NVRI SEMINAR SERIES 2014

This seminar series is a publication of seminar papers presented by staff and visiting scientists to the National Veterinary Research Institute, Vom during 2014

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INTRODUCTION

In keeping with the mandate of the Institute, research into economically important diseases of livestock remained the main thrust of activity in 2014 despite the precarious financial situation pervading the country. Working in concert, the management and staff of the Institute devoted time and resource to conduct basic and applied research geared towards treatment, prevention and control of endemic, emerging and re emerging diseases. The results and findings from these researches were presented during seminars in the Institute. It is the compilation of these seminars that is presented to you in this compendium "The seminar Series 2014."

In this series, presentation covers topical issues like Ebola Virus disease in man and animals; what it is, it's origin, transmission, clinical manifestations and methods of prevention and control. The role of frugivorous bats in the epidemiology of Duvenhage and rabies viruses in Nigeria was presented with highlights on potential public health implications for health and livestock workers. Molecular and serological techniques were used to characterize and determine the nature and prevalence of rabies virus in dogs in Niger and Plateau states of Nigeria. The prevalence remains disturbingly high despite annual anti rabies vaccination campaigns and mass public enlightenments. Molecular phylogeny of the viral isolates from dogs in Niger state reveals that there may be a new viral lineage circulating among dogs in Nigeria.

To ensure Nigerians are kept abreast with quality of products they consume, Spectrophotometric analysis of meat/offal was undertaken to determine the level of heavy metal (Cadmium) as residues in kidney and liver of cattle slaughtered for human consumption. It was shown that the level of this metal in slaughtered animals was within the allowable safe limit of the International standard by the Food and Agricultural Organization (FAO)/World Health Organization (WHO). Similarly, a metagenomic profiling of viral communities in raw and potable water in Jos and environ was also undertaken. It identified the presence of Adenoviruses in potable water which reveals the shortcoming in microbial quality standards of water meant for consumption.

To improve on the field diagnosis of Newcastle disease in poultry and to enable farmers and Veterinarian make informed decision on time, a comparison of a new Lateral flow technique with the haemagglutination inhibition test was undertaken. The new lateral flow technique appears to be more sensitive than the HA. However, there is need to test this new technique on a larger scale before recommending it to users. The prevalence of trypanosomosis in cattle,

isolation and antimicrobial sensitivity of *Salmonella gallinarum* from day old chicks, pathology of *Salmonella enterica* serovar *Gallinarum* in quails and the role of multi walled carbon nanotubes in oxidative stress and damage to hepatocytes and spermatocytes in Wistar rats were some other vital researches featuring in this report.

Finally, the research finding on the effects of *Euphorbia hirta* and *Acalypha wilkesiana* (plants) in albino rats carried out by the 2014 Interns of the African Education Initiative (NEF/NVRI) Toxicology Internship Programme suggests that the decoctions of the two plants contain vital phytochemicals like tannins and flavonoids making the plants candidates for further research as remedy for ailments of man and animals.

Undoubtedly this Seminar Series presents an array of research findings using novel methodologies geared towards fulfilling the mandate of the Institute. It further attests to the indefatigable spirit of the staff for research excellence.

Dr. P. A. Okewole

Chairman,

Seminar and Publications Committee

EFFECT OF *EUPHORBIA HIRTA* AND *ACALYPHA WILKESIANA* DECOCTIONS IN ALBINO RATS AND BHK-21 CELL LINE

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INTRODUCTION

Many people in developing countries depend on plants as remedy for their ailments and that of their animals including diarrhoea. Diarrhoea cases have increased over the years due to antibiotic resistance (WHO, 2013). Plant extracts have been successfully used for treatment of diarrhoea (Begum *et al.*, 2013). The presence of phytochemicals such as tannins, flavonoids and alkaloids has been linked to antidiarrhoeal activities of medicinal plants. Despite their therapeutic merits, some constituents of medicinal plants have been found to be deleterious (Ping *et al.*, 2012). Toxicity of potential drug candidates on vital body organs accounts for the withdrawal of about one-third of all these potential drugs. Toxicological studies provide information for safe use of medicinal plants. Evaluation of the effects of plant extracts *in vitro* provides further insight into their specific cellular toxicity.

Seminar presented 21st August, 2014 at NVRI Auditorium

Ethnobotanical surveys in Plateau and Sokoto States have indicated the use of *Euphorbia hirta and Acalypha wilkesiana* in diarrhoea treatment (Offiah *et al.*,

2011; Etuk *et al.*, 2009). *E. hirta* has also reportedly been employed in the treatment of skin infections, wound healing, asthma and snake bites. *Euphorbia hirta* is a medicinal herb which belongs to the genus *Euphorbia* and family Euphorbiaceae. Common names include; asthma weed, Hausa name-Nonon kurchiya, Yoruba-Emi-ile, Igbo-Ahuhia ugwa.

*Acalypha wilkesiana (*Euphorbiaceae-family, genus Acalypha) is commonly called copperleaf and Joseph's coat. In Southern Nigeria, the expressed juice or decoction of the leaves of *Acalypha wilkesiana* is used in traditional health care practice, for the management of: gastrointestinal disorders, fungal skin infections, hypertension, diabetes mellitus (Ikewuchi *et al.*, 2009), bacterial infections (Oladunmoye, 2006). The leaf poultice is used in the treatment of headache, inflammation, colds and malaria.

Reported studies on these plants involved the use of organic solvent extraction whereas the use of the plant in traditional medicine is as decoctions. This scanty information on the decoction therefore necessitates further study on the toxicity profile of the plants.

The aim of this work was therefore to determine the effect of sub-acute administration of *E. hirta* and *A. wilkesiana* decoctions in albino rats and cytotoxicity in BHK-21 cell line

METHODOLOGY

Plant samples (*E. hirta* and *A. wilkesiana)* were obtained from the Agricultural Services and Training Centre (ASTC) Vom, and Dagwom farm National Veterinary Research Institute, Vom respectively. The plants were identified, authenticated at Forest Herbarium Jos with Voucher number FHJ 145 and FHJ 146 for *A. wilkesiana* and *E. hirta* respectively. *E. hirta* whole plant and *A.wilkesiana* leaves were washed, boiled and sieved and the liquid portion dried in an oven. Phytochemical screening was done using standard methods of

Trease and Evans (1989). Acute toxicity test was done according to OECD, (2001) revised up and down procedure for acute toxicity testing.

For cytotoxicity testing, sterile plant extracts were prepared to obtain a 200µg/ml solution and serially diluted to obtain 100, 50 and 25µg/ml. BHK-21 cells were propagated to a monolayer into a total number of twenty (20), 25 cm^3 tissue culture flasks using Earl's Minimum Essential Medium (EMEM) containing 10 % New Calf Serum, and Antibiotics (Freshney, 2010). The flasks were inoculated with the extracts and sterile Phosphate Buffered Saline as control. Observations of inoculated cell lines were done at 12hrs interval for 72 hrs using an inverted light microscope at x10 magnification and results recorded.

For the *in vivo* studies, Wistar rats of both sexes weighing 210-230g were divided into four groups of eight animals per group. Groups I, II and III received 300, 600 and 1200mg/kg of the plant extracts respectively, while the control group was administered distilled water for 14 days. On the 15^{th} day, the rats were fasted overnight and euthanized with chloroform. Blood samples were collected by exsanguination. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Protein, Creatinine, Urea and Albumin were analyzed using Randox reagent kits while Sodium, Potassium and Chloride concentrations were determined using Teco Diagnostic kits, Haematological analysis was carried out using Abacus haemoautoanalyzer. Histopathological examination of kidneys and liver were done after processing using standard methods.

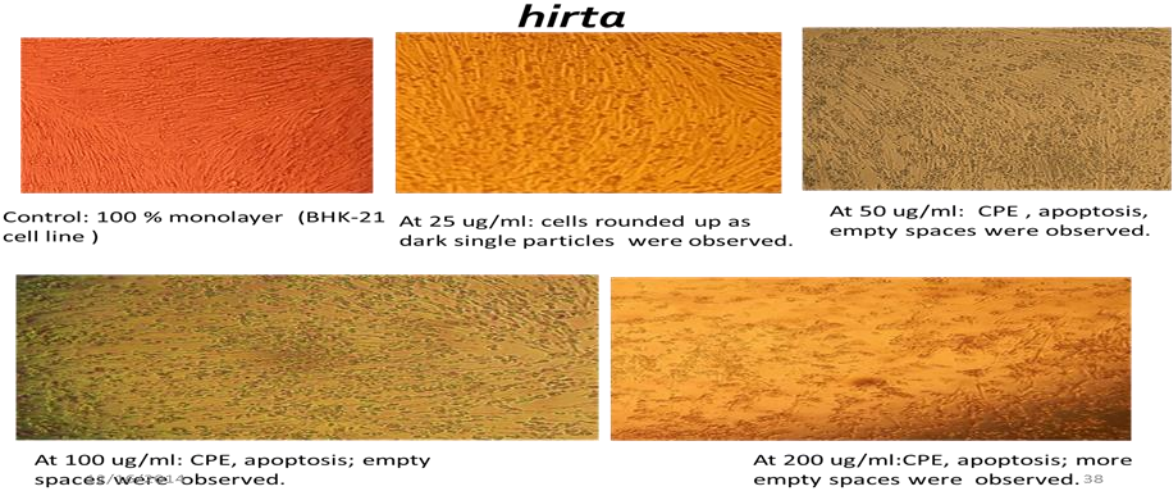
Data were expressed as mean ± standard error of mean. Comparative analysis of means was done using ANOVA with Tukey's-b post hoc test. Using Statistical Package for Social Sciences (SPSS Version 20) p values less than 0.05 were considered significant.

RESULTS

Table 1: Phytochemical profile of *E. hirta* and *Acalypha wilkesiana* decoction

Phytochemical	E. hirta	*A. Wilkesiana*
Saponin	+	+
Tannin	-	+
Flavonoid	+	+
Steroid	-	+
Carbohydrate	+	+
Cardiac glycosides	+	+
Resins	+	-
Alkaloid	-	-
Anthraquinones	+	-

Plate 1. CPE after 72 hours of inoculation with *E. hirta*



Control: 100 % monolayer (BHK-21 cell line)

At 25 ug/ml: cells rounded up as dark single particles were observed.

At 50 ug/ml: CPE , apoptosis, empty spaces were observed.

At 100 ug/ml: CPE, apoptosis; empty spaces were observed.

At 200 ug/ml:CPE, apoptosis; more empty spaces were observed.

Table 2: Results of acute toxicity and Median Lethal Dose (LD_{50})

Day	Number of rats	Mortality
1	3	0
2	3	0
3	3	0

Table 3: Effect of *E. hirta extract* on serum enzymes activity

Group	Dosage (mg/kg)	AST (u/l)	ALT (u/l)	ALP (u/l)
1	**300**	31.48 ± 2.91^{c}	11.53± 0.28^{b}	168.13 ± 29.96^{a}
2	**600**	22.48 ± 0.53^{b}	13.68 ± 1.07^{c}	358.80± 130.53^{b}
3	**1200**	25.00 ± 0.34^{b}	15.06 ± 0.25^{c}	302.07 ± 63.42^{a}
Control	**-**	**15.61 ± 0.50^{a}**	**7.07 ± 0.18^{a}**	**338.22±173.53^{b}**

Table 4: Effect of *E. hirta extract* on serum total protein and Albumin concentrations

Group	Dosage (mg/kg)	Total Protein (mg/ml)	Albumin (g/L)
1	**300**	198.47 ± 14.71^{ab}	45.24 ± 7.89^{a}
2	**600**	190.47 ± 17.80^{ab}	40.48 ± 5.77^{a}
3	**1200**	208.48 ± 14.73^{b}	46.88 ± 5.54^{a}
Control	**-**	**183.37 ± 9.85^{a}**	**56.94 ± 23.00^{a}**

Table 5: Effect of *E. hirta extract* on serum urea and creatinine concentrations

Group	Dosage (mg/kg)	Urea (mmol/L)	Creatinine (µmol/L)
1	**300**	262.35 ± 76.91[a]	168.07 ± 6.13[ab]
2	**600**	243.71 ± 51.41[a]	188.93 ± 5.90[b]
3	**1200**	270.60 ± 27.05[a]	280.71 ± 22.39[c]
Control	**-**	**132.00 ± 109.16[b]**	**128.61 ± 7.40[a]**

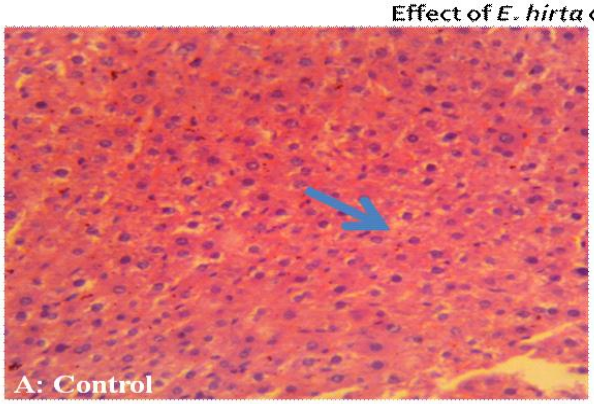
Table 6: Effect of *E. hirta extract* on serum electrolytes concentration

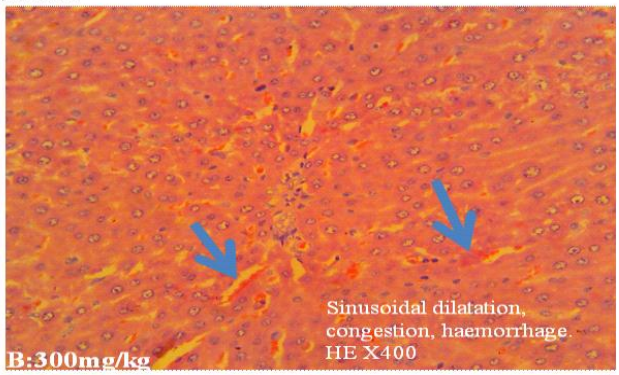
Group	Dosage (mg/kg)	Cl^-(mEq/L)	K^+(mEq/L)	Na^+(mEq/L)
1	**300**	215.23 ± 11.37[a]	6.71 ± 1.79[a]	1238.41 ± 152.70[a]
2	**600**	206.39 ± 29.79[a]	7.63 ± 0.52[a]	1382.40 ± 134.21[a]
3	**1200**	198.19 ± 35.66[a]	7.88 ± 1.40[a]	1370.12 ± 165.03[a]
Control	**-**	**199.40 ± 47.27[a]**	**8.22 ± 0.81[a]**	**1312.11 ± 47.86[a]**

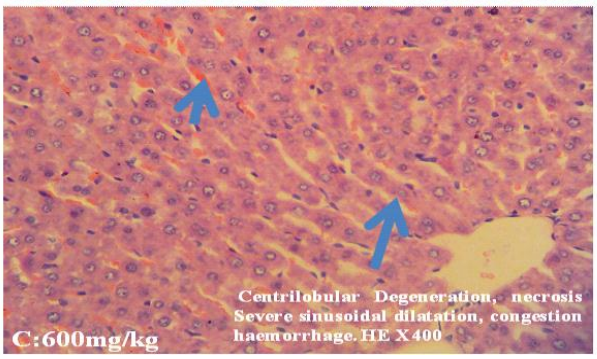
Table 7: Effect of *E. hirta extract* on haematological indices

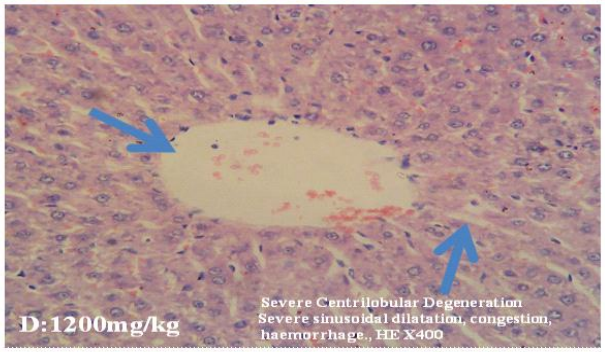
Treatment	Haematological parameter				
	RBC(10^{12}/L)	**Hb(g/L)**	**PCV (%)**	**PLT(10^9/L)**	**WBC(10^9/L)**
300 mg/kg	8.53±0.69	173.67±7.67	48.18±1.66	738.37±97.70	8.08±2.13
600 mg/kg	8.21±0.64	163.75±17.63	45..38±4.63	699.25±122.55	7.17±1.86
1200 mg/kg	8.49±0.68	170.14±8.13	47.06±1.39	745.14±118.87	6.99±1.53
Control	8.17±0.26	166.75±5.33	46.89±1.51	719.87±96.94	7.17±3.19

Effect of *E. hirta* on liver

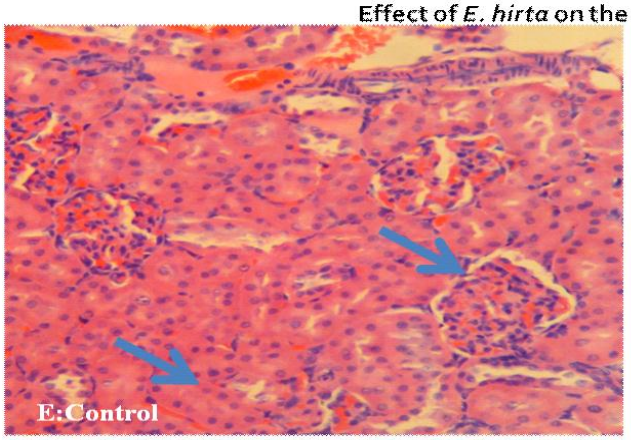


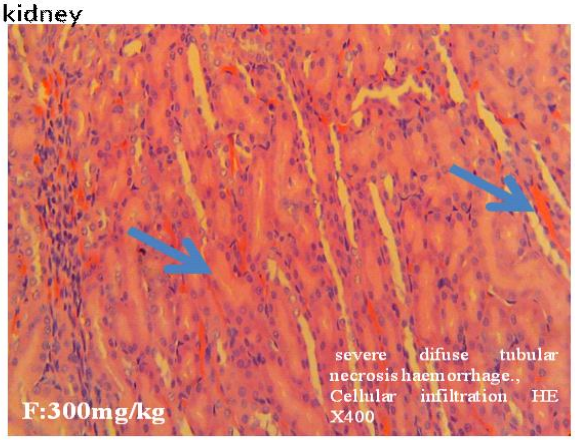


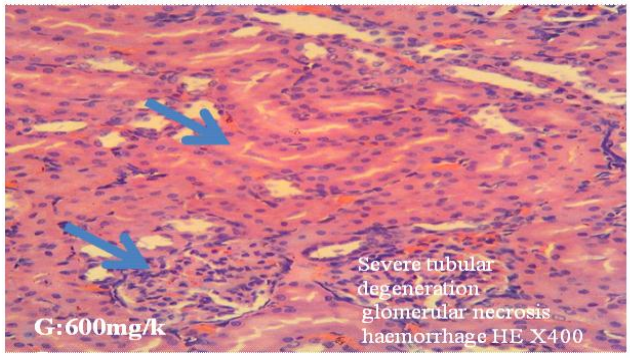




Effect of *E. hirta* on the kidney







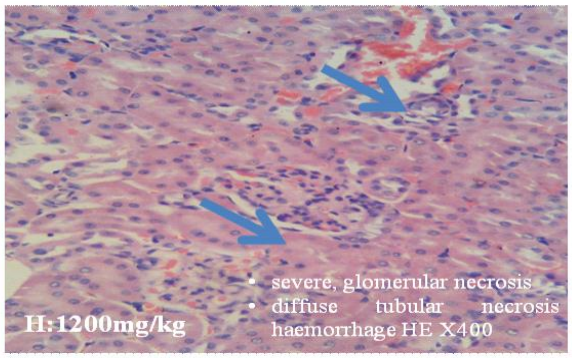


Plate 2. CPE after 72 hours of inoculation with *A. wilkesiana*

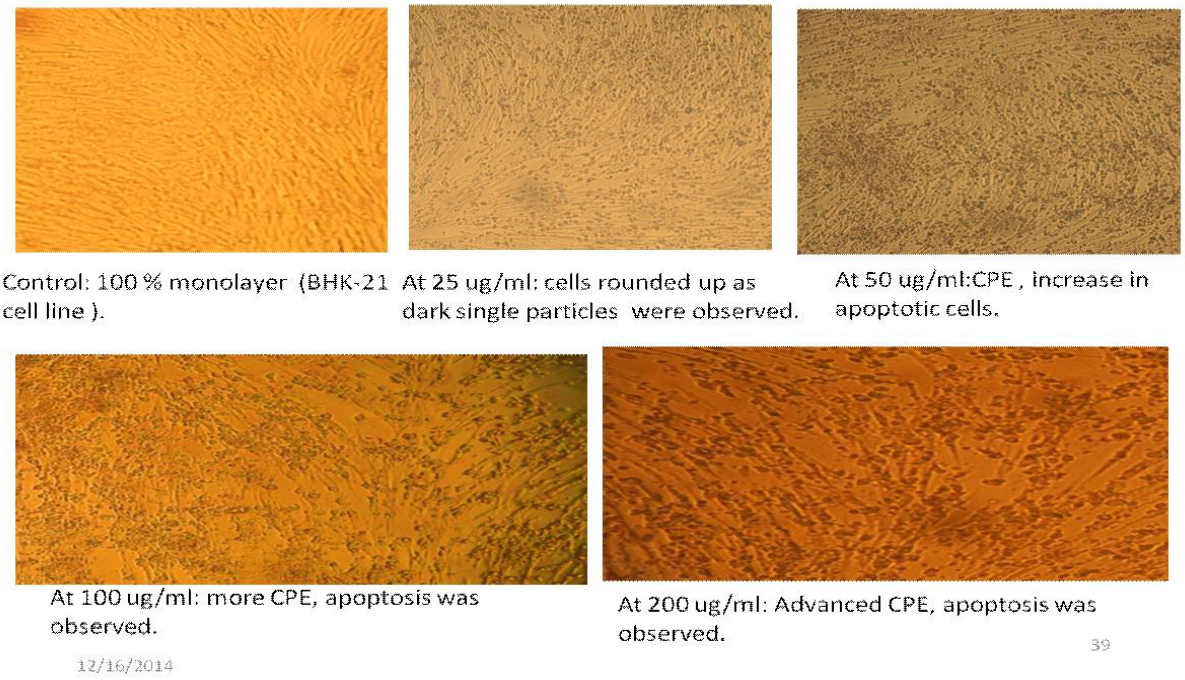


Table 8: Effect of *A. wilkesiana extract* on serum enzymes activity

Group	Dosage (mg/kg)	AST (u/l)	ALT (u/l)	ALP (u/l)
1	**300**	22.42 ± 1.93*	6.74 ± 1.96	236.18 ± 73.66*
2	**600**	26.23 ± 2.42*	6.29 ± 1.80	254.71 ± 69.38*
3	**1200**	27.17 ± 5.27*	11.58 ± 1.74*	371.42 ± 131.86
Control	**-**	20.35 ± 1.52	5.89± 0.75	380.88 ± 130.74

Table 9: Effect of *A. wilkesiana extract* on serum total protein and Albumin concentrations

Group	Dosage (mg/kg)	Total Protein (mg/ml)	Albumin (g/L)
1	**300**	186.96 ± 9.59*	41.36 ± 3.19*
2	**600**	192.94 ± 14.45*	46.31 ± 2.47*
3	**1200**	197.54 ± 14.13*	46.47 ± 4.49
Control	**-**	180.35 ± 6.33	49.98 ± 6.04

Table 10: Effect of *A.wilkesiana extract* on serum urea and creatinine concentrations

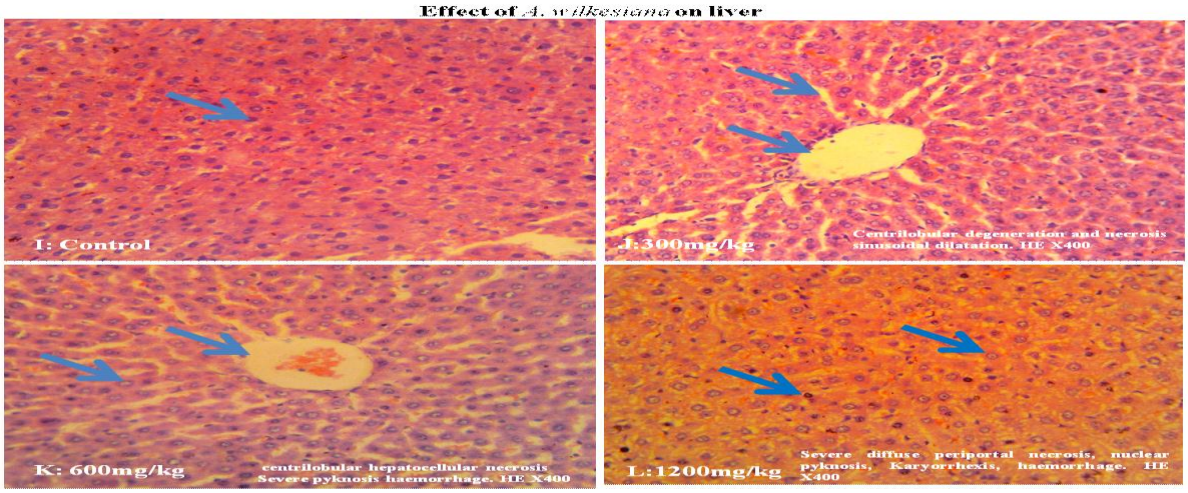
Group	Dosage (mg/kg)	Urea (mmol/L)	Creatinine (µmol/L)
1	**300**	1685.76 ± 223.50	8.71 ± 1.55*
2	**600**	1725.428 ± 56.09	9.06 ± 0.81*
3	**1200**	1632.876 ± 168.67	10.04 ± 0.86*
Control	**-**	1527.10 ± 130.05	6.66 ± 1.78

Table 11: Effect of *A. wilkesiana extract* on serum electrolytes concentration

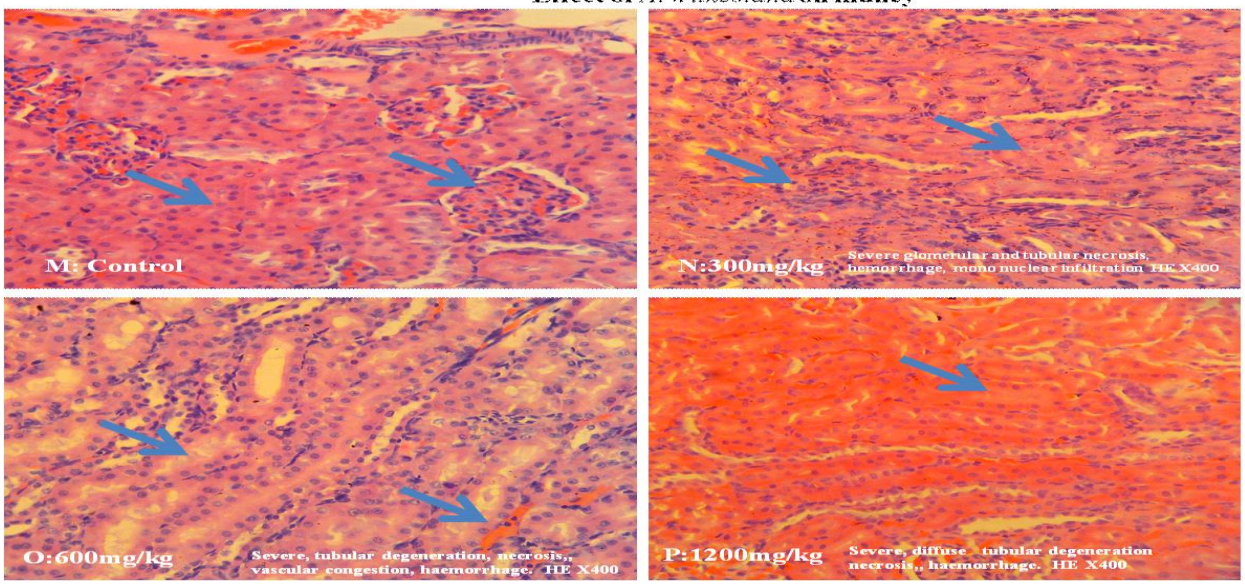
Group	Dosage (mg/kg)	Na^+(mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)
1	**300**	8.82 ± 0.14*	8.43 ± 0.50	217.32 ± 11.28
2	**600**	8.89 ± 0.18*	8.50 ± 0.61	232.38 ± 9.29
3	**1200**	9.00 ± 0.7	8.93 ± 1.46	236.55 ± 7.53*
Control	**-**	8.66 ± 0.19	8.28 ± 0.24	213.25 ± 2.26

Table 12: Effect of *Acalypha wilkesisna* on some hematological parameters

Treatment	Haematological parameter				
	RBC(10^{12}/L)	**Hb(g/L)**	**PCV (%)**	**PLT(10^9/L)**	**WBC(10^9/L)**
300 mg/kg	8.3 ± 0.9	165.5±13.3	45.9±2.5	798.8±86.9	8.0 ± 2.2
600 mg/kg	8.7 ± 0.4	172.0±7.6	47.9±1.6	837.3±150.5	7.6 ± 1.8
1200 mg/kg	8.7 ± 0.7	171.9±13.7	47.0±2.3	812.1±115.4	8.3 ± 0.9
Control	8.2 ± 0.3	166.8±5.3	46.9±2.5	719.9±96.9	7.1 ± 2.9



Effect of *A. wilkesiana* on kidney



CONCLUSION

The results of our study suggest that the decoction of both *Euphorbia hirta* and *Acalypha wilkesiana* contain vital phytochemicals like tannins and flavonoids. However both plants are hepatotoxic and nephrotoxic at doses of 300, 600 and 1200 mg/kg, as correlated with *in vitro* toxicity studies on BHK – 21 cell lines at 25, 50, 100 and 200µg/ml

RECOMMENDATIONS

Further studies should use lower doses of the decoction and examine the effect of sub chronic and chronic exposure. The lipid profile should be determined in subsequent studies. Advanced chromatographic and spectrometric methods should be used to characterize particular phytochemicals present in both plants to determine their active components. Immuno-histochemistry techniques should be included in subsequent studies to understand the molecular mechanisms of toxicity or therapy.

Although these plants are widely used in herbal medicine for the treatment of diarrhoea, caution should be exercised in their use.

ACKNOWLEDGEMENT

Dr. M. S. Ahmed (EDVR) for hosting and partly sponsoring the toxicology internship programme, Prof. Nduaka Chudy (President) and Board of AEI (NEF) for sponsoring the programme. All the Mentors and Divisions that hosted the 2014 NEF Toxicology Internship Programme for their invaluable contributions.

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EBOLA VIRUS DISEASE IN ANIMALS AND MAN

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INTRODUCTION

Ebola Virus Disease (EVD) was first recognised in Zaire (Democratic Republic of Congo) in 1976 and named after river Ebola. It presents as severe viral haemorrhagic fever with body temperature >38.6^{o}C following an incubation period of 2-21 days (CDC, 2014). Other clinical signs include severe diarrhoea, vomiting, bleeding and ultimately death. The virus replicates in almost all tissues of the body and is shed in all body fluids including stool, vomitus, saliva, sweats, mucous membranes, and it has tropism for skin etc. In its severity merely touching an infected person or a dead body is infectious and morbidity is almost 100% while mortality may reach 90%.

This highly infectious disease is caused by a filamentous enveloped RNA virus in the family filoviridea consisting of Ebola and Marburg virus. Other members of Ebola virus group are Reston, Ebola Zaire, Ebola Sudan, Ebola Tai forest (Ivorian strain) and Bundibuyo (Figure 1). Recent outbreak of EVD in West African has caused about 6800 deaths in six countries including 8 from Nigeria (18464 cases) as at 15th December 2014. Infection has also spread outside Africa to USA and Spain (WHO, 2014).

Seminar presented 26th August, 2014 at NVRI Auditorium

EVD IN ANIMALS AND MAN

Ebola is a zoonotic viral disease but there are no scientific proofs of its natural animal reservoir. This is unlike other zoonoses like Lassa from rats, HPAI from waterfowls and HIV-2 from non-human primates.

What is known among many unknowns of EVD, particularly the Zaire strain is that the animal reservoirs dwell in the tropical rain forest of Central and West Africa (Figure 2). Many suspected animals include, bats (genus Rousettus), tortoise, porcupine and arthropods etc. Highly susceptible non-human primates include Monkeys, Chimpanzees and Apes (Figure 3). These animals like human are dead end hosts (Jahrling *et al.*, 1996; Leroy, 2005; WHO, 2009)

Infection can be directly through contact with unknown animal reservoirs or infected primates who also dies of the infection. Human to human transmission often occurs amongst family members and healthcare workers in hospital settings lacking in appropriate Personal Protective Equipment (PPE) and infection control practices. Dead bodies of Ebola victims are infectious including funeral rites of washing, touching and kissing dead bodies.

DATELINE OF EVD AND RELATED VIRUS OUTBREAKS

DATE	EVENT	REMARK
1967	Marburg in lab. Workers Germany.	Source of virus were African green monkeys from Africa
1976/9	Ebola in Zaire and Sudan respectively	Source unknown but related to contact with animals in the forest
1989	Ebola-Reston in Reston, Virginia USA	Traced to Monkeys (macaca fascicularis) imported from Philippine
1994	Ebola Ivorian (Tai) in Ivory coast	Source was a necropsy on chimpanzees
1995	Ebola in Kikwit, DRC (EBO-Z)	Index case had exposure to burrowing species of plant and animals; Mortality was 90 – 100%.
1996	Ebola in Mayibout area in Gabon, (EBO-Z)	Hunters, butchers and eaters of infected Chimpanzee (including family) got sick and died
2008	Ebola-Reston in Philippines	1st known Ebola-Reston in pigs. Pig farm/slab workers had antibodies but were asymptomatic.
2012	Ebola in Luwero district in Sudan (EBO-Z)	6 infected, 3 deaths (50%) mortality.
2014	Ebola in Guinea, Sierra Leone, Liberia, Nigeria, Senegal and Mali (**EBO-Z**)	18464 infected, 6800 deaths (37% mortality rate) as at 15th December 2014.

Adapted from CDC, 2014

LABORATORY DIAGNOSIS

- Key to early warning, emergency preparedness and management
- Blood specimen should be collected and handled with full PPE
- Inactivate and analyse specimen in BSL 3 facilities (ELISA, RT-PCR) (pan Filoviridae and strain specific primers are commercially available) Yoshikawa *et al.* (2003).
- Higher biosafety level (BSL4) is required for live virus research

PREVENTIVE MEASURES

- Hunters and wildlife enthusiasts including Veterinarians to be cautious while handling wild animals particularly sick animals
- Healthcare workers must religiously use PPE when attending to sick persons
- Regular hand washing (running water with soap and detergents)
- Good sanitary practices (avoid hand to nose and mouth habits)
- Avoid contact with infected persons, sick or dead
- Better public health infrastructures that can respond effectively to disease emergencies and infection control.
- EVD management include fluid and electrolyte therapy, hyper immune serum and monoclonal antibodies grown on tobacco leaves may be useful.

CONCLUSION

"We need to better understand how dreadful zoonotic diseases like Ebola first crosses over from animals to human so we can prevent this from happening" – J. Epstein

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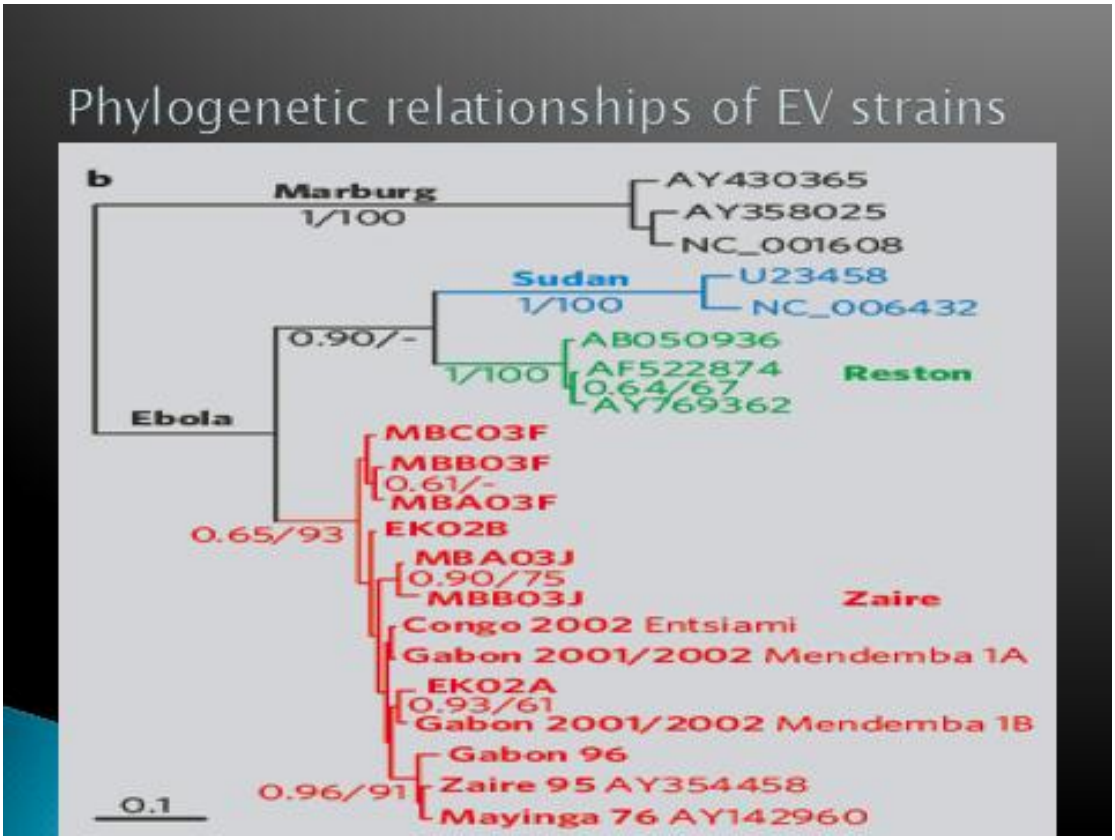

Figure 1: Phylogenetic relationship of Ebola Virus strains (Leroy *et al.*, 2005)

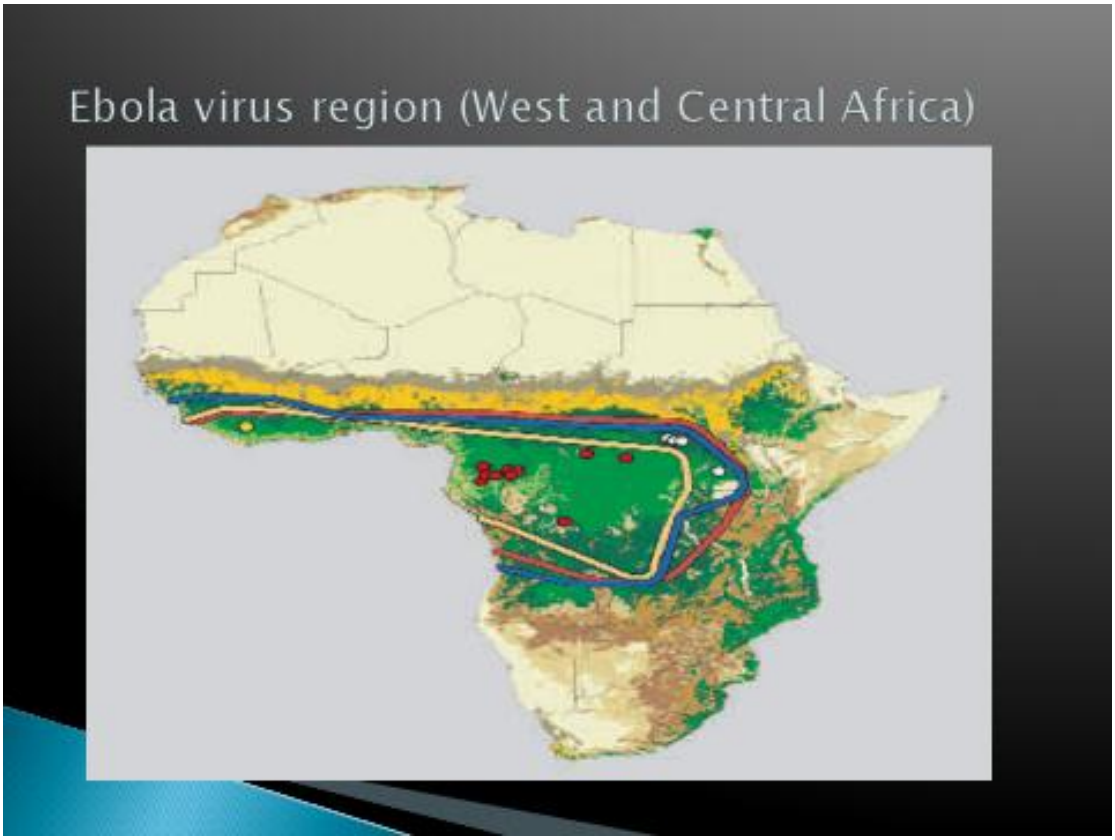

Figure 2: Ebola Virus region (West and Central Africa) (Leroy *et al.*, 2005)

Figure 3: Likely reservoirs and susceptible hosts of Ebola Virus

DETECTION OF CADMIUM (CD) RESIDUE IN KIDNEY AND LIVER OF SLAUGHTERED CATTLE IN SOKOTO CENTRAL ABATTOIR, SOKOTO STATE, NIGERIA

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INTRODUCTION

Heavy Metals are those elements which have density more than 5,000 kg/m^3 (Hawkes 1997), atomic weight 63.546g to 200.590g (Kennish, 1992) and a specific gravity greater than 4.0 (Connell and Miller, 1984). Although heavy metals contaminate ground water, soil, pasture (vegetation), and air, these metals when consumed by animals and human tend to bio-accumulate in their tissues and organs, and excess accumulation will lead to severe health hazard (Kennish, 1992).

Historically the fall of Roman Empire was believed to be as a result of utensils coated with lead, which resulted in lead poisoning in the Empire (Andrada *et al.*, 2006).

Once the environment is contaminated with heavy metals, they remain so for years and increase the chances of becoming toxic to humans and animals (Chin *et al.*, 2008). Recent studies have shown that poor handling of some modern products such as paints, petrol, battery, engine oil, (Bastarche, 2003), mercury amalgam dental filling (Chin *et al.*, 2008), cosmetics (Hardy *et al.*, 1998), and ground water residues (Ghosh *et al.*, 2004) resulted to chronic exposure to heavy metals.

Food crops and pasture grown on soil containing toxic heavy metals or irrigated with water containing heavy metals accumulate heavy metals in them

and serves as a source of heavy metals exposure to animals and humans (Ward and Savage, 1994). Some occupations that involve direct contact of the workers with heavy metals like, painting, dental surgery, and welding, plumbing, mechanics etc (Olah and Tolgyessy, 1985) are at greater risk of exposure to heavy metal poisoning. Indiscriminate dumping of waste materials, and sewage water containing heavy metals on land gradually increase the toxic concentration of heavy metals in the soil and these are increasingly taken up by the plants and finally find its way into the food chain causing severe health hazard to both animals and human (Haiyan and Stuanes, 2003).

Animals reared on pasture fertilized with materials containing heavy metals or fed with feed containing heavy metals become a good source of heavy metal residues in their edible products such as meat, fish, eggs, milk etc.

Heavy metal contamination in meat and other edible tissues is a matter of great concern for food safety. Some heavy metals are toxic in nature and even at relatively low concentrations can cause adverse effects (Santhi *et al.*, 2008). Different researchers have reported the instances of contamination of heavy metals in meat products during processing (Santhi *et al.*, 2008). Heavy metals residues were found in meat and meat product of livestock fed with contaminated feed and reared in proximity to environment polluted with heavy metals (Miranda *et al.*, 2005). This is of serious public health problem that needs an immediate attention of health regulatory authorities and researchers as well. The objective of this paper is to determine the level of cadmium in kidney and liver of cattle slaughtered for human consumption in Sokoto Nigeria

MATERIALS AND METHOD

Study Area

The study area is Sokoto central abattoir, which is located in Sokoto North local government area of Sokoto State Nigeria. Sokoto state is geographically located in the North Western part of Nigeria, between longitudes 4°8'E and

6°54' E and latitudes 12° N and 13°58'N. The State rank second in the Nigeria livestock population with an estimated 3 million cattle, 3 million sheep, 5 million goats, 4,600 camels, 52,000 donkeys and host of other species of local and exotic poultry species (MOCIT, 2002; Mamman, 2005).

Sample Collection and Preservation:

Samples (kidney and liver) were purchased from randomly selected slaughtered cattle from Sokoto central abattoir for a period of seven weeks. The age of the selected slaughtered cattle was determined. About 100 g of liver from any lobe and a whole kidney (either right or left) of each selected animal were packed in a sterile polythene bags, properly labelled and transported to Veterinary Public Health and Preventive Medicine laboratory, where it was frozen and stored at 20°C. The samples were transported to National Research Institute for Chemical Technology Zaria, Kaduna State, Nigeria for further processing and analysis.

Processing of Samples

Digestion of Samples

Liver and kidney samples were dried at 45° C. Individual samples were crushed into fine powder using mortar and pestle, and 1.0g was weighed into porcelain crucible. The crucible and the fine powdered samples were ignited in a muffle furnace at 500°C for six to eight hours, removed from the furnace and allowed to cool in desiccators, and weighed again. The difference between the weight of the crucible and ash and the weight of the crucible alone was used to calculate the percentage ash content of the sample. Then, 5cm^3 of 1M trioxonitrate (v) acid (HNO_3) solution was added to the left-over ash and evaporated to dryness on a hot plate and returned to the furnace for heating again at 400°C for 15-20 minutes until perfect grayish-white ash was obtained. The samples were then allowed to cool in desiccators. To the cooled ash 15 cm^3 hydrochloric acid (HCl)

was then added to dissolve it and the solution was filtered into 100 cm^3 volumetric flask. The volume was made to the 100cm^3 with distilled water.

Spectrophotometry Techniques for cadmium

In the prepared liver and kidney samples, cadmium was determined using AA-6800, Shimadzu atomic absorption spectrophotometer according to manufacturer's instructions. The concentration of lead in each sample was determined using the calibration curve (Szkoda and Żmudzki, 2005).

RESULTS AND DISCUSSION

All the samples (100%) tested positive for Cadmium. The mean Cadmium concentration in kidney is higher among the age group≥9 years with the concentration of 0.0526mg/kg, and it is lower among the age group of 0-2years with the concentration of 0.0194. While in the liver, the mean Cadmium concentrations was also higher among the age group of ≥9 years with the concentration of 0.0243 mg/kg and lower mean Cadmium concentration was found among the age group of 0- 2 years with the concentration of 0.0162 mg/kg .

Generally the mean Cadmium concentration in kidney was higher than in liver in all age groups, this is similar to the report of Zasadowski *et al.* (1999). But this result was contrary to the findings of Akan *et al.* (2010) who recorded high concentration of Cadmium in the liver than the kidney. From this study, the concentration of Cadmium increases with the increase in the age of cattle this is also similar to the findings of Zasadowski *et al.*(1999) who reported low concentration of Cadmium in cattle whose ages are less than 2 years and high concentration among the ages of cattle group greater than 2 years.

The mean concentration of Cadmium from this study is lower compared with the findings of Zasadowski *et al.* (1999), who reported mean concentration of Cadmium in liver and kidney of cattle less than 2 years to be 0.159mg/kg and 0.4265 mg/kg respectively. And mean concentration of Cadmium in liver and kidney of cattle whose ages are greater than 2 years to be 0.263mg/kg and

1.703mg/kg respectively. This shows that the contamination of environment with Cadmium in the study area (Sokoto) was less compared to other studies (Zasadowski *et al.* 1999, Irfana *et al* 2004 and Akan *et al.* 2010)

According to the results of this study, there was a significant difference in the concentration of Cadmium in liver and kidney in the different age groups $P \leq 0.05$. Accumulation of Cadmium in the liver and kidneys associated with age has also been found in other studies on cattle (Lopez Alonso *et al.*, 2002).

Table showing mean concentration of cadmium (Cd) (mg/Kg) in Liver and kidney samples of different age groups of cattle

Age Range (years)	Mean conc of Cd		Permissible limit by FAO /WHO (mg/kg)
	kidney (mg/kg)	liver (mg/kg)	
0-2	0.0194	0.0162	0.5
3-5	0.0366	0.0186	,,
6-8	0.0479	0.0175	,,
≥9	0.0526	0.0243	”

Cadmium has high ability to migrate from the soil to Plants (Peterson and Alloway, 1979), this may be the main source of cattle exposure to this element. Sokoto, Kebbi and Zamfara States from where these animals are purchased for slaughtered at Sokoto Central abattoir are agricultural areas of North Western

part of Nigeria, so Cadmium in the soil can originate mostly from chemical substances and fertilizer used in agricultural practices. Other possible sources of contamination may include motor vehicles, emissions from local industrial premises, fertilization of soil with sludge, dumping of industrial wastes, etc., which may contaminate soil, plants, water, and air.

Generally the concentration of Cadmium in liver and kidneys of slaughtered cattle at Sokoto Central abattoir were below permissible level of 0.5 mg/kg and 1 mg/kg in liver and kidney respectively as recommended by FAO/WHO (2000), which means bovine carcasses slaughtered at Sokoto are safe for human consumption.

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PREVALENCE OF ANIMAL TRYPANOSOMIASIS IN SOME PARTS OF CENTRAL SENATORIAL ZONE OF PLATEAU STATE, NIGERIA

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INTRODUCTION

Trypanosomes are flagellated protozoa that belong to the genus Trypanosoma. They cause diseases that are generally referred to as trypanosomiasis. These protozoa can infect all classes of vertebrates: Fish, amphibians, reptiles, birds and mammals (Connor and Vanden Bossche,2004). African animal trypanosomiasis (AAT) affects livestock in Sub-Saharan Africa, (Cheneret *et al;* 2006). In Nigeria, trypanosomiasis is probably one of the most important diseases of cattle, and one of the predominant obstacles to profitable animal production particularly in the middle belt region which provide good breeding grounds for tsetse flies because of the thick vegetation (Lamorde, 1989). Jos, Mambila and Obudu highlands which had been hitherto declared as tsetse and trypanosomiasis free zones are now infested (Ahmed *et al,* 2005). Thus the Jos Plateau attracted over the years a constant in-flux of cattle which often results in permanent settlement of Fulani herdsmen. The objective of this study is to determine the prevalence of AAT in Jos Plateau.

MATERIALS AND METHODS

Sample collection

Blood samples were collected at random from cattle in the rural areas of the two local government areas which have high cattle population. Five (5) militres of blood were collected from the jugular vein and

transferred into bijou bottles containing EDTA salts.

It was packaged in flasks containing ice pack and was transported to Parasitology laboratory of National Veterinary Research Institute Vom.

LABORATORY INVESTIGATION

Wet Preparation

Blood samples were processed using the standard trypanosamal detection methods according to the following procedures: Wet preparation, Haematocrit Centrifugation Technique (HCT) (Woo, 1970) Buffy Coat Technique, (BCT) (Murray *et al*; 1977) and the stained thin and thick blood films.

Ethical Consideration

The study was carried out with the full approval of cattle keepers, the Plateau state ministry of Agriculture, the National Veterinary Research Institute, (NVRI) and the Nigerian Institute for Trypanosomiasis Research (NITR) Vom.

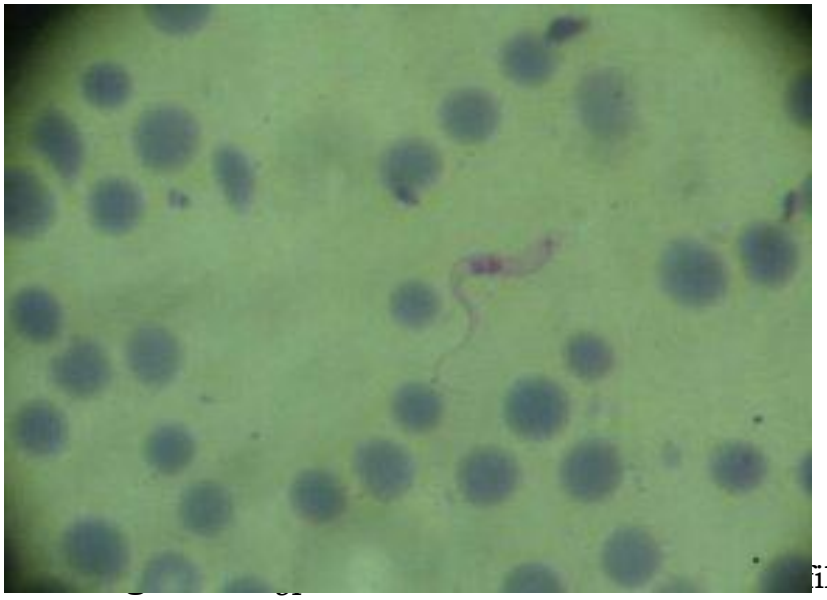
RESULTS

Table 1: Prevalence of Bovine Trypanosoma species in the two Districts

District	No sampled	No. Positive (%)	Chi square (x^2)	P
Bokkos	55	0(0.0)	5.238	0.022
Daffo	55	5(4.5)		
Total	110	5(4.5)		

In table 1 above, equal number of bovine blood samples were taken in the two districts (Bokkos & Daffo). The positive samples were all from Daffo district

based on the analysis. No single positive sample was detected from Bokkos district. There is a significant difference in the infection rate. P<0.05



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Trypanosomal vivax as seen in a Giemsa stained blood film

DISCUSSION

The study area became a converging place for the Fulani pastoralists since the crisis in some parts of the Plateau persisted. Also, the animal rearing activities in the study area is basically on extensive method where there is little restriction to the movement of animals (Majekodunmi *et al.*, 2013). The Standard Trypanosome Detection Method (STDM) is a combination of different techniques used to avoid misdiagnosis of the infection. Buffy coat technique detected most of the infection than others because it has the ability to concentrate the available parasite(s). The Murray method is regarded as one the best microscopic examination techniques for the detection of trypanosomes in blood samples (Murray *et al.* 1977). However, this technique is less sensitive and therefore cannot diagnose the earlier stages of the infection. Furthermore,

the Murray method cannot accurately distinguish the different species of trypanosomes. In addition, microscopy is laborious and time consuming as individual slide has to be examined. The prevalence rate is in agreement with earlier works (Kalejaiye *et al.,* 2004, Omotainse *et al*; 2004). Although these workers obtained higher infection rates of 11.5 and 8.5% respectively, they were able to prove that the buffy coat technique gave a higher percentage over other techniques. Although the prevalence rate obtained in this study is quite low, as compared to 8.4% by Enwezor *et al*, (2009) in a grazing reserve in Kaduna State, Nigeria, possible reasons could be attributed to the vectors and the presence of the trypanosomes in those areas and the time of sampling. Although, this is lower than the results obtained by Kalejaiye *et al*. (2004) when a percentage prevalence of 11.5% was obtained in seven villages in Bokkos Local Government of Plateau State. This could probably be likened to wider study area.

CONCLUSION

This study also suggests that *T. congolense* is the most prevalent species in the study area. The lower prevalence in animals indicates a low transmission of the parasites in the study area. Hence further research be taken in the area to check relapse.

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PATHOLOGY OF *SALMONELLA ENTERICA* SEROVAR GALLINARUM IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

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INTRODUCTION

The genus *Salmonella is a member* of the Family *Enterobacteriaceae* and consists of Gram-negative, non-spore forming bacilli (Popoff *et al.*, 2003). *Salmonella enterica* serovar Gallinarum (*Salmonella* Gallinarum) is a non-motile host adaptive *Salmonella* that causes fowl typhoid (FT), a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and pathological lesions (Parmer and Davies, 2007). With the continuous expansion of poultry farming in Nigeria, FT has gained ground as a major disease of poultry which can cause heavy economic losses in poultry through mortality (Agbaje *et al.*, 2010). Quails are ideally suited for avian research, because of their small size and require little cage space for rearing. They are easy to raise and are suitable for genetic studies since they rapidly attain sexual maturity (Haruna *et al.*, 1997). Quails are birds that every household can keep without stress (Huss *et al.,* 2008). Although quail farming contributes to the alleviation of protein- deficiency in the diets of people in developing countries, they have

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largely been neglected as a livestock species. Most farmers have focused mainly on chicken production. Thus, quail's actual contributions to food production have been greatly ignored and or underestimated by policy makers in the agricultural sector in developing countries. Nigerians are interested in quail production especially for medicinal reasons and so quail farming should be encouraged with a special focus on the traditional subsistence rearing system as practiced by resource-limited farmers (NVRI, 2008).

Quail meat and eggs are known for high quality protein, high biological value and low caloric value, thus making them good choices for hypertension prone individuals (NVRI, 2008). There is a scarcity of information on microscopic lesions of FT in chickens and quails, and most of the lesions described are from field cases which may be complicated by other bacterial and or viral agents in chickens talk less about the quails (Chadfield *et al.,* 2003). The Japanese quail has the potential of filling some of the gap in the protein needs of Nigerians, it is therefore important to investigate diseases that can interfere with production such as FT in order to control them.

This study evaluates the gross and histopathology of *Salmonella enterica* serovar Gallinarum (*Salmonella* Gallinarum) in Japanese quail *(Coturnix coturnix japonica).*

METHODOLOGY

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria. A total of 160, four-week old Japanese quails (108 males and 52 females) were obtained from the Poultry Unit of National Veterinary Research Institute, Vom, and used for the experiment. They were randomly divided into four groups (A, B, C and D) of 40 birds each. The quails were allowed to acclimatize for two weeks. They were kept in mesh cages of 120 × 140 × 120 cm in size in an enclosed house with the litter changed every week throughout the experimental period. They were fed for two weeks on commercial chick mash, followed by commercial layer

mash throughout the period of the research which lasted 120 days. They were fed and provided with water *ad libitum* during this period.

Before infection, cloacal swabs were collected from all the quails in order to confirm if they were free from *Salmonella* organism. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods (Parmer and Davies, 2007). The bacterium, *Salmonella enterica* serovar Gallinarum, was obtained from the bacterial culture bank of the Central Diagnostic Laboratory, NVRI Vom. The lyophilized bacterium from the culture bank was reactivated by sub-culturing on blood agar (BA) and MacConkey agar (MCA). The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative). Three colonies were scooped and inoculated into 20 ml of nutrient broth and this was incubated for 24 hours at 37^{o}C after which a ten-fold dilution was carried out in test tubes. The colony counts from the test tubes were determined. Challenge of the quails was done orally. Group A quails received a dose of 1×10^6 organisms/0.2 ml of nutrient broth, group B quails received a dose of 1×10^4 organisms/0.2 ml of nutrient broth, while group C quails received a dose of 1×10^2 organisms/0.2 ml of nutrient broth. Group D quails served as control and were not challenged with the bacterium, but were given bacteria-free nutrient broth.

Mortality was recorded. Post-mortem examination was conducted on dead birds, and those not dead at the end of the experiment, were euthanized by cervical dislocation. Thereafter, gross lesions were observed, recorded and photographed. Samples of the liver, spleen, heart, intestine, lung, ovary, pancreas and caecal tonsil showing gross lesions were taken and fixed in10% buffered neutral formalin for at least 48 hours. The fixed tissues were dehydrated in graded concentrations of alcohol (70%, 80%, 90% and 100%) using an automatic tissue processor (STP 120). The tissues were cleared with xylene, embedded in molten paraffin wax block and labelled appropriately

(Oladele *et al.,* 2008). Tissue sections of 5 µm thick were made from the embedded tissues. The sectioned tissues were mounted on a grease-free, clean glass slide, dried at room temperature and stained with haematoxylin and eosin (H & E) stains. The slides were studied using light microscope at ×40 and ×100 magnifications in order to make microscopic morphological diagnoses of the lesions on the slides. Photomicrographs of the lesions on the slides were taken, and labelled appropriately (Hossain *et al.*, 2006).

RESULTS AND DISCUSSION

Mortality was observed in all infected groups (A, B and C), with mortality rates of 35%, 30% and 30%, respectively (Table 1). No mortality was recorded in group D (control).

Table 1: Mortality rate of Japanese quails after infection with *Salmonella enterica* serovar Gallinarum

Day after infection	Mortality rate per group			
	Group A (10^6)	**Group B (10^4)**	**Group C (10^2)**	**Group D (control)**
5	3	-	-	-
7	4	3	3	-
8	3	3	3	-
9	2	1	2	-
10	1	2	1	-
11	1	1	1	-
15	-	1	-	-
18	-	1	1	-
20	-	-	1	-
Total	**14 (35%)**	**12(30%)**	**12(30%)**	-

Table 2: Gross lesions of quails in different groups experimentally infected with *Salmonella enterica* serovar Gallinarum

		Number and percentage of birds with lesions in each infected group		
Organ	**Gross lesions**	**GroupA (10^6)**	**Group B (10^4)**	**Group C (10^2)**
Liver	Hepatomegaly	5(12.5%)	5(12.5%)	4(10%)
Liver	Congested	19(47.5%)	26 (65%)	17(42.5%)
Liver	Bronze colouration	2(5%)	1(2.5%)	1(2.5%)
Liver	Friable	-	-	2(5%)
Lung	Congested	19(47.5%)	26(65%)	17(42.5%)
Lung	Edematous	3(7.5%)	6(15%)	5(12.5%)
Spleen	Splenomegaly	6(15%)	4(10%)	3(7.5%)
Kidney	Swollen	-	1(2.5%)	-
Ovarian follicle	Egg yolk peritonitis	1(2.5%)	1(2.5%)	-
Ovarian follicle	Congested	1(2.5%)	2(5%)	1(2.5%)
Peritoneum	Hydroperitoneum (Ascites)	1(2.5%)	-	-

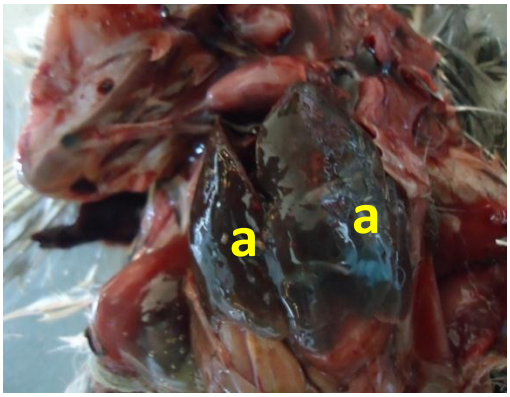

Plate I: Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^4 of *Salmonella enterica* serovar Gallinarum. Note hepatomegaly and bronze liver (a)

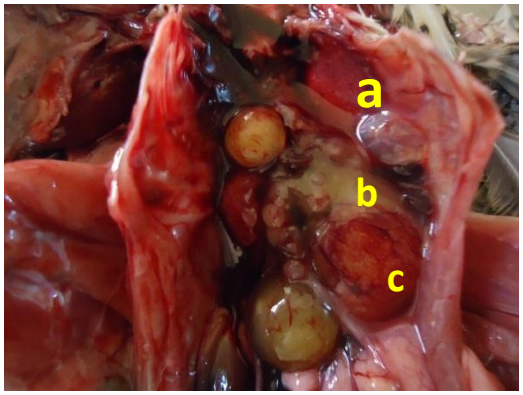

Plate II: Japanese quail *(Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note congested lung (a), egg yolk peritonitis (b) and congested ovarian follicle (c)

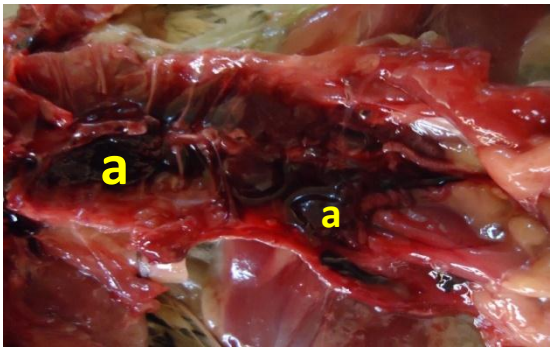

Plate III: Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica serovar* Gallinarum. Note enlarged and

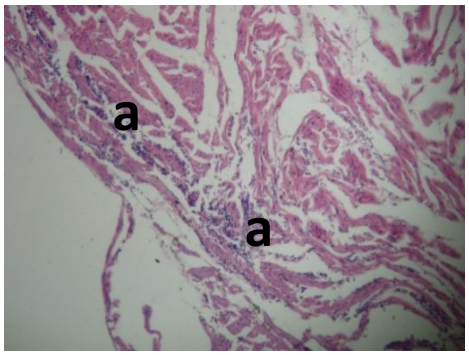

Plate IV: Photomicrograph of the heart of Japanese quail experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note generalized cellular infiltration of the myocardium

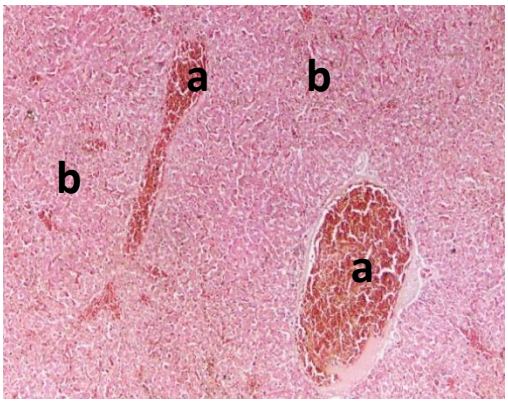

Plate V: Photomicrograph of liver of Japanese quail experimentally infecteted with 10^6 of *Salmonella enterica serovar Gallinarum.N*ote severe congestion (a) and

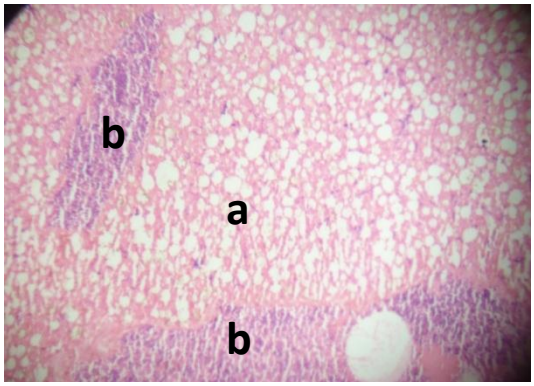

Plate VI: Photomicrograph of liver of Japanese quail experimentally infecteted with 10^6 of *Salmonella enterica serovar Gallinarum.N*ote generalized vacuolation due to fatty degeneration (a) and cellular infiltration (b) H&E x40

s re greement 10- 1996) in his n death, implying that the infection was peracute to acute. The mortality started five days post-infection and persisted till twenty days post-infection. The high mortality recorded in this experiment indicated that Japanese quails are susceptible to *Salmonella enterica* serovar Gallinarum like the chickens. The gross lesions and histopathological findings observed in Japanese quails (*Coturnix coturnix japonica*) in this study were consistent with previous studies in chickens (Garcia *et al.*, 2010). The gross lesions of hepatomegaly, splenomegaly, swollen kidneys, congested liver, lung and ovarian follicle, bronze liver, egg yolk peritonitis, edematous lung and hydroperitonuem (ascites),observed in this study were similar to those reported by previous researchers in chickens (Beyaz *et al.*, 2010; Garcia *et al.,* 2010). The gross lesions are highly indicative of septicaemic infection. The histopathogical findings (cellular infiltration of the liver and heart, vacuolation of hepatocytes and congested liver and lung) in this study were also similar to previous works in chickens (Hossain *et al.*, 2006; Nwiyi and Omadamiro, 2012).

CONCLUSION

This study demonstrates that *Salmonella* Gallinarum caused a septicaemic disease with distribution of the organism in major organs and characterized by high mortality, congestion and enlargement of various organs, in Japanese quails (*Coturnix coturnix japonica*). The pathological findings in this study

indicated that *Salmonella* Gallinarum produces disease in quails similar to that in chickens.

RECOMMENDATIONS

Further study should be carried out on haematogical and serum biochemical changes in Japanese quails infected with *Salmonella enterica* serovar Gallinarum to see what transpire at sub-clinical level of the infection. Further study should be carried out to observed the clinical signs in Japanese quails infected with *Salmonella enterica* serovar Gallinarum.Those keeping quails should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in the quail farm.

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ISOLATION AND ANTIMICROBIAL SUSCEPTIBILTY OF SALMONELLA GALLINARUM IN DAY OLD BROILER CHICKS OBTAINED FROM SOME HATCHERIES AROUND JOS, PLATEAU STATE.

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INTRODUCTION

Salmonella is a genus of rod-shaped, Gram-negative bacteria. There are only two species of *Salmonella; Salmonella bongori* and *Salmonella enterica*, of which there are about six sub-species and innumerable serovars. The genus belongs to the same family as *Escherichia*, which includes the species *Escherichia coli* (Ryan *et al.,* 2004; Rebecca *et al.,* 2000).

Salmonellae are found worldwide in both cold-blooded and warm-blooded animals, and in the environment. *Salmonella* species are facultative intracellular pathogens. Many infections are due to ingestion of contaminated food. They can be divided into two groups—typhoidal and non typhoidal *Salmonella* serovars. Non typhoidal serovars are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and are zoonotic (Jantsch *et al.*, 2011).

Seminar presented 2nd October, 2014 at NVRI Auditorium

Fowl typhoid, caused by *Salmonella* Gallinarum is a septicemic contagious bacterial infection in poultry, causing economic losses, due to performance moderation, and mortality in chicks of all ages (Arun-Prasad *et al.*, 2011). The eradication of this disease is extremely problematic in countries where the ambient temperature necessitates the use of open-sided housing (Chaudhari *et al.,* 2012). Where treatment is necessary a variety of drugs may be effective, including amoxicillin, tetracyclines, potential sulphonamides, spectinomycin, enrofloxacin and other fluoroquinolones. None, however, is capable of totally eliminating infection from a flock. If it is decided to treat an outbreak of salmonellosis, this should always be on the basis of pretreatment sensitivity testing of the organism involved by laboratory test. Although treatment might seem to be effective, a number of birds may become carriers and further antibiotic-resistant *Salmonella* strains or *Escherichia coli* might appear (Mark *et al.,* 2008).

The rough mutant strain *Salmonella* Gallinarum 9R developed 50 years ago has been examined due to its protective efficacy against FT. However, the use of live *Salmonella* Gallinarum 9R vaccine is limited to layer breeds older than 6-weeks It is associated with several disadvantages such as insufficient protection, low growth rate, and residual virulence that can cause hepatitis and splenic lesions in chicks. Therefore, a vaccine that can be safely administered to chickens (especially at a young age) to obtain desired immune responses and offer sufficient protection from FT is needed (Chaudhari *et al.,* 2012).

This study was carried out to determine the presence of the disease in our local hatcheries.

MATERIALS AND METHOD

The samples were collected from four different hatcheries. Two of the hatcheries are located in the eastern part of Jos East Local Government Area, one in the northern part of Jos South Local Government Area and the forth is in the northern part of Barkin Ladi local Government Area. All are situated in Plateau State, Nigeria.

Sample Size and Sampling

Four hundred samples of cloacae and conjunctivae swabs each were collected from apparently healthy broiler day old chicks from four different hatcheries (100 from each hatchery). Also, 20 moribund/dead birds and 20 dead-in-shell eggs were examined from each hatchery totalling 80 each of moribund/dead and dead-in-shell by harvesting the liver, heart, spleen and retained yolk sacs post-mortem aseptically. The samples from each hatchery were collected two weeks apart. Organs from each bird were pooled. This was done in a careful manner to avoid mixing the organs thus minimizing contamination. These organs were placed in separate sterile polythene bags and placed in a crucible and crushed carefully to avoid perforation in order to prevent contamination. The processed tissues were then inoculated into selenite-F broth and incubated at between 37-42^{o}C for 24 hours. These were further incubated on solid media for 24 hours at a temperature of 37^{o}C aerobically.

Serotyping and Anti-biotic Resistance

These tests were carried out at Padova, a reference laboratory, Italy. The antibiotic types used were similar to the ones commonly prescribed by Nigerian clinicians.

RESULTS

Results of the study are as presented on Table I depicting the four hatcheries and the quantity/type of sample collected from the hatcheries. The colonies of *Salmonella* Gallinarum appeared on the media used (*Salmonella-Shigella* agar) as colorless to grey, smooth, moist and entire, as described by Calnek (1997).

Biochemically, *Salmonella* Gallinarum was found to ferment Mannitol, Maltose, Dulcitol, and Glycerol and delayed for Rhaminose. Other sugars used include; Glucose, Sucrose, Urea and Lactose as shown: Motility negative, Glucose negative, Dulcitol positive, Glycerol positive, Mannitol positive, Maltose positive, Rhaminose (positive) and H_2S from TSI negative.

The serovar of *Salmonella* Gallinarum isolated (sero-typed) is the Gr. D1, 1. 9. 12 strain which demonstrates the existence of one serovar in the study area. The infectivity rate of the apparently healthy day old broiler chicks was 3.75%, dead/moribund was 17.3% and the dead-in-shell was 13.75%.

Table I: Total Percentage Isolation of *Salmonella* Gallinarum from all Samples tested from Hatcheries A-D

Chick type	**No. tested**	**No. positive (%)**	**No. negative (%)**
Apparently Chicks	400	15 (3.75)	385 (96.25)
Dead/Moribund Chicks	80	14 (17.3)	66 (82.5)
Dead-in-shell eggs	80	11 (13.75)	69 (86.25)
Total	**560**	**40 (7.14)**	**520 (92.86)**

X^2 of test of association between four Hatcheries and *S. gallinarum* = 18.22, d f= 2, P value =0.0016, at P < 0.05. (Fisher exact test)

The overall isolation rate of *Salmonella enterica var* Gallinarum from the four hatcheries was 7.14%, with highest percentage in the dead/moribund (17.5%), followed by the dead-in-shell (13.75%) day old broiler chicks and the apparently healthy broiler day old chicks (3.75%).

From the sensitivity test results, it appears that the organism isolated (*Salmonella* Gallinarum) was susceptible to most of the antibiotics used, which demonstrate that a wide range of antibiotics can be used in treating the infection fowl typhoid caused by *Salmonella* Gallinarum.

Table 2.0: Standard Zones of Diameter Interpretation of the Antibiotics used For Testing Antimicrobial Resistance of Isolates using the Disc Diffusion Method

S/NO	Antibiotic Type	Antibiotic Concentration	Resistance Zone (mm)	Intermediate Zone (mm)	Susceptible Zone (mm)	Mean Inhibiti zones (mm)
1	Colistin	10ug	≤ 8	9 – 10	≥11	12.41
2	Trimet/Sulfamethoxazole	23.75ug+1.75ug	≤ 10	14 – 17	≥16	27.06
3	Kanamycin	30ug	≤13	14 – 17	≥18	25.94
4	Gentamycin	10ug	≤12	13 – 14	≥15	24.06
5	Cefotaxime	30ug	≤14	15 – 22	≥23	29.88
6	Amoxicillin/Clavulan	20ug+10ug	≤8/4	16/8	≥32/16	34.29
7	Ceftazidime	30ug	≤14	15 – 17	≥18	31.71
8	Ac. Nalidixic	30ug	≤13	14 – 18	≥19	27.18
9	Tetracycline	30ug	≤14	15 – 18	≥19	26.59
10	Ampicillin	10ug	≤11	15 – 16	≥17	25.35
11	Streptomycin	10ug	≤11	12 – 14	≥15	16.29
12	Triple-Sulfa	0.25ug	≤10	11 – 15	≥16	09.47
13	Chloramphenicol	30ug	≤12	13 – 17	≥18	26.36

14	Cefalotin	30ug	≤14	15 – 17	≥18	22.16
15	Enrofloxacin	5ug	≤12	13 – 17	≥18	19.06
16	Ciprofloxacin	5ug	≤15	16 – 20	≥21	23.47

DISCUSSION

Salmonellae are among the ubiquitous organisms in nature (Kwaga, 1987). *Salmonellae* have been isolated in Nigeria around Zaria, Ibadan and in many other parts of the country (Calnek, 1997). Due to the ubiquitous nature of *Salmonella* it can be transmitted trans-ovarially, also the sperm of cocks could also serve as a source of the organism (Seifert, 1996). Prasad *et al.*, (2011) also reported the organism in chicks thus, these statements support the present study.

The organism can be diagnosed based on isolation and identification, biochemical and serology using specific anti-sera (Polyvalent O and H) using slide agglutination tests and by the use of fluorescent antibody techniques (FAT). *Salmonella* Gallinarum has almost similar biochemical reactions with *Salmonella* Pullorum but these can be biochemically differentiated by the following; Pullorum ferments glucose, and delays in the fermentation of maltose while Gallinarum does not ferment any of the above mentioned sugars (David *et al.,* 1998).

The finding of *Salmonella Gallinarum* in healthy doc shows that the organism can be transmitted by day old broiler chicks, thus proving the significance of day-old broiler chicks in the transmission of the infection (fowl typhoid) to other susceptible birds. It is also interesting to note that only the Gr. D1, 1. 9. 12 strain is present in the study area. The reason behind this could be due to the vaccine strain used in that area.

The sensitivity of the organism isolated shows that antibiotic resistance is quite minimal, because it was sensitive to many antibiotics.

It is possible that the poultry or hatcheries were positive due to poor sanitary practices or lack of strict bio-security. There was no proper disinfection of equipment, lack of deeps at their entrances, the workers were not professionally dressed and lots of surrounding bushes were evident at their surroundings. The two hatcheries had dumping pits of waste including dead-in-shell and dead/moribund birds within the surrounding of the pens or hatcheries.

This result demonstrates that day old broiler chicks play a significant role in the distribution of fowl typhoid in commercial poultry production. This result agrees with Seifert, (1996), Komarov, (1932), Maartnaglia, (1929) and Prasad *et al.,* (2011) who all reported a case of *Salmonella* Gallinarum each other than pullorum disease causing death in chicks.

The study has proved that only one strain of *Salmonella* Gallinarum is common in the selected area of study. This will make it easy to control by the use of vaccines protective against that strain.

Further molecular studies could also be carried out to ascertain other findings associated with the *Salmonella* Gallinarum isolated from the hatcheries studied.

CONCLUSION

From this study, it can be deduced that the isolation rate suggests that day old broiler chicks can serve as a source of fowl typhoid, even when they are coming directly from the hatcheries as apparently healthy chicks. The industry must therefore be constantly vigilant to maintain the highest standards of management and disease security at breeding farms, hatcheries, rearing farms feed mills and processing plants in order to prevent the introduction and spread of *Salmonella* both from established serovars and also from new or emerging strains.

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METAGENOMIC PROFILE OF THE VIRAL COMMUNITIES IN RAW AND TREATED WATER IN JOS AND ENVIRONS

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Viruses are ubiquitous in nature with every species of microorganism both vertebrates and invertebrates likely to harbor numerous viruses. These viruses can be found not only within cellular organisms but also in the environment. Oceans, rivers, lakes, and air all contain virions released from infected hosts. Metagenomic studies of the oceans (Williamson et al., 2008), arctic lakes (Lopez-Bueno et al., 2009), stool samples (Kurokawa et al., 2007), and other environments (Vega Thurber et al., 1998) suggest that known viruses are found in unsuspected locations and that a large number of uncharacterized viruses exist in nature.

Recently, versatile metagenomic techniques have been developed to profile all viruses present in an environmental or clinical sample by sequencing virion-associated nucleic acids (Thurber et al., 2009). Simultaneously, the approaches allow comparisons of multiple genomes including those viruses that cannot be cultured. Metagenomics has been used to explore the virus populations in diverse biomes and unique niches throughout the world.

The surveillance of environmental waters used for the purpose of consumption, recreation, cultivation of fish or other activities that have direct relationship with human health needs constant monitoring for pollution. Fecal and urinary pollution of animal origin may constitute a health risk for humans and the industrial contamination when water is not properly treated. Drinking water treatment as pertaining to public supplies involves series of barriers in a treatment chain that varies based on the requirements of the supply and the nature and vulnerability of the source. The procedures involve coagulation and

flocculation, filtration and oxidation. The most common oxidative disinfectant used is chlorine. This provides an effective and robust barrier to pathogens and provides an easily measured residual that can act as a marker to show that disinfection has been carried out, and as a preservative in water distribution

There is therefore a public health requirement for additional parameters that indicate the presence of viruses in waters. Human adenoviruses (HAdV) have been proposed to fill this gap both as indicator and as source tracking organisms (Hundesa et al., 2006), since HAdV have a high stability under environmental stress, such as UV radiation, temperature, chlorine concentration and pH variation, including sewage treatment procedures (Fong and Lipp, 2005). The levels of HAdV found in untreated water match the numbers of the usual faecal indicator *E. coli* (EC), and possibly outnumber intestinal enterococci (IC) (Bofill-Mas et al., 2006). Therefore the development of a simple efficient method for the concentration and quantitation of HAdV is of high interest in respect of indicators, of instruments for source tracking of faecal pollution, and for carrying out risk assessment studies of polluted waters.

Drinking water safety is judged on the basis of national standards or international guidelines. The most important of these are the WHO Guidelines for Drinking-Water Quality. These are revised on a regular basis and are supported by a range of detailed documents describing many of the aspects of water safety. The Guidelines are now based on Water Safety Plans that encompass a much more proactive approach to safety from source-to-tap.

This study was designed to assess the viral profile of water from raw and treated water from Jos and its environs.

MATERIALS AND METHODS

Samples collection site: Untreated water (raw water) was obtained from five locations that serve as feeders to the water treatment plants for the residents of Jos: Lamingo, Shen, Manje Y, NIPSS and Vom dams between March – April 2012. Lamingo and Shen dams provide services to Jos and Bukuru metropolis and the surrounding communities for household and industrial consumption. NIPSS and Vom are also processed for household use and industrial purposes due to the number of institutions located within the area. Besides, some of the dams are also used for irrigation of vegetables for both local consumption and export to other states. Treated water meant for consumption was also collected from the treatment plants for microbiological analysis. Samples were kept at 4^{o}C for 24 h before processing.

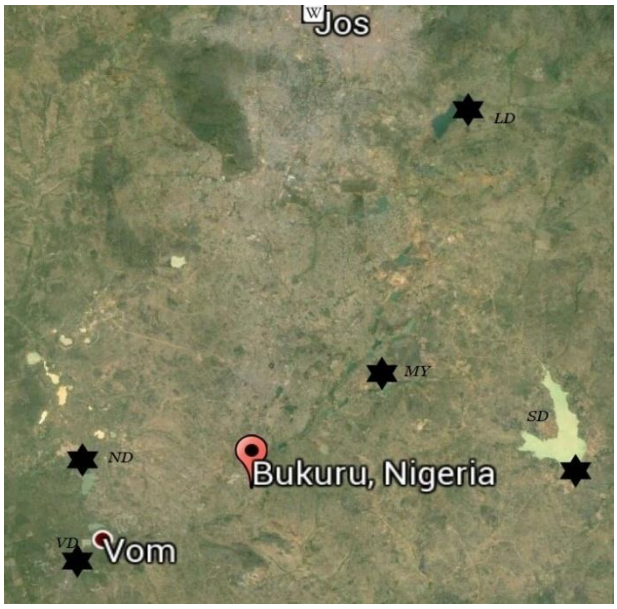

Figure 1: Google map of Jos and environs showing the location of the dams were raw water was collected for this study (LD: Lamingo dam, SD: Shen dam, MY: Manje Y, ND: NIPPS dam and VD: Vom dam).

Enrichment and concentration of virion populations by skimmed milk flocculation: Pre-flocculated skimmed milk solution (1% (w/v)) was prepared by dissolving 10g skimmed milk powder (Marvel™) in 1 L artificial seawater and carefully adjust the pH to 3.5 with 1 N HCl. To every 5 liters of water collected, 100 ml of the artificial seawater solution was added. The samples were stirred

for 8 hr at room temperature and followed by another 8 hr stand on the bench to allow flocs to sediment by gravity. The supernatants were carefully removed using a vacuum pump without disturbing the sediments. The final volume was transferred into a centrifuge cups and centrifuged at 7000 x g for 30min at 4oC. The supernatant was carefully removed and the pellet resuspended in 8 ml of phosphate buffer at pH 7.5. Once the pellet was dissolved the phosphate was added to a final volume of 10 ml and the concentrate stored at -20^{o}C.

Nucleic acid extraction and amplification

Aliquots of the concentrates from both raw and treated water from all the locations were filtered through 0.22 µm and treated with DNase 1000U (Roche®) to remove non-virion associated DNA by incubating at 37^{o} C for 1 hr. DNA and RNA from all the samples were separately extracted using Qiagen DNeasy® Blood and tissue kit (Qiagen, Maryland. USA) and QIAamp® Viral RNA mini Kit (Hilden, Germany) following the manufacturer's protocol, respectively. DNA was then used in the SISPA method which was performed as previously described by Braham et al. (2009). The RNA amplification involves incubation of 10 µl of the extract with 2µl (10uM) of the random SISPA-A primer for 5 min at 65^{o} C and chilled on ice. A reaction mix of 8 µl containing 4 µl of First strand buffer (Invitrogen; Carlsbad, CA), 2 µl solution containing each dNTPs, 1 µl RNase inhibitor (Qiagen Hilden, Germany) and 1 µl AMV reverse transcriptase (Promega; Madison. WI). The reaction was incubated at 25^{o} C for 10 min and at 37^{o} C for 1 hr and chilled on ice. After chilling on ice 0.5 µl of 3' – 5' exo-Klenow fragment (Fermentas, Lithuania) were added and the reaction was incubated at 37^{o}C for 1 hr and the enzyme inactivated at 75^{o}C for 10 min. The resultant product (cDNA) was used for PCR amplification using 2.5 µl of the cDNA, 0,5 µl of the SISPA-A (5' – GTTCCCAGTCACGATCNNNNNN-3'), 2.0 µl SISPA-B (5' - GTTCCCAGTCACGATC - 3'), 25 µl DreamTaq® PCR master mix (thermo scientific, England) and nuclease free water, 20 µl. PCR condition was carried out with the initial 3 min denaturation step at 94oC; followed by 5 cycles of 94^{o} C for 30 s, 37^{o} C for 15 s, 40^{o} C for 15 s, 45^{o} C for 15 s, 50^{o} C for 15 s, 55^{o} C for 15 s, 72^{o}C for 2min, and 35 cycles of 94^{o} C for 30 s,

55° C for 30 s, and 72° C for 2min; and a final extension step at 72° C for 10 min.

Analysis

Each amplified sample was further processed as described for shotgun library preparation in GS FLX 454 technology. The sequencing reads were trimmed to remove SISPA primers and barcodes, and only reads with a length greater than 50 bp were retained. Low complexity repeats were masked using Repeatmasker (RepeatMasker Open-3.0.1996-2010 http://www.repeatmasker.org) and sequences with more than 50% repeats were excluded. The sequences in each pool were assembled using the Newbler assembler version 2.5.3 with default settings (Roche. Genome Sequencer FLX Data Analysis Software Manual.Mannheim, Germany: Roche Applied Science, 2007). Contiguous sequences (contigs) and reads which did not assemble into contigs were categorized using BLASTN and BLASTX homology searches against the non-redundant nucleotide and amino acid databases from NCBI (version June 2011). Taxonomic classification of each contig/read was investigated using MEGAN 4.0

RESULTS

Untreated (raw) and treated water were collected from different locations in Jos and its environs. Water was concentrated and flocculated and DNase treated in other to capture both RNA and DNA viruses. Total nucleic acid were isolated and reverse transcripted followed by deep sequencing (Figure2).

A. **B.**

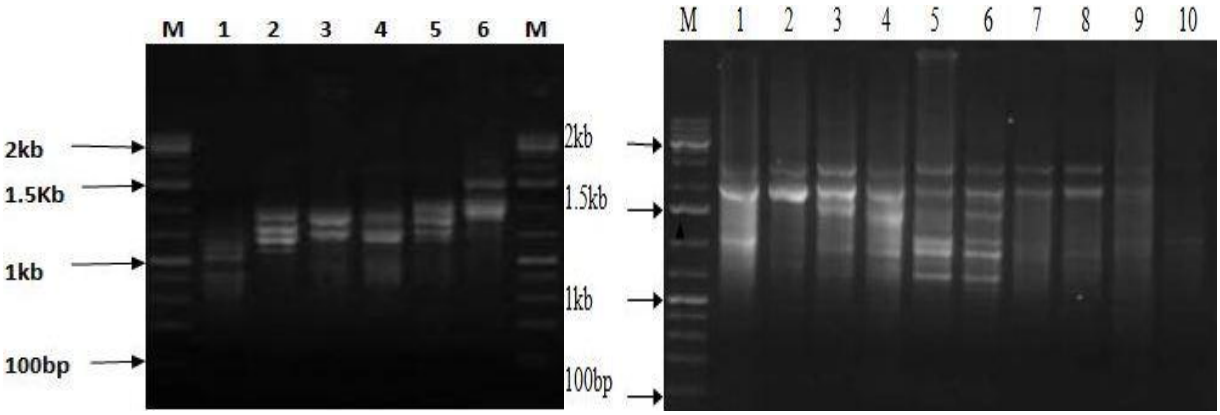

Figure2: The use of SISPA for obtaining the sequence of unknown viruses from water; **A** and **B** indicates RNA and DNA viruses amplification respectively.

We detected 6 viruses known to infect humans from the samples (Table1). These viruses include human adenovirus, a well-studies indicator of human fecal contamination as well as other viruses.

Table1: Viruses discussed in this study

Family	**Virus name**	**Viral type**	**Genome size**	**PCR Type**
Asfarviridae	Asfavirus-like virus	dsDNA	170-190kb	Nested
Adenovidae	Human Adenovirus	dsDNA	36-44kb	Nested
Astroviridae	Human Astrovirus	ssRNA(+)	6.8kb	Nested
Parvoviridae	Adeno-associated virus Human bocavirus	ssDNA	5.2kb	Nested
Picornaviridae	Human parechovirsu 1 Human parechovirus 4	ssRNA(+) ssRNA(+)	7.2-8.4kb	Nested

DISCUSSION

The quality of drinking water and possible associated health risks vary throughout the world. Marked variations in levels of contamination also occur more locally, often as a result of human, agricultural and industrial activities. The differences in health risks that these variations represent lead to different priorities for the treatment and provision of drinking water. Microbial contamination of drinking water remains a significant threat and constant vigilance is essential, even in the most developed countries (Allander *et al.,* 2001).

Pioneering work in viral metagenomics has led to advances in methods for capturing virus particles, sequencing their nucleic acid and computational analysis of metagenomic data. The outcome of several metagenomic studies from oceans, lakes, human stool samples and waste water have consistently revealed the notion of a large number of uncharacterized viruses that exist in nature (Wooley *et al.*, 2010).

The presence of Adenovirus in this study reveals the shortcoming in microbial quality standards of water for consumption (Cantalupo *et al.,* 2011). There is evidence from a number of countries of consumers rejecting microbially safe public supplies, because of problems with discolouration and chlorine tastes, in favour of more expensive and microbiologically less satisfactory local supplies or bottled water. There is little point in making a considerable investment in providing safe public supplies if water is not accepted by consumers. In particular, this can lead to poorer consumers, who are more likely to receive unacceptable water supplies, paying more for their water than better off consumers. Delivering safe and acceptable water, therefore, is a key target in improving public health in many developing countries (Kumar *et al.*, 2013).

There also remains a need for high quality research in a number of areas such as water quality standards, monitoring for contaminants and health associated risk of untreated water. Increased knowledge has shown the complexity of

many of the issues that are related to drinking water and health (Cantalupo *et al.,* 2011).

Overall, however, it is evident that the supply and maintenance of safe drinking water remain key requirements for public health and industrial use since infectious diseases can be transmitted through contaminated water.

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MULTI WALLED CARBON NANOTUBES INDUCE OXIDATIVE STRESS AND DAMAGE TO HEPATOCYTES AND SPERMATOCYTES IN MALE WISTAR RATS

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INTRODUCTION

Carbon nanotubes (CNTs) are a class of molecules that have attracted the attention of researchers worldwide in recent years. This is because of their unique architecture and immense potential in nanomedicine. They are novel tools for the delivery of drugs to target tissues (Beg et al, 2011). Their larger inner volume and biocompatibility make CNTs a superior nanomaterial for drug delivery (Prato et al, 2008). Although literature survey on CNTs suggests that this novel nanocarrier has multiple biomedical applications especially in cancer therapy, proven success in its use for different purposes may not translate to the use of CNTs in medicine for humans. This is because; carbon nanotubes must be proven safe. Unfortunately, inadequate data on its toxicity profile have put a question mark on its successful use. Some researchers have reported multi walled carbon nanotubes to be biocompatible and safe (Foldvari and Bagonluri, 2008). In contrast, others have reported carbon nanotubes to

induce toxicity even at low doses (Patlolla et al, 2012). This contrasting information requires further investigation. Therefore, this research work sought to use biochemical parameters to examine toxicity effects in liver and epididymis associated with *in vivo* exposure to nanotubes in male Wistar rats.

Seminar presented 30th October, 2014 at NVRI Auditorium

MATERIALS AND METHODS

Forty (40) adult male Wistar rats (140-160 g) were procured from the Central Animal House, College of Medicine University of Ibadan, Nigeria for the study. The rats were acclimatized for 1 week and housed eight (8) per cage in plastic cages placed in a well-ventilated rat house, provided feed and water *ad* libitum. Multi walled carbon nanotube (MWCNT) synthesized by NanoLab Inc. (Newton MA, USA) was obtained from Jackson State University, United States of America and used for administration. MWNCNT was administered intraperitoneally every morning for 5 days. Group I served as control and received normal saline. Group II, III, IV and V were administered 0.25, 0.50, 0.75 and 1.0 mg/kg MWCNT. Detailed observations were recorded prior to administration of MWCNT.

All animals were observed twice daily for mortality. Cage-side observations were made daily during the study and any abnormal findings were recorded. The rats were sacrificed after 24 hours of the last MWCNT administration. Blood was collected in non-heparinized tubes and allowed to clot. Serum was then separated by centrifugation of the clotted blood at 3000 rpm for 10 minutes with a table centrifuge. The liver was excised and processed for histopathological examination. Fresh caudal epididymis from each animal was

processed to determine the sperm count and morphology. Homogenates (from liver and epididymis) were used for biochemical analysis.

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT) and Bilirubin were analyzed in serum using Randox reagent kits according to manufacturer's instructions. Lipid peroxidation was determined using the method of Varshney and Kale (1990). The method of Beutler *et al.*, (1963) was followed in estimating the level of reduced glutathione (GSH). Catalase activity was determined according to the method of Sinha (1971). Glutathione-S-transferase activity was determined according to Habig *et al.*, (1974). Glutathione peroxidase assay was determined according to Rotruck *et al* (1973). The level of SOD activity was determined by the method of Misra and Fridovich (1972). Data were expressed as mean ± standard error of mean. Comparative analysis of means was done using ANOVA at 0.05 level of significance with Tukey's-b post hoc test. Using Statistical Package for Social Sciences (SPSS Version 20)

RESULTS

Table 1: Effect of MWCNT administration on serum markers of liver damage

	ALT	**AST**	**ALP**	**Bilirubin**	**GGT**
Control	67.92±0.12	80.00±0.23	103.74±5.75	12.96±0.02	17.08±1.98
0.25mg/kg	70.00±0.07	124.00±0.10*	119.37±10.42	17.01 ±0.02*	18.24±4.16
0.50mg/kg	82.08±0.06*	172.00±0.27*	126.27±8.24*	29.43±0.09*	22.00±2.22*
0.75mg/kg	89.58±0.09*	188.67±0.28*	142.83±8.24*	20.79±0.02*	25.48±3.66*
1.0 mg/kg	87.50±0.17	184.00±0.28*	140.76±19.25	18.36±0.03*	19.40±3.05

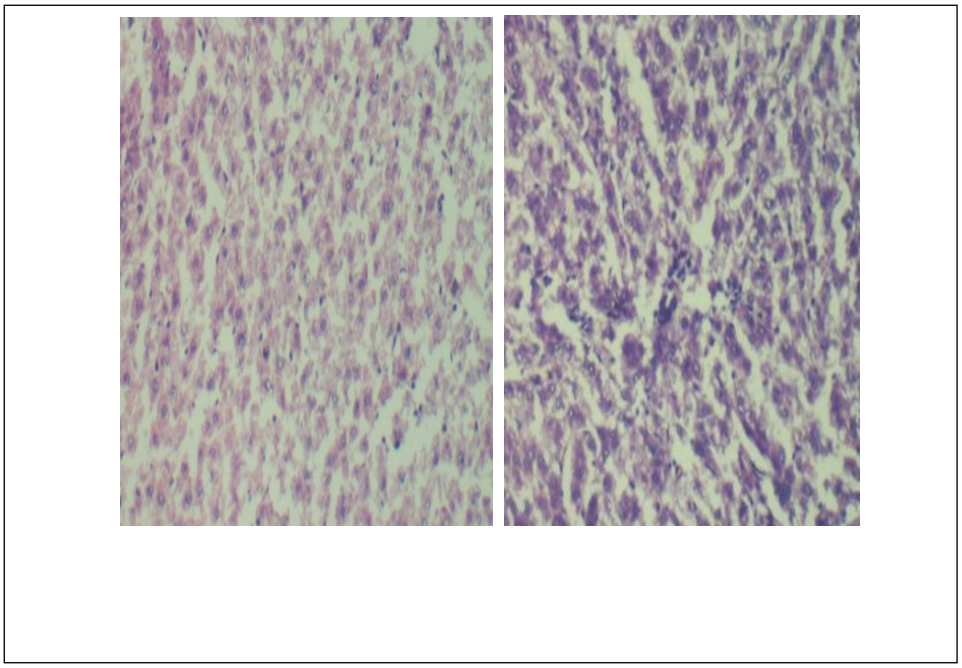
Table 2: Effect of MWCNT administration on oxidative stress markers in liver homogenates

	Superoxide dismutase (SOD)	**Catalase**	**Malon-Dialdehyde**	**Reduced Glutathione (GSH)**	**Hydrogen peroxide generate**
Control	8.67±0.04	76.86 ± 4.97	0.4778±0.09	29.25±1.24	11.06±0.63
0.25mg/kg	6.25±0.03	81.97 ± 1.18*	0.705±0.17*	26.95±1.10*	12.00±0.54*
0.50mg/kg	5.27±0.02*	90.17 ± 9.53*	0.975±0.36*	24.63±0.45*	12.69±0.77*
0.75mg/kg	5.28±0.03*	105.25±21.25*	1.361±0.43*	25.06±2.30*	13.15±0.68*
1.0 mg/kg	5.13±0.04*	110.94±12.43*	1.375±0.51*	25.94±3.41	10.19±0.77

Table 3: Effect of MWCNT administration on oxidative stress markers in epididymis homogenates

	Superoxide dismutase (SOD)	**Catalase**	**Malon-Dialdehyde**	**Reduced Glutathione (GSH)**	**Hydrogen peroxide generate**
Control	4.13±0.02	60.97 ± 6.78	0.172±0.01	23.60±0.20	17.25±0.20
0.25mg/kg	10.19±0.02*	63.18 ± 1.81	0.183±0.01	22.19±0.83*	17.38±0.14

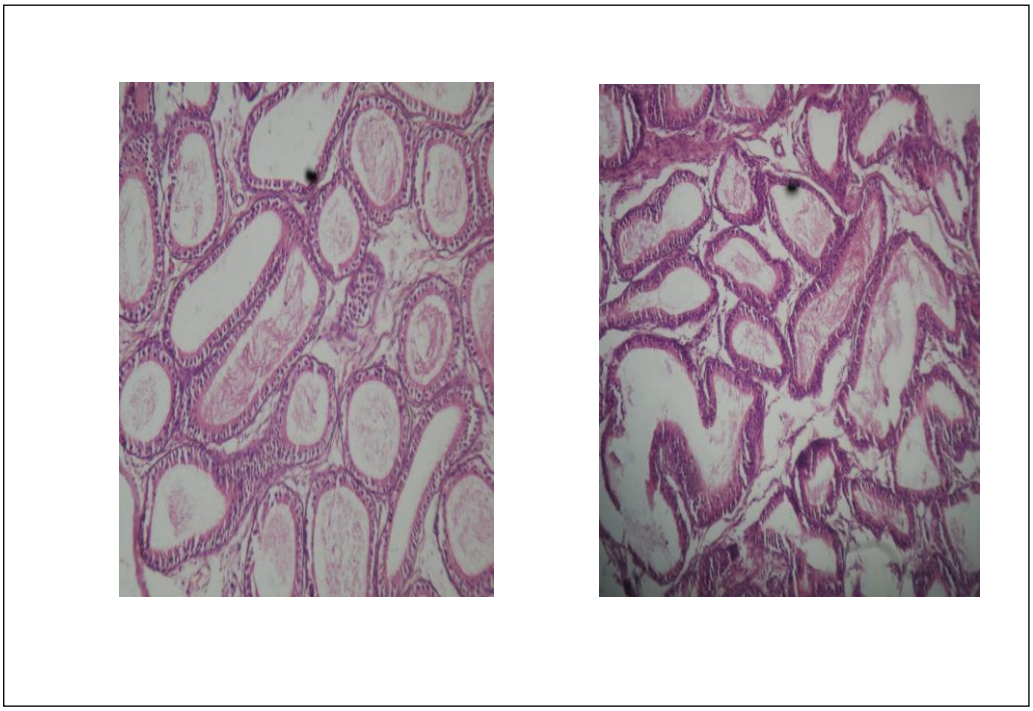
0.50mg/kg	12.99±0.03*	63.94 ± 2.12	0.230±0.02*	21.55±0.43*	18.81±0.69*
0.75mg/kg	15.56±0.02*	70.21 ± 2.77*	0.262±0.02*	20.95±0.46*	18.69±1.25*
1.0 mg/kg	18.50±0.02*	72.02 ± 7.19*	0.166±0.06	20.75±0.32*	18.31±0.55*



Control

0.75 mg/kg administered

Figure 1: Histopathology of the Liver on MWCNT Administration



Control

0.75 mg/kg administered

Figure 2: Histopathology of the Epididymis on MWCNT Administration

DISCUSSION

The increase in serum transaminases (γ-GGT, ALT, AST) suggested damage to liver, possibly through reactive oxygen species (ROS) mediated oxidation of hepatocyte membrane lipids. ROS can oxidize membrane lipids causing damage to membranes and release of cellular constituents (Pantarotto *et al.,* 2004). Increased lipid membrane oxidation on MWCNT administration was evidenced by increased levels of malondialdehyde in liver homogenates, suggesting ROS mediated damage. Malondialdehyde is a product of ROS induced oxidation of membrane lipids (Chen and Yu, 1994). There was also a decrease in glutathione levels with increased dose compared to control, suggesting ROS mediated oxidative stress causing a reduction in cellular glutathione levels to counteract the generated ROS. Glutathione is a known antioxidant in cells which gets depleted during oxidative stress (Sevin et al, 2013). Superoxide dismutase is an enzyme which serves as a first line of defense against ROS mediated oxidative stress. The decrease in SOD observed in the administered groups could be because ROS generated overwhelmed physiological SOD enzymes resulting in denaturation of the enzyme (Comhair et al, 2005).

Histopathological examination of the liver of rats administered 0.75 mg/kg of MWCNT revealed diffuse hepatic vacuolation, diffuse cellular infiltration by mononuclear cells and periportal aggregation of inflammatory cells, while the epididymis of the same animals showed hyperplasia of epithelial cells lining the duct of the epididymis with the lumen containing few spermatozoa.

In conclusion, although Multi walled carbon nanotubes have been reported to be biocompatible and safe, showing promise in targeted drug delivery and nanomedicine, the results of our study shows that MWCNTs induce damage to

hepatocytes and spermatocytes at doses of 0.25, 0.50, 0.75 and 1.0 mg/kg. It is possible that the ability of these nanotubes to penetrate cell membranes alters the transmembrane potential of the cell, resulting in oxidative stress mediated toxicity and activation of proteins implicated in inflammation. This possible mechanism of MWCNT induced toxicity provides evidence for the role of reactive oxygen species in the pathogenesis of diseases.

We recommend that further studies using lower doses of MWCNT should be conducted to examine the effect of sub chronic and chronic exposure. Biomarkers of oxidative stress including malondialdehyde, glutathione, superoxide dismutase, catalase, glutathione –S- transferase, should be included among the Assays carried out in the Biochemistry Division of NVRI. Research in the Institute should examine the role of reactive oxygen species (ROS) and oxidative stress in the pathogenesis of veterinary diseases and the effect of antioxidant phytochemicals in the amelioration of veterinary diseases.

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DOG ECOLOGY, DOG BITE CASES AND MOLECULAR CHARACTERIZATION OF RABIES VIRUS IN SLAUGHTERED DOGS IN NIGER STATE, NIGERIA

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INTRODUCTION

Dog ecology involves studies on dog population density, dog population structure, and pattern of dog ownership (Cleaveland *et al*, 2006) in addition to dog to human interactions. Rabies is a major public health problem in most parts of the developing world, where the domestic dog plays a principal role as a reservoir and transmitter of the disease to humans (WHO, 1992). Therefore better insight into dog ecology would be a useful tool for designing appropriate rabies control strategies in the dog population. In Nigeria, the first laboratory confirmed human and canine rabies was at Rabies Laboratory Yaba in 1912 and 1925 respectively (Boulger and Hardy, 1960). Since then reports of rabies in both animals and humans has been documented. Rabies is a viral disease which causes severe encephalitis and affects all warm-blooded animals causing

over 55,000 human deaths worldwide annually (WHO, 2010). The disease is caused by the rabies virus (RV) which belongs to the genus *Lyssavirus* of the family Rhabdoviridae, in the order Mononegavirales of virus classification (ICTV, 2009). The rabies virion encodes five proteins; the glycoprotein (G), the lipoprotein (L), nucleoprotein (N), phosphoprotein (P) and an RNA-dependent RNA polymerase matrix protein (M) (Warrell and Warrell, 2004). It is these viral proteins when targeted and sequenced using RT-PCR that gives the intrinsic

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and extrinsic relatedness of the RABV and other *Lyssaviruses* (Kissi *et al,* 1995).

Currently cases of dog bite and rabies is occurring in almost all states in Nigeria. Additionally, slaughter of dogs amongst Nigerian ethnic groups is on the increase (Ajayi *et al*, 2006; Dzikwi, 2008; Garba *et al*, 2010). For an effective control of rabies in any location, the causative virus has to be known, the principal reservoir host (dog) has to be studied and the ecosystem that permit their interaction (between agent and host) has to be investigated (dog ecology). Studies on dog ecology in Nigeria have been attempted by some workers (Okoh, 1986; Oboegbulem and Nwakonobi 1989; El-Yuguda *et al.,* 2007; Hambolu *et al*, 2014). However, there is paucity of information on dog ecology and rabies in Niger State in spite of an estimated dog population of over 199,812 (RIM, 1992), hence this study.

MATERIALS AND METHOD

Study area/Study design

A cross-sectional study was conducted in Niger State, Nigeria, between January-July 2012. The state is located between Latitudes 8° 30' N and 11° 30' N and between Longitudes 3° 30' and 7°20'E, comprising of three senatorial

zones (Bida, Kontagora and Minna) with 26 local government areas. The state has a total human population of over 3.9 million (NPC, 2006) with abundant livestock resources having 199,812 dog populations in the state (RIM, 1992).

Sample size: Sample size was determined based on previous 28% prevalence report of rabies antigen in brains of slaughtered dogs (Garba *et al*, 2010) from Sokoto and Katsina states and using the mathematical expression as described by Mahajan (1997).

Questionnaire survey on dog ecology and management

A total of 300 structured questionnaires were distributed in the three senatorial districts of Niger State. The questionnaire comprises of 4 sections with a total of 40 questions on demography of respondents, dog population, feeding and health management and dog bite cases. Four major towns; Bida, Kontagora, Suleja and Minna were conveniently selected. In every selected town, a major road that crosses the town was first identified and from one end towards the other, every third street radiating from the road were selected from the left and right hand sides of the road using systematic randomization method. In the same way every third house/compound (on the left and right) in the selected streets were used to administer the questionnaires to the most senior and enlightened member of the family in the households. Of the 300 questionnaires distributed 237 were returned. Of the 237 returned, 30 were discarded due to lack of merit, 207 were actually used. Data were entered into Microsoft excel 2010 spread sheet, cleaned and coded then Imported into SAS statistical program version 9.3 to generate the frequencies of variables. Information generated was summarized into tables and chart using Microsoft office tools.

Data collection on hospital records of dog bite and laboratory confirmation of dog rabies

A total of nine General Hospitals and two tertiary health providers (all government owned) were randomly selected in the state using simple

randomization, the selected hospitals were visited across Niger State. Data from the records of dog bite cases between January 2006 and July 2013 in these hospitals were retrieved. All records of cases reported to the clinic with dog bite or suspected rabies were considered as dog bite cases and included in the research. Information sought in the cases included date of bite, sites of bite, sex and age of victims. Similarly, the records of rabies confirmatory diagnosis at NVRI, Vom-Nigeria between January 2006 and July, 2013 were retrieved. These data were analyzed using descriptive statistics and information/results were presented in tables.

Dog brain samples collection, shipment and Laboratory analyses

A total of 471 dog brain samples were collected from the three senatorial (Bida, Minna and Kontagora) zones of the state where dogs are being slaughtered for human consumption.

Samples were initially shipped on ice pack to virology laboratory, Department of Veterinary Public Health and Preventive Medicine, A.B.U – Zaria and stored at -20^0 C until used. Later samples were shipped on dry ice to Rabies Program, Center for Disease Control and Prevention (CDC), Atlanta, Georgia, USA for further analyses. Direct Fluorescent Antibody Test (FAT) as described by Dean *et al* (1996) and Direct rapid immunohistochemistry test (DRIT) as described by Neizgoda and Rupprecht (2006) respectively were conducted on dog brain samples. Samples that were positive and questionable by FAT and DRIT were used for RT-PCR and sequencing/phylogenetic analyses as previously described by David *et al* (2007) with some modification as recommended in the RT-PCR protocol designed by CDC, USA (CDC, 2012)

RESULTS

Results indicated that there is a population ratio of 1:5.4 dogs to humans and 1:1.9 female dogs to male ratio with an estimated 732,476 dog population in Niger State (table 1). Most of the dogs (58.6%) are kept for security reasons, but

52% of dogs are not housed/confined and majority of the dogs stray in the night (52.4%) and evenings (23.8%) at homes. Responsibility to dogs in terms of welfare, mostly (61.5%) lies on everybody in the family with 61% of dogs being fed on family left over. About 30.4% of dogs are never vaccinated and 31% of the respondents (or their family members) have been inflicted with dog bite but in only 28.1% of cases received post-exposure-prophylaxis (PEP). Only 59% respondents took their dogs to clinics when sick. Hospital records of dog bite cases shows that about 47% of cases were in children below the age of 15 years with 66.8% of victims being males (table 2). Also, 81.2% of bites were inflicted on the legs with the highest proportion of 17.8% (40 out of 223) cases of bites seen in the year, 2012. No rabies confirmatory diagnosis at NVRI, Vom from Niger State throughout the study period. Of the 471 dog brains analyzed, only 3(0.63%) were positive for rabies antigen all belonging to Africa 2 phylogroup and have 98 to 99% identity with those found in Burkina Faso, Chad and Niger Republics (figure 1). Two of the 3 variants have never been reported in Nigeria and one of the 2 forms a new lineage.

DISCUSSION

Based on the results, there is an increase in dog population density from 1:12.4 dog to human ration in 1991 to 1:5.4 in the present report. This Suggest more interaction between dogs to humans thereby increasing possible risk of contracting rabies in Niger State. Male dogs are prone to contracting rabies than females (Garba *et al,* 2005), thus having over 65% of dogs being males will increase the risk of human rabies. Keeping of over 58% of dogs for security purposes may be as a result of increased insecurity in the country and indeed the study area over the last two decades. Having 52% of dogs not confined will favour straying/free roaming; this was evident when over 75% of dogs strayed in the nights and evenings. Responsibility to dogs has been shown to lie on everybody (61.5%) in the household and over 61% of dog's feed are from the family leftover. This will certainly increase scavenging of dogs for food and increase risk of stray/free roaming dogs to human interaction with increase

exposure to dog bite. This was evident when 31% of the respondents or members of their households shown to have been inflicted with dog bite in the present report. The retrival of 223 dog bite cases in the hospital records affirmed the occurrences of dog bite in the study area. The occurrence of over 47% of dog bite cases in children below the age of 15 years has supported the assertion of the World Health Organization that children below the age of 15 years are vulnerable to rabies world over (WHO, 2010). Occurrence of over 66% of bites on males may be due to outdoor activities of males in the study area which expose them more to meeting stray dogs than the female counterparts. The occurrence of over 81% of bites on the legs may enhance misdiagnosis of rabies due to possible long incubation period that may exist. Victims may attempt to cleanse the bite site and never care for PEP after few days or weeks. However, after several months or years when the virus is in the brain (if the biting dog was rabid), most of the relatives of or even the victim will not associate the present ailment to the previous dog bite and rabies may be misdiagnose for evil spirit or any neurologic disease.

Detection of 3 (0.64%) positives out of 471 dog brains screen has confirmed the presence of rabies in the study area. The low proportion (0.64%) of positive may be because the samples were not suspect materials but from apparently healthy looking dogs destined for human consumption. But the implication of this finding is the possible exposure of dog butchers, pet owners, hunters with dogs and the Veterinarians to unrecognized rabies carrier dogs due to their occupational manipulations. It was shown that only one of the 3 positive rabies variants was previously reported in Nigeria but the other 2, one was previously reported in Niger republic and the other closely related to those clusters reported in Chad republic and it forms a new lineage. The presence of these foreign variants may be due to dog trade and slaughter.

CONCLUSIONS

- Dog population and structure will enhance the spread of dog bite/rabies in Niger State
- New lineage '276 Nigeria 2012' has emerge in Nigeria and Nigeria 4 sub-group is hereby proposed
- Trans-boundary spread of rabies variants through trade and slaughter of dog is further established (252 is a variant from Niger republic).

RECOMMENDATIONS

- Further dog ecological studies in urban and rural areas of Niger State should be conducted
- Field and laboratory based surveillance of rabies in transit, trade(slaughtered), own and stray dogs in the state and West African sub-region is proposed

Table 1: Dog population and structure in Niger State, Nigeria

	Variables	**Frequency**	
		No.	**%**
a.	**No. of compounds visited**	207	
	No. of persons	1965	
	No. of dogs	366	
	Man to Dog ratio	5.4 : 1	
	Estimated Human*: Dog population	3,955,372* : 732,476	
b.	**Sex distribution of dogs**		
	Male	238	65
	Female	128	35
	Male to Female ratio	1.9 : 1	
c.	**Age distribution of dogs**		
	< 1 year old	120	32.8
	1 – 5 year old	155	42.3

> 5 year old	91	24.9
d. **Breed of dog**		
Native	88	60.3
Exotic	37	25.3
Mixed	21	14.4

Key: No. = number, % = percentage, * = estimate based on NPC census, 2006

Table 2: Sex and age distribution of dog bite victims in Niger State, Nigeria January, 2006 – July, 2013

Variable	Frequency	Specific rate
Sex		
Male	149	66.8%
Female	74	33.2%
Total	223	100%
Age		
< 15 year old	103	46.2%
> 15 year old	120	53.8%
Total	223	100%
Site of bite		
Leg	181	81.2%
Hand	32	14.3%
Face	5	2.5%
Multiple areas	5	2.5%

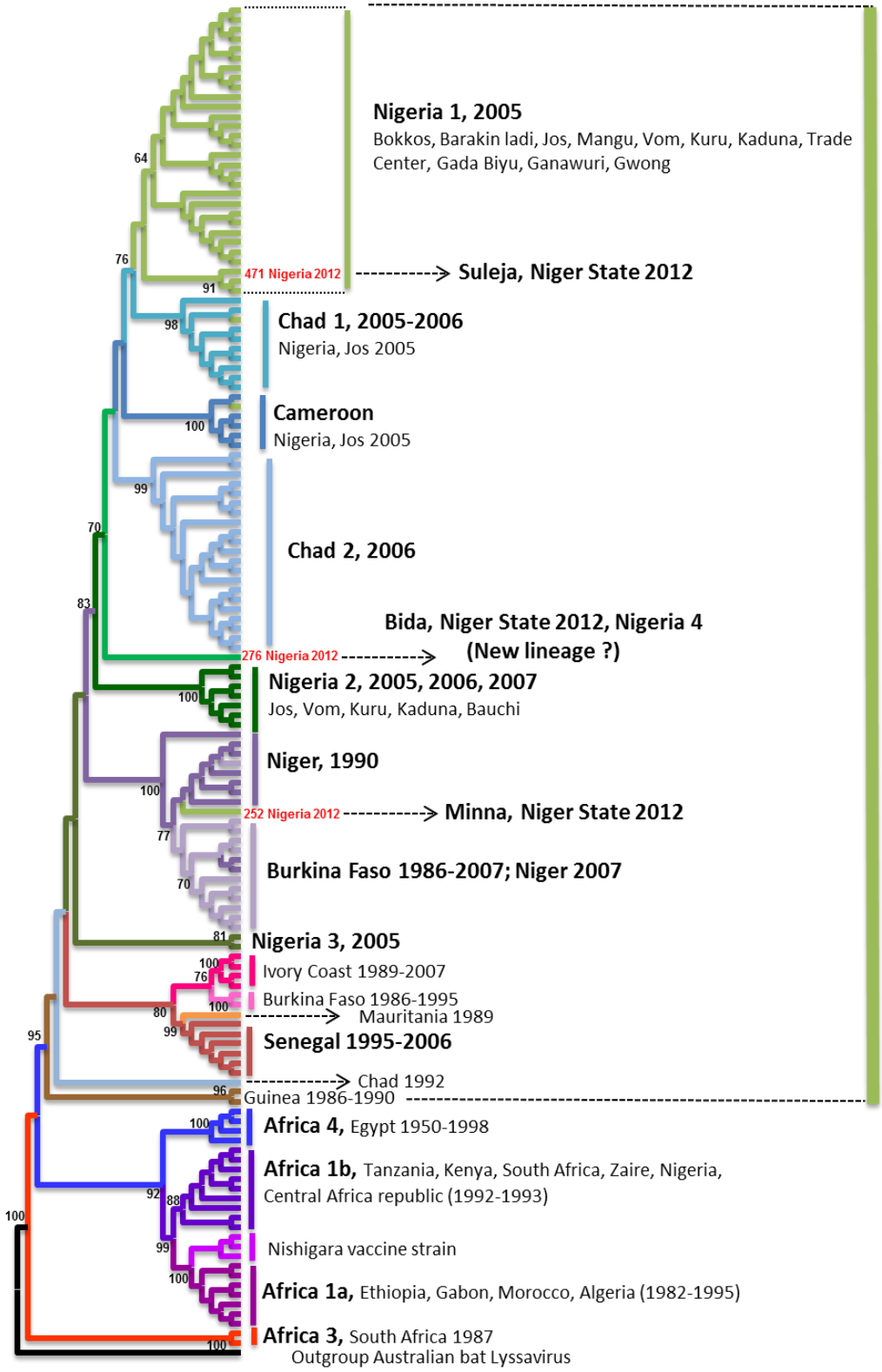

Figure 1: Phylogenetic tree of 3 positive samples (labelled in red colours) with the rest of Africa

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PREVALENCE OF RABIES ANTIGEN IN APPARENTLY HEALTHY DOGS SLAUGHTERED FOR HUMAN CONSUMPTION IN PLATEAU STATE.

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INTRODUCTION

Rabies has almost 100% case fatality rate, it is an incurable though a preventable viral zoonotic disease that causes acute encephalomyelitis in all warm blooded mammals and survivors are extremely rare (Alvarez *et al.*, 1996). It is one of the most important and widespread zoonotic diseases and a global dilemma (Blancou, 1988). The disease accounts for the death of over 50,000 people worldwide, Africa and Asia were apportioned 95% of these fatalities (WHO, 2010).

Current trend in rabies epidemiology indicates that the disease is spreading in many regions, mainly owing to increasing density and mobility of human and animal host populations (Krebs *et al.,* 1999). In Nigeria, dog meat consumption is very common in states such as Plateau, Akwa Ibom, Cross River, Kaduna, Kebbi and Ondo. Dog "suya" (barbequed dog meat) is sold publicly in the dog eating areas (Ajayi *et al.,* 2006). In some areas such as Jos, only local and seasoned connoisseurs easily distinguish restaurants where dog and other

conventional meats are sold and there seems to have been little or no local effort to evaluate the risk of rabies transmission (Ajayi *et al.,* 2006).

Rabies is caused by an enveloped, single-stranded, non-segmented, "bullet" shaped and negative sensed RNA virus that belongs to the family; *Rhabdoviridae* and the genus; *Lyssavirus*, the disease is characterised by an acute and almost fatal encephalomyelitis in individuals who have been infected by a rabid animal (Paez *et al.,* 2002). Once clinical signs of rabies develop, it is invariably fatal and there is no cure (WHO, 2006). For rabies diagnosis, the direct fluorescent antibody (DFA) test is most frequently used and the standard test approved by World Health Organisation (WHO) and Office International des Epizootics (OIE) (Dean *et al.,* 1996). This test is performed post-mortem on brain tissue from animals suspected of being rabid. The aim of this study was to detect the presence of rabies virus antigen in the brains of apparently healthy dogs slaughtered for human consumption, in Plateau State Nigeria.

METHODOLOGY

Study Areas

Plateau State is located in Nigeria's middle belt. With an area of 26,889 square kilometres, and has an estimated population of about 3.5 Million people (Blench *et al.*, 2003).

Three local government areas, Jos South, Kanke and Shendam known for dog trade and dog meat consumption, were conveniently selected for the study (fig 1)

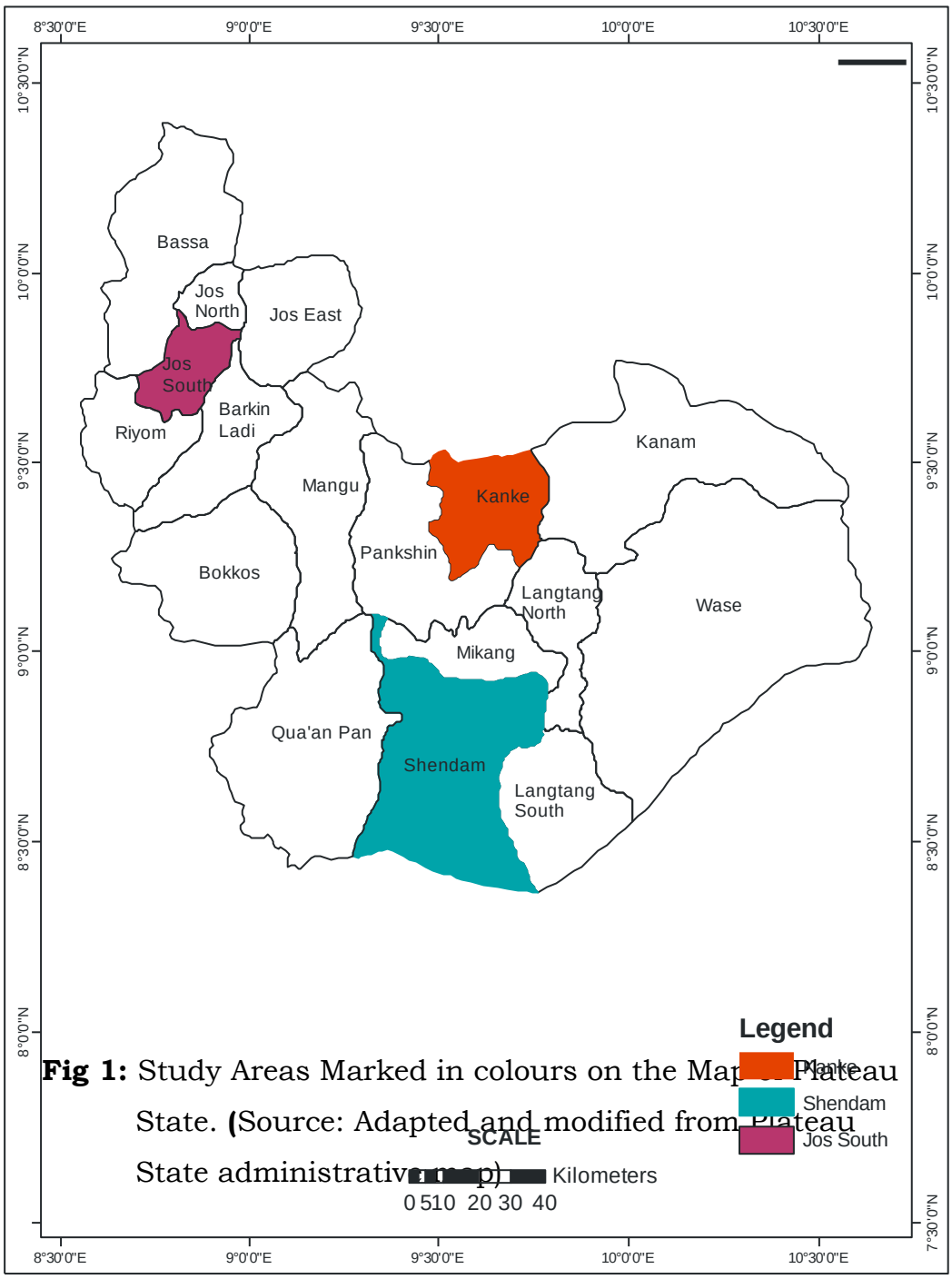

Fig 1: Study Areas Marked in colours on the Map of Plateau State. **(**Source: Adapted and modified from Plateau State administrative map)

Sample Collection and Processing

Sampling method: Convenient random sampling as described by Mike (2011) was employed.

Sample size

a. The sample size for dog brains was determined using the formula by Thrusfield (2007)

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Dog brain sample collection

A total of 203 dog heads were purchased from dogs bought and slaughtered in dog markets in three local government areas spread across the three senatorial zones in the State over a period of five months (June to October, 2012). One hundred and forty-six (146) dog heads were purchased from Jos South LGA, 48 from Kanke LGA and nine (9) from Shendam LGA; all the dogs were adults. Information on the source, sex and breed were obtained.

Heads of the slaughtered dogs were purchased were transported to the Rabies Laboratory of the National Veterinary Research Institute (NVRI) Vom, their brains were removed as described by Atanasiu (1975). Samples of hippocampus and cerebellum were obtained, placed in pre-labelled EDTA free bottles and stored at –20°C in the Rabies Laboratory of NVRI Vom, until they were processed.

All the samples were later transported on ice pack to the Viral Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for fluorescent antibody test (FAT).

Laboratory Analysis:

Fluorescent antibody test (FAT) procedure

This was carried out as described by Dean *et al.* (1996). Using light diagnostic rabies fluorescent antibody conjugate (FITC ANTI- RABIES MONOCLONAL

GLOBULIN) LOT No. 9C03711 reagent from FUJIREBIO Diagnostics Inc. Malvern, P.A. 19355, USA was used and the working reagents dilution prepared according to the manufacturer's recommendation as described by Flamand *et al.* (1980). A known rabies positive dog brain sample (positive control) and a known negative dog brain sample (negative control) were included in the test as controls. Impression smears of the samples were made on glass slides, air-dried and fixed in cold acetone for 30 minutes. The smears were air dried, stained with the anti-rabies conjugate and incubated for 30 minutes at 37°C. The slides were then washed twice in working phosphate buffered saline (PBS), for five minutes each then, air dried and examined by a fluorescence microscope (MEIJI TECHNO, MT 6000 microscope, Saitama, Japan) at X20 magnification. The presence of clear cut rounded dots or clusters of apple green fluorescence on a dark background indicated the presence of rabies antigen.

RESULTS

Rabies Antigen in Dog Brain

Of the 203 samples of dog brains collected, 10 (4.93%) were positive for rabies virus. Five (4.95%) of the male dogs and 5 (4.90%) of the female dogs tested positive for rabies virus antigen (Table 1)

Of the 146 dog brain samples collected from Jos south LGA, 5 (3.42%) were positive. For Kanke LGA, 48 dog brain samples were collected, 4(8.33%) were positive and of the 9 dog brain samples collected from Shendam LGA, 1 (11.11%) was positive (Table 1).

Of the thirty-three dogs sourced from Zamfara State, 3(9.10%) were positive while from Plateau State 23 dogs were slaughtered but 2 (8.60%) were positive. Dogs sourced from Chad Republic, Kano, Borno, Bauchi, and Yobe states had one positive sample each. Thirty one dogs were sourced from Niger Republic 19 sourced from Niger state, none was positive for the rabies virus (Table 1).

Table 1: Distribution of dog brain positive for rabies antigen by Sex, LGA and Source of specimens in Plateau State Nigeria

Characteristic	Number Sampled	Number of positive samples (%)
Sex		
Males	101	5(4.95)
Females	102	5(4.90)
Total	203	10(4.93)
L.G.A		
Jos South	146	5(3.42)
Kanke	48	4(8.33)
Shendam	9	1(11.11)
Total	203	10(4.93)
Source (Reported by processors)		
Kano State	46	1(2.17)
Zamfara State	33	3(9.10)
Plateau State	23	2(8.60)
Niger State	19	0(0.00)
Borno State	13	1(7.60)
Bauchi State	18	1(5.55)
Yobe State	12	1(8.33)
Niger Republic	31	0(0.00)
Chad Republic	08	1(12.50)
Total	**203**	**10(4.93)**

DISCUSSION

The presence of rabies antigen in the brain of slaughtered dogs in this study agreed with earlier studies that apparently healthy dogs could carry rabies virus in their saliva for a long period without showing signs of the disease Fekadu (1975). In general, many reported cases in Africa and elsewhere showed that clinically rabid dogs may not die but recover and continue to live

as carriers Fekadu (1975). This study, established a prevalence of 4.93% of rabies virus in slaughtered dogs in Plateau State using the direct fluorescent antibody test (FAT). Jos South LGA has the largest number of specimens which can be attributed to daily slaughtering of dogs at the dog market ("Kasuwan Kare") with the exception of Tuesdays and Sundays. Unlike in Kanke LGA where majority of the dogs are slaughtered only on Thursday which is the market day for the Dawaki market and Saturday is the market day for the Amper market. There is no dog market in Shendam LGA; the dogs are usually slaughtered in the houses of the dog butchers. Obviously from the results of this study some of the dogs imported into Plateau State are carriers of the rabies virus. The following researchers: Garba (2007) in Sokoto State, Akombo (2009) in Benue State, Sabo, (2009) in Plateau State, Aliyu *et al.,* (2010) in Adamawa State, Audu (2011) in Kaduna State, and Isek (2013) in Cross-Rivers State, reported the presence of rabies virus in slaughtered dogs. The findings of this work is similar to the work of Audu (2011) in Kaduna State, and Isek (2013) in Cross-Rivers State, who reported prevalence of 6% and 3.4% respectively.

CONCLUSION

It was concluded from this study that rabies antigen was present in the brain of dogs slaughtered for human consumption in Jos South, Kanke and Shendam LGA of Plateau State of Nigeria.

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SEROLOGICAL EVIDENCE OF DUVENHAGE VIRUS ANTIBODIES IN BATS AND RABIES VIRUS ANTIBODIES IN HUMANS IN NIGER STATE, NIGERIA

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INTRODUCTION

The genus *Lyssavirus* is a group of single-stranded, negative-sense RNA viruses that cause rabies. Rabies virus (RABV) is the prototype *lyssavirus*, and all subsequently discovered *lyssaviral* species are collectively known as the rabies-related *lyssaviruses* (Markotter *et al*, 2013).The genus *Lyssavirus* contains up to 11 genotypes (ICTV, 2009) and the 12th member Shimoni bat virus and the 13th member Bokeloh bat lyssavirus were newly proposed in 2010 (Kuzmin *et al*, 2010) and in 2011 (Freuling *et al*, 2011) respectively. The first member classical rabies virus (RABV) genotype 1 has a worldwide distribution, while Lagos bat virus (LBV) genotype 2, Mokola virus (MOKV) genotype 3 and Duvenhage virus (DUVV) genotype 4 have distribution restricted to Africa (ICTV, 2009). All *Lyssavirus* cause illness in humans indistinguishable from classical rabies except *Lagos bat virus* (Smith, 1996). The DUVV was first discovered in 1970 when a South African farmer (after whom the virus is named) died of a rabies-like encephalitic illness after being bitten by a bat (Tignor *et al*, 1977).

Seminar presented 13th November, 2014 at NVRI Auditorium

Human fatalities due to Duvenhage virus have been reported in South Africa (Paweska *et al.,* 2006), and Amsterdam in 2007. *Nycteris thebaica* is the only bat species linked with DUVV infection. More recently, Virus-neutralization assays indicated DUVV-neutralizing antibodies in 30% of the sera collected

from this population of *N. thebaica* in Swaziland making the 6th reported evidence of DUVV (Markotter *et al*, 2013).

The virus neutralization methods (rapid fluorescent focus inhibition test or RFFIT and fluorescent antibody virus neutralization or FAVN) are the current reference methods prescribed by WHO and OIE (Smith *et al,* 1996; OIE methods, 2000; Stantic- Pavinic *et al,* 2006).

So far no report of DUVV was documented in Nigeria or West Africa. However, serological evidence of rabies virus antibodies has been reported in 15.93% of unvaccinated dogs in South-western Nigeria (Aghomo *et al*, 1987). Furthermore rabies neutralizing antibodies have been reported in 28.7% of unvaccinated humans in South-western Nigeria (Ogunkoya *et al*, 1990). About 10 million people receive post-exposure treatments each year after being exposed to rabies-suspect animals (WHO, 2006). The serological evidence of DUVV and RABV neutralizing antibodies in bats and humans respectively in the study area is hereby reported.

MATERIALS AND METHODS

A total of 262 fruit – eating bats (Epomorphorus gambiense) were captured by shooting using local shot guns with low density bullets (by hunters) from roosting sites across Niger State Nigeria between March and July, 2012. A 1.5 to 2ml of blood was collected from bats through heart puncture. Sera samples were collected by centrifugation at 3000 rpm for 10 minutes, sera collected and transferred to pre-labeled vacutainers and stored refrigerated until shipped. Similarly, 2mls of blood samples each were collected from 185 human volunteers in rabies occupational risk group during same period in the study area. A consent form and a questionnaire were filled by each volunteer prior to blood collection. Human samples were processed as earlier described for bats. The 162 bat sera and 185 human sera with the questionnaires were shipped to Rabies Unit, Centers for Disease Control and Prevention (CDC), Atlanta, USA. A modified and standard RFFIT tests were conducted as described by Smith *et al*

(1996) on bat and human sera respectively in accordance with the CDC SOP for RFFIT (CDC, 2006). End point titration was conducted on the human positive samples and potency of the rVNA was calculated based on Reed and Maunch method (Reed and Muench, 1938) as described in the CDC SOP using the expression below:

$$\text{Number of IU/ml} = \frac{\text{End-point titer of the test serum}}{\text{End-point titer of the reference}} \times \text{2 IU / ml in the reference serum}$$

Statistical analysis

Data were analyzed using descriptive statistics and results presented in tables

RESULTS AND DISCUSSION

Of the 162 bats sera collected, 17 samples were not processed and 2 samples were cyto-toxic to the cell line. Only 144 samples were analyzed for the presence of Duvenhage virus and only 3 out of 144 (2.1%) viable sera samples showed detectable antibody titres for Duvenhage virus (table 1). All the 3 samples were from male bats. This is the first report of presence of DUVV antibody in Nigeria. All the 3 positive samples were from Epomorphorus spp (fruit eating) of bats, probably because they constituted over 95% of the total bat species sampled for this study. Serological evidence of circulating Lagos bat virus in fruit eating bats (*Eldelon helvum*) has previously been reported (Dzikwi *et al*, 2010). However, this is the first report of serological evidence of DUVV antibody in fruit eating bat (*Epomorphorus gambiense*), all the previous reports were identified to have occurred in small insectivorous bats (*Nycteris thebaica*) (Markotter *et al*, 2013).

Table 1: Bat species with detectable antibodies against Duvenhage virus by Modified RFFIT test.

Species	No. Bats	Serum	Serum	No.	No.	No.

	captured	collected	Not done	Cyto-toxic	done	Positive (%)
Epomophorus	254	151	11	2	138	3
Micropteropus	13	11	5	0	6	0
Total	**267**	**162**	**17**	**2**	**144**	**3 (2.1)**

The human sera RFFIT results revealed that about 16.4% of rabies occupational risk humans had some reasonable titre of rabies virus neutralizing antibodies (rVNA) in their body with higher specific rates seen in butchers and pet owners (tables 2). This suggests prior exposure of these individuals to rabies viral antigen either at once or repeatedly in smaller doses due to their occupation or hobby. The detection of rabies antibodies in unvaccinated dogs and humans has since been reported by some workers in Nigeria (Aghomo *et al.,* 1987; Ogunkoya *et al.,* 1990). It has been shown that 25% each of dog butchers and pet owners, 20% hunters and 14.8% dog meat consumers tested had detectable rabies virus neutralizing antibodies (rVNA) (table 3). This shows that dog butchers and pet owners have greater tendencies of being infected with rabies.

Table 2: RFFIT rabies antibody detection on dog butchers and others

Volunteers	Total number collected	Cyto-toxic	Negative	Positive (%)
Butchers	12	4	6	2 (25)
Dog meat consumers	113	32	69	12 (14.8)
Pet owners	24	8	12	[1]*4 (25)
Hunters	6	0	5	1 (20)
Veterinarians	6	0	4	*2 (8.7)

Anim. Personnels	Hlth.	25	1	21	*2 (13.8)
Total		185	44	117	23 (16.4)

Key: * = they were previously vaccinated, [1]* = 1 previously vaccinated

However, few of these positives (5 out of 23 positives) in the present study, from the questionnaires results had history of anti-rabies vaccination over 10 years earlier with high titres presently (table 3). This may suggest that the presence of rVNA in these individuals may be due to previous vaccinations received. Particularly that some reports have indicated the presence of rVNA in an unvaccinated fox trapper with high titre of 2.30IU/ml in Alaska (Follman *et al,* 1994) and in some villagers in Peru (Gilbert *et al*, 2012) .

Table 3: Rabies antibody titre for all the RFFIT positive human volunteers in Niger State, Nigeria

S/no.	Sample no.	Group	Sample date	Titer IU/ml	Location of volunteer

1.	001H	DB	26 May 2012	5	0.05	Bida
2.	007H	DC	26 May 2012	7	0.07	Bida
3.	019H	DC	26 May 2012	11	0.11	Bida
4	020H	DC	26 May 2012	11	0.11	Bida
5.	031H	DC	27 May 2012	65	0.65	Minna
6.	032H	DB	27 May 2012	65	0.65	Minna
7.	040H	PO	28 April 2012	8	0.08	Minna
8.	045H	DC	28 April 2012	13	0.13	Minna
9.	048H	DC	24 May 2012	42	0.42	Bida
10.	049H	PO	24 May 2012	11	0.11	Bida
11.	073H	DC	24 May 2012	29	0.29	Bida
12.	076H	DC	24 May 2012	11	0.11	Bida
13.	092H	DC	31 May 2012	13	0.13	Minna
14.	096H	PO**	1 June 2012	7	0.07	Minna
15.	108H	DC	1 June 2012	11	0.11	Bida
16	111H	HT	2 June 2012	13	0.13	Bida
17.	114H	DC	2 June 2012	7	0.07	Bida

18.	127H	PO	5 June 2012	13	0.13	Minna
19.	139H	DC	22 June 2012	70	0.7	Bida
20.	167H	AH**	20 July 2012	50	0.5	Minna
21.	177H	VET*	20 July 2012	50	0.5	Minna
22.	181H	AH*	20 July 2012	17	0.17	Minna
23.	182H	VET*	20 July 2012	540	5.4	Suleja

Key: DB = Dog butcher, DC = Dog consumer, PO = Pet owner, HT = hunter, AH = Animal health personnel, VET = Veterinarian, * = Vaccinated, ** = Bitten & Vaccinated, H = Human sera. Reference serum titre used was 200.

CONCLUSION

This study concludes that there is serological evidence of DUVV in Nigeria and fruiting eating bats (E. gambiense) is a possible reservoir host for DUVV.

RECOMMENDATION

This study recommends further survey for lyssaviruses in bats in Niger State and screening/ vaccination of rabies occupational risks groups in the state as well public awareness.

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A COMPARISON OF LATERAL FLOW TECHNIQUE AND HAEMAGGLUTINATION INHIBITION TEST IN THE DETECTION OF NEWCASTLE DISEASE ANTIBODIES IN COMMERCIAL POULTRY IN JOS SOUTH LOCAL GOVERNMENT AREA, PLATEAU STATE

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INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease that affects many species of domestic and wild birds (Al-Garib *et al.*, 2003). The disease is said to be a major factor affecting the development of poultry industry in Africa (Ambali *et al.*, 1995). Newcastle disease is caused by Newcastle disease virus (NDV) which belongs to the Family, Paramyxoviridae and Genus, Rubulavirus (Alexander, 1997). Newcastle disease virus is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds (Aldous and Alexander, 2001). Routine diagnosis of ND usually requires two distinct steps. First, it is necessary to establish that the virus isolated is NDV and not any other avian paramyxovirus or influenza virus (Alexander, 1997). The second is whether or not the virus isolated is vaccinal or enzootic since a variety of live vaccines are used in the phase of an outbreak which is likely to interfere in the virus typing experiments (Alexander, 1997). A wide range of

tests may be used to detect antibodies to NDV in poultry sera and tests based on neutralization or enzyme-linked immunosorbent assay (ELISA) reactions have been used to support the diagnosis of ND (OIE, 1996). However, currently, the haemagglutination inhibition (HI) test is the most widely used (Jordan, 1990). It is regarded as the gold test for the diagnosis of ND (OIE, 1996). Newcastle disease virus may be confirmed by HI test using specific NDV antiserum (OIE, 1996). Though, HI test is the well-known and the traditional test for diagnosis of ND and determination of antibodies against NDV, there is however, a new technique designed for this purpose, it is called the Lateral flow technique, also known as the rapid test (Allan and Gouph, 1984; CELFD, 1999).

The lateral flow technique is a membrane based assay that provides visual evidence of the presence of an analyte in a liquid sample (CELFD, 1999). It is also known as 'dip-stick' or immunochromatographic strip tests. It is sensitive and its operation is simple and quick (CELFD, 1999). This study therefore, focuses on the comparison of LFT and HI test in the detection of ND antibodies in commercial poultry in Jos South LGA, Plateau State.

METHODOLOGY

The sample size was calculated from an expected prevalence of 51·9% based on the highest prevalence obtained from a previous study in Plateau State by Musa *et al.* (2009) at 5% accuracy and a total of 407 sera were collected from commercial chickens in Jos South LGA, Plateau State. Convenience sampling procedure was used to select nine districts under Jos South L.G.A. These districts were; Federal low cost, Rantya, Bukuru, Rayfield, Da zarmangada, Dung village, Dadinkowa, Gyel and State low cost. Furthermore, 1- 3 farms were also randomly selected from each of the districts and 10 to 20 birds were selected at random from each flock, depending on the number of farms in each

district and the flock size, respectively. A total of 34 copies of structured questionnaire was administered to 34 farmers. The birds were categorized into chicks (0 to less than 6 weeks of age), pullets (6 to 18 weeks of age) and layers (19 weeks of age and above). The farmers' awareness of ND, clinical signs of ND noticed by the farmers, vaccination against ND and other information were obtained from the farmers. Two millilitres of blood was collected aseptically via the wing vein of each bird. The samples were labelled appropriately. The blood samples were kept in a slanting position at room temperature to allow for clotting and sera formation. The sera were separated by transferring into two types of labeled sterile bottles; one for lateral flow test which was carried out immediately and the other bottle for haemagglutination inhibition test which was stored frozen at -20^{0}C and sent in a cold pack to Regional Laboratory for Avian Influenza and other Transboundary Animal Diseases at the National Veterinary Research Institute (NVRI), Vom, Plateau State. Chi-square test followed by tukey's post hoc test were used to determine the association between various numbers of birds positive for Newcastle disease virus antibodies using both haemagglutination inhibition test and lateral flow tests. They were analyzed using Graphpad prism version 4.0 window. Values of $p \leq 0.05$ were considered significant for these two tests.

RESULTS AND DISCUSSION

From the total of 407 birds tested, the LFT showed that 367 (98.66%) layers and 34 (97.14%) were positive for ND Abs, while the HI test showed that 362 (97.31%) layers and 32 (91.42%) pullets were also positive for ND Abs (Figure 1). In this study, the LFT detected the presence of ND antibodies in a higher number (98.5%) than the HI test which detected presence of ND antibodies in 96.6% of the birds tested. This result can be compared with the previous work carried out by Martin *et al.* (2009), on the evaluation of the immunochromatography assay in the serodiagnosis of tuberculosis, in comparison with X-ray diagnosis. This result is also in line with the previous

work carried out by Bautista *et al.* (2011) on the detection of *Salmonella* sp. using LFT (immunochromatographic assay) in comparison with isolation of *Salmonella* sp. on XLT4. The LFT detected *Salmonella* sp. as early as 18–48 hours during pre-enrichment and enrichment with a sensitivity of 94.7 % and specificity of 96.8 % while isolation on XLT4, which required an overnight incubation step for the presumptive isolation and identification of *Salmonella sp.* had a sensitivity of 93.8 % and specificity of 89 % .

In this study, the LFT and HI test showed no significant association ($p \geq 0.05$) between the presence of ND Abs and the type of bird tested; although a higher percentage of the layers showed presence of ND Abs than the pullets in these two tests (Figure 1). This may be due to the fact that continual boosting of immunity with ND vaccine over time in layers, as a result of their economic value, may increase their ND antibody titre than the pullets. In this study, the LFT can be said to be a suitable alternative to the conventional HI test. This is in agreement with the work carried out by Rauw *et al.* (2009).

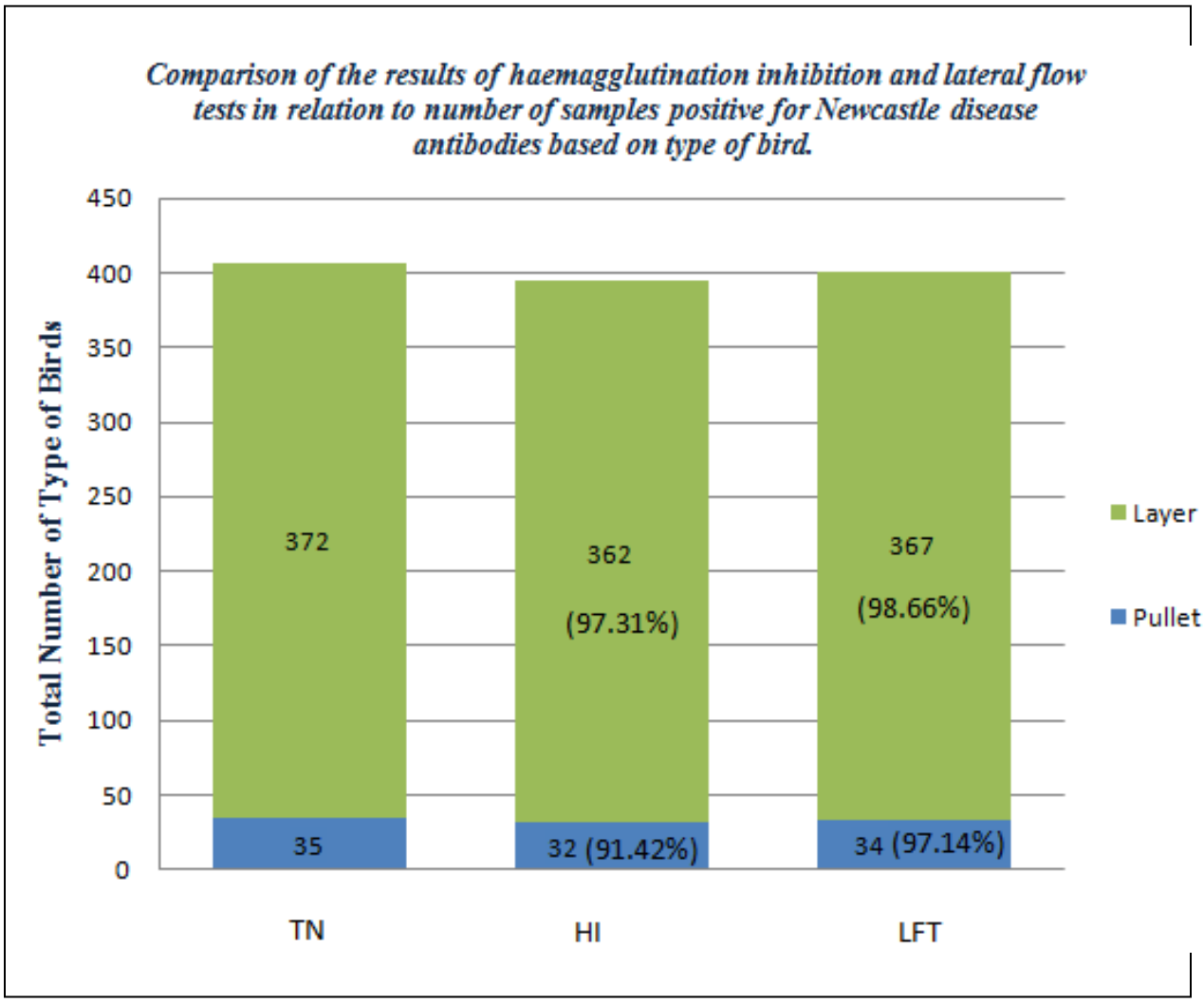

Figure 1

HI: P = 0.0916, RR = 0.3519 (CI = 0.1236 to 1.003); OR = 0.2947 (CI = 0.07714 to 1.126)

LFT: P = 0.3642, RR = 0.4239 (CI = 0.07130 to 2.521); OR = 0.3706 (CI = 0.04025 to 3.411)

Keys

TN = Total number of birds tested

HI = Haemagglutination inhibition test

LFT = Lateral flow test

P = p value, RR = relative risk, CI = confidence interval, OR = odd ratio

CONCLUSION

In this study, LFT detected 98.5% of ND antibodies while the HI test detected 96.8% of the total number of birds tested. The LFT can therefore, complement HI test for rapid detection of ND antibodies in commercial chickens.

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