OCCURRENCE OF RABIES VIRUS AMONG APPARENTLY HEALTHY DOGS AND BATS SLAUGHTERED FOR MEAT IN SOME SELECTED LOCAL GOVERNMENTS OF ENUGU STATE.

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DECLARATION

I hereby declare that the work described herein is original work and has not been previously submitted for any degree to any University or similar institution

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CERTIFICATION

This is to certify that EZE UKAMAKA UCHENNA (PG/M.Sc./ 10/57743), a postgraduate student in the department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka has satisfactorily completed the requirements for research work for the Degree of Master of Science in Veterinary Medicine. The work embodied in this dissertation is original and has not been submitted part or in full for any other degree of this or any other University. The Dissertation has therefore been approved for award of Master of Science Degree in the Department of Veterinary Medicine, University of Nigeria, Nsukka.

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DEDICATION

This is dedicated to my lovely husband Obiechina Eze, my children Chidiebuebe Ihuoma, Ogugua Chizaram, Munachimso (my special M.Sc. gift from God), and

My Dad late Mr. Alphonsus Alutaoji.

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ABSTRACT

This work was undertaken to investigate whether or not rabies virus is present in the brains and saliva of apparently healthy dogs and bats slaughtered for human consumption in Enugu State, Southeastern Nigeria; attempt to establish possible exposure potential of humans to rabies virus through handling, processing, selling, buying, and consumption of dog meat and to establish the genotype of the viral isolates if any. A total of 100 questionnaires were distributed to those that sell dog or dog meat. One hundred and fifty two dog heads and fifty nine bat heads were collected from markets, restaurants and bars where these animals are slaughtered and consumed as delicacy in the study area and processed accordingly. The points of collection were georefrenced using global positioning system (GPS). Sex and age of dogs were noted at points of collection. Eighty seven percent of the dog meats sold were sourced from dogs bought from homes and markets while the rest (13%) were sourced from stray dogs. Eighty one percent of the respondents reported eating dog meat because it cures some diseases including reducing high blood pressure while the rest (19%) perceive it just as any other meat. Specifically, 50% was of the opinion that the meat of rabid dog enhances male libido. Twelve percent of respondents had been bitten by suspected rabid dog in the course of the business yet only one person (2%) indicated that he received full course of anti-rabies vaccination in a hospital. Seventy one percent of the respondents had slaughtered and eaten suspected rabid dogs. Out of the 152 dog brain samples examined, 18 (12%) were positive for rabies virus antigen using FAT while 17 (11%) were positive using MIT. Nine (17.6%) out of 51 male and nine (8.9%) out of the 101 female dog heads examined were positive for rabies virus antigen. seventeen (13%) of the 131 adult dog brains tested positive for rabies virus antigen, while 1(5%) of the puppy brains tested positive. There was no significant association ($p > 0.05$) between gender or age and the occurrence of rabies virus antigen in the brain of dogs. There was no significant association ($p > 0.05$) between occurrence of rabies virus antigen and senatorial zones. Out of a total of 10 salivary glands

from positive brains that were analyzed by FAT, all (100%) tested positive for rabies virus antigen. All the 59 (100%) bat brain samples tested were negative for rabies virus antigen using FAT. All the rabies virus isolates from dog brain samples belonged to genotype 1 rabies virus (true rabies virus). The geographic information system (GIS) revealed that the positive dog brain samples were collected from 5 out of the 6 local government areas (LGAs) sampled and from 7 locations within the 5 LGAs; Udenu (1 location), Enugu North (1), Nsukka (2), Igbo-Etiti (2), and Udi (1). This study has shown that some of the apparently healthy dogs slaughtered for meat in Enugu state carry rabies virus and secrete the virus in their saliva without showing clinical signs. All the isolates belonged to genotype 1 rabies virus. No rabies virus antigen was found among apparently healthy bats slaughtered for meat in Enugu state. Furthermore, this work has revealed that those involved in dog trade or butchering have high exposure potential to rabies virus antigen

CHAPTER ONE

INTRODUCTION

1.1. Background of study

Rabies is an acute, almost inevitably fatal zoonotic and re-emerging disease affecting all mammals including humans (Krauss *et al*, 2003). It is one of the neglected endemic zoonotic diseases in Nigeria. Besides poliomyelitis and pox, rabies is one of the longest -known infectious diseases in human history (Krauss *et al*, 2003).

The aetiologic agent of rabies is rabies virus. Rabies and rabies-related viruses belong to the Order Mononegavirales which are viruses with non-segmented negative-stranded RNA genomes with antimessenger polarity (CDC, 2008). They are of the family Rhabdoviridae which are viruses with distinct ñbulletò shape and Genus lyssavirus (CDC, 2008). They are neurotropic, single-stranded, RNA viruses capable of producing fatal encephalitis in a wide variety of mammalian species (Nadin-Davis 2002; WHO, 2005).

It is debatable whether there are rabies free countries including the canid rabies-free isolated islands because of the more recently discovered bat rabies problems. Fruit-eating bats (flying foxes, suborder megachiroptera) were identified in Australia as rabies carriers and have caused two human deaths (Krauss *et al,* 2003). Also in Europe where only insectivorous bats are known to occur, three human cases of rabies acquired from bats have been recorded since 1997 (Krauss *et al*, 2003).

One of the first grand success of the Pasteurôs germ theory of disease was the development of rabies vaccine which was the first successful scientifically developed vaccine for prevention of human disease (Oboegbulem, 1994; Real and Child, 2005).There have been development of safer and more successful vaccine treatments, better and more effective vaccines and yet rabies remains the most important zoonotic and re-emerging disease worldwide. Every year, more than 55,000 people die of rabies and more than

95% of human deaths occur in Asia and Africa (WHO, 2005). More than 15 million people worldwide receive a post ï exposure preventive regimen to avert the disease every year and this is estimated to prevent 327,000 rabies deaths annually (WHO, 2005).

In Nigeria, the first recorded case of human rabies was in 1912, and in 1925 canine rabies was diagnosed at Yaba rabies laboratory (Oboegbulem, 1994). Every year in Nigeria, about 10,000 people are exposed to rabies (Okoh, 2007). A concomitant rise in rabies in both dog and human population in Nigeria portends danger (Osinubi *et al*, 2003).

The most widely used and accepted test for rabies is the direct immunofluoresence test (FAT) on acetoneï fixed smears of hippocampus, cerebellum or medulla oblongata (OIE, 2000). Reverse transcriptase-polymerase chain reaction (RT-PCR) is rapid and sensitive for the detection of rabies virus (RV) and rabies- related viruses (RRV). (Heaton *et al*, 1997).

In North America and Europe, wild life species are the most important reservoirs of rabies (Cliquet and Picard-Meyer, 2004). In Africa, canine rabies is still an important risk for human (Nel *et al*, 1993). The existence of independent rabies cycle in wild animals complicates the situation.(Cliquet and Picard ï Meyer,2004). Although dog is the predominant vector of rabies in Nigeria (Oboegbulem, 1994), rabies has been reported in wildlife species (lynx,civetcat,monkeys,insectivorous bats,chimpanze,lion,hyrax) (Anon, 1969 : Anon, 1972; Isoun *et al* 1973; Okoh, 1986 ; Enurah *et al,* 1988; Ogunkoya *et al* 2008; Dzikwi *et al*, 2010a).

Thousands of dogs are vaccinated annually with Flurry low egg passage vaccine strains; but prevalence of the disease in domestic animals has not declined (Nottidge, 1994; Ogunkoya *et al*, 2003).

In Nigeria, particularly in Maiduguri, a survey of the apparently healthy dogs revealed that 32% were carriers of the rabies virus (Baba, 2006) ; in another study carried out in the Northwestern states of Nigeria the results revealed that 23.1% were positive for rabies (Garba *et al* 2010). A study carried out by

Aliyu *et al* (2010), showed 44% prevalence in Adamawa state. A study carried out in Ibadan (western Nigeria) in 1990 suggested the presence of RABV-neutralizing antibodies in the sera of fruit bats but no rabies virus antigen was isolated from their brain samples (Aghomo *et al*.,1990). A Lyssavirus surveillance done by Dzikwi *et al* (2010a) on fruit bats (*Eidolon helvum*) in Northern Nigeria revealed the presence of lyssavirus neutralizing antibodies in bats sera. Of the 140 sera tested, 27(19%) neutralized Lagos bat virus and two of these additionally neutralized Mokola virus, but no virus was isolated from their brain samples.

In the USA, Canada, and Western Europe, where canine rabies has been controlled, dogs are responsible for very few cases of the disease. Rather, human rabies develops from bites of wild animals (especially bats, squirrels, rats, raccoons, skunks and foxes) or occurs in travelers bitten by dogs elsewhere in the world (Dato *et al*., 1995; Smith, 1996).

The GIS application in the last few years has revealed itself to be an indispensable tool for surveillance activities both for the geo-referencing and the geostatistical analysis of the data obtained in order to elaborate spatial model like the distribution of disease outbreak. Moreover, importance of pathogens responsible for disease both in animal and in man requires a careful plan of control and monitoring within the infested territories. The construction of archives of geo-referenced data is fundamental for managing the phenomenon on a vast scale and checking eventual infection hotsbeds in good time to avoid possible epidemics (Ramirez *et al*, , 2004).

1.2. Statement Of Research Problem

Rabies remains a global zoonosis of major public health, agricultural and economic significance. The pattern of rabies in Nigeria still implicates dogs as the principal vector of the disease (Tomori, 1980). Dogs are listed as only moderately susceptible but are without any doubt the animals most likely to spread

infection to human beings in Africa and Asia (Nadin-Davis *et al.,* 2008). Rabies is one of the neglected zonootic diseases endemic in Nigeria (Ehizibolo, 2008).

There have been reported cases of dogs recovering from clinical rabies. (Fekadu and Baer, 1980; Aghomo *et al*, 1886). This suggests that virus carrying and virus excreting healthy dogs are assumed to exist. The implication of healthy carriers, in-apparent infection and recovery from clinical rabies is that they compound human exposure to unrecognized carriers (Oboegbulem, 1994; Ajayi *et al.*, 2006).

Dogs are the major animal reservoirs in the developing regions, wild life maintains cycle of infection even in developed countries and new viral aetiologic agents continue to emerge (Ruppretch *et al*., 2006). In Nigeria, although the epizootiology of rabies has been described by several authors (Adeyanju and Addo, 1973; Umoh and Belino, 1979; Ogunkoya *et al*, 1984; Aghomo *et al*, 1986; Okoh, 1989; Bello *et al*, 2007), there is no information on the occurrence of rabies virus antigen in apparently healthy dogs and bats in Enugu state. Records from the National Veterinary Research Institute (NVRI) Vom, show that rabies has been diagnosed in ground Insectivorous bats and other wild animals such as squirrel, lion, monkeys, chimpanzee, between January, 1986 to January 2005 (Ogunkoya, 2008).

Human consumption of bats is practiced in certain parts of Enugu state (Ogui, Ngwo, Okpatu, Ukana, Awhum), but the public health implication of this practice has not been determined. One problem in Nigeria is the lack of field data on the conflicts, risks and impacts of diseases from interaction among wildlife, human and domestic animals.

1.3.Justification

Nigeria is particularly important for lyssavirus surveillance because the initial isolation of Lagos bat virus and Mokola virus occurred in the country (Dzikwi *et al.*, 2010a). Repeated isolation of rabies virus from healthy dogs for a period of more than seven years has been reported (Fekadu, 1975). Furthermore, in a study carried out in Ethiopia, it was observed that dogs inoculated with rabies virus later recovered from

clinical rabies indicating that the virus carrying and virus excreting healthy dogs could exist (Fekadu and Baer, 1980). In general, many reported cases in Africa and elsewhere show that clinical rabid dogs may not die but recover and continue to live as carriers (Arko *et al*., 1973, Fekadu, 1975; Bell, 1975).

Consequently, in addition to brain samples, it has been recommended that samples of salivary glands should also be submitted for rabies diagnosis (Fekadu, 1975). A study carried out by Wosu and Anyanwu (1990) showed that 16.1% of non-vaccinated and apparently healthy dogs in Nsukka environment have rabies virus antibody in their sera. Bats are recognized as important reservoirs of many emerging infectious agents, including lyssaviruses (Dzikwi *et* al .,2010a) and are reservoirs and vectors for six out of the seven genotypes of lyssavirus characterized so far (WHO, 2004). Bearing in mind that commercially available rabies biologics do not provide reliable protection against LBV, MOKV, and West Caucasian bat virus (WCBV) (Hanlon *et al*., 2005), it is therefore imperative to enforce surveillance of lyssaviruses in apparently healthy dogs and bats for a better understanding of the epidemiologic situation, circulation pattern and public health significance in order to establish control strategy in Enugu State in particular and Nigeria in general.

1.4.**Aim**

To determine the occurrence of rabies virus antigen among apparently healthy dogs and bats slaughtered for meat in some parts of Enugu state.

1.5. Specific objectives include the following:

- To detect rabies virus antigen in the brains of apparently healthy dogs slaughtered for meat.
- To determine the presence of rabies virus antigen in the salivary glands of those dogs whose brains have been tested.
- To determine the presence of rabies virus antigen in bats hunted, slaughtered or killed for meat.
- To demonstrate possible exposure potential of humans to rabies virus antigen through selling and consumption of dog meat.
- To establish the genotype of the viral isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of rabies in Africa

Rabies was first confirmed in Kenya in 1912. Since then, the disease has largely existed in varied degrees of occurrence (Adedeji *et al*., 2010). Spatial and temporal distribution of cases of animal rabies are well documented (Borus, 1996). The rabies problem in Kenya has been greatest in Machakos district where the disease has persisted endemically for over 40 years (Kitala *et al*., 2000). Dog has been the principal animal reservoir for rabies. The 1980's witnessed a dramatic upward swing in the number of cases reported annually. Over the years an enzootic pattern that covered most parts of the country emerged.

In 1996, Borus reviewed available data that showed rabies as an emerging microbial threat in Kenya (Borus, 1996). Mongoose rabies in South Africa was recognized in 1932 which differs from classic dog rabies (Swanepoel, 2004). Although, an inadequately characterized lyssavirus was isolated from a bat trapped in a survey in 1963, before the existence of rabies-related viruses was known. Awareness of lyssaviruses other than rabies viruses in South Africa dates from the identification of DUVV in 1970 (Swanepoel, 2004; Cohen *et al*., 2007). Since the 1970s, most human rabies cases in South Africa have occurred in KwaZulu-Natal Province, where the major animal vector is the domestic dog (Cohen *et al*., 2007). Before the report of Cohen *et al*. (2007), the most recent 2 laboratory-confirmed human rabies cases in Limpopo Province occurred in 1980 and 1981. DUVV was discovered in 1970 when it caused fatal rabies like disease in a person bitten by an unidentified Insectivorous bat about 150 km northwest of Johannesburg, South Africa (Paweska *et al*., 2006). In 1981, the virus was isolated from what is believed to have been a *Miniopterus schreibersi* insectivorous bat caught in daylight by a cat in Makhado town (formerly LouisTrichardt) in Limpopo Province, South Africa, and in 1986 the virus was recovered from an insectivorous bat, *Nycteris thebaica*, trapped in a survey in Zimbabwe (Paweska *et al*., 2006).

In Nigeria, it dated back to the 1950s. Lagos bat virus (LBV) was also isolated from frugivorous bats (*Eidolon helvum*) on Lagos Island in 1956 (Kemp *et al*., 1972; MMWR, 1983). Mokola virus (MOKV) was first isolated from shrew (*Crocidura* spp.) in Nigeria in 1968 (Kemp *et al*., 1972; MMWR, 1983). Mokola virus (MOKV) was believed to have caused rabies-like disease in 2 persons in Nigeria in 1969 and 1971, shortly after its initial discovery in shrews in 1968, but no cases of human infection have subsequently been recognized (Familusi and Moore, 1972; Familusi *et al*., 1972; Paweska *et al*., 2006).

With the exception of MOKV, all lyssavirus genotypes and putative genotype have been isolated exclusively or most frequently from chiropteran species. MOKV has never been isolated from these species, but only from terrestrial mammals (Sabeta *et al*., 2007). Since then, > 20 isolates of this lyssavirus have been found throughout Africa (Cameroon, Central African Republic, Ethiopia, South Africa, and Zimbabwe) (Kemp *et al*., 1972; Familusi *et al*., 1972; Sabeta *et al*., 2007).

2.2. **Epidemiology of rabies.**

Rabies is a fatal zoonosis characterized by encephalomyelitis. It is a disease of public health concern because the fatality rate is almost 100% (Rupprecht *et al*., 1995). The epidemiology of the disease is influenced by events at a cellular and molecular level, as well as overt dynamics of different lyssavirus infection with local host species distribution, abundance, demographic, behavioral ecology, dispersal and interactions with other species (Cliquet and Picard-meyer, 2004; Ruppretch *et al.,*2006). The disease exists in two epidemiological patterns; urban rabies involving dogs and cats and sylvatic rabies involving wild carnivores and bats (Paez *et al.,* 2003). Sylvatic rabies occurs mostly in developed countries like in North America were canine rabies have been eradicated (WHO, 2005). Urban rabies occurs mostly in less developed countries where the dog is the principal host and vector giving rise to a greater opportunity for spill-over infection into human population. This pattern of rabies is by far more dangerous to humans

accounting for an estimated 99% of all recorded human cases and 92% of all human post exposure treatment (Pastoret *et al.*, 1995).

2.3. Structure and Molecular Mechanism of the rabies virus:

The rabies virus has been classified as a member of the genus Lyssavirus (from Greek, lyssa meaning ñfrenzyò) within the Rhabdoviridae family (from Greek rhabdo meaning ñrod-shapedò) and order mononegavirales. Each virus is bullet-shaped and approximately 180nm long and 75nm wide. The lipid bilayer membrane is coated with numerous 10nm spikes composed of glycoprotein. Inside the membrane is a lining of matrix protein and a core containing the ribonucleoprotein complexes.

Fig 2.1: longitudinal and cross-sectional schematic view of rabies virus (Albertini *et al*, 2006)

The genome of the rabies virus is made up of approximately 12,000 nucleotides of single stranded RNA (Smith *et al.,* 1999). This contains five genes which code for each of the five viral proteins. The largest is the L protein, or Large (244kDa) polymerase. It is a multifunctional protein, but its main activity is as a polymerase which allows the genomic RNA to be copied during virus multiplication within the cell. Between 30 and 60 copies of the L protein form the ribonucleoprotein core with the RNA genome and two other proteins N (approximately 1,800 copies) and NS (approximately 900 copies) (Cliquet and Picard-Meyer ,2004). The N protein is a 55kDa protein which is the main structural component of the

ribonucleoprotein core and binds tightly to the RNA allowing it to be packed in an orderly helical structure. The NS protein is a highly phosphorylated protein whose role has not yet been fully determined but is suspected to be involved in viral RNA transcription and/ or replication (Bourhy *et al.*,1993; Baer *et al.,*1994). The other two proteins encoded for by the rabies genome are the M (or matrix) protein, which has been shown to be crucial in the ability of a newly formed virus to bud from the host cell membrane, and transmembrane G protein, which is involved in host cell attachment and invasion (Baer *et al.,*1994). Also the G protein is the main target for antibody therapy against rabies, as it is the only exposed protein on the virus and binding of antibody to it can block its function in the host cell infection (Smith *et al.,* 1999).

2.4. Demarcation criteria in the *Lyssavirus* genus

Until 1956 and the first isolation of rabies-related viruses in Africa, the rabies virus was believed to be unique and antigenically distinct from other members of the *Rhabdoviridae* family (Tordo, 2004). This warranted the creation of the genus *Lyssavirus* (Greek Lyssa: rabies) for viruses responsible for rabies-like encephalitis. The genus was at first divided into four serotypes (1-4) by antigenic cross-reactivity with sera and monoclonal antibodies, which corresponds to the following species: serotype 1, rabies virus (RABV); 2, Lagos bat virus (LBV); 3, Mokola virus (MOKV); and 4, Duvenhage virus (DUVV). Further isolation of new bat Lyssavirus in Europe, then Australia and the progress in genetic characterization of several genes (N,P, and G) supported the delineation of seven genotypes (1-7), confirming and expanding the antigenic data from 4 to 7 which are as follows:-1, RABV; 2,LBV; 3,MOKV; 4,DUVV; 5,European bat Lyssavirus 1 (EBLV-1); 6, European Lyssavirus 2 (EBLV-2) and 7, Australian bat Lyssavirus (ABLV). Within each genotype, sublineages correspond to variants circulating in specific geographical regions and animal hosts (WHO, 2004). The genotypes further segregate in two phylogroups including genotypes 1,4,5,6 and 7 (phylogroups I) and 2 and 3 (phylogroups II).

Viruses of each phylogroup differ in their biological properties (pathogenecity, induction of apoptosis and cell receptor recognition) (Badrane, 2001; Nadin-Davis, 2002).

It is important to note that bats are reservoirs and vectors for six out of the seven genotypes characterized so far; the precise reservoir/vector for genotype 3 (MOKV), remains to be determined (WHO,2004). For the other five genotypes, bats are the exclusive vectors, and only genotype 1, RABV, also includes terrestrial vectors (mainly carnivores). Genotype 1 corresponds to the classical rabies virus and is spread in domestic or wild animals worldwide. Genotype 2-7 have a narrower geographical and host-range distribution. This classification will evolve, particularly as surveillance for bat lyssaviruses is reinforced (Botvinkin, 2003). Four recent isolates of bat Lyssavirus in central Asia, East Siberia and Caucasian region need to be characterized as new genotypes (Botvinkin, 2003). Aravan virus (ARAV),Khujand virus (KHUV), Irkut virus (IRKV) and West Caucasian bat virus (WCBV). Also two rabies related viruses isolated from insects in Nigeria equally need to be characterized as new genotypes: Obodhiang virus and Kotokan virus (Nottidge, 2012). Lyssaviruses show a broad antigenic cross-reactivity at the nucleocapsid level, mainly because of sequence conservation of the N protein: amino acid identities range from 78% (MOKV and EBLV-2) to 93% (DUVV and EBLV-1) (WHO, 2004). This allows the use of similar reagents for diagnosis by immunofluorescence. The ectodomain of the G protein (carrying the main antigenic site) is more variable and cross-neutralization exists among lyssaviruses of the same phylogroup (amino acid identities in the ectodomain >74%), but not between phylogroups (amino acid identities in the ectodomain <62%). Experimental evidence obtained so far indicates that vaccine strains (all belonging to genotype1 within phylogroup 1) are ineffective for protection against infection by lyssaviruses from phylogroup II (Botvinkin, 2003). Rabies vaccines that are currently used do not have cross ï neutralizing activity against members of phylogroup 2 (Nel, *et al* 2005).

2.5. Host range

The increasing incidence of dog and wild life rabies in Africa (king, 1993) is causing concern, not only for public health but also for the conservation of some endangered canids (Macdonald, 1993). In order to control rabies effectively, we need to identify which animals are reservoirs and by what mechanisms the disease is maintained in reservoir population. The rabies virus infects a wide range of mammalian hosts, including raccoons, skunks, foxes, coyotes, bats, domesticated dogs and cats, human beings. While infection can occur in any warm-blooded animal, some (such as foxes, coyotes and wolves) are highly susceptible, while others (such as opossums and birds) are much less susceptible. Laboratory reared rodents are rarely, if ever infected, hence bites by laboratory mice or rats are usually not considered exposure, (except rodents used in rabies diagnosis or research) (Okoh, 1986a). Rodents represented <1% of all species tested for rabies in the United States (Childs *et al*., 1997), and virtually all of those tested and found rabid were large species such as woodchucks (*Marmota monax*) and beavers (*Castor Canadensis)*. An extreme underrepresentation of small terrestrial mammals was obvious in this dataset. However, the factors contributing to the size ï bias are confounded by reliance on human observations of suspicious animal behavior, the problems inherent to mixing small body-size with RABV transmission by bite, the financial constraints that limit diagnostic laboratories to testing only specimens from animals directly involved in a human or domestic animal exposure to rabies virus (Winkler, 1991), and the public health communityôs general assumption that rodents, with the exception of large-bodied species, carry little risk of transmitting RABV (Real and Childs, 2005). However, rodent species examined are fully susceptible to rabies infection, can transmit RABV by bite, and have been implicated in human rabies infection (Winkler, 1991). In some countries rodents have been implicated in the maintenance of RABV, but critical data to support these contentions are elusive (Okoh, 1986 b; Summa *et al.,* 1987). Small terrestrial mammals usually account for the greatest number of individuals and biomass in mammalian

community, and potentially these populations could benefit from rabies epizootics reducing predation pressure. However, virtually nothing is known of the direct effect of rabies on small mammal population within a community. Therefore, any discussion of the community ecology of rabies is severely limited by the profound, possibly insurmountable, deficit of any representative information on wildlife rabies; most notably rabies among smaller mammals (Real and Childs, 2005).

The susceptibility of bats appears to vary from place to place. Apart from the vampire bats of Central and South America, rabies has been reported in 26 out of 40 species of insectivorous and fungivorous bats in North America (Smith, 1996). Bat rabies is most commonly reported among domesticated dogs in underdeveloped nations, especially in parts of Africa and Asia. However, in nations where canine rabies has been controlled through mass vaccination protocols, wild life rabies accounts for the vast majority of reported cases (WHO, 1999). In 1995 only 8% of sited cases in the United States were in domestic animals, while more than 50% were in raccoons, 22% in skunks, 10% in bats and 6% in foxes (WHO, 1999).

2.6. Global distribution of the rabies.

Rabies is worldwide in distribution except for few island nations: Australia, New Zealand and Antarctica (Rupprecht *et al*., 2006). No account of global rabies distribution can proceed without mentioning humans and the domestic dog. Domestic dogs serve as the major reservoir and principal source of RABV transmission to human and other animals in many countries of Asia, Africa, and South America (WHO, 1999). Other domestic and wild animals are typically infected through secondary transmission of RABV variants maintained by dogs, or in many locations, variants of RABV maintained by wild carnivore hosts traceable to a domestic dog origin (WHO, 1999).

In Asia, the main transmission route for rabies is a rabid dog bite, between 94% and 98% of rabies deaths are due to canine bites (Dodet and Meslim, 1997). Over the last few years, there has been a decrease in

mortality rates in countries where efficient disease control measures have been implemented, e.g. Thailand and Vietnam (Kamoltham *et al*., 2003). In other countries, such as Peopleôs Republic of China and the Philippines, the number of cases has increased over the years. There are several possible reasons for this increase; an increase in rabies surveillance, an increase in dog populations, inadequate post-exposure treatment, and the lack of effective rabies vaccines (Yongxin and Qing 2001; Xianchum *et al*., 2005). Most people (usually children) who die of rabies have not been treated or have not received adequate treatment because of the high cost of vaccines and rabies immunoglobulins. Furthermore, both rabies immunoglobulins and vaccines are not always available. In Asia, dog populations can be divided into three groups; Dogs with owners, community dogs and stray dogs.

Data from Thailand for the year 2000 showed that 54% of human cases of rabies were caused by dogs with owners. However, 47% were caused by stray dogs (Puanghat, 2001). It is much more difficult to implement control measures (particularly with the parenteral antirabies vaccination) for strays. Until recently, very few studies of rabies isolates had been conducted in Asia, but over the last few years four new isolates have been described in insectivorous bats. The Aravan virus was isolated in *Myotis blythiiin* Kyrgyzstan (Arai *et al* 2003) in 1991 and Khujand virus in *Myotis mystacinus* in Tajikistan in June 2001 (Kuzmin, 2002). The Irkut virus, which is phylogenetically similar to Gt4 and Gt5, detected in a *Murian leucogaster* in 2002 in eastern Siberia and the West Caucasian bat virus was isolated in a *Miniopterus schreibersii* in the Caucasus. West Caucasian bat virus is phylogenetically similar to the cluster of Gt2 and Gt3 and is the most distinct lyssavirus detected so far (Botvinkin, 2003).

In the 1950ôs, canine rabies was endemic in the Unites States of America (USA), but carefully planned control measures and parenteral vaccination have brought it under control (Cliquet and Picard-Meyer, 2004). In the USA and Canada today, rabies reservoirs are to be found among wildlife. Different epidemiological cycles exist, the following species parentheses indicates a percentage of the total number

of rabies cases; raccoons (*Procyon lotor*) (40%), skunks (*Mephitis mephitis*) (30%), bats (approximately 17%) and red and grey foxes (*Vulpes vulpes* and *Alopex lagopus*) (approximately 6%). These species are infected with several rabies virus variants (Gtl). Usually, the geographical distribution of the virus strains which belong to a particular species is delimited. The virus variants circulating in the raccoon are located along the Atlantic coast as far as the Appalachians, those circulating in the skunk are found in California and in the North Central and South Central States, and those circulating in the grey fox (*Urcocyon cinereoargenteus*) are found in Alaska, Arizona and Texas (Cliquet and Picard-Meyer, 2004). On the border between Texas and Mexico, coyotes (*Canis latrans*) and unvaccinated dogs maintain rabies cycle with canines, on the other hand, the cycle maintained by insectivorous bats is distributed throughout the USA (Rupprecht *et al*., 2006). One study has shown that cats and dogs are usually infected by the most common wild reservoir species (McQuiston *et al*., 2001). Since the 1950s, at least 39 human cases of rabies linked to infection by a rabid bat have been recorded in the USA, and in most cases it was unclear how the victims were infected because most had no memory of being scratched or bitten. The species most often involved are the eastern pipistrelle (*Pipisrellus subflavus*) and the silver-haired bat (*Lasionycteris noctivagans*) (Krebs *et al*., 2002). Spillover infection has been observed from other wild animals into domestic carnivores. In the same way, the viruses present in bats are sporadically found in cats and dogs (McQuiston *et al*., 2001). Since 1990, these viruses have been implicated in 92% of indigenous human cases of rabies (Krebs *et al*., 2002).

In Latin America there are two epidemiological cycles, both with Gtl (Cliquet and Picard-Meyer, 2004). The first cycle is terrestrial and is maintained by dogs; canine rabies being enzootic in most Latin American countries, including the Caribbean islands. The second is aerial and is carried by bats. Several species of haematophagous bats, the main one being *Desmodus rotundus* (vampire bat) (Warner *et al*., 1999), represent the principal transmission vector of RV to domestic carnivores and livestock, causing

considerable economic losses (Martinez-Burnes *et al*, 1997). In historical terms, the presence of RV in Latin America was suggested for the first time in insectivorous bats in Brazil in approximately1910,and confirmed in Trinidad in 1931. In Latin America, as in the USA, several variants of RV exist (Smith, 1996). Sporadic cases are recorded in humans and the risk of transmission from vampire bats to people is estimated at 0.96 cases for every 100 inhabitants in Arizona region in Brazil (Cliquet and Picard-meyer, 2004). Insectivorous bats can also infect humans; these species represent an important reservoir for rabies, particularly in urban environments where as haematophagous bats on the other hand are found in rural environments, close to livestock breeding areas (Favi *et al*., 2002).

In Europe, the main reservoir and vector of rabies, since the late 1930, has been the red fox (*Vulpes vulpes*), followed by the raccoon dos (*Nyctereutes procyonoides*) in Central and Baltic Europe. In Estonia, the raccoon dog is the primary infected species, accounting for more than 44% of the rabies cases in 2003 (Anon, 2003). With the exception of Islands (i.e Britain, Ireland) and large peninsulas (Norway, Sweden), most European countries have become infected. At the end of the 1970s, the farthest limits of the rabies wave to the North, South and West were the Netherlands, Italy and France, respectively. Turkey is the only European country where the domestic dog is the principal vector of rabies. However, a molecular analysis of different isolates (Johnson *et al*, 2003) suggests that there has been recent spill over from the domestic dog to the fox, which could imply the development of an endemic cycle in wild life. Cliquet and Picard-Meyer, (2004) reported that seven European countries are free of rabies as a result of oral vaccination programmes: Finland and the Netherlands since 1991; Italy since 1997; Switzerland since 1998; France since 2000; Belgium and Luxembourg since 2001. Since the first case of rabies in bats was recorded in Europe in 1954, Gt5 (EBLV-la) and Gt6 (EBLV-2b) have been detected throughout the continent. The EBLV-1 strain seems to be more prevalent, infecting approximately 95% of all bats testing positive for the presence of the virus between 1977 and 2000 (Fooks *et al*., 2003). Bats infected with

EBLV-1 and EBLV-2 have been reported throughout Europe, from Russia to Spain, particularly in coastal regions (Cliquet and Picard-Meyer, 2004). Isolates of EBLV-2 have been recorded in central Europe, Switzerland, Eastern Europe and the Ukraine (Johnson *et al*., 2003), whereas EBLV-1 strains are distributed principally throughout Western Europe countries. The EBLV-1a strain has been found in Germany, the Ukraine, Russia, Poland, the Netherlands and Denmark, while EBLV-b has been reported from France, Spain and the Netherlands (Allworth *et al*., 1996; Serra-cobo *et al.*, 2002).

2.7. Distribution of rabies in Africa

In Africa, the dog is the principal vector and reservoir of rabies (Blancou, 1988; Rupprecht *et al*., 2006). Dogs represent more than 75% of rabid animals in most African countries and most of the reported human infections occur in children under ten years of age (Cliquet and Picard-Meyer, 2004). Although sporadic rabies cases in wild mammals have been documented throughout the African continent, the disease has not been extensively studies in these countries. This is understandable, because applying control measures and educating the general public is difficult when there are so many other public health priorities, e.g., acquired immune deficiency syndrome (AIDS), Malaria, and tuberculosis. Canine rabies has existed in Algeria, Morocco, Tunisia and Egypt for centuries and in each of these countries, more than 100 cases a year are reported in dogs (Cleaveland *et al*., 2003).

In Tunisia, a national rabies control programme was implemented in 1982. Mass campaigns of parenteral dog vaccination were conducted every two years at first, then, with the rapid dog population turnover, annually, with quality vaccines produced locally. The number of rabies cases decreased accordingly (Black *et al*., 2000). In 1999, 178 cases of rabies were recorded in animals and there was one case in humans.

In morocco, in 2002, there were 446 cases of rabies in animals and 23 cases of human mortality. These human cases occurred mainly in rural and suburban areas and were transmitted by dogs. It is important to

note that 86% of the people who died of rabies between 1995 and 2001 in Morocco and not received any anti-rabies vaccines treatment (Cliquet and Picard-Meyer, 2004). Canine rabies is present in all of the countries situated between the Sahara desert and the Equator. The role of jackals and hyenas (*Crocuta crocuta*) is occasionally important in some areas, but as spillover from dogs (i.e. additional to canine rabies) rather than an independent disease cycle (Cliquet and Picard-Meyer, 2004). The area between Sudan and Guinea presents the same epizootic characteristics in the south as those that exist in the sub-Saharan zone to the north, but with the addition of accidental infection of wild species (monkeys, bats, rodents, etc.).

Nigeria occupies a special place, having been the source of numerous RRV isolates from shrews (Sorex spp.) and bats infected by Lagos virus and Mokola virus (MOKV).

In Tanzania, it has been demonstrated that the number of cases of human rabies is clearly underestimated, with a recent study by Cleaveland *et al*., (2003) suggesting that there are actually 4.9 human deaths per 100,000 inhabitants, 100 times more than the number officially recorded.

Rabies in Ethiopia is primarily a disease of dogs. However, many people receive post exposure anti-rabies treatment annually all over the country. Most people are at increased risk of being exposed to rabies, as man-dog contact is very common in the country (Eshetu *et al*., 1999). Studies by Kitala *et al*, (2001) in Kenya showed that the dog population is growing with poorly supervised and inadequate vaccination against rabies.

In South Africa, Angola, Botswana, Namibia, Zambia and Zimbabwe, canine rabies represents an important risk for humans (Cliquet and Picard-Meyer, 2004). The situation is complicated by independent rabies cycles in wild mammals and by the presence of RRV (Gt 2, 3 and 4) in wild cats (Nel *et al*. 1993; Hofmeyr *et al*., 2004). In South Africa (Nel *et al*. 1997), Zimbabwe (Bingham *et al*. 1999a, b) and

Botswana (Johnson *et al*. 2004), two distinct RABV variants have been identified from specimens of over 30 different carnivore species belonging to four families of Carnivora: Viverridae, Canidea, mustelidae, and Felidae. The two virus variants are maintained by independent cycles; one primarily associated with canids, originating with, and primarily involving domestic dogs, but also affecting several species of jackal (genus *Canis*), and bat-eared foxes (*Otocyon megalotis*); and the second cycle associated with viverrids and genets (genera *Cyntictis, Herpestes, Suricata, Genetta, Civettictus*) (Nel *et al*, 1997; Von Teichman *et al.,* 1995). The viverrid subtype of RABV probably arose recently from spillover of the canid subtype and each of these RABV ñbiotypesò have crossed species barriers to infect other mammals, with no evidence of genetic modification in the virus recovered from non-reservoir host species (Nel *et al*, 1997). Jackal populations support the epidemic of canine RV generally associated with the domestic dog. The same RV found in canines has also been discovered in the bat-eared fox (*Otocyon megalotis).* Several variants of this strain also exist. One is the canine biotype closely related to the European strains of RV, which is maintained by members of the Canidae family, the bat-eared fox, jackal species and the domestic dog (Cliquet and Picard-Meyer, 2004). The incidence of canine rabies is increasing in sub-Saharan Africa, and few successful dog vaccination programmes have been implemented in the last twenty years (Cleaveland *et al*., 2003). Some attempts at oral vaccination have been conducted in jackals (Bingham *et al*., 1999 b), free-ranging African wild dogs (*Lycaon pictus*) (Knobel *et al*., 2002; Hofmeyr *et al*., 2004) and domestic dogs (Aubert, 1992). Lagos bat virus (Genotype 2) Mokola virus (Genotype 3) and Duvenhage virus (Genotype 4) have been isolated in Africa. The isolation of Mokola virus has been reported sporadically and rather infrequently from only a small number of African countries. This is the only lyssavirus that has never been isolated in a bat. However, surprisingly, it has been isolated in a number of other different hosts, such as shrews, cats, dogs and rodents (Cliquet and Picard-Meyer, 2004). Mokola virus antibodies have been found in dogs, sheep and goat (Kemp *et al*, 1972; Ogunkoya *et al*,

1990 and Nottidge *et al*, 2007). To date, RRV of genotypes 2, 3 and 4 has never been isolated outside of Africa, with the exception of one case of rabies in the frugivorous bat (*Rousettus aegyptiacus*), reported in France in May 1999. This bat had been imported from Africa to France through Belgium, and molecular data demonstrated that the virus was an LBV (Cliquet *et al*., 1999).

2.8. Distribution of Rabies in Nigeria

Evidence of the long history of rabies is contained in the fact that every Nigerian Language has a name for rabies in dogs (Nuru, 1973; Oboegbulem, 1994). The first diagnosis of canine rabies in Nigeria was done in 1925 by the Rabies laboratory unit at Yaba, by the demonstration of Negri bodies (Oboegbulem, 1994). Although there have been reported cases and outbreaks of rabies in Nigeria since then (Anon, 1927; Owolodun, 1969; Okoh, 1983; Onunkwo *et al*., 1980; Ezebuiro *et al*, 1980; Fagbemi *et al*., 1981; Ogunkoya, 1986; 1990; Ezeokoli and umoh, 1987; Kwasari and Baba, 2002; Bello *et al*. 2007; Dzikwi *et al*, 2010 b). Information on rabies death is scarce due to misdiagnoses and under reporting (Ehizibolo *et al*., 2008). In recent decades, the frequency of rabies has increased in Nigeria (Kwasari and Baba, 2002). Controlling this disease will require a deeper understanding of its biology (Okonko *et al*, 2010). When interpreting rabies case data for epidemiologic analysis, we must distinguish between the concept of maintenance (the ability of local populations to support a disease cycle) and persistence (the presence of > 1 infected local population) in a host metapopulation (Okonkwo *et al*., 2010). Many animal and human cases are probably unreported especially in rural areas where veterinary and hospital facilities are inadequate. Data from diagnostic laboratories and field surveys show that over 94% of rabies outbreaks in Nigeria involve domestic dogs (Ezeokoli and Umoh, 1987; Ogunkoya, 2008). Available records (Oboegbulem, 1994) have indicated that from 1928 to 1990 the rabies diagnosis unit of NVRI Vom has diagnosed a total of 3770 rabies positive cases from dogs, cats, domestic and wild animals. Of this number 3555 (94.3%) were dogs, 114 (3.0%) were in cats and 101 (2.7%) in other domestic and wild

animals. Data collected from the records of Rabies Diagnostic Unit of NVRI Vom between January 1991 and December 2005, showed that out of the 2141 brain samples submitted for rabies diagnosis, 1039 (48.5%) were positive for rabies. Out of which 1039 (99.1%) were in dogs, 5(0.5%) in cats while 3(0.29%) and 1 (0.10%) cases were in other domestic and wild animals respectively (Garba *et al*., 2008). Strains of rabies virus were isolated from a monkey and two hyraxes in Ibadan (Annual reports, University of Ibadan Virus Research Laboratory, 1969) and one each from a Caracal lynx (Okoh, 1986 b) and a Civet cat (Enurah *et al*., 1988) at the Ibadan and Jos Zoological gardens respectively. In addition, two rabies related viruses, mokola virus isolated from wild shrews caught in Ibadan (Shope et al., 1970; Kemp *et al*., 1972) and Lagos bat virus isolated from fruits bats in Yaba, Lagos (Boulger and Porterfield, 1958) known to exist in Nigeria are still in circulation among apparently healthy dogs (Dzikwi *et al*., 2010b). Bats are recognized as important reservoir of many emerging infectious agents, including lyssavirus in Nigeria. Dzikwi *et al*., (2010a) showed that during lyssavirus surveillance to determine the presence of lyssavirus-neutralizing antibodies in bats sera in northern Nigeria, 19% of the 140 sera samples tested neutralized Lagos bat virus. Chalmers and Scott (1969) have proposed a model in which rabies virus survives by adaption to preferred host (Dog) such that a mutually non-detrimental ecological balance or ñclimaxò is established. In the ñcanid climaxò in Africa, they suggest that the dog suffers in-apparent infection or transient illness, and recovered animals become ñhealthy carriersò capable of transmitting the disease to other animals. Occasionally infection of the climax hosts may result in overt disease and death and spill over into the less adapted wild-life or human hosts which result in epidemics. There are some evidence to support this model. The evidence includes:

(a) The well adapted existence in West Africa of óOloufatoô a non-fatal disease of dogs which has been identified as rabies (Chalmers and Scott, 1969).

(b) Occasional reports of disease being transmitted from apparently healthy carriers exist in Nigeria and other parts of Africa (Fekadu, 1972; Ogunkoya *et al*., 1990; Baba, 1999; Ajayi *et al*., 2006; Garba *et al*., 2010; Dzikwi *et al*., 2010b). More work is needed to confirm this hypothesis. Alternative to the ñclimaxò hypothesis is that rabies can be continually transmitted from infected to susceptible dog before the sick dog dies. The long and variable incubation period ensures that the virus can exist in a host for up to one year before clinical disease and death occurs. This allows time for new susceptible hosts to accumulate. Transmission is further facilitated by the peculiar property of the virus that forces the diseased animals to actively seek to bite other animals (Ezeokoli and Umoh, 1986). The habit of dogs to congregate and fight during the mating season also enhances transmission (Nawarthe, 1980). Regardless of the exact method of maintenance, the domestic dog is the primary source of human infection in Nigeria (Nawarthe, 1980; Ogunkoya *et al*., 2003; Okoh, 2007). Human exposure is more likely to occur through bites from owned unvaccinated dogs then stray dogs, although because of their tendency to roam far and wide, stray dogs appear to serve as primary source of spread from one community to the other (Ezebuiro *et al*, 1980; Ezeokoli and Umoh, 1986). Human exposure is more common in cities and large cosmopolitan areas than villages in Nigeria, perhaps because of cultural practices in most village communities which limit existence of stray dogs, and ensure rapid destruction of dogs showing aberrant behaviour (Nawarthe, 1980; Ezeokoli and Umoh, 1987).

The history of rabies in Enugu state appears to be very long as evidenced by the fact that every community of the state has a name for rabies in dogs, for example, Udi people call it Nkita ara, Nsukka people call it ugodu era, Ngwo people call it nchita era, Ezeagu people call it nkuta era. A seroepidemiological survey of rabies virus antibodies in non vaccinated dogs in Nsukka environment carried out by Wosu and Anyanwu, 1990, showed that out of 254 serum samples collected from non vaccinated and apparently healthy dogs of local breeds 16.1% had antibodies against rabies virus. The

prevalence rates of rabies antibodies in, less than 3 months, 3-6 months, and over 6 months old dogs were 17.5%, 7.3% and 22.8% respectively. This was the first documented report of rabies antibodies in non vaccinated dogs in Eastern Nigeria. It was suggested that the antibodies were most probably due to the non virulent prototype rabies or rabies-related virus strains. 83.9% of susceptible dogs in the population suggested a mandatory regular vaccination of all dogs in interest of public health and control of the disease. The study highlighted the need for more studies on epidemiology of rabies and rabies-related viruses in Nigeria.

2.9. Transmission.

Exposure to rabies virus occurs when an animal or person comes into physical contact with saliva from rabid animal or with an environment containing live rabies virus (WHO, 2005). However, exposure does not always lead to contraction of the disease (Baer and Tordo, 1994). The type of infected animal, as well as the location and severity of the bite all appear to influence the chance of infection. For instance, if left untreated, severe wolf bite on the face results in 80% to 100% mortality, while a more superficial dog bite on the leg only results in a 3% mortality rate (Baer and Tordo, 1994). In general the proximity of the bite to the head determines the risk, presumably because the virus faces a more direct route to the central nervous system. The risk of exposure to the victim is most likely dependent on the amount of virus present in the saliva (NIAID, 1998). Despite the fact that rabid dogs only present a modest risk when compared to wolves or even cats, the vast majority of human deaths worldwide (99%) results from dog bite (WHO, 1999). This is due to imbalance of cases which occur in countries where canine rabies has not been controlled and humans and dogs live in very close contact (CDC, 1998). On the other hand, in developed nations where canine vaccinations are well established, rabies cases are dramatically reduced in number, and reported bites are almost always from wild animals such as raccoons and skunks (Hanlon *et al.,* 1998; WHO 2005*).* While bites accounts for the vast majority (about 99.8%) of rabies cases, other

forms of transmission has been reported, including contamination of mucus membranes, faulty vaccines, corneal transplants and aerosol transmission (CDC, 1998). Movement of animals provides a route for transfer of pathogens between animals and the spread of disease to a new area. Movement of animals occur for different reasons such as pets, laboratory purposes, food, farming and hunting trades as well as for conservation, reintroductions and rehabilitation. These trades are large, increasingly globalised and without adequate disease surveillance (Daszak and Cunningham, 2003). Globally, outbreaks resulting from wildlife trade have caused hundreds of billions of dollars of economic damage (Karesh, 2005).

2.10. Generalized Pathogenesis

Rabies is almost always caused by the bite of a rabid animal (Ruppretch *et al*., 2006). Lyssa viruses are transmitted from peripheral to the central nervous system (CNS) by progressive centripetal retrograde trans-synaptic spread from infected sensory and motor neurons to the spinal cord ganglia and brain (Dietzschold *et al*., 2005). The mechanism by which the rabies virus infects a cell is similar to that of many other viruses. The infection is started when G protein projections on the virus interact with the cell membrane of the host. After host cell attachment via the viral G protein, the virus is adsorbed into the cell via engulfment with the plasma membrane. Once inside the cell, the viruses congregate inside the endosomes which quickly drop in pH. As the pH changes the conformation of the G protein changes such that it causes the viral membrane to fuse with the endosomal membrane. This leads to the expulsion of viral proteins and RNA into the cytoplasm. Once in the cytoplasm, the viral L protein transcribes five mRNAs from the RNA genome using the free nucleotides from the host cell cytoplasm. These mRNAs are 5ô-capped and poly-adenylated, allowing them to be translated into their corresponding five viral proteins using the cellôs translation machinery. These proteins also undergo post-translational modification within the host cell, including the glycosylation of G protein and phosphorylation of the NS protein (Baer and Tordo, 1994). The viral RNA genome is replicated using a complex made up of the L

and NS proteins (Finke and Conzelmann, 2005). All the viral components congregate in one location of the cytoplasm, which leads to the characteristic staining seen in the diagnostic test which looks for Negri bodies (Dietzschold *et al*., 2005). At this site the ribonucleoprotein complex is formed and it, along with M and G proteins, are recruited to the host cell membrane. At the membrane, M protein is implicated in the bundling of the virus into a particle and initiating the budding off process which releases new viron, ready to infect a nearby cell. Inside the neuron virus spread can occur very fast, as viral particles within the cell can simply travel the length of the axon before budding off, therefore being able to cover a large distance in less time and with fewer replication cycles. This may be one reason why the disease can spread so quickly from the peripheral to central nervous system (Baer and Tordo 1994). One thing that is extraordinary about the rabies virus is its high affinity for nervous tissue, the precise mechanism for passage of virus from nervous system to glandular sites such as the salivary gland during the infection process has not been determined (Ruppretcht *et al*, 2006). After multiple rounds of CNS replication, virus spreads centrifugally to the salivary glands and other innervated sites (Finke and Conzelmann, 2005; Rupprecht *et al*., 2006). Pathology associated with infection is subtle and typical of non suppurative viral encephalomyelitis. Histologic changes include; perivascular cuffing, gliosis and neuronophagia (Dolma and Charlton, 1987; Dunlop *et al*., 1996). The prodromal period typically lasts between two and ten days, and is characterized by nonspecific symptoms which can be mistaken for common cold or flu. These include; headache, fever fatigue, sore throat, cough and gastrointestinal discomfort. Occasionally, the individual experiences pain or numbness at the site of the bite (Dietzschol *et al*. 2005).

2.11. Clinical Signs of Rabies

Generally, the first clinical signs of rabies may be nonspecific and include lethargy, fever, vomiting, and anorexia. Signs progress within days to cerebral dysfunction, cranial nerve dysfunction, ataxia, weakness,

paralysis, seizures, difficult breathing, difficulty swallowing, excessive salivation, abnormal behavior, aggression, and/or self-mutilation. (Haig, 1976; CDC, 2008).

According to Kahn *et al* (2005), the clinical course may be divided into 3 phases- prodromal, excitative, and paralytic/end stage. However, this division is of limited practical value because of the variability of signs and the irregular lengths of phases. During the prodromal period, which lasts ~1-3 days, animals show only vague CNS signs, which intensify rapidly. The disease progresses rapidly after the onset of paralysis, and death is almost always inevitable. Some animals die rapidly without obvious clinical signs.

The term ñfurious rabiesò refers to animals in which aggression (the excitative phase) is pronounced. ñDumb or paralytic rabiesò refers to animals in which the behavioral changes are minimal, and the disease manifests principally by paralysis.

Furious Form:

This is the classic ñmad-dog syndrome,ò although it may be seen in all species. There is rarely evidence of paralysis during this stage. The animal becomes irritable and, with the slightest provocation, may viciously and aggressively use its teeth or claws. The posture and expression is one of alertness and anxiety, with pupils dilated. Noise invites attack. Such animals lose caution and fear of other animals. Carnivores with this form of rabies frequently roam extensively, attacking other animals, including people, and any moving object. They commonly swallow foreign objects, e.g., feces, straw, sticks, and stones. Rabid dogs may chew the wire and frame of their cages, breaking their teeth, and will follow a hand moved in front of the cage, attempting to bite. Young pups can seek human companionship and are overly playful, but bite even when petted, usually becoming vicious in a few hours. Rabid skunks may seek out and attack litters of puppies or kittens. Rabid domestic cats and bobcats can attack suddenly, biting and scratching viciously. As the disease progresses, muscular incoordination and seizures are

common. Death results from progressive paralysis.

Paralytic Form:

This is first manifest by paralysis of the throat and masseter muscles, often with profuse salivation and inability to swallow. Dropping of the lower jaw is common in dogs. Owners frequently examine the mouth of dogs and livestock searching for a foreign body or administer medication with their bare hands, thereby exposing themselves to rabies. These animals may not be vicious and rarely attempt to bite. The paralysis progresses rapidly to all parts of the body, and coma and death follow in a few hours.

Species Variations:

Cattle with furious rabies can be dangerous, attacking and pursuing humans and other animals. Lactation ceases abruptly in dairy cattle. The usual placid expression is replaced by one of alertness. The eyes and ears follow sounds and movement. A common clinical sign is a characteristic abnormal bellowing, which may continue intermittently until shortly before death.

Horses and mules frequently show evidence of distress and extreme agitation. These signs, especially when accompanied by rolling, may be interpreted as evidence of colic. As in other species, horses may bite or strike viciously and, because of their size and strength, become unmanageable in a few hours. People have been killed outright by such animals. These animals frequently suffer self-inflicted wounds.

Rabid foxes and coyotes often invade yards or even houses, attacking dogs and people. The abnormal behavior that can occur is demonstrated by the fox that attacks a porcupine; finding a fox with porcupine quills can, in most cases, support a diagnosis of rabies.

Rabid raccoons and skunks typically show no fear of humans and are ataxic, frequently aggressive, and active during the day, despite their often crepuscular nature. In urban areas, they may attack domestic pets.

In general, rabies should be suspected in terrestrial wildlife acting abnormally. The same is true of bats that can be seen flying in the daytime, resting on the ground, attacking people or other animals, or fighting.

2.12. Diagnosis of rabies

2.12.1 Collection of brain samples

Usually the brain is collected following the opening of the skull in a necropsy room, and the appropriate samples are collected preferably Ammonôs horn, thalamus, cerebral cortex and medulla oblongata (OIE, 2000).

2.12.2. Identification of the agent

Clinical observation may only lead to a suspicion of rabies because signs of the disease are not characteristic and may vary greatly from one animal to another. The only way to undertake a reliable diagnosis of rabies is to identify the virus or some of its specific components using laboratory tests. As rabies virus is rapidly inactivated, refrigerated diagnostic specimens should be sent to the laboratory by the fastest means available. Shipment conditions must be considered to be part of the órabies diagnostic chainô and should follow international guidelines. Several laboratory techniques may be used, and have been detailed and standardized in the fourth edition of the WHOôs *Laboratory Techniques in Rabies* (WHO, 1996). The methods vary in their efficiency, specificity and reliability. They are classically applied to brain tissue, but they can also be applied with variable sensitivity and specificity to other organs (e.g. salivary glands). In the brain, rabies virus antigen is particularly abundant in the thalamus, Pons and medulla. It is recommended that a pool of brain tissues that includes the brain stem should be collected and tested (Bingham & Van der Merwe, 2002). The most widely used test for rabies diagnosis is the fluorescent antibody test (FAT), which is recommended by both WHO and OIE, and is sensitive, specific and cheap. Precautions should be taken when handling central nervous system tissues from

suspected rabies cases. Gloves should always be worn and precautions must be taken to prevent aerosols. Cutting tools, scissors and scalpels, should be used with care to prevent injury and contamination.

2.13. Laboratory tests

2.13.1. Fluorescent antibody test (FAT)

The most widely used test for rabies diagnosis is the FAT, which is recommended by both WHO and OIE. This ógold-standardô test may be used directly on a smear, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of mice that have been inoculated for diagnosis. The FAT gives reliable results on fresh specimens within a few hours in more than 95ï 99% of cases. The FAT is sensitive, specific and cheap. The sensitivity of the FAT depends on the specimen (the degree of autolysis and how comprehensively the brain is sampled) (Barrat & Aubert, 1995), on the type of lyssavirus and on the proficiency of the diagnostic staff. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue, that includes the brain stem, are fixed in 100% high-grade cold acetone for at least 20 minutes, air dried and then stained with a drop of specific conjugate for 30 minutes at 37°C. Anti-rabies fluorescent conjugates available commercially are either polyclonal or monoclonal antibodies (MAbs), specific to the entire virus or to the rabies nucleocapsid protein, conjugated to a fluorophore such as fluorescein isothiocyanate (FITC). FAT slides should then be examined for specific fluorescence using a fluorescent microscope and filter appropriate for the wavelength of the fluorescent conjugate used, for instance FITC, the most commonly used, is excited at 490 nm and re-emits at 510 nm. Aggregates of nucleocapsid protein are identified by specific fluorescence of bound conjugate. It is recommended that two independent trained operators read each FAT slide. Fluorescent antibody conjugates may be made locally, but should be fully validated for specificity and sensitivity before use, including its ability to detect lyssaviruses other than rabies.

In cases of inconclusive results from FAT, or in all cases of human exposure, further tests on the same sample or repeat FAT on other samples are recommended. This is particularly important where sample autolysis is confirmed or suspected.

2.13.2. Mouse inoculation test (MIT)

This test detects the infectivity of a tissue suspension in laboratory animals. They should be used if the FAT gives an uncertain result or when the FAT is negative in the case of known human exposure (OIE, 2000). Three -to-ten mice, 3ï 4 weeks old (12ï 14 g), or a litter of 2-day-old newborn mice, are inoculated intracerebrally. The inoculum is the clarified supernatant of a 10ï 20% (w/v) homogenate of brain material including brainstem (e.g. cortex, Ammonôs horn, thalamus, and medulla oblongata) in an isotonic buffered solution containing antibiotics. Mice should be anaesthetised when inoculated. The mice are observed daily for 28 days, and every dead mouse is examined for rabies using the FAT. For faster results in newborn mice, it is possible to check one baby mouse by FAT on days 5, 7, 9 and 11 post-inoculation. Any deaths occurring during the first 4 days are regarded as nonspecific (due to stress/bacterial infection etc.).

Once a validated and reliable cell culture unit exists in the laboratory, consideration should be given to replacing the mouse inoculation test with cell culture whenever possible as it avoids the use of live animals, is less expensive and gives more rapid results. However, advantages of MIT are that when the test is positive, a large amount of virus can be isolated from a single mouse brain for strain identification purposes and that it can be easily and practicably applied in situations where skills and facilities for other tests (e.g. cell culture) are not available. MIT may also detect viruses other than rabies virus (OIE, 2000).

2.13.3. Molecular techniques

Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription PCR (RT-PCR), PCR-ELISA, hybridisation *in situ* and real-time PCR are used as rapid and sensitive additional techniques

for rabies diagnosis (Fooks *et al.,* 2009). The principle of lyssavirus-specific PCRs is a reverse transcription of the target RNA (usually parts of the N gene) into complementary DNA followed by the amplification of the cDNA by PCR. Although those molecular tests have the highest level of sensitivity, their use is currently not recommended for routine post-mortem diagnosis of rabies (WHO, 2005) due to high levels of false positive or false negative results without standardisation and very stringent quality control. Nevertheless, they are useful for confirmatory diagnosis, as a first step in virus typing.

2.14. Serological tests

Serological assays are not suitable for diagnosis of rabies infections in humans and animals as virus-specific antibodies in serum tend to appear on average 8 days after the onset of clinical symptoms. They are mainly used to evaluate the immune response to human and animal rabies vaccines. The gold standard is the virus neutralisation test. Virus neutralising antibody titres directly correspond to the level of protection. Virus neutralisation assays are also the prescribed tests for international trade and travel with pets. Both FAVN (Fluorescent Antibody Neutralization Test) and RFFIT (Rapid Fluorescent Focus Inhibition Test) are approved for determination of the titre of virus neutralizing antibodies. (Rabies-Bulletin-Europe, 2006).

2.15. Control of rabies in dogs

Canine rabies can be eliminated, as has been demonstrated in North America, Western Europe, Japan and many areas in South America (WHO, 2004). However, canine rabies is still widespread, occurring in over 80 countries and territories, which are predominantly in the developing world (Cliquet and Picard-Meyer, 2004). In more than 99% of all human rabies cases, the virus is transmitted from dogs; half of the global human population lives in canine rabies endemic areas and is considered at risk of contracting rabies (WHO, 2004). Effective animal vaccines that provide a considerable duration of immunity have been developed and mass parenteral vaccination programmes remain the mainstay of canine rabies control (WHO, 2003). Dog destruction alone is not effective in rabies control. The principal challenge is effective delivery of vaccines to ensure adequate vaccination coverage in the reservoir dog population. Studies coordinated by World Health Organization on dog populations have shown that, in many communities in Africa, Latin America and Asia, a substantial proportion (at least 60 ï 75%) of the total dog population is accessible for parenteral immunization (WHO, 2003). During the last two decades, a significant reduction

in human rabies has been achieved in Mexico, United States of America and the Caribbean by the programme for the elimination of canine rabies initiated and coordinated by the Pan American Health Organization/WHO Regional Office for the Americas (WHO,2003). In contrast, over the past two decades rabies has been increasing in parts of sub-Saharan Africa and Asia, attributed to rapidly growing dog populations and increasing urbanization, density and mobility of human populations (WHO, 1984). To ensure effective coverage, vaccination programmes should consider the local ecology of the dog population, involve coordination of related sectors and incorporate culturally appropriate education efforts (WHO, 2004; OIE, 2010). Canine rabies control programmes should incorporate three basic elements, with priorities varying according to the prevailing social, cultural and economic factors. The basic elements are: (a) epidemiological surveillance; (b) mass vaccination; and (c) dog ecology and population control. They require community participation, managerial skills and legislation (OIE, 2010).

Epidemiological surveillance of rabies is the basis for any programme of rabies control (WHO, 1984). Rabies should be a notifiable disease within national health and veterinary system (WHO, 2004). Epidemiological data should be collected, processed, analyzed and disseminated rapidly between sectors and different administrative levels. Surveillance of areas in which laboratory confirmed cases in animals are reported should be encouraged. Attempts should be made to isolate viruses for characterization of prevalent strains (CDC, 1998). Reporting of laboratory confirmed human rabies cases alone may lead to a severe underestimation of the true number of human cases, resulting in a low priority being given to rabies control. Therefore data on the number of humans suspected as being rabid based on clinical evaluation should also be reported (WHO, 2004). The number of people seeking and receiving post-exposure prophylaxis should be reported to provide additional epidemiological information on disease burden and to evaluate the effectiveness and cost-benefit of rabies control programme (CDC, 1998). There is need to adopt or establish systems of rabies reporting, especially for the investigation of rabies outbreaks and identification of the rabies virus strains involved, in view of increased international travel and transfer of animals (WHO, 2004).

Mass canine vaccination campaigns have been the most effective measure for controlling canine rabies (Matter, 2000). The organization of the campaigns is based on inter-sectoral collaboration, community participation and strong media support. The success and sustainability of these campaigns depends on political commitment, acquisition and supply of canine vaccines by the ministries of health, free delivery of these vaccines, and local-level commitment in the planning and execution of the campaigns and effective coordination and supervision of the campaigns by the health services. At least 70% of the dog population in each community should be vaccinated in areas where canine rabies is endemic (WHO, 2004). Rabies vaccination campaigns are generally conducted annually but more frequent campaigns may be required in areas where population birth and death rates are high (Cleaveland *et al*., 2006). All dogs and cats, when presented, should be immunized, regardless of their age, weight or state of health. Given the high birth rates of many populations, particular attention should be paid to ensuring adequate vaccination coverage of puppies (Dodet and Meslim, 1997). In order to apply strategic planning and management, an estimate of the dog population and evaluation of a mass vaccination campaign is required. WHO has produced guidance for estimating dog population size (Matter, 2000). All personnel handling dogs during vaccination campaigns should receive pre exposure prophylaxis. Registration and permanent identification of vaccinated dogs is recommended (WHO, 2003). Three basic approaches to mass vaccination campaigns have been adopted especially in canine rabies endemic area: house to house visits, fixed vaccination posts in well-recognized sites within the community, and mobile teams which set up temporary vaccination posts (Dodet and Meslim 1997; Cleaveland *et al*., 2006). Oral vaccination of dogs offers a new approach that may significantly improve dog vaccination coverage (especially of free-roaming and poorly supervised dogs) when applied either exclusively or in combination with parenteral vaccination. As dog accessibility to vaccination by the parenteral route is one of the major obstacles for canine rabies control in many different parts of the world (Dodet amd Meslim, 1997; WHO, 2003).

Acknowledging the limitations of the parental route for canine rabies elimination; WHO stimulated studies on oral vaccination of dogs and the development of safer and effective vaccines and baits for such vaccination (WHO, 1993; WHO, 2004). Although the preferential vaccine for dog immunization should be parenteral, oral vaccination should be used whenever there is high population of inaccessible dogs (Dodet and Meslim, 1997). Further field studies to evaluate economy, efficiency and effectiveness, and demonstrate safety are encouraged and strategies for vaccine bait distribution must be studies further as new innovations are required for economical distribution (WHO, 1993; WHO, 2004).

2.16. Control of rabies in terrestrial wildlife species

Until 1970s, wild life rabies control was focused on efforts to decrease population levels of rabies vector species (Rupprecht *et al*., 2006). The objective of wildlife culling is to lower population densities below the threshold necessary to maintain the disease. Culling techniques include hunting, trapping, poisoning and den gassing (OIE, 2004). Studies on the effect of culling reservoir species on the control of rabies show that there are only very few examples where such methods alone have either eliminated the disease or have prevented its spread to previously uninfected areas (Rupprecht *et al*., 2006). Any predator control technique should be directed not toward extinction of a reservoir species but toward reduction of a species population to a level too low to support an epizootic of rabies (Uhaa *et al*., 1992; OIE, 2010). Many limitations of cost and danger restrict the effective wide scale use of trapping, poisoning, gassing and hunting. The resilience of these Carnivora to persecution, their high reproductive potential, together with the capacity of the environment to provide food, water and shelter, most often render population control efforts futile (WHO, 2005). A more promising approach would be to combine population reduction with immunization, as has been successfully applied in Canada in 1999 to stop the advancing raccoon rabies epizootic (OIE, 2004). Promising results have been obtained in experiments on immunization of foxes with vaccine (European commission, 2005) and the procedure has also been applied in field conditions

namely in Europe and Canada. Vaccination has received increasing attention as a potential means of wildlife rabies control (European Commission, 2002). Attempts in Europe to trap wild carnivores and to release them after parenteral vaccination were rapidly abandoned, though such trap-vaccinate-release procedures are still used with apparent success in some areas of Canada. It appears more promising to lure the wild mammal into vaccinating itself. This is possible when oral vaccines are included in baits targeted at the principal host species (Uhaa *et al.* 1992; European Commission, 2005). As in Europe, the first rabies control measures applied in the USA consisted of attempts to reduce the reservoir populations, but these were measures applied in the USA, but these were unsuccessful (Smith, 1996). Oral vaccination has proved to be effective in significantly reducing the number of rabies cases in red and grey foxes, as well as in raccoons and coyotes, but oral vaccination is much more difficult in the USA than in Europe, where the number of reservoir animals is limited and the surface areas to be covered by vaccination are much smaller (WHO, 2003; Randall *et al*, 2004). As far as rabies control in wild terrestrial animals is concerned, the only efficient and cost-effective technique thus far has been oral vaccination (Aubert, 1992; Randal *et al*., 2004).

2.17. Bat rabies control

Vampire bat-transmitted paralytic rabies of cattle can be controlled by vaccination of cattle. The only presently available approach to control in the vector species is culling (Cliquet and Picard-meyer, 2004). This can be achieved by administration of anticoagulant to vampire bats, either by direct application of the substance on the backs of captured bats or by the intramuscular injection of warfarin to cattle. Non-specific methods that indiscriminately destroy haematophagous, frugivorous, nectarivorous and insectivorous bat species must be avoided. Approaches to control the transmission of insectivorous bat rabies to people should include education of the public to avoid potentially infectious contact with bats, to seek proper treatment after exposure and to prevent bats from establishing colonies in certain sensitive

buildings (e.g. hospitals and schools) (Rupprecht *et al*., 1995; Rupprecht, 2003). Preventive immunization of populations living in highly endemic areas should be considered (WHO, 2004).

It is recommended to provide education to avoid direct contact with wild life in general, and with abnormally behaving and sick animals in particular. Any person bitten by a wild animal, including bats, musk seek medical attention. Translocation of wildlife for any purpose, except conservation, should be banned or strongly discouraged.

Another possibility is the principle of population control by interrupting the reproduction cycle through the use of hormones (WHO, 2004; OIE, 2004).

2.18. Control of Rabies

The ultimate solution to the rabies problem is in the control and eventual elimination of the disease from animal population, prevention in human beings, livestock and in the reservoir host is based primarily on breaking the cycles of transmission in the reservoir hosts and secondarily on protection of these alternate hosts from exposure or infection (Baran and Frith, 1988; Okonko *et al*., 2010).

2.19.Rabies Post-exposure prophylaxis

2.19.1. General considerations

All people exposed to rabies should promptly and thoroughly cleanse their wound(s) and apply appropriate antiseptics. Professional assistance is advised. This should be followed, if careful medical assessment requires it, by a complete vaccine series using a potent and effective vaccine that meet WHO criteria and passive immunization in category III exposure (WHO, 2005). Rabies vaccines for human use that meet WHO requirements for production and control are safe and effective and are free from the neuroparalytic adverse reactions associated with nerve tissue-derived products. Pregnancy, infancy, old age and concurrent illness are not contraindications for rabies post-exposure prophylaxis in the event of

an exposure (WHO, 2005; Khawplod *et al*., 2006). Prolonged incubation periods have been associated with human rabies; therefore people who present for treatment even months after a possible rabies exposure should be evaluated and treated as if the event had occurred recently. Factors that should be considered in deciding whether or not to initiate post-exposure prophylaxis include: Nature of the contact or injury; presence of rabies in the area where the contact occurred or where the animal responsible originates; Availability of the animal for laboratory examination or observation; species of the animal; clinical status of the animal responsible; Vaccination history of the animal, and type and timing of vaccine used (Cleaveland *et al.,* 2002; WHO, 2005). The decision to administer post-exposure prophylaxis after an exposure to an apparently animal should be based on careful risk assessment by a qualified medical professional. The risk assessment should consider the criteria outlines above. A history of rabies vaccination in an animal is not always a guarantee that the biting animal is not rabid. Animal vaccine failures may occur because of improper administration or poor quality of the vaccine, poor health status of the animal, and the fact that one vaccine those not always provide long-lasting protection against infection in dogs (Cleaveland *et al*., 2002). Whether a dog bite was provoked rather than unprovoked should not be considered a guarantee that the animal is not rabid as it can be difficult to understand what an attacking dog considers provocation for an attack (Cleaveland *et al*, 2002). If the animal involved in the exposure is a potential rabies vector in a rabies-endemic region, initiation of post-exposure treatment is indicated (WHO, 2005). Prophylaxis should never await the results of laboratory examination, nor should the responsible animal be observed for signs of rabies prior to starting post exposure prophylaxis, (Willoughby *et al,* 2004). Wound treatment and administration of rabies biological, including a passive immunization product, when required, and vaccine, should be started as soon as possible after exposure (WHO, 1999). Immediate humane killing of the animal and examination of the brain at a reliable laboratory should be performed whenever possible. If the species involved is unlikely to be infected with

rabies, treatment may be deferred pending the outcome of laboratory testing, provided that results can be obtained within 48 hours (WHO, 1993; WHO 2003). If the attacking animal is a pet dog or cat that is available, it should be kept under observation for 10 days, preferably under the supervision of a veterinarian. Prophylaxis can be discontinued if the dog or cat remains healthy for at least 10 days after the exposure occurred (WHO, 2005). The natural history of rabies in mammals other than dogs or cats is not fully understood and therefore the 10-day observation period may not be applicable. Humans exposed to other species of mammals suspected to be rabid, including bats and other wild animals involved in the transmission of rabies should therefore receive post-exposure prophylaxis unless the animal can be captured, humanely killed and immediately examined at a reliable laboratory (Willoughby *et al*., 2004; WHO, 2005).

2.20. Geographic information system (GIS)

A Geographic Information System (GIS) is a system for capturing, storing, analysing and managing data and associated attributes which are spatially referenced to the earth. It is a computer system capable of integrating, storing, editing, analyzing, sharing and displaying geographically referenced information. In a more generic sense, GIS is a tool that allows users to create interactive queries, analyse the spatial information and edit data (Adewale and Olugasa., 2005; Olugasa *et al.,* 2009).

GIS technology can be used for scientific investigation, resource management, asset management, environmental impact assessment, urban planning, cartography and routing planning. For example a GIS might allow emergency planners to easily calculate emergency response times in the event of a national disaster or a GIS might be used to find wetlands that need protection from pollution (Olugasa *et al.,* 2009) .

GIS currently combines accurate, high quality digital maps with an abundance of additional information and powerful tool sets for data processing and analysis. It also makes it possible to exchange specialized information through the web. The main advantage of GIS is its ability to provide different information on the basis of maps. The first epidemiological studies carries out using GIS technology, have shown that these systems are able to investigate the in-depth mechanism of infectious diseases dissemination, and predict their spread and also analyze the consequences of bioterrorism acts (Adewale and Olugasa., 2005)

Effective surveillance and detection of animal diseases can be achieved with the use of GIS. It is able to localize hotspots in the event of rapidly expanding outbreaks. It also provides new analytical tools for the early recognition and evaluation of epidemiological characteristics of an outbreak, and subsequently for the improvement of prevention and control measures (Ramirez *et al,*2004)

Surveillance for disease events or conditions in animal populations has an implicit spatioï temporal component. Data generated within surveillance system permit changes in the health status of population overtime and space to be identified, facilitating the planning, implementation and evaluation of disease condition programme (Ramirez *et al,* 2004). GIS technology can increase the efficiency and effectiveness of surveillance systems through three functions, namely data input, analysis and outputs (Olugasa *et al.,* 2009).

2.21. Dog meat consumption and problems associated with it.

Even with the global criticism on dog slaughter as a social animal from international organizations such as the World Society for the Protection of Animals which goes against dog meat consumption and the torture of dogs caged and farmed for their meat (WSPA,2013); Some tribes in Nigeria still consider the consumption of dog meat as their cultural norms. However, majority of people and tribes consider the consumption of dog meat as unethical, prohibitive and forbidden on both religious and social grounds.

Dog trade and dog meat consumption in Nigeria is very common and a dog meat consumption rate of 80.6% has been recorded among dog traders in Niger state (Garba *et al*; 2013). The average number of dog slaughtered per day in the same state was 4 (Garba *et al*; 2013). According to Ogunkoya (2008), the illegal trade in dogs, destined for the commercial exchange for food source across the Cameroon-Nigerian border is common. Studies on twenty-four canine virus isolates from Plateau State of Nigeria revealed IV genetic variants (GV) in which GV I to III shared antigenic relatedness with isolates found in Cameroon, Chad and Benin republic (David *et al*., 2008). The forth GV was believed to be from another Northern African country (David *et al*., 2008) suggesting trans-border spread of rabies from the dog trade. The slaughter and consumption of dog meat has been reported worldwide. The practice of slaughter and consumption of dog meat has been reported in most continents and countries across the globe. These include China, Mexico, Rome, South Korea (Schwabe, 1979; Rupert, 2002; Anthony, 2009); India, Indonesia, (Anon, 2004; Mao, 2010; Shepherd, 2012); as well as in Africa like Cameroon, Ghana and Nigeria (Simmons, 1994). While some people speculate domestic dogs are only eaten for specific rituals (Eric and Oliver, 1982); others claimed it is a delicacy (Mao, 2010) but Douglas (2009) reported that the dog meat has also been used as a survival food in times of war and/or other hardships. In the reports of Murray (2007) and Willy (2007) they said dog meats are believed to have medicinal powers.

In Nigeria, rabies virus antigen has been found in the brains and salivary glands of apparently healthy dogs slaughtered for meat (Baba, 2006, Garba *et al*, 2010, Aliyu *et al* 2010)In a report from Vietnam; 5 human rabies patients did not have any history of dog or cat bites, but they had an experience of butchering dogs or cats, or consuming their meat (Anh et al., 2011). Rabies virus was also detected in 2 of the 100 sick dogs from slaughterhouses (Anh et al., 2011). These indicate that humans could become exposed and infected with rabies virus through this practice.

Despite the international perceived risk of rabies, the trade, slaughter and consumption of dogs in sub-Saharan West African countries is on the increase and may represent a source of human rabies exposure and infection. In addition researched literatures on rational for dog consumption are scanty except news paper tales. It is thus imperative to understand the social and cultural drivers of dog meat slaughter and consumption as a necessary step in preventing rabies transmission (Garba *et al*; 2013).

CHAPTER THREE

Materials and Methods

The study was made up of (i) a cross-sectional survey using a structured questionnaires (ii) collection of materials from the markets (iii) virus identification and isolation from brain tissues and salivary glands of dogs and bats (iv) GIS appreciation (v) data analysis.

3.1. Study Area

Enugu State is located between latitude 6^{o} 45^{l} and 7^{o}N and longitude $7^{o}12.5^{l}$ and 7^{o} 36^{l} W in the Southeast geopolitical zone of Nigeria. It covers a total land area of 7617.82 sqkm. It has three Senatorial zones and 17 local government areas. The State lies on low table land surrounded by streams, rivers and a number of wavering hills collectively known as Udi hills. The State is bordered in the north by Kogi and Benue States, in the south by Abia State, in the west by Anambra State and in the east by Ebonyi State (Anon, 2008).

Two senatorial zones were used for the study. The zones used were Enugu West and Enugu North senatorial zones. These areas were chosen by purposive sampling method, where dog markets, bars and restaurant exist in which dogs are slaughtered as delicacy for human consumption in the state. The specific locations sampled were; Enugu West ï Ezeagu, Udi town, Ebe, Okpatu, Awhum, 9^{th} Mile Corner, and Ngwo. Enugu North ï Nsukka town, Orie Orba, Ogbede, Ozalla, Aku, Ukehe, Ekwegbe, and Edeoballa,

Fig3.1: Dogs restrained for sale at Nkwo Ogbede and Orie orba Dog Markets

3.2. Questionnaire survey

A questionnaire survey was carried out using a structured questionnaire. The questionnaires were validated in the department of Veterinary Public health and Preventive Medicine, University of Nigeria, Nsukka.. A total of 100 copies of questionnaires were given to those that sell dog or dog meat. The questionnaires were divided into two parts. Part 1 covered the respondent bio-data, while part two was about information on dog selling business, consumption rate, possible exposure to rabies virus and frequency of rabies outbreak.

3.3. Sample Collection

A total of one hundred and fifty two heads of dogs were bought from dog traders who slaughter dogs in the dog markets and also from restaurants and bars where dogs are slaughtered as a delicacy for human consumption in the study area. Sex and age of dogs were noted at point of collection. Aging of dogs was done using Dentition, dogs under the age of six months were grouped as puppies, while those that were six months old or above were considered as adults (Wiggs and Lobprise, 1997; Bellow, 2011).

The points of collection were geo-referenced using a global positioning system (GPS) as described by Olugasa *et al* (2009).

3.4 Brain removal and sampling.

All the heads were taken to the Post Mortem Room, Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka. The brain samples (hippocampus, brain stem, cerebellum and cerebrum) and salivary glands of dogs were harvested as previously described by Aliyu *et al* (2010). Essentially, the heads were held firmly in a vice fitted on the operation table with the roastral end of the head facing downward, and the dorsal surface of the head facing the operator. A midline incision was made on the dorsal surface of the head using scalpel and blade. The skull was then exposed by dissecting away the skin, aponeurosis and temporal muscles and reflecting them laterally. The skull was sawed using hacksaw by making two latero-medial cut through the occipital bone, then the temporal bones and finally joining these two lateral cuts at a midpoint just above the eyes. The calvarium was lifted off by the aid of a strong thumb forceps, the meninges and the optic nerves were cut with the help of rat tooth forceps and a pointed scissors. The two parts of head were then untied from the vice and turn upside down and using a scalpel blade, the brain sample was then transferred onto a polythene bag and then placed on the table to remove the hippocampus, cerebrum, the cerebellum and the brain stem. All the samples were stored at -20^{o}C until analyzed.

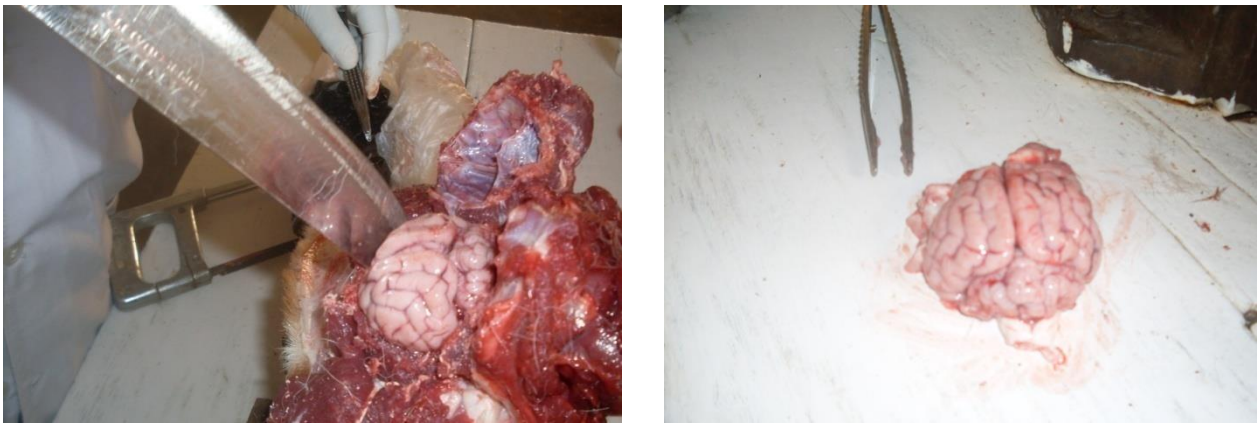

Fig 3.2: Harvesting of brain sample

3.5. Laboratory investigations

Samples were transported to National Veterinary Research Institute, Vom (NVRI) in plastic coolers with ice packs. The samples were analyzed at the Central Diagnostic Division of NVRI, Vom, to determine the presence of rabies antigen using direct fluorescent antibody test (DFA) according to the method described by Dean *et al*., (1999) and CDC, (2008); and isolation of the rabies virus using the intracerebral inoculation of 10% suspension of homogenized suspended brain materials taken from various segment of the brain as previously described by Koprowski (1967).

3.5.1. Direct Fluorescent Antibody technique (DFA):

A monoclonal antibody labeled with fluorescein-isocyothionate rabies antibody (monoclonal antibody FITC-conjugate) was used. The conjugate was titrated to determine the working dilution using the laboratoryøs standard operating procedure (SOP) and was stored in a freezer. A known positive dog brain sample (positive control) and a three weeks old normal mouse brain sample (negative control) were used as controls. Thin impression smears of the test brain specimens were made at the centre of properly labeled glass slides. Similarly, smears of the positive and negative controls were made on different slides, also properly labeled. The smears were air-dried and fixed in cold acetone contained in coplin jars for thirty minutes in a freezer. The slides were brought out and air-dried for about three minutes and the smears encircled with wax pencil. The slides were placed on a moist chamber to prevent drying. 150ul of the diluted aliquot of the rabies monoclonal antibody FITC-conjugate (DFA reagent) was added to cover the smears within the encircled areas of the slides in the moist chamber and incubated for thirty minutes at 37^{o}C. Afterwards the slides were rinsed four times in phosphate bufferd saline (pH 8.5) for about five minutes to remove the unbound DFA reagent in the smears. The stained smears were examined with X20 magnification for the presence of clusters of apple green fluorescence under fluorescent microscope indicating the presence of rabies antigen while the contrary indicated absence of rabies antigen. The

findings in the test sample smears were compared with the findings in the positive and negative control smears.

3.5.2. Mice inoculation test (MIT):

All positive samples were isolated in mice, using the technique summarized by Koprowski (1967) cited by Atanasiu (1975) and Oboegbulem (1994). One gram of the brain tissue was ground in 9mls of phosphate buffered saline (pH7.2) to make a 1 in 10 dilution. The mixture was poured into a 15 mls test tube and centrifuged at 3000rpm for 10 minutes. The supernatant was poured into a sterile container and a drop of antibiotics (penicillin-streptomycin combination) added to prevent any bacterial growth. Thereafter, 0.03mls of the supernatant was inoculated intracranially to 3 weeks old suckling mice. Each sample was given to a particular family which was made up of about 8 to 12 mice. Inoculated mice were observed for 28 days for signs of central nervous system (CNS) abnormalities such as prostration, hunch back, hind limb paralysis, running around the cage aimlessly, etc or death. Deaths from day 1 to 4 were regarded as non-specific for rabies and may be due to stress or bacterial infection. Brains of those that died or showed clinical signs within 5 to 28 days after inoculation were removed and stored at -20^{o}C. At the same time, a smear from the brain samples were made and tested for rabies using DFA.

Thereafter the viral genotypes of the confirmed positive samples were determined using reverse transcriptase polymerase chain reaction (RT-PCR) as described by Heaton *et al.*, (1997).

Fig 3.3: Intracranial inoculation of suckling mice

3.5.3. Reverse transcriptase polymerase chain reaction (RT-PCR):

Seventeen street rabies viruses isolated in mice and one DFA positive sample were subjected to RT- PCR. The single tube method using multi enzymatic liquefaction of tissue (MELT^{TM}) Total nucleic Acid Isolation System was used in the isolation, extraction and purification of the RNA according to manufacturerôs instructions (Applied Biosystems). Challenge Virus Standard (CVS) ï positive RNA was used as the positive control in all the semi nested RT-PCR. The positive RNA were obtained, following the intra-cerebral inoculation of mice with CVS. The mouse brains were confirmed as DFA positive prior to RNA extraction as earlier described. While extracting RNA from a panel of isolates, negative mouse brain was also extracted at the same time in order to provide a negative control. The negative mouse brains originated from normal uninfected mice. The RNA was quantified using the Nanodrop machine (following the manufacturerôs instructions). The RNA was diluted in HPLC purified water ensuring that it was not weaker than 1ng/εl. This was divided into aliquots of 5εl and stored at -80°C until used. A hemi-nested reverse transcriptase PCR (hnRT-PCR) assay that uses a cocktail of primers capable of detecting

the six established genotypes of rabies and rabies-related viruses was conducted according to the method of Heaton *et al* (1997) and Nadin-Davis (2002). These genotypes included classical rabies represented by ñPò Pasteur virus, Lagos bat virus ñLò, Mokola virus ñMò, Duvenhage virus ñDò and European lyssaviruses 1 and 2 ñEò. The primers were obtained from Inqaba Biotec Industries, South Africa. The primer designs were based on previous work of Heaton et al (1997). These primers were designed to recognize regions with high degree of homology between the nucleoprotein encoding genes. They detected the partial regions on the nucleoprotein gene of the rabies virus as described by previous authors (Heaton *et al* 1997; Ito *et al* 2001). The expected amplicon size was 405bp and this when sequenced could be used to differentiate between genotypes.

Positive PCR results were observed in the form of bright band corresponding to 405bp of a 100bp marker. The amplicons sequencing was carried out at Inquaba laboratory Pretoria South Africa.

3.6. Collection of bat samples, processing and laboratory investigation.

A total of Fifty nine (59) heads of bats were bought from hunters who set traps for these bats, bars and restaurants in Okpatu and Awhum which were the only places were bats are slaughtered for human consumption in the study area. All the heads were taken to the laboratory. The whole brains were harvested, processed and tested for rabies virus antigen using DFA as previously described for dogs.

Fig 3.4. Species of bats *(Tadarida nigeriae and Eldolon helvum*) consumed in the study area.

3.7. Data analysis

Results were presented in percentages and Chi square (χ^2) test was used to determine association between categorical variables. Significance was accepted at $P < 0.05$. Geographic Information System used in the analysis of the coordinate was ArcView 3.3 (Ramirez *et al*, 2004).

CHAPTER FOUR

RESULTS

4.1. Questionnaire Responses

Out of the 100 copies of questionnaire distributed, there were a total of 58 (58%) respondents who completed and returned their questionnaires. Many of the respondents who sell and consume dog meat were not resident in Enugu State.

Sex distribution, age, educational qualification and dog selling business experience of respondents are presented in table 4.1. Thirty four (58%) and 22 (38%) of the respondents were males and females, respectively, while the remaining 2 (4%) did not indicate their sex. Three (3%) of the respondents were below 25 years of age, 33 (57%) were between 25 and 44 years, while the remaining 20(34%) were between 45 years and above. The educational qualifications of the respondents showed that 70% had primary to secondary certificate, 10% had diploma certificate, 9% had university degree and the remaining 11% had no formal education. Fifty one (88%) of the respondents reported that they have been in the business for more than one year while the remaining 7 (12%) have not stayed up to a year in the business.

Information on dog business, dog meat consumption rate, vaccination status of dog traders, possible exposure to rabies virus and frequency of rabies outbreak in dog market is presented in table 4.2. Eighty seven percent of the dog meats sold were sourced from homes and markets while the rest (13%) were sourced from trapped stray dogs. Ninety five percent of the respondents (dog traders) reported that people sell dogs for slaughter due to financial constraints while the remaining 5% was because of the dog eating culture of the community where they hailed from. Three quarter (75%) of the respondents do not ask for anti- rabies vaccination history of dog before purchase while the rest (25%), asked for anti-rabies

vaccination history. Eighty one percent of the respondents reported that they eat dog meat because they believe that it cures some diseases including reduction of high blood pressure, while the rest (19%) said that they perceived it just as any other meat. Specifically, 50% were of the opinion that the meat of rabid dog enhances male libido.

Vaccination status of dog sellers, possible exposure to rabies virus and frequency of rabies outbreak in the dog market are presented in table 4.3.While 84% of the respondents admitted that they had never been vaccinated against rabies; 16% claimed that they were vaccinated. About one fifth (22%) of the respondents indicated that they had been bitten by dogs in the course of the business. Sixteen percent of the respondents who had been bitten by dogs reported that those dogs did not die within 10 to 21days after the bite. Twelve percent of respondents reported that they had been bitten by suspected rabid dogs in the course of the business yet only one of them (2%) indicated that he received full course of anti- rabies vaccination in a hospital, the other 88% used herbal concoction to treat themselves. Seventy one percent of the respondents reported that they had slaughtered and eaten suspected rabid dogs. Twenty three (39%) of the respondents had encountered suspected rabid dogs in the course of their business; of these, 19(83%) stated that it occurred more between October to December while the remaining 4 (17%) reported that they encounter it more between April to June.

Table 4.1: Distribution of Sex, Age, educational qualification and dog selling business experience of 58 dog traders and butchers responding to a questionnaire survey in Enugu State, Nigeria.

Characteristics	Frequency (%)
Sex	
Male	58
Female	38
No response	4
Age (years)	
Below 25	2
25-34	14
35-44	19
45-54	13
55 and above	7
Educational qualification	
First School Leaving Certificate	47
West African School Certificate Education	23
University Degrees	9
No formal Education	11
Dog seller's Experience	
Less than 1 year	12
1-4 years	22
5-9 years	36
10- 19 years	17
20- 29 years	8
30 years and above	5

Table 4.2: Information provided by respondents on dog selling business, consumption rate, and reason for dog meat consumption

Characteristics	Frequency (%)
Source of dog meat	
Household dogs	36
Primary market	51
Trapped stray dogs	13
Why people sell dogs for Slaughter	
Due to Sickness	1
Frequent complaints of bite	0
Financial constraints	95
Breed for sale	4
History of ARV during purchase	
Ask for ARV history	25
Do not ask	75
Reason for dog meat consumption	
It is medicinal (malaria and high blood pressure)	81
Relishes it but not medicinal	19
Encountered and recognized rabid dogs	
Yes	71
No	29
Consumption of suspected rabid dog	
Eaten suspected rabid dog	71
Never eaten rabid dog	29
Reason for consuming rabid dog meat	
Enhances male libido	50
No reason	50

Table 4.3: Information on the vaccination status of dog sellers, possible exposure to rabies virus and frequency of rabies outbreak in the dog market

Characteristics	Frequency (%)
ARV status of dog traders and butchers	
Vaccinated	16
Not vaccinated	84
Dog bite in the course of the business	
Bitten	22
Not bitten	78
What happened to the dog?	
Did not die 10 to 21 days after the bite	16
Was sold off	25
Killed immediately	18
Escaped	41
Died within 10 to 21 days	0
Suspected rabid dog bite	
Bitten	12
Not bitten	88
Method of treatment	
Herbal concoction	88
Anti- tetanus toxoid	7
Black stone	3
Full course post- exposure ARV	2
Occurrence of suspected rabid dog	
January ï March	0
April - June	17
July ï September	0
October - December	83

4.2. STUDY LOCATIONS AND SAMPLE DISTRIBUTION.

The coordinates of locations within the different Local Government in the study area from where samples are collected is presented in table 4.3. Out of the 16 locations sampled, the greater proportion of the samples 83 (55%) were obtained from four locations; Orie Orba (28 samples), Ngwo (22), Edeoballa (15) and Nkwo Ogbede (18) located respectively in Udenu, Enugu North, Nsukka, and Igbo-Etiti Local Government Areas (LGA). The balance of the samples totalling 69 (45%) ranging from 3-9 samples per site was collected from the remaining 12 sites within the six LGAs sampled.

Table 4.4. COORDINATES OF THE SAMPLED AREAS IN ENUGU STATE

Source	LGA	Number of samples	Elevation(Ft)	Longitude (E)	Latitude (N)
Orie Orba	Udenu	28	1404	007^{O} 27.471ô	06^{O} 51.540ô
Nsukka Town	Nsukka	4	1537	007^{O} 23.766ô	06^{O} 50.855ô
Ngwo	Enugu north	22	1409	007^{O} 26.927	06^{O} 25.337ô
Ezeagu	Ezeagu	6	1895	007^{O} 17.419	06^{O} 26.253
Ebe	Udi	8	1188	007^{O} 23.033	06^{O} 28.872
9^{th} Mile Corner	Udi	8	1156	007^{O} 24.385	06^{O} 26.014
Ozalla	Igbo-Etiti	5	1531	007^{O} 24.169	06^{O} 43.556
Edeoballa	Nsukka	15	1686	007^{O} 25.294	06^{O} 47.709
Ekwegbe	Igbo- Etiti	8	1769	007^{O} 25.778	06^{O} 42.947
Nkwo Ogbede	Igbo-Etiti	18	1204	007^{O} 22.659	06^{O}40.465
Udi Town	Udi	6	1420	007^{O} 23.951	06^{O}19.393
Ukehe	Igbo-Etiti	5	1547	007^{O}24.914	06^{O} 39.645
Aku	Igbo-Etiti	9	1203	007^{O} 20.057	06^{O} 41.848
Ukopi	Igbo-Etiti	3	1631	007^{O} 25.212	06^{O} 41.735
Okpatu	Udi	4	1504	007^{O} 24.792	06^{O} 33.260
Awhum	Udi	3	1394	007^{O} 24.834	06^{O} 32.095

4.5: Areas of sample collection

The locations of sample collection within Local Government Areas (LGA) in the study area are presented in figure 4.1. Dog head samples were collected from 16 different sites located within six LGAs; Udenu (1 location), Nsukka (2), Enugu North (1), Ezeagu (1), Udi (5), and Igbo-Etiti (6 locations).

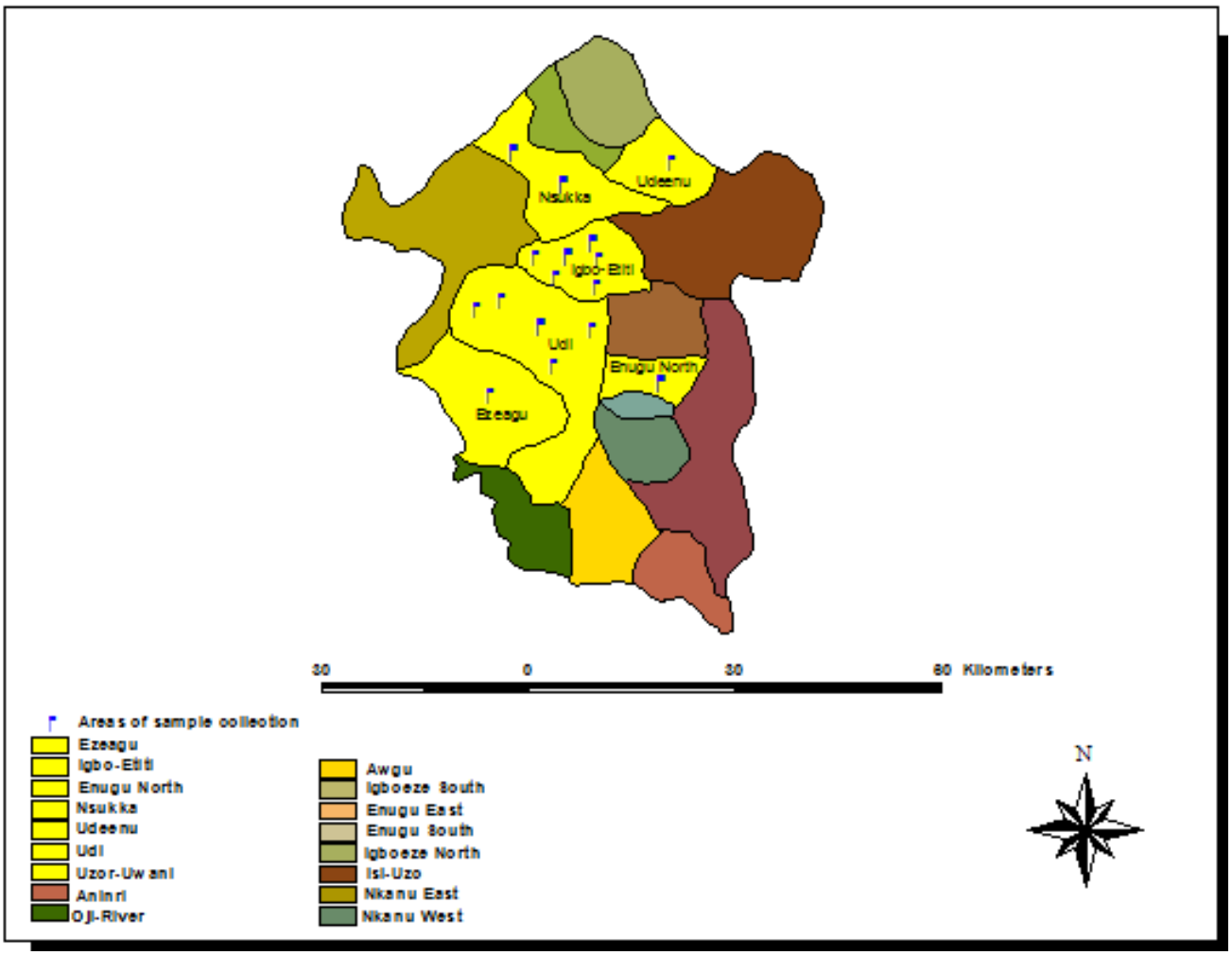

Figure 4.1: Map of Enugu State showing areas of sample collection.

4.4. RESULT OF THE DFA ANALYSIS OF APPARENTLY HEALTHY DOG BRAIN AND SALIVARY GLAND SAMPLES.

The DFA results of dog brains and salivary glands collected from different locations within Enugu North and Enugu West senatorial zones are presented in table 4.4. Out of a total of 152 dog brain samples collected from the study area, 18(12%) tested positive for rabies virus antigen. A total of 10 salivary glands from positive brain samples analyzed by DFA were all (100%) positive for rabies virus antigen. Positive dog brain samples were detected in 7 out of the 16 locations sampled. The percentage positive samples recorded were; Orie Orba (21%), Nsukka Town (25%), Ngwo (18%), Ebe (13%), Edeoballa (13%), Nkwo Ogbede (11%) and Aku (22%) No positive sample was recorded in Ezeagu, 9th Mile Corner, Ozalla, Ekwegbe, Udi, Ukehe, Ukopi, Okpatu, and Awhum.

Table 4.5: Distribution of DFA results according to the locations of sample collection

Source of sample	Brain			Salivary gland		
	Number of samples	Number positive	% positive	Number of samples	Number positive	% positive
Orie Orba	28	6	21	4	4	100
Nsukka Town	4	1	25	0	-	-
Ngwo	22	4	18	2	2	100
Ezeagu	6	0	0	0	-	-
Ebe	8	1	15	0	-	-
9th mile corner	8	0	0	0	-	-
Ozalla	5	0	0	0	-	-
Edeoballa	15	2	13	2	2	100
Ekwegbe	8	0	0	0	-	-
Nkwo Ogbede	18	2	11	1	1	100
Udi Town	6	0	0	0	-	-
Ukehe	5	0	0	0	-	-
Aku	9	2	22	1	1	100
Ukopi	3	0	0	0	-	-
Okpatu	4	0	0	0	-	-
Awhum	3	0	0	0	-	-
Total	152	18	12	10	10	100

Fig 4.2: DFA NEGATIVE SLIDE

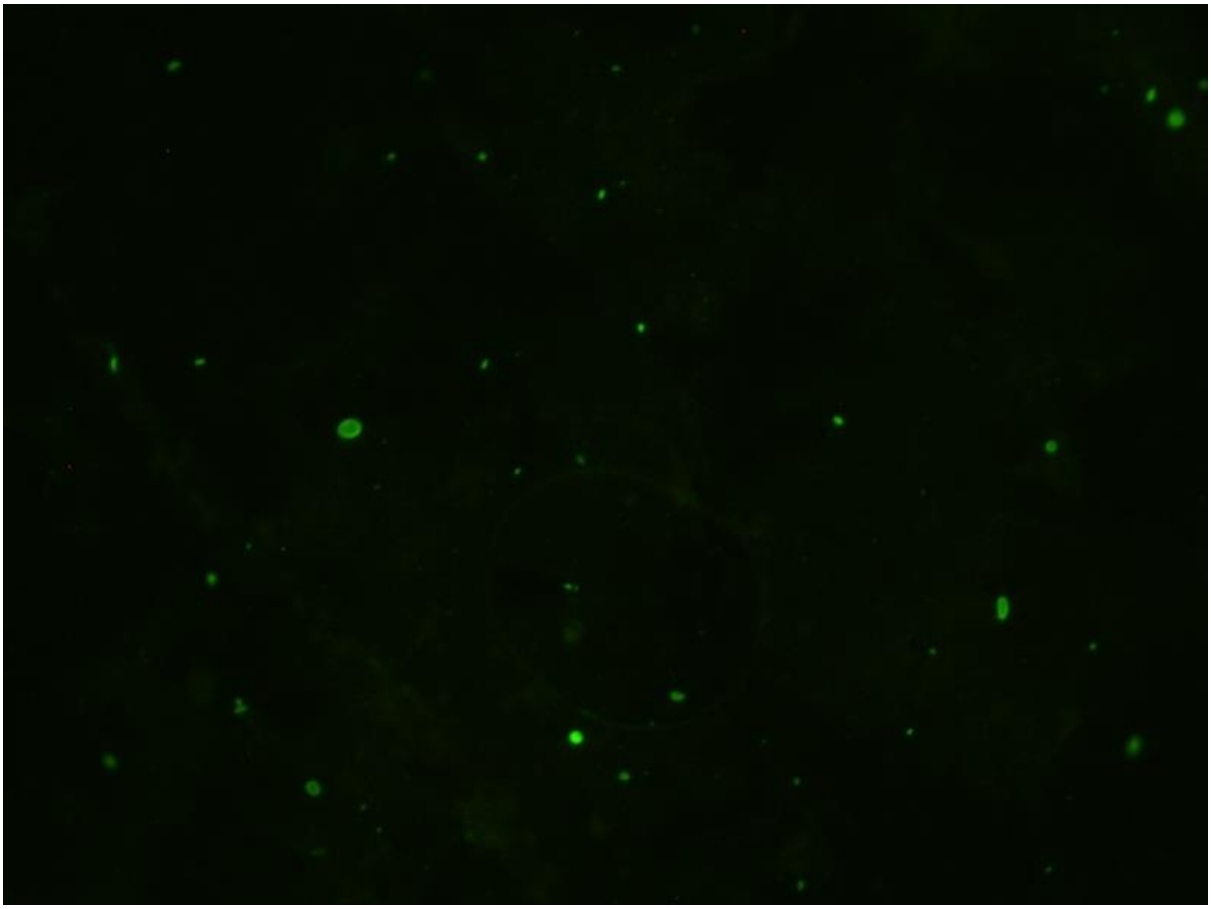

Fig 4.3: DFA POSITIVE SLIDE

4.5: Distribution of DFA positive samples into local government areas.

The locations within the LGAs where rabies positive dog brain samples were collected are shown in figure 4.4 The positive dog brain samples were collected from 5 out of the 6 LGAs sampled and from 7 locations within the 5 LGAs; Udenu (1), Enugu North(1), Nsukka (2), Igbo-Etiti (2), and udi (1).

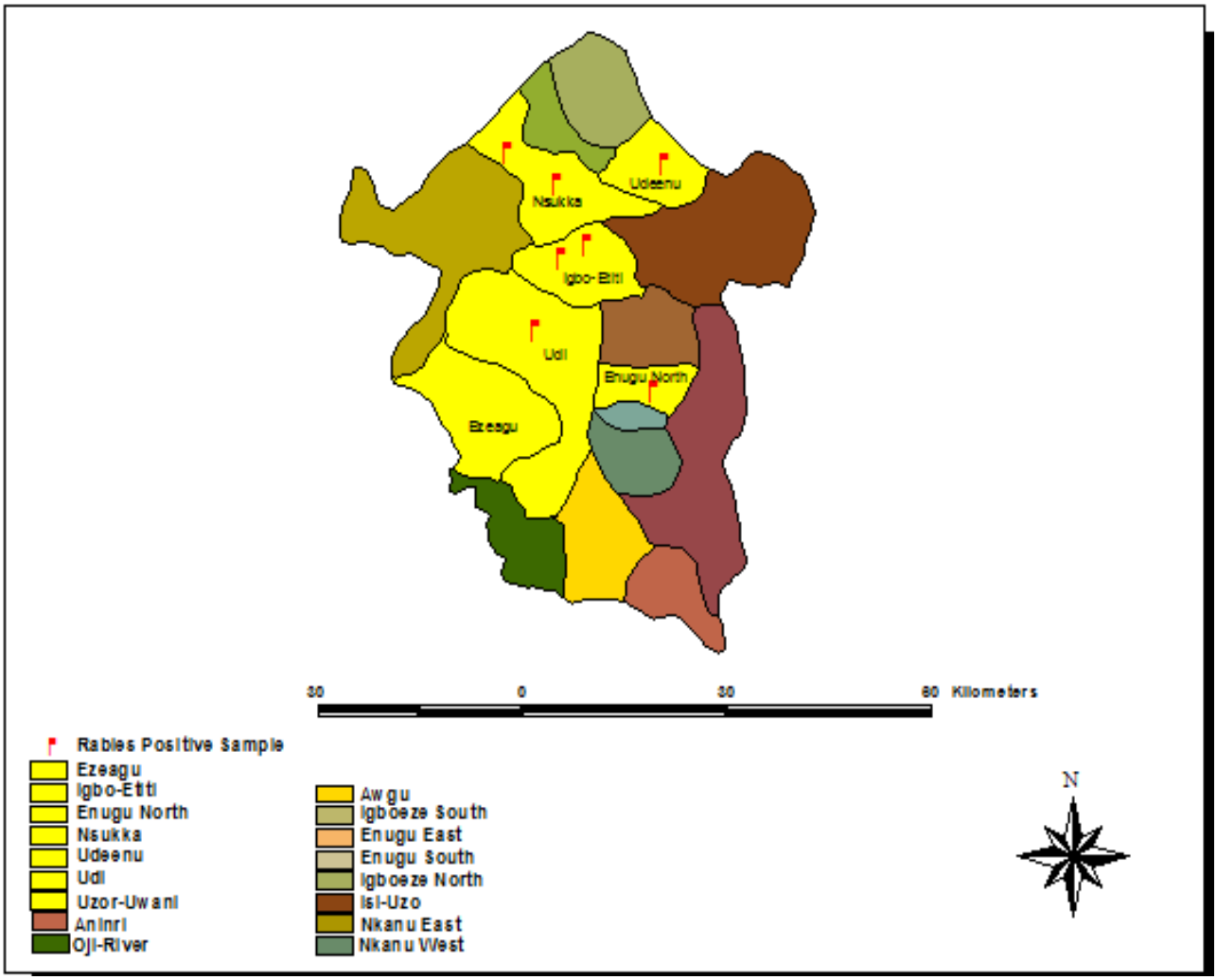

Figure 4.4: Map of Enugu State showing clusters of rabies positive sample within the study area.

4.6: Distribution of DFA Negative samples into local government areas.

The locations within LGAs where rabies negative dog brain samples were collected are shown in figure 4.5. Negative dog brain samples were collected from 3 out of the 6 LGAs sampled and 9 locations within the 3 LGAs; Igbo-Etiti (4), Udi (4) and Ezeagu (1).

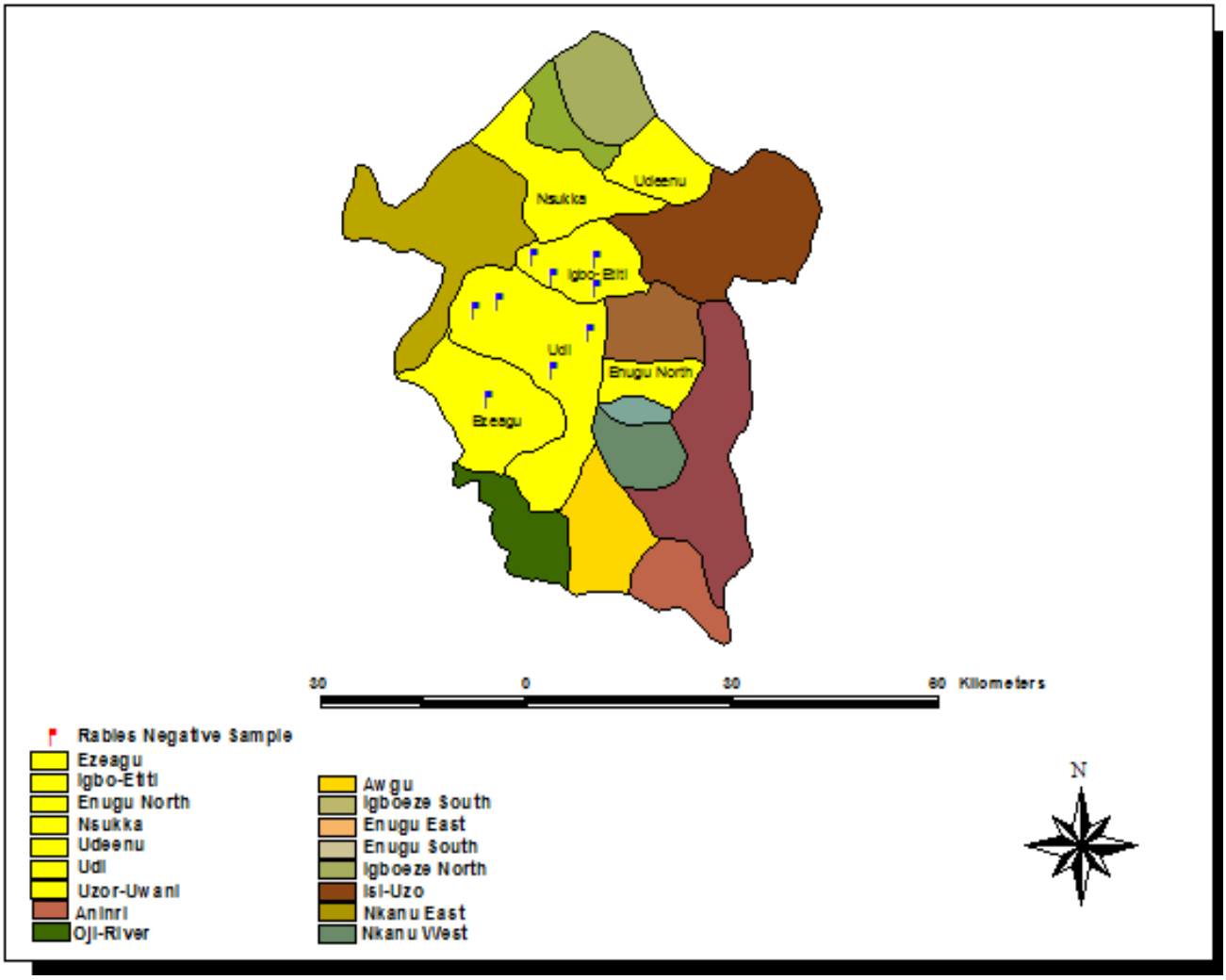

Figure 4.5: Map of Enugu State showing clusters of rabies negative samples within the study area.

4.7. MIT result

Out of the 18 DFA positive samples that were inoculated in Mice, 17 (94%) were positive.

Table 4.6: MIT result of the DFA positive samples.

TOTAL NO OF DFA POSITIVE SAMPLES TESTED	TOTAL NO OF MIT POSITIVE SAMPLES.
18	17 (94%)

Note: ( ) percentage positive.

4.8. Sex distribution of DFA positive samples.

The sex distribution of the DFA positive dog brain samples is shown in table 4.6. Nine (18%) out of 51 male and nine (9%) out of the 101 female dog heads examined were positive for rabies virus antigen in their brain. There was no significant [$p > 0.05$] association between gender and presence of rabies virus in the brain of apparently healthy dogs.

Table 4. 7. Sex Distribution of DFA positive dog brain samples

Sex	Total number of samples tested	Number positive	% positive	χ^2	P value
Male	**51**	**9**	**18**	**2.468**	**NS**
Female	**101**	**9**	**9**		
Total	**152**	**18**	**12**		

4.9: Age distribution of DFA positive samples.

The age distribution of the DFA positive dog brain samples is presented in table 4.7. One hundred and thirty one of the dog brain samples belong to adults while 21 were puppies and 17(15%) and 1(5%) respectively tested positive for rabies virus antigen by DFA.

There was no significant [$p > 0.05$] association between age and presence of rabies virus antigen in the brain of apparently healthy dogs.

Table 4.8: Age Distribution of DFA positive dog brain samples

Age group	Total number no samples tested	Number positive	% positive	G^2	P value
Puppy	21	1	5	1.19	NS
Adult	131	17	15		
Total	152	18	12		

4.10. Geographic distribution of rabies virus antigen in the study area

Geographic distribution of rabies positive dog brain samples is shown in table 4.8. Five (9%) out of 57 samples collected from Enugu West Senatorial zone tested positive for rabies virus antigen while 13 (14%) out of 95 samples collected from Enugu North Senatorial zone tested positive for rabies virus antigen. There was no significant [$p > 0.05$] association between distribution of rabies virus antigen and senatorial zones.

Table 4. 9: Geographic distribution of rabies positive dog brains in the study area

Senatorial zones	Total number no samples tested	Number positive	% positive	G^2	P value
Enugu West	57	5	9	0.823	NS
Enugu North	95	13	14		
Total	152	18	12		

4.11: RABIES VIRUS ANTIGEN SURVIELLANCE IN SLAUGHTERED BATS USING DFA

The DFA result of the bat brain samples tested is presented in table 4.9.Out of the 59 bat brains tested, 20 were insectivorous bats (*Tadarida nigeriae),* while 39 were frugivorous bats (*Eldolon helvum).* None tested positive for rabies virus antigen.

Table 4. 10: DFA result of the different bat species tested

Species of bat	Total number of sample	Number positive	% positive
Tadarida nigeriae	20	0	0
Eldolon helvum	39	0	0
TOTAL	59	0	0

4.12. RT-PCR CHARACTERIZATION OF THE RABIES VIRUS.

All (100%) of the viral isolates from dog brain samples belonged to genotype 1 rabies virus (true rabies virus).

CHAPTER FIVE

DISCUSSION

The 58% response to the questionnaire recorded in this study is similar to the finding of Garba *et al*., 2013 in Niger State. This relatively poor response to the questionnaire was due to the fact that most of the dog dealers and dog meat sellers were unwilling to release information spontaneously and freely. Most of those that refused vehemently to respond or provide any information were the uneducated ones who misunderstood the intention of the research. From the respondent bio-data, it was observed that as many as 5 (9%) of the respondents were university graduates. These graduate respondents understood better the issues raised by the questionnaire and thus provided more reliable information. Also, 88% of the respondents have been in the business of dog trading for more than one year and are assumed to have acquired long enough experience to provide reliable responses to most of the questions in the questionnaire. The involvement of graduates in dog business recorded in this study contrasts with the report of *Garba et al*, 2013, that none of the dog traders encountered in Niger State had tertiary education. It may be that dog business is sufficiently financially rewarding and thus is attracting and engaging some of the graduates that abound in the south eastern part of Nigeria and providing them means of sustenance and livelihood. It was observed that more males (58%) than females (38%) were involved in dog trading. This may be because handling of dogs requires courage and aggression, and these two traits are known to be more characteristic of males than females. Fifty seven percent of the respondents were between the ages of 25 to 45. This age bracket constitutes the bulk of the workforce of the nation, and the fact that greater numbers of them (88%) have been in this business for more than one year, shows that the business is lucrative. This is further supported by the finding that 95% of respondents are in the business because of financial gain, and are reported to sell their dogs with an average profit margin of 45% per dog depending on the market trend.

The high rate (81%) of dog meat consumption in the study area agrees with earlier work done by Garba *et al,* 2013, who recorded consumption rate of 80.6% in Niger State. The other reason for dog meat consumption apart from being delicious is that it is believed to have some medicinal properties. This report is in accordance with what was reported earlier for Nigeria dog meat consumers (Murray, 2007; Willy, 2007; Garba *et al,* 2013). However, these claims are still subject to verification. It is necessary to note that dog market and dog meat consumption are new important parameters that need more understanding and thus, should now be considered in the epidemiological study of rabies in Nigeria.

Sixteen percent of the respondents claimed to have received anti ï rabies vaccination, this information is unreliable as only one (2%) respondent indicated that he received full course of anti-rabies vaccination in a hospital after a dog bite, the rest did not know what the full course should even be.

The study has revealed that there is high exposure potential to rabies among dog traders. About 13 (22%) bitten by apparently healthy dogs and 12% by suspected rabid dogs in the course of the business. This group of people is at a high risk of being infected with rabies. Surprisingly, they are aware of rabies and can identify a suspected rabid dog, but they do not take any precautionary measure against rabies. And when exposed through dog bite, even with the 12% category three exposure which requires full post ï exposure anti rabies vaccination, their attitude and response did not change. Whatever happened to the victims 3months, 6months or 1 year is unknown as nobody focuses on it and may never be linked to the exposure. As a result, the consequences of these exposures cannot be established and still remains an eclipse in the study of rabies in Nigeria. A greater percentage 71% of dog dealers can recognize rabid dog (those biting others in the same cage, biting cages, attacking people, drooling saliva, etc), the dog sellers buy and keep them in separate cages and then sell them at higher prices to buyers who use some of it parts for specific medicament or cultural practices. Additionally, 71% out of the 58 respondents reported that they consume suspected rabid dog meat. Half of the 41 respondents do so because of the belief that it

enhances male libido. A report from Vietnam revealed that 5 human rabies patients did not have any history of dog or cat bites, but they had an experience of butchering dogs or cats, or consuming their meat (Anh *et al*., 2011). Rabies virus was also detected in two of the 100 sick dogs from slaughterhouses (Anh *et al*., 2011). In 2010, a study in Sokoto and Katsina State from Northwestern Nigeria, found that 28% of apparently healthy dogs slaughtered for human consumption had rabies virus antigen in their brains (Garba *et al*., 2010). Earlier findings from north eastern Nigeria (Borno State) revealed 31% of apparently healthy dogs slaughtered for human consumption had rabies antigen in their brains (Ajayi *et al*., 2006), indicating that humans could become exposed and infected.

Fifty one percent of dog traders sourced their dogs from other markets within and outside Nigeria, 36% from homes while the remaining 13% were from trapped stray dogs, this report is in contrast with the reports of Garba *et al.*, 2013, who reported that all the butchers in Niger state, Northen Nigeria sourced their dogs from household. It is worthy to note that some rabies virus isolates found in Nigeria (David *et al*., 2008), Burkino-Faso (De Benedictis *et al*., 2010) and Vietnam (Anh *et al*., 2011) were associated with transboundary spread from Cameroon, Mauritania and China respectively. Furthermore, Ogunkoya (2008) reported that dog traders illegally import dogs from Cameroon to Nigerian for consumption purposes. Therefore, the risks associated to dog trade/slaughter, caging and torture of dogs destined for human consumption are serious problems that need government intervention at all levels. The low level of education of most (58%) of the dog traders may affect the strategic interventions for the control of rabies, especially, with the current high rate of unemployment in the country and good profit margin of the business.

Eighty three percent of the respondents claimed to encounter suspected rabid dog more between Octobers to December. This view agrees with the rabies upsurge after the dog breeding season (April and

September) because of their habit to congregate and fight during these mating seasons (Nawarthe, 1980; Oboegbulem, 1994).

Direct fluorescent antibody (DFA) test used in this study for rabies virus detection is the gold-standard test recommended by WHO and OIE for the diagnosis of rabies and has been used by several researchers (Aghomo *et al,* 1990; OIE, 2000; Ogunkoya et al, 2003; Baba, 2006; Aliyu *et al*,2010; Garba *et al*, 2010). It is a quick and accurate diagnostic test that can detect rabies viral antigens present in the tissues of infected host even before Negri bodies are formed in the brain (OIE, 2000). Sellerôs staining method which is based on the demonstration of Negri bodies in the brain is another diagnostic test available for diagnosis of rabies (Oboegbulem, 1994). It has the merits of being simple, readily available and cheaper cost compared to DFA. However, false negative results of about 18% have been recorded with this test. This was attributed to the fact that the test is satisfactory in advanced cases since Negri bodies which the test demonstrates in positive rabies cases accumulate more in the brain during clinical rabies (Atanasiu, 1975; Garba, 2011). Mouse inoculation test (MIT) was equally used in this study as a confirmatory test and for virus isolation. Even though this method has excellent sensitivity and is able to detect false-negative results in DFA technique, which occurs in a very small percentage (0.043%) of cases, its drawback includes the need to sacrifice relatively large numbers of mice and the long period (at maximum 21 days) required for a final diagnosis (Zanoni *et al*,1990). Rabies Tissue Culture Inoculation Test (RTCIT) using mouse neuroblastoma cells is a preferred alternative to MIT because it gives a conclusive result within few days and does not involve the use of live animals (Zanoni *et al*, 1990). Reverse transcriptase polymerase chain reaction (RT-PCR) test was used in this study as a first step in typing the viral isolates (WHO, 2005).

Eighteen (12%) of the dog brain samples tested in this study were positive using DFA, while 17 (94%) of the DFA positive samples were positive using MIT. The apparent concurrence between the two test

results would seem to suggest that the handling and preservation of the samples in this study was adequate to preserve the viability of the virus in the samples. The presence of rabies virus antigen in apparently healthy dogs in this study agrees with earlier reports that apparently healthy dogs could excrete rabies virus in its saliva for a long period without showing symptoms of the disease (Arko *et al* 1973; Bell, 1975; Fekadu,1975; Fekadu and Baer,1980; Baba, 2006; Ogunkoya *et al*, 2008; Aliyu *et al,* 2010; Garba *et al*, 2010). This finding therefore suggests possible carrier state of rabies among stray and owned dog population of the area and thus collaborates reports that death of infected dogs is no longer a criterion for ascertaining rabies infection (Aghomo et al, 1990; Ogunkoya *et al*, 2003; Aliyu *et al*, 2010). It may be possible that in future, these apparently healthy dogs may develop the disease and come down with clinical rabies. However, irrespective of the clinical outcome in these dogs, they pose serious danger to healthy animals and humans. Furthermore, results of this study lends credence to the recommendation by Chhabra *et al* (2007) that in rabies endemic countries, treatment should be started immediately after an animal bite on the presumption that biting animal is rabid unless proven otherwise.

All dogs that tested positive for rabies virus in the brain samples also tested positive for rabies virus in their salivary glands. This is not in agreement with the findings of Aliyu *et al*, 2010 who recorded only 34% positive salivary gland cases. The presence of viruses in the salivary glands suggests that infection would have been transmitted if those dogs had bitten susceptible hosts. However, transmission of infection is further facilitated by the peculiar property of rabies virus that forces the diseased animals to actively seek to bite other animals (Ezeokoli and Umoh, 1986).

The results of this study also revealed that the rabies virus isolates were virulent and caused clinical rabies in mice. This therefore may imply that some of the rabies infected dogs could have developed clinical rabies if they were not slaughtered prematurely. On the other hand, this finding could be explained in relation to the ñCanid Climaxò model in Africa proposed by Chalmer and Scott (1969) where rabies virus

survives by adaptation to preferred host (dog) such that a mutually non-detrimental ecological balance or ñClimaxò is established. They suggested that the dog suffers in-apparent infection or transient illness, and recovered animal becomes ñhealthy carrierò capable of transmitting the disease to other animals.

Prevalence of infection was non- significantly ($p > 0.05$) higher in males (18%), adults (15%), Enugu North (14%) compared to female (18%), puppies (5%) and Enugu West (9%), respectively. The inability to demonstrate significant differences in sex-, age- and location- related prevalence may be due to the fact that the sample size studied was not large enough and thus confounded the results. The higher infection rate in males compared to females agrees with the findings of earlier workers (Ajayi *et al,* 2006; Baba, 2006; Aliyu *et al*, 2010). This may be because male dogs tend to congregate around bitches in the community and fight among themselves to get access to the females and equally establish their territory. This behavior increases the chance of infected male dog transferring the virus to other dogs. Similarly, the higher prevalence in adults than puppies is in agreement with other reports (Baba, 2006; Aliyu *et al*, 2010). This may be due to the fact that adults were more exposed to infection due to their nature of movements and wider activities, than puppies that have more restricted movement. Also the presence of maternal antibody in the puppies could be a contributing factor to the lower prevalence. Furthermore, the higher prevalence of rabies in Enugu North senatorial zone may be attributed to the fact that the two major dog markets are located in the area. Dog trading activities are more and chances of bringing in healthy carriers are greater than in Enugu West senatorial zone were only selected bars and restaurants are involved in dog meat selling business.

The distribution of slaughtered dogs and rabies positive dog brain samples according to locations which were georerefrenced using GIS, revealed that the highest number of rabies positive samples were from Nsukka (25%), Aku (22%), Orie Orba market (21%), followed by Ngwo (18%), Ebe (15%), Edeoballa (13%),and Nkwo Ogbede (11%). It is to be expected therefore, that if there would be rabies outbreak in

the study area, it would be primarily from Nsukka, Aku, and Orie Orba, and possibly Ngwo, Ebe, and Nkwo Ogbede. Among these areas with potential risk of rabies outbreak, Orie Orba is particularly significant and strategic because dog sellers bring dogs from different parts of the country (kogi, Jos etc) to the place for sales and could thus serve as a nidus for the spread of rabies. Some other risk factors that would favour rabies outbreaks in these areas include; coming in contact with a rabid dog meant for slaughter or those that may escape or even the more complicated healthy carriers. It will therefore be necessary for surveillance of rabies in Enugu to be strengthened in these areas.

No rabies virus antigen was isolated from the fifty nine bats tested. This agrees with earlier works done on by Aghomo *et al* (1990) and Dzikwi *et al* (2010a) who could not isolate rabies virus from the brain samples of fruit bats in western and Northern Nigeria, respectively. However, the number of bats tested in this present study was considered small to make a conclusive judgment.

The PCR and gene typing results have confirmed that the viruses isolated in the brains and salivary glands in this study were classical rabies virus and not rabies related viruses. This agrees with earlier reports of Aghomo *et al*, 1990 and Okoh (2008) that no rabies related virus has been isolated from dog brains examined for Lyssavirus in Nigeria. The question is, if rabies has been proven to have 100% morbidity and mortality, then why were these dogs able to carry this virus and still looking normal? There have been arguments that there is a carrier state of rabies in dogs (Arko et al,1973; Fekadu, 1975; Bell,1975; Fekadu and Baer,1980, Ogunkoya *et al*, 1990; Aghomo *et al,* 1990; Baba, 2006; Ogunkoya, 2008; Aliyu et al, 2010; Garba *et al*, 2010). This study has therefore confirmed that carrier state do exist in dogs. This therefore suggests that anyone who comes in contact with the saliva of these healthy carriers will be infected and could develop clinical rabies.

CONCLUSION

This study has shown that some of the apparently healthy dogs slaughtered for meat in Enugu state carry rabies virus antigen and secrete the virus in their saliva without showing clinical signs. In other words, healthy carriers do exist and thus pose danger to susceptible animals and humans who may come in contact with them. All the isolates belonged to genotype 1 rabies virus (RABV1) and thus still implicates dog as the major reservoir of rabies in Nigeria.

The consequences of these carrier state of rabies in dogs is yet to be fully elucidated in Nigeria, because these dogs were living with some people before they were sold, and some people buy them before slaughtering them or reselling them to someone that will slaughter them for meat. Therefore, it is a long connection of problems, where does it end? What is the dog population in Nigeria, what percentage is in carrier state? Since this is unknown, rabies endemicity in Nigeria should be revisited.

RECOMMENDATIONS

- Pre-exposure anti rabies vaccination of veterinary doctors, DVM students, VTH workers, dog handlers and dog sellers should be reinforced.
- Students who use dogs as experimental animal should vaccinate them and also be very careful while handling them. And in case of bite (provoked or non provoked) post exposure treatment should be instituted immediately until the animal is proven to be free of rabies
- Further studies should be done to ascertain the sequence of the isolated viruses from apparently healthy dogs.
- In Nigeria, rabies should be studied in relation to peopleôs culture and peculiar practices such as selling, buying, handling, slaughtering and consumption of suspected rabid dog.
- Further works on rabies should be done on bats, wild animals and rodents in Enugu state.
- Awareness campaign should be done amongst those involved in dog trade and butchering.
- The attention of Nigerian government should be drawn to the risks associated with dog trading.

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APPENDIX

Questionnaire:

UNIVERSITY OF NIGERIA, NSUKKA

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY MEDICINE

DOG SELLERS AND CONSUMERS QUESTIONNAIRE

URBAN [ ] RURAL [ ]

LGA CODE ---------- WARD CODE--------- REF. NO-------

Please the information you give is confidential and will be treated as such. Do not give your name.

Please tick the option that best describes your answer

Section A

Respondent Bio-data

1. Sex:

Male [ ]

Female [ ]

2. Age:

Below 25 years [ ]

25 -34 years [ ]

35-44 years [ ]

45-54 years [ ]

55 years and above [ ].

3. Formal education:

Primary [ ]
Secondary [ ]
Diploma [ ]
Degree [ ]
None [ ]

Section B
Please thick only one answer in each question

1. How long have you been in this business of selling dog meat?

Less than 1 year [ ]

1-4 years [ ]

5- 9 years [ ]

10-19 years [ ]

20-29 years [ ]

30 years and above [ ]

2. Why are you in this business?

Because by our culture people eat dog meat [ ]

There is ready market [ ].

3. Where do you get the dogs you sell?

Household dogs [ ]

Trapped Stray dogs [ ]

Primary markets [ ]

4. Why do people sell dogs for slaughter?

Due to sickness [ ]

Frequent complaint of bites [ ]

Due to financial constraints [ ]

Breed for sale [ ]

5. Do you ask for history of the dog before purchase?

Yes [ ]

No [ ]

6. If yes, what history do you ask for?

Vaccination [ ]

Deworming [ ]

Any other information [ ]

Do not ask for any history [ ]

7. What category of dog do you buy?

Stray [ ]

Domestic/ owned dog [ ]

Both [ ]

8. Do you eat dog meat?

Yes [ ]

No [ ]

9. Why do you eat dog meat?

Like any other meat [ ]

Because of local belief that it cures some sickness such as malaria[ ]

It helps to reduce high blood pressure (BP) [ ]

No reason [ ]

10. Have you been vaccinated against rabies?

Yes [ ]

No [ ]

11. If yes where?

Chemist shop [ ]

Private Nurse [ ]

Hospital [ ]

12. How long ago have you been vaccinated?

Less than 2years ago [ ]

2 ï 5 years ago [ ]

More than 5 years ago [ ]

13. Have you been bitten by a dog before you started this business

Yes [ ]

No [ ].

14. Have you been bitten by a dog since you started this business

Yes [ ]

No [ ]

15. If yes, how were you treated?

With local herbs [ ]

With magic stone [ ]

Anti-tetanus injection [ ]

Only first aid treatment of wounds e.g. soap and water [ ]

Anti-rabies post-exposure vaccination [ ]

16. If by anti- rabies post- exposure vaccination, how many times were you given?

One [ ]

Two [ ]

Three [ ]

Four [ ]

Five [ ].

17. If by local herb, please can you name the herb?

é é

18. What happened to the dog that bit you?

Was alive more than 3months after the bite [ ]

Is still alive [ ]

Was sold off [ ]

Killed immediately

Escaped [ ]

19. Have you seen a mad dog since you started this business?

Yes [ ]

No [ ]

20. Have you been bitten by a mad dog since you started this business?

Yes [ ]

No [ ]

21. Will you buy and kill a mad dog for meat?

Yes [ ]

No [ ]

22. Why? Because

It is believed that the meat or brain of a mad dog will prevent rabies in person bitten [ ]

There is nothing wrong with eating the meat and brain of a mad dog [ ]

It makes the male who eats it sexually stronger.[ ]

No reason [ ]

If others, please specify é ..

23. How frequent do you encounter mad dog in this business?

Occasionally [ ]

Often [ ]

Very often [ ]

Never [ ]

24. When do you come across mad dogs more? During

January -March [ ]

April - June [ ]

July - September [ ]

October - December [ ].

No specific time [ ]

25. Have you seen anybody who died after a dog bite?

Yes [ ]

No [ ]

26. 1f yes, after how long did the person die?

Less than 1 month [ ]

1 - 3 months [ ]

4 - 6months [ ]

More than 1 year [ ]