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ANTIPSYCHOTIC EFFECT OF AQUEOUS STEM BARK EXTRACT OF *Amblygonocarpus andongensis* IN WISTAR ALBINO RATS

[1]EBBO, Abdullahi Aliyu., [2]ELSA, Abdullahi Teleh., [3]ETUK, Emmanuel Udok., [4]LADAN, Mohammed Jengebe and [5]SAGANUWAN, Saganuwan Alhaji

[1]Department of Veterinary Pharmacology, Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria.

[2]Department of Veterinary Medicine, Surgery and Theriogenology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria.

[3]Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria.

[4]Department of Chemistry, Faculty of Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Sokoto State, Nigeria.

[5]Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, PMB 2373, Makurdi, Benue State, Nigeria.

Corresponding Author: Saganuwan, S. A. Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, PMB 2373, Makurdi, Benue State, Nigeria. Email: pharn_saga@yahoo.com Phone: +234 703 9309 400; +234 802 7444 269

ABSTRACT

The study of antipsychotic effect of the aqueous stem bark extract of Amblygonocarpus andongensis was carried out on amphetamine induced psychosis in 42 Wister albino rats weighing between 105 and 305.2g using two indices: feeding and locomotor activity. Twelve out of the 42 rats were divided into two groups; six per group. Group 1 and 2 received 1.5mg/kg body weight of oral amphetamine. Oral chlorpromazine (0.5mg/kg) was administered to group 2 rats in addition. The remaining 30 rats were divided into 5 groups: A, B, C, D, and E, each group comprised 6 rats. All the groups received 1.5 mg/kg body weight of amphetamine but E received 0.5mg/kg oral chlorpromazine in addition. However, B, C and D received 450, 900 and 1350mg/kg bodyweight of Amblygonocarpus andongensis aqueous stem bark extract. Feeding and locomotor activities were measured in groups 1 and 2 and A, B, C, D and E rats respectively. The result showed that there were significant differences in feeding and locomotor parameters between groups 1 and 2 and among groups A, B, C and E ($p<0.05$) except group E. In amphetamine psychotic model test, group 2 animals have reduced feeding and locomotor activity as compared to group 1. Conclusively, Amblygonocarpus andongensis has a dose dependent reducing effect on feeding and locomotor activity at 135mg/kg body weight as compared to chlorpromazine (0.5mg/kg) in amphetamine induced psychosis in Wister albino rats. Hence both Amblygonocarpus andongensis and chlorpromazine may have pharmacokinetic effect on amphetamine and therefore maybe used to treat psychosis induced by amphetamine.

Keywords: Antipsychotic, effect, Amblygonocarpus, andongensis, Wister rat

INTRODUCTION

Psychosis is the term used to describe a mental state in which the individual experiences a distortion or loss of contact with reality, clouding of consciousness (Joel *et al.,* 1996). The mental state is characterized by the presence of features such as depression, anxiety, sleep disturbance, social withdrawal and impaired role functioning during a psychotic episode (Joel *et al.,* 1996). Psychosis can be caused by a number of conditions. These include organic causes such as drug intoxication, metabolic and schizoaffective disorder (Szasz, 1960).

Psychotic mental illnesses are of major social and public health importance. These conditions affect a significant number of individuals in our communities. About two percent of people experience a psychosis episode at some stage in their life (Beekman *et al.,* 1999). An estimated 80 % of those affected by psychotic disorder experience their first episode between the ages of 16 and 40 years (Beekman *et al.,* 1999). It has been postulated that the onset of course of psychosis is determined by an underlying vulnerability of psychosis coupled with the impact of environmental stresses, which may then trigger active psychotic symptoms. This is the so-called stress/vulnerability model for psychosis (Ayd and Blackwell, 1970).

The use of drugs is the most important in the management of psychosis (Ross, 1996). The current effective antipsychotic agents are tricyclic antidepressants such as: phenthiazines, thioxanthenes, benzodiazepines, as well as butyrophenones and its congeners. Other drugs include heterocyclic and experimental benzamides (Ross, 1996). All these drugs block D_2 dopaminergic receptors and inactivate dopamine neurotransmission in the forebrain. Some also interact with D_1

dopaminergic, $5HT_2$ serotonergic and alpha-adrenergic receptors (Ross, 1996). Although low potent chlorpromazine has more sedative, hypertensive and autonomic side effects (Ross, 1996).

Because of side effects of these antipsychotic drugs, there is need to investigate our indigenous herbs that have long standing claims of antipsychotic properties by our indigenous traditional medical practitioners. More so, Orji *et al.,* (2003) reported that Nigeria has an interesting rich flora ranging from mangrove swamps and rainforest in the south to the savanna and thorn bush regions in the north.

Since there has been a renewed interest in the use of traditional medicine in the last decade (Ross, 1996), the need to investigate our indigenous plants for the antipsychotic properties is not out of place. Therefore, this study was designed to investigate the antipsychotic effect of aqueous stem bark extract of *A. andongensis* in wistar albino rats.

MATERIALS AND METHODS

Plant Materials: *A. andongensis* stem bark used for the experiment was collected from Anka town in Zamfara State, and identified by a botanist in the herbarium of Biological Science Department, Usmanu Danfodiyo University, Sokoto, Nigeria where a Voucher specimen was kept. The stem of the plant was washed and the bark separated, air-dried and pulverized using mortar and pestle.

Extraction: One hundred (100) grammes of the pulverized air-dried bark of *A. andongensis* was dissolved in 500 mls of distilled water in a conical flask. The mixture was shaken vigorously for 6 hours and allowed to stand for 24 hours. It was then filtered with Whatman (No. 1) filter paper and the filtrate was evaporated at 50^{o} C in a desiccator (Eduardo *et al.,* 2000).

Experimental Animal: Forty-two (42) Wistar albino rats of either sex weighing between 105 to 305.2 g were used for the study. They were acquired from the Animal house, Zoological Garden, Usmanu Danfodiyo University, Sokoto and housed in cages in Pharmacology Department Research Laboratory. They were acclimatized for two weeks, fed on pellets of growers marsh poultry feed (Vital feeds®) and allowed access to water. Twelve out of the 42 rats were used to test for psychotic model of amphetamine. Whereas the remaining 30 rats were used for psychotic and antipsychotic model of amphetamine and *A. andongensis* respectively.

Confirmation of Amphetamine as Psychotic Model: The method of Oscar *et al.* (2004) was adopted. Twelve out of the 42 rats of either sex were divided into two groups, 1 and 2. After having weighed each of the twelve rats, the rats in group 1 and 2 were treated orally with 1.5 mg/kg body weight of amphetamine and their physical, somatic locomotive and behavioral responses were observed and recorded. Stereotype locomotive activity such as sudden quick, jerky movement (agitation) and feeding habits were recorded (Psychotic model). The rats from group 2 received chlorpromazine at 0.5mg/kg 30 minutes post administration of amphetamine but they were observed for abnormal behaviors (Antipsychotic model). This was to access the reliability of the models used for the experiment.

Antipsychotic Test with *Amblygonocarpus andongensis* Aqueous stem bark Extract: Thirty (30) Wister albino rats of either sex weighing between 105 g and 305.2 g were randomly distributed into 5 groups of 6 animals per group, labeled A (negative control), B, C, D, and E (positive control).

The rats from group A - E were treated orally with amphetamine to induce psychosis at the dose of 1.5 mg/kg body weight using a blunt ended canula. After the induction of psychosis in all the 30 rats, the rats in group B - D were treated with 450, 900 and 1350 mg/kg of the extract and the group E rats received chlorpromazine at the dose rate of 0.5 mg/kg body weight 30 minutes post amphetamine administration. The physical, somatic and behavioral changes observed from all the groups were recorded.

Statistical Analysis: The data were analyzed using one-way ANOVA followed by Turkey Kremer's multiple comparison tests (Petrie and Watson, 2002).

RESULTS

Confirmation of Amphetamine as Psychotic Model: Less than 10 minutes post administration of 1.5 mg/kg body weight of oral amphetamine, all the rats from groups 1 and 2 showed behavioural and somatic changes. There were repetitive stereotype locomotive activities, reduction in general activity level, anorexia, reduced feed intake, pupilary dilation and recumbency. But in group 1, appetite was restored 271.17 $\pm$ 1.2 minutes post administration of amphetamine but group 2 had reduced duration of action of psychotic effects characterized by agitation and loss of appetite that was restored after a short period of time (144.5 $\pm$ 1.34 min) as compared to those of group 1 (271.17 $\pm$ 1.2) (Figure 1.)

Anti-psychotic Effect of *Amblygonocarpus andongenesis* Aqueous Stem Bark Extract: The results of antipsychotic effect of *andongensis* extract in wistar albino rats revealed significant difference ($P < 0.05$) in parameters (feeding and agitation) among groups A, B and C, except group D that received highest dose of the extract and group E that received chlorpromazine where the differences were not increased significantly ($P > 0.05$) (Table 1). Nevertheless, *andongensis* extract started showing antipsychotic effect using feeding as a parameter 204.5 $\pm$ 2.5, 190.1 $\pm$ 0.7 and 150 $\pm$ 1.0 min post administration of the extract in groups A, B and C respectively (Figure 2). But onset of action of the extract and chlorpromazine in group D and E rats were 150 $\pm$ 1.0 and 145 $\pm$ 2.21 minutes respectively.

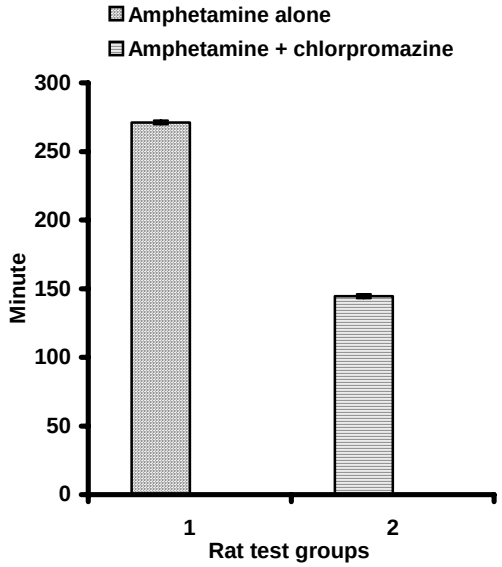

Figure 1: Atenuating effect of chlorpromazine on amphetamine induced psychosis (using feeding and agitation)

The effect of the extract lasted for about 65 ± 3.11, 80 ± 2.34, 120.17 ± 2.56 and 125 ± 2.61 minutes in animals of groups B, C, D and E respectively (see table 1 fig 2). The periods taken before restoration of appetite were 270 ± 3.03, 205 ± 2.38, 90.17 ± 2.71, 149.83 ± 2.01 and 145 ± 2.2 minutes in groups A, B, C, D and E respectively (Table 1).

Table 1: Antipsychotic Effect of *Amblygonocarpus andongensis* in Albino Rats Using Feeding Parameter

Parameters	Experimental rats				
	A	B	C	D	E
Onset of action (min)	-	204.5 ± 2.4	190.1 ± 0.7	150 ± 1.0*	145 ± 2.21*
Duration of action (min)	-	65 ± 3.11	80 ± 2.34	120.17 ± 2.56*	125 ± 2.61*
Restoration of appetite (min)	270 ± 3.03	205 ± 2.38	90.17 ± 2.71	149.83 ± 201*	145 ± 2.2*

Key: P > 0.05; A = Amphetamine; B = Amphetamine + 450 mg/kg of extract; C= Amphetamine + 900 mg/kg of extract; D = Amphetamine + 1350 mg/kg of extract; E = Amphetamine + Chlorpromazine*

DISCUSSION

The observation of the increased stereotyped locomotory activity (in form of quick, sudden movement, sudden halting and restlessness), anorexia upon administration amphetamine to group 1 and 2, and A - E rats (fig. 1 and 2) agreed with what Mark and Athina (2000) reported. They reported that amphetamine can induce psychotic activities in animal models. However, in group 2 animals feeding and agitation were reduced 150 minutes post chlorpromazine administration. But group 1 animals resumed feeding 271 minutes post amphetamine administration.

In the phase of the antipsychotic testing of the extract, the rats in group A (amphetamine alone) went off feed immediately, and could not resumed feeding until after 270 ± 3.03 minutes (Table 1). The result is in concordance with what was obtained (271.17 ± 1.2) in group 1 animals that were used to confirm the reliability of amphetamine model of psychosis in this experiment. This also agrees with the finding in human that weight loss in obesity following amphetamine treatment is almost entirely due to its anorectic effect but also due to increased metabolism (Joel *et al.,* 1990).

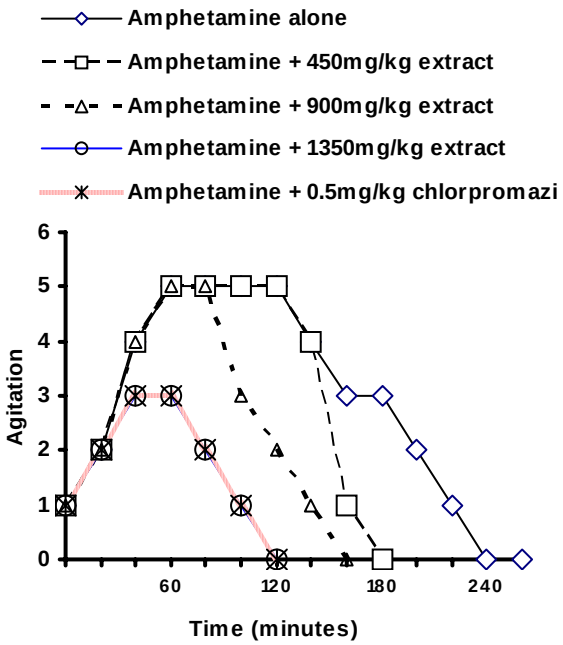

Figure 2: Antipsychotic effect of Amblygonocarpus andongensis using agitation parameter

The resumption of feeding 270 minutes post amphetamine treatment agrees with the observation of Silverton (1992) and Bray (1993) that tolerance to amphetamine is very rapid and thus continuous quest for higher dose. The decrease from 270 to 205 minutes in period of anorexia in group B rats given 450 mg/kg of the extract was significant (P<0.05) suggesting the antianorexic effects of the extract even at low doses. The group C and D rats that received 900 and 1350mg/kg limit doses of the extract recorded significant decrease in duration of restoration of appetite (P < 0.05). The restoration of appetite of the group E rats that received chlorpromazine (0.5 mg/kg orally) following 1.5 mg/kg oral amphetamine treatment was not statistically significant as compared to those of group D (P > 0.05) (Table 1).

Also, agitation of the rats reduced in duration with increase in the dose of the extract following amphetamine administration. Group D animals (1350 mg/kg) firstly becoming calmed and

lastly group B. That is as the dose of the extract increased, the period of calming effect also increased.

The highest antipsychotic effect (agitation) displayed by amphetamine (Fig. 2) for a period of 160 minutes at a dose rate of 1.5mg/kg body weight agrees with the report of Lees (1991) that the effect of amphetamine on the central nervous system (CNS) includes increase alertness (agitation), wakefulness and feeling of euphoria in man. He reported that the application of the agitative effect of amphetamine has been in the treatment of overdose with CNS depressants i.e. analeptic effect. So because of the agitative effect of amphetamine, physical work capacity is improved and sleep is prevented (Lees, 1991). The psychotic effect shown by amphetamine in our study is supported by the report of Tripathy (2003) that alertness, increase concentration, attention span, talkativeness, euphoria and increased work capacity are the central effects of amphetamine as fatigue is allayed.

Nonetheless, the uniformity in antipsychotic effects of chlorpromazine in group 2 and E may be suggestive of the pharmacokinetic effect of chlorpromazine and andongensis on amphetamine as shown by subsequent decrease in psychotic effect of amphetamine in wistar rats (Figures 1 and 2). However, the decrease in the psychotic effect of amphetamine due to Amblygonocarpus extract administration at 450, 900 and 1350 mg/kg in groups B, C and D may suggest the antagonistic effect of the extract of *A. andongensis* aqueous stem bark on amphetamine. Hence, it is used in the treatment of psychosis by the tradomedical practitioner of Northern Nigeria.

Conclusion: In conclusion, the aqueous stem bark extract of *Amblygonocarpus andongensis* has dependant antipsychotic effect at a rate of 1350 mg/kg body weight in comparison with chlorpromazine (0.5 mg/kg). Although, chlorpromazine is more potent than *Amblygonocarpus andongensis.* More so, both chlorpromazine and andongensis may have pharmacokinetic effect on amphetamine.

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EFFECT OF LEAF EXTRACTS OF *Draceana aborea* L. AND *Vitex doniana* SWEET ON THE LARVAE OF *Anopheles* MOSQUITO

[1]NNAMANI, Catherine Vera, [2]OSELEBE, Happiness Ogba and [1]OGBONNA, Anthony
[1]Department of Applied Biology, Ebonyi State University, Nigeria
[2]Department of Crop Production and Land Scape Management Ebonyi State University, Nigeria

Corresponding Author: Nnamani, C. V. Department of Applied Biology, Ebonyi State University, Nigeria. Email: nnamanikate2007@yahoo.com Phone: +234 8037786269

ABSTRACT

The leaf extracts of Draceana aborea and Vitex doniana of Agavaceae and Verbenaceae families respectively, were tested on the larvae of anopheles mosquito for their botanical insecticidal effects. The results of the investigation showed that the minimum percentage mortality concentration (MPMC) of these leaf extracts on the test organisms were at 7.5ml/20ml and 10ml/20ml as the starting points for D. aborea and V. doniana, respectively. Findings equally revealed that the combination of D. aborea and V. doniana leaf extracts exerted synergistic effects on these organisms at 5.0ml/20ml, whereas the use of the D. aborea and V. doniana extracts separately resulted in reduced efficacy. Analysis of variance showed that, there was no significant difference (P = 0.01) between the synergy and the individual treatments of the leaf extracts on these organisms. Preliminary phytochemical screening showed the presence of flavonoids, free phenolics, condensed tannins, pseudotanins, triterpenes, glycosides and saponins which have some insecticidal effects on their targeted organisms. These findings represent one of the steps in identifying plants, with insecticidal properties from the rich Bioresources in the Mosaic of the Low-Land Rainforest vegetation zone of Southeastern Nigeria.

Keywords: *Draceana aborea, Vitex doniana, Leaf* extract, Botanical insecticide, Phytochemical, *Anopheles* mosquito

INTRODUCTION

Mosquito has perhaps attained the greatest public nuisance than any other arthropod in recent time. This is because of responsible for 273 million clinical cases and 1.13 million deaths annually in Sub-Sahara Africa (USAID, 2006). Synthetic insecticide which inhibits or kills insects has now become popular target for elimination by environmental conservationists. This is because they are considered to be important sources of man-made pollutants and are detrimental to the environment and the surrounding biodiversity (Clark, 2000). Their negative impacts on the environment are becoming of global concern, such problems like environmental contamination, bioaccumulation, residues in foods and feeds and pest resistance.

More recently, attention has been turned to plant extracts as alternative sources of insecticidal compounds, against the synthetics. Among the botanical insecticides used are Rotenone and Azadirachtin. Rotenone acts as a stomach poison and can effectively control leaf feeding insects such as beetles and aphids. Rotenone is produced from the roots of *Lonchocarpus* sp. grown in South America. Its mode of action is the inhibition of cellular respiration in nerve and muscle cells causing rapid cessation of feeding. Rotenone is useful against leaf feeders; because it degrades rapidly in the air and sunlight (Anne, 2005). Hence it is used as fish poison in water management. Azadirachtin acts as an insect repellent, feeding deterrent and growth regulator. Its active ingredient Azadirachtin is extracted from *Azadirachta* seed that has both insecticidal and fungicidal activities (Anne, 2005). The insecticidal properties derived from these plants have prompted the examination of other plants for new insecticidal compounds.

Dracaena arborea belongs to the family Agavaceae and the genus is composed of about 500 species of woody stemmed plants mostly occurring in the tropics and subtropics. *Vitex doniana* belongs to the family Verbenaceae. This large genus is distributed throughout the tropics and subtropics (Onochie *et al.,* 1964). The preponderance of these plants within the mosaic of the lowland rainforest vegetation zone provides an adequate means of their utilization as cheap sources of biological agents for study on the production of bio-insecticides. Moreover, it will serve as a cheaper means of treatment for low income earners and rural dwellers who cannot afford high cost of modern insecticides.

The aims of this study were to determine the effect of *D. arborea* and *V. doniana* leaf extracts on the larvae of Anopheles mosquito. To compare the mortality rate of these extracts to that of synthetic insecticide (pest ox). To determine the synergistic effect of the two plants and finally evaluate the minimum percentage mortality concentration (MPMC) of these plant extracts.

MATERIALS AND METHODS

Preparation of Plant Extract: The leaves of the *D. arborea* and *V. doniana* plant were collected, washed and oven dried at 50 o C for 40 minutes. Twenty grams of each of the pulverized leaves of *D. arborea* and *V. doniana* were introduced into 200 ml of 95% ethanol and left for 24 hours. At the end of this duration it was filtered and the solutions collected were stored at 4 o C pending use. Synergy extract was prepared by the combination of 10 g each of the *D. arborea* and *V. doniana* pulverized leaf and treated as above. Pest Ox a synthetic insecticide was used as a reference.

Preparation of Test Organisms: One albino rat was bought, shaved and allowed to stay in a netted cage with a container of stagnant water. Anopheles mosquitoes were equally introduced into the cage to have a blood meal on the albino rat and lay eggs which formed the sources of our larvae. Four concentrations of 5.0ml, 7.5ml, 10.0ml and 12.5ml of leaf extracts were introduced into 20ml of water along side with 100 larvae of Anopheles mosquitoes collected with a syringe respectively. Three replications each, of these were allowed to stand for 3 hours. Fifty larvae of the target organism, which were not subjected to any treatment but ordinary pond water served as control. Mortalities were expressed as mean percentage of three replications per treatment. Treatments were analyzed for significant differences using analysis of variance (ANOVA).

Preliminary phytochemical screening of the two leaves was done in the Department of Phamarcognocy University of Nigeria Nsukka.

RESULTS AND DISCUSSION

The results of the phytochemical screening for the leaves of the two plants were done. The result revealed the presence of high levels of free phenolic and glycosides in the two leaves and moderate levels of flavoniods and triterpenes in *V. doniana*. There were complete absence of Stroides and Alkaloids in the tow leaves (Table 1).

Table 1: Phytochemical composition of the leaves of *D. arborea* and *V. doniana*

Compounds	*D. arborea*	*V. doniana*
Flavoniods	+ + +	+ +
Free phenolics	+ + +	+ + +
Condensed tannins	+ + +	+ +
Pseudotanins	+ + +	–
Steroids	–	–
Alkaloids	–	–
Triterpenes	+ + +	+ +
Glycosides	+ + +	+ + +
Saponins	+ +	+

** Legend + + + + =Excessive, + + + = High, + + =Moderate, + = Low and - = Absent*

The percentage mortality rate and the minimum percentage mortality concentration (MPMC) of the leaf extracts on the test organisms were 17.666, 13.222, 19.666 and 70.111 at 7.5mg/20ml, for *D. arborea* and *V. doniana* and 10.0mg/20ml, 5.0ml/20ml for synergy and pest ox respectively (Table 2).

The mortality of the test organism to *D. arborea*, *V. doniana*, synergy and (pest ox) chemical insecticide were evaluated by observation. The results showed that the extracts exhibited a moderate toxicity or low killing effect on the test organisms. The toxic effects however increased with increase in the concentration of the leaf extract. It also showed that among the treatments, pest ox has the highest mortality rate of 70.111 at the least concentration of 5.0ml/20ml followed by synergy 19.666 at 5.0 ml / 20 ml (Table 2). This was in line with the result of Aliero (2003), who reported that, the exposure of Anopheles mosquito larvae to undiluted extracts of seed oil, leaf and back of crude extracts of *Azadirachta indica* for 12 hours led to 100, 98, 48% mortality respectively. The minimum percentage mortality concentration (MPMC) of *D. arborea* was 7.5ml/20ml and that *of V. doniana* was 10.0ml/20ml, respectively as indicated figures with asterisk in table 2. The mortality effect of these plant extracts can be compared with the lethal death (oral LD50) caused by rotenone, pyrethrins, sabadilla, and azadrachtin plant extracts which have oral Lethal death ranging from 60mg/kg to 4,000mg/kg (Addor, 1995) .

It is important also to note that *when D. arborea* and *V. doniana* were combined (synergy), a high mortality effects were recorded at a concentration of 5 ml / 20ml (Table 2) . Statistical analysis of variance showed that, there was no significant difference ($P > 0.01$) between the synergy and the individual treatments of the leaf extracts on these organisms (Table 3).This means that these plants extract exerted synergistic effect on the test organisms. Total mortality was consistently positively correlated with insecticidal concentrations and the duration of exposure. This was in agreement with the work of Essam *et al.* (2005) who showed that the effect of *Callitris glaucophylla* extracts on the development of *Aedes aegypti* was higher as the concentration in increases. The effects of these plant extracts on the targeted organisms have further confirmed the insecticidal potentials of some metabolic compounds produced by plants that will be environmental friendly.

Phytochemical screening confirmed the presence of flavonoid, phenolics, tannins, pseudotanins, triterpenes, glycosides and saponins. This agreed favourably with the report of Isman (1997), who reported that, natural defense of plants against herbivory consist almost of mixtures of closely related compounds, rather than a single toxicant alone. He further pointed out that Rotenone contains six or more insecticidal isoflavonoids, glycosides and tannins which caused the death of insects.

However further studies on the stability and phytotoxicity of these bio-active compounds could enhance our knowledge and facilitate its large scale production for commercial use.

Table 2: Mortality rate of anopheles mosquito larvae exposed to leaf extracts of *D. arborea* and *V. doniana*

Plants	Concentration					
	5.0ml/20ml	7.5mg/20m	10.0mg/20ml	12.5mg/m	15mg/20ml	Control
D. arborea	6.333	17.666*	33.666	48.333	52.222	0.000
V. doniana	3.111	5.333	13.222*	35.111	46.666	0.000
Synergy	19.666*	30.333	46.333	58.222	60.000	0.000
Pest Ox	70.111*	96.666	-	-	-	0.000

Table 3: Deviation from mean on mosquito larvae mortality exposed to leaf extracts of *D. arborea* and *V. doniana*

Plants	Concentration					
	5.0ml/20ml	7.5mg/20m	10.0mg/20ml	12.5mg/ml	15mg/20ml	Control
D. arborea	6.333± -0.090	17.666*0.951	33.666 ± 0.331	48.333±0.740	52.222±0.441	0.000
V. doniana	3.111±0.000	5.333±0.596	13.222*±0.341	35.111±0.32	46.666±0.411	0.000
Synergy	19.666*±9.666	30.333±0.490	46.333±0.521	58.222±0.454	60.000±0.4	0.000
Pest Ox	70.111*±0.275	96.666±0.033	-	-	-	0.000

Conclusion: In conclusion, the effect of *Dracaena arborea* and *Vitex doniana* leaf extracts in this study have shown and suggested that these plants have potential bio-actives properties on Anopheles mosquito larvae, although the insecticidal activity of pest ox on the test organism was the highest and causes 100% mortality within a short period of time. These botanicals could be more environmentally friendly and cheaper and should be harnessed.

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HAEMOPARASITES OF CAMELS *(Camelus dromedarius)* IN MAIDUGURI, NIGERIA

[1]JOSHUA, Kamani., [3]TURAKI, Aliyu, Usman., [2]EGWU, Godwin Onyeamaechi., [2]MANI, Aliyu, Usman., SAIDU, Mohammed Kida., [3]ABDULLAHI, Jibrin Gimba and [2]KUMSHE, Hussaini Adam

[1]Parasitology Division, National Veterinary Research Institute, PMB 01, Vom, Plateau State, Nigeria
[2]Department of Veterinary Medicine, University of Maiduguri, Nigeria
[3] National Veterinary Research Institute Laboratory, Maiduguri Outstation, Borno State, Nigeria

Corresponding Author: Joshua, K. Parasitology, Division National Veterinary Research Institute, PMB 01, Vom Plateau State, Nigeria. Email: shapumani@yahoo.com Phone +2347035517715; +2348052052829

ABSTRACT

A study was conducted to determine the prevalence and significance of haemoparasite of camels slaughtered in Maiduguri abattoir. Blood samples were collected aseptically from camels before slaughter noting age and sex of animals. The samples were processed for packed cell volume (PCV) and thin smear stained with Geimsa stain according to standard procedure. An overall prevalence of 14.2 % (n = 16) of the 113 animals examined was recorded in this study. Theileria camellensis was most prevalent (n = 9 or 8.0 %) followed by Trypanosoma evansi (n = 4 or 3.5 %) and mix infection with both T. evansi and T. camellensis (n = 3 or 2.7 %). There was no significant difference (P>0.05) between male and female camels, however, there was significant difference between young and adult camels (P < 0.05) using student t-test at 95 % confidence interval. All the parasites seen in this study significantly (P < 0.01) affected the packed cell volume of the animals when compared to PCV of non infected animals. The haemogram shows marked macrocytic normochromic cells. Further work on the pathogenesis and effects of haemoparasites of camel is required. This is the first report of haemoparasites of camel in this region of Nigeria.

Keywords: Prevalence, Haemoparasite, *Theileria camellensis, Trypanosoma evansi,* Camel, Abattoir, Tropics

INTRODUCTION

The national camel population has been estimated at 92,494 as at 2000 (Felsner, 2002). Recently there has been a steady increase in the number of camels slaughtered for meat in Maiduguri and other cities in the region due to increasing cost of cattle and the decline in other livestock species. Daily 16- 25 camels are being slaughtered at the Maiduguri central abattoir (MANR, 2002). The camel's ability to utilize the scanty fodder resources of the arid zones of the world for body maintenance, growth and milk production makes this animal a potentially important source of food (Shalash, 1979; Squires, 1979). There is the need to improve the management practice of camels for maximum productivity (Knoess, 1977). Gastrointestinal and blood parasites are known to affect the health of camels leading to anemia, wasting and death in heavy infection (Jorgen and Brian, 1990). Mahran (2004) reported a prevalence of 21.1 % of blood parasites in camels in Egypt. There is paucity of information on haemoparasites of camels and their significance on health and productivity in Nigeria. This study was undertaken to determine the prevalence of haemoparasites of camels slaughtered in Maiduguri, Borno state in the semi-arid region of Nigeria.

MATERIALS AND METHODS

Maiduguri is the capital of Borno state, the most north-easterly state in Nigeria, with an area of 69,435 sq. km. It lies between latitude 10°N and 13°N and longitude 12° and 15°. Mean day temperature is 38°C. Relative humidity is generally low throughout the State, ranging from as low as 13 % in the driest months of February and March to the highest values of 70 to 80 % in the rainy season months of July and August. The rainy season lasts for less than eighty days in the extreme north; the mean annual rainfall is about 600 mm or less than 500 mm in the extreme north around Lake Chad. Droughts are endemic and rainfall tends to have been in decline since the 1960s. The semiarid nature of the Sahel and northern Sudan savannah consist mainly of open acacia tree vegetation which can no longer support the livestock population of the state. There is increase in camel raring in this region to meet up with the increasing animal protein need in the area.

Blood samples were collected aseptically from camels before slaughter in the mornings in Ethylene Diamine Tetra-acetic Acid (EDTA) tubes and transported to the laboratory on ice. Blood from each sample was introduced into a plain glass microhaematocrit tube, one end of the tube was sealed using plasticin, and the tubes were spun for 5 min at 13000 × *g* in a Microhaematocrit centrifuge (Hawksley). Packed Cell Volume (PCV) was measured using a haematocrit reader (Hawksley) and the buffy coat was examined for motile blood parasites. A thin blood smear was prepared from each blood sample, air-dried, fixed in methanol for 2 – 3 min, stained in 5 % Giemsa stain with added Azur II (2 g/l of undiluted stain) and rinsed in buffered water.

The smears were examined at ×100 magnification (oil immersion) on a Nikon microscope; at least 50 fields were searched per slide. Presence of haemoparasites was recorded; identification was carried out to genus and species level.

RESULTS AND DISCUSSION

Sixteen (14.2 %) of the 113 camels examined were positive for blood parasites. Of these animals 4(3.5 %) were positive for *T. evansi*, 9 (8.0 %) for *T. camellensis* and 3(2.7 %) had mixed infection of *T. evansi* and *T. camellensis.* 11(28.9 %) of the 38 young camels were positive with only 5(6.7 %) of 75 adult camels (Table 1). Nine (13.2 %) of the 68 males and 7(15.6 %) of the 45 females were positive (Table 1). The packed cell volume (PCV) ranges from 09 %-43 %. All animals positive for blood parasites have PCV values less than 20 %. There is a significant difference (P<0.05) between PCV of none infected and infected camels using students t-test at 95 confidence interval (Table 2). Hemogram of infected animals reveals marked macrocytic normochromic anaemia.

Table 1: Prevalence of Haemoparasites of Camels According to Sex and Age Group

Parameters	Age		Sex		Total
	Adult	Young	Male	Female	
Number examined	75	38	68	45	113
Number positive	5	11	9	7	16
Percent	6.7	28.9	13.2	15.6	14.2
T. evansi	1	3	3	1	4
T. camellensis	3	6	4	5	9
T. evansi + T. camellensis	1	2	2	1	3

There was no significant difference in prevalence between male and female animals. However, there was a significant difference (P < 0.05) in prevalence between young and old camels. Haemoparasites are responsible for lowered production and a constraint to successful introduction of improved breeds of animals in different parts of the tropics and subtropics. They also cause decreased performance of indigenous breeds of livestock (Jorgen and Brian, 1990). Generally camels are less susceptible to most blood parasites of other domestic livestock. *T evansi* was reported in 15 % of camels examined in Ethiopia (Richard, 1976) and 7.8 % in camels raised under traditional management condition in Kenya (Chemuliti *et al.,* 2003) were higher than 4 % seen in this study. Camel trypanosomosis (Surra) usually appear as a chronic (sub acute) debilitating ailment, but the acute form is rare. The first signs of the disease are a drop in production (milk yield) and the tendency of pregnant females to abort. There is loss of appetite and the animals become very emaciated. Most of the camels positive for *T. evansi* were weak, emaciated with pale mucus membrane at the time of slaughter.

The effect of *T. evansi* on PCV was marked suggesting the possible effect of the parasite on the red blood cells leading to haemolysis. Some of the smears reveal high parasitaemia with macrocytic normochromic cell. *T. camelensis* also had severe effect on the PCV suggesting that it is pathogenic in camels (Table 2).

Table 2: Haemoparasites Status and Mean PCV Values of Camels

Haemoparasite status	Number of animals	Mean PCV
No parasite seen	97	33 ±6.5
Trypanosoma evansi	4	15 ± 5.1
Theileria camellensis	9	21 ± 8.1
T. evansi + T. camellensis	3	19± 7.6

Combination of *T. evansi* and *T. camelensis* also markedly affected the PCV of infected animals. Generally there is paucity of information on diseases of camels in Nigeria with particular reference to parasitic infections. There is need to further study the pathogenesis of these parasites to clearly understand their pathological effects.

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DETERMINATION OF HYDROPHILE-LIPOPHILE BALANCE (HLB) OF BOVINE MUCIN FOR POSSIBLE EMULSIFYING PROPERTIES

MOMOH, Mumuni Audu and ADIKWU, Micheal Umale
Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka

Corresponding Author: Momoh, M. A. Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Email: jointmomoh@yahoo.com Phone: 234 8037784357

ABSTRACT

The Hydrophile-Lipophile balance (HLB) of bovine mucin was evaluated. Mucin was processed from the small intestine of freshly slaughtered cow via precipitation with chilled acetone, air-drying and pulverization. Series of emulsion were formed with bovine mucin and paraffin oil, in varying ratios, the most stable emulsion with the least creaming level was found to be mucin-oil ratio of 1:9, after a period of 7 days and the HLB value of mucin calculated according to standard methods. The HLB value of mucin was 8.4. These HLB value fell within the range of 8 – 18, that is characteristics of oil-in-water (o/w) emulgents.

Keywords: Hydrophile-Lipophile balance, Bovine mucin, Emulsifying properties

INTRODUCTION

The type of emulsion produced, oil-in-water (o/w) or water-in-oil (w/o), depend primarily on the property of the emulsifying agent. The balance between the hydrophilic and lipophillic moieties of surface-active molecules has been used as the basis for a more rational means of selecting and classifying agents than the empirical methods traditionally used (Pharmaceutical Codex, 1979). This characteristic is referred to as the Hydrophile-Lipophile Balance (HLB); and in the system, each emulsifying agents is assigned a number between 1 and 20. (Graffin, 1954) define HLB of a surfactant as the moles of the hydrophilic group divided by 5. In the case of non-ionic substances, the number is calculated from the hydrocarbon chain length and the number of polar groupings. In this study mucin was evaluated for possible emulsifying property.

Mucin or mucus glycoproteins are a family of polydisperse molecules, which carry out multiple tasks at mucosal surface throughout the body. They contribute to the mucus gel barrier and are part of the dynamic, interactive mucosal defensive system (Corfield *et al.,* 2002). Mucus is very high molecular weight, carbohydrate rich protein with up to 80 % *O*-glycosidically linked carbohydrate. Several studies carried out on mucus glycoproteins from many organs have suggested that these macromolecules consist of subunits held together by interchain disulphide bonds and further stabilized by non-covalent interaction. The end result of multiple interconnections is an extended and random network, which imparts to mucus secretions their characteristic property of viscoelasticity. Evidence that S-S bond play an important structural role has been provided by demonstrations that thiol-group reagents decrease the viscosity and increase the solubility of the native mucus secretions (Forstner *et al.,* 1979) and in some cases decrease the molecular weight of purified mucin. Researchers observed that the sedimentation coefficient of a large porcine gastric mucin decreased from 335 mol. wt. 2.3 x 10^6 to 145 mol. wt. 25 x 10^5 after treatment with 0.2 M of 2- mercaptoethanol (Carrigan and Bates, 1973). Somewhat similar but less dramatic effects have been observed after reduction of bronchial mucus glycoproteins (Forstner *et al.,* 1979).

MATERIALS AND METHODS

Extraction of Bovine Mucin: The small intestines of freshly slaughtered cow were obtained from the Nsukka abattoir and dissected starting from the beginning of the jejunum to the ileocaecal sphincter. The intestines, sectioned into short lengths, were flushed through with chilled saline, and the mucosal surface was exposed by longitudinal dissection. By using a microscope slide, the mucus layer was gently scraped off in to chilled saline. The mucus was precipitated using chilled acetone and dried. The resultant flakes were pulverized using end runner mill' machine and stored in an air-tight container until used.

Test for Sugars: Three drops of freshly prepared Felling's solution A and B were added to 1 mg of 1 % w/v aqueous dispersion of bovine mucin and then heated in a boiling water bath for 5 min and observed.

Test for Carbohydrates

a. Reduction test: Two drops of 1 % iodine solution was added to 1 ml of the (1 % w/v) of bovine mucin and then observed for blue-black colouration.

b. Molisch's test: This reaction is a general test for the presence of carbohydrate and other organic compounds that could form furfuradehyde or hydroxymethyl furfuradehyde in the presence of conc. sulphuric acid. For the test, two drops of α-

naphthol solution was added to 2 ml of the bovine mucin dispersion and mixed thoroughly. Then 1ml of conc. sulphuric acid was gently poured down the side of the tube and observed.

c. Tollen's reagent test: The silver ions in a solution containing silver ammonia complex are reduced to metallic silver. By aldehydes, reducing sugars, polyhydroxyl phenols, formic acid and other reducing agents. Tollen's reagent prepared as 1 ml of 5 % silver nitrate solution was treated with a few drops of 5 % sodium hydroxide solution. A volume of aqueous ammonia just enough to redissolve the precipitate was added to 3 drops of the bovine mucin dispersion and the mixture warmed in a boiling water bath for a few minutes. The colour of the precipitate formed was observed.

Test for Proteins

a. Biuret test: Two drops of water and 1 ml of dilute sodium hydroxide were added to 20 mg of bovine mucin. Two drops of 1 % copper sulphate solution was added and the solutions shaken thoroughly after each drop and observed.

b. Xanthoproteic reaction: Two drops of concentrate nitric acid were carefully added to 2 % dispersion of bovine mucin. A white precipitate was formed, which turns yellow on heating. The contents of the test tube were cooled and three drops of ammonia solution added and the precipitate observed.

Test for Fixed Oils: A drop of the acetone extract was placed on a filter paper. The solvent was allowed to evaporate and the filter paper observed carefully.

Solubility Profile of Bovine Mucin: The solubility of bovine mucin in several solvents was determined by dispersing 100 mg of the bovine mucin in definite volume of each solvent- acetone, ethanol, water, sodium hydroxide, hydrochloric acid and ammonium hydroxide at different temperature.

Preparation of the Emulsion: Oil-in-water (o/w) emulsions were prepared using liquid paraffin as the oily phase and various combinations of Tween 65 and bovine mucin powder. The proper quantity of the gum was weighted and distributed in the oil phase contained in stainless steel cup. The calculated quantity of Tween 65 was added to the oil as an aqueous dilution, and the total volume of 120 ml of the product was made by the addition of distilled water. The final emulsion contained 30 % liquid paraffin. Emulsification was effected by mixing the above mixture for 5 min with a Silverson homogenizer (L.2R 2900, Erweka, England).

Determinations of HLB Value: The HLB of the bovine mucin was determined according to the method described by Graffin and modified by some group (Graffin, 1954). Series of emulsions were prepared with varying ratios of Tween 65 and the mucin powder. The percentage of the total emulsifying agent (mucin and Tween 65) was fixed at 1 % in all cases. The first series of emulsions contained emulsifier's blends of ratios ranging from 9:1 to 1:9 respectively. The (HLB) of the mucin is then calculated using the equation below. HLB = R – (H x S) / N; Where R = "required HLB" of liquid paraffin, H = HLB of Tween 65 used, S = is the percent of Tween 65 expressed as a decimal fraction and N = is the percent of mucin powder expressed as a decimal fraction.

RESULTS AND DISCUSSION

Physicochemical Properties of Bovine Mucin: Physiochemical tests performed on the bovine mucin showed that carbohydrates, proteins and trace amounts of fats were present (Table 1). In both wet and dry states, the mucin is light- brownish in colour, almost tasteless and has a pleasant meaty odour.

Bovine mucin when dispersed in water gives a slight viscous dispersion. This is unlike gelatin - a typical purified animal protein that swells in cold water with a resultant colloidal solution when heated. Bovine mucin disperses with little difficulty in cold water. The bovine mucin is not soluble in the ethanol and acetone but showed some level of solubility in 0.1 M sodium hydroxide, ammonium hydroxide and sulphuric acid. It showed a greater level of solubility in dimethylsulphoxide (DMSO) especially at elevated temperature (Table 2).

Table 1: Physiochemical properties of bovine mucin

Test	Observation	Inference
carbohydrate	+++++	Present
protein	++ + +	Present
fats	+	Trace amount

Key: *+ Present in trace amount; + + + + copiously present*

Table 2: Solubility profile of bovine mucin

Temp (°C)	Solvents					
	Acetone	Ethanol	0.1M NaOH	0.1M H_2SO_4	0.1M NH_4OH	DMSO
25	-	-	-	-	-	+
30	-	-	-	-	-	+
35	-	-	-	-	-	+

Key: - *Quite insoluble, + slightly soluble*

Table 3: Organoleptics properties of bovine mucin and its emulsion

Parameter	Organoleptics properties of bovine mucin	Organoleptics properties of mucin emulsion
Colour	Light brown	White
Odour	Characteristics	Odourless
Texture	Very fine	Pourable

Properties of Bovine Mucin Emulsion: The most stable emulsions in the series were determined, by evaluating primarily on the basis of degree of creaming. The heights of the separated layers (in cm) were recorded and the percentage calculated. In

almost every case, the best emulsion of a given series showed a lesser degree of creaming than a poorer one in the series (Table 3) (Udeala and Uwaga, 1981).

Table 4: Determination of HLB value of bovine mucin

S/N	Mucin: Tween 60	Level of Creaming (cm)	% Creaming
1	1:9	28.00	23.32
2	2:8	24.14	20.10
3	3:7	52.22	43.50
4	4:6	54.26	45.20
5	5:5	70.00	58.31
6	6:4	55.00	45.82
7	7:3	63.64	53.02
8	8:2	62.00	51.65
9	9:1	63.90	53.23

% creaming = *Level of creaming (cm) / initial volume x 100.* ***Note:*** *The initial volume is taken as 100 cm*

Series of emulsion were formed with bovine mucin and paraffin oil, in varying ratios, the most stable emulsion with the least creaming level was found to be mucin-oil ratio of 1:9, and the HLB was calculated to be 8.4. The HLB value 8.4 (Table 4) is within the acceptable range of 8 -16 which are best fitted for oil/water emulsifying agents (Adikwu *et al.,* 1992). The organoleptics properties show that bovine mucin exhibited good emulsifying properties as exemplify by the odourless nature of the emulsion and easy pourability. Ideally emulsion should exhibit the rheological properties of plasticity and easily re-disperse with gentle shear stress and good pourability (Aulton *et al.,* 1998). Oily substances can be emulsified by mucin and this could be a possible mechanism whereby drugs are absorbed through the villi of the small intestine in animal and human (Guess, 1981). The pharmaceutical importance for determining the HLB of a material or substance intended to be used as a pharmaceutical emulgent is based on the fact that physically stable emulsions are best achieved by a condensed layer of emulgent at the oil/water interface and that the complex interfacial films formed by a blend of an oil-soluble emulsifying agent with a water soluble one produces a satisfactory emulsion (Corfield *et al.,* 1973). This result revealed that bovine mucin had emulsifying properties as evidenced by its ability to form good and elegant emulsions, and therefore can be used as an excipient in pharmaceutical preparation if properly harnessed.

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EVALUATION OF BURNS HEALING EFFECTS OF NATURAL HONEY, DERMAZINE CREAM® AND THEIR ADMIXTURE

[1]MOMOH, Mumuni Audu, [2]NWACHI, Uche Emmanuel and [1]ERAGA Sylvester
[1]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka
[2] Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka

Corresponding Author: Momoh, M. A. Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Email: jointmomoh@yahoo.com Phone: +2348037784357

ABSTRACT

The healing effect of natural honey was evaluated in vivo using the excision wound healing model. Unpurified honey was used to treat burns inflicted on the experimental rats. The healing effects of the honey were compared to that of dermazine, honey-dermazine mixture, and methylated spirit. The burns healing agents all shown a progressive decrease in the wound, the healing effect of honey was more than any of the other agents used, with 100% healed in the 15th day, dermazine attained 100% by 21st day, indicating that natural honey has healing property than formulated dermazine, methylated spirit gives 61% by 21st day.

Keywords: Honey, Dermazine®, Burns, Healing, Admixtures

INTRODUCTION

The therapeutic potential of uncontaminated, pure honey is grossly underutilized. It is widely available in most communities and although the mechanism of action of several of its properties remains obscure and needs further investigation, the time has now come for conventional medicine to lift the blinds off this 'traditional remedy' and give it its due recognition." Mostly this was in reference to reports of the use of honey as a wound dressing (Zumla and Lulat, 1989). The ancient usage of honey as a wound dressing has been reviewed (Beck and Smedley, 1944), but there have been only some very brief reviews, with little clinical detail, of the literature reporting modern usage of this rediscovered therapy for wounds (Wood *et al.,* 1997). Because of the increasing interest in the use of alternative therapies, especially as the development of antibiotic resistance in bacteria is becoming a major problem (Greenwood, 1995), and because of the increase in reported usage of honey as a wound dressing in recent times.

Honey is a highly concentrated sugar solution produced by honey bees, primarily from the nectar of plants. It is composed of carbohydrates (sugars), water, enzymes, amino acids, pigments, pollen, wax, and other trace constituents from both bees and plants. Honey has been used in the treatment of burns and wounds for many centuries, with documents describing this use dating back to 1700 BC.

A number of properties inherent to honey might contribute to its ability to fight infection and promote healing. Its high sugar content allows it to draw infection and fluid from wounds by a process called “osmosis.” Honey prevents bacterial growth through its acidic pH and through the work of an enzyme that produces small amounts of hydrogen peroxide. Its ability to keep the area around a wound moist and protected promotes fast healing and prevents scarring.

Wound is defined as a break in the continuity of tissue from violence or trauma while healing is the restoration to normal condition especially of an inflammation or a wound it follows that wound healing has to do with the restoration of a break in the continuity of a tissue.

According to (Taber, 1965), wound healing is an important biological process that involves tissue repair and regeneration. Monocytes-derived macrophages are usually attracted to the site and these release a number of growth factors and cytokines which are important in maintenance of inflammatory reaction, irritation, maturation and control of wound healing process. Wound healing can be classified into any of three types- healing by first intention healing by second intention or healing by third intention, depending on the nature of the healed wounds. Neutrophils appear first in the wound to clear contaminating bacteria. Bacterial infection is the major factor affecting wound healing. Bacteria directly invade wound producing inflammation and fluid exudation, which interferes with healing. In addition, bacteria toxins cause tissue damage and delays fibroplasias as well as collagen synthesis (Thomas, 1997). When skin integrity in an animal is compromised by surgery or accidental trauma, infections agents have access into the wound to cause contamination and infection of the soft tissue locally. The infectious agents could also gain entry into the blood stream, which carries it to distant organs (Harris, 1994).

MATERIALS AND METHODS

Spatula, dermazine cream, metylated-spirit(KP), gauze (Agary), cotton wool (Tender), ethyl ether, razor blade, Bunsen burner, normal saline, Eusol solution were used for the study.

Animals: Mature Wister albino rats of both sex weighing between 120 – 180 g obtain from the

Table 1: Percentage reduction of burns in (mm)

Healing Agents	Day							
	0	3	6	9	12	15	18	21
Honey	(0%)	50%	60%	75%	95%	100%	-	-
Darmazine cream	0%	40%	55%	66%	84%	91%	94.5%	100%
Honey plus Darmazine cream	0%	52.5%	60%	70%	88%	100%	-	-
Methylated spirit	0%	10%	20%	22%	32.5%	47.5%	55%	61%

Department of Biochemistry, University of Nigeria and fed on 'check marsh' were used for the study. After the purchase, all the rats were allowed to equilibrate in standard and conditioned animal houses at the Department of Biochemistry, University of Nigeria for a period of two weeks before use.

Preparation of Burns Site in Experimental Animals: The burns site was prepared following the excision wound model (Karl *et al.,* 2001). The animals were anaesthesed with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circle of diameter 15 mm was marked on each of the two sides of the skin surface. Then circular burns were then made on the marked areas of the skin surface with the aid of red-hot spatula. The area was measured immediately by tracing out the burned area using a transparent tracing paper and the squares counted.

Preparation of the Honey Extract: Honey was obtained from the comb of the beehive, *Apis melifera* L. the honey was warmed to 80 ^{o} C and allowed to stand. The impurities which rose to the surface were skimmed off, and the liquid was passed through a calico. The clear viscous liquid so obtained was diluted with water to bring its viscosity to the Pharmaceutical codex standard of 1.355 to 1.360 g at 20 ^{o} C.

Treatment of Burns : Treatment started shortly after the burns was made by applying Honey, Darmazine, Honey plus Darmazine mixture and methylated spirit topically to the four groups of rats after dressing the wound with Eusol solution. The burn areas were measured while the animals were under anaesthesia on the 0, 3, 6, 9, 12, 15, 18, 21 day after the burnt.

Analysis of Data: The data were graphically analyzed using plot of wound diameter Vs healing rate. The student's t-test was also used to test the significance of the result obtained.

RESULTS AND DISCUSSION

The results of the healing effects of the various testing agents are presented in Table 1. There was a general decrease in burnt area upon application of the honey, dermazine and there admixture with time. By 3rd day 50 % of the burns have healed with honey and 100% healed by day 15th (Table 1). The group that received honey-dermazine gave 40 % healed by 3rd day and 100% day 15th. Darmazine gave 40 % healed by 3rd day and 100 % at the 21st day, while methylated spirit dressing alone shows no much improvement within the first 15th day. The percentage wound healing effectiveness was as follows; honey, honey-dermazine, dermazine and methylated spirit (Table 1). It has been noted that dressing wounds with honey allows early grafting on a clean clear base.

Honey is also an ideal first-aid dressing material, especially for patients in remote locations when there could be time for infection to have set in before medical treatment is obtained: it is readily available and simple to use. It would be particularly suitable for first-aid treatment for burns, where emergency dousing or cooling frequently involves the use of contaminated water which then leads to heavy infection of the traumatized tissue. As well as providing an immediate anti-inflammatory treatment the honey would provide an antibacterial action and a barrier to further infection of the wound.

Gyang (1986) observed that rapid cleansing and chemical or enzyme debridement resulting from the application of honey to wounds have also been reported (Gupta *et al.,* 1992), with no scar forming on burns (Subrahmanyam, 1994). Several other authors have noted the cleansing effect of honey on wounds (Hutton, 1966). The result of this study is an indication that natural honey could be used as an alterative in wound dressing.

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A SURVEY OF THE GUT PARASITES OF RODENTS IN NSUKKA ECOLOGICAL ZONE

OKOYE, Ikem Chris and OBIEZUE, Rose Nduka Nwanyo
Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria

Corresponding Author: Okoye, I. C. Department of Zoology, University of Nigeria, Nsukka, Nigeria. Email: ikemchriso@yahoo.co.uk; Phone: +2348069284633

ABSTRACT

A survey of gut parasites of rodents was undertaken in Nsukka ecological zone. Out of the 87 rodents caught and examined, 47 (54.0%) were positive for helminth parasites. The prevalence rates for the various rodents examined were 60.0% for Xerus erythropus (squirrels); 59.3% for Cricetomy sp. (giant rats) and 48.9% for Rattus rattus (house rats). The difference in prevalence rates amongst the rodents was statistically insignificant (P>0.05). The parasites isolated were 2 Cestode species- Hymenolepis sp (17.2%) and Raillietina sp; 3 nematode species- Trichuris muris (9.2%),, Ascaris sp (2.3%), Cyathostomum sp (4.6%) and one Acanthocephalan- Moniliformis morniliformis.(6.9%).

Keywords: Rodents, *Xerus erythropus, Cricetomy* sp, *Rattus rattus,* Gut parasites, *Hymenolepis* sp, *Raillietina* sp, *Trichuris muris, Ascaris* sp, *Cyathostomum* sp, *Moniliformis morniliformis*

INTRODUCTION

Rodents are hosts to a number of ectoparasites such as lice, mites and ticks that can transmit viral, bacterial and protozoan parasites to man and animals Soliman *et al.,* 2001). In addition, they can harbour many different protozoan and helmintic endoparasites (Mafiana *et al.,* 1997; Mahid, 2003). Other than the tremendous economic losses to agriculture owing to their pestiferous nature, rodents survive and proliferate in close association with humans in households, agricultural and commercial places, thus making them interesting subjects for research. Although there are several reports on rodent parasites in other parts of the world, documented studies in Nigeria are wanting. Rodents are the most successful of modern mammals other than man. They make up the largest order of mammals, with over 40% of mammalian species belonging to the order Rodentia (Carleton and Musser, 2005). Their success is probably due to their small size, short breeding and ability to gnaw and eat a wide variety of foods. There are about 2,277 species of rodents, about 42% of all mammal species (Wilson and Reeder, 2005). Rodents are important in many ecosystems because they reproduce rapidly, and can function as food source for predators, mechanisms for seed dispersal and as disease vectors. Humans used rodents as a source of fur, as model organism in animal testing for food and even in detecting landmines. Furthermore, rodents may act as reservoir hosts for important human parasitic diseases. The importance of rodents as a source food for man and their possible roles as a source of pathogens therefore necessitates a study of their parasites.

The helminth parasites of rodents are the cause of disease in man and other farm animals and these have resulted in great mortality of them. And because of this, there is a great need for more research on helminth parasites of rodents. Thus, knowledge of these parasites of rodents in a community is of both veterinary and public health importance. In this paper, we present our findings on the biodiversity of parasites in rodents caught in Nsukka ecological zone.

MATERIALS AND METHODS

Study Area: The study area is Nsukka local Government Area of Enugu State, Nigeria. Nsukka is located at latitude $6^{o}51^{o}$N and longitude 7^{o} 27^{1}E. The landmass and topography is characterised by hill and grasslands where rodents are widely distributed. Community clusters were chosen by random sampling for the present study.

Sample Collection: A total of 87 rodents made up of 27 giant rats (*Cricetomys* sp); 45 house rats (*Rattus rattus*) and 15 squirrels (*Xenus erythropus*) were used for this study. The giant rats and squirrels were obtained by trapping in bushes within the study area while the house rats were caught in living houses and food stores. Those caught alive were kept in iron cages at the University of Nigeria Nsukka Zoological Garden prior to dissection.

Examination for Gut Parasites: The body cavities of the rodents were slit open from throat to the anus to expose the internal organs. The organs of interest viz. oesophagus, stomach, small and large intestines were severed in the above order and placed in clean, white Petri-dishes and immersed in 5% formal saline. Parasites isolated were identified using guide by Bush *et al.* (2001).

RESULTS

Out of the 87 rodents examined, 47 were infected by different helminth parasites. The overall prevalence rate was 54.0 % (Table 1).

Table 1: Prevalence of helminth parasites of rodents in Nsukka ecological zone

Rodent species No Exam.	*Hymenolepis* sp.	*Raillietina* sp.	*Trichuris muris*	*Ascaris* sp.	*Cyathostomium*	*M. morniformis*	Total
***Cricetomys* sp 27**	54	3	3	1	2	2	16(59.3)
***Rattus rattus* 45**	78	5	5	1	1	2	22(48.9)
***Xenus erythropus* 15**	2	-	4	-	1	2	9(60.0)
Total 87	15(17.2)	8(9.3)	12(13.8)	2(2.3)	4(4.6)	6(6.9)	47(54.0)

The helminth parasites were demonstrated in the small and large intestines while the oesophagus and stomach of all the rodents were free of parasites. The prevalence rates for the various rodents examined were 60.0% for *Xerus erythropus* (squirrels); 59.3 % for *Cricetomy* sp. (giant rats) and 48.9 % for *Rattus rattus* (house rats). The difference in prevalence rates amongst the rodents was statistically insignificant (P>0.05). The parasites isolated were 2 Cestode species- *Hymenolepis* sp. (17.2 %) and *Raillietina s*p; 3 nematode species- *Trichuris* m*uris* (9.2 %), *Ascaris sp.* (2.3 %), *Cyathostomum* (4.6 %) and one Acanthocephalan- M*orniliformis morniliformi* (6.9 %).

A total of 48 out of the 87 rodents were males and 39 were females. Prevalence rate of parasites in males was 56.3 % while 51.3 % of the females were infected (Table 2). The difference in infection rates by sex of rodents was statistically insignificant (P > 0.05).

Table 2: Prevalence of helminth parasites by sex of rodents in Nsukka ecological zone

Helminth sp.	Males n=48	Female n=39	Total n=87
Hymenolepis sp	9 (18.8)	6(15.4)	15(17.2)
Railietina sp	4(8.5)	4(10.3)	8(9.2)
Trichuris muris	7(14.6)	5(12.8)	12(13.8)
Ascaris sp	2(4.2)	0(0.0)	2(2.3)
Cyathostomum	3(6.3)	1(2.6)	4(4.6)
M. morniliformis	4(8.3)	2(5.1)	6(6.9)
Total	27 (56.3)	20 (51.3)	47 (54.0)

DISCUSSION

The study has revealed the extent of parasite biodiversity in Nsukka ecological zone which is vast and diverse. The public health implications is important when it is remembered that all the rodents studied also serve as veritable sources of animal protein in the study area and neighbouring Benue and Kogi States.

The helminth parasites recorded in this study are similar to those of related studies elsewhere (Mafiana *et al.,* 1997; Ajayi *et al.,* 2007). There was however the striking absence of the cestode- *Taenia taeniaformis* (lyst) demonstrated in the above surveys.

The result of the present survey has shown the cestode parasite, *Hymenolepis sp*. (17.2 %) and the nematode, *Trichuris muris* (9.2 %) as the most important parasites of rodents in Nsukka ecological zone. The rate of infections demonstrated among the males (56.3 %) and female (51.3) rodents by sexes that expose them to helminth parasite infection.

Despite heavy infection with intestinal parasites, and marked hepatic tissue damage owing to severe capillariasis and strobilovercus larva infection, all rats appeared healthy and agile, suggestive of a well- established rat host-parasite relationship. In view of the diversity and zoonotic nature of rat parasites, and the impoverished conductions prevailing in communities where Rattus spp survive and proliferate, they can readily facilitate parasite transmission to humans and other susceptible animal hosts.

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PRELIMINARY SURVEY OF ECTOPARASITES OF CHICKEN IN AWKA, SOUTH-EASTERN NIGERIA

IKPEZE, Obiora Osegboka., AMAGBA, Immaculata Chioma and ENEANYA, Christine Ifeoma
Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Corresponding Author: Ikpeze, O. O. Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Email: ikpezevet@yahoo.com Phone: +2348035838255

ABSTRACT

A total of 4650 domestic chickens, comprising 1410 cocks (30.3 %), 2550 hens (54.8 %) and 690 chicks (14.8 %), displayed for sales between February and May 2008 at Eke-Awka market in Anambra State, south-eastern Nigeria were systematically examined for ectoparasites. Most of the cocks originated from the North while the hens and chicks were raised on nearby farms in Anambra and Enugu States. Overall, ectoparasites infested about 40.5 % of the chickens examined. The wing louse, Lipeurus caponis moderately infested 1935 chickens (41.61 %). The shaft louse, Menopon gallinae, extensively infested 2205 (31.90 %), while the fluff louse, Gonoicotes gallinae, lightly infested 471 (7.07 %) chickens. The sticktight flea, Echidnophaga gallinacea, attacked the head region of 3087 (69.37 %) while the symptoms of scaly leg mite, Knemidocoptes mutans, was observed on 1679 (27.70 %) of the birds, respectively. Market survey revealed a depreciation of about 10 to 20 % in the selling prizes of the affected chickens, a positive indication that ectoparasites on chickens are associated with financial losses incurred by operatives of the poultry industry in Nigeria.

Keywords: Ectoparasites, Chickens, *Lipeurus caponis, Menopon gallinae, Gonoicotes gallinae, Echidnophaga gallinacea*

INTRODUCTION

Poultry Industry, being an important sector of Nigerian livestock production is expected to play a vital role in national development through generation of revenue. About 85 % and 5 % of Nigeria's poultry population estimated at 190 million were reared extensively in rural and urban areas, respectively, while 10 % were managed intensively nation-wide (FAO, 1989). Common ectoparasites of chicken are lice, fleas, ticks and mites which spend their entire life cycles on the host (Saidu *et al*., 1994). Lice have been reported to be the most common and widely spread ectoparasites of chickens (Benbrook, 1965). In Western Nigeria, *Menacanthus cornuthus*, *M. pallidulis*, *Anyriodea powellion*, *Menopon gallinae*, *Numidulipenes tropicalis* and *Gonoicotes gallinae* species have been isolated from chicken (Fabiyi, 1988).

The adverse effects of lice infestation include irritation, reduced mating potentials in cocks, reduced egg laying in hens, and loss of weight in broilers, pullets and cockerels (Soulsby, 1982). The tropical chicken flea or stick-tight flea (*Echidnophaga gallinacea*) which attaches to the combs, wattles and around the eyelids, induce irritation, restlessness and anaemia by their biting and sucking activities, and affected chickens may become blind when their nictitating membranes are damaged (Soulsby, 1982). It has been severally observed that heavy infestation of the chicken by the soft tick, *Argas persicus* (Fowl tick) caused extensive loss of blood, emaciation, retarded growth, lowered egg production, loss of protective feathers, marked sluggishness, and diarrhea (Adene and Dipeolu, 1975). The common red chicken mite (*Dermanyssus gallinae*), the northern fowl mite (*Ornithonyssus sylvarium*), the tropical fowl mite (*Ornithonyssus bursa*) and the scaly-leg mite (*Knemidocoptes mutans*) are known to feed constantly on blood and lymph of chickens, thereby causing anaemia, depluming of feathers and scaly legs (Soulsby, 1982).

The present study was conducted to further identify the species of ectoparasites on chickens as well as assess their economic impact. The result will enable the stake holders in the poultry industry fully appreciate the importance of ectoparasites and be motivated to implement feasible control programme in order to reduce the associated economic losses.

MATERIALS AND METHODS

Area of Study: Eke-Awka market, the study area, is the busiest daily market in Awka, the capital of Anambra State, south-eastern Nigeria. Poultry-line in the market receives indigenous cocks from northern states of the country, as well as exotic broilers, spent hens, pullets and cockerels raised on different farms in Enugu and Anambra States. The chickens are displayed for sale in expanded metal cages which have provisions for feed and water. Buyers examined the chickens, handling them to assess their weights during the bargain. Poultry-line remains a bee-hive of activities as hoteliers, operators of restaurants and housewives haggle over the prizes of chickens on daily basis. This study was carried out between February and May 2008.

Sample Population of Chickens: Each poultry cage contains between 5 to 10 adult chickens (cocks,

Table 1: Ectoparasites on chickens at Awka, Anambra State, south-eastern Nigeria

Chickens	Sample examined		Chickens infested with different ectoparasites (as percentages of examined)									
			Wing louse *L. caponis*		Shaft louse *M. gallinae*		Fluff louse *G. gallinae*		Sticktight flea *E. gallinacea*		Scaly leg mite *K. mutans*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cocks	1410	30.32	96	6.8	294	20.8	84	6.0	963	68.3	551	39.1
Hens	2550	54.84	1242	48.7	1911	74.9	387	15.2	1590	62.4	1128	44.0
Chicks	690	14.84	597	86.5	0	0.0	0	0.0	534	77.4	0	0.0

broilers and spent hens) and 15 to 20 chicks (pullets and cockerels). Five birds were taken at random from each cage in a row and examined for ectoparasites. The head, combs, eyelids, wattles, neck, feathers, breast, back, wings, shafts, legs and other external surfaces of the chicken were thoroughly examined. About 4650 chickens were sampled during the study period.

Collection and Identification of Ectoparasites: Several specimen bottles, each containing 75 % alcohol, to which few drops of 5 % glycerine had been added, were labeled with different parts of the chicken as well as the types of chicken examined. Thus ectoparasites from cocks, hens and chicks, as well as from different predilection sites, were properly distinguished from each other. Glycerine was to prevent the specimen from becoming brittle as the alcohol evaporated. The ectoparasites were pulled out of the chickens with either the pin-forceps or blunt forceps; sufficient care being taken to prevent damage to the morphological features needed for subsequent identification of the ectoparasites. Preserved specimen were taken to the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka and emptied in well-labeled Petri dishes. Lactophenol was added for clearance and each ectoparasite was examined under the stereoscopic microscope. Entomological Keys (Ikeme, 1976) was used for the identification of the ectoparasites. The predilection sites, antennae, and presence or absence of mandibles, shape and number of bristles aided in the identification of louse species (Ikeme, 1976). Digital photos of typical symptoms of arthropod infestation, together with the incriminating arthropods were taken. Descriptive statistics was done using SPSS Version 11.

RESULTS

The different types of domestic chickens sold at Eke-Awka market include cocks, spent hens and chicks. A total of 4650 chickens comprising 1410 cocks (30.3 %), 2550 spent hens (54.8 %) and 690 chicks (14.8 %) examined during the study period were observed to be infested to varying degrees by different types of ectoparasites (Table 1). The wing louse, *Lipeurus caponis* affected 1935 chickens comprising 96 cocks (6.8 %), 1242 hens (48.7 %) and 597 chicks (86.5 %). The shaft louse, *Menopon gallinae* was identified from 2205 chickens (31.90 %), made up of 294 cocks (20.8 %) and 1911 hens (74.9 %). The fluff louse, *Gonoicotes gallinae* infested 471 adult chickens composed of 84 cocks (6 %) and 387 hens (15.2 %). Both *M. gallinae* and *G. gallinae* were apparently absent from young chicks. Typical symptoms of feather-loss due to *M. gallinae* infection, and egg knits of *M. gallinae* on feather are shown in Figures 1c & d respectively, while digital photographs of *G. gallinacea*, *M. gallinae*, and *L. caponis* from affected chickens are shown in Figures 2a, b and c respectively.

The sticktight flea, *Echidnophaga gallinacea,* attacked 3087 chickens, made up of 693 cocks (68.3 %), 1590 hens (62.4 %) and 534 chicks (77.4 %). The distribution of the sticktight flea was mainly on the head region of affected chickens, especially on the wattles, combs and around the eyelids. Sticktight flea infestation of the head of chicken is shown in Figures 1a, while the digital photograph of *E. gallinacea* from an affected chicken is presented in Figure 2d.

Scaly leg mite, *Knemidocoptes mutans*, infection were evident on the legs of 1679 (27.70 %) adult chickens comprising 551 cocks (39.1 %) and 1128 hens (44 %). Lesions of scaly leg appeared to be restricted to adult chickens (Figure 1b), an indication of the progressive nature of the infection.

The predilection sites and relative intensity of infestation of ectoparasites on infested chickens is presented on Table 2. Lice were mostly found beneath and on the wings, on body surfaces and feather shafts of affected chickens. *Liperus caponis* moderately infested the wings and body surfaces of affected birds. Concentrations of *M. gallinae* were mostly on the shafts than beneath the wings and body surfaces, while *G. gallinae* lightly infested body surfaces and wing feathers. *E. gallinacea* were found mainly on the head of affected chickens; the wattles, eyelids and combs being heavily infested. Very few of the affected chickens were blind due to damaged nictitating membranes. Overt symptoms of *K. mutans* infestation were on the legs of affected cocks and spent hens. Young chicks that may have been infested with *K. mutans* were yet to show symptoms of scaly legs, which require time to develop (Urguhart *et al.*, 1988).

DISCUSSION

The different types of chickens namely, cocks, spent hens, and chicks on sale in Eke-Awka market is an indication that poultry production is an important sector of livestock industry in Nigeria, and may play a vital role in national development through generation of revenue at individual and cooperative levels. However, the incidence of ectoparasites appears to limit full realization of this goal.

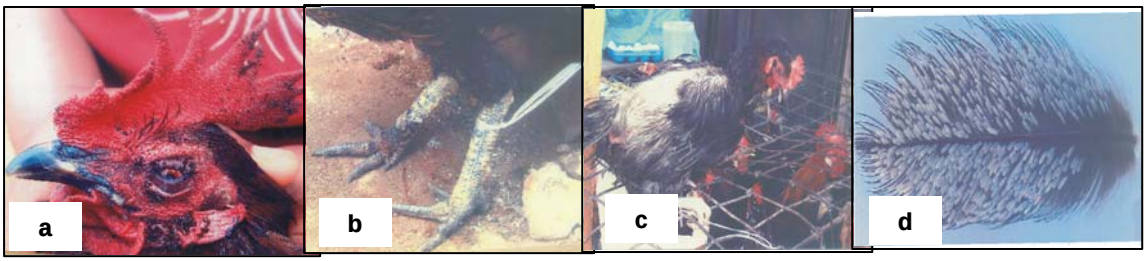

Figure 1: Sites of ectoparasites infestation in birds from Awka, Anambra State, Nigeria. a: *Echidnophaga gallinacea*-infested comb, wattle and eyelids of a cock. **b:** Scaly-leg due to *Knemidocoptes mutans*. **c:** Feather-loss due to *Menopon gallinae,* and **d:** Egg knits of *Menopon gallinae* on wing feather.

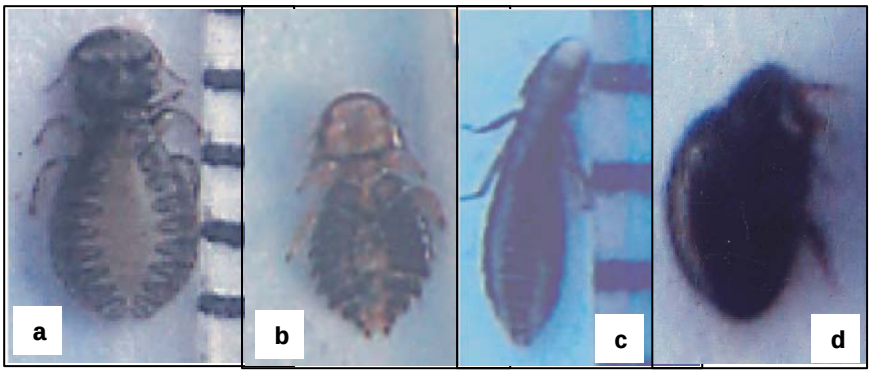

Figure 2: Species of ectoparasites infesting chicken from Awka, Anambra State, Nigeria. a: *Goniocotes gallinae* (≡ 3mm long), **b:** *Menopon gallinae* (≡ 3mm long). **c:** *Lipeurus caponis* (≡ 4mm long) and **d:** *Echidnophaga gallinacea* (≡1.2mm long).

Table 2: Predilection sites and intensity of ectoparasites on chickens at Awka, south-eastern Nigeria

	Ectoparasites	Combs	Wattles	Eye-lids	Wings	Body surfaces	Shafts	Legs
Lice	***Lipeurus caponis***	-	-	-	++	++	+	-
	Menopon gallinae	-	-	-	++	+	+++	-
	Gonoicotes gallinae	-	-	-	+	+	+	-
Fleas	***Echidnophaga gallinacea***	+++	+++	+++	-	-	-	-
Mites	***Knemidocoptes mutans***	-	-	-	-	-	-	+++*

+ = Light (with ≤ 5 ectoparasites), ++ = Moderate (with 6 to 10 ectoparasites), +++ = Heavy (with ≥ 11 ectoparasites)
** Symptoms of scaly leg*

This study showed that about 48.7 % and 86.5 % of chickens infested with the wing louse, *L. caponis*, were hens and growing chicks respectively. Hens also constituted about 74.9 % and 15.2 % of the adult chickens infested by the shaft louse, *M. gallinae* and the fluff louse, *G. gallinae* respectively (Table 1). This finding corroborated earlier reports of *M. gallinae* and *G. gallinae* on chickens in Nigeria (Fabiyi, 1988). The effects of lice infestation included feather loss (Figure 1c), reduced egg-laying, weight loss in chicks and broilers and reduced mating in cocks (Soulsby, 1982).

The symptoms of the sticktight flea (*E. gallinacea*) infection were obvious in about 69 % of the chickens examined in the market (Table 1). These fleas buried their fascicles in the hosts' skin and remained in place on the combs, wattles and around the eyelids of affected chickens (Figure 1a) induced irritation, restlessness and anaemia by their biting and sucking activities.

Heavy bleeding was observed as these fleas were being pulled out of the combs, wattles and around the eyelids of infested chickens. Ulcerations of the combs and wattles were evident on severely infected birds. *E. gallinacae* is known to possess a patch of spinelets on the inner side of its metacoxa and prefers to attach to areas with few feathers, such as the comb, wattles, and around the eyes of the host as we observed in this study. Roberts and Janovy (2000) observed that the infestation caused ulcers, into which the female deposited the eggs. The larvae hatched in the ulcers and then dropped to the ground to develop off the host, as in most other fleas. We also noticed that some cocks with nictitating membrane damaged had gone blind. Wild birds have been implicated in the epizootiology of *E. gallinacea* infestation in Nigeria (Mbaya *et al.*, 2007).

The scaly leg mite *Knemidocoptes mutans* has been reported to burrow into the tissues under the scales of the legs and by its feeding activity leads to thickened, encrusted and unsightly scaly appearance of chicken legs (Urguhart *et al.*, 1988). The mites caused irritation and inflammation, with the result of powdery material formed, the accumulation of which raised the scales on the legs. Scaly leg is highly contagious. Accumulation of the crust may interfere with flexion of the joint and cause lameness. Severe infection may cause arthritis or loss of toes. We observed overt symptoms of scaly leg on about 27.7

% of the chickens examined (Figure 1b). The development of scaly leg may be progressive and age-dependent, since the symptoms were apparently absent from young chicks.

The soft tick *Argas persicus* has been demonstrated from wild birds in Northern Nigeria (Mbaya *et al*., 2007), but it is quite surprising that ticks were not encountered in this study. On handling, most of the chronically infected chickens appeared underweight, compared with their non-infected counterparts. The annual loss in poultry production, caused by external parasites in the tropical and temperate regions of the world, has been estimated at one billion US dollars (Akinwunmi *et al*., 1978).

The fact that most cocks sold in Awka market were derived from the northern states of the country is a strong indication that ectoparasites of chickens may be a country-wide problem. This paper therefore calls for a coordinated nation-wide survey of commercial poultry farms to establish the actual socio-economic impact of ectoparasites of chickens. Meanwhile, sanitary management practices involving routine use of proven insecticides and acaricides are essential for on-the-farm control of ectoparasites on chickens, as well as the diseases they vector in Nigeria.

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MOSQUITO FAUNA OF A TROPICAL MUSEUM AND ZOOLOGICAL GARDEN COMPLEX

[1]ONYIDO, Angus Ejidike., [1]OZUMBA, Nwora Abiodun., [2]EZIKE, Venantius Izuka., [2]CHUKWUEKEZIE, Okechukwu Chimaeze., [2]NWOSU, Emmanuel Okechukwu., [1]NWAORGU, Obioma Comfort and [1]IKPEZE Obiora Osegboka

[1]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria.
[2]National Arbovirus and Vectors' Research Centre, 17/33 Park Avenue, GRA Box 104, Enugu, Nigeria.

Corresponding Author: Ozumba, N. A. Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria. Email: nwaozumba@yahoo.com Phone: +234 80 23239056

ABSTRACT

The mosquito fauna of Museum and Zoological Garden Complex (JZC), a major tourist attraction in Jos Metropolis of Nigeria, was studied. The choice of the complex was out of public health curiosity. A total of 627 mosquitoes comprising 4 genera, Aedes, Culex, Coquilletidia and Eretmapodites, and 9 species were caught in two different study trips. Five species, namely, Aedes aegypti, A. africanus, A. vittatus, Culex quinquefasciatus and Eretmapodites chrysogaster, caught by human bait method are known variously to be involved in the transmission of yellow fever and other viral diseases. Culex quinquefasciatus had the highest frequency followed by Aedes aegypti. Simpson's dominance and Shannon-Wiener diversity indices of 0.4942 and 0.4550 were respectively recorded for the whole mosquitoes sampled by the human bait method. C. quinqefasciatus was the most frequent species with diversity values of 0.4444 (Simpson's) and 0.1174 (Shannon-Wiener), followed by A. aegypti with 0.0455 (Simpson's) and 0.1431 (Shannon-Wiener). Ecological statistics demonstrated a highly significant difference in diversity between samples in March, during the dry season, and June in the rainy season (P < 0.001). The presence of man-biting mosquitoes in JZC constitutes apparent public health danger and calls for regular surveillance and control operations on such disease vectors in the complex.

Keywords: Mosquitoes, Zoo Complex, Public Health, *Aedes, Culex, Eretmapodites, Coquilletidia*

INTRODUCTION

Mosquitoes are of remarkable importance in the transmission and dissemination of both human and animal diseases. Amongst the veterinary and medically important arthropods, mosquitoes rank first in the spread of such diseases as yellow fever, encephalitis, dengue fever, malaria, filariasis as well as other viral and bacterial diseases (Chandler and Read, 1961; Gillet, 1972; Gordon and Lavoipiere, 1979; Service, 1980). Jos Museum and Zoo Complex (JMZ) with a simulated natural ecological setting for the animals is one of the major tourist attractions in the town and therefore provides good site for the study of the ecology and distribution of insect disease vector species.

The study of species diversity in an ecological community takes account of the total number of species encountered in the sample, expressed as richness, and how the species abundances are distributed among the species, expressed as evenness (Fisher *et al*., 1943). Another measure, Shannon-Wiener Index of Diversity, which combines species richness and evenness as a single value is expressed as $H = (N \log N - \sum fi \log fi)/N$, where fi is the abundance and N the total number of individuals in the species (Ogbeibu, 2005). A greater number of species, as well as a more even distribution among species will therefore increase species diversity as measured by Shannon-Wiener Index (Lloyd and Ghelardi, 1964). The probability of picking two organisms at random that are different species, known as Simpson's Dominance Index, is expressed as $C = \sum (Pi)^2$ or $\sum (ni/N)^2$, where ni = number of individuals of the *ith* species, N is the total number of individuals for all species and Pi being the proportional abundance of *ith* species i.e., $Pi = ni/N$ (Krebs, 1972). Simpson's dominance indices is weighted towards the abundance of the commonest species and gives relatively little weight to the rare species, and ranges in value from 0 (low diversity) to a maximum of $(1–1/S)$, where S is the number of species (Fisher, 1943). The formula $\sum (ni/N)^2$ refers to a finite population where all of the members have been counted. Since ecologists work with infinite populations where it is impossible to count all members, an unbiased estimator of Simpson's Index, which is expressed as $D = \sum ni\,(ni–1)/\,N\,(N\text{-}1)$, where ni is the total number of individuals in the *ith* species, and N is the total number of individuals, has been developed for sampling from infinite natural population (Ogbeibu, 2005).

The major aim of this study was to sample the mosquito species in Jos museum and zoological garden complex (JZC) and determine if there was any significant difference in species diversity and dominance between adult mosquitoes sampled during parts of the dry and rainy seasons of the year. This surveillance involved the collection, analysis, consolidation and evaluation of data, and its prompt dissemination which represented an overall intelligence or disease-accounting system designed to

permit disease control authorities to be alerted early to the presence of disease problems and the effects of the disease on the population they serve (WHO, 1975).

MATERIALS AND METHODS

Study Area: The study was carried out in a tropical museum and zoological garden complex located in Jos, Plateau State, Nigeria. Jos is a cosmopolitan town and the capital of Plateau State of Nigeria. It houses many Federal and State Government ministries and parastatals. It is also a University Town. The neighbouring towns of Bukuru, Kuru and Vom have important federal establishments such as the National Institute for Policy and Strategic Studies, Nigerian Veterinary Research Institute, Nigerian Institute for Trypanosomiasis Research, Federal School of Laboratory Technology, National Root Crop Research Institute, Plateau State Polytechnic etc. These towns and this establishment are short distances from Jos metropolis, thus contributing to the teaming population. Jos is situated on a rocky plateau, about 1,300km above sea level with many rocky outcrops and patchy woodland bushes. It has huge mineral deposits, especially tin ore. Thus in addition, Jos attracts many mineral miners, as well as a large population of traders from different parts of Nigeria. Apart from a few hotels, the Museum and Zoological Garden Complex is the major tourist centre in the Jos metropolis. The Museum houses the famous Nok cultural materials as well as those of Gwosh, Nupe, Gwari, Igbo, Yoruba and Hausa tribes of Nigeria. Within the museum section, are decorative aquaria with water fountains and clay pots. Some of these have gone out of use, but still retained water throughout the year, thus providing good breeding places for mosquitoes.

The climate is neither too cold nor too hot, and thus attracts many foreigners from other parts of the world. It has two major seasons, the dry season between November and March, and the rainy season between April and October. The early dry season is characterized by cold harmattan period between November and February. Mean annual rainfall is 140 cm. The mean monthly temperatures vary between 20º C in the month of August and 25º C in the month of April. The relative humidity varies between 40% in January and 80% in July. Jos has a few streams and rivers flowing across the city. In the dry season, some of the streams dry up leaving breeding pools for mosquitoes and other aquatic organisms. Sometimes the rivers and steams flow sluggish and are heavily polluted with human and animals wastes such that snail intermediate hosts of helminths are common phenomena.

The Zoological section is located beyond a primary forest with giant trees that provide shade for the animals. Rock outcrops and forest patches in the Zoo provide the animals with natural ecological settings. Animals in the Zoo included members of the chimpanzee families, wild cats, jackal, drills, monkeys and baboons as well as lions, antelopes and duikers. Others include the rocky pythons, tortoises and turtles, crocodiles, various wild birds including eagles, owls, kites, peacocks and doves. The Zoological garden is traversed by a seasonal river that dries up almost entirely in the dry season, leaving small water pools and puddles for the breeding of mosquitoes. In addition, this river is usually heavily polluted by organic materials of plants, human and animal origin.

Ethical Considerations: The study was carried out by the staff of national arbovirus and vectors; research Centre, Enugu, under the auspices of the Federal Ministry of Health as a regular surveillance measure against Arboviruses. Formal letter of intent was written to the Curator of the museum who gave written consent before the work commenced. All the mosquito scouts were employed based on their personal consent. They were given adequate training and necessary health education. Also, each participant was given yellow fever vaccination at least ten days prior his involvement in the study. All health conditions of the mosquito scouts were taken care of by the Federal Government.

Mosquito Sampling Techniques: Field trips were made to the Jos Museum and Zoological Garden in the months of March and June 2005. Each trip lasted four days. Adult mosquitoes were collected at the Zoological Garden between 16.30 – 20.30 hours (4.30 – 8.30pm), using Voluntary Human Bait Method. In each occasion, three mosquito scouts were deployed. The scouts sat on low stools positioned at strategic areas such as tree shades, with their hands and legs exposed to mosquito bites, by rolling up the sleeves of their shirts and trousers to their elbows or knees respectively. Thereafter, with the aid of torchlight, mosquitoes alighting to bite on the exposed parts of the body were trapped with vials. Each mosquito was kept separately in a vial carefully stoppered with cotton wool, from where it was transported to Enugu laboratory for sorting and identification. Larvae were collected with ladles from ponds and artificial-water containers, which were mainly concrete troughs, providing water for the animals. No attempt was made to estimate the larval density as this initial effort was primarily to identify the mosquito fauna of the area. Mosquito eggs were collected by using a set of 25 and 26 CDC ovitraps in March and June respectively. The traps were set randomly near animals' shades and collected two days later. The paddles were wrapped in blank white duplicating sheets of paper and sent to the laboratory of the National Arbovirus and Vector's Research Centre, Enugu, for examination. Egg-positive paddles were soaked in water for two days to hatch-out the larvae. The larvae were reared to fourth instar stage or adult for proper identification. The soaked paddles were allowed to dry under room temperature, for about five days before re-soaking to ensure that all viable mosquito eggs hatched-out. Soaking and drying were repeated three times thereafter. All mosquitoes collected were identified by disease vectors taxonomist at the National Arbovirus and Vectors' Research Centre, Enugu, where voucher specimens were preserved.

Table 1: Species composition of mosquitoes caught with different trapping methods in Jos Museum and Zoo Complex

Mosquito genera and species	Number sampled with various trapping methods				
	Ovitrap	**Ladle**	**Human bait**	**Total**	**Percentage**
Aedes aegypti	304	119	32	455	72.6
A. africanus	-	-	5	5	0.8
A. vittatus	-	-	1	1	0.15
A. luteocephalus	5	-	-	5	0.8
Culex quinquefasciatus	-	49	100	149	23.8
Coquilletidia metallica	-	-	4	4	0.6
Eretmapodites quinquevittatus	-	-	5	5	0.8
E. inornatus	-	-	2	2	0.3
E. Chrysogaster	-	-	1	1	0.15
Total	309	168	150	627	100.0

Table 2: Mosquitoes sampled from eggs using CDC ovitraps in Jos Museum and Zoo Complex

S/No.	**Number of traps per animal house per time**	**Associated animal housing**	**Mosquitoes sampled**					
			March		**June**		**Total**	
			Species	**No.**	**Species**	**No.**	**No.**	**%**
1	1	Civet Cat	*A. aegypti*	2	*A. aegypti*	10	12	3.9
2	1	Black Kite	*A. aegypti*	9	*A. aegypti*	20	29	9.4
3	1	Barn Owls	-	0	*A. aegypti*	50	50	16.2
4	1	Serval and Crackal	-	0	*A. aegypti*	60	60	19.4
5	1	Peacocks and Duikers	-	0	-	0	0	0.0
6	1	Rock python	-	0	*A. aegypti*	14	14	4.5
7	1	Mona monkeys	*A. luteocephalus*	4	*A. aegypti*	16	20	6.5
8	1	Porcupine	-	0	-	0	0	0.0
9	1	Hornbill bird	-	0	*A. aegypti*	6	6	1.9
10	1	Black-faced monkeys	-	0	*A. aegypti*	30	30	9.7
11	1	Drill	-	0	*A. aegypti*	14	14	4.5
12 – 15	4	Lion	-	0	*A. aegypti*	13	13	4.2
16	1	Horse	-	0	*A. aegypti*	1	1	0.3
17 – 18	2	Chimpanzee	-	0	*A. aegypti*	30	30	9.7
19	1	Baboon	-	0	-	0	0	0.0
20	1	Mangabey	-	0	*A. aegypti*	6	6	1.9
21	1	Crocodile	*A. luteocephalus*	1	-	0	1	0.3
22	1	Tortoise *and* Baboon	-	0	-	0	0	0.0
23	1	Spotted hyaena	-	0	-	0	0	0.0
24	1	Stripped hyaena	-	0	*A. aegypti*	3	3	1.0
25	1	Doves	-	0	*A. aegypti*	20	20	6.5
26	1	Bateleus	-	0	-	0	0	0.0
Total				16		293	309	99.9

Ecological Statistics: Data on mosquito composition were analyzed quantitatively to determine the total abundance, percentage abundance of each species identified during the study period, as well as determining Shannon-Wiener diversity index (H) and Simpson's dominance index (C) for the area. Shannon-Wiener index (H) was used in calculating *t'* (Ogbeibu, 2005), to test for significant differences in diversity and dominance of mosquito species.

RESULTS

A total of 627 mosquitoes (Table 1), comprising 9 species from 4 genera; *Aedes, Culex, Coquilletidia and Eretmapodites* were caught during the sampling period. Also, 309 mosquitoes, consisting of 304 *A. aegypti* and 5 *A. luteocephalus* were collected as eggs; 168 mosquitoes, comprising 119 *A. aegypti* and 49 *C. quinquefasciatus* were collected as larvae; whereas 150 mosquitoes made up of 8 species namely: *A. aegypti, A. africanus, A. vittatus, C. quinquefasciatus, C. metallica, E. quinquevittatus, E. inornatus* and *E. chrysogaster* were collected as adults. On the whole, *A. aegypti* had the highest dominances of 455 mosquitoes, representing 72.6% of the total mosquito population. This was followed by *C. quinquefasciatus,* 100 mosquitoes, 23.8% of the total catch, with also the highest catch from human bait collections.

As illustrated in Table 2, CDC ovitraps were set close to animal sheds or cages to trap engorged female mosquitoes seeking oviposition sites after feeding on these animals. Mainly *A. aegypti* was collected from traps associated with reptiles, birds and mammals. This was an indication that *A. aegypti* virtually feeds on all animals. Amongst the sheds housing the rock python (*Python sabae*), crocodiles (*Crocodylus niloticus*) and tortoise (*Geochelone pardalis*), it was only from the tortoise shed that there was no collection. Amongst the aviary housing black kites (*Falco peregrinus*), barn owls (*Tyto alba*), peacocks, hornbills (*Upupa epops*), doves and eagles, only the eagles' aviary did not yield egg-positive paddles.

Table 3: Mosquitoes sampled from larvae, using ladles in Jos Museum and Zoo Complex

Mosquito species	Breeding place	*Number sampled*		
		March	June	Total
Aedes aegypti	Artificial containers and concrete scoops	0	119	119
Culex quinquefasciatus	Polluted river pools and ponds	10	36	49
Total		10	158	168

Table 4: Mosquitoes sampled as adults using voluntary human bait method in Jos Museum and Zoo Complex

Mosquito species	Number sampled			
	March	June	Total	Percentage
Aedes aegypti	0	32	32	21.3
A. africanus	1	4	5	3.3
A. vittatus	46	1	1	0.7
Culex quinquefasciatus	0	54	100	66.7
Coquilletidia metallica	0	4	4	2.7
Eretmapodites quinquevittatus	0	5	5	3.3
E. inornatus	0	2	2	1.3
E. chrysogaster	0	1	1	0.7
Total	47	103	150	100.0

Among the sheds housing civet cats (*Viverra civetta*), serval (*Leptailurus serval*) and crackal (*Caracal caracal*), mona monkeys, black-faced monkeys (*Cercopothecus ascanius*), lions (*Panthera leo*), horses (*Equus caballus*), chimpanzee (*Pan troglodytes*), baboons (*Papio cynocephalus*), mongabey (*Cercocebus attarimus*), hyaenas (C*rocuta crocuta* and *Hyaena hyaena*) and porcupines, no mosquitoes were trapped near the porcupine and baboon sheds. The groups of animals, servals and crackals, barn owls, chimpanzees and black-faced monkeys appeared to have attracted the mosquitoes most, with 60, 50, 30 and 30 mosquitoes respectively. Doves seemed to attract the *A. aegypti* mosquitoes. Few eggs of *A. luteocephalus* were caught from some animals units.

Eight (32%) of the 25 ovitraps set in March were positive and yielded 16 mosquito larvae. This gave an average of 2 eggs per egg-positive paddle and also represented 5.2% of the total eggs trapped during the study. In June, 18 (69.2%) of the 26 ovitraps set were positive with eggs and yielded 293 larvae, giving an average of 16 eggs per-positive paddle and this represented 94.8% of the total mosquitoes caught as eggs.

From Table 3, a total of 168 mosquito larvae, comprising 49 *C. quinquefasciatus* and 119 *A. aegypti* were collected with ladle. All the *Aedes* larvae were collected in June in artificial containers only, whereas, *Culex* larvae were collected in March and June from polluted river pools and ponds, a fact indicating the affinity of *Culex* to polluted water for oviposition.

A total of 151 adult mosquitoes were collected through human bait method. In the dry period of March, 1 *A. africanus* and 46 *C. quinquefasciatus* were collected giving a total of 47 mosquitoes (Table 4). One hundred and three (103) adult mosquitoes, comprising *C. quinquefasciatus* (54), *A. aegypti* (32), *Eretmapodites* spp. (8), *A. africanus* (4), *C. metallica* (4), and *A. vittatus* (1) were caught in June.

In March and June, 36 man-hours were spent in each occasion for collecting the mosquitoes. This translated to 1.3 and 2.9 mosquitoes per man-hour respectively. For *C. quinquefasciatus* and *A. aegypti* it was 1.6 and 0.9 respectively. Computations for diversity and dominance indices for adult mosquitoes sampled at Jos Zoo Complex are shown in Table 5. Simpson's diversity values of 0.9584 and 0.3773 and Shannon-wiener diversity values of 0.0448 and 0.5499 were recorded for the adult mosquitoes during the months of March in the dry season, and June during the wet season, respectively. A Simpson's and Shannon-Wiener values of 0.4942 and 0.4550 were recorded for the whole adult mosquitoes sampled with human bait method (Table 5). *C. quinquefasciatus* was the most frequent species with diversity values of 0.4444 (Simpson's) and 0.1174 (Shannon-Wiener). This was followed by *A. aegypti* with 0.0455 (Simpson's) and 0.1431 (Shannon-Wiener). Ecological statistics demonstrated a highly significant difference in diversity between samples in March and June ($P<0.001$).

DISCUSSION

The choice of Jos Museum and Zoological Garden Complex (JMZ) for this study was out of public health curiosity. Apart from the fact that the complex serves as a good holiday resort for visitors, the human and animal populations provided a regular blood-meal source for the mosquitoes. Such constant interaction between animal and human populations on one side and mosquito population on the other had the potential for diseases transmission to visitors and staff of the Zoological Garden and Museum. Also the attempt to fashion the Zoological Garden as close as possible to natural conditions does not only make it ecologically habitable for the animals but also for these disease vector species.

Historically, epidemics of yellow fever had been recorded in 1952 and 1953 in Okwoga district of Jos was postulated to be as a result of northward

Table 5: Computations for species diversity and dominance indices for adult mosquitoes sampled in March and June in Jos Zoo Complex (JZC) Nigeria

Jos Zoo Complex	fi	fi *log* fi	fi $\log^2$ fi	Pi	$(Pi)^2$ *or* $(ni/N)^2$	ni (ni-1)/N (N-1)	Pi log Pi	Pi In Pi	$Pi(In\ Pi)^2$	*Shannon-Wiener diversity index* $H = (N \log N - \Sigma fi \log fi) / N$ *or* $-(\Sigma Pi \log Pi)$	*Simpson's dominance index* $C = \Sigma (ni/N)^2$
March and June											
1 ***Aedes aegypti***	32	48.1648	72.4952	0.2133	0.0455	0.0444	-0.1431	-0.3296	0.5092	0.1431	0.0455
2 ***A. africanus***	5	3.4948	2.4428	0.0333	0.0011	0.0009	-0.0492	-0.1133	0.3854	0.0492	0.0011
3 ***A. vittatus***	1	0.0000	0.0000	0.0067	0.0001	0.0000	-0.0146	-0.0335	0.1679	0.0146	0.0001
4 ***Culex quinquefasciatus***	100	200.00	400.00	0.6667	0.4444	0.4429	-0.1174	-0.2693	0.1089	0.1174	0.4444
5 ***Coquilletidia metallica***	4	2.4082	1.4499	0.0267	0.0007	0.0005	-0.0420	-0.0967	0.3505	0.0420	0.0007
6 ***E. quinquevittatus***	5	3.4948	2.4428	0.0333	0.0011	0.0009	-0.0492	-0.1133	0.3854	0.0492	0.0011
7 ***E. inornatus***	2	0.6021	0.1812	0.0133	0.0002	0.0001	-0.0249	-0.0575	0.2482	0.0249	0.0002
8 ***E. chrysogaster***	1	0.0000	0.0000	0.0067	0.0011	0.0000	-0.0146	-0.0335	0.1679	0.0146	0.0011
Σ	150	258.1647	479.0119	1.0000	0.4942	0.4897	-0.4550	-1.0467	2.3234	0.4550	0.4942
March											
1 ***A. africanus***	1	0.0000	0.0000	0.0213	0.0005	0.0000	- 0.0356	-0.0819	0.3155	0.0356	0.0005
2 ***A. vittatus***	46	76.4868	127.1791	0.9787	0.9579	0.9574	-0.0092	-0.0211	0.0005	0.0092	0.9579
Σ	47	76.4868	127.1791	1.0000	0.9584	0.9574	-0.0448	-0.103	0.316	0.0448	0.9584
June											
1 ***Aedes aegypti***	32	48.1648	72.4952	0.3110	0.0965	0.0944	-0.1577	-0.3632	0.4245	0.1577	0.0965
2 ***A. africanus***	4	2.4082	1.4499	0.0390	0.0015	0.0012	-0.0547	-0.1261	0.4096	0.0547	0.0015
3 ***A. vittatus***	1	0.0000	0.0000	0.0100	0.0001	0.0000	-0.0195	-0.0450	0.2084	0.0195	0.0001
4 ***Culex quinquefasciatus***	54	93.5493	162.0642	0.5240	0.2749	0.2624	-0.1470	-0.3385	0.2186	0.1470	0.2749
5 ***Coquilletidia metallica***	4	2.4082	1.4499	0.0380	0.0015	0.0012	-0.0546	-0.1254	0.4084	0.0546	0.0015
6 ***E. quinquevittatus***	5	3.4948	2.4428	0.0490	0.0024	0.0020	-0.0637	-0.1468	0.4442	0.0637	0.0024
7 ***E. inornatus***	2	0.6020	0.1812	0.0190	0.0003	0.0002	-0.0332	-0.0765	0.3015	0.0332	0.0003
8 ***E. chrysogaster***	1	0.0000	0.0000	0.0100	0.0001	0.0000	-0.0195	-0.0450	0.2084	0.0195	0.0001
Σ	103	150.6273	240.0832	1.0000	0.3773	0.3614	-0.5499	-1.2665	2.6236	0.5499	0.3773

Key: *fi = Abundance of species, N = total number of individuals, Pi = Proportion of individuals found in the ith species, In = the Natural (Naperian) logarithms ($\log_e$), $(ni/N)^2 = (Pi)^2$*

transmission of yellow fever virus from the epidemic foci in Eastern Nigeria (Monath, 1972). The yellow fever virus exists normally in animal reservoir (monkey), in which it is maintained by several forest mosquitoes, with man acquiring the infection by frequenting forests or their neighbourhood where he becomes exposed to the bites of infected wild mosquitoes. An infected person subsequently returns to the village where the virus is transmitted to non-infected persons by domesticated species of mosquitoes.

Of the nine mosquito species collected, four were *Aedes*, three were *Eretmapodites* species, while one species each of *Culex* and *Coquilletidia* were recorded. Only *Aedes* and *Culex* were caught in the March and June surveys respectively. With the exception of *A. luteocephalus* captured as eggs only, the others were caught in adult forms by human bait method. This indicated that the mosquitoes fed on man were attracted to man. Human bait collections have been reported to be the most reliable method of detecting and monitoring the anthropophilic mosquito species (Service, 1977).

The hours of collection, 4.30 – 8.30pm is also the period of the crepuscular activities of these mosquitoes and corresponds to the peak visiting hours to the JMZ, especially between 4.00 – 6.00pm. This means that during any epidemic, considerable population of holiday makers could be bitten by infected mosquitoes and infected persons will subsequently transport the infections to their cities and villages, where others would be affected.

Of all the mosquitoes collected, the presence of *A. aegypti, A. africanus, A. vittatus, C. quinquefasciatus, E. chrysogaster* were of apparent health danger. In Nigeria, *Coquilletidia* and *Eretmapodites* species have not been known to be involved in disease transmission, although *Eretmapodites chrysogaster* and *Coquilletidia metallica* are known to be capable of transmitting yellow fever and West Nile fever viruses respectively (Gillet, 1972; Service, 1980). Although this situation has not been reported in Nigeria, close watch is required on any mosquito that feeds on or is attracted to man. *Aedes* subgroup, especially *A. aegypti, A. africanus, A. vittatus* and *A. luteocephalus* have since been known to feed on man and other animals and are involved in the transmission of many viral infections, especially *A. aegypti* which is the principal vector of viral infections such as dengue fever virus, encephalitis virus, haemorrhagic fever virus, chikungunya, Rift Valley and Uganda S. viruses (Gillet, 1972; Service, 1980). The capacity of *A. aegypti* to do this depends on its ability to feed on virtually every moving animal (Snow and Boreham, 1978). This might explain why the eggs were collected virtually from all animal sheds. *A. vittatus* and *A. africanus* are equally important vectors of yellow fever and other viral diseases. In 1941, in the Sudan, where a serious outbreak of yellow fever occurred, it was shown that *A. vittatus* was the important vector. *A. vittatus* is a rock-pool breeder and is well known to be involved in peri-urban transmission of yellow fever in Nigeria. Service (1974), in a survey of yellow fever vectors in North-West Nigeria showed that only *A. aegypti* and *A. vittatus* were the dominant vectors.

In East Africa, sylvan yellow fever virus was passed from monkey to monkey by *A. africanus* (Gillet, 1972). This species is a forest mosquito which breeds in tree holes often at high levels. The females become active soon after sunset and feed throughout the night on monkey living in the higher foliage of the forest (Gordon and Lavoipierre, 1979). These forest monkeys come to the ground to raid plantations, such as banana plantations situated on the forest fringes or clearings in the forest, in these circumstances the monkeys are exposed to day-biting mosquitoes, *A. simpsoni,* which breeds chiefly in leaf-axils of the banana plants.

In Nigeria, it was reported that the role of *A. simpsoni* was probably continued by *A. africanus* and *A. aegypti* which are found in large numbers in rural dwellings and forest fringes (AVRU 1979a; 1979b). The *A. simpsoni* found in Nigeria seems to be non-man biting variants – *A. lilii* and *A. bromeliae* (Huang and Ward, 1981). With the Zoological Garden sheltered by the primary forest trees, the monkey populations, the abounding vector population supported by the favourable ecological conditions and the ever-increasing population of visitors, is a pointer to the immediate need for constant surveillance and control of mosquitoes to make the complex safe for tourists and staff.

Culex quinquefasciatus also caught in the Museum Complex is a cosmopolitan mosquito. *Culex quinquefasciatus* is night-biting and serves as the most important vector of the nocturnally periodic form of the filarial worm, *Wuchereria bancrofti,* heavy infection of which leads to elephantiasis (Chandler and Read, 1961; Gillet, 1972; Gordon and Lavoipierre, 1979). Besides the capacity of mosquitoes as vectors of public health diseases, they constitute serious nuisance by their frequent and menacing bites. All-day biting mosquitoes with crepuscular peaks as *Aedes* group under heavy infestation may scare away some tourists within the Zoo - Museum Complex, whereas night-biting species including the *Culex* group will certainly affect the performance of the zoo staff on night-duties. This may result in loss of many man-hours spent in seeking medical treatment. Mosquitoes affect also the productively of the zoo animals, not only by possible transmission of some bacterial and viral diseases to them (Andrews and Pereira, 1976), but also by their bites and sucking of blood from them. This may lead to aneamia, restlessness and loss of condition amongst these animals thereby directly or indirectly affecting their general well-being and productivity.

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STUDIES ON THE REPRODUCTIVE POTENTIAL OF HOMOPLASTIC AND HETEROPLASTIC PITUITARY HORMONES IN *Heterobranchus bidorsalis* (GEOFFROY SAINT HILAIRE, 1809)

[1]NWOKOYE, Charles Ononuju., [1]NWUBA, Lucy Afuluenu and [2]EYO, Joseph Effiong
[1]Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra State
[2]Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka, Nigeria.

Corresponding Author: Nwokoye, C. O. Department of Crops Science and Horticulture, Faculty of Agriculture, Anambra State University, Igboriam Campus, Anambra State. Email: aquafishc@yahoo.com Phone: +2348035489844.

ABSTRACT

Artificial induce breeding of gravid Heterobranchus bidorsalis was carried out using two hormonal materials – homoplastic and heteroplastic hormones. The study which involved 10 trials was carried out with 60 gravid female and 20 mature male. The broodfish used for the study were 18 months hatchery produced H. bidorsalis. The hormonal treatments led to the following results in terms of percentage weight loss (3.16 and 3.06%); fertilization rate (9522.77 ± 348.13 and 8,857.93 ± 255.57); and hatchability (9,180.13 ± 343.37 and 8,476.83 ± 345.95) for homoplastic and heteroplastic hormones respectively. The mean numbers of dead eggs were 396.10 ± 19.15 for homoplastic hormone injected catfish and 425.53 ± 17.09 for those injected heteroplastic hormone. Recorded deformed of larva were low (35.80 ± 1.11 and 34.27 ± 1.43) respectively for catfish injected homoplastic and heteroplastic hormones. Survival of hatchlings was high (99.61 and 99.59 %) for gravid catfish injected homoplastic and heteroplastic hormones respectively. There was no significant difference (P < 0.05) in weight of pre and post female spawners. Although the two tested hormones investigated were effective inducers, homoplastic hormone is recommended as it recorded better results.

Keywords: Homoplastic and Heteroplastic hormones, Induced spawning, Hatchability, *Heterobranchus bidorsalis*

INTRODUCTION

Nigeria is a coastal state with lots of fisheries resources both marine and inland waters. About 12 million hectares and 500,000 hectares (Gaffar, 1996), was estimated to be suitable for aquacultural development in freshwater and marine environments. The growing aquaculture industry has led to the high demand for fish fingerlings. Capture and culture fisheries play major roles in fish production contributing an average of 84.2 % of the total domestic output between 1990 and 1994 (CBN, 1994). Gaffar (1996) reported that out of 650,000 metric tons of total annual fish output in Nigeria, 350,000 metric tons were produced locally with Inland water and aquaculture accounting for 110,000 metric tons and 18,000 metric tons respectively. Ogbe and Odiba (1996) reported that between 1990 and 1994, Nigeria's fish output experienced a negative growth (-0.6 %) averaging 298.8 thousand metric tons per annum against annual demand of 1.5 million metric tons.

Otubusin (1996) reported that Food and Agricultural Organization (FAO) indicated that to maintain the present per caput fish consumption levels of 13.0kg per year, 91 million metric tons of food fish would be required. Such an increase can only be achieved through aquaculture. Farming culturable fish species under controlled environment has proved be a successful method of increasing fish supply. In a national diagnostic survey of water resources of Nigeria carried out in 1983, it was revealed that about 200 ha of ponds are under cultivation with another 836 ha under construction and about 2700 ha proposed for execution (Ita *et al.,* 1985). In 1994, a national survey of aquaculture development in Nigeria was conducted by the Nigeria Institute of Freshwater Fisheries Research (NIFFR), New Bussa. The survey showed that there had been immense awareness of profitability of fish farming in the country within the last one decade. It was noted that 80% of all the existing fish farms in every state of Nigeria were developed within the last decade (Ita, 1996). The survey also revealed that out of 80 % of fish hatcheries identified in different parts of the country, 48% were government owned. FAO (1990) reported that the major constrain of fish farming in Nigeria is the inadequate availability of quality and fast growing fish seed. Based on a 1992 United Nations Development Project (UNDP) assisted base line study (Fish Network, 1994) the total fingerlings requirement of Nigeria was 250,000 million while the domestic production stood at 7.2 million.

Catfish is one of the most sought after culturable food fish in Nigeria. It is very popular with fish farmers and consumers and commands very good commercial value in Nigerian markets (Oladosu *et al.,* 1993; Anyinla *et al.,* 1994; Ezenwaji, 1985). The catfish are very important to the sustainability of the aquaculture industry in the country.

Despite the break through reported for artificial propagation of catfish (Richter and Van der

Hurk, 1982; Madu *et al.,* 1987; Madu *et al.,* 1989) the demand for the fish seed still exceeds supply. Various types of fish have been induced to spawn using various hormonal materials (Nwadukwe, 1993; Eyo, 1996, 1997, 2000; Nwuba and Aguigwo, 2002). Some of the hormonal materials include HCG (Eyo, 2002), clomiphene citrate (Aguigwo, 1991), Pituitary extract (Janssen, 1985; Haniffa *et al.,* 2000) and ovaprim (Abol-Munafi *et al.,* 2006).

The present study compared the effects of Homoplastic and Heteroplastic hormones on artificial breeding of *Heterobranchus bidorsalis* with the following objectives. Comparison of the level of ovulation inducement of the two hormonal materials, establishment of spawn efficacy of *H. bidorsalis* injected with the hormonal materials, determination of percentage hatchability and survival of the eggs and larva respectively and establishment of cost benefit of the hormones

MATERIALS AND METHODS

The study Area: This study which lasted 70 days (May – July, 2002) was carried out using fish hatchery facilities at the Aquafish farm, Ihudim, Ihiala, Anambra State. The farm which covers an area of 5 hectares has the following developed structures and facilities: indoor fish hatchery complex with 10 incubation concrete tanks (1 x 1.5 x 1 m) each. 20 earthen nursery ponds (10 x 15 x 1.2 m). Three brood stock ponds (20 x 10 x 1.2 m) each. Seven production ponds (30 x 80 x 1.5 m) each. A borehole and 5000 gallon concrete reservoir. A perennial river – Ulasi is located about 300 m from the farm.

Brood Fish Procurement and Management: Broodfish used for the study were 18 months old domesticated *H. bidorsalis* fingerlings produced at Aquafish hatchery. All broodfish were selected by external morphological characteristics using the methods described by Ayinla and Nwadukwe (1988). Female brood fish were selected on the basis of ovarian biopsy of the oocytes as described by Legendre (1986). The 60 selected gravid females and 20 mature males had weight range of 310 to 550 grams. The female broodfish were kept separate from the males in earthen ponds (10 x 15 x 1.2 m). They were fed Aquafish pelleted fish feed (35 % Crude Protein) twice daily (7 and 5 pm) on 5 % of total fish biomass, 7 days of the week. The brood stock were acclimated in their new environment (10 x 15 x 1.2 m earthen fish pond) for 15 days at mean temperature of 28 ± 2^{O}C and normal photoperiodic regimes (12 hour light and 12 hour darkness).

Experimental Design and Induced Spawning: Three treatments replicated trice with three fish per replicate was used. Two hormonal materials (homoplastic and heteroplastic hormones) were used. Control fish were administered 1 ml of O.6 % saline solution.

Ten induced spawning trials were carried out using 60 gravid females and 20 mature males. Prior to each trial, pituitary gland was extracted from mature fishes for homoplastic and heteroplastic hormones respectively (Viveen *et al.,* 1985). Each gland was then transferred into a glass tube containing acetone. The acetone was decanted after eight hours and refilled. This was kept in a cool place for 24 hours after which it was finally decanted, dried and stored pending use.

Hormone Injection: Before hormone injection, vitellogenic females were randomly seined out from the ponds and kept singly in aerated 50 litres aquarium with 25 litres of aerated water for 12 hours. This was to allow them remove their gastro-intestinal content (Viveen *et al.,* 1985).

The injection of hormonal materials was done between six and seven pm during each experiment. During each trial the acetone dried pituitary was macerated in a porcelain mortar with a known volume (1 ml /kg body weight of fish) of 0.6 % saline solution. It was allowed to settle after which the supernatant was drawn with 5 ml hypodermic syringe with 0.6 mm gauge needle. The weighed gravid fish were then covered with towel and injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. After withdrawal of the needle, the fish was finger rubbed to avoid backflow of the injected fluid. The control fish were injected 0.6 % saline solution. The injected fish were returned separately into their respective 50 litre aquaria.

Stripping, Fertilization and Incubation: Stripping took place 10 h after injection at a mean temperature of 28 ± 2^{O}C. This was carried out by holding the fish at the head and tail by an assistant. The ovulated eggs oozed out on slight pressure by thumb towards the tail onto a plastic bowl. Incisions were then made on the sperm sac which was collected minutes prior to stripping by sacrificing the mature male. Milt was squeezed over the eggs. The two sex products were then mixed with plastic spoon. To this, 0.6 % saline solution was added and further agitated by spoon. One mature male was used for 3 females. The whole process took 3 minutes to accomplish.

Incubation of the fertilized eggs was carried out in 1 x 1.5 x 1 m concrete tank that was partitioned into three equal compartments. It was equipped with water flow through facilities.

Nylon mesh net (1 mm) was suspended above the floor and the fertilized eggs were spread in single layers on it for incubation. Water parameters were monitored. Temperature was measured by centigrade thermometer, pH was monitored using Hanna Hep pH meter and optimum oxygen level was maintained with RESUN LP-100 low noise air-pump.

Sample of 200 eggs was taken from each of the treatments at random and incubated in aerated aquaria (36 x 24 x 18 cm). Dead eggs were removed after 10 hours (Nwadukwe, 1993).

Table 1: Effect of hormonal treatment on the weight before and after spawning of *H. bidorsalis*

Hormone	Mean wt. before spawning (g)	Mean wt. after spawning (g)	Mean wt. loss	% wt. loss	T-value	P-value
Homoplastic hormone	423.83 ± 14.19	410.42 ±13.92	13.09	3.16	0.67	0.25
Heteroplastic hormone	424.43 ± 12.39	411.43 ± 11.97	12.34	3.06	0.76	0.23

Table 2: Effect of hormonal treatment on the number of fertilized eggs of *H. bidorsalis*

Hormone	Mean number of fertilized eggs	% fertilized	F-value	P-value
Homoplastic hormone	9,522.77 ± 348.13	96.01	13.18	0.001
Heteroplastic hormone	8,851.93 ± 255.57	95.42	13.18	0.001

Table 3; Effect of hormonal treatment on the number of dead eggs after fertilization in *H. bidorsalis*

Hormone	Mean number of dead eggs	% of dead eggs	F-value	P-value
Homoplastic hormone	396.10 ± 19.15	3.99	235.32	0.00
Heteroplastic hormone	425.53 ± 17.09	4.55	235.32	0.00

Table 4: Effect of hormonal treatment on hatchability of eggs at same environmental variables for *H. bidorsalis*

Hormone	Mean number of eggs fertilized	Mean number of eggs hatched	% Hatchability	F-value	P-value
Homoplastic hormone	9522.77 ± 348.13	9,180.13 ± 343.37	96.40	15.86	0.001
Heteroplastic hormone	8,857.93 ± 255.57	8476.83 ± 345.95	95.70	15.86	0.001

Percentage hatchability and larval deformity were calculated (Haniffa and Sridhar, 2002). The number of eggs released was calculated following the gravimetric method (Lagler, 1982; Legendre, 1986).

The nylon meshed net was removed with the egg shells while the hatched larvae clustered at dark corners of the incubation tank. Three days after hatching, post-yolk fry were fed to satiation with incubated *Artemia naupli.* Percentage survival was calculated at the end of 5 days.

Data Analysis: The data collected for the trials were pooled together and analysed for central tendencies using descriptive statistics. Analysis of variance with F-LSD post ad hoc test was used to separate differences in treatment means. Multiple regression and correlation statistics was used to establish linear relationships between variables. All analysis were carried out using Microsoft Excel 2006. The output is presented in tables.

RESULTS

Effect of Hormonal Treatment on Body Weight of Pre and Post Spawners of *H. bidorsalis:* The effects of the two hormonal materials of gravid female *Heterobranchus bidorsalis* are presented in Table 1. The mean pre-spawning weight was 423 ± 12.39 g for gravid *H. bidorsalis* injected with heteroplastic hormone. There was non significant difference ($P < 0.05$) in weight of female fishes before hormonal treatments. Similarly, all gravid *H. bidorsalis* recorded no significant weight loss after spawning. The non significant weight difference was 410.42 ± 13.92 g for homoplastic hormone and 411.4 ± 11.97 g for females injected with heteroplastic hormone. The T- value was not significantly different for the hormonal treatments.

The percentage weight loss emanating from spawning as induced by hormonal treatments were 3.16 % for fishes injected with homoplastic hormone and 3.06 % for those administered ovaprim. Although homoplastic hormone recorded higher percentage weight loss, the result was not significantly different ($P. > 0.05$) from that of heteroplastic hormone.

Effect of Hormonal Treatment on Number of Fertilized Eggs: Gravid female *H. bidorsalis* injected with homoplastic hormone recorded higher number of eggs (9522.77 ± 348.13) while females injected with heteroplastic hormone recorded (8857.93 ± 255.57).

Also, the percentage fertilization was higher for homoplastic injected *H. bidorsalis* (96.01 %) while those injected heteroplastic hormone recorded (95.42 %) (Table 2). The analysis of variance test of the number of fertilized eggs indicated significant difference ($P < 0.001$) for the hormonal treatments.

Effects of Hormonal Treatment on Number of Dead Eggs after Fertilization in *H. bidorsalis:* Gravid female *H. bidorsalis* injected with homoplastic hormone recorded lower mean number of dead eggs (396.10 ± 19.15). Those injected with heteroplastic hormone recorded (425.53 ± 17.09).

Furthermore, the percentage dead eggs were lower (3.99 %) in homoplastic injected female *H. bidorsalis* than that of gravid *H. bidorsalis* injected heteroplastic hormone (4.55 %). The analysis of variance test of number of dead eggs showed significant difference ($P < 0.001$) for the hormonal treatments (Table 3).

Effects of Hormonal Treatment on Hatchability of Eggs at Same Environmental Variables for *H. bidorsalis:* The effects of different hormonal treatments on the hatchability of gravid *H. bidorsalis* are presented in Table 4.

Table 5: Effect of hormonal treatment on larval deformities of *H. bidorsalis* under similar environment

Hormone	Mean number	Mean number of deformed larvae	% deformity	F-value	P-value
Homoplastic hormone	9180.13 ± 343.37	35.80 ± 1.11	0.39	86.63	0.001
Heteroplastic hormone	8,476.83 ± 245.95	34.27 ± 1.43	0.40	86.63	0.001

Table 6: Effect of hormonal treatment on percentage survival of *H. bidorsalis* larvae under unvaried environment

Hormone	Mean number of larvae	Mean number of hatchlings	% survival
Homoplastic hormone	9,180.13 ± 343.37	9,144.33	99.61
Heteroplastic hormone	8,476.83 ± 245.95	8,442.56	99.59

Table 7: Costs Benefit of hormonal treatment on *H. bidorsalis*

Hormone	Total wt. of fish (g)	Cost of hormone ₦
Homoplastic hormone	12,715	6,350.00
Heteroplastic hormone	12,733	6366.00

Gravid *H. bidorsalis* injected with homoplastic hormone recorded higher number of hatched eggs (9180.13 ± 343.37). Female *H. bidorsalis* administered heteroplastic hormone recorded (8476.83 ± 345.95). Similarly, percentage hatchability was 96.40 % for female *H. bidorsalis* injected homoplastic hormone and 95.70 % for those injected heteroplastic hormone. The analysis of variance test of eggs hatchability showed significant difference ($P < 0.001$) for the hormonal treatments.

Effects of Hormonal Treatment on Larval Deformities of *H. bidorsalis:* Record of deformed larva arising from hatchlings of *H. bidorsalis* injected with hormonal materials are shown in table 5. Gravid female of *H. bidorsalis* injected with homoplastic hormone recorded higher mean number of deformed larva (35.80 ± 1.11). Females of *H. bidorsalis* injected with heteroplastic hormone recorded lower number of deformed larva (34.27 ± 1.43).

However, percentage deformity was (0.39 % and 0.40 %) respectively for female *H. bidorsalis* injected with homoplastic and heteroplastic hormones respectively. Analysis of variance test for deformed eggs, showed significant difference ($P < 0.001$) for the hormonal treatments.

Effect of Hormonal Treatment on Percentage Survival of *H. bidorsalis* Larvae Under Unvaried Environment: Gravid female *H. bidorsalis* injected with homoplastic hormone recorded percentage survival of 99.61% while those injected heteroplastic hormone recorded 99.59 % (Table 6).

Cost Benefit of Hormonal Treatment in *H. bidorsalis:* Comparative costs of hormonal materials used on *H. bidorsalis* are shown in Table 7. Gravid female of *H. bidorsalis* which weighed a total of 12.72 kg were injected homoplastic hormone worth N6350 while female *H. bidorsalis* that weighed a total of 12.73 kg were administered heteroplastic hormone worth ₦ 6366:00K.

DISCUSSION

Effects of Hormonal Treatment on Body Weight of Pre and Post Spawners: Results presented in table 1 indicated non significant difference ($P > 0.05$) in weight of female spawners before hormonal treatment and after spawning. The non significant weights were 410.42 ± 13.92 g and 411.43 ± 1.97 g for female injected with homoplastic and heteroplastic hormones respectively. The non significant differences may be as a result of the fact that the ovarian weight is usually a negligible fraction of the somatic (body) weight. de Graaf *et al.,* (1995) reported similar finding for *Clarias gariepinus* breed, using induced breeding technique.

Delince *et al.* (1987) reported that a spent ovary of *C. gariepinus* represented about 10 – 20 % of its initial weight. Viveen *et al.* (1985) reported about 700 eggs per gram in *C. gariepinus* and noted that the quantity of ovulated eggs was between 15-20 % of its own body weight. In another study, Eyo and Mgbenka (1992) established linear relationship between fecundity, ovarian weight, length, GSI and somatic weight of *C. gariepinus.* This relationship is important in estimating fecundity from ovarian weight, length, GSI and somatic weight, hence facilities required from successful spawning trials.

Effects of Hormonal Treatment on Number of Fertilized Eggs: The result in Table 2 indicated that spawners injected with homoplastic hormones recorded significantly higher number of fertilized eggs (9522.77 ± 348.13 eggs) fertilized eggs arising from injection of heteroplastic hormone was (8857.93 ± 255.57 eggs). In another study, using HCG to induce breed *Channa punctatus,* Haniffa and Sridhar (2002), had fertilized egg output ranging from 1253 ± 126 eggs for *C. punctatus* weighing 65 – 85 g injected 3000 IU HCG. The difference in egg output of Haniffa and Sridhar when compared to this study may be due

to difference in species, weight of spawners and the hormonal material used. In another study Oladosu *et al.* (1993) induce breeding *H. bidorsalis* (mean weight 707.72 ± 4.28.22) with carp pituitary recorded mean egg out put of 33460 ± 20.571 eggs. This study reported lower fertilized eggs output than Oladosu *et al.* may be due to weight difference in spawners used.

Effect of Hormonal Treatment on the Number of Dead Eggs after Fertilization in *H. bidorsalis:* This study showed that there was significant difference ($P < 0.05$) in the number of dead eggs recorded, for female fish injected with the hormonal materials.

The female fish injected homoplastic hormone recorded 396.10 ± 19.15 while those administered heteroplastic hormone had 425.53 ± 17.09. The percentage of dead eggs was 3.99 % for females injected homoplastic hormone and 4.55 % for those injected heteroplastic hormone. In another study, Nwadukwe (1993) induce breeding *Heterobranchus longifilis* with frog (*D. occipitalis*) pituitary hormone recorded higher dead eggs percentage (29 %). The lower percentage achieved in the present study may be attributed to the efficacy of the hormones.

Effect of Hormonal Treatment on Hatchability of *H. bidorsalis:* Results on Table 5, showed higher hatchability (9,180.13 ± 343.37 larva or 96.40 %) of eggs for female *H. bidorsalis* injected with homoplastic hormone while those administered heteroplastic hormone recorded slightly lower number of hatched larva (8,476.83 ± 345.95 larva or 95.70 %). In a similar study Fagbenro and Adebayo (2004), using homoplastic hormone suspension on *H. bidorsalis* reported hatchability percentage of 86 %.

Effect of Hormonal Treatment on Larval Deformities of *H. bidorsalis:* In this study spawners injected homoplastic hormone recorded slightly higher number of deformed larvae (35.80 ± 1.1) when compared with those injected heteroplastic hormone (34.27 ± 1.43). Percentage deformity followed the same pattern (0.12 % and 0.39 % respectively. In a similar study, Nwadukwe (1993) using frog pituitary to induce breed *H. longifilis* reported higher percentage deformity of 7.17 ± 1.72. The higher percentage deformity recorded by Nwadukwe may be Nwadukwe may be attributed to the low potency of frog pituitary.

Effect of Hormonal Treatment on Percentage Survival of *H. bidorsalis* under Unvaried Environment: Percentage survival of hatchlings was 99.61 and 99.59 % respectively for gravid *H. bidorsalis* injected homoplastic and heteroplastic hormone suspension. The result recorded in this study is similar to those reported by earlier studies. Salami *et al.* (1994) induce breeding *C. gariepinus* with non-piscine pituitary extract (HCG) reported a better survival rate due to HCG administration than carp pituitary extracts.

Similarly, in another study Nwadukwe (1993) using frog pituitary extract to induce breed *H. longifilis* reported a survival rate of 66 to 90% for hatchlings after one week. The slight difference in these results when compared with that of this study may be attributed to species of fish and hormonal material used.

Cost Benefit of Hormonal Treatment in *H. bidorsalis:* Table 7 showed the comparative costs of the hormonal materials used homoplastic hormone which recorded better result in the parameters investigated cost same (₦ 6350:00K for 12.7 kg of fish injected). Although the two hormones tested were effective inducers, homoplastic hormone is recommended as it recorded better results.

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SPECIES COMPOSITION AND ABUNDANCE OF MOSQUITOES OF A TROPICAL IRRIGATION ECOSYSTEM

OGUOMA, Victor Maduabuchi and IKPEZE, Obiora Osegboka
Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Corresponding Author: Ikpeze, O. O. Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Email: ikpezevet@yahoo.com Phone: +2348035838255

ABSTRACT

Exophagic-anthropophilic mosquitoes were collected during the April 2007-January 2008 planting season in four designated millet and guinea-corn irrigation fields sampled in Gezawa Agro-ecological Zone of North-central Nigeria. Gezawa-1, Gezawa-2, Ketawa and Jogana irrigation fields contributed about 31.2 %, 24.8 %, 22.8 % and 21.2% respectively, to the number of mosquito species collected in the zone. There was preponderance of Anopheles gambiae complex (20.7 %) over Culex quinquefasciatus (11.8 %), C. pipiens fatigans (9.0%), A. funestus complex (7.0 %), Aedes aegypti (6.9%), A. albopictus (6.6 %), C. pipiens pipiens (5.7 %). C. tigripes (5.0%), A. pharoensis (3.7 %), A. africanus (3.6%), A. taylori (3.4%), A. coustani (3.3 %), A. luteocephalus (2.9 %), A. vittatus (2.8 %), A. rhodesiensis (2.1 %), Mansonia (2.0 %), A. simpsoni (1.9 %) and Psorophora species (1.6 %). A Shannon-Wiener and Simpson's diversity values of 1.1431 and 0.0925 were recorded for the mosquito species in Gezawa Agricultural Zone. A. gambiae had the highest Shannon-wiener diversity and Simpson's dominance indices of 0.1415 and 0.0427 respectively. There was no significant difference between species diversity for the four irrigation fields (P>0.001). Vector control must be carried out in the irrigation fields to reduce the number of these out-door biting mosquitoes, since total reliance on ACTs and ITNs could not offer full protection against malaria to farmers in Gezawa irrigation fields.

Keywords: Mosquitoes, Composition, Diversity, Dominance, Tropical ecosystem, Irrigation

INTRODUCTION

Millet (*Panicum miliaceum*) and guinea corn (*Sorghum spp*) are the major crops that are mostly grown by peasant farmers in the northern parts of Nigeria. As a result of prolonged dry season in Gezawa ecological zone in North-central Nigeria, irrigation is practiced to boost production of these crops. The demand for irrigation has led to the proliferation of mosquito fauna in the irrigation system. Despite the on-going program of artemisinin-based combination therapy (ACT) and insecticide treated bed-net (ITN) administration in Gezawa Local Government Area (LGA) of Kano State, the increasing number of malaria cases at Gezawa general Hospital prompted us to carry out this surveillance of man-biting mosquitoes in the irrigation fields where over 70% of the farmers in Gezawa work during the planting season. According to WHO (1975), such surveillance involved the collection, analysis, consolidation and evaluation of data, and its prompt dissemination which represented an overall intelligence or disease-accounting system designed to permit disease control authorities to be alerted early to the presence of disease problems and the effects of the disease on the population they serve.

The study of species diversity in an ecological community takes account of the total number of species encountered in the sample, expressed as richness, and how the species abundances are distributed among the species, expressed as evenness (Fisher *et al*., 1943). A better measure known as Shannon Index or Shannon-Wiener Index of Diversity, which combines species richness and evenness in a single value is expressed as $H = (N \log N - \sum fi \log fi)/N$ where fi is the abundance and N the total number of individuals in the species (Ogbeibu, 2005). A greater number of species, as well as a more even distribution among species will therefore increase species diversity measured by Shannon-Wiener Index (Lloyd and Ghelardi, 1964). Another measure, the probability of picking two organisms at random that are different species, is known as Simpson's Dominance Index, and is expressed as $C = \sum (Pi)^2$ or $\sum (ni/N)^2$, where ni = number of individuals of the *ith* species; N being the total number of individuals for all species; Pi being the proportional abundance of *ith* species i.e., $Pi = ni/N$ (Krebs, 1972). Simpson's dominance indices is weighted towards the abundance of the commonest species and gives relatively little weight to the rare species, and ranges in value from 0 (low diversity) to a maximum of $(1–1/S)$, where S is the number of species (Fisher, 1943). The formula $\sum (ni/N)^2$ refers to a finite population where all of the members have been counted. Since ecologists work with infinite populations where it is impossible to count all members, an unbiased estimator known as Simpson's Index, denoted by $D = \sum ni\ (ni–1)/N\ (N-1)$ has been developed for sampling from infinite natural population, where ni is the total number of individuals in the *ith* species, and N is the total number of individuals (Ogbeibu, 2005). The major aim of this study was to use natural counts of the number of species, and the number of individuals in each of the species of mosquitoes sampled from four millet and guinea-corn irrigation fields in Gezawa Agricultural Zone of North-central Nigeria to determine the composition and abundance of mosquitoes in a

tropical irrigation eco-system. The study will be helpful in understanding the epidemiology of mosquito-borne diseases in an endemic area, thereby aiding in vector control.

MATERIALS AND METHODS

Study Area: The study area, Gezawa irrigation system, is in Gezawa, Kano State, North-central Nigeria. Gezawa has a purely rural setting with limited health intervention. Majority of the natives cultivate millet and guinea corn. The adoption of irrigation practices has created different mosquito fauna in irrigated ditches, bed-pools, puddles and hoof-prints, which may guarantee all-year breeding of the mosquito in the area, thereby constituting a major factor in the epidemiology of malaria in Gezawa.

Mosquito Breeding Habitats: Mosquitoes larvae and adults breeding habitats in the irrigated millet and guinea corn fields were observed on weekly basis, from May to December 2007. Irrigation ditches, bed-pools, puddles, and hoof-prints were also observed from April 2007 to January 2008.

Collection of Larval Mosquitoes: Mosquito larvae observed in bed-pools and puddles were collected using ladles; those in hoof-prints were scooped with plastic spoons and those in ditches were sampled with well nets. Subsequent collections of the available larvae were made into sampling vials with rubber stoppers. The vials were appropriately labeled with place of collection and type of habitat. Each vial holds collections from only one habitat sampled. There was no attempt to exhaust all the larvae in a habitat in cases of heavy breeding but to collect representative sample as far as was possible.

Collection of Adult Mosquitoes: Adult mosquitoes were sampled by hanging a black clothe, coated with hair cream (made up of mineral oil, petroleum jelly, anhydrous lanolin, coconut oil, microcrystalline wax, collagen and fragrance) on scarecrows that acted as effective attractant to biting mosquitoes. Adult mosquitoes hovering around the baited-scarecrows were collected, especially in the evenings, using a 20cm diameter well net sampler.

Identification of Larval and Adult Mosquitoes: Adult mosquitoes collected were brought to the Department of Pathology laboratory at Gezawa General Hospital (GGH) and released into a glass jar containing chloroform. All mosquito species were observed under dissecting microscope for identification using standard morphological keys (Hopkins, 1952; Smart *et al*., 1956; Service, 1976; Gilles and Coetzee, 1987). Voucher specimens were also deposited with the pathology department at GGH, Gezawa.

Ecological Statistics: Data on mosquito composition were analyzed quantitatively to determine the total abundance, percentage abundance of each species identified during the study period, as well as determining Shannon-Wiener diversity index (H) and Simpson's dominance index (C) for the area. Shannon-Wiener index (H) was used in calculating *t* (Ogbeibu, 2005) to test for significant differences in diversity and dominance of mosquito species.

RESULTS AND DISCUSSION

Five mosquito genera sampled from the four irrigated millet and guinea-corn fields were *Anopheles, Aedes, Culex, Mansonia* and *Psorophora*. The 18 species from different fauna as well as from different irrigation fields are shown in Table 1. Five species of *Anopheles*, four of *Culex*, seven of *Aedes*, each of *Mansonia* and *Psorophora* were encountered in the study area. *A. gambiae complex, A. funestus complex, A. aegypti, A. albopictus* and *A. taylori* were found in puddles; *A. albopictus* was found in hoof-prints while *A. gambiae complex, A. funestus complex, A. coustani, C. quinquefasciatus, A. aegypti, A. africanus* and *Psorophora* spp., were found in bed pools during the rainy season which lasted for a short period of time. All mosquito vector species encountered in the study were found in the irrigation ditches while only *Psorophora*, was not found in millet and guinea corn fields. Gezawa-1 contributed about 31.2 % of all the species collected, while Gezawa-2, Ketawa and Jogana contributed 24.8%, 22.8% and 21.2 % respectively. From Table 1, it could be observed that *A. gambiae complex* contributed 20.7 %, *A. funestus complex* (7.0 %), *A. pharoensis* (3.7 %), *A. rhodesiensis* (2.1 %), *A. coustani* (3.3%), *C. quinquefasciatus* (11.8 %), *C. pipiens pipiens* (5.7%), *C. pipiens fatigans* (9.0 %), *C. tigripes* (5.0%), *A. aegypti* (6.9 %), *A. albopictus* (6.65), *A. simpsoni* (1.9%), *A. africanus* (3.6 %), *A. taylori* (3.4 %), *A. luteocephalus* (2.9 %), *A. vittatus* (2.8%), *Mansonia* spp., (2.0 %) and *Psorophora* spp., (1.6 %) to the total number of mosquitoes sampled.

During the preliminary surveillance of the irrigated fields, we observed that our head region mostly attracted the mosquitoes, perhaps due to the colour of hair, fragrance of the hair cream, exhaled carbon dioxide and body temperature. This led us to conclude that a combination of visual, olfactory and physical stimuli were effective attractants to man-biting mosquitoes encountered in the irrigated field, and therefore we adopted a black clothed robot, coated with hair cream, as mosquito attractant. In the four crop fields, *A. gambiae complex* was the most frequently encountered species and this could possibly explain the cause of some malaria cases we observed at the Gezawa General Hospital, where administration of ACTs and ITNs was in progress. It has been reported that more of *A. arabiensis* (66.7%) than *A. gambiae s. s.* (6.7%) was present at Yola in the dry-savannah of north-eastern Nigeria (Umaru *et al.,* 2007). However, Service (1993) established that *A. arabiensis* s.s is a dry-savannah zoophilic and anthropophilic as well as exophagic and endophagic mosquito. The *A. gambiae complex* was recovered in the millet and guinea corn fields and water bodies but their presence in puddles, hoof-prints and bed-pools was limited, perhaps as a result of the fast drying-up of water in these artificial habitats. During the peak of the rains the presence of *A. gambiae complex* was prolonged for more than 2 weeks in puddles and bed-pools.

Table 1: Man-biting mosquitoes sampled from Gezawa irrigation Zone, North-central Nigeria

Mosquito species		Mosquito fauna					Individuals of mosquito species collected								Total	
							Gezawa-1		Gezawa-2		Ketawa		Jogana			
		Millet and guinea-corn fields	Irrigation ditches	Puddles	Bed-pools	Hoof-prints	No.	%	No.	%	No.	%	No.	%	No.	%
1	***Anopheles gambiae***	+	+	+	+	-	83	31.1	71	26.6	62	23.2	51	19.1	267	20.7
2	***A. funestus***	+	+	+	+	-	24	26.7	15	16.7	30	33.3	21	23.3	90	7.0
3	***A. pharoensis***	+	+	-	-	-	11	22.9	5	10.4	15	31.3	17	35.4	48	3.7
4	***A. rhodesiensis***	+	+	-	-	-	8	29.6	9	33.3	4	14.8	6	22.2	27	2.1
5	***A. coustani***	+	+	-	+	-	17	40.5	11	26.2	7	16.7	7	16.7	42	3.3
6	***Culex quinqefasciatus***	+	+	-	+	-	57	37.3	40	26.1	31	20.3	25	16.3	153	11.8
7	***C. pipiens pipiens***	+	+	-	-	-	21	28.8	25	34.2	12	16.4	15	20.5	73	5.7
8	***x. pipiens fatigans***	+	+	-	-	-	44	37.9	32	27.6	19	16.4	21	18.1	116	9.0
9	***C. tigripes***	+	+				19	29.7	16	25.0	12	18.7	17	26.6	64	5.0
10	***Aedes aegypti***	+	+	+	+	-	32	35.9	15	16.9	25	28.1	17	19.1	89	6.9
11	***A. albopictus***	+	+	+	-	+	29	34.1	20	23.5	21	24.7	15	17.7	85	6.6
12	***A. simpsoni***	+	+	-	-	-	6	24.0	10	40.0	7	28.0	2	8.0	25	1.9
13	***A. africanus***	+	+	-	+	-	11	23.4	15	32.0	12	25.5	9	19.1	47	3.6
14	***A. taylori***	+	+	+	-	-	9	20.5	7	15.9	11	25.0	17	38.6	44	3.4
15	***A. luteocephalus***	+	+	-	-	-	5	13.2	8	21.0	12	31.6	13	34.2	38	2.9
16	***A. vittatus***	+	+	-	-	-	12	32.4	6	16.2	7	19.0	12	32.4	37	2.8
17	***Mansonia* spp.**	+	+	-	-	-	8	30.8	9	34.6	2	7.7	7	26.9	26	2.0
18	***Psorophora* spp.**	-	+	-	+	-	7	33.3	7	33.3	5	23.8	2	9.5	21	1.6
Σ		17	18	5	7	1	403	31.2	321	24.8	294	22.8	274	21.2	1292	100.0

Table 2: Computations for species diversity and dominance indices for mosquitoes sampled from Gezawa Irrigation Zone, North-central Nigeria

Mosquito species (*S* = 18)		*fi*	*fi log fi*	*fi log² fi*	*Pi*	*(Pi)² or (ni/N)²*	*ni(n-1)/N(N-1)*	*Pi log Pi*	*Pi In Pi*	*Pi (In Pi)²*	Shannon-Wiener diversity index	Simpson's dominance index
1	***Anopheles gambiae***	267	647.88	1571.08	0.2067	0.0427	0.0426	-0.1415	-0.3259	0.5137	0.1415	0.0427
2	***A. funestus***	90	175.88	343.72	0.0697	0.0049	0.0048	-0.0806	-0.1856	0.4945	0.0806	0.0049
3	***A. pharoensis***	48	80.70	135.68	0.0372	0.0014	0.0014	-0.0532	-0.1224	0.4030	0.0532	0.0014
4	***A. rhodesiensis***	27	38.65	55.32	0.0209	0.0004	0.0004	-0.0351	-0.0808	0.3127	0.0351	0.0004
5	***A. coustani***	42	68.18	110.67	0.0325	0.0011	0.0010	-0.0484	-0.1114	0.3816	0.0484	0.0011
6	***Culex quinqefasciatus***	153	334.26	730.25	0.1184	0.0140	0.0139	-0.1097	-0.2526	0.5390	0.1097	0.0140
7	***C. pipiens pipiens***	73	136.02	253.45	0.0565	0.0032	0.0032	-0.0705	-0.1624	0.4665	0.0705	0.0032
8	***C. pipiens fatigans***	116	239.48	494.39	0.0898	0.0081	0.0080	-0.0940	-0.2164	0.5216	0.0940	0.0081
9	***C. tigripes***	64	115.60	208.79	0.0495	0.0024	0.0024	-0.0646	-0.1488	0.4472	0.0646	0.0024
10	***Aedes aegypti***	89	173.50	338.21	0.0689	0.0047	0.0047	-0.0800	-0.1843	0.4931	0.0800	0.0047
11	***A. albopictus***	85	164.00	307.67	0.0658	0.0043	0.0043	-0.0778	-0.1791	0.4872	0.0778	0.0043
12	***A. simpsoni***	25	34.95	48.86	0.0193	0.0004	0.0004	-0.0331	-0.0762	0.3008	0.0331	0.0004
13	***A. africanus***	47	78.59	131.41	0.0364	0.0013	0.0013	-0.0524	-0.1206	0.3996	0.0524	0.0013
14	***A. taylori***	44	72.31	118.84	0.0341	0.0012	0.0011	-0.0500	-0.1152	0.3892	0.0500	0.0012
15	***A. luteocephalus***	38	60.03	94.84	0.0294	0.0009	0.0008	-0.0450	-0.1037	0.3657	0.0450	0.0009
16	***A. vittatus***	37	58.02	90.99	0.0286	0.0008	0.0008	-0.0441	-0.1016	0.3613	0.0441	0.0008
17	***Mansonia* spp.**	26	36.79	52.06	0.0201	0.0004	0.0004	-0.0341	-0.0785	0.3062	0.0341	0.0004
18	***Psorophora* spp.**	21	27.77	36.71	0.0162	0.0003	0.0003	-0.0290	-0.0668	0.2753	0.0290	0.0003
∑		*N* = 1292	2542.61	5122.94	1.0000	0.0925	0.0918	-1.1431	-2.6323	7.4582	1.1431	0.0925

Table 3: Computations for species diversity and dominance indices for mosquitoes sampled from Gezawa 1 and Gezawa-2 irrigation fields in Gezawa Agricultural Zone, North-central Nigeria

Irrigation field		*fi*	*fi* log *fi*	*fi log*^{2} *fi*	*Pi*	*(Pi)* ^{2} or *(ni/N)* ^{2}	*ni (ni-1)/N (N-1)*	*Pi log Pi*	*Pi In Pi*	*Pi(In Pi)* ^{2}	Shannon-Wiener diversity index H = (*N* log *N* − *Σ fi* log *fi*) / *N* or −*(Pi log Pi)*	Simpson's dominance index C = *Σ* (*ni*/ *N*)^2
Gezawa-1												
1	***Anopheles gambiae***	83	159.28	305.677	0.206	0.04244	0.04201	-0.1413	-0.325	0.5142	0.1413	0.04244
2	***A. funestus***	24	33.12	45.719	0.060	0.00360	0.00341	-0.0733	-0.169	0.4750	0.0733	0.00360
3	***A. pharoensis***	11	11.45	11.929	0.027	0.00073	0.00068	-0.0423	-0.097	0.3522	0.0423	0.00073
4	***A. rhodesiensis***	8	7.22	6.524	0.020	0.00040	0.00035	-0.0339	-0.078	0.3061	0.0339	0.00040
5	***A. coustani***	17	20.92	25.738	0.042	0.00176	0.00168	-0.0578	-0.133	0.4221	0.0578	0.00176
6	***Culex quinqefasciatus***	57	100.08	175.736	0.141	0.01988	0.01970	-0.1199	-0.276	0.5411	0.1199	0.01988
7	***C. pipiens pipiens***	21	27.77	36.713	0.052	0.00270	0.00259	-0.0668	-0.154	0.4545	0.0668	0.00270
8	***x. pipiens fatigans***	44	72.31	118.841	0.110	0.01210	0.01167	-0.1054	-0.243	0.5359	0.1054	0.01210
9	***C. tigripes***	19	24.30	31.069	0.047	0.00221	0.00211	-0.0624	-0.144	0.4394	0.0624	0.00221
10	***Aedes aegypti***	32	48.16	72.495	0.080	0.00640	0.00612	-0.0877	-0.202	0.5103	0.0877	0.00640
11	***A. albopictus***	29	42.41	62.019	0.072	0.00518	0.00501	-0.0823	-0.189	0.4984	0.0823	0.00518
12	***A. simpsoni***	6	4.67	3.633	0.015	0.00022	0.00019	-0.0274	-0.063	0.2646	0.0274	0.00022
13	***A. africanus***	11	11.45	11.929	0.027	0.00073	0.00068	-0.0423	-0.097	0.3522	0.0423	0.00073
14	***A. taylori***	9	8.59	8.195	0.022	0.00048	0.00044	-0.0365	-0.084	0.3205	0.0365	0.00048
15	***A. luteocephalus***	5	3.49	2.443	0.012	0.00014	0.00012	-0.0230	-0.053	0.2347	0.0230	0.00014
16	***A. vittatus***	12	12.95	13.975	0.030	0.00090	0.00081	-0.0457	-0.105	0.3689	0.0457	0.00090
17	***Mansonia* spp.**	8	7.22	6.524	0.020	0.00040	0.00035	-0.0340	-0.078	0.3061	0.0340	0.00040
18	***Psorophora* spp.**	7	5.91	4.999	0.017	0.00029	0.00026	-0.0301	-0.069	0.2822	0.0301	0.00029
Σ		*N* =403	601.30	944.158	1.000	0.10056	0.09818	-1.1121	-2.559	7.1784	1.1121	0.10056
Gezawa-2												
1	***Anopheles gambiae***	71	131.43	248.076	0.221	0.04884	0.04838	-0.1449	-0.334	0.5036	0.1449	0.04884
2	***A. funestus***	15	17.64	20.748	0.047	0.00221	0.00204	-0.0624	-0.144	0.4394	0.0624	0.00221
3	***A. pharoensis***	5	3.49	2.443	0.015	0.00023	0.00019	-0.0274	-0.063	0.2646	0.0274	0.00023
4	***A. rhodesiensis***	9	8.59	8.195	0.028	0.00078	0.00070	-0.0435	-0.100	0.3580	0.0435	0.00078
5	***A. coustani***	11	11.45	11.929	0.034	0.00116	0.00107	-0.0499	-0.115	0.3887	0.0499	0.00116
6	***Culex quinqefasciatus***	40	64.08	102.664	0.125	0.01563	0.01519	-0.1129	-0.260	0.5405	0.1129	0.01563
7	***C. pipiens pipiens***	25	34.95	48.856	0.078	0.00608	0.00584	-0.0864	-0.200	0.5076	0.0864	0.00608
8	***x. pipiens fatigans***	32	48.16	72.495	0.099	0.00980	0.00967	-0.0994	-0.229	0.5295	0.0994	0.00980
9	***C. tigripes***	16	19.27	23.198	0.050	0.00250	0.00234	-0.0650	-0.150	0.4487	0.0650	0.00250
10	***Aedes aegypti***	15	17.64	20.748	0.047	0.00221	0.00204	-0.0624	-0.144	0.4394	0.0624	0.00221
11	***A. albopictus***	20	26.02	33.853	0.062	0.00384	0.00370	-0.0749	-0.172	0.4794	0.0749	0.00384
12	***A. simpsoni***	10	10.00	10.000	0.031	0.00096	0.00087	-0.0468	-0.108	0.3740	0.0468	0.00096
13	***A. africanus***	15	17.64	20.748	0.047	0.00221	0.00204	-0.0624	-0.144	0.4394	0.0624	0.00221
14	***A. taylori***	7	5.91	4.999	0.022	0.00048	0.00041	-0.0365	-0.084	0.3205	0.0365	0.00048
15	***A. luteocephalus***	8	7.22	6.524	0.025	0.00063	0.00055	-0.0400	-0.092	0.3402	0.0400	0.00063
16	***A. vittatus***	6	4.67	3.633	0.019	0.00036	0.00029	-0.0327	-0.075	0.2984	0.0327	0.00036
17	***Mansonia* spp.**	9	8.59	8.195	0.028	0.00078	0.00070	-0.0435	-0.100	0.3580	0.0435	0.00078
18	***Psorophora* spp.**	7	5.91	4.999	0.022	0.00048	0.00041	-0.0365	-0.084	0.3205	0.0365	0.00048
∑		*N* =321	442.66	652.303	1.000	0.09918	0.09643	-1.1275	-2.598	7.3504	1.1275	0.09918

Key: *fi = Abundance of species, N = total number of individuals, Pi = Proportion of individuals found in the ith species, In = the Natural (Naperian) logarithms (log_e), (ni/N)* ^{2} *= (Pi)* ^{2}

Table 4: Computations for species diversity and dominance indices for mosquitoes sampled from Ketawa and Jogana irrigation fields in Gezawa Agricultural Zone, North-central Nigeria

Irrigation field		*fi*	*fi* log *fi*	*fi log*^{2} *fi*	*Pi*	*(Pi)* ^{2} or *(ni/N)* ^{2}	*ni (ni-1)/N (N-1)*	*Pi log Pi*	*Pi In Pi*	*Pi(In Pi)* ^{2}	Shannon-Wiener diversity index H = (*N* log *N* − Σ *fi* log *fi*) / *N* or −*(Pi log Pi)*	Simpson's dominance index C = Σ (*ni*/ *N*)^2
Ketawa												
1	***Anopheles gambiae***	62	111.13	199.185	0.211	0.04452	0.04390	-0.1426	-0.328	0.5108	0.1426	0.04452
2	***A. funestus***	30	44.31	65.457	0.102	0.01040	0.01010	-0.1011	-0.233	0.5315	0.1011	0.01040
3	***A. pharoensis***	15	17.64	20.748	0.051	0.00260	0.00234	-0.0659	-0.152	0.4517	0.0659	0.00260
4	***A. rhodesiensis***	4	2.41	1.450	0.014	0.00020	0.00014	-0.0259	-0.060	0.2551	0.0259	0.00020
5	***A. coustani***	7	5.91	4.999	0.024	0.00058	0.00049	-0.0389	-0.090	0.3338	0.0389	0.00058
6	***Culex quinqefasciatus***	31	46.23	68.949	0.105	0.01103	0.01080	-0.1028	-0.237	0.5333	0.1028	0.01103
7	***C. pipiens pipiens***	12	12.95	13.975	0.041	0.00168	0.00153	-0.0569	-0.131	0.4183	0.0569	0.00168
8	***x. pipiens fatigans***	19	24.30	31.069	0.064	0.00450	0.00397	-0.0764	-0.176	0.4836	0.0764	0.00450
9	***C. tigripes***	12	12.95	13.975	0.041	0.00168	0.00153	-0.0569	-0.131	0.4183	0.0569	0.00168
10	***Aedes aegypti***	25	34.95	48.856	0.085	0.00723	0.00697	-0.0910	-0.209	0.5165	0.0910	0.00723
11	***A. albopictus***	21	27.77	36.713	0.071	0.00504	0.00488	-0.0816	-0.188	0.4967	0.0816	0.00504
12	***A. simpsoni***	7	5.91	4.999	0.024	0.00058	0.00049	-0.0389	-0.090	0.3338	0.0389	0.00058
13	***A. africanus***	12	12.95	13.975	0.041	0.00168	0.00153	-0.0569	-0.131	0.4183	0.0569	0.00168
14	***A. taylori***	11	11.45	11.929	0.037	0.00137	0.00128	-0.0530	-0.122	0.4021	0.0530	0.00137
15	***A. luteocephalus***	12	12.95	13.975	0.041	0.00168	0.00153	-0.0569	-0.131	0.4183	0.0569	0.00168
16	***A. vittatus***	7	5.91	4.777	0.024	0.00058	0.00049	-0.0389	-0.090	0.3338	0.0389	0.00058
17	***Mansonia* spp.**	2	0.60	0.181	0.007	0.00005	0.00002	-0.0151	-0.035	0.1723	0.0151	0.00005
18	***Psorophora* spp.**	5	3.49	2.443	0.017	0.00029	0.00023	-0.0310	-0.069	0.2822	0.0310	0.00029
Σ		*N* =294	393.81	557.655	1.000	0.09569	0.09222	-1.1307	-2.603	7.3104	1.1307	0.09569
Jogana												
1	***Anopheles gambiae***	51	87.09	148.705	0.186	0.03460	0.03409	-0.1359	-0.313	0.5262	0.1359	0.03460
2	***A. funestus***	21	27.77	36.713	0.077	0.00005	0.00561	-0.0857	-0.197	0.5062	0.0857	0.00005
3	***A. pharoensis***	17	20.92	25.738	0.062	0.00384	0.00364	-0.0749	-0.172	0.4794	0.0749	0.00384
4	***A. rhodesiensis***	6	4.67	3.633	0.022	0.00048	0.00040	-0.0365	-0.084	0.3204	0.0365	0.00048
5	***A. coustani***	7	5.91	4.777	0.025	0.00063	0.00056	-0.0400	-0.092	0.3402	0.0400	0.00063
6	***Culex quinqefasciatus***	25	34.95	48.822	0.091	0.00828	0.00802	-0.0947	-0.218	0.5228	0.0947	0.00828
7	***C. pipiens pipiens***	15	17.64	20.748	0.055	0.00303	0.00281	-0.0693	-0.159	0.4627	0.0693	0.00303
8	***x. pipiens fatigans***	21	27.77	36.713	0.077	0.00593	0.00561	-0.0857	-0.197	0.5062	0.0857	0.00593
9	***C. tigripes***	17	20.92	25.738	0.062	0.00384	0.00364	-0.0749	-0.172	0.4794	0.0749	0.00384
10	***Aedes aegypti***	17	20.92	25.738	0.062	0.00384	0.00364	-0.0749	-0.172	0.4794	0.0749	0.00384
11	***A. albopictus***	15	17.64	20.748	0.055	0.00303	0.00281	-0.0693	-0.159	0.4627	0.0693	0.00303
12	***A. simpsoni***	2	0.60	0.181	0.007	0.00005	0.00003	-0.0151	-0.035	0.1723	0.0151	0.00005
13	***A. africanus***	9	8.59	8.195	0.033	0.00109	0.00096	-0.0489	-0.113	0.3840	0.0489	0.00109
14	***A. taylori***	17	20.92	25.738	0.062	0.00384	0.00364	-0.0749	-0.172	0.4794	0.0749	0.00384
15	***A. luteocephalus***	13	14.48	16.131	0.048	0.00230	0.00209	-0.0633	-0.146	0.4426	0.0633	0.00230
16	***A. vittatus***	12	12.95	12.975	0.044	0.00194	0.00176	-0.0597	-0.137	0.4293	0.0597	0.00194
17	***Mansonia* spp.**	7	5.91	4.777	0.025	0.00063	0.00056	-0.0400	-0.092	0.3402	0.0400	0.00063
18	***Psorophora* spp.**	2	0.60	0.181	0.007	0.00005	0.00003	-0.0151	-0.035	0.1723	0.0151	0.00005
Σ		*N* =274	350.25	466.251	1.000	0.07745	0.07990	-1.1588	-2.665	7.5057	1.1588	0.07745

Key: *fi = Abundance of species, N = total number of individuals, Pi = Proportion of individuals found in the ith species, In = the Natural (Naperian) logarithms (log_e), (ni/N)* ^{2} *= (Pi)* ^{2}

A. funestus complex (7.0 %) was sparse in the field, except the irrigation ditches, during the dry season. *An. funestus* had earlier been incriminated as the major malaria vector in the savannah areas in Nigeria (Service, 1963). This is in contrast with our result but we opine that the phenomenon of 'climate change' might be a common factor responsible for the observed variation. Faye (1995) reported that reduced precipitation and drought adversely affected the population dynamics of *A. funestus*. The Presence of *C. quinquefasciatus, C. fatigans, A. aegypti* and *A. albopictus* is worrisome, as these have been earlier identified as potential vectors of yellow fever in Nigeria (Bang, *et al*., 1980; Bang, *et al*., 1981). It is important to note that these mosquito vectors were recovered in both the millet and guinea corn fields and from irrigation ditches throughout the year; therefore their public health significance need not be overemphasized. The study incorporated the surveillance of irrigation ditches because each millet and guinea corn field was juxtaposed to an irrigation ditch where some other minor crops were grown. All the mosquitoes encountered in this study were found breeding in the irrigation ditches, an indication that the over 70% of farmers who carry out more than 80% of their daily activities in the fields were exposed to the risk of mosquito bites. Computations used for diversity and dominance indices for Gezawa Irrigation Zone; Gezawa-1 and Gezawa-2; Ketawa and Jogana irrigation fields are shown in Tables 2, 3 and 4 respectively. These Tables also summarized the diversity and dominance indices for Gezawa as a whole, and each of the four irrigation fields, respectively. A Shannon-Wiener and Simpson's diversity values of 1.1431 and 0.0925 were recorded for the mosquito species in Gezawa Agricultural Zone. *A. gambiae* had the highest frequency of occurrence followed by *C. quinquefasciatus*. *A. gambiae* had the highest Shannon-wiener diversity and Simpson's dominance indices of 0.1415 and 0.0427 respectively. Shannon-Wiener diversity and Simpson's dominance indices for Gezawa-1 (1.1121, 0.1006), Gezawa-2 (1.1275, 0.0992), Ketawa (1.1307, 0.0957) and Jogana (1.1588, 0.0775) were recorded in this study. There was a close similarity in the respective diversity and dominance indices in the four irrigated fields. Statistical *t*-test was used to confirmed no significant difference in the diversity and dominance indices between mosquito samples from the four irrigation fields (P>0.001). Since the presence of these vectors is of apparent danger to public health, much attention should therefore be targeted at vector control in the irrigation fields to complement the on-going provision of ACTs and ITNs in the farming communities of Gezawa LGA.

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EFFECTS OF HYDROGEN ION CONCENTRATION AND SALINITY ON THE SURVIVAL OF JUVENILE *Clarias gariepinus* (BURCHEL, 1822)

IYAJI, Florence Oyibo
Department of Science Laboratory Technology, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria.

ABSTRACT

Experiments were conducted to determine the effects of the interactions of two environmental parameters, pH and salinity on the survival of juvenile Clarias gariepinus (4 – 12 g) under laboratory conditions. The pH values used were 3, 3.5, 4, 7 and 10 while salinity values varied thus: 0, 5, 10, 15 and 20 ppt in each of the pH values. Three and 3.5 pH had lethal effects in all salinities. The corresponding Lt_{50} values obtained at 5 and 10 ppt were higher than those obtained at 0, 15 and 20 ppt. At 4 and 7 pH, mortalities occurred only in the higher salinities, 15 and 20 ppt. At 10 pH, Lt_{50} values at salinities 10, 15 and 20 ppt were considerably low. The lower LT_{50} values recorded at 3, 3.5 and 10 pH with the higher salinity values indicated the lethal effects of the interactions of both parameters on fish.

Keywords: Hydrogen ion concentration, Salinity, Survival, *Clarias gariepinus*

INTRODUCTION

Aquatic organisms are directly affected by the physicochemical parameters of their environment. Boyd (1979), working on water quality in warm water aquaculture stated that aquatic organisms live in an environment foreign to man and consequently, one which is poorly understood. The development of high intensity fish culture which has lagged considerably behind that of the land animals like poultry and cattle may be attributed to lack of understanding. According to Kutty (1979) information on the various levels of environmental parameters and their interactions within the system is needed in formulating culture practices. Fry (1971) summarized the effects of environmental factors and grouped them into five categories namely; lethal, controlling, limiting, masking and corrective. The lethal factors restrict the range of the environment in which the organism can exist, beyond which metabolism is destroyed. Controlling and limiting factors both govern metabolic rates while masking and directive factors are exploited by the organism to maintain its being through organic regulation. Quite often, during the extreme fluctuations of a factor, the organism is exposed to the lethal factor for a short time and the capacity at which it resists the inclement condition would decide its survival. The correct information on the interaction of the parameters on culturable fish species would lead to effective utilization of the vast coastal swamplands, estuaries and lagoons with productivity comparable to that of the sea and inland lakes (Denyoh, 1967; Ezenwa and Ayinla, 1994).

Various studies carried out on the effect of pH and salinity on fish include those of Swingle (1961), Carter(1964), Jordan and Lloyd (1964), Parry *et al.* (1968), Calabrase (1969), Clay (1977), Chervinski (1983), Riiri (1986), Wilkie and Wood (1994; 1996), Norm (2001), Saha *et al* (2002), Fashina-Bombata and Busari (2003). Carter (1964) acclimated brown trout to full strength sea water (salinity 35ppt) and exposed them to alkaline solutions; a pH value of 9.5 gave a median lethal period of 20 hrs whereas fish at a pH of 9.5 at lower salinity value survived more than 5 days. Further investigation by Jordan and Lloyd (1964), confirmed that survival times of fish in lethal alkaline solutions were considerably less than those of rainbow trout in freshwater at similar pH values. Parry *et al.* (1968), experimenting with channel catfish, *Ictaluturus punctatus* juveniles in brackish water ponds (pH 3.5 - 4.5), demonstrated the species survival and growth in salinities ranging from 2 ppt to 11 ppt. Salinity tolerance limit of the catfish age six months to one year in similar pH values was found to be 12 ppt.

Aleem (1987) listed some stenohaline fresh water species like *Tilapia zillii, Oreochromis niloticus* and *Clarias gariepinus* as potential culture species in saline waters because of their ability to respond favourably in brackish waters with their characteristic low pH values. The possibility of the culture of *Clarias gariepinus* in brackish water environment has not been fully explored in Nigeria with numerous costal systems and enormous potential for aquaculture (Ezenwa and Ayinla, 1994). It has been reported that Nigeria spent $ 267,156,521 to import 356,217 metric tones of fish between 1993 and 1999 (FDF, 2000). The successful acclimation of the stenohaline fish to brackish water environment will significantly increase fish production and reduce importation and thereby help conserve resources.

In this work, attempts were made to study the effects of various levels of pH/Salinity interactions on the survival of juveniles of *Clarias gariepinus*. This is with a view to determining the safe levels of these parameters for the introduction of this fish species in the coastal brackish water environment for aquaculture production, not only in Nigeria but also in other parts of the world.

MATERIALS AND METHODS

Fish: Juvenile *Clarias gariepinus* (size range: 4 – 12 g, 9 – 15 cm total length) were bred and raised in the hatchery at African Regional Aquaculture centre,

(ARAC) Aluu, Port Harcourt. The fish were fed with a mixture of powdered milk and ground shrimps in addition to NIOMR formulated feed (35 % protein) twice daily at 5 % body weight for two months before being used in the experiments. Feeding stopped 24 hours before the experiment.

Water: Saline water was collected from the open river at Buguma, River State, Nigeria (S=18 – 25 ppt). To obtain the required salinities, the saline water was mixed with fresh water and the values checked with a salinometer. To obtain the required pH, sulphuric acid or sodium hydroxide solutions were added gradually dropwise to water medium while the values were read using a pH meter model 191 CP-20 digital. Dissolved Oxygen (DO) was measured using the oxygen meter YS1 model 51B while Ammonia ($N\text{-}NH_3$) was measured using a spectrophotometer (Bausch and Lomb spectronic mini 20) at 410 nm wavelength. Temperature was measured using mercury in glass thermometer.

Experiment: Ten litre plastic troughs containing six litres of water were used. These were continuously aerated to keep the dissolved oxygen between 6 – 7 mg/l. The pH was constant at 3, 3.5, 4, 7 and 10 while salinity varied between 0 ppt and 20ppt in each pH value. Six juveniles of *Clarias gariepinus* were introduced into each trough. Observations were made between the time of fish introduction to a maximum period of one week, after which the experiments were terminated. Mortality was recorded at the exact period it occurred. Fish were considered dead when the opercula movement stopped and they failed to respond to touch stimuli. The methods followed were as described by Fry (1972), Kutty (1979) and Alabaster and Lloyd (1982).

RESULTS AND DISCUSSION

Table 1 summarizes the details of Lt_{50}'s obtained from arithmetic, probit and geometric mean calculations of time to death of fish exposed to various pH/salinity concentrations. The Lt_{50} obtained increased as the salinity decreased. At the lower pH values of 3, 3.5, Lt_{50} obtained at 0 ppt were considerably less than those obtained in 10 ppt and 5ppt. In 4 and 7 pH, complete mortalities were recorded only at higher salinities of 15 ppt and 20 ppt, and at pH 10, mortality occurred at 10 ppt salinity. At 0 ppt, no mortality was recorded in each case. At pH 10, the Lt_{50} obtained at higher salinities were less than those in 4 and 7 pH.

In all pH tested, complete mortalities were observed in salinities of 15 ppt and 20 ppt. Table 1 summarized the relationship between Lt_{50} and pH values in various salinity concentrations and between salinity and Lt_{50} in different pH values respectively.

Dissolved oxygen (DO) concentration ranged between 6 - 7.5 mg/l. Mean ammonia ($N\text{-}NH_3$) concentration was 2.0 ± 0.0036mg/l while Temperature ranged between 24.8 - 27.3 ºC. The series of experiments on the effects of pH and salinity combinations on the survival of *Clarias gariepinus* juveniles revealed that pH 3, 3.5 and 10 were lethal to fish in all salinity concentrations. This observation is in line with those of Brett (1979); Holmes and Donaldson (1969) who attributed the death of fish to the destruction of the gills and skin tissues by the lethal effects of pH. These tissues are responsible for the processes of osmoregulation and active ion exchange in fish.

The Lt_{50} obtained at pH 3, 3.5 at 5 ppt and 10ppt were considerably higher than those obtained in the same pH values at 0ppt as shown in Table 1. According to Roberts (1981) the body salt concentration of teleost fish is about 7.5 %. The saline concentrations in which the fish were exposed are close to the body salt concentration. Roberts (1981) further explained that bronchial ionic uptake in fish increases with the external salt concentration up to a point when the mechanism becomes saturated. Fish in 5 ppt and 10ppt salinities could be said to be in the saturated range. Thus, although the tissues were destroyed, fish did not expend energy in maintaining body salt concentration, the saline solution being about the same with the body salt concentration (Norm, 2001), hence their longer survival rate.

The lower Lt_{50} obtained at 0ppt could also be explained in the light of failure of the affected tissues to regulate body salt-water balance. The fresh water fish have higher body salt concentration than the surrounding water environment which they maintain through osmoregulation. The direct transfer of stenohaline freshwater fish into acid/alkaline or saline water elicits a stress response and the extent of the response depends on many variables such as season, temperature, saline concentration, acid/alkaline concentrations, species and size of fish (Swann, 1999). The major problem faced in 0ppt is that of excess water intake. As fish strife to regulate and maintain the body salt/water balance, much energy is expended leading to weakness and early death.

At pH values of 4 and 7 mortality observed at 15 ppt and 20ppt could be due to effects of salinity alone as fish did not die at these pH values even at 0ppt.

At pH 10, in higher salinities of 15ppt and 20ppt, the Lt_{50} obtained were less than those in pH 4 and 7 in the same salinity values. The low Lt_{50} at pH 10 in higher salinities was in line with Carter, (1964) who reported that trout acclimated to full strength seawater and exposed to high pH values had less survival rate than those found in freshwater. According to Saha *et al.* (2002), *Clarias batrachus* survived in alkaline water of pH 10 for several days in freshwater environment. Saha *et al.* (2002) further explained that the restriction of oxygen uptake due to pathological changes at the gill lamellae was the cause of death in freshwater teleost exposed to varying alkaline pH water in higher salinities. Wilkie and Wood (1994; 1996) also suggested that the acute increase in plasma pH, possibly due to disturbances of acid-base balance at higher salinities could be the primary cause of death of fish in higher pH waters.

Table 1: Median lethal time (LT_{50}) of juvenile *Clarias gariepinus* (4 – 12 g) exposed to various pH/salinity concentrations, taken from arithmetic plots, probit plots and geometric mean calculations

pH Values	Salinity Values														
	20 ppt			15 ppt			10 ppt			5 ppt			0 ppt		
	LT_{50}	GM	P	LT_{50}	GM	P	LT_{50}	GM	P	LT_{50}	GM	P	LT_{50}	GM	P
pH3	31	32.50	31.6	50	51	46.7	219	219.4	218	260	265	263	46	46	49
pH3.5	33	34.80	33.1	58	59	57.5	750	760	758.57	1000.9	1116.8	1000	168	173	169
pH4	39	40	39	424	425	416	ND	ND	ND	ND	ND	ND	ND	ND	ND
pH7	46.77	48	47.35	385	388	380	ND	ND	ND	ND	ND	ND	ND	ND	ND
pH10	19.95	21	21.8	30	30	31	63	63.3	60.25	ND	ND	ND	ND	ND	ND

Key: *Lt_{50} Median lethal time, P: Probit mean, GM: Geometric mean*

The mode of toxicity of hydroxyl ion concentration is similar to those of hydrogen ion concentration. Apart from destroying the gill and skin tissues of the fish as earlier mentioned, both also affect the oxygen carrying capacity of the blood haemoglobin (Lagler *et al.*, 1977).

From the results obtained and the foregoing discussion, it is important to note that at lower pH levels of 3.5 -4, salinity must be between 5-8ppt for the survival and growth of the juveniles of *Clarias gariepinus* since this range is within the body salt concentration. Fresh water of 0ppt and higher salinities of 10ppt and above would be lethal to fish at such pH levels. More investigations need to be carried out on the interaction of pH levels and salinity concentrations at closer ranges and on graded fish in order to establish the actual safe levels/concentrations for the different sizes of the fish species.

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PREVALENCE OF URINARY TRACT INFECTIONS (UTI) IN SEXUALLY ACTIVE WOMEN OF ABAKALIKI, EBONYI STATE, NIGERIA

ANI, Ogonna Christiana and MGBECHI, Edna Kelechukwu
Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Corresponding Author: Ani, O. C. Applied Biology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Phone +234 8076688913

ABSTRACT

A research to investigate the prevalence of urinary tract infections in sexually active women (18 – 41 years) from selected health care centres in Abakaliki was carried out. Attempt was made to fined out the number of treated cases, aetiologic agents and age range with highest incidence of urinary tract infections over the study period (2004 – 2005). Medical records of urinary tract infected women from the selected health care centres were reviewed. The prevalence of urinary tract infections was high (1232) among the study group and Escherichia coli was implicated as the principal causative agent of these infections. The high prevalence recorded in this study makes it necessary for women to be adequately educated on matters affecting their reproductive health. There is also the need for government of the state to provide improved, adequate and affordable health care services in the communities.

Keyword: Prevalence, Urinary, Tract infection, Sexually active women

INTRODUCTION

Urinary tract infection (UTI) is the infection of any part of the urinary tract. The urinary tract consists of the kidneys, ureters, bladder and urethra. Any part of these structures can become infected but bladder and urethra infections are the most common (Anon, 2006). The bladder infection is known as cystitis while that of the urethra is known as pyelonephritis and is more serious.

The two types of UTI are lower UTI which is an infection of the lower part of the urinary tract (the bladder and urethra) and upper UTI which is an infection of the upper part of the urinary tract (kidneys and ureters). The upper UTI is potentially more serious than the lower one because there is a possibility of kidney damage.

Most UTIs are caused by bacteria that can live in the digestive tract, the vagina or around the urethra. Infection occurs when bacteria enter the normally sterile urinary system and multiply there. They produce enzymes which help them feed on tissues of the host and thus damage them (Adult Helath Advisor, 2005).

Bacteria can enter the urinary system through the urethra or more rarely through the blood stream (Macnair, 2006). Poor toilet habits, pregnancy in women and prostate enlargement in men can predispose one to infection. The aetiologic and clinical presentation of UTI is similar in both industrialized and developing nations but the range of infections varies form place to place (Latif, 2004). Women are more prone to UTIs than males.

According to Macnair (2006), about 11 % of girls and 4% of boys have UTI before they reach the age of 16. About 40 – 50 % of women and 12 % of men have UTI at some time of their lives. It is believed that sexual activity may be responsible for high infection rate in sexually active women as the problem begins once the women become sexually active. Mercola (2001) also reported that as many as 60 % of women contact UTI at some point in their live and that at least a third of the women with UTI will experience a recurrent infection during the following year. UTIs are most common in sexually active women and increase in people living with diabetes. A common cause of UTI is an increase in sexual activity such as vigorous sexual intercourse with a new partner. This leads to what is known as honeymoon cystitis. Women are more prone to UTI than males because their urethra is much shorter and closer to the anus than in males. Hence bacteria from the anus can pass easily into the urinary tract. Females also have three openings in a very small area (the rectum, the vagina and the meatus of the urethra). Women also lack the bacteriostatic properties of prostatic secretions that are present in males. They are also susceptible to recurrent UTIs because they do not secrete a certain blood group antigens (Thompson, 2006).

Some common symptoms of UTI are frequent urinations, frequent urge to urinate, pain/burning sensation in urethra when urinating, discomfort at lower abdomen, soreness in the lower abdomen and back. When the infection is well developed and had spread up to the kidney and uterus, back pains, chills, fever, nausea and vomiting may be experienced (Janice, 2006). The infection can be diagnosed by testing clean urine sample for white blood cells and other components. Urine may also be cultured to allow the growth of any bacteria and subsequent identification. Traditional treatment is with the use of antibiotics.

This research was carried out in order to find out the real situation of urinary tract infections among sexually active women in Abakaliki with the aim of determining the number of recorded cases in notable hospitals and private diagnostic laboratories

in the town, the aetiologic agents among sexually active women and the age class or age with the highest incidence.

MATERIALS AND METHODS

Study Area: Abakaliki is the capital of Ebonyi State in the South – eastern part of Nigeria. It is densely populated and relatively developed with such basic amenities as pipe borne water, electricity, transport and communication services. The populace is made up of farmers, civil servants, businessmen and women, students and politicians. Notable hospitals that serve most populace within and around the town are Ebonyi State University Teaching Hospital (EBSUTH), Federal Medical Centre (FMC), General and private hospitals. Private diagnostic laboratories such as St. Luke's and Anchor – C render good health services to the populace.

Study Population: The study population includes women between 18 and 41 years within the study area. The study period of two years was undertaken to provide sufficient data for meaningful analysis and minimize confounding variables that may arise due to changing practices over time.

Data Collection: The study involved the review of medical records of the study population using their admission and discharge registers, clinic and laboratory records with the consent of laboratory scientists and nurses on duty. Such records contained the rate of infection, the ages of women infected and causal organisms implicated.

Data Analysis: The results of the research were analyzed using Measures of Central Tendencies to find the age/age class that had highest infection (modal age).

RESULTS

A total number of 1232 cases were treated over the years and the year 2005 had a higher prevalence of 696 (56%) (Table 1). Among the hospitals and laboratories, Federal Medical Centre recorded the highest prevalence of 552 (44.8 %) while Ebonyi State University Teaching Hospital recorded the least prevalence of 143(12.4 %) (Table 1). The modal class was 18 – 23 which recorded 38 % of the UTI cases and the modal age was 22 years. This was followed by age class 24 – 29 which had 20.2 %. 30 – 35 that had 18.8 % and the age class 36 – 41 years had the least (12.1 %) (Table 2). Cumulatively, January – March period had the highest number of cases (396) followed by April – June (335), October – December (269) while July – September quarter had the least (132) (Figure 1). The order of prevalence rate of causal organisms of UTI was *E. coli* (67.2 %) > *Pseudomonas* spp (12.1 %) > *Staphylococcus aureus* (1.2 %) *Klebsiella* spp (3.7 %) > *Schistosoma haematobium* (2.9 %) > Amoeba spp (2.1 %) > *Streptococcus faecalis* (0.5 %) > *Blastomyces* spp (0.3 %) (Figure 2).

Table 1: Prevalence of UTI among different hospitals/establishments from Jan 2004 – December 2005 in Abakaliki, Ebonyi State

Hospitals/ establishment	cases in 2004	cases in 2005	Total cases	% cases
Federal Medical Centre (FMC)	207	345	552	44.8
Ebonyi State University Teach Hospital	67	76	143	12.0
St. Luke's Private diagnostic laboratory	91	144	235	19.1
Anchor – C. Private diagnostic Laboratory	171	131	302	24.4
Total	533 (43%)	696 (56%)	1232	

Table 2: Prevalence of UTI by age among sexually active Women in Abakaliki, Ebonyi State

Age class	Year 2004	Year 2005	Total case of infection	Prevalence
18 – 23	226	253	479	38.9
24 – 29	166	206	372	30.2
30 – 35	76	155	231	18.8
36 – 41	68	82	150	12.1
Total	596	696	1232	100

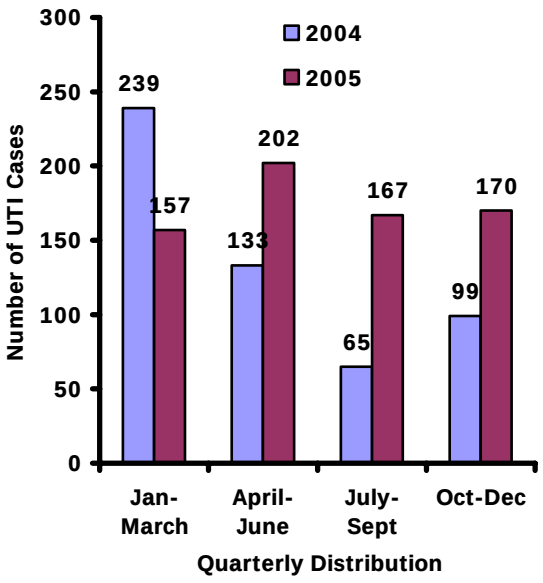

Figure 1: Quarterly Prevalence of UTI Among Sexually Active Women in Abakaliki, Ebonyi State

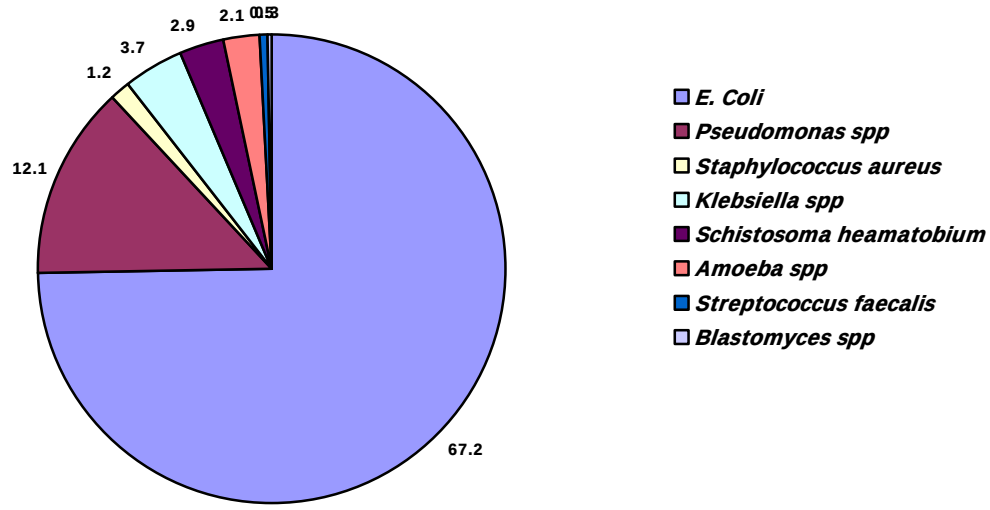

Figure 2: Casual organisms implicated by laboratory findings

DISCUSSION

One thousand two hundred and thirty – two urinary tract infection cases were treated in the study area in two years under review.

The prevalence of this infection in this study was quite high and this agrees with the report of Chevins (2001) that this infection was a common medical complaint among women in their sexually active years. The age class 18 – 23 had the highest prevent rate of 38.9 %. At this age range, women/girls tend to live active sexual life and promiscuity is sometimes on the increase. This also agreed with APUA (2003) that women at the peak of their sexual active years had high prevalence of urinary tract infection. Women at this age range also feel ashamed of going for medical check – ups and therefore indulge in one form of self medication or the other or even prefer to die in silence (Ehinmidu, 2003).

The year 2005 had a higher rate of infection (56.5 %) than 2004. This could be attributed to the fact that literacy level and health awareness of the populace/masses keep improving year after year. As this happens, more women get aware of their health needs, become bolder to face their problems and hence pay more visits to the hospitals and private laboratories for accurate diagnosis and treatment more than the preceding years.

The overall prevalence of UTIs in the years under review was highest in the January – March quarter (396). The major occupation of the people of the study area is farming and October – December quarter is a time of harvest. The farmers therefore tend to concentrate on harvesting and selling of their products neglecting their health needs. By the first quarter of the year when such activities may have lessened, they then think of taking care of their health needs. This was in consonance with Latif (2004) that people in developing countries often present themselves for care with more severe illness and often after complications have developed.

The order of prevalence of causal organisms in this study (*E. coli*> *Pseudomonas spp* > *Staphylococcus aureus* etc) contrasted the report of Ehinmidu (2003) of *(Pseudomonas aeruginosa* > *Staphylococcus aureus* > *E. coli*) although the same organisms were identified. The result, however agreed with Chevins (2001), Jawetz and Adelberg (2001) and Nyberg *et al* (2004) that *E. coli* was the primary causal agent of most urinary tract infections.

From the findings of this research, it was evident that urinary tract infection was prevalent in sexually active women. On the basis of this therefore, there is need for improved, adequate and affordable health care services in the communities especially in matters of reproductive health. Promiscuity and girl – child marriage should be discouraged while masses should be educated on the importance of personal hygiene in order to help them elevate their health status and manage themselves properly.

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ISOLATION AND CHARACTERIZATION OF *Pasteurella multocida* FROM CAPRINE PNEUMONIC LUNGS

UGOCHUKWU, Emman Ikenna
Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. Email: emmaugochukwu@yahoo.com
Phone: +234 7039071914

ABSTRACT

By conventional microbiological methods, investigation was carried out in Nsukka and Enugu areas of Enugu State, Nigeria to determine the percentage frequency of occurrence of Pasteurella multocida from caprine pneumonic lungs in Nsukka and Enugu areas of Enugu State, Nigeria. In this study that spanned 12 years, a total of 350 pneumonic lung samples were collected from West African Dwarf goats, Sokoto Red goats and Fulani goats slaughtered in Nsukka and Enugu Municipal abattoirs in Enugu State. By cultural, biochemical and physiological attributes, four (4) of the isolates were characterized as Pasteurella multocida Both gross and histopathological lesions of the pneumonic lung specimens from which this aerobic bacterium was isolated were correlated with the organism. Inspite of the low percentage frequency (1.14%) of isolation of Pasteurella multocida in this study, attention is drawn to the pathogenic potential of this organism for goats and other livestock in this part of Nigeria.

Keyword: Isolation, Characterization, *Pasteurella multocida*, Pneumonic, Caprine

INTRODUCTION

One of the major limiting factors in the successful rearing of goats and sheep in Nigeria is disease. This is rather disturbing since, goats contributes 40% of the meat consumed in Nigeria (Ademsun, 1986) and is said to adapt physiologically to harsh environmental conditions (Silanikove, 2000) of tropical forest belt of West Africa (Olapade and Onwuka, 2006).

Endoparasitism and respiratory diseases have been incriminated as being responsible for most of the morbidity and mortality in small ruminants (Ojo, 1971; Ojo, 1976; Ikede, 1977; Ikede, 1978; Okoh and Kaldras, 1980). Bacteriological studies on the occurrence and distribution of bacteria and mycoplasma in the pulmonary lesions of goats and sheep in Western Northern Nigeria have shown high frequency of occurrence of *Pasteurella hemolitica, multocida, soaphylococcus* species and *Mycoplasma arginini* (Ikede, 1977; Falade *et al.,* 1977; Ikede, 1978). The finding of Ikede (1977) in University of Ibadan farm confirmed that respiratory diseases were the major cause of death in farmed sheep and goats.

Not only was animal performance greatly affected in terms of poor growth, reduced productivity, debility and lowered resistance (Abubakar *et al.*, 1980) but also caprine pneumonia is globally a threat to the goat industry due to high mortality.

Worse still, concomitant secondary bacterial infection has been found to complicate a fatal viral disease of sheep and goats in West Africa sub region known as *Peste des Petit Ruminants,* (Ugochukwu, 1983).

In this investigation, the isolation, culture and identification of *P. multocida*, a potentially pathogenic aerobic bacterium and their possible role in the pathogenesis of caprine pneumonia are detailed.

MATERIALS AND METHODS

Animals: Animals used for this investigation were goats mainly of West African Dwarf breed, Sokoto Red and Fulani breeds slaughtered at Nsukka and Enugu Municipal abattoirs in Enugu State, Nigeria.

Sampling: Pneumonic lung samples were randomly collected from goats suffering from different clinical respiratory diseases. A total of 350 lungs were examined for pathological changes and all pneumonic lung specimens were aseptically collected in Bijou bottles with a pair of flamed thumb forceps and a pair of flamed scissors both dipped in methylated spirit. Each specimen was carefully numbered and labelled.

Microbial Studies: With a wire loop, an inoculum was obtained from the specimen bottle containing the pneumonic lung sample; a wet mount was made on a dry clean slide and examined under the microscope for the presence of aerobic bacterial organisms. Furthermore, the inoculum obtained from the pneumonic lung samples was streaked over blood and chocolate agar plates. The inoculated blood agar plates were incubated at 37^{O}C for 24 hours in a glass jar using candle flame to reduce the degree of aerobiosis. The two objectives of this primary inoculation were, first to cultivate the organisms and secondarily to obtain discrete colonies. After 24 hours of aerobic incubation, the plates were brought out and the type and amount of growth of colonies were studied. The size, shape, colour and character of the colonies were recorded. Subsequently, the colonies were stained using Grain-stain technique and examined for microscopic morphology.

The following physiological, enzymatic and biochemical tests were conducted on each blood agar plates using standard bacteriological techniques. These tests include: Motility test, growth in nutrient

agar, growth in McConkey agar, growth under increased CO_2 tension, growth under anaerobic condition, catalase test, oxidase test, indole production test, hydrogen sulphide production, urease production, Nitrate reduction test, Oxidation-Fermentation (O – F) test and Sugar test in which glucose, lactose, sucrose or mannitol were incorporated separately.

RESULTS

Lung specimen from which *P. multocida* was isolated did not show significant histopathological changes except for the cellular infiltration involving mainly the neutrophils.

From physiological and biochemical tests, four out of a total of 153 aerobic bacteria isolates were characterized to be *P. multocida.* No haemolytic species was recovered out of the 350 pneumonic lung examined bacteriaologically, only (1.14% were positive for *P. multocida* (Figure 1). Physiological and biochemical characteristics of *P. multocida* isolated from caprine pneumonic lungs are shown on Table 1.

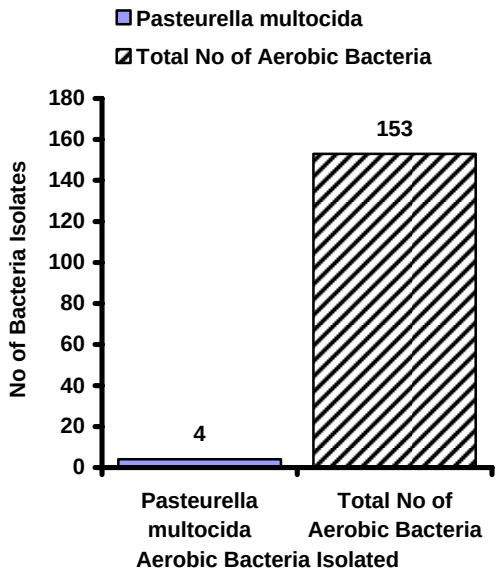

Figure 1: Prevalence of Pasteurella multocida from caprine pneumonic lungs

DISCUSSION

Previous investigations in Northern and Western parts of Nigeria led to successful isolation of *Staphylococcus aureus, S. epidermidis, Escherichia coli, Proteus species, Pseudomonas aeruginosa, Streptococcus species, Corynebacterium pyogenes* and *P. haemolytica* from goats (Ojo, 1976: Ikede, 1977).

In this investigation centred on the isolation of Pasteurella from caprine pneumonic lungs, the four species were biochemicallly and physiologically characterized to be *P. multocida*. Incidentally, *P. haemolytica* was not isolated in this study in this part of the country. The failure to isolate *P. haemolytica* in this bacteriologic investigation cannot be effectively explained until further investigation to determine the prevalence of this species in this part of the country has been fully explored.

P. multocida is a potential pathogenic bacteria organism, which has been incriminated, in both human and animal infections where it causes often times severe respiratory abnormalities that can terminate in death (Dritz *et al*., 1996).

The successful isolation of *P. multocida* in this investigation is interesting not only because of its traditional role as a disease-causing aerobic bacteria but also because of its toxigenicity (Hall *et al* 1987; Cheville and Rimler, 1989) making it a highly invasive, pathogenic and virulent microorganisms.

Its ability to initiate infection in different anatomic structures of the body in man as well as in animals has been documented (Dritz *et al*, 1996).

Its toxin has been reported to have deleterious effects on organs systems and immuno-responsiveness (Hall *et al,* 1987; Cheville and Rimler, 1989).

Although the percentage isolation was relatively low (1.15%), the possible role of *P. multocida* in the aetiology and pathogenesis of caprine pneumonia should not be under estimated. Although, it may be found occasionally as a normal inhabitant of the respiratory system, experimental evidence has shown that under certain conditions associated with debilitation, nutrition and climatic factors, this organism may singly or in concert with other organisms flare up to cause severe infections with high morbidity and mortality. The neutrophilic infiltration in pneumonic lung samples from which *P. multocida* was isolated is not characteristic but rather a common finding in bacterial infection.

The isolation of *P. multocida* from caprine pneumonic lungs, in this investigation, is more disturbing in the light of the prevalence of a highly fatal viral disease of goats in this parts of West Africa sub region, since recent works have implicated concomitant secondary bacterial infection in the severity, pathogenesis and pathology of *Pestes Des Petit Ruminants* (PPR) (Ugochukwu, 1983; Ugiochukwu, 1985, Ugochukwu and Agwu, 1991).

However, further scientific work is needed to clearly elucidate the precise role of *Pasteurel;la multocida* and indeed other species of *Pasteurella* in the pathology of caprine pneumonia in this unique geo-climatic region of the world.

Moreover, more research is needed on the risk factors due to cross infection to human population considering the fact that goats are kept in close proximity to human population in cultural setting of this part of Nigeria under bacteriological investigation.

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Table 1: Detailed physiological and biochemical properties of Isolates of *Pasteurella*

Isolate Number	Hemolysis (sheep blood)	Moitlity	Growth in MacConkey agar	Growth in nutrient agar	Growth under increase CO_2	Growth under anaerobic condition	Catalase	Oxidase	Indole production	H_2S production	Urease production	Nitrate reduction	O-F	Acid in sugars: Glucose Lactose	Maltose	Sucrose	Mannitol
1	-	-	-		+	-	+	+	+	-	-	+	F	+	-	+	+
2	-	-	-	+	+	-	+	+	+	-	-	+	F	+	+	+	+
3	-	-	-		+	-	+	+	+	-	-	+	F	+	-	+	+
4	-	-	-		+	-	+	+	+	-	-	+	F	+	+	+	+

Key: + indicates positive reaction; - indicates negative reaction; F indicates that carbohydrate break-down is fermentative

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ANTAGONISTIC EFFECTS OF CEFTRIAXONE AND SULPHADIMIDINE ON KETAMINE AND THIOPENTONE ANAESTHETICS IN NIGERIAN LOCAL DOG

SAGANUWAN, Alhaji Saganuwan
Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, PMB 2373, Makurdi, Benue State, Nigeria.
Email: pharn_saga2006@yahoo.com Phone: +234 8027444269, +234 8026745993

ABSTRACT

Antagonistic effects of ceftriaxone and sulphadimidine on ketamine and thiopentone anaesthetics were studied in Nigerian local dogs. Twenty – four Nigerian local dogs were used for the study. The dogs were divided into six groups: A, B, C, D, E, and F, with four dogs per group. Groups A and B were intravenously administered 17 mg/kg and 20 mg/kg body weight f thiopentone and ketamine respectively. But groups C and D were administered 23 mg/kg and 100mg/kg body weight of ceftriaxone and sulphadimidine respectively in addition to 20 mg/kg body weight of ketamine. However, groups E and F were administered intravenous dose of 23 mg/kg and 100 mg/kg of ceftriaxone and sulphadimidine respectively in addition to 17 mg/kg weight body of thiopentone. Vital parameters such as determination of anesthesia; onset and duration, temperature, respiratory and heart rates were recorded. The results of onset of duration of anesthesia revealed significant difference (P< 0.05) among group E, F and A animals. Although the result of onset anesthesia revealed significant difference between group A and B animals (P < 0.05). The values of respiratory rate revealed significant difference between the animals in groups D and B as well as groups F and A (P < 0.05). The values of heart rate showed significant difference between group C and D animals as well as between E and A animals (P < 0.05). Conclusively, sulphadimidine and cerftriaxone caused decreased duration of both anesthesia and onset of anesthesia when either was co-administered with ketamine or thiopentone in Nigerian local dogs. Sulphadimidine also caused increased respiratory rate, but cerftriaxone caused decreased and increased heart rate if co-administered with ketamine and thiopentone respectively. More so, thiopentone had higher durations of both anesthesia and onset of anesthesia in comparison with ketamine. So thiopentone is more potent than ketamine.

Keywords: Antagonistic, Anesthetics effect, Thiopentone, Katemine, Sulphadimidine, Cerftriaxone, Dog

INTRODUCTION

Thiopentone is a short acting barbiturate that is used for induction and/or maintenance of anesthesia, preoperative sedation and emergency management of seizure in both human and veterinary medicine (Gary and Tresznesk, 1983). It has half – life of 5 – 15 minutes by intravenous or rectal routes with between 75 and 85 % of the drug bound to plasma protein mostly albumin (Gary and Tresznesk, 1983; Tripathy, 2003). Thiopentone diffuses readily into the central nervous system (CNS); because of the lipid solubility, a small portion is excreted unchanged in urine and the terminal time is approximately 11.15 hours (Gary and Tresznesk, 1983; Laurence *et al,* 1999). Single or intermittent intravenous injection of thiopentone, concentration of 2.5 % in aqueous solution is used to reduce pain. However, injections are mostly made safe when saline or 5 % dextrose I water is used (Kennedy and Longnecker, 1996). Estimated fatal dose of thiopentone is 1g/kg body weight (Dreisbach and Robertson, 1987). Thiopentone and sulphadimidine have same binding site and hence thiopentone could easily displace sulphadimidine (Saganuwan *et al.,* 2005).

Ketamine a dissociative anesthetic induces profound analgesia, immobility, amnesia with light sleep and feeling of dissociation from ones owns body and the surrounding (Tripathy, 2003). The primary part of action is in the cortex and subcotical areas not in the reticular activating system (which is site of action of barbiturates) (Tripathy, 2003). It has rapid onset and recovery time but causes cardiovascular stimulation, increased cerebral blood flow and emergence reaction impair recovery (Trevor and white 2003). Katemine is the only intravenous anesthetic that possesses analgesic properties and produces cardiovascular stimulation. The peak increases occur in 2 – 4 minutes (*Waltzing et al.,* 2004)

Sulphadimidine has maintained an active place in the armamentry of antimicrobial drugs use in veterinary medicine (Bevill, 1982). It is 79 % plasma protein bound with half-life of 7 - 13 hours and is 60 – 90 % excreted as acetylated derivatives (Bevill, 1982). But Saganuwan *et al.* (2003) reported half-life of 15.4 ± 2.85 h in Nigerian local dogs. Sulphadimidine is known to cause decreased packed cell volume, total bilirudin and alkaline phosphate (Saganuwan, 2006a).

Cerftriaxone is a parenteral, semi synthetic third generation cephalosporin obtained from fungus cephalosporin (Tripathy, 2003) found in the sea sewage outfall in *Sardinia* (Bywater, 1991). It has activity against gram positive cocci (Prescott, 2000) but stronger activity against enterobacteriaceae, including penicillin's producing strains (Huber, 1988).

Table 1: Effects of cerftriaxone and sulphadimidine on anesthetic potency of thiopentone and katemine in Nigeria local dog

Time	Treatment groups					
	A	B	C	D	E	F
Onset of	17.50 ±	14.25 ±	11.75 ±	9.25 ±	5.33 ±	7.25 ±
Anesthesia	0.37	0.96	3.95	2.23	0.58	4.03*
Anesthesia	1872.50 ±	1440 ±	720 ±	1095 ±	1155 ±	1664.50 ±
	1077.40	189.74	259.23	79.37	552.9*	427.44*

Key: *A = thiopentone, B=ketamine, C=ceftriaxone,/ketamine, D = sulphadimidine/ketamine, E = ceftriaxone /thiopentone, F=sulphadimidine/thiopentone*

Table 2: Effects of cerftriaxone and sulphadimidine am vital parameters of Nigerian local dogs an aesthesia with katemine and thiopentone

Vital parameters	Treatment groups					
Temperature (°C)	39.35 ±	39.35 ±	37.30 ±	36.37 ±	37.38 ±	37.0 ±
	1.86	0.39	0.65	0.65	0.75	0.62
Respiratory	60.87 ±	23.4 ±	28.50 ±	33.0 ±	50.98 ±	15.05 ±
rate(/min)	15.66	2.30	5.26	11.01*	29.79	2.27*
Heart rate	85.15 ±	182.05 ±	146.12 ±	148.25 ±	143.33 ±	131.50 ±
(blat/min)	32.39	9.21	10.1*	41.33	3.05*	13.80

Keys: ** =significance difference in comprise with thiopentone; . =significance different in comparism with katemine*

It has extensive protein binding hence permitting once or at most twice daily dosing (Prescott, 2000).the binding capacity of cerftrixaxone differs somewhat between animal species (Baggot, 2001).however cerftriaxone may cause eosinophilia, hypobilirubiaemia, hypochloraemia increased bicarbonate ion concentration (Saganuwan, 2006b), hypoprorienaemia and hyperkakemia (Saganuwan, 2006c) in Nigeria local dogs. It has half-life of 0.85h and 25mg/kg body weight. cerftrixaxone is given 12-24 hourly (Prescott, 2000).however 50mg/kg bodyweight of cerftrixaxone for four days caused increased bilirudin (Saganuwan, 2006c)and hepatotoxicity in Nigeria local turkey (Saganuwan and Azubike, 2008).

Because drugs combinations are commonly used for preanaesthetic medication and general anesthesia, the potential exists for drug interaction to occur (Baggot, 2001).in view of this, the present study was designed to investigate whether sulphadimidine and cerftriaxone have ability to antagonize the anesthetics effects of thiopentone and Kate mine in Nigerian local dogs.

MATERIALS AND METHODS

Experimental Animals: Twenty- four Nigerian local dogs of either six weighing between 7 and 10 kg were used for the study. The dogs were obtained from a dog breeder who kept their mother for house security. They were fed daily with boiled rice beans meat and bone. The animals were divided into six groups A, B, C, D, E and F having four dogs per group.

Drug Administration: thiopentone sodium served as first control was intravenously administered at the dose rate of 17mg/kg body weight of into cephalic vein of four out of 24 dogs (group A). While 20 mg/kg body weight of ketamine served as second control was administered intravenous into cephalic vein of another 4 dogs (group B). Groups C and D were administered intravenous cerftriaxone and sulphadimidine at the dose rate of 23mg/kg and 100 mg/kg bodyweight respectively. Furthermore, 20 mg/kg body weight of katemine was intravenously administered in addition, to all the animals in groups C and D. However, group E and F animals were administered intravenous thiopentone sodium at the dose rate of 17 mg/kg body weight after post administration of 23mg/kg and 100mg/kg weight of cerftriaxone and sulphadimidine respectively. All the animals were observed for anaesthetic effects. Vital parameters such as duration of anesthesia, temperature, respiratory rate and heart rate were measured after 10 minutes intervals using thermometer and pericardial stethoscope for respiratory and heart rates. Duration of anesthesia and onset of anesthesia were recorded.

Statistical Analysis: Vital parameters, duration of an aesthesia and onset of anesthesia were express as means ± SD, test for significance between mean parameters in respect of control and experimental values were performed using student t-test. Bar charts were also used to present the result (Peltries and Watson, 2002).

RESULTS

The result of duration of onset of anesthesia of experimental animals from group D (9.25 ± 2.23s*) revealed significance difference in relation to the result from group B14.25 ± 0.96s) which was the control ($p < 0.05$) between the result of animals from group E (5.33 ± 0.58s*) and F (7.25 ± 4.03s*) in relation to the result from group A (17.50 ± 0.36s) (Table 1).

But the results of duration of an aesthesia of animals from Groups E (155 ± 552.90s*) and F 1664.50 ± 427.44s*) revealed significance difference ($p < 0.05$) in relation to that of animal from group a (1872.50 ± 1077.40s). Nevertheless, there was no significance difference ($p < 0.05$) between the result

of animals from groups C and Din relation to the result from group B animals. The result of onset of duration of an aesthesia of animals in the group A (17.50 ± 0.37s) in comparison to those of animals in Group B (14.25 ± 0.96s) was significantly difference ($p < 0.05$).

There was no significance difference between the temperatures values from the animals in group C and D in comparism with that of animals in group B ($p < 0.05$), more so there was no significance difference between the temperatures values of animals from group E and F in relation to that of animals from group A ($p < 0.05$). However, there was no significance different between the respiratory values of animals from group C in relation from that of group B animals as there was there lack of significant different between the values of animals from group E and A ($p > 0.05$). Although there was significant different in respiratory values of the animals of group F and A ($p < 0.05$) (Table 2).

The value of heart rate of animals from group C in comparism with that of animals from group B was significantly different ($p < 0.05$) as there was significant different ($p < 0.05$). Nonetheless, there was no significant difference in the value of heart rate between the animals of the group D and B as well as between group F and A ($p > 0.05$) (Table 2).

The duration of onset of anesthesia and duration of anesthesia were both highest in group A as duration anesthesia was lowest in group C and the onset in group of anesthesia was lowest in group E. Furthermore the duration of anesthesia was high in group B as compare in group F, then followed by group E, D and C in that order. Although the duration of onset of anesthesia was higher in group B as compare to that of group C, then followed by the Group D, F and A also in that order.

DISCUSSION

The significant difference ($p < 0.05$) between the duration of onset of anesthesia of animals from the group D (9.25 ± 2.23s*) and B (14.25 ± 0.96s) as well as group E (5.33 ± 0.58*) and F (7.25 ± 4.03s*) in relation to group A (17.50 ± 0.37s) (table1) are suggestive of the ability of sulphadimidine and ceftriaxone to interact with katemine and thiopentone, thereby decreasing the onset of duration of anesthesia of ketamine and thiopentone respectively. This agrees with the report of Saganuwan *et al* (2005) that the major processes, which determine the pharmacokinetic behaviour of a drug absorption, distribution, metabolism and excretion, are capable of being affected by co-administration of other drugs. The finding of this study is in line with earlier reports of (Gary and Tresnezsk, 1983) that the main type of interaction occurs when one drug competes with another for binding site. However, the significant difference ($p < 0.05$) in duration of anesthesia in group E (1155 ± 552.90s*) and F (1664. 50 ± 427.44s*) animals in relation to group A (1872.50 ± 1077.40s) animals is suggestive of the ability of cerftriaxone and sulphadimidine to cause decrease in duration of anesthesia (Table 1). Saganuwan *et al. (*2005) had earlier reported that thiopentone displaces sulphadimidine since the two drugs have the same binding site which is chiefly albumin. Hence, caution should be exercised when either sulphyadimidine or ceftriaxone is used pre-surgically with ketamine or thiopentone during the induction or maintenance of anesthesia in dog. Although, Saganuwan *et al.* (2005) reported that thiopentone increased the apparent volume of distribution of sulphadimidine therefore, increasing the half – life of Sulphadimidine. Nonetheless, lack of significant difference ($p>0.05$between the of anesthesia of animals in group C and D in comparism with duration of anesthesia of group B animals (Table 1) is suggestive of the inability of ceftriaxone and sulphadimidine to caused significant decreased in duration of anesthesia of ketamine. this may be attributable to the possibility of ceftriaxone and sulphadimidine having the same binding site with ketamine and so increasing ketamine excretion.

Lack of significant difference ($p > 0.05$) between the temperature value of animals in group C and D in comparism to group B animals, as well as animals from groups E and f in comparism to group A animals (table 2) was suggestive of the inability of ceftriaxone and sulphadimidine to cause significant increased in body temperature when co-administered with ketamine and thiopentone respectively.

Lack of significant difference between the respiratory value of the animals from group C and B ($p < 0.05$) and the animals from group E and A (Table 2) was suggestive of the inability of ceftriaxone to cause significant increase in temperature. This agreed with report of the Tripathy (2003) that respiration is not depressed in ketamine anesthesia even though ketamine was co-administered with ceftriaxone. The significant difference ($p < 0.05$) in respiratory value between group D and B animals as well as group F and A animals (Table 2) was suggestive of the ability of sulphadimidine to cause increased and decreased respiratory rates when used in combination with ketamine and thiopentone respectively. Ordinarily, respiratory rates are a good indicator of condition of animals and the dosage of the drugs (Booth and Mcdonald, 1992). Hence respiration should be adequately monitor in anesthesia patients by either decreasing the dosed of anaesthetic agent or by using respiratory machine.

The significant decrease ($p<0.05$) and increase in the value of heart rates of animals from group C and E in relation to the animals from group B and A respectively (Table 2)showed the ability of ceftriaxone to cause both decreased and increased heart rate. My finding agreed with the report of Tripathy (2003) and Booth McDonald (1992)that heart rates, cardiac output and blood pressure are elevated by Ketamine due to sympathetic stimulation even though ceftriaxone was co-administered with ketamine. Drugs or disease states that reduce cardiac output decrease liver blood flow and may change hepatic clearance of drug with a high

extraction ratio (E_H>0.6) (Nies *et al.*, 1973). Hence, ceftriaxone may be used to treat ketamine and thiopentone over dose and vice versa. Lack of significant difference in heart rate of group D and F animals in relation to group B and A animals showed the inability of sulphadimidine to cause increased heart rate when used in combination with either ketamine or thiopentone respectively (Table 2).

The highest duration of onset of anesthesia (1872.50 ± 1077.40s) displayed by group A animals in comparison to animals from the other groups (Table 1) agrees with the finding of Brodie *et al*. (1952) that, it is mainly redistribution of thiopentone rather than elimination by hepatic transformation that determines the duration of anesthetic effect. However, the duration of anesthesia (1440 ± 189.74s) observed from group B animals (Table 1) agrees with the finding of Baggot (2001) that the duration of anesthesia produced by a single intravenous dose of ketamine relates mainly to distribution and party depending on the size of the dose and to biotransformation. My finding is in concordance with the finding of Baggot (2001) that 5 mg/kg body weight of ketamine administered intravenously produced anesthesia of 588.0±102 seconds in dog. Since 20mg/kg body weight of ketamine administered to group B dogs produced duration of anesthesia (1440 ± 189.74s) double fold more than the duration of anesthesia (588.0 ± 102s) reported by Baggot (2001) when 5mg/kg was administered to dogs, the anaesthetic effect of ketamine may be dose dependent. Therefore, the higher the dose of the intravenous ketamine, the longer the duration of ketamine anesthesia. This relative increased dose-response phenomenon of ketamine may give ketamine superiority over thiopentone. Hence, ketamine can be used alone in contrast to thiopentone (Laurence *et al.,* 1999). To prolong anesthesia of ketamine one half of the initial dose may be administered (Hardman *et al.,* 1996). The half-life of ketamine in dog is 1 hour (Baggot, 2001). Nevertheless, the duration of onset of anesthesia and duration of anesthesia of ketamine and thiopentone were both decreased by ceftriaxone and sulphadimidine respectively in groups C, D, E and F animals in relation to groups B and A animals (Table 1).

Conclusion: Sulphadimidine and ceftriaxone caused decreased duration of both anesthesia and onset of anesthesia if either is co-administered with ketamine or thiopentone in Nigeria local dog. Sulphadimidine also caused increased respiratory rate as ceftriaxone caused decreased and increased heart rate if co-administered with ketamine and thiopentone respectively. More so, thiopentone is more potent than ketamine, but ketamine has relative dose-response higher than thiopentone.

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