoods, were randomly screened for haemoglobin genotype while 6,540 were screened for G6PD deficiency. No blood transfusion had been administered to any of these subjects for at least three months before the tests. A questionnaire was designed to obtain a subject's name, age, sex, residence, consanguinity of parents and family history of blood diseases were recorded.

Blood Collection: Blood samples (5 ml) were collected by venipuncture into sample tubes containing EDTA (1.0 ± 0.15 mg/ml of blood) as anticoagulant and then rocked gently to mix and used for both haemoglobin genotype and G6PD deficiency determinations.

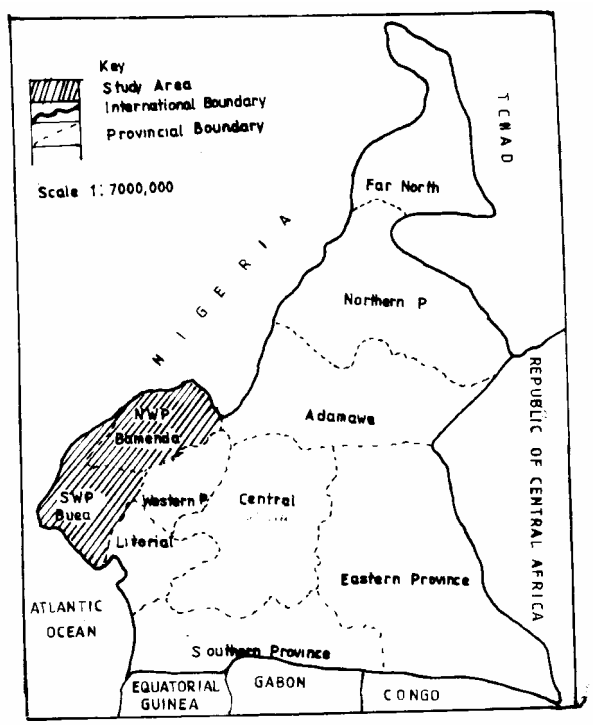

FIG.1: A MAP OF CAMEROON INDICATING THE STUDY AREAS SOUTH WEST AND NORTH WEST PROVINCES

Preparation of Haemoglobin Lysate and Haemoglobin Genotype Determination: Uncoagulated whole blood was centrifuged at 3,000 x g for ten minutes to separate the red blood cells from the plasma. The upper layer was aspirated out while the sediment was washed three times by re-suspending in equal volumes of normal saline (0.85 % w/v) and then re-centrifuged at the same speed. The packed cells were re-suspended in an equal volume of normal saline. About 20 μl of the suspended cells was then mixed with 80 μl of distilled water in a 1:4 dilution to lyse the cells and release the haemoglobin. The solution was gently shaken for two minutes and then centrifuged at 3,000 x g for 20 minutes. The resulting supernatant, haemoglobin lysate, was used for the genotype test while the precipitate was discarded. The Hb lysate was stored in the refrigerator (2 – 4 ^{o}C) until used within four days. Alternately, 20 μl of uncoagulated whole blood was mixed with 60 μl of distilled water and the resulting haemoglobin lysate spotted directly for haemoglobin genotype determination. Haemoglobin genotypes were determined by cellulose acetate membrane electrophoresis (CAME) of Evans (1971) as modified by Uzoegwu and Onwurah (2003).

Determination of G6PD Deficiency: The reaction mixture contained glucose-6-phosphate (0.01M), $NADP^+$ (0.01 M), saponins (0.02 M), phosphate buffer, pH 7.4 and distilled water. The activity of G6PD was determined by fluorescent spot test as described by Beutler *et al.* (1996). Fluorescence was produced during the reduction of $NADP^+$ to NADPH.

This reaction is coupled with oxidation of glucose-6-phosphate to 6-phosphogluconolactone and catalysed by G6PD. Specimen with G6PD activity of <20 % of normal (severe deficiency) do not fluoresce as the small amount of NADPH formed is reoxidised by glutathione present in the reagent. Presence of fluorescence indicated normal cells while weak fluorescence indicated slight deficiency.

Data Analysis: The prevalences of sickle haemoglobin and G6PD deficiency were estimated from the carrier frequency using Hardy-Weinberg equation. Difference in gender distribution was tested using student's t–test, while ANOVA and Fischer's exact test were used for analysis of age distribution and interaction between the two genetic defects.

RESULTS

Sickle Cell Disease and G6PD Deficiency Awareness: The levels of awareness about SCD and G6PD deficiency in the studied populations, gleaned from the questionnaire answers and post-lecture questions, were extremely low [North West population (12.6 % : 0.3 %) and South West population (12 % : 0.1 %)]. The North West population therefore was more knowledgeable about SCD and G6PD deficiency genes than South West population.

Incidences of Sickle Cell Disease: The haemoglobin genotype distribution in 4,042 and 8,428 volunteers screened for haemoglobin shows that the North West population exhibited lower incidence of both sickle and HbC genes (26.15 % and 0.15 % respectively) while the South West manifested higher sickle and HbC genes of 35.32 % and 0.21 % respectively (Table 1).

Frequencies of Haemoglobin Gene Mutation: Table 2 shows the haemoglobin gene frequencies calculated according to the method of Burn (1976) by applying the Hardy-Weinberg equation. The ratio of the percentage gene frequencies of male and female is 0.9 : 1. The estimated birth incidence of children with HbSS, SC and CC were 2.1×10^{-3} (2.1/1000 live births), 2.0×10^{-4} (0.2/1000 live births) and 5.4×10^{-7} (0.00054/1000 live births) and 8.1×10^{-3} (8.1/1000 live births), 1.9×10^{-4} (0.19/1000 live births) and 2.5×10^{-7} (0.00025/1000 live births) for the North West and South West populations respectively (Table 3).

Prevalence of G6PD Deficiency: G6PD deficiency was classified as completely deficient homozygotes and slightly deficient heterozygotes. G6PD deficiency was detected in 252 NW subjects (10.4 %) and 507 SW subject (12.4 %) with male : female percentages of 9.2 % : 2.4 % and 10.8 % : 3.0 % respectively for the NW and SW populations respectively. Of the females, 0.7 % had severe enzyme deficiency (homozygotes) and 0.5 % had moderate enzyme activity in the NW population while 1.2 % had severe enzyme deficiency (homozygotes) and 0.3 % had moderate enzyme activity in the SW population (Table 5).

Interaction of G6PD Deficiency with Different Haemoglobin Genotype: Co-inheritance of G6PD deficiency and HbS genes was detected in 3 (3.79 %) and 5 (2.89 %) of NW and SW subjects respectively (Table 6). The interaction of the two genetic defects was calculated and found not to be significant ($P > 0.01$) for the NW and SW populations respectively.

DISCUSSION

Limited information is available on the current distribution of sickle and G6PD deficiency genes in most West African populations. This study provided the much needed information on the subject matter. The availability of adequate information on the subject matter could invariably help respective government health ministries to plan for adequate health care provision. In Cameroon, it was discovered, that the frequencies of occurrence of sickle and glucose-6-phosphate dehydrogenase deficiency genes were high. Consequently, the abnormalities manifest diverse adverse effects on the populations studied. The overall high frequency of sickle (32.20 %) and G6PD deficiency (11.61 %) genes revealed by this study was not surprising in view of common consanguineous marriages contracted in the study area as well as the high malaria endemicity usually associated with high frequencies of sickle gene (Allison, 2004). High HbS gene has been reported to be confined to populations living in malarious areas while low frequencies were seen in tribes living in areas with low malaria transmission (Allison, 1954). For instance, in Africa, high HbS gene frequencies are confined to the malaria belt north of South Africa as well as south of the Sahara while low frequencies occur in the non-malarious highlands in East Africa and parts of West Africa (Allison, 2002). The overall frequency of these genetic defects could not be compared with those of the other eight provinces of Cameroon, since no such data were available.

Haemoglobin C gene is known to be polymorphic in West Africa attaining heterogeneous frequencies approaching 20% in northern Ghana and Burkina Faso (Modiano *et al.,* 2001). The rarity of this gene in Cameroon as revealed in this study could be corroborated by the fact that its frequency declined in all directions from northern Ghana and Burkina Faso (Allison, 2002) to 0.49% in Nigerian population (Uzoegwu, 2006).

The high frequency of G6PD deficiency (11.61 %) in malaria endemic populations of Cameroon corroborates the role malaria play in the distribution of G6PD genes in most malaria endemic areas in the world (El-Hazmi and Warsy, 1994). The percentage gene frequencies for G6PD deficiency of hemizygous males were computed to be 9.21 % and 10.85 % for the NW and SW populations respectively. Although the electrophoretic mobility was not carried out to ascertain the G6PD variant, the common African variant G6PD A^{-} (Beutler, 1994) was assumed

Table 1: Haemoglobin Genotype Distribution in North and South Western Cameroon

Sex	AA		AS		AC		SS		SC		CC		Total
	M	F	M	F	M	F	M	F	M	F	M	F	
North West population	1,390	1,609	385	517	2	2	59	76	2	0	0	0	
Total incidence (%)	2,999		902		4		135		2		0		4,042
	74.20		22.32		0.10		3.33		0.05		0.0		
South West population	2,679	2,760	1,309	1,321	7	5	181	160	4	1	1	0	
Total incidence (%)	5,439		2,630		12		341		5		1		8,428
	64.53		31.21		0.14		4.05		0.06		0.01		
Sex total	4,069	4,369	1,694	1,838	9	7	240	236	6	1	1	0	12,470
Incidence (%)	32.60	35.04	13.60	14.70	0.07	0.06	1.93	1.90	0.05	0.01	0.01	-	100
Group total	8,438		3,532		16		476		7		1		12,470
Total incidence (%)	67.67		28.32		0.13		3.82		0.06		0.01		100

Table 2: Gene Frequencies in the NW and SW Populations

Haemoglobin Genotype	Gene Frequency and Percentage (%)	
	North West Population	South West Population
A	0.8541 (85.41%)	0.8116 (81.16%)
S	0.1451 (14.51%)	0.1880 (18.80%)
C	0.0007 (0.07%)	0.0005 (0.05%)
Total	1 (100%)	1 (100%)

Table 3: Population Probabilities for Haemoglobinopathies in the NW and SW Populations

Haemoglobin Genotype	Gene Frequency	Population Probability	
		North West Population	South West Population
AA	p^2	0.7295	0.6587
AS	2 pq	0.2479	0.3052
AC	2 pt	1.3×10^{-3}	0.0353
SS	q^2	2.1×10^{-3}	8.1×10^{-3}
SC	2 qt	2.0×10^{-4}	1.9×10^{-4}
CC	t^2	5.4×10^{-7}	2.5×10^{-7}

Table 5: Prevalence and Gene Frequency of G6PD Deficiency in NW and SW Populations of Cameroon

Sex	North West Population (%)	Gene Frequency (%)	South West Population (%)	Gene Frequency	Total	Gene Frequency
Males	9.21 % (223/2420)	0.0921	10.85% (447/4120)	0.1085	10.24% (670/6540)	0.1024
Females	1.20 % (29/2420)	0.0120	1.46% (60/4120)	0.0146	1.36% (89/6540)	0.0136
Severe	0.74 % (18/2420)	0.0074	1.2% (49/4120)	0.0119	1.02% (67/6540)	0.0102
Moderate	0.45% (11/2420)	0.0045	0.3% (11/4120)	0.0027	0.34% (22/6540)	0.0034
Total	10.41 % (252/2420)	0.1041	12.30 (507/4120)	0.1230	11.61% (759/6540)	0.1161

Table 6: Interaction of G6PD Deficiency with Different Haemoglobin Genotype

Hb Genotype	North West population			South West population		
		G6PD deficient			G6PD deficient	
	Nº Investigated	Nº	%	Nº Investigated	Nº	%
HbA	173	22	12.72	334	51	15.27
HbS	79	3	3.79	173	5	2.89
HbC	-	-	-	-	-	-

to be the variant in Cameroon. A greater proportion of male subjects were deficient compared to their female counterparts probably due to a higher inactivation of normal X-chromosome in heterozygous females. Since G6PD deficiency is sex linked, severe enzyme deficiency occurred in hemizygous males and homozygous females while heterozygous females have normal or moderately lower enzyme level (Beutler, 1990).

Plasmodial parasite densities were reported to be lower in G6PD deficient Tanzanian children than in children in other resident areas where chemoprophylaxis was not used against plasmodial parasites (Allison and Clyde, 1961). Furthermore, it was observed that G6PD deficient cells do not efficiently support malaria parasite growth in culture and that high frequency of G6PD deficiency are confined to malarious parts of the world (Allison,

2002). These observations are further supported with the high G6PD deficiency frequency as observed in this study. The significantly lower frequencies of sickle gene in the North West as compared to that in the South West populations of Cameroon may be attributed to differences in the environmental factors and malarial endemicity, rather than in the affinities defined by tribal (Foy *et al.,* 1954) and linguistic (Lehmann and Raper, 1949) factors or even blood group antigens (Allison, 1954) in the two populations. Similar occurrence of sickle differences was reported in the population of central Greece, which had 16.0 % malaria frequency (Choramis *et al.,* 1952) as compared to 32.0 % observed in the Chalkhide peninsular of northern Greece (Deliyannis and Tavlarakis, 1955). These areas were notorious for malaria before it (malaria) was controlled. But however, the former had lower malaria than the later populations (Allison, 2002). The higher incidence of sickle cell anaemia (4.05 %) manifested in the south western population of Cameroon, when compared with that of the north western population (3.33 %) could therefore implicate higher incidence of sickle cell trait in South West (31.21 %) then North Western (22.32 %) provincial populations as observed in this investigation. Higher frequency of sickle heterozygotes is capable of generating more haemoglobin incompatible marriages particularly in populations with limited knowledge of SCD and its control. A risk of the reproduction of children with sickle cell anaemia could be high from such mismatched marriages. Our results on the frequencies of the haemoglobinopathies in this study confirmed the rarity of HbC gene in Cameroon.

The population probabilities for HbAA, AS, AC, SS, SC and CC as shown in Table 7 indicated that one in every four hundred persons may be sickle cell anaemia patients while one in every five thousand persons could have HbSC disease and one in every two million could suffer HbCC disease in the NW population. In the SW population one in two hundred can suffer SCD, one in every six thousand persons could have HbSC disease and one in every four million could suffer HbCC disease. However, high rate of ignorance, consanguinity and other forms of intermarriages can disturb the Hardy-Weinberg equilibrium. Since this study was hospital-based, conducted on supposed malaria patients and subjects with minor illnesses, there may therefore be a slight difference when compared to the study in a general population devoid of any restrictions. Both genes were found to interact even though they are located on separate genes, in consonance with the reports of some scientists (Obaid *et al.,* 2001; Hassan *et al.,* 2003). Their interaction could possibly influence the survival of the carriers by protecting them against malaria. It is important for African clinicians to be aware of RBC disorders in their population. Known oxidative drugs linked to clinically important haemolysis in G6PD deficient subjects should be avoided unless G6PD deficiency has been ruled out. By keeping to this principle, unnecessary complications and unfruitful diagnostic evaluations may be avoided.

Recommendations: As effective management of SCD involves blood transfusion and iron chelating agents, may be too expensive for most developing countries particularly now that HIV infection is much higher. It is therefore cogent to assert that health education; knowledge of ones genotype through effective genotype screening should form a critical part of management. For the prospective control of sickle cell disease, proper enlightenment and genetic counseling on the disease are very essential. Health educators must create the awareness of the disease and methods of control and management in the population. Compulsory screening will probably inform people of their genotypes. SCD being an autosomal recessive genetic disorder, one of the important preventive measures is to avoid inbreeding between sickle heterozygotes. Education on the implication of glucose-6-phosphate dehydrogenase deficiency and the subsequent screening will ensure reduction in the problems associated with this enzyme deficiency, particularly in malaria endemic country like Cameroon.

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REFERENCES

ALLISON, A. C. (1954). The Distribution of the Sickle Cell Trait in East Africa and Elsewhere, and its Apparent Relationship to the Incidence of Subtertian Malaria. *Transactions of Royal Society of Tropical Medicine and Hygiene, 81:* 312 – 318.

ALLISON, A. C. (2002). The Discovery of Resistance to Malaria of Sickle Cell Heterozygotes. *Biochemistry and Molecular Biology Education*, *30:* 279 – 287.

ALLISON, A. C. (2004). Two Lessons from the Interface of Genetics and Medicine. *Genetics,* 166: 1591 – 1599.

ALLISON, A. C. and CLYDE, D. F. (1961). Malaria in African children with deficient erythrocyte G6PD. *British Medical Journal*, *5236:* 1346 – 1349.

ANGASTINIOITIS, M. MODELL, B., ENGLEZOS, P. and BOULYENKOV, V. (1995). Prevention and control of haemoglobinopathies. *Bulletin of World Health Organization,* 73: 375 – 386.

ARYA, R., LAYTON, D. M. and BELLINGHAM, A. J. (1995). Hereditary Red Cell Enzymopathies. *Blood Review*, 9: 165 – 175.

BEUTLER, E. (1959). The Haemolytic Effect of Premaquine and Related Compounds: A Review. *Blood*, *14:* 103 – 139.

BEUTLER, E. (1990). The Genetics of G6PD Deficiency. *Semin Haematology*, 27: 137 – 164.

BEUTLER, E. (1994). G6PD Deficiency: Clinical Manifestations, Genetics and Treatment *Blood*, *84(11):* 3613 – 3623.

BEUTLER, E., VULLIAMY, T. and LUZZATTO, L. (1996). Haematologically Important Mutations: G6PD, *Blood Cells and Molecular Diseases*, *22:* 49 – 56.

BURN, G. W. (1976). The Science of Genetics. 3rd Edition, Collier Macmillan Publications, London.

CHEESBROUGH, M. (2000). *District laboratory Practice in Tropical Countries* Part 2. University Press, Cambridge, pp 271 – 340.

CHORAMIS, C., ZERVOS, N., CONSTANTANIDES, B., ZANNOS, L. and SKLAVOUNOVA, S. (1952). Sickle Cell Trait in the Lake Copais Region of Central Greece. *Schweitzer Medicine Wonchenchr*, *82:* 709 – 712.

DELIYANNIS, G. A. and TAVLARAKIS, N. (1955). Sickling Phenomenon in Northern Greece. *British Medical Journal, 4934:* 299 – 301.

EL-HAZMI, M. A. F. and WARSY, A. S. (1994). The Frequency of Glucose-6-Phosphate Dehydrogenase Phenotypes and Sickle Cell Genes in Al-Qatif Oasis. *Annals of Saudi Medicine,* 14(6): 491 – 494.

EVANS, D. I. K. (1971). Haemoglobin Electrophoresis on Cellulose Acetate Using Whole Blood Samples. *Journal of Clinical Pathology, 24: 8*77 – 878.

FOY, H., KONDI, A., TIMMS, G. L., BRASS, W. and BUSHRA, F. (1954). The Variability of Sickle Cell Rates in Tribes of Kenya and Southern Sudan. *British Medical Journal*, 4857: 294 – 297.

HASSAN, M. K., TAHA, I. J. Y., AL-NAAMA, L. M., WIDAD, N. M. and JASIM, S. N. (2003). Frequency of Haemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Barsa. *Eastern Mediterranean Health Journal*, *9(1/2):* 132 – 147.

KAR, B. C, AGRAWAL, K. C. and PANDA, A. (1990). Sickle Cell Haemoglobin, G6PD Deficiency and Malaria in Western Orissa. *Journal of the Association of Physicians of India*, *38(8):* 555 – 557.

KOCH, A. A., OLNEY, R. S. and YANG, Q. (2000). Sickle Haemoglobin Allele and Sickle Cell Disease. *American Journal of Epidemiology, 9:* 839 – 845.

LEHMANN, H. and RAPER, A. B. (1949). Distribution of Sickle Cell Trait in Uganda and its Ethnological Significance. *Nature*, 164: 494 – 495.

LUI, T. Z, LIN, T. F., HUNG, I. J, WEI, J. S. and CHIU, D. T. Y, (1994). Enhanced Susceptibility of Erythrocytes Deficient in Glucose-6-Phosphate Dehydrogenase in Alloxan, GSH-induced decrease in Red Cell Deformability. *Life Sciences. 55:* l55 – 60.

MODIANO, D., LUONI, G., SIRIMA, B. S., LANGRANCOTTI, A., PETRARCA, V., CRUCIANI, F., SIMPORE, J., CIMINELLI, B. M., FOGLIETTA, E., GRISANTI, P., BIANCO, I., MODIANO, G. and COLUZZI, M. (2001). The Lower Susceptibility of *Plasmodium falciparum* Malaria to Fulani of Burkina Faso (West Africa) is Associated with Low Frequencies of Classic Malaria-Resistance Genes. *Transactions of Royal Society of Tropical Medicine and Hygiene*, *95:* 149 – 152.

OBAID, A. D., HASSAN, M. K. and AL-NAAMA, L. M. (2001). Sickle Cell and Glucose-6-Phosphate Dehydrogenase Deficiency Genes in Abu Al-Khasib District of Southern Iraq. *Medical Journal of Barsa University, 19:* 12 – 18.

TROYE–BLOOMBERG, M., PERLMANN, P., MINCHEVA, N. L. and PERLMANN, L. (1999). Immune Regulation of Protection and Pathogenesis in *P. falciparum* Malaria. *Parasitologia*, *41(1 – 3):* 131 – 138.

UZOEGWU, P. N. and ONWURAH, A. E. (2003). Correlation of Lipid Peroxidation Index with Sickle Haemoglobin Concentration in Malaria-Positive and Negative of AA, AS, and SS individuals from the University of Nigeria, Nsukka Community. *Journal of Biological Research and Biotechnology*, *1(1):* 97 – 144.

UZOEGWU, P. N. (2006). The Possible Role of Diet in Mitigating Oxidative Stress Induction during Plasmodial Infection. *Bio-Research*, 4(2): 201 – 206.

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