PREVALENCE OF RABIES ANTIGEN IN THE SALIVA AND BRAINS OF APPARENTLY HEALTHY DOGS SLAUGHTERED FOR HUMAN CONSUMPTION IN ABIA STATE, NIGERIA

BY

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NIGERIA

MAY, 2014

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(MSc./VET-MED/1154/2011-2012)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADAUTE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLEDMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTERS DEGREE IN VETERINARY MEDICINE.

DEPARTMENT OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

MAY, 2014

DECLARATION

I hereby declare that the work in this thesis titled "**Prevalence of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption in Abia State, Nigeria"** was performed by me in the Department of Veterinary Medicine under the supervision of Professors A.B.Ogunkoya, S.U. Abdullahi and Dr. B.V. Maikai.

The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Philip Paul MSHELBWALA	_____	_____
Name of student	Signature	Date

CERTIFICATION

This thesis" **PREVALENCE OF RABIES ANTIGEN IN THE SALIVA AND BRAINS OF APPARENTLY HEALTHY DOGS SLAUGHTERED FOR HUMAN CONSUMPTION IN ABIA STATE, NIGERIA" by** Philip Paul MSHELBWALA, meets the regulations governing the award of the degree of Masters of Science in Veterinary Medicine, of the Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

Professor A.B. Ogunkoya Chairman ,Supervisory Committee	_____ Signature	_____ Date
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DEDICATION

This thesis is dedicated to the loving memory of my teacher and mentor late Professor M.M. Aliyu.

ACKNOWLEDGEMENTS

All glory and thanks to Almighty God who made all the provisions and gave me the strength to carry out this study.

I wish to express my profound gratitude to my loving parents Mr. Paul Bukar Mshelbwala and Mrs. Jummai Paul Mshelbwala, for their prayers, encouragement and immeasurable support.

To my siblings, Arhyel, Lynda and Zainab, I say a big thank you, for their concern, encouragement and prayers.

Special thanks to my supervisors, Prof. A.B. Ogunkoya who gave me fatherly advice, free access to his office and needed materials; Prof. S.U.Abdullahi, for his guidance, support, critical input, constructive criticism and advice and Dr.(Mrs). B.V. Maikia, for her guidance, Critical input .encouragement, support and advice.

Many thanks to; Dr. U.C. Nlebedum, of the Animal World Veterinary Clinic, Umuahia (my former employer) and his family, who gave me free accommodation, feeding, space in their clinic to ensure my samples are kept in good condition and assisted me financially when I ran short of funds ;Dr. G. Y. George, who was always there to receive my samples for onward delivery to NVRI; Vom, Mrs. Serah Olaleye, of the rabies laboratory, NVRI, Vom, who assisted me with Fluorescent Antibody Test; Drs.Ajoke, Solomon, Garba who assisted with dRIT; final year students(Adaeze and Ogo) of the College Veterinary Medicine ,Umudike, who took me to various dog markets and interpreted my questionnaire to dog meat butchers and consumers ;Dr. Chininye, who accompanied me several times to Ndoro and Umueze for sample collection. Centers for Disease Prevention and Control (CDC), Atlanta, for donating the dRIT Kits used in this research.

To my teachers, in Ahmadu Bello University, Zaria, Professors. A.K.B, Sackey, L.B. Tekdek, C.A.Kudi, D.A.Y. Adawa, P.A. Abdu, A. Z.Hassan and Drs. S. Okieyeto, S.N.A Saidu and B. Jahun, I say a big thank you, for their encouragement and advice.

To my friends, U. Okoye, P. Philip, Drs. M.T Angani, K.O. Abah, A.M. Bappah, E.T. Onyinche, S.T. Ogundare, R.G. Otolorin,B.O. Omontese, H.Y. Daya,I.J Gosomji and D.F. Audu for their input and criticism. I say a big thank you. May God see them through, in all their endeavors.

Finally to Dr.(Mrs).Queenlilia Nwankocha, Deputy Director Zonal Veterinary Clinic,Umuahia, Abia State, who gave me the laptop used in putting this research work together, God bless you abundantly.

ABSTRACT

The study was carried out in four Local Government Areas: Umuahia North, Isiala Ngwa North, Osisioma, Ekwauno of Abia State, to detect the presence of rabies antigen in the saliva and brain tissues of apparently healthy dogs slaughtered for human consumption. Two rapid diagnostic tests, Rapid immunochromatographic test (RICT) and Direct Rapid Immunohistochemistry Test (dRIT) and the gold standard test, Fluorescent Antibody Test (FAT) were used to detect the presence of rabies antigen in the saliva and brains of slaughtered dogs. A structured questionnaire was designed and administered to dog meat butchers and consumers in the selected areas to access their level of awareness of rabies infection and demonstration of exposure potentials. A total of 100 saliva samples were obtained from 64 females and 36 male dogs from the selected areas before slaughter. One hundred brain samples were also obtained from the dogs after they had been slaughtered. The saliva samples were subjected to RICT while the brain tissues were subjected to FAT and dRIT respectively. A total of 100 questionnaire were administered to respondents comprising of 81 dog consumers and 19 processors. Five (5%) of the saliva and brain tissues samples were each positive for all the tests. All the positive cases were detected in the female dogs. There was no statistical association ($p>0.05$) between sex and rabies status of the dogs sampled. Despite the fact that majority of the processors (73.7%) and consumers (71.6%) had prior knowledge of rabies, on exposure to dog bite , 72.8% and 70.4% respectively sought traditional methods of treatment. None of the butchers found out the vaccination status of the dogs before slaughter and all of them believed the business was lucrative. Dog meat butchers engaged in practices that further exposed them to dog bite and were not aware that post exposure prophylaxis exists for victims of dog bite. They also believed that

dog meat had a curative effect on malaria and high blood pressure, enhances libido and provide spiritual protection. In this study, there was total agreement between the RICT, dRIT and the conventional, FAT, as all the samples tested positive with the use of all the tests. This study has established the presence of rabies antigen in apparently healthy dogs, there was a high level of awareness of rabies by consumers and processors in the study area. Rabid dogs were not seen as a public health concern but rather associated with medicinal and spiritual values. This belief may pose a health hazard and may militate against the control of rabies in the state.

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ABBREVIATIONS

AIDS	-	Acquired immune deficiency syndrome
°C	-	Degree Celsius
CDC	-	Centers for Disease Control and Prevention
CSF	-	Cerebrospinal fluid
dRIT	-	Direct Rabid Immunohistochemical Test
FAT	-	Fluorescent Antibody Test
HIV	-	Human Immunodeficiency Virus
IFN	-	Interferon
LGAs	-	Local Government Areas
MAb	-	Monoclonal Antibody
MIT	-	Mouse Inoculation Test
NVRI	-	National Veterinary Research Institute
OIE	-	Office International des Epizooties
PEP	-	Post exposure prophylaxis
RFFIT	-	Rabies Tissue Culture Inoculation Test
RGP	-	Rabies Virus Glycoprotein
RICT	-	Rapid Immunochromatographic Test
RNA	-	Ribonucliec Acid
RNP	-	Ribonucleoprotein
RT	-	Reverse- Transcription polymerase chain reaction
TPBS	-	Tween phosphate buffer saline

WHO - World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Rabies is an acute, contagious and highly fatal disease of all warm blooded animals caused by rabies virus (Bourhy *et al*., 1993; Ogunkoya *et al.,* 2003). The disease affects all warm-blooded animals including man (Radostits *et al*., 1995). Rabies infection has a case fatality rate of almost 100 %, accounting for about 55,000 human deaths annually with most cases occurring in developing countries of Asia and Africa (WHO, 2011).

Rabies was first documented in Nigeria in humans in 1912 and in dogs in 1925 (Boulger and Hardy,1960) and since then many authors (Umoh and Belino,1979; Fagbami *et al*.,1987; Harry *et al.*,1984; Okoh, 1984) have established that the disease is endemic in the country with more reports (WHO, 2006; 2010;Mshelbwala *et al.*, 2013a; Hambolu *et al*., 2014) showing that the prevalence of the disease is on the increase. The disease is caused by a virus of the family Rhabdoviridae and genus Lyssavirus , now called Lyssa Virus 1(RABV gen1). It manifests in three classical stages, namely prodromal, excitatory stage and paralytic or silent stage (Idachaba *et al*., 2009).

The saliva of rabid dog has been documented to contain high concentration of rabies virus and serve as a medium for transmission of the infection (Bishop *et al.,* 2002; CDC, 2007; WHO, 2008) but the virus may also be found in the tears, urine, serum and other body fluids of infected animals (Wolfgan,1999). A case of bite is considered an exposure only when the virus is inoculated into a new wound of a susceptible animal (Beran ,1981; Ogunkoya *et al*., 2003).

Although non-bite exposure can occur through licks or splash of infected saliva into the mucus membrane (Audu, 2011); however, 98 % of human rabies deaths have been documented to have been caused by almost always bite of a rabid dog (Rupprecht, 2008).

Rabies is found in most countries of the world except for few countries that claimed to be free due to their geographical location and elimination or strict control programme, however the emergence of bat rabies has affected the status of these countries (Kuzmin *et al*., 2008; Kuzmin, 2010; Dzikwi *et al*., 2010a). The disease is rated as the 11th world killer disease and large proportion of the deaths are from Africa and Asia (Nishizono *et al*., 2008; Okoh, 2008; Rupprecht, 2008; WHO, 2008; Mshelbwala *et al* .,2013).

1.2 Statement of Research Problem

Rabies, though a disease is preventable through vaccination, still accounts for the death of over 50,000 people worldwide, Global Alliance for Rabies Control report that the death is over 70,000 with 10 million treated for bites from potentially infected dogs. Majority of the cases occur in Africa and Asia although, the true incidence in Asia and Africa is likely to be higher than is reported as most cases are not reported (WHO, 2010), while 98% of the cases were caused by rabies infected dog bite (Rupprecht, 2008).Records from National Veterinary Research Institute (NVRI) Vom, Rabies Diagnostic Laboratory (1986-2005) showed prevalence of rabies in dogs in Nigeria is 98.3% and thus dogs play a vital role in epidemiology rabies in this country (Ogunkoya, 2008).

Worldwide, evidences abound supporting the fact that dogs shed rabies virus in their saliva (Nottidge, 1994; Fekadu, 1988). Fekadu *et al.* (1983) have established that rabies infected dogs that recovered shed rabies virus in their saliva intermittently and this shedding continued for

about seven months while the dog remained apparently healthy. The above represent a carrier status which was previously described in Nigeria (Anon, 1932; Audu, 2011). Carrier state in rabies might play a significant role in the perpetuation and survival of the virus and may become a source of rabies outbreak (Fekadu, 1988). Inapparent infection and recovery from clinical disease with resultant persistent or intermittent shedding of rabies virus have affected the overall effort in rabies eradication and control in most parts of the world (WHO., 2005; Ajayi *et al*., 2006; Nishizono *et al*., 2008;OIE, 2008).

It has been suggested that shedding of rabies virus in saliva may endanger dog handlers, butchers, owners, veterinarians and other dogs in the community (Nishizono *et al*., 2008; Ogunkoya, 2010; Audu, 2011; Garba *et al.,* 2013).

Evidences supporting existences of carrier rabies in dog population have been documented (Fekadu *et al*., 1983). In addition to that serological evidences of lyssa virus and recovery have also been established (Ogunkoya, 1990). Rabies virus has been isolated from the saliva of apparently healthy unvaccinated dogs (Fekadu, 1975; Aghomo, 1986). More recently rabies antigen have been found in the brain of apparently healthy dogs slaughtered for human consumption in Nigeria (Ajayi*et al*., 2006; Garba *et al*., 2008; Aliyu *et al.,* 2010, Audu,2011). Salivary isolates have been found to be about 6% in apparently healthy dogs sold for human consumption in Kaduna State (Audu, 2011).

The most dangerous aspect of carrier dogs is that bites from such dogs are usually not recognized as an exposure that will stimulate appropriate post-exposure treatment. Consequently, human deaths occur from exposure buried in an erratic incubation period where dog show no sign suggestive of rabies (Ogunkoya *et al*., 1984).

A ten years retrospective study of dog bite cases reported to Zonal Veterinary Clinic, Umuahia, Abia state, south eastern Nigeria by Mshelbwala *et al*. (2013b), revealed that all the suspected cases of rabies were not sent for confirmatory diagnosis due to the distance to diagnostic facilities and the cost of transportation.

Some brain tissue samples received for diagnosis in Nigeria and other developing countries of Africa get decompose due lack of storage facilities, inadequate transportation, proximity from the place of exposure to laboratories where diagnosis can be conducted, lack of rapid test; especially when exposure occur in rural areas (Ogunkoya, 2010), which may result in misdiagnosis and loss of human life.

1.3 Justification of the Study

Abia State shares border with Cross River State. Recently, 8 people died in Cross River State and the cause was traced to dog bite (OIE, 2012). Those dogs were disposed off before reports got to Veterinary authorities. Probably those dogs must have been consumed before the onset of clinical sign thereby precluding confirmatory diagnosis (OIE, 2012).

Abia State, has markets for dogs and the business in on the increasing, virtually every community in the state, consume dog meat as a result of the value placed on the meat by members of the community. Dog consumption in the eastern part of Nigeria attracts high population of dogs from different parts of the country and neighboring countries (OIE, 2012; Ogunkoya, 2008). Work on rabies in apparently healthy dogs have been carried out in different parts of the country (Ajayi et al.,2006; Garba *et al.*, 2008; Aliyu *et al.*, 2010; Audu, 2011). A study conducted in 1500 clinically healthy dogs in Nigeria led to the isolation of rabies virus in

their saliva (Aghomo *et al.*, 1989). Also many healthy dogs in Nigeria were seropositive to lyssa virus (Aghomo *et al.*,1989; Ogunkoya, 1990; Nottidge *et al*., 2007; Dzikwi *et al*., 2010b). All these facts are proofs supporting the existence of carrier rabies in Nigeria. The role of carrier dogs in the epidemiology of rabies in the country is yet to be properly defined.

Earlier workers agreed that dog marketing and dog consumption carry their peculiar risks because of the presence of rabies antigen in the brain and saliva of many of such dogs involved in the selling, slaughtering and consumption cycle (Audu, 2011). However, information on rabies in Abia State in general is lacking despite rabies outbreak in neighboring state of Cross River.There is, therefore, a need to find out the status of rabies in the state, especially carrier status. For rabies eradication and control to be achieved in Abia State, an in-depth understanding of the role played by dogs that are carries of rabies in this area is a pre-requisite for an effective control programme.

Rapid and accurate laboratory diagnosis of rabies in humans and other animals is essential for timely administration of post-exposure prophylaxis (PEP) (Helmick, 1983). If the animal is not rabid, prompt diagnosis may save a patient from unnecessary physical and psychological trauma, as well as financial burden (Helmick, 1983). For rabies surveillance, control and eradication program to succeed, proper diagnosis is fundamental.

Lembo *et al*., 2006 had 100% sensitivity and specificity when he compared dRIT and FAT in the diagnosis of rabies in Tanzania under field and laboratory conditions. To the best of my knowledge, this is the first study to compare the rapid immune chromatographic test (RICT) using saliva to that of the direct rapid immunohistochemistry test (dRIT) and direct fluorescent antibody test (FAT) using brain tissues. The dRIT test has also undergone extensive evaluation

in other countries and 100% correlation was found with FAT (Madhusudana *et al.*, 2012; Dürr .,*et al*, 2008; Tao *et al.*, 2008). It is against this background that this study was designed with the aim of evaluating two rapid diagnostic tests suitable for use in Nigeria with the gold standard for rabies diagnosis.

1.4. Aim of the Study

The aim of this study was, to determine the prevalence of rabies virus antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption in Abia State.

1.5. Objectives of the Study

The specific objectives of the study were to:

1. determine the prevalence of rabies virus in the brains of apparently healthy dogs in Abia State;
2. determine the percentage of dogs shedding rabies virus in their saliva ;
3. find out the level of awareness of rabies infection among processors and consumers of dog meat in Abia State; and
4. evaluate two rapid diagnostic tests suitable for use in Nigeria with the gold standard for rabies diagnosis.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Rabies

Rabies is a transmissible viral disease that affects humans and all mammals. It is a fatal disease caused by the virus which has been associated with dog bite for more than 3,000 years and the oldest disease known to Medical Sciences (Rupprecht *et al*.,1987; Wilkkinson, 1988; Bishop *et al*.,2002; Swanepoel, 2005). It causes acute, progressive encephalitis and it is considered universally fatal (Coetzee and Nel, 2007).

Rabies, an ancient scourge is one of the oldest diseases known to be transmitted to man from animals (Oboegbulem, 1994). In ancient Babylon the code of Hammurabi (2,300 B.C) stipulate the payment of fine by the owner of a mad dog when human death results from the bite of his dog (Oboegbulem, 1994). In Mesopotamia the Sumerian "Law of the city of Eshnuna"(1,885 B.C) has the following reference" "if a dog is mad and the authorities have brought the fact to its owner, if he fails to confine it and the dog bites a man and it results in death, then the owner will pay two-thirds of a mina(40 shekels) of silver. If the dog causes the death of a slave following bite, its owner shall pay 15 shekels of Silver" (Kaplan and Kaprowski, 1980). In the Homer's Iliad (9^{th}century B.C), Achilles called Hector a "rabid dog". Aristotle in 400 B.C. stated, though erroneously", that man was preventing from contracting rabies from the bite of a mad dog. In the first century B.C, Celsius recommended preventive measures for victims of dog bite, which included immediate excision of the bite tissue and cauterization of the bite wound with a hot iron (Oboegbulem, 1994).

People of ancient Greece, Rome, and Egypt believed rabies was caused by evil spirits, because, suddenly a friendly animal becomes aggressive on contracting the disease. The Greeks called the disease lyssa (frenzy) and the Romans called it rabere (to rage). It is from the Roman word that modern English and French name, rabies is derived (Oboegbulem, 1994).

In 1808, Zink recognized and confirmed the infectious nature of rabies by reproducing the disease in normal dogs with the saliva of a rabid dog. The neurotropism of the causative agent was demonstrated by Louis Pasteur in 1881; he found that the poison of rabies (virus) could be obtained in its pure form in the brain and spinal cord of infected animal (Oboegbulem, 1994).

The first scientific feat in rabies immune-prophylaxis was achieved by Pasteur in 1885. This major breakthrough was discovered that by repeated passage of the causative agent in rabbits. The street virus, which had a long and variable incubation period was changed into a fixed form with a short and constant incubation of 4 to 6 days .The fixed virus in the nerve tissue suspension was found particularly suitable for preparation of vaccine he successfully used in dogs. In 1885, he tried the vaccine on one Joseph Meister who had been severely bitten by a rabid dog. Following vaccination, he survived and became the Janitor (Cleaner) at the Pasteur Institute. Thus rabies became the first human disease for which scientifically developed vaccine was successful (Oboegbulem, 1994).

In Africa, the first major outbreak of rabies in local dogs was recorded in 1884 in Ethiopia. The first case of rabies in an exotic animal was reported in an imported dog from England at Port Elizabeth, South Africa, in 1893 (Rollinson, 1956).

In West Africa, the first report originated in French –speaking zone when a disease of dog known locally as “Oulon fato” was identified as rabies by Bouffard in 1912. Early existence of

rabies can be inferred from the fact that the various ethnic groups in Africa know the disease and have vernacular names for the disease “ara nkita”(Igbo) “ciwon haukan kare”(Hausa) “digbolugi”(Yoruba)” idat ebau”(Efik/Ibibio)(Umoh and Belino,1979; Ogunkoya, 2010) and Chuchukela (Bura).

In Nigeria, available records showed that, it was first officially reported in human as contained in the Annual Report for Southern Nigeria in the year 1912.Two cases (Nigerian woman and European woman), were reported, one in Eket , and the other at Bony(Boulger and Hardy,1960). The first laboratory diagnosis of rabies in Nigeria was made by the Rabies Laboratory at Yaba in 1925 (Oboegbulem, 1994). The earliest investigation of dog rabies was made by Smith (1928), when he described the clinical signs of five out of six cases notified during that year. Between 1928 and 1990, the Rabies Investigation Laboratory at National Veterinary Research Institute (NVRI), Vom, had confirmed a total of 3,770 cases of rabies in dogs, farm animals and some wildlife. During the same period, 637 human rabies were confirmed at Yaba Rabies Laboratory, since then, rabies has remained one of the most important endemic zoonoses and of serious concern (Oboegbulem, 1994; Atuman *et al* .,2014a).

In humans, rabies is an ongoing scourge that have continued to exact unnecessary large toll on human life (Bishop, 2002).It is estimated to cause over 30,000 deaths in India alone, while the WHO estimates that over 55, 000 persons yearly. Global Alliance for Rabies Control report that the death is over 70,000 with 10 million treated for bites from potentially infected dogs. The transmission of rabies virus through the saliva of infected animals has the highest case of any known infection. Once the agent enters the nervous system of the host, the resulting encephalitis is very fatal. The availability of safe and efficacious vaccines and immunoglobulin has prevented

many fatalities. It has been estimated that over 10 million people receive post-exposure treatment annually after potential exposure to dog bite (Bishop, 2012).

People with high occupational risk of exposure to the infection, such as Veterinarians, laboratory personnel working with rabies virus, dog meat butchers, and wild life handlers, should ensure they receive PEP, through the administration of three doses of vaccine into the deltoid muscles at days 0,7, and 28 (Mshelbwala *et al*., 2012 ;WHO, 2014). Variations in few days in the timing of the second and third does not affect the immune response (Bishop, 2002).

2.1. 1 Aetiology of rabies

Rabies is caused by the virus of the genus Lyssavirus and family Rhadoviridae and it is a single stranded RNA virus (Timoney *et al*., 1988; Wunner *et al*., 1995). Rabies virus belongs to the order Mononegavirales, viruses with a no segmented, negative-stranded RNA genomes. Within this group, viruses with a distinct "bullet" shape are classified in the Rhabdoviridae family which includes at least three genera of animal viruses, Lyssavirus, Ephemerovirus, and Vesiculovirus. The genus Lyssavirus includes rabies virus, Lagos bat, Mokola virus, Duvenhage virus, European bat virus 1 and 2 and Australian bat virus (CDC, 2013).

2.1.2 Structure of the virus

Rhabdoviruses are approximately 180 nm long and 75 nm wide. The rabies genome encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). All rhabdoviruses have two major structural components: a helical ribonucleoprotein core (RNP) and a surrounding envelope. In the RNP, genomic RNA is tightly encased by the nucleoprotein. Two other viral proteins, the phosphoprotein and the large protein

(L-protein or polymerase) are associated with the RNP. The glycoprotein forms approximately 400 trimeric spikes which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP and may be the central protein of rhabdovirus assembly (Langevin *et al*,1982; CDC, 2013).

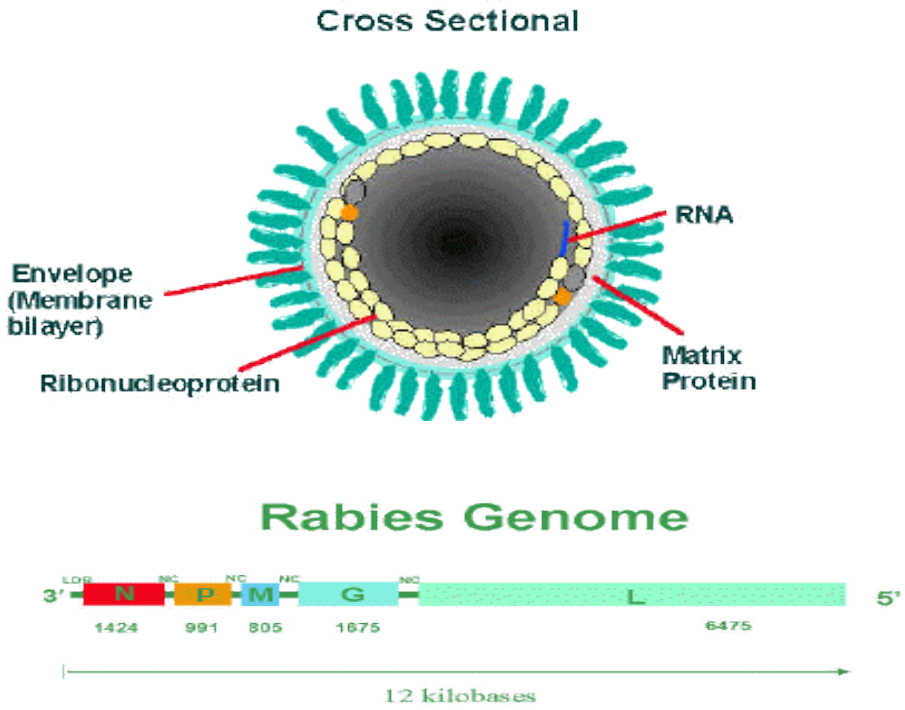


Figure 1: Cross section of the virus and genome.

Source: Centers for Disease Control and Prevention (CDC), Atlanta Georgia, USA
http://www.cdc.gov/rabies/transmission/virus.html

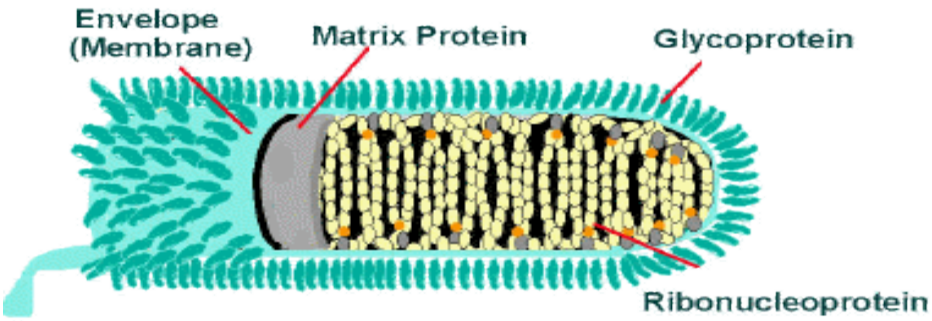


Figure 2: Longitudinal section of the rabies virus showing the bullet shape structure.

Source: Centers for Disease Control and Prevention (CDC), Atlanta Georgia , USA
http://www.cdc.gov/rabies/transmission/virus.html

Rhaddoviruses are rod shaped as the name implies and have one end that is rounded and the other flattened they are often referred to as bullet shaped. They have an envelope derived from the host plasma cell membrane. The rabies virus has single strands of RNA that is anti-sense to messenger RNA needed to code for viral proteins. This implies that the RNA cannot code directly for protein synthesis and must be copied to positive strand mRNA. As a result, the virus must carry its own RNA-dependent RNA polymerase (Warrel and Warrel, 2004). The virus has five proteins (Hunts, 2007). These are:

2.1.2.1 *G (surface) proteins-*

Rabies virus glycoprotein (RGP) is the only surface exposed viral protein, it assembles into trimmers (in the endoplasmic reticulum and its ectodormain protrudes from the lipid envelope(Guadin *et al.*,1992 ; Largevin *et al.*,2002). The RGP is a viral attachment protein responsible for host cell receptor recognition and low pH induced fusion of the viral envelope with endosomal membranes (Guadin *et al.*, 1993; Tuffereau *et al.*, 1998). It is also the primary target of the host

humeral and cellular immune response (Celis *et al*.,1998). RGP can assume three conformational states: The Native state (N) present at the viral surface and responsible for receptor binding; Activated hydrophobic state(A) required for the intereaction of RGP with its target membrane during fussion, and the fussion inactive conformation (Maillard and Gaudin, 2002).

2.1.2.2 *M (matrix) protein*

A peripheral membrane protein that lines the inner surface of the viral membrane, though this remains controversial, may serve as a bridge between the outer surface (G) protein and nucleocapsid (Warrel and Warrel, 2004). The nucleocapsid is the infectious ribonuceoprotein core of the virus. It is a helical structure that lies within the membrane and has a striated appearance (Jackson, 2002;Hunt, 2007)

2.1.2.3 *Large (L) proteins*

Together with the Phosphoprotein form the RNA polymerace and transcriptase. It has a molecular weight of about 240 kiloDaltons and its gene is up to 60% of the genome (Hunt, 2007).

2.1.2.4 *Phosphoprotein (P) proteins*

This is an interferon (IFN) antagonist counteracting transcriptional activation of the type 1 IFN(Brzozka *et al*., 2006).

2.1.3 Epidemiology of rabies

Rabies is widely distributed across the globe, with only a few countries (mainly islands and peninsulas) being free of the disease and accounts for over 55,000 human deaths (WHO, 2013). Most of the human fatalities occur in Asia and Africa where dog rabies remains endemic and thus the main source of human exposure (Rupprecht, 2008 , WHO, 2013). Bat rabies maintains a global viral circulation and a source of public health concern in areas where canine rabies has been controlled (Kuzmin *et al*., 2006). Basically, two types of rabies epidemiology are known, urban rabies where dog is the main host and sylvatic rabies, where the disease circulates in the wildlife (Bishop *et al*.,2002; Swanepoel, 2005, Atuman *et al.*, 2014a). In Nigeria, urban rabies is the most important (Opaleye *et al*., 2006; Ogunkoya, 2008; Nottidge and Omobawale, 2010). Rabies was first documented in Nigeria in humans in 1912 and in dogs in 1925 (Bougler and Hardy, 1960). Many authors have continued to report cases of rabies, confirming the disease is endemic in Nigeria. (Oboegbulem, 1994; Ogunkoya, 2006; Garba *et al*, 2005; Garba *et al*, 2008; Mshelbwala *et al*., 2013a).

2.1.4 Transmission of rabies

Animals and humans, acquire rabies infection following a bite by a rabid animal, which is the mode by which at least 99% of the infections are acquired. Rabies has infrequently occurred through aerosol transmission and via the oral and nasal routes (Woldehivet, 2002; Hemachudha *et al*., 2002). Therefore, it is possible that human rabies transmission occurs through contamination of skin abrasions, open wounds, the conjunctiva and oral mucosa with infectious saliva of a rabid dog (Hemachudha *et al*., 2002). Rabies infection occurring through the handling

and skinning of rabid animals has been reported (Tarig *et* al., 1991) and more recently through solid organ transplantation (Tarig *et* al., 1991; Srinivasan *et al*., 2005; CDC, 2013).



Plate I: Severe facial lacerations in a child caused by dog bites.
Source: (Bishop *et al*., 2002).

2.1.5 Pathogenesis of rabies

Bites by rabid animals generally introduce the virus through the skin into the muscle and subcutaneous tissues (Warrell and Warrel, 2004; Jackson, 2010). The glycoprotein spikes on the surface of the virus particles are the major determinants for rabies virus neuropathogenicity because of its role in binding to specific cellular receptors. Some early studies showed that the virus may replicate in muscle fibers before invading the nervous system (Charlton *et al*., 1997), and thus suggesting that this process amplifies the virus for axonal transport into the peripheral

nerves (Murphy and Bauer, 1974). Other studies revealed that the rabies virus attaches to nerve cells through acetylcholine receptors at the neuromuscular junction (Lentz *et al*., 1982; Lewis and Lentz, 2000). Once in peripheral nerves, the virus travels towards the central nervous system (CNS) via the motor and sensory axons. Within the CNS, the virus infects neurons and dendrites and then neuronal cell surfaces and synapses (Woldehiwet, 2002; Warrel and Warrel, 2004). Virus dissemination occurs through retrograde axoplasmic flow, cell to cell transmission via synaptic junctions and free passage of virus within intercellular spaces (Iwasaki, 1991). These processes result in the migration of rabies virus along peripheral nerves towards the CNS (Tsiang, 1993). Centrifugal spread of the virus from CNS in somatic and autonomic nerves deposits virus in many tissues including skeletal and cardiac muscle, adrenal glands, kidney, retina, cornea, pancreas and nerves around hair follicles (Jackson and Park, 1999). Productive viral replication with budding from plasma membranes takes place predominantly in the salivary glands in readiness to infect other mammals (Warrel and Warrel, 2004).

2.1.6 Laboratory diagnosis of rabies

Handling of suspected rabies materials for diagnostic purpose should be done with uttermost care (Meslin and Kaplan, 1996). Personnel working in rabies laboratory should ensure they are vaccinated and their immunological status checked every six months (Kaplan, 1996). Rabies diagnosis based on clinical sign is not reliable as there are no pathognomonic signs (Trimarchi and Smith, 2002). Laboratory diagnosis of rabies employs the use of various histological and virological methods (Woldehivet, 2005).

2.1.6.1 Histology

Adlochi Negri, in 1903 observed the presence of eosinophilic inclusions in rabies –infected tissue now referred to as Negri bodies and considered as definitive diagnosis of rabies (Woldehivet, 2005). Negri bodies are typically round or oval in shape, eosinophilic with basophilic granules (Meslin and Kaplan, 1996). Negri bodies are effectively demonstrated in histological sections or fresh bilateral smears of sample from the hippocampus (Ammon's horn), brain stem and cerebellum after staining with sellers, haematoxylin and eosin of Mann. This method has however been superseded with other methods like fluorescent antibody test (FAT) (Dean *et al*., 1996) because of 50%-80% reliability in detecting antigen in infected animals (Jogai *et al*., 2000).

2.1.6.2 Fluorescent antibody test

Fluorescent Antibody Test (FAT) is the current Office International des Epizooties (OIE) and World Health Organization (WHO) recognize method for rabies diagnosis (WHO, 1992; Dean *et al*., 1996; OIE, 2013) because it is reliable and sensitive. It is quick and result is obtained within two hours, however its sensitivity can be reduced when testing autolysed samples (Albas *et al*., 1999).However, its major limitations are the cost of acquiring, maintaining a fluorescent microscope and human capacity, especially in Africa. This test maybe 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 95-99% of cases (Shankar, 2009). The sensitivity of the FAT depends on the specimen (the degree of autolysis and how comprehensively the brain is sampled, on the type of lyssavirus and on the proficiency of the diagnostic staff (Shankar, 2009). Sensitivity may be lower in samples from vaccinated animals due to localization of antigen,

which is confined to the brainstem (Bingham and van der Merwe, 2002). For direct rabies diagnosis, smears prepared from a composite sample of brain tissue that includes the brain stem, are fixed in high-grade cold acetone and then stained with a drop of specific conjugate (Bourhy *et al*., 1989). Anti-rabies fluorescent conjugates may be prepared in the laboratory (Shankar, 2009). Those available commercially are either polyclonal conjugates specific to the entire virus or specific to the rabies nucleocapisid protein, or they may be prepared from a mix of different MAbs (Bourgeon and Charlton, 1960). In the FAT, the specific aggregates of nucleocapsid protein are identified by their fluorescence (Bourhy *et al*.,1989). The specificity and sensitivity of these anti-rabies fluorescent conjugates for locally predominant virus variants should be checked before use (Shankar, 2009). The FAT may be applied to glycerol-preserved specimens. If the specimen has been preserved in aformalin solution, the FAT may be used only after the specimen has been treated with a proteolytic enzyme. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue (Shankar, 2009).

2.1.6.3 *Immunohistochemistry tests*

These are immunochemical methods for detecting rabies antigen in formalin fixed sections. Rabies specific monoclonal antibody as primary antibodies and specie specific antibodies conjugate with peroxidase or avidin-biotin as secondary antibodies are used (Fekadu *et al*., 1988). Studies have shown that the detection of rabies antigen using peroxidase labeled antibodies is sensitive in detecting Negri body as FAT (Kotwal and Narayan, 1985). Higher sensitivity has been demonstrated by Bourgeon and Charlton (1987). It can be performed easily on archived materials (Jogai *et al*.,2000).

2.1.6. 4 *Direct rapid immunohistochemistry test*

Recently, a direct rapid immunohistochemical test (dRIT) was developed by the Centers for Disease Prevention and Control (CDC) for the detection of rabies virus using immunoperioxidase technique (Niezgoda and Rupprecht, 2006). The test uses concentrated and purified bionylated anti-nucleocapsid monoclonal antibody againts rabies virus. The antibody reagent is made visible with 3-amino-9-ethycabazole after incubation with a Streptavidin - peroxidase complex. The result can be read with the aid of a light microscope in less than an hour. The Direct Rapid Immuno-histochemical Test (dRIT) is an alternative post-mortem technique to the FAT it employs the use of cocktail of anti-nucleocapsid on brain impressions. This test is as sensitive as FAT and avoids the use of expensive fluorescent microscopes, it can be performed at a bench top and does not require the use of sophisticated equipment (Niezgoda and Rupprecht, 2006; Alexandre *et al*., 2012). It does not employ the use of electricity and can be used under field condition, however, under human exposure it should not be used as primary diagnostic tool to rule out rabies (Niezgoda and Rupprecht, 2006).

2.1.6.5 *Mouse inoculation test and tissue culture*

Following human exposure, the WHO Expert Committee of rabies recommends that all human samples that are negative to FAT must be confirmed by a backup test such as MIT (WHO, 1992) or Rabies Tissue Culture Inoculation Test (RTCIT). The MIT was first used for confirmatory diagnosis of rabies infection and for protection of assays of rabies vaccine as early as 1935 (Webster and Dawson, 1935). Recently cell culture has replaced MIT in many developed countries; while in developing countries of Africa MIT remain the only confirmatory method. The cell culture test is widely used to isolate rabies virus from the brain and salivary glands. The RTCIT test has advantage over MIT because it is more sensitive, less expensive, easily carried

out and performed in good time (4 days) in contrast to 30days for MIT (Webster and Casey,1996). The murine neuroblastoma(C-1300) cell line has been shown to be most susceptible for the isolation of either fixed or street rabies virus(Crick and King, 1998; Rudd and Trimarchi,1987).

2.1.6.6 *Serological test*

The Fluorescent Focus Inhibition Test (RFFIT) and fluorescent antibody virus neutralization test(FAVNT) are currently utilized in many diagnostic and research laboratories for detection and titration of antibodies against rabies(Cliquet *et al*.,1998). These methods however, require laboratories capable of handling live rabies virus.

2.1.6.7 *Rapid immune-chromatographic test*

The RICT is based on the principles of immunochromatography using a gold labeled monoclonal antibody (MAb). Purified anti- Nucleoprotein MAbs are immobilized in a test zone of the nitrocellulose membrane, while purified goat anti-mouse IgG are immobilized in the control zone of the membrane to capture unbound MAb. The sample, once added in the sample pad, migrates through the gold MAb pad, the test zone and the control zone respectively. It is a method suitable for field diagnosis of rabies, before submission for confirmatory diagnosis in areas distant to where FAT can be done (Zhang *et al*., 2006). It has advantage over other tests as it is rapid, cheap, both saliva and brain can be used, little training is needed for personnel and cheap to purchase (Alexandre ., *et al*., 2012; Mshelbwala *et al*., 2013).

2.1.6.8. *Reverse-transcription polymerase chain reaction*

The RT-PCR is used when further characterization of rabies virus is necessary or in situation when highly putrefied specimens, fluid samples such as saliva and cerebrospinal fluid(CSF) which are unsuitable for FAT and when virus isolation and histology are not possible (David *et al.*,2002). It has also been used to confirm rabies infection in humans where other tests could not be readily applied (Hughes *et al*., 2004).

2.1.6.9 *Differential diagnosis of rabies*

Conditions like canine distemper, foreign body lodgement in the mouth and esophagus, cerebral babesiosis,cerebral cystercercosis, tetanus and erhlichosis can be clinically confused with rabies(Bishop *et al*., 2002; Ogunkoya, 2006) .In humans it can be confused with conditions like tetanus, hysterical pseudo-hydrophobia (Warrel,1996).

2.1.7 Management

There is no known treatment for rabies such that rabid animal are recommended to be euthanized (Ogunkoya, 2006; Rupprecht, 2008). However, in humans the approach to management of rabies normally should be palliative as the disease is fatal. In unusual circumstances, a decision may be made to use an aggressive approach to therapy for patients who present at an early stage of clinical disease. No single therapeutic agent is likely to be effective, but a combination of specific therapies could be considered, which include rabies vaccine, rabies immunoglobulin, monoclonal antibodies, ribavirin, interferon-α, and ketamine. Corticosteroids should not be used. As research progresses, new agents may become available in the future for the treatment of human rabies (Rupprecht, 2008; Jackson*et al*., 2013). A 15 -year- old girl was reported to have

recovered from clinical rabies following treatment (Rodney *et al*.,2005); however, on attempt to use same treatment regimen on another victim of rabies failed (Rupprecht, 2008).

2. 1.8 Prevention of rabies

Prevention of rabies can be achieved in both animals and humans by vaccination (Audu, 2011). Animal vaccination is the single most efficient way of rabies control in domestic animals (Ogunkoya, 2006). Safe and efficacious animal and human vaccines have been developed and are becoming available to those in need, although their cost is very high for most victims of dog bite on individual level. The development of effective animal vaccine for rabies, mass vaccination is the major integral component of rabies control. However, rabies elimination require additional components, including effective community involvement and policy makers, dog population evaluation and management, surveillance and legislation (Meslin and Briggs, 2013). Education and promotion of responsible dog ownership is critical. Vaccine failure, variation in incubation period and abortive rabies are factors militating against effective rabies control (Nottidege and Omobowale, 2010).

Post exposure prophylaxis for victims of rabies exposure is highly effective in the prevention of rabies if administered within 14 days after exposure (Ogunkoya, 2010). Wounds sustained from bites are washed with plentyof water and soap and five doses of immunoglobulin is administered to the patient over 28- day period (Warrel and Warrel, 2004). Pre-exposure prophylaxis for humans at high risk as a result of occupational hazard or trip to endemic areas is advocated (Nottidge and Omabawale, 2010; Ogunkoya, 2010). Recently, attempt was made towards the development of rabies DNA vaccine using Evelyn Rokitnicki Albelseth (ERA) strain, the result from the study revealed that intramuscular and intra-dermal routes of administration were

significant. Suggesting glycoprotein gene from ERA rabies virus strain may be ideal candidate for DNA vaccine enhancement (Osinubi*et al*., 2009).

2. 1.9 Control of rabies

Rabies control can be achieved by active immunization of animals and strict quarantine regulations (Okoh, 2000; OIE, 2008; Mshelbwala *et al*., 2013b). Highly effective, safe and thermostable inactive veterinary vaccines are still currently available, and are in use (Barth *et al*., 1985; Swanepoel *et al*., 1993). However, in Nigeria live vaccines are still used for dogs (Okoh, 2000; Opaleye *et al*., 2006).

2.1.10 Rabies in apparently healthy dogs

Gel diffusion tests carried out on 125 serum samples for detection of rabies antibody from apparently healthy dogs revealed 3.2% positive cases (Afshar *et al*., 1972). Out of the 101 salivary gland and saliva samples collected from apparently healthy dogs for rabies precipitating antigens, six samples of salivary gland and one of saliva were positive. These results suggested that in-apparent or abortive infection with rabies virus might occur in dogs. (Afshar *et al*., 1972, Bell *et al*., 1973). Fekadu (1975), reported the isolation of rabies virus from the saliva of five symptomless unvaccinated dogs. Three dogs were alive and asymptomatic up to 72 months after the first isolation of rabies virus from their saliva. The isolated virus was highly neurotropic and produced a great number of large Negri bodies in experimental animals. In Nigeria, many workers have reported the presence of in-apparent rabies in dogs. Out of the 463 serum samples obtained from non-vaccinated dogs, neutralizing antibodies were detected in 142 (30%); this is a clear indication of rabies exposure in the dog population. In the survey carried out by Aghomo

et al. (1989), in 1500 unvaccinated dogs and apparently healthy dogs, rabies virus was isolated from four dogs.

2.1.11 *Rabies situation in Nigeria*

Rabies was first officially reported in humans in 1912 (Boulger and Hardy, 1960).While the first canine rabies was diagnosed in 1925 at Yaba Rabies Laboratory (Oboegbulem, 1994). Subsequently reports on the incidences increased (Anonymous, 1932; Fagbami *et al.*, 1980; Ogunkoya *et al*., 1984). The discovery of rabies related virus like mokola and Lagos bat virus further complicates the epidemiology of rabies in Nigeria. Currently, rabies has been reported in almost all the six geopolitical zones of the country (Ajayi *et al*., 2006; Garba *et al*., 2008; Audu, 2011; Abubakar and Bakeri,2012; Isek, 2012; Hambolu, 2013; Mshelbwala *et al*., 2013a)and more recently by Hambolu *et al*.(2014). This is a clear indication that it is endemic, in Nigeria.However, ignorance about the public health importance, lack of responsible dog ownership as well as lack of political will and commitment to control and eradicate the disease in Nigeria has continued to worsen the situation in Nigeria (Bata *et al*., 2011).

Governmental and non-governmental organizations tend to place more emphasis on diseases like Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome (HIV/AIDS), tuberculosis and malaria. Very little or no attention is given to rabies which also poses a great economic burden and in addition, is almost always fatal (Nottidge and Omobowale, 2010).

The following have been suggested as strategy for an effective control of rabies in Nigeria, public health education, surveillance and monitoring, subsidy on pre and post-exposure

prophylaxis, mandatory vaccination for people at high risk, annual mass vaccination and control of stray and free roaming dogs.

2.1.12. *Dog meat consumption*

Every year, an estimated 25 million dogs are killed for human consumption (Anon, 2012).Dog meat refers to the flesh and other edible parts derived from dogs. Human consumption of dog meat has been recorded in many parts of the world, including East and Southeast Asia, West Africa, The Philippines, Europe, and pre-Columbian America.(Schwabe,1979). Dog meat is consumed in China, Korea, Vietnam, and in Switzerland (Simmons, 1994; Rupert, 2002; Murray, 2007; Anthony, 2009). Dog meat has also been used as survival strategy in times of war and other hardships (Douglas, 2007).

Some cultures see the consumption of dog meat to be a part of their cuisine, while others consider consumption of dog to be inappropriate and a taboo on both social and religious grounds (Garba *et al*., 2013). Cultural globalization has increased international criticism, (particularly from Western countries, as well as organizations such as the World Society for the Protection of Animals) against dog meat consumption and the torture of dogs caged and farmed for their meat (Anonymous, 2012). In response to these criticisms, proponents of dog meat consumption have argued that distinctions between livestock and pets are subjective, and that there is no difference in eating the meat of different animals (Eric and Olivier 1982; William, 2002). Historical cultural records in China have, however, noted how Chinese variations on Buddhism have preached against the consumption of dog meat, which is held to be one of the five 'forbidden meats'. Eating dog is also forbidden under both Jewish (Nicholas and Michael, 2000) and Islamic dietary laws (Willy, 2007). In Nigeria, dog trade is on the increase (OIE,

2012). Potential risk associated with the processing of dog meat have also been highlighted by recent workers (Audu, 2011; Garba *et al.*,2013; Odeh *et al*., 2014;Atuman *et al*., 2014b). Nutrient obtained from dog meat have been analyzed (Table 2.1).

Table 2.1. Nutritional value per 100 g of Dog meat

Energy	1,096 kJ (262 kcal)
Carbohydrates	0.1 g
Dietary fiber	0 g
Fat	20.2 g
Protein	19 g
Water	60.1 g
Vitamin A equiv.	3.6 µg (0%)
Thiamine (vit. B_1)	0.12 mg (10%)
Riboflavin (vit. B_2)	0.18 mg (15%)
Niacin (vit. B_3)	1.9 mg (13%)
Vitamin C	3 mg (4%)
Calcium	8 mg (1%)
Iron	2.8 mg (22%)
Phosphorus	168 mg (24%)
Potassium	270 mg (6%)
Sodium	72 mg (5%)
Ash	0.8 g

Percentages are roughly approximated using US recommendations for adults. Source: Ann, 1999

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

Abia State is located in the southeastern part of Nigeria. It lies within latitudes 4° 40' and 6° 14' north of the equator, and longitudes 7° 10' and 8° east of the Greenwich meridian. It shares common boundaries with Ebonyi State to the north, to the South and Southwest with Rivers State, and to the east and southeast with Cross River and Akwa lbom States respectively. To the west is Imo State and to the northwest is Anambra State. The state covers an area of about 5,243.7 sq. km which is approximately 5.8 per cent of the total land area of Nigeria. It has seventeen Local Government areas(LGAs), namely: Aba North, Aba South, Isiala Ngwa North, Isiala Ngwa South, Ukwa West, Ukwa East, Obingwa, lkwuano, Bende, Arochukwu, Ohafia, lsuikwuato, Umuahia North, Umuahia South, Ugwunagbo, Osisioma and Nnoch (Fig 3). Umuahia is the state capital. This study was carried out in eight slaughter slabs located in four LGAs namely; Umuahia North, Isiala Ngwa North, Osisioma and Ikwuano. Figure 1 shows the points where samples were collected during the study..

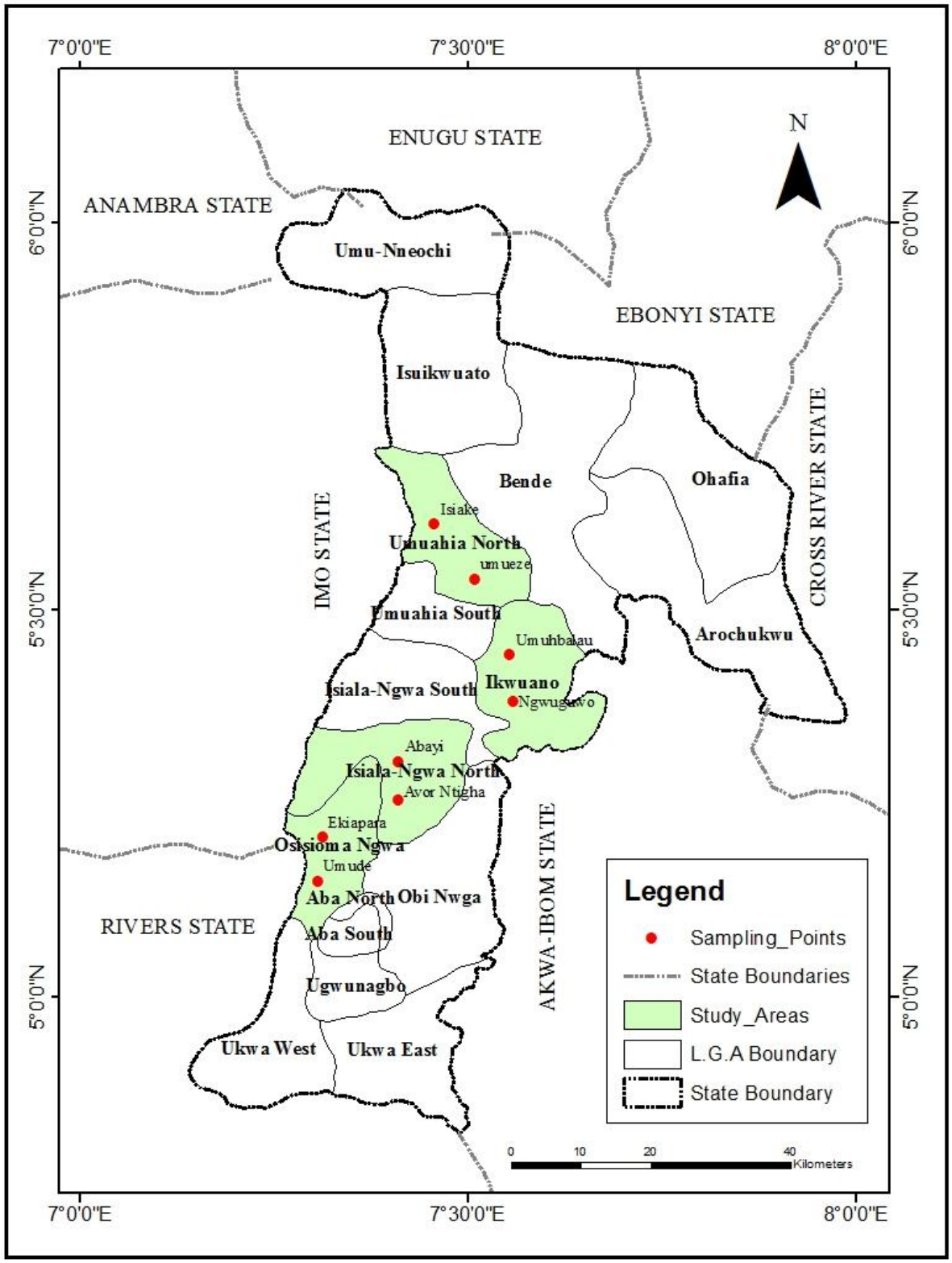


Figure 3: Map of Abia State, showing study areas
Source: Adapted and Modified from the Administrative Map of Abia State.

3.2 Period of Sample Collection

Samples were done collected within a period of three months (April- June, 2013). Slaughter time of dog for Ikwuano LGA was 7 to 9 am, while it was 12 pm upwards (except Sundays which was 10 pm to 11pm) in the other LGAs.

3.3 Sample collection and handling

Purposive sampling as described by Kudi (2013) was employed. The most accessible units of the population were used. Live adult dogs bought or brought for slaughter were sampled in these designated Local Government Areas. Local arrangement was made with dog meat butchers in the selected areas within the LGAs to placed calls across to me, whenever they have dogs available for slaughter. A total of 25 dogs were collected from each LGA. The formula by Trusfield (2007) was used to calculate the sample size. A total of one hundred samples each of saliva and brains were collected during the period of study from the various slaughter points within the study area. Information on the source and sex of dogs presented for slaughter was obtained before slaughtered and was entered into the record book. The saliva was obtained before slaughter while the brain was collected after. Each slaughter point was visited once daily, except in occasion of emergency slaughter during burial ceremonies. The whole brain was removed, placed in a labeled polytene bag, placed into an ice pack, it was then transported and stored in thermo cool® deep freezer at Animal World Veterinary Clinic, Umuahia, before being transported to National Veterinary Research Institute (NVRI), Vom for, FAT and Rabies laboratory of the Department of Veterinary Medicine, ABU, Zaria, for dRIT

3.4 Use of Questionnaire

A structured questionnaire (Appendixes 1 and 2) was prepared and administered to one hundred (100) dog meat processors and consumers of dog meat within the study areas. The questionnaire which was in two parts was administered in the form of interview. Part one ,was administered to butchers to obtain information on their demography; rate of contacts with dogs, practice of dog butchering, vaccination status, knowledge about rabies; frequency of dog bite and method of treatment following bite; attitude towards rabid dog, reason for consuming dog meat, and practice on sighting a rabid dog.

The second part was administered to dog meat consumers, to obtain information about their demography; knowledge about rabies; vaccination status; frequency of dog bite; method of treatment following bite; attitude towards rabid dogs, reasons for consuming dog meat. Practice on sighting a rabid dog, and knowledge of someone who died of dog bite. The questionnaire was administered to those who were willing to participate in the study. Data obtained from the questionnaire survey were collated and subjected to statistical analysis using SPSS version 17.

3.5 Calculation of Sample Size

Thrusfield (2007) $N = \frac{Z^2P(1-P)}{d^2}$

Where N= Sample size

Z= 1.96 Standard normal value for desired confidence(normal distribution table)

Prevalence (6%) Audu (2011)

d=Allowable error (5%)

$$\frac{1.96^2 \times 0.06(1-0.06)}{0.05^2} = 86.7$$

The sample size was increased to 100 in order to minimize sampling error and increase precision

3.5.1 Saliva sample collection

Dog brought for slaughter was restrained by the butcher, its mouths muzzled, the sterile swab stick was then used to collect saliva from the oral cavity. The swab was then inserted into an assay buffer tube and stirred to ensure a good sample extraction. The immune chromatographic test cassette was removed and placed horizontally. Using a sterile dropper, 3-4 drops of the extracted sample was dipped into the sample hole in the cassette and the result was interpreted within 5-10 minutes, as described by the manufacturer (BioNote, Inc). One hundred saliva samples were obtained and used for the test.

3.5.2 Brain sample collection

A total of one hundred (100) dog head samples were collected from the study areas. The labeled head samples were then placed in polythene bags and were transported in ice pack to the laboratory for direct FAT. Brain was extracted on the field using the method described by Kaplan and Koprowski (1980). The FAT was carried out using the facilities of the NVRI, Vom, and Plateau State while dRIT was done at the rabies laboratory of the Department of Veterinary Medicine, ABU, and Zaria.

3.5.3 Processing of the brain

Each of stored brain samples (hippocampii, cerebrum, cerebellum and gasserian ganglion) was removed from the deep freezer and was allowed to thaw and then subjected to Fluorescent Antibody Test as described by Dean *et al.* (1996).

3.5.4 Procedure for florescence antibody test

Rabies direct fluorescent antibody assay FATT (Monoclonal antibody-conjugate) reagents from Fujirebio Diagnostic Inc Malvern (P.A 19355) were used and the working (reagent) dilution after titration was achieved at 1:40 in accordance with the manufacturers recommendations and as described by Flamand *et al.* (1980).

A small fraction of the brain sample was smeared using wire loop on one part of a slide and then was air dried and fixed in cold acetone for one hour at -20^{o}C. The slides were air dried and then the rabies conjugate was applied at 1:40 and incubated for 30 minutes at 37^{o}C in a humid chamber after which excess conjugate was removed from the slides by rinsing it with 7.4 pH PBS solution about 3-5 minutes and was allowed to air dry. The cover slips were mounted with buffered glycerol mounting medium and the slides were examined using a fluorescence microscope within 2 hours after staining. When brilliant apple-green fluorescence color or greenish yellow objects are exhibited against a black background the test slide is positive. If no specific apple-green fluorescence is exhibited the test slide is negative. As described by CDC, 2011.

3.5. 5. Direct rapid immunohistochemistry test

Following FAT at NVRI, the same brain samples were transported to rabies laboratory of the Department of Veterinary Medicine, Ahmadu Bello University Zaria and stored at -20˚C in deep freezer. All the brain samples were subjected to dRIT as described by Lembo *et al*. (2006).

1. A routine touch impressions of each sampled brain tissue were made on labeled glass microscope slides (including the standard positive and negative controls).

2. Slides were arranged in slides holder, air-dry for 5 minutes at room temperature.

3. Slides were then immersed in 10% buffered formalin at room temperature for 10 minutes. Dish I

4. Slides were then removed and dip-rinsed several (10 X) times to wash off any excess fixative in wash buffer Tween phosphate buffer saline TPBS (PBS with 1% tween 80). Dish II.

5. Slides were then immersed in 3% hydrogen peroxide (H_2O_2) for 10 minutes. Dish III.

6. Excess hydrogen peroxide was removed by dip-rinsing slides in TPBS, Dish IV. Slides were then Transferred into the next rinse Dish V containing TPBS (after dip-rinsing, excess buffer were shake off from slides edges using wipe papers surrounding the impression). A slide at a time was removed and wiped and were arranged on a wet paper towel on laboratory flat table top.

7. Slides were then incubated in a humidity chamber (I used a moistened paper towel, on laboratory bench top covering with 96 well plastic cover) at room temperature after adding primary antibody – (biotinylated anti-rabies mAb) for10 minutes (primary antibody usually 2-3 drops were added using plastic pipettes).

8. After incubation with primary antibody, excess conjugate were shake off. Slides dip-rinsed in TPBS, Dish V (excess TPBS were shake off and blot buffer from slides edges surrounding the impression using wipe paper). This same wash buffer was used through step 10.

9. Slides were placed back on the wetted paper towel then incubated with Streptavidin – peroxidase complex (2-3 drops were added) in humidity chamber as described in step 7 above at room temperature for 10 minutes. After incubation, slides were shake- off.

10. Slides were then dip-rinsed with TPBS, in Dish V (excess buffer shake off and blotted from slide edges surrounding the impression).

11. Slides were then incubated with peroxidase substrate, amino-ethylcarbizole (AEC) – the working dilution was prepared just prior to use. Enough of this substrate was added to the slide by drop to cover the impression in a humidity chamber (as in step 7 above) at room temperature for 10 minutes. After incubation, shake off excess substrate.

12. Slides were then dip – rinsed in de-ionized/distilled water, Dish VI.

13. Then counter stain with Gills Hematoxylin (diluted 1:2 with de-ionized/distilled water) for 2 minutes Dish VII.

14. Immediately after the 2 minutes counter staining, the slides were dip-rinsed (10 times) with de-ionized/distilled water in Dish VIII. A second dip-rinse of slides with fresh deionized/distilled water (Dish IX) was made to ensure removal of excess stain.

15. Slides were then transferred to fresh distilled water Dish X. Slides were mounted with cover slips using water-soluble mounting medium.

16. Slides were viewed by light microscopy, using a x20 objective to scan the field, and a x40 objective for higher power magnification.

17. Rabies viral antigen appeared as red colored particles/inclusions under a bluish neuronal background indicated the samples was positive. A complete bluish background suggested that the sample was negative.

3.6. Data Analysis

In this study, data generated were analyzed using the Statistical Package for Social Science (SPSS) Version 17.0 to carryout descriptive analysis. Chi-square was used to test for association between categorical variables. Sex, rabies status, risk factors; age, period involved in butchering, level of education dog bite. A value of $p<0.05$ was considered significant.

The prevalence of rabies antigen in the saliva and brains was calculated using the formula.

$$\text{Prevalence} = \frac{\text{Total number of samples positive}}{\text{Total number of samples collected}} \times 100$$

CHAPTER FOUR

RESULTS

4. 1 Dog Trade

In Abia State, market days hold once weekly in various communities, during which dogs are sold for consumption. Two slaughter points were selected in each Local Government Areas (LGAs), totaling eight (8) slaughter points in four LGAs (Figure 4). Dogs were slaughtered on daily bases in Umuahia North and Ikwuano, while in Osisoima and Nsialangwa North dogs were slaughtered 3 times weekly. Most of the dogs were sourced from the local community and were adult dogs, the sources of some was difficult to ascertain (Figure 4). Dogs were sold between 6,000-10,000 Naira based on size and sex. There are placed in small enclosure and transported from one community to the other (Appendix 7). An oral interview with one traditional healer, at Bende road, Umuahia North, revealed that, parts from a known rabid dog is most sorts for, because of its medicinal and spiritual value.

4.2. Slaughter Areas and Number of Dogs Sampled

Brain and saliva samples were collected from eight slabs namely Umueze (16), Isiake (9), Umuhbalu(17), Ngwuguwo(8), Avor Ntigha (19), Abayi (6), Umudi (12), Ikaipara (13) (Figure 4). More sample were obtained at Umueze, Umuhbalu , Avor Ntigha and Ikaipara, butchers in these areas were very cooperative, some placed calls across before slaughter, conversely in the other locations, there was no maximum cooperation from butchers as they believe the process of sample collection will waste their time of processing and preparation.

4.3. Results of Samples Obtained Based On Location

Two samples of saliva and brain tissue were positive from the samples obtained in Umuahia North, 2 from Isialangwa North, 1 from Ikwuano, while none of the samples obtained from Osisioma was positive (Table 4.1).

4.4. Sex and Breeds of Dogs Sampled

Sixty-four of the saliva and brain tissue samples used in this study came from female dogs out of which (7.8%) were positive for rabies antigen using RICT, FAT and dRIT, while 36 were from male dogs and none was positive (Table4.3). Indigenous breeds of dogs were the highest of the dogs sampled in the population totaling 96(96%), mixed breed were 3 (3%) while only one (1%) was exotic. Of the 96 indigenous breeds tested (5.2%) were positive for rabies antigen using the three diagnostic tests (Table 4.4).

4.5. Results of Rapid Immunochromatographic Test

The study revealed that there were more females dogs (64%) and only 36 dogs (36%) were males. Out of the 64 females samples (7.8%) were positive by Rapid Immunochromatographic Test (Appendix 3).

4.6. Result of Fluorescent Antibody Test

A total of 100 brain samples were submitted for FAT, out of which, only 5 was positive for the present of rabies antigen and were all from female dogs(Appendix 4).

4.7. Result of and Direct Rapid Immunohistochemistry Test

The study revealed that of the 100 brain samples subjected to dRIT, five were positive for rabies antigen (Appendix 5).

4.8. Comparing results of the Three Tests Used

Using Rapid Immunochromatographic Test, five out of the 100 dogs slaughtered were positive an indication that they were shedding rabies virus in their saliva. FAT and dRIT were also positive for the same samples that were positive for RICT, signifying a total agreement between the entire test used. The positive samples were from Umuahia North, Ikwuano and Isianlangwa North. None of the samples obtained from Osisioma was positive (Figure 5).

Table 4.1: Distribution of saliva and brain samples by location where it was collected, in Abia State.

Location	No of samples	No Positive (%)
Umuahia North	25	2 (8)
Ikwuano	25	1 (4)
Isianlangwa North	25	2 (8)
Osisioma	25	0 (0)
Total	100	5 (5)

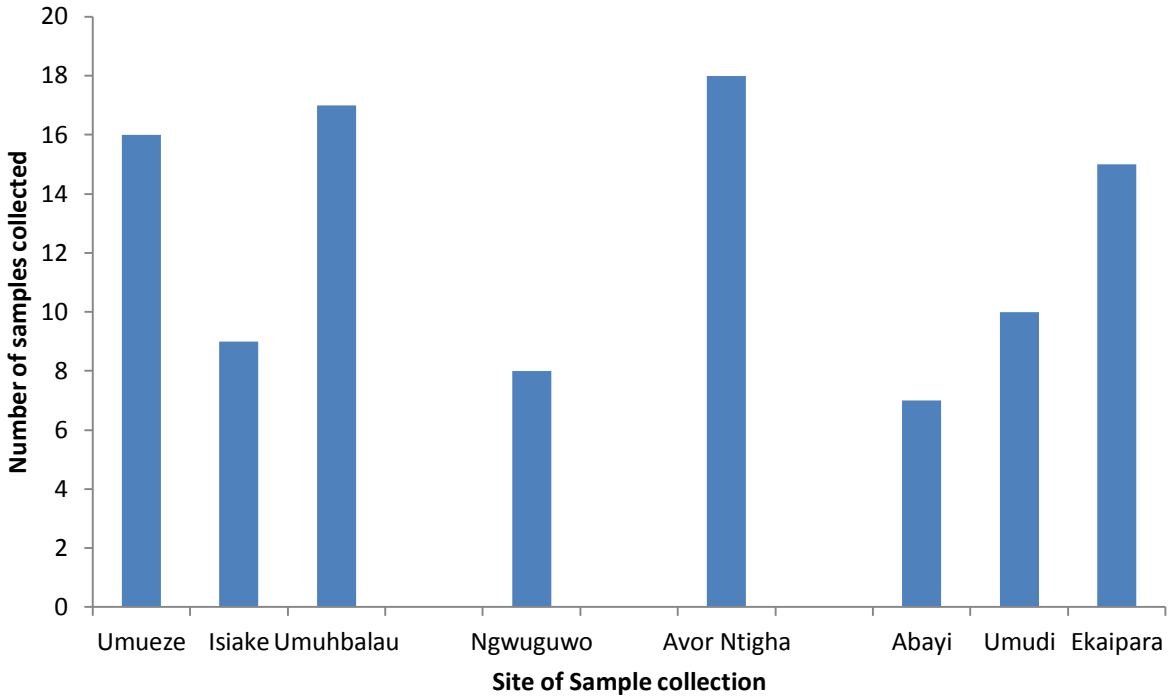


Figure 4: Slaughter areas and number of dogs sampled, Abia State.

Table 4.2: **Distribution of dog samples according to sex and positive cases to rabies antigen in Abia State, Nigeria.**

Sex	Number tested	Number positive (%)	
Male	36	0	(0)
Female	64	5	(7.8)
Total	100	5	(5)

Table 4.3: Distribution of rabies positive samples according to breeds in Abia State, Nigeria.

Breed	Number tested	Number positive (%)
Indigenous	96	5 (5.2)
Mixed	3	0 (0)
Foreign	1	0 (0)
Total	100	5 (5)

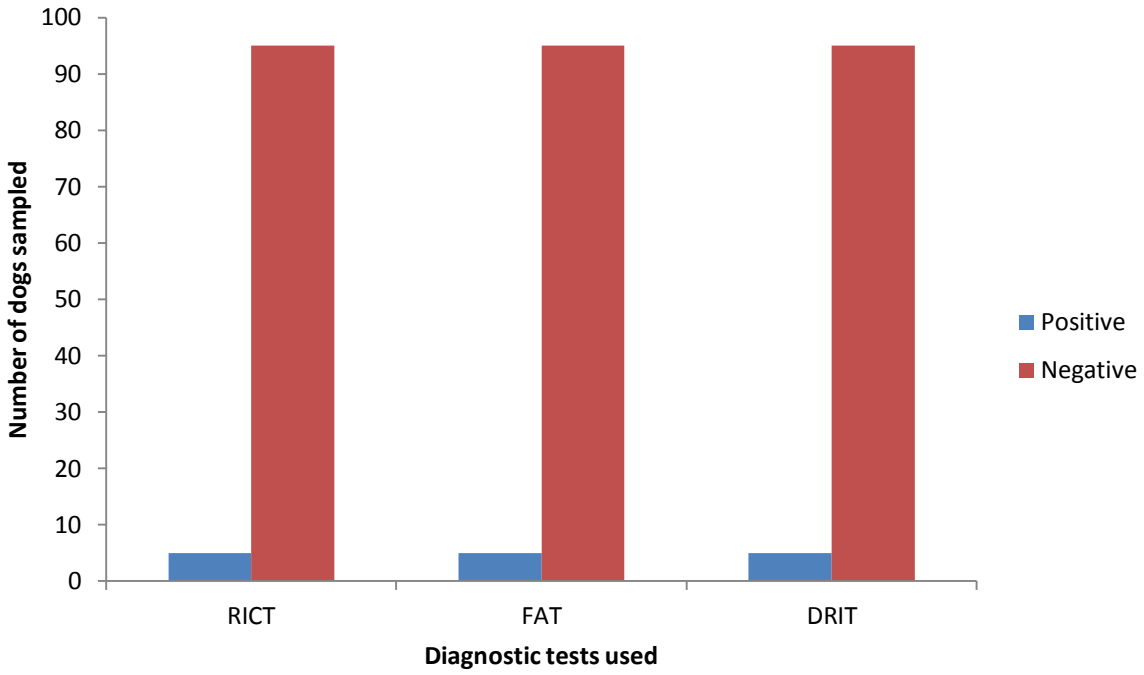


Figure 5: Comparing results of rapid immune-chromatographic test(RICT), fluorescent antibody test(FAT) and direct rapid immunohistochemistry test(dRIT) for the detection of rabies antigen.

4.9. Demographic Data of Butchers, Awareness of Rabies Infection and Risk Assessment

Nineteen butchers participated in the study comprising of 18(94.7%) males and 1(5.3%) female (Figure 6). Eleven of the respondents (57.9%) aged between 41-60; those within the range 20-40 were 7(36.8%) while only 1(5.3%) respondent was less than 20 years (Figure 7).

Analysis of the level of education showed that 13(68.4%) had primary education, 5(26.3%) respondents had no formal education while only 1(5.3%) had tertiary certificate (Figure 8). All the respondents had butchering as their major occupation and believed the business was very lucrative.

Information of how long the respondents had been in dog meat business and whether or not they consumed dog meat themselves revealed that 18 respondents (94.7%) consumed dog meat while only one (5.3) did not consume the meat (Table 4.4). One dog meat butcher (5.3) was less than a year in the business, 11(57.9%) respondents were between 1-5 years in the business, 4(21.1%) respondents were between 6-10 years, only one dog meat butcher (5.3%) was 11-15 years in the business and 2 (10.5%) respondents were above 20 years in dog meat business (Figure 9).

Out of the nineteen respondents, 14 (73.7%) had knowledge of rabies, while 5(26.3%) did not know about rabies, 13(68.4%) knew rabies was contracted through dog bite while 6(31.6%) did not know its mode of transmission (Figure 10).

None of the butchers was previously vaccinated against rabies and none found out the vaccination status of the dogs they slaughtered. Eighteen (94.7%) were previously exposed to dog bite during the course of slaughtering while 1 (5.3%) had never been bitten (Table 4.5). Following dog bite, 13 (72.2%) sought for traditional method of treatment while 5 (27.8%) reported to the hospital (Table 4.6). When asked what they did before reporting to the hospital,

3.7% applied kerosene at the site of bite, 54.3% washed the area with soap and water and covered the affected part with a piece of cloth, 13.6% did nothing, while 28.4% employed methods not well enumerated (Figure 11).

Analysis of practice on sighting a rabid dog revealed that 15 (78.9%) butchers had seen a rabid dog while 4 (21.1%) had never seen a rabid dog. Ten (52.6%) butchers killed the dog and ate its meat, 2(10.5%) stoned the dog while 7(36.8%) ran away from the rabid dog (Figure 12). Dog meat butchers handled dogs in a cruel manner (Plate II) and most handled dogs using their bare hands (Plate III).

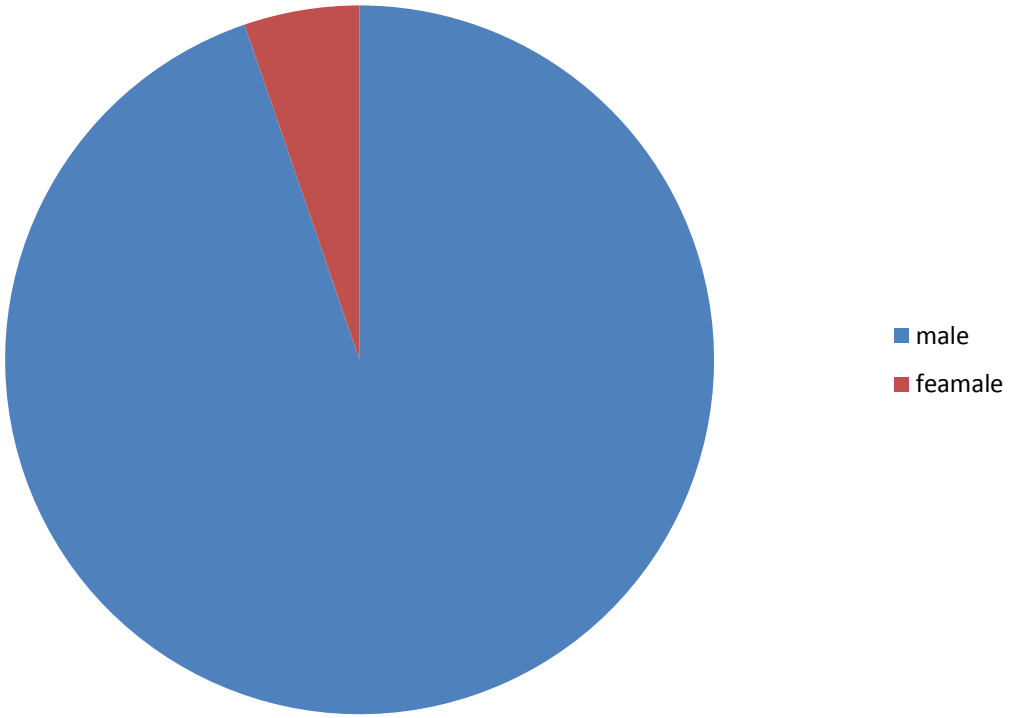


Figure 6: Sex distribution of dog meat consumers, in Abia State.

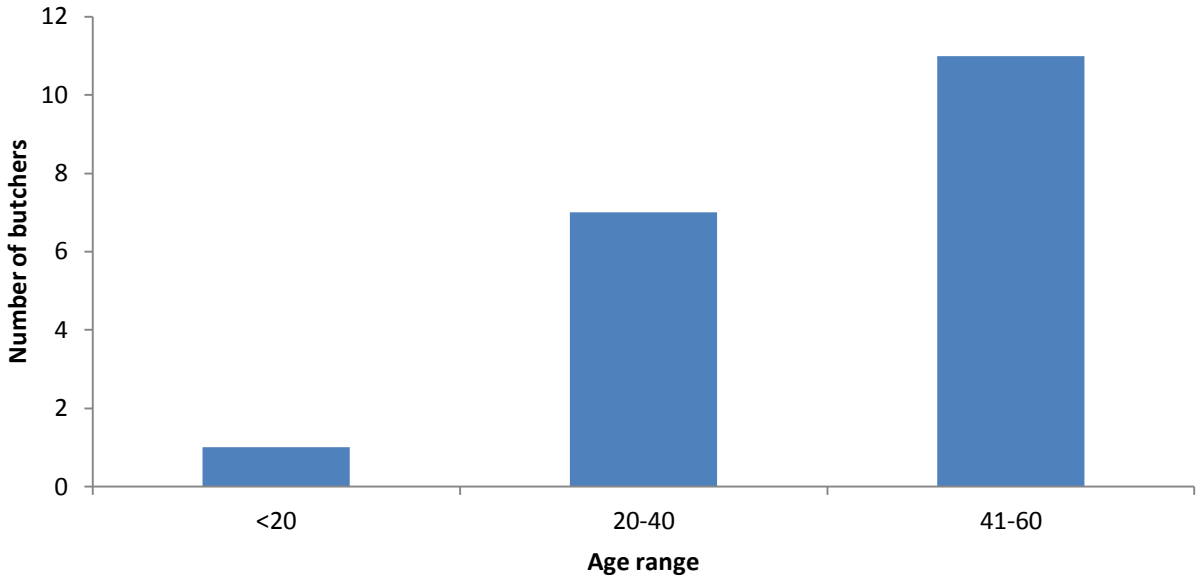


Figure 7: Age distribution of dog meat butchers in Abia State, Nigeria.

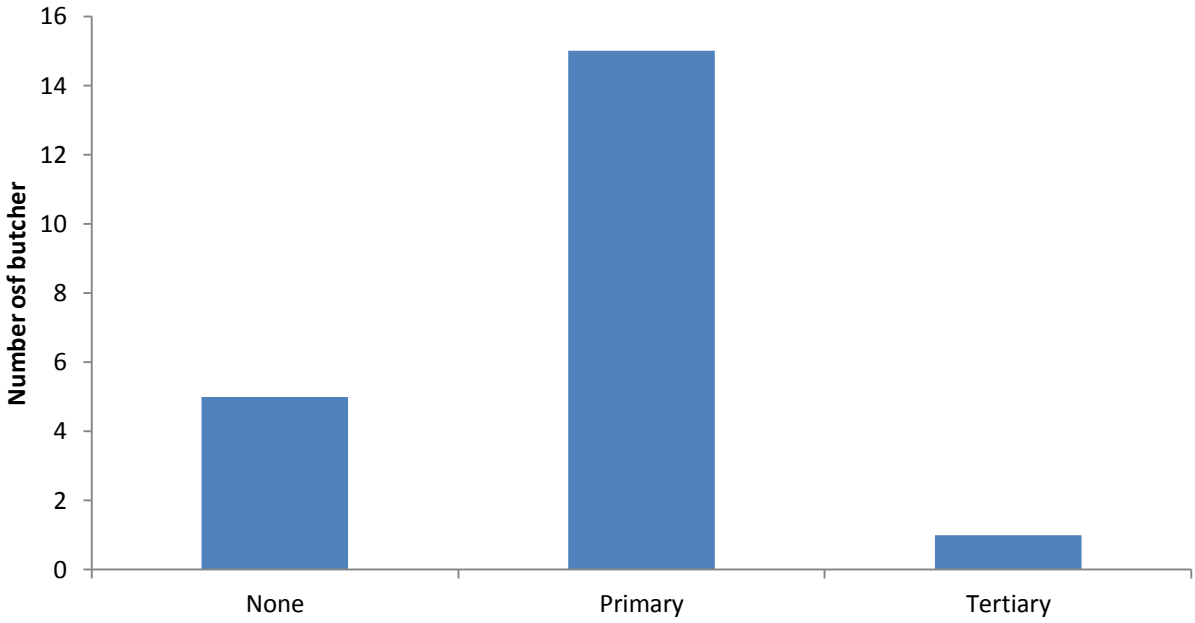


Figure 8: Educational qualifications of respondents, in Abia State.

Table 4. 4. Dog meat consumption among butchers in Abia State.

Response	Number of butchers	Percentage
Yes	18	94.7
No	1	5.3
Total	19	100

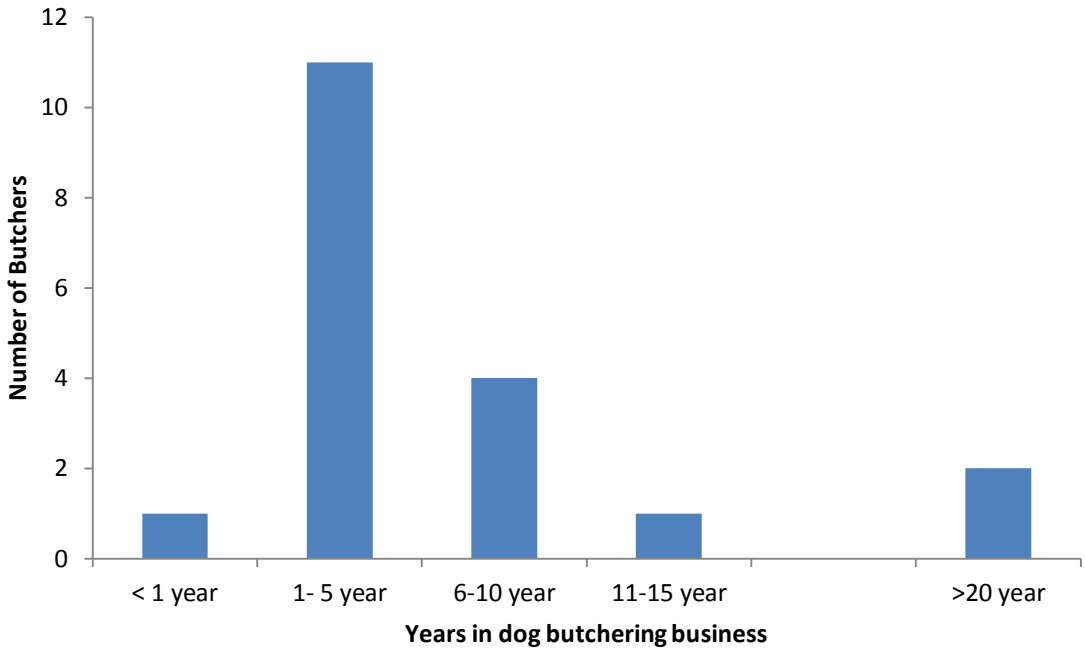


Figure 9: Number of years dog butchers were involved in dog butchering business, in Abia State.

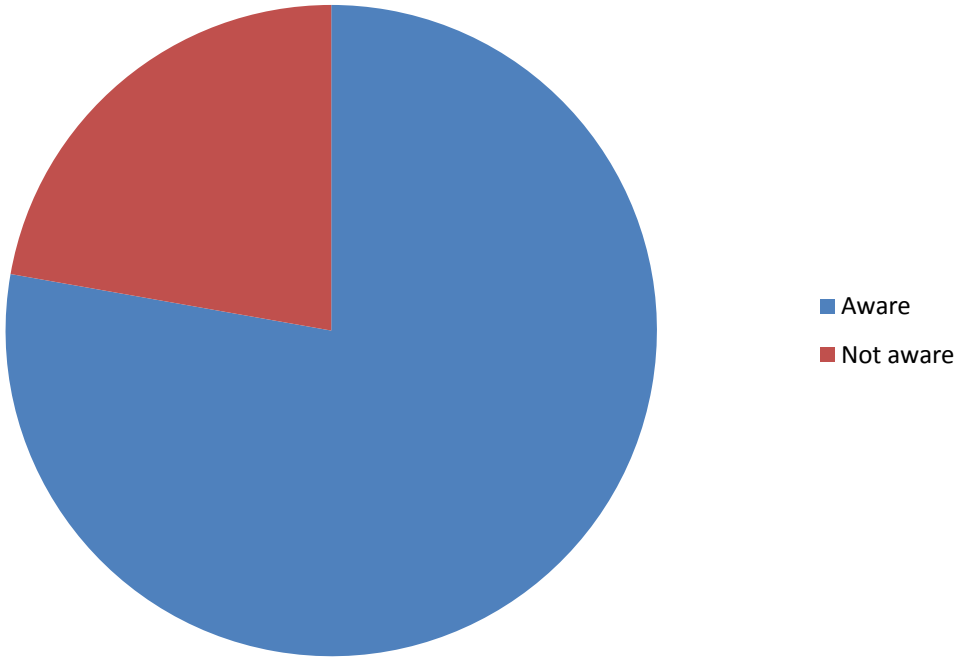


Figure 10: Awareness of rabies infection among dog butchers in Abia State, Nigeria

Table 4.5: Dog meat butchers previously bitten during slaughtering processes, in Abia State.

	Number of butchers	Percentage
Once bitten	18	94.7
Never bitten	1	5.3
Total	19	100

Table 4.6. Method of treatment by dog butchers following of dog bite, in Abia State.

Method of treatment	Number butchers	Percentage
Hospital	5	27.8
Traditional	13	72.8
Total	18	100.0

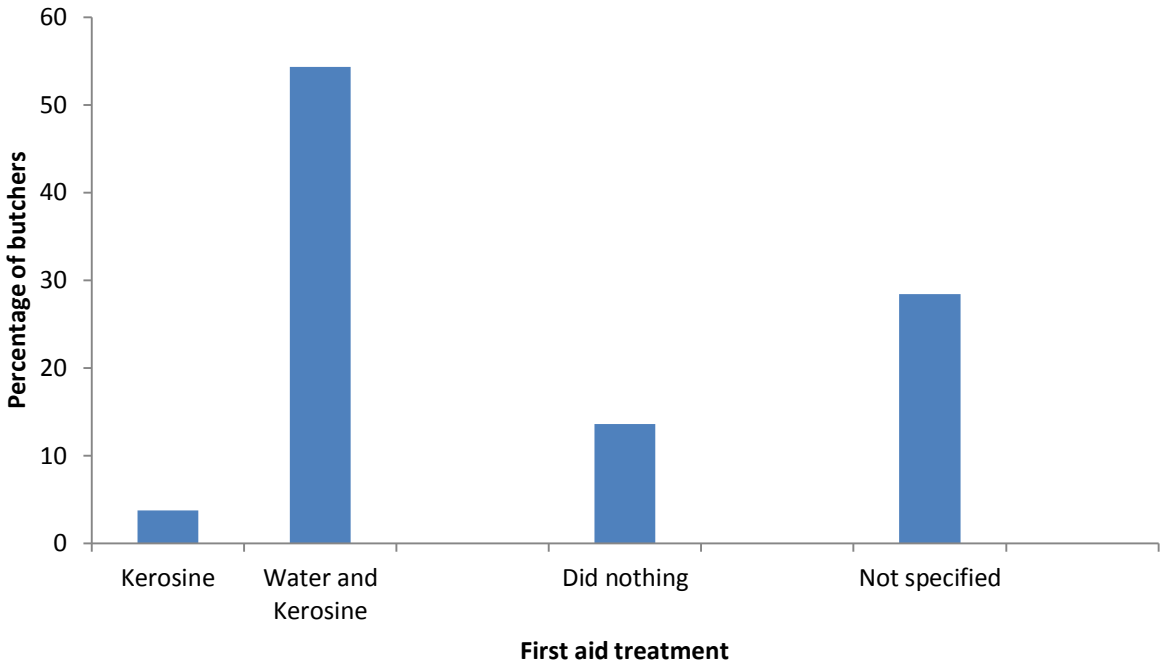


Figure 11: First aid treatment given to victims of dog bite before reporting to the hospital, in Abia State.

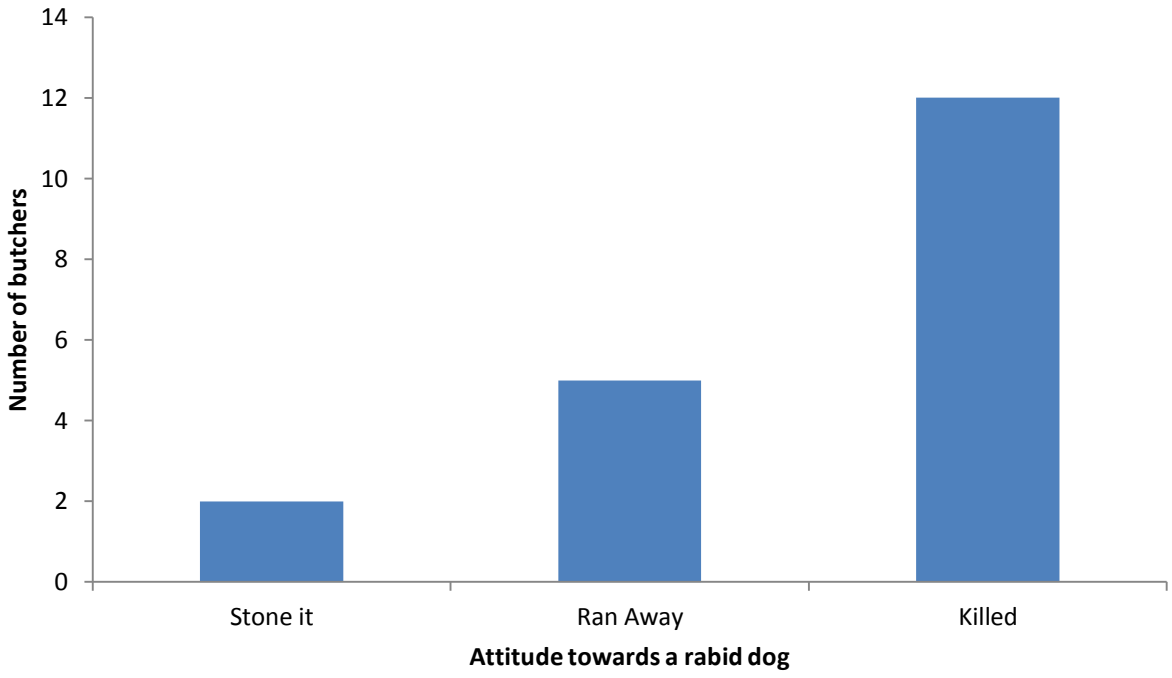


Figure 12: Attitude of dog meat consumers towards rabid dog in Abia State.



Plate II: Common restraint method by dog butchers at Isiake, Umuahia North, Abia State, Nigeria

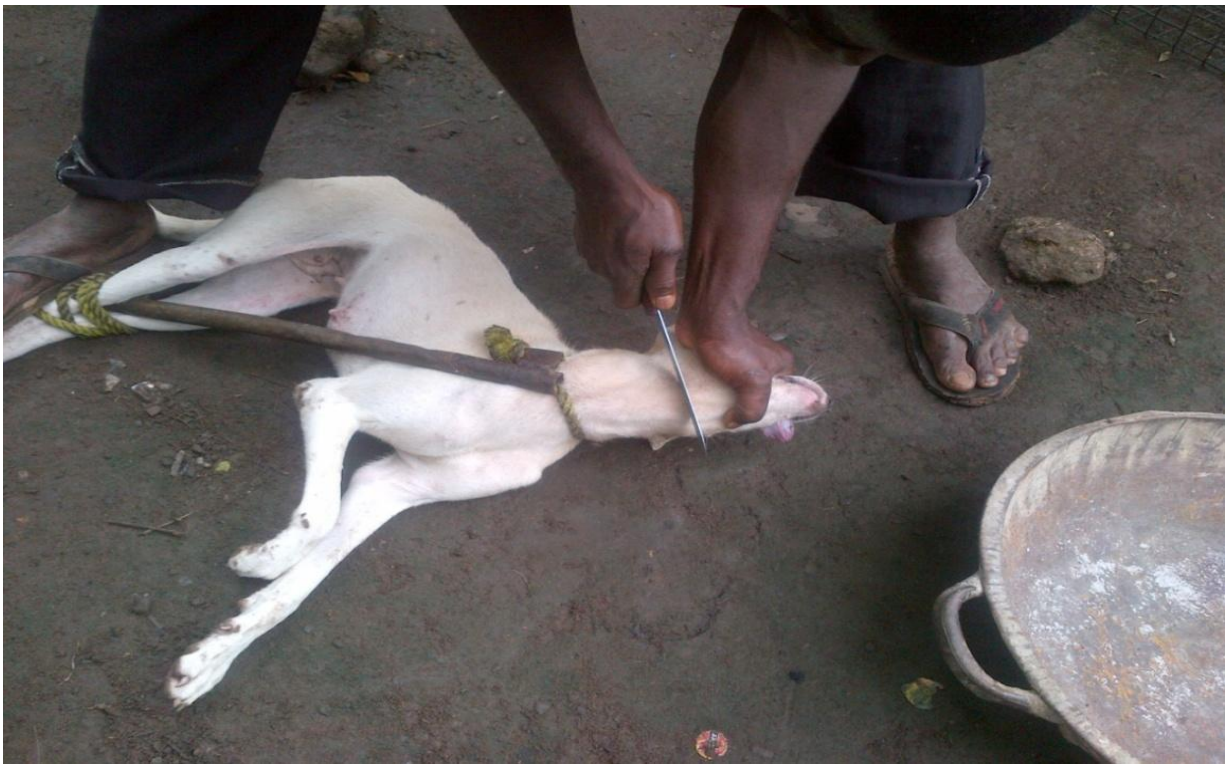


Plate III: Dog butcher using his bare hands to muzzle the mouth of dog, at Umuhbulu, Abia State.

4.10. Demographic Data of Dog Meat Consumers in Abia State, Reason for Consuming Dog Meat and Awareness of Rabies

Eighty one dog meat consumers participated in the study comprising of 62(76.5%) male and 19(23.5%) females (Figure 13). Eleven (13.6%) were less than 20 years of age, those between the age range of 21-40 were 44(54.3%), 23(28.4%) were between the ages 41-60 while 3(3.6%) were above 60 years of age **(**Table 4.7). Analysis of the level of education showed that 37(45.7%) had primary education, 31(38.3%) had secondary education, 7(8.6%) had tertiary education while 6(7.4%) had no formal education (Figure 14). `

Information of the occupations of dog meat consumers showed that 11(13.3%) were farmers, 16(19.8%) traders, 15(18.5%) were students, 10(12.3%) civil servants, 7(8.6%) hunters while 18(22.2) were not engaged occupations not specified (Table 4.8). Knowledge about rabies and how it is contracted and attitude towards rabid dog revealed 58(71.6%) knew what rabies is and how it is contacted while 23(28.4%) did not know what rabies is and how it is contacted (Figure 15). Sixty-one (75.3%) had seen rabid dog while 20(24.7%) had not seen a rabid dog before. Fifty-four (66.7%) killed the rabid dog and ate its meat, 24(29.6%) ran away on sighting the rabid dog while 3(3.7%) stoned the rabid dog on sighting it **(**Figure 16).

Respondents had various reasons for consuming dog meat. Four-seven (58%) believed it was medicinal and can cure of malaria and high blood pressure, 19 (23.5) eat dog because they said it was very delicious, 7 (8.6%) took it for spiritual protection ,while 8 (9.9%) said it enhanced libido **(**Figure 17). Information of the religious affiliation of respondents revealed that 71(87.7%) were Christians, 7 (8.6%) Traditionalist and 3(3.7%) Muslims (Figure 18). On whether or not they knew of someone who died from dog bite, 9 (11.1%) knew, while 72 (88.9%) did not know anyone (Figure 19).

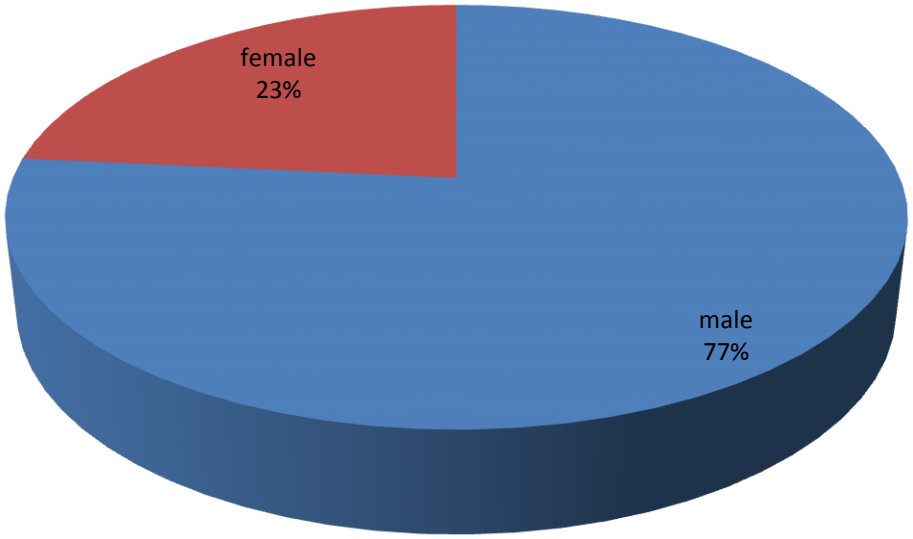


Figure 13: Sex of dog meat consumers in Abia State, Nigeria

Table 4.7. Age distribution of dog meat consumers in Abia State,Nigeria

Age	Dog meat consumers (%)
<20	11(13.6)
21-40	44(54.3)
41-60	23(28.4)
>60	3(36)
	81(100)

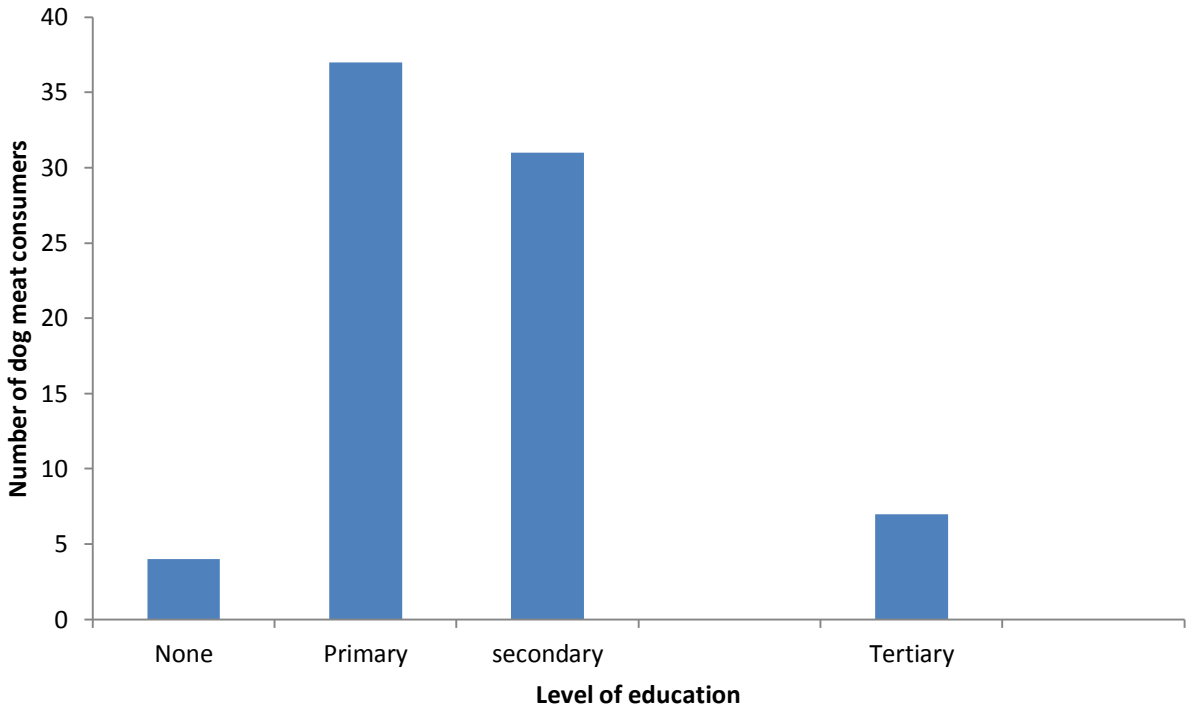


Figure 14: Educational level of dog meat consumers, in Abia State,Nigeria

Table 4.8. Occupation of dog meat consumers and dog meat preparation in Abia State, Nigeria.

Occupation	Number of meat consumers (%)	Meat preparation
Civil servant	10(12.3)	Cooked
Student	15(19.8)	Cooked
Farmer	11(13.6)	Cooked
Trader	16(19.8)	Cooked
Hunter	7(8.6)	Cooked
Other	18(22.2)	Cooked
Total	81(100)	

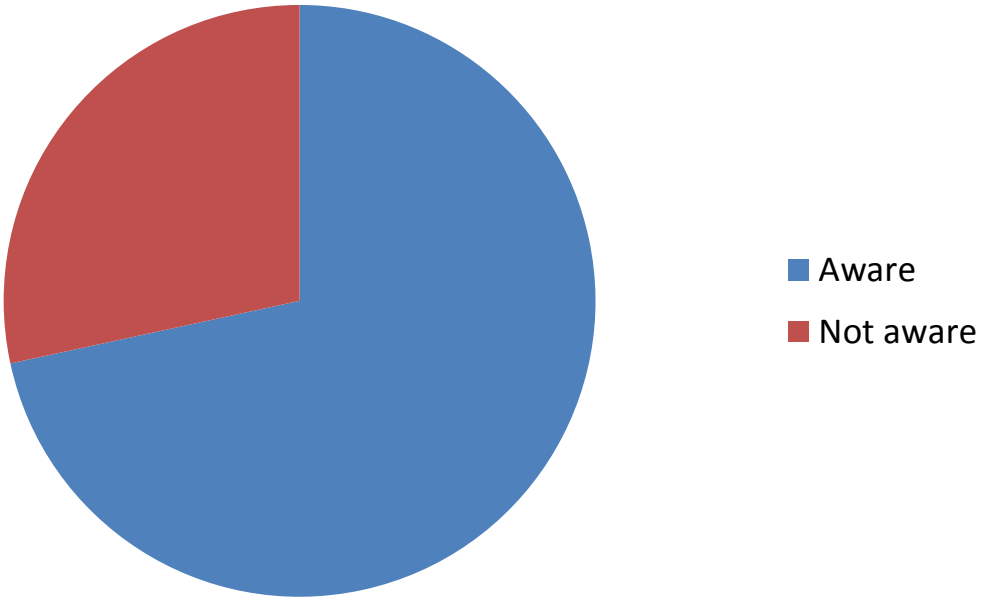


Figure 15:Knowledege of dog meat consumers about rabies and how it is contacted, in Abia State,Nigeria

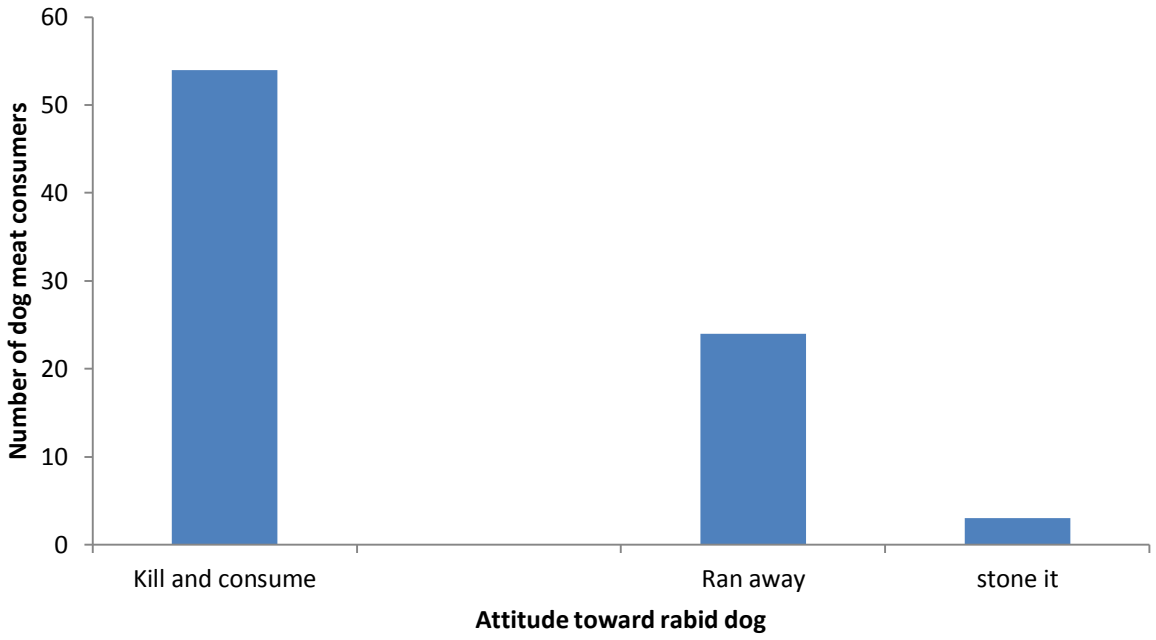


Figure 16 : Dog meat consumers attitude towards rabid dog in Abia State, Nigeria

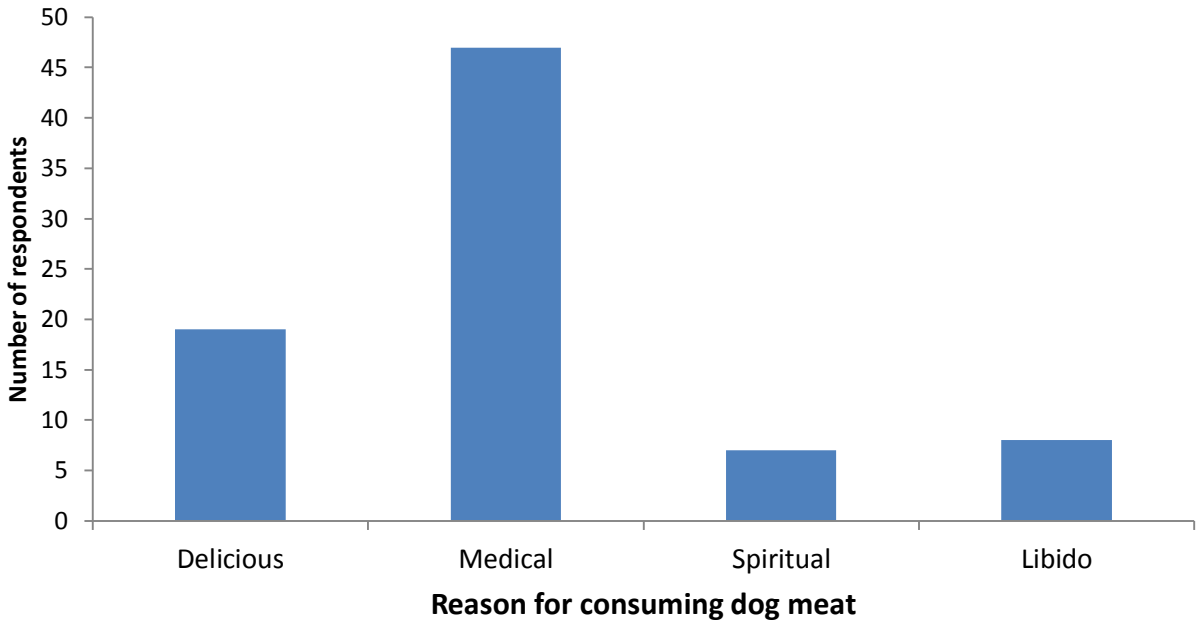


Figure 17: Reasons for consuming dog meat in Abia State,Nigeria

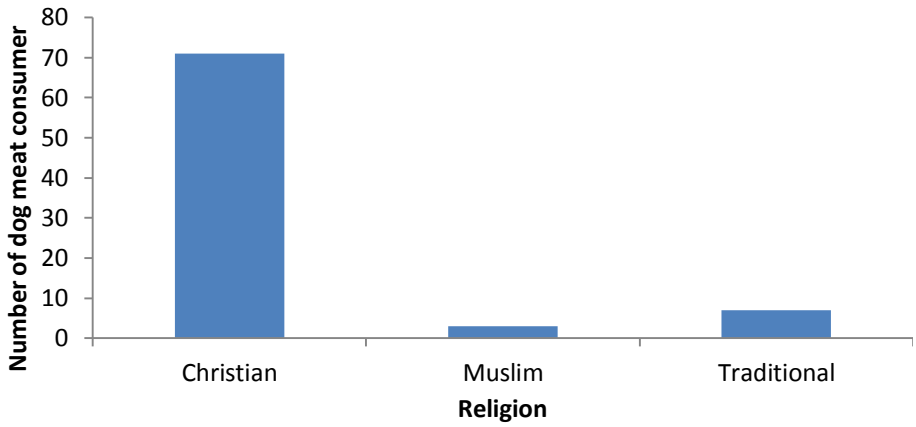


Figure 18: Religious affiliations of dog meat consumers in Abia State, Nigeria

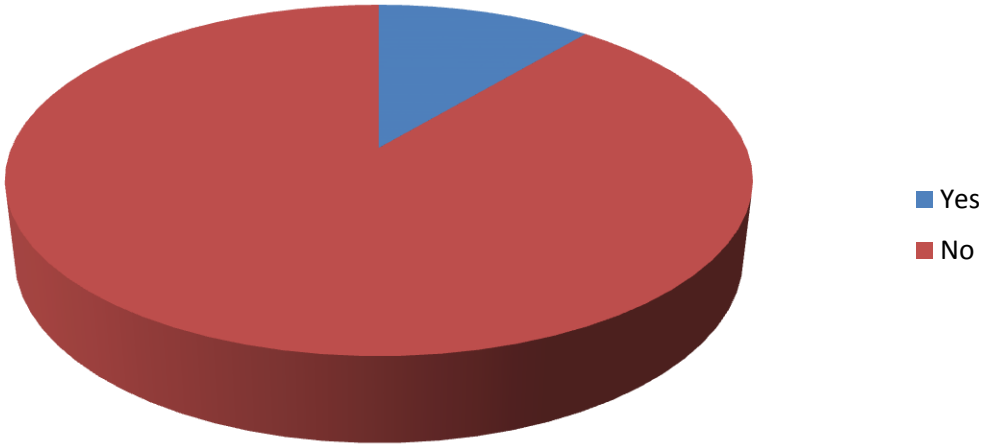


Figure 19: Knowledge of someone who died from dog bite in Abia State, Nigeria

Table 4.9 Risk Assessment for dog meat Butchers in Abia State, Nigeria.

Risk factors	Number of butchers previously bitten	Number of butchers never bitten	X^2(P Value)
Sex			
Male	17	1	(0.80)
Female	1	0	
Age			
<20 years	1	0	(0.40)
20 – 40 years	6	1	
41 – 60 years	1	1	
Education Level			
None	4	1	(0.23)
Primary	13	0	
Tertiary	1	0	
Length of involvement			
1 < year	1	0	
1 – 5 years	10	1	
6 – 10 years	4	0	(0.94)
11 – 15 years	1	0	
> 20 years	2	0	

CHAPTER FIVE

DISCUSSION

Results from this study, revealed that dog marketing, and related businesses have become well integrated in the culture of the people of Abia State, and was found to have stood the test of time. Apart from the fact that there is wide spread of dog markets in various communities, the marketing dynamics have revolve into a stable time table of rotation and structured to go round in all the communities. Market days hold every seven days, on rotational bases, during which dogs are transported in undersized cages from one community to the other. From the result obtained, few dogs (5%) involved in the trade were found to be carriers of rabies virus, that continue to excrete the virus in the saliva. This practice of moving dogs from one place to the other may aid in the spread of rabies virus and may enhance the spread of the virus and consequently, will make the control and eradication of rabies in Abia State, difficult if intervention measures are not put in place.

In general, dog meat was more expensive compared to other meats sold. Because of its curative effect on malaria and high blood pressure and provides spiritual protection, and enhanced male libido. Results obtained from the interview with some users, it was apparent that users of parts from these clinically ill dogs (rabid dogs) was claimed to be effective for whatever it is used. For example, male organs of such dog will sale many folds than non-clinically ill dog. This believes of users and readiness to pay higher costs makes clinically rabid dog to be sought for seriously. Butchers do all they could to restrain such dogs without precaution; this may further expose them to dog bite. There is potential for virus transfer if fluids or nervous tissues of the infected animals

come into contact with breaks in the skin before the meat is cooked or if the fluid split to the eyes of other processors.

The dog market boom has been laid on almost 100% profit made from each dog by butchers because of the value attached to the meat by dog meat consumers. This high yielding profit was found to be the point of attraction to many young jobless youths, who are mostly secondary school certificate holders. This is contrary to the report by Audu (2011), who reported the participation of first degree holders in the business in Kaduna State.

In this study, three laboratory procedures recommended for rabies diagnostic and research investigations` by the WHO and OIE were employed (WHO, 2014), a total of 5% prevalence of rabies antigens in the saliva and brain tissues of apparently healthy dogs slaughtered for human consumption within four LGAs of Abia State was established, If the result obtained from this study, reflects the true situation within the natural dog population in Abia State, It could be within epidemiological reasoning to assume that, yet undefined human population, who live in the same environment with such dogs are constantly exposed to rabies antigen within the dog-human interface with possible consequences. Unfortunately, the consequence of human exposure to such rabies antigenic challenges was not considered in this study. Similar studies conducted in Sokoto, Kastina and Borno States, in northern Nigeria, have established higher prevalence of rabies antigens in the brain tissues of apparently healthy dogs (Garba *et al*., 2008; Ajayi *et al*, 2010;Aliyu *et al*.,2010). The higher prevalence observed in the above states (28%-40%), when compared with 5% obtained in Abia State, could not be easily explained. However, in this study, dogs in the markets were sourced within the state, without external influence. It is evident that cross- border and inter-state dog trade is not yet prominent in Abia State, unlike

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northern dog market , which were found to originate from neighboring states and countries like Chad, Niger, Cameron and Benin (David *et al*., 2008; Garba *et al*.,2013). However, a 5% rate of carrier dogs obtained in this study is enough a problem capable of leading to a possible exposure potentials for all involved in selling, butchering, processing, consuming, and those with casual contact with such dogs. It is therefore, necessary to restrict the influx of dogs into Abia State, in order to keep the number of carrier dogs low for easy control and eradication of rabies.

The age, sex, breed, and location of dogs slaughtered during the course of the study were also considered. In this study, more females were slaughtered than males; this finding differs from the findings of Aliyu *et al*. (2010) and Ajayi *et al*. (2006) in Northern Nigeria. The reason for attributing more females may be due to the practice of using male dogs for burial ceremony in some parts of the state, which makes the prices of male dogs go higher than females and also scarce. There was no statistical association between sex and rabies status of the dogs sampled (> 0.05). All the dogs sampled were sourced from their neighborhood; this finding is in agreement with that of Garba *et al*. (2013); this is an indication that these dogs actually lived a normal life and continued to excrete the virus in their saliva without showing clinical signs. This form of rabies is the dangerous type as people are only aware of the violent form. All the positive case came from indigenous breeds, this can attributed the cost and interest as exotic breeds are expensive and most dog meat consumers believe their meat is not delicious when compared to the indigenous breeds.

Result obtained from this study have shown total agreement in the results of the entire tests used, although previous works have been carried out to evaluate dRIT with FAT under laboratory condition and dRIT with FAT, using post mortem samples. However, in this study we compared

two rapid tests with the gold standard test for rabies, under field condition using antemortem and post mortem samples.

The use of RICT and dRIT in diagnosis will reduce the number of human deaths due to rabies and financial loss incurred during PEP, because the results obtained is prompt and will determine if PEP is to be giving or not. Tests are simple to conduct under field condition, they do not require the use of specialized equipment, and dRIT can be performed on samples preserved under different conditions (Lembo *et al.*, 2006). However, RICT can be used on ante-mortem samples, this in turn save the lives of innocent dogs that are killed for post mortem diagnosis. Dogs serve various purposes in Nigeria, such as security, hunting, herding, in event such dogs are killed for rabies diagnosis and are found to be negative, the listed purposes are defeated and resulting in waste.

Additionally, the light microscope used in dRIT is cheaper compared to the costs of acquiring and maintaining a fluorescent microscope used in the FAT test; it shows dRIT is less expensive. It is therefore more suitable for use in low income countries like Nigeria, especially in rural areas where rabies diagnosis is not available. Both rapid test are cheap and can be use under field conditions.

In this study, one female was captured as a butcher and was pregnant at the time of sample collection, this finding is contrary to reports of earlier workers in some northern states of the country (Audu, 2011; Garba *et al.*, 2013), who recorded no female participation dog butchering. The presence of a female butcher in this study, can be attributed to the fact that the study was carried out in South- East Nigeria, where sex is not a barrier to business affiliation, as against their study in Northern Nigeria, where some business are strictly for male. The consequence of

this finding is that her bravery to restrain dogs could expose her and the unborn child to infective dog bite.

Majority of the butchers had only primary education (68.4%), 26.3% had no formal education, while, only (5.3%) had tertiary education. This low level of formal education may be responsible for the manner in which they handled the dogs brought for slaughter. Majority of the butchers (94%) consume dog meat themselves, because of the spiritual and medicinal purpose.

Despite the fact that majority of high risk group (butchers and consumers) had knowledge of rabies, none was previously vaccinated against rabies. When exposed to dog bite majority (72.8%) were treated by traditionalist because of the trust that had been established from generation to generation, which is rooted on spiritualism and myth of the people of the state. Even though the practice has no scientific proof, it seems reassuring to them, cheap and affordable and in consonance with the community belief and spiritualism. Traditional methods of treatment is seen as the best option for treatment for victims of dog bites, as they said it was more effective as against the orthodox treatment. Traditional healers claimed it was 100% effective, however it has not been established whether treatment for victims of dog bite commenced following onset of clinical signs, this could be attributed to the low level of education by the respondents. This finding agrees with the report of Ogunkoya (2010). Although some (27.8%) of the respondents reported to the hospital, but the first aid treatment given to them before reporting to the hospital could further complicate the situation, as some applied kerosene to the affected area and covered the wound with a piece cloth. This practice could be as a result of low level of education among respondents. Those seen to have recovered from the

wound sustained and living a normal life following the use of herbs and concoctions could have sustained bites from uninfected dogs, as not all dog bites result in infection.

Dog butchers brutally man-handle dogs without adequate protection against rabies and some even use their bare hands to try and clamp the dog's mouth shut as their killing it, which leaves them exposed to dog saliva (which may be infected with rabies virus). Almost all dog butchers have very low, if any, formal education and are oblivious to the seriousness of rabies. This practice is capable of increasing their chances of exposure and infection to rabies as accidental bite and salivary contamination of wound is very possible. Loses of lives through infection was not always properly documented in Nigeria, not to talk of those infected by apparently healthy dogs either through handling or consumption. However, related cases of fatality through apparently healthy dogs have been reported in Vietnam by Anh *et al*. (2012) in their report, five human rabies patients had no previous history of dog or cat bite but were involved in dog meat butchering. In this study knowledge of people who died from dog bites and some the butchers and consumers who died seemly of rabies but no investigation was carried out to ascertain the sources of infection, more work is required in this area.

The practice of handling dogs in a cruel manner before slaughtering and crowding animals in a small under size cages should be a challenge to the Veterinary profession and animal right groups, as it goes contrary to the Veterinary oath which is aimed at preventing animal suffering. Majority of the butchers had only primary education (68.4%), 26.3% had no formal education.

Majority of the dog meat consumer (77%) were males, this can be attributed to the fact that spots where dog meat are consumed are associated with alcohol consumption and some women cook the meat in their houses.

The result from this study revealed that almost all occupations were represented. Both civil servants and students consumed dog meat in the study area with students as the majority. Suggesting a wide acceptance of dog meat by all members of the community .This finding is contrary to the report of Garba *et al*. (2013) in Niger State. Who reported a higher dog meat consumption rate among civil servants than other occupations. There was high level of responses among Christians than traditional worshipers, this may be attributed to the fact that Abia State is a Christian dominated state and traditionalists are secretive, and will not divulge information freely.

Majority of the respondents killed and consumed rabid dog, In general, dog meat was more expensive compared to other meats sold. Because of its curative effect on malaria and high blood pressure and provides spiritual protection, and enhanced male libido. Results obtained from the interview with some users, it was apparent that users of parts from these clinically ill dogs, claimed it was effective for whatever it is used. For example, male organs of such dog will sale many folds than non-clinically ill dog. These believe of users and readiness to pay higher costs make clinically rabid dog to be sought for seriously. Butchers do all they could to restrain such dogs without precaution; this may further expose them to dog bite. There is potential for virus transfer if fluids or nervous tissues of the infected animals come into contact with breaks in the skin before the meat is cooked or if the fluid split to the eyes of other processors. A report from Vietnam revealed that five human rabies patients had no previous history of dog or cat bite but

were involved in dog meat butchering (Anh *et al.*, 2011). These further points to risk associated with dog meat slaughtering. Majority of the butchers had only primary education while only one had tertiary education. This can be attributed to the manner in which they handled the dogs brought for slaughter.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study has established a presence of rabies antigen in the saliva and brain tissues of 5% of apparently healthy dogs slaughtered for human consumption in Abia State, Nigeria.

2. There was high awareness of rabies infection among dog meat butchers (73.1%) and consumers (71.6%) in Abia State, although they were not aware of the dump form of rabies.

3. There was agreement between results of RICT, dRIT and FAT for all the samples tested.

6.2 Recommendations

1. Surveillance and control of dog rabies in Abia State be improved while vaccine for humans and animals be made more available.
2. Further studies on the rabies virus isolates obtained from Abia State should be carried out;
3. Use of RICT in Veterinary Clinic in cases of dog bite to relieve victims of psychological trauma and take appropriate action to save the lives dogs that are killed for FAT;
4. Use of dRIT for postmortem diagnosis of rabies where facilities for FAT are not readily available;
5. Evaluation of traditional methods of treatment employed by victims of dog bite.

6. Annual canine anti-rabies vaccination campaign should be carried out to reduce rabies endemicity in Abia State;
7. Government should assist in subsidizing the cost of acquiring canine rabies vaccine so as to help poor people vaccinate their dogs annually;
8. Human antirabies vaccine should be made available and affordable for use in health centers in Abia State; and
9. Further studies should be carried out to find out the consequence of exposure to rabies virus by butchers in Abia State.

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APPENDIX 1

DEPARTMENT OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

RESEARCH QUESTIONNAIRE

AWARENESS OF RABIES INFECTION AMONG DOG MEAT PROCESSORS IN ABIA STATE, NIGERIA

Kindly provide the answers to the questions below. All information provided will be used for academic purposes solely and will be treated with confidentiality.

S/No. ----------

Location----------

Part A (Dog meat processors).Please tick where appropriate

1. Age: <20years ☐ 20-40 ☐ 40-60 ☐ >60 ☐
2. Religion…….
3. Sex : Male☐ Female ☐
4. Educational level: None ☐ Primary☐ Secondary ☐ Tertiary ☐
5. How long have you been involved in dog meat business? <1year ☐ 1-5 years ☐

   5-10years ☐ 10-15years ☐ 15-20years ☐ >20years☐
6. Why are you in dog meat business?.....
7. Type of dog commonly slaughtered Local ☐ Not local ☐
8. Where do you source the dog you slaughter……
9. How much do you pay per dog…….
10. Do you eat the meat you process? Yes ☐ No ☐
11. If yes to question 7 above, How do you eat the meat? Raw ☐

    Cooked☐ Both raw and cooked ☐

Others: Specify----------

12. Do you know what rabies is? Yes ☐ No ☐

13. If yes to question 9 above, how is it contacted?----------

14. Have you been vaccinated against rabies before? Yes ☐ No ☐

15. If no to question 11 above, are you aware that vaccine exists for humans exposed to dog bite?

    Yes ☐ No ☐

16. Do you find out if a dog was vaccinated before slaughtering? Yes ☐ No ☐

17. Have you ever been bitten by a dog? Yes ☐ No ☐

18. If yes to question 14 above, where was the wound treated? Hospital ☐ Locally at ☐ home

    Others: Specify----------

19. What did you do to the would before reporting to the hospital

    ……..

18. Have you ever seen a mad dog before? Yes ☐ No ☐

19. If yes to question 16 above, what did you do on sighting a mad dog?

    Stoned it ☐ Ran away Killed it ☐

    Others: Specify----------

20.Do you know that a dog can be prevented from becoming mad if it is vaccinated against rabies?

    Yes ☐ No ☐

APPENDIX 2

AWARENESS OF RABIES INFECTION AMONG DOG MEAT CONSUMERS IN ABIA STATE, NIGERIA

Kindly provide the answers to the questions below. All information provided will be used for academic purposes solely and will be treated with confidentiality

Part B (Dog meat consumers).Please tick where appropriate

1. Age: <20years ☐ 20-40 ☐ 40-60☐ >60 ☐
2. Religion……
3. Sex : Male☐ Female ☐
4. Educational level: Non☐ Primary ☐ Secondary ☐ Tertiary ☐
5. Occupation: None ☐ Civil servant ☐ Military ☐ Farmer ☐

   Trader ☐

   Others: Specify----------
6. Do you eat dog meat? Yes ☐ No ☐
7. How long have you been involved in eating dog meat? <1year ☐ 1-5 years ☐

   5-10years ☐ 10-15years ☐ 15-20years ☐ >20years ☐
8. Why do you eat dog meat?.....
9. Preferred type of dog meat commonly eaten: Local ☐ Not local ☐
10. Preferred way of eating dog meat: Raw ☐

    Cooked ☐ Both raw and cooked ☐

    Others: Specify----------
11. Do you know what rabies is? Yes ☐ No ☐
12. If yes to question 10 above, how is it contacted?----------

13. Have you been vaccinated against rabies before? Yes ☐ No ☐

14. If No to question 12 above, are you aware that vaccine exists for humans exposed to dog bite?

Yes ☐ No ☐

15. Have you ever been bitten by a dog? Yes ☐ No ☐

16. If yes to question 14 above, where was the wound treated? Hospital ☐ Locally at ☐ home

Others: Specify----------

17. Do you know anyone who died of dog bite in your community? Yes ☐ no ☐

18. Have you ever seen a mad dog before? Yes ☐ No ☐

19. If yes to question 14 above, what did you do on sighting a mad dog?

Stoned it ☐ Ran away ☐ Killed it ☐

Others: Specify----------

20. Do you know that a dog can be prevented from becoming mad if it is vaccinated against rabies?

Yes ☐ No ☐

APPENDIX 3

Rapid Immunochromatographic Test Result

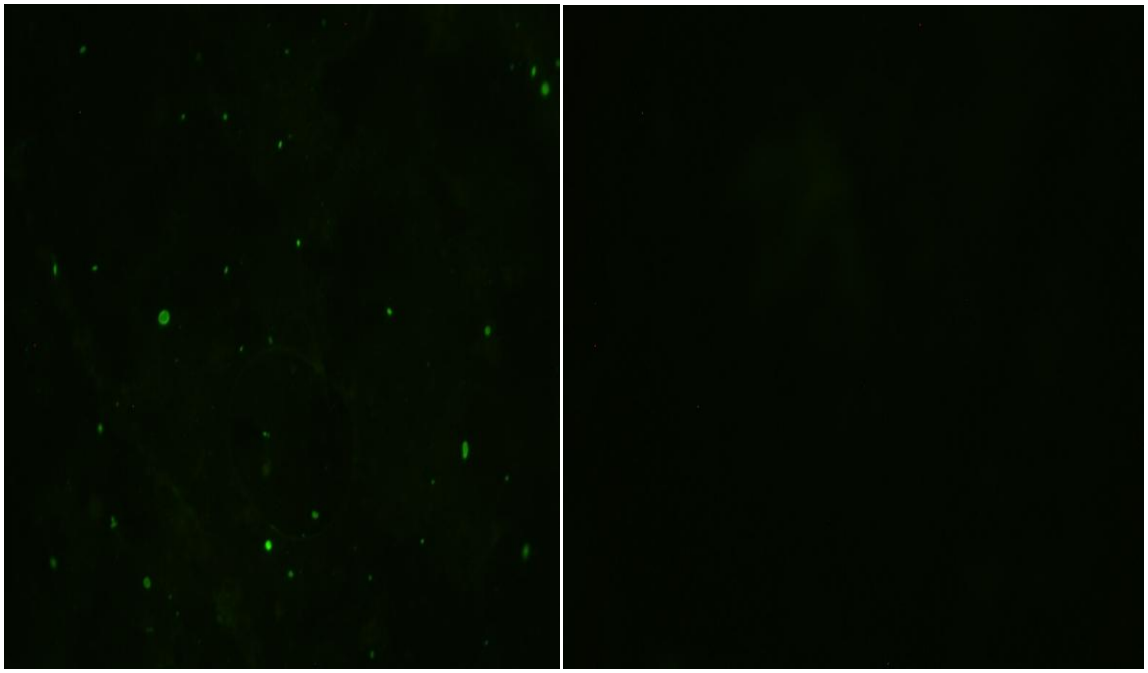
Positive **Negative**



APPENDIX 4

Results of Fluorescent Antibody Test

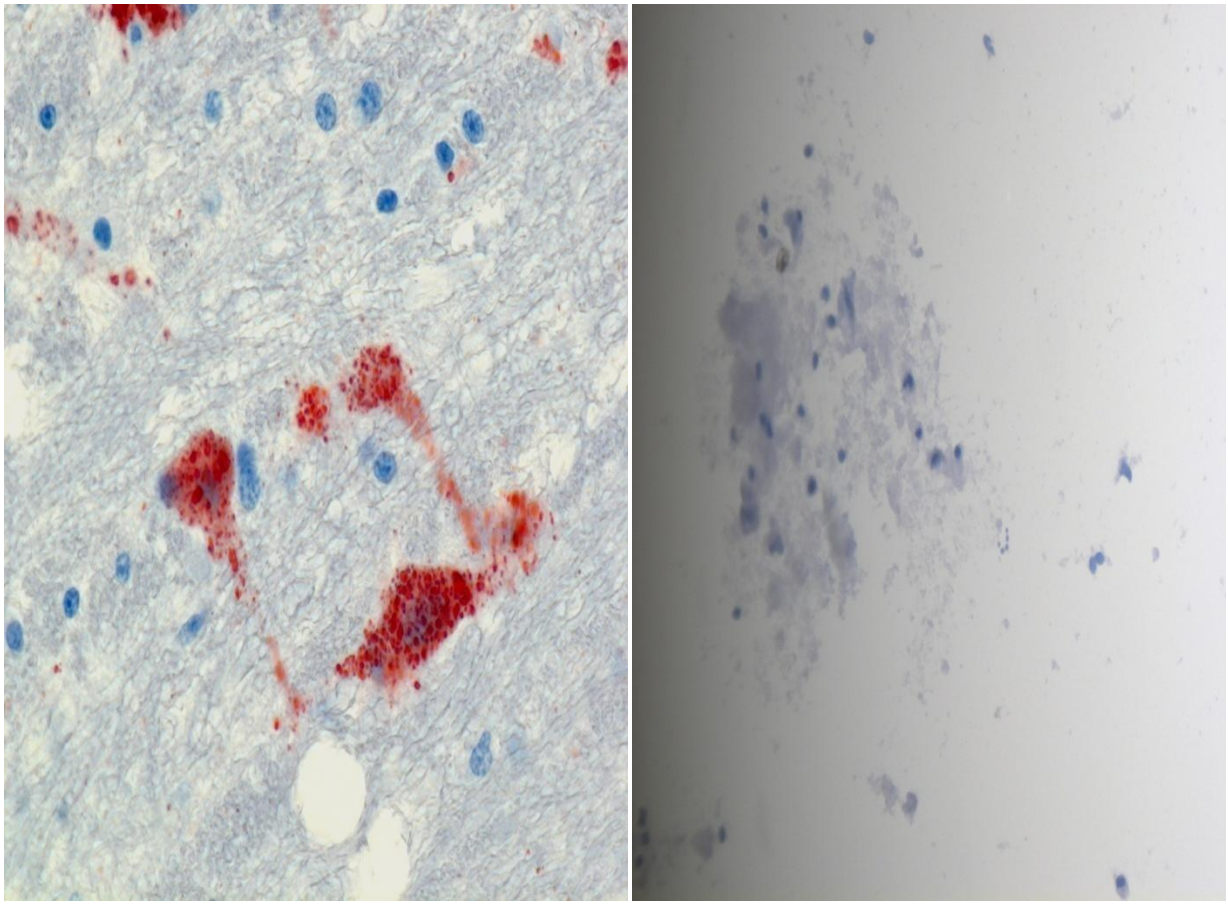
Positive **Negative**



APPENDEX 5

Direct Rapid Immununo-histochemistry Test, Results

Positive **Negaive**



APPENDIX 6

Traditional treatment, for victim of dog bite at Umeze, Abia State.



Site of dog bite



Traditional treatment

APPENDIX 7

Dog brought from the market, about to me slaughtered at Ndoro, Ekwuano.



APPENDIX 8

Dogs and goats displayed for sale at Ndoro Market, Abia State

