SERO-CONVERSION AMONG RABIES VACCINATED DOGS IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA

BY

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Attestation

I declare that the work in the dissertation entitled "SERO-CONVERSION AMONG RABIES VACCINATED DOGS IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA"**,** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, under the supervision of Prof J. Kabir and Dr. B. V. Maikai.

The information derived from the literature and the works of other investigators have been duly acknowledged in the text and a list of references provided. No part of this Dissertation has previously been submitted for another degree or diploma at any University.

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Certification

This is to certify that the work presented in this dissertation was carried out by Dr. Mustapha Tukur in the Department of Veterinary Public Health of Ahmadu Bello University, Zaria under our supervision and is approved for the award of the degree of Master of Public Health (Vet. Epidemiology) of the Ahmadu Bello University, Zaria.

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Dedication

I dedicate this work to God almighty, who created and sustained us out of his bounty, and with whom all things are possible.

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List of Abbreviations/Acronyms

ARV	Anti- Rabies Vaccine
BC	Before Christ
CCO	Cell Culture Origin
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
ELISA	Enzyme linked Immunosorbent Assay
iELISA	Indirect Enzyme linked Immunosorbent Assay
HEP	High Egg Passage
INF	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International Unit
LEP	Low Egg Passage
LGA	Local Government Area
MLV	Modified Live Virus
FELTP	Nigeria Field Epidemiology and Laboratory Training Programme
NVRI	National Veterinary Research Institute
NTO	Nervous Tissue Origin
OIE	Organization International des Epizootics
OR	Odds Ratio
ORV	Oral Rabies Vaccine
PCR	Polymerase Chain Reaction

RGP	Rabies Virus Glycoprotein
RNA	Ribonucleic Acid
TCO	Tissue Culture Origin
USA	United States of America
WHO	World Health Organization

Summary

Preventive vaccination against rabies virus is a highly effective means for rabies prevention but some animals do not reach the minimal prevention threshold level for neutralizing antibodies. To assess sero-conversion among dogs that were given rabies vaccination in Kaduna metropolis of Kaduna State Nigeria, a quantitative indirect enzyme–linked immunosorbent assay (i-ELISA) was used to detect rabies virus anti-glycoprotein antibodies in sera from 300 apparently healthy rabies vaccinated dogs at the four state veterinary clinics between March and May 2015. Of the 300 dogs, 276 (92.0%) consisting of 33 (11.9%) young dogs, 180 (65.2%) adult dogs and 63 (22.8%) old dogs were immune to rabies virus (antibody titre >0.5IU per ml), while 24 (8%) were not immune. The prevalence of rabies anti-glycoprotein antibody was higher with the adult dogs (96.8%) than among the old dogs (92.6%) or the younger dogs (71.7%). The prevalence was also higher among cross bred dogs (95.2%) than Exotics (91.0%) and Local (90.3%) breeds. Male dogs had a higher rabies anti-glycoprotein antibody, 173 (95.0%) than the female ones, 103 (87.3%). Local and foreign anti-rabies vaccines were used and both achieved success at stimulation of antibody production but the rate of antibody generation was slightly higher with the use of local (92.4%) than foreign (91.0%). At bivariate level, age and sex of the dog were significant factors associated with rabies anti-glycoprotein stimulation while breed and vaccine type were not. On multivariate logistic regression, age remained independent predictor of sero-conversion in rabies vaccinated dogs. The result in this study has shown that there is high anti-rabies immunity among dogs vaccinated against rabies virus at the state veterinary clinics in Kaduna. Hence, there is the need to ensure sustenance of the annual rabies vaccination campaign in the state and the

country at large in order to achieve the desired 80% immunity required to guide against the occurrence of an outbreak.

Keywords: Dogs, Antibody, Immunity, Kaduna, Rabies, Vaccination, ELISA, Serum.

CHAPTER 1

1.0. INTRODUCTION

1.1 Background of the Study

Rabies virus causes acute encephalitis in all warm-blooded hosts, including humans and the outcome is almost always fatal. Rabies with a timeline history of over 2300 years BC is 100% preventable, yet over 55,000 people are reported dead annually due to rabies [1]. Most of the deaths are in developing countries, India and Africa. The disease is worldwide in distribution except for a few island countries and areas that have historically been free of the disease. Such areas are Britain, Ireland, New Zealand, Japan, Taiwan, Pacific Island and Hawaii, the continent of Australia and Antarctica [2]. Nearly all rabies free countries are surrounded by a large body of water, which provides natural protection[3].

In Nigeria, all published reports have incriminated dogs as the principal reservoir and the transmitter host for rabies to other animals and human[4,5,6]. The first officially documented report of human rabies in Nigeria was in 1912 and that of canine rabies was diagnosed in 1925 at Yaba Rabies Laboratory[4]. Reliable data on rabies are scarce in many parts of the world, making it difficult to assess its full impact on human and animal health[7].

Preventive vaccination against rabies virus is a highly effective method for preventing rabies in humans and animals[8]. The minimal threshold level for neutralizing antibodies following rabies vaccination accepted by WHO/OIE is 0.5 IU/ml[9]. Despite proper vaccination some animals do not reach the threshold[8]. A study in 1982 reported cases of canine rabies in vaccinated dogs in Nigeria from 1970-1980, 14 cases of rabies in vaccinated dogs were reported in various parts of Nigeria[10]. Available records indicate that the rabies diagnostic laboratory unit of the National Veterinary

Research Institute (NVRI) Vom, Nigeria have diagnosed a total of 3770 cases of rabies in dogs, cats and other animals between 1928 and 1990[10] . Of this number, 3555 (94.3%) were in dogs, 114 (3%) in cats, while 101(2.7%) were in other domestic and wild animals.

It is estimated that there are about 4.5 million dogs in Nigeria. Controlling rabies in dogs through vaccination reduces the risk of human exposure. Dogs are vaccinated at 3 months of age. Puppies less than 3 months are generally excluded from rabies vaccination programmes on the assumption that they have immature immune systems and maternal antibodies [11].

The aim of vaccination is to reduce the population of susceptible animals. If about 70% of the dogs in the population are immunized, outbreaks of rabies will be prevented[12]. Vaccination has recently been used to reduce the incidence of rabies by 97% in Tanzania.

1.2 Statement of Research Problem

Rabies is an encephalomyelitis which is almost always fatal with an underestimation of about 60.000 human deaths each year [13]. Humans are mostly contaminated after being bitten by rabid animals among which, the dog is the most important vector and reservoir of the disease. Rabies remains a disease of public health concern in most developing countries, especially in the Indian subcontinent, Africa and Latin America. This situation has led the WHO to initiate a number of programmes aimed at the control of canine rabies by mass vaccination. These programmes have resulted in the control of enzootic dog rabies in many urban areas but have never been effective in eliminating the disease in the majority of these countries[14, 15].

Pasteur's development of a successful vaccine treatment for patients exposed to rabies was among the first grand successes of the Germ theory of Diseases and this success has been celebrated widely in literature and films[16]. Despite the development of even safer and more successful treatment, the availability of better and more effective vaccines, rabies still remain the most important and devastating viral zoonotic disease worldwide.

Cases of rabies in vaccinated dogs have been reported for lower egg passage (LEP) Flury strain vaccine in many countries including the USA[17], France[18], Kenya[19] and Nigeria[20, 21].

Several studies based on the evaluation of rabies antibody responses after mass vaccination of dogs yielded contradictory results. In Peru, 12 months after mass vaccination under field conditions with inactivated cell cultured-derived rabies vaccine, 97% of dogs had rabies neutralizing antibody titres of >0.5 IU/ml [22] . While it was70% in Thailand[23] and 73% in Tunisia at 4 weeks post-vaccination at the peak of the immune responses[24].

1.3 Justification

Canine rabies is endemic and occurs throughout the year in all parts of Nigeria; with dog forming the major source of infection to man, and about 10,000 persons exposed to rabies annually in the country[25]. Vaccination against rabies has been shown to be an efficient way for its control[26] and statistics have shown that successful immunization of 70% - 80% of dog population in country places rabies under control[12]

While a significant reduction in human rabies associated with domestic dog rabies has been achieved in Mexico, South America and Caribbean over the last two decades, dog-associated human rabies has increased in parts of sub-Saharan Africa in the same period[27]

There have been several reports of rabies in Nigeria occurring in dogs vaccinated with LEP flurry-strain anti-rabies vaccine[28, 29] but dog vaccination remains the most important means of controlling rabies in the country.

Reliable data on rabies are scarce in many parts of the world, making it difficult to assess its full impact on humans and animals[7]

Rabies outbreaks have been reported with increasing frequency in Kaduna State. In 2013, residents of the Nigeria Field Epidemiology and Laboratory Training Program (NFELTP) went to the state to investigate reported rabies outbreak involving people and livestock earlier bitten by a stray dog.

Although many studies have been conducted on rabies in Nigeria, information on rabies anti-glycoprotein antibody levels (herd immunity) among vaccinated dogs in Kaduna metropolis is not available in the literature. This limits informed public health decision making on rabies control for the city.

1.4 Research Questions

1. What proportion of dogs becomes protected against rabies following vaccination in Kaduna metropolis?

2. What are the types of rabies vaccine used for dog vaccination in Kaduna metropolis?

3. What are some of the factors related to dog, or vaccine administration that are associated with sero-conversion following rabies vaccination?

1.5 Aim

To determine the sero-conversion rate and associated factors among dogs vaccinated against rabies in Kaduna metropolis.

1.6 Objectives

The objectives of the study were to:

1. estimate the proportion of dogs vaccinated against rabies that sero-converted.
2. determine the types of vaccine used for rabies vaccination in Kaduna metropolis.
3. assess factors that are associated with sero-conversion following rabies vaccination in dogs.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Sero-conversion

Sero-conversion refers to the development of detectable specific antibodies in the serum as a result of infection or immunization[30].

B-cells contribute to adaptive immunity by secreting antibodies, and the response of B-cells to an antigen is usually measured by analyzing the specific antibody produced in a humoral immune response. This is most conveniently achieved by assaying the circulating antibody that accumulates in the blood[31].

2.2 Factors affecting sero-conversion

2.2.1 *Influence of vaccine types and potency*

As far as inactivated virus vaccines are concerned, besides individual variation, the level of neutralizing antibodies in serum correlates positively with the antigenic value of the vaccine[32]. The influence of the antigenic value of the vaccine on the level of neutralizing antibodies has been demonstrated in domestic population of owned dogs in Switzerland[33]. Where it showed that in owned dogs, higher titres were generally obtained with inactivated vaccines than with live (and less potent) vaccines.

2.2.2 *Influence of the route of vaccination*

Since Pasteur, the route of vaccination has been subcutaneous. Fuenzalida in 1967 demonstrated that intramuscular route resulted in higher neutralizing antibody titers in dogs[34].

However, the advantage of the intramuscular route diminishes with high potency vaccines[32] and the use of adjuvant confers a longer lasting immunity, which can be obtained with smaller quantity of antigen[35]. Despite the use of smaller quantities of antigen and a reduced vaccination schedule (less frequent boosters), the neutralizing antibody levels reached after one, two or three years with adjuvant vaccines are equivalent to those reached with non-adjuvant vaccines given according to the small schedule (two injections of vaccine the first year, with animal booster).

2.2.3 *Influence of age*

The relationship between the age of animals and protection from challenge was confirmed in a laboratory study in three to five months old puppies. Three months after vaccination with flury LEP vaccine, ten of forty puppies had antibody titres below 1/5[36] . A survey in France showed that beyond three months of age, older dogs produced higher antibody titre following vaccination[37].

2.2.3 *Influence of health and breeding status of dogs*

A study was conducted in France to compare the proportion of individuals developing neutralizing antibodies in 64 dogs after administration of adjuvanted and non-adjuvanted vaccines[38]. This rate may vary considerably depending on the category of dogs (bred for laboratories, belonging to individuals in France or uncontrolled in Tunisia). The rate drops from 100% to 59% in the case of semi- stray dogs as compared to laboratory dogs. Urban dogs exhibited better rates than the uncontrolled dogs in Tunisia[39].

Pet dogs which had received one subcutaneous dose of rabies vaccine exhibited a better neutralizing antibody response when they did not suffer from aneamia. In 440 pets under quarantine in Hawaii, Sasaki and colleagues demonstrated that those with internal parasites had significantly lower levels of neutralizing antibodies than those without parasites[40].

2.3 Rabies vaccines

There have been many types of anti-rabies vaccines produced since the original vaccine was developed from the spinal cord of experimentally infected rabbits by Pasteur[41]. The first vaccine for dogs was developed by Umeno and Doi in 1921 using phenolised rabbit brain.

Koprowski and Cox in 1948 developed a modified live rabies vaccine of chick embryo origin and such vaccines were shown to produce immunity for at least 3 years[42].

Rabies vaccines for dogs have cycled from inactivated nervous tissue origin (NTO) vaccines to modified live virus (MLV) vaccines and now back to inactivated vaccines but primarily of tissue culture origin (TCO) [41].

The vaccines that are available today for use in dogs can be divided into 3 groups: modified live virus (MLV) vaccine, the inactivated nervous tissue origin (NTO) vaccine and the inactivated cell culture origin (CCO) vaccines. The availability of vaccines in each group varies from one country to another depending on whether the vaccines are produced locally or imported[41]. A total of 108,360,216 animal vaccines were produced from 1986 to 1989 in countries that engaged in domestic vaccine production for animal use. The immunogenicity of modified life virus vaccine is enhanced if the vaccines are given intramuscularly instead of subcutaneously[43] but for safety reasons the site should be confined to muscles of the thigh.

Cases of rabies in vaccinated dogs have been reported for low egg passage (LEP) flurry strain vaccine in many countries. A possibility exists that the vaccine failure in Nigeria could be due to rabies - related viruses[44].

Since routine rabies vaccines does not protect against rabies related viruses[45], their existence in the canine population in Nigeria, Zimbabwe , South Africa and other African countries could hamper the rabies control effort. A vaccination failure rate of 2.5 per 10,000 doses of anti rabies vaccine (ARV) has been reported in the former Anambra state, Nigeria[21].

Since 1972, WHO has been coordinating research on the immunization of wildlife against rabies for resent years, attempt have been made to reduce the dog and wildlife population in the hope that this would break the chain of natural transmission of rabies but experience have shown that this does not work. Even the killing of animals on the scale required to control rabies is no longer tolerable in modern society hence the only technique available today for control of rabies in those animals that remain in physical reach is oral vaccination (ORV) [46].

Immunization with inactivated vaccine by oral or enteric route has so far not given applicable result but trials with oral and enteric live virus vaccines have shown that a number of strains are highly effective when applied directly on the tongue and buccal mucosa or when ingested with appropriate bait.

2.4 Rabies antigenic immune response

Antigenic stimulation caused by diseases of bacterial, viral or even protozoan origin, or the immunization induced by appropriate vaccines, are both associated with increased immunoglobulin synthesis. In infectious diseases, the classes of immunoglobulin G and M (IgG and IgM) respectively are known to predominate. After primary stimulation, the first serum antibody detected is IgM, which is gradually replaced in succeeding weeks by IgG. In contrast, the immediate response to secondary antigenic stimulation is predominantly production of IgG.

However, the sequence of immunoglobulin response can be altered by the nature and dosage of antigen, the use of adjuvant, and the route and timing of administration.

2.5 Rabies

The name rabies is derived from the Latin word 'rabere', meaning 'rage'[47]. It is the oldest infectious disease known to medical sciences, associated with animal bites and dated back as far as three centuries ago[47]. Rabies is a fatal zoonotic viral infection of all mammals caused by the rabies virus.

This neuropathic virus, one of the first ever recognized zoonotic diseases causes acute, progressive viral encephalomyelitis[48]. Genomic sequencing of the virus has established two major clades, one comprising those isolated from terrestrial animals around the world and the other containing viruses isolated from bats and raccoons in the Americas[49].

Domestic dog accounts for more than 95% of human cases of rabies[50] and is an occupational health hazard in veterinary medicine[51]. Transmission is by viral laden saliva, usually by biting, with incubation period of 3-6 weeks, although longer incubation period have been reported (up to

25 years in humans)[52]. The virus reaches the brain via peripheral nerves, but also infects the salivary glands. Hematogenous spread does not occur.

Clinical signs are variable, but consistent findings are behavior changes and progressive paralysis. The behavioral changes are related to progressive neuropathy, particularly the region responsible for emotional control. Dogs may become aggressive ('rabid form'), antisocial, hyper-excitable, fearful (appendix 1), or mentally depressed and wander aimlessly ('dumb form') eventually collapse and respiratory paralysis occur, leading to death (appendix 2).

Once symptoms appear, rabies is almost inevitably fatal[53].

2.6 Rabies virus

The rabies virus belongs to the family Rhabdoviridae and genus lyssavirus. It is a non segmented, single stranded negative sensed RNA virus. It has only one genotype, and was the first of the seven Lyssavirus genotypes to be identified. There are other six rabies related genotypes and they include Aravan virus, Australian bat lyssavirus, Duvenhage virus, European bat lyssavirus 2, Irkut virus, Khujant virus, Lagos bat virus, Mokola virus, Kotokon and West Caucasian bat virus[54, 55].

Rhabdoviruses are rod shaped as the name implies and have one end that is rounded and the other flattened and is often referred to as bullet shaped (figure 1). They have an envelope derived from the host plasma cell membrane. The rabies virus has singled strand 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[56]. The virus has five proteins[55]. These are given below:

2.6.1 *Glycoprotein (G) proteins*

Rabies virus glycoprotein (RGP) is the only surface exposed viral protein, it assembles in trimmers[57] in the endoplasmic reticulum[48] and its ectodormain then protrudes from the lipid envelop. The RGP is viral attachment protein responsible for host cell receptor recognition and for low pH induced fusion of the viral envelope with endosomal membranes[59, 60]. It is also the primary target of the host hormonal and cellular immune responses[61]. 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 interaction of RGP with its target membrane during fusion, and the fusion inactive conformation[62].

2.6.2 *Matrix (M) protein*

Is a peripheral membrane protein that lines the inner surface of the viral membrane (figure 2). Though this remains controversial, it may serve as a bridge between the other surface (G) protein and nucleocapsid[56]. The nucleocapsid is the infectious ribonucleoprotein core of the virus. It is a helical structure that lies within the membrane and has a striated appearance[55, 63].

2.6.3 *Nucleoprotein (N) proteins*

This is the major structural protein and covers the RNA genome. It protects the genome from nucleases and holds it in a conformation that allows transcription[55].

2.6.4 *Large (L) Proteins*

Together with the Phosphoproteins form the RNA dependent RNA polymerase and transcriptase. It has a molecular weight of about 240 kilo Daltons and its gene is up to 60% of the genome[55].

2.6.5 *Phosprotein (P) proteins.*

This is an interferon (IFN) antagonist counteracting transcriptional activation of type 1 IFN[64].

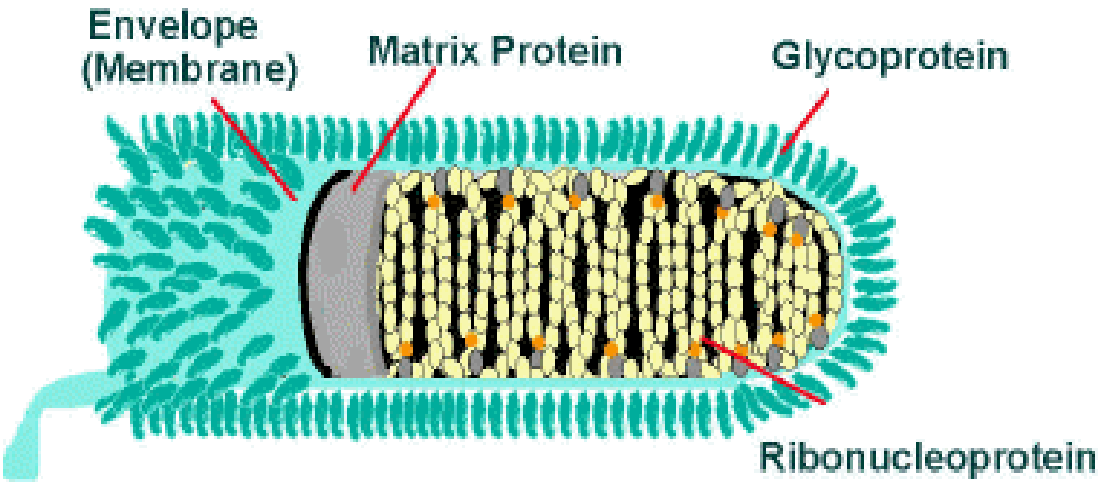

Figure 1: Diagram of the section of a rabies virion

Source: CDC_INFO

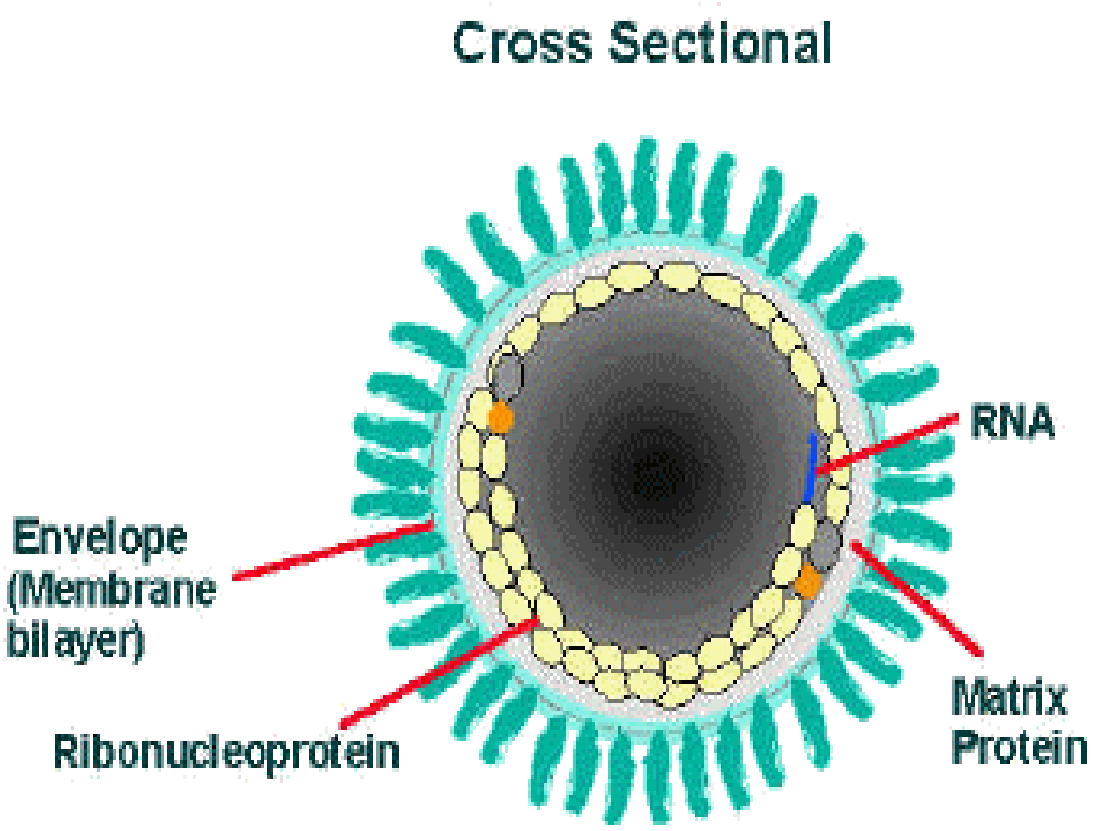

Figure 2: Cross-sectional diagram of the rabies virion

Source: CDC_INFO

2.7 Transmission

The virus is transmitted mostly through contact with saliva of infected animals[63.] Most mammals infected by the virus can transmit the disease to humans e.g infected dogs, cats, bats, foxes, skunks, raccoons[63].

2.7.1 *Transmission through bite*

Bites by rabid animals generally inoculate virus-laden saliva through the skin into muscle and subcutaneous tissues[55, 63, 65, 66]. In most cases, the biting animal is uncharacteristic in behavior and attacks without provocation or runs into seclusion[67].

Having gained access to peripheral nerves, it travels in a retrograde direction within the axoplasm. When the virus reaches the central nervous system, there is massive replication on membranes within neurons. Direct transmission of virus occurs from cell to cell across synaptic junctions[63]. At the onset of illness when evidence of neuronal dysfunction appears, there is little or no apparent histopathological change[63, 67].

2.7.2 *Transmission through aerosols*

Transmission in this case via inhalation of contaminated air and infection is through a break in the mucus membrane of the respiratory system. Transmission in this form is common in people that explore caves populated by bats[55].

2.7.3 *Transmission through organ transplants*

Transplant surgery has served as a major source of infection in humans. Cases of patients coming down with rabies following organ transplant have been reported in countries like India, Iran, Thailand, United States of America and France[68, 69]. Organs like Kidneys, liver and cornea

transplanted form infected donors that died of unidentified neurological disorders served as source of infection to recipients[70]. Contamination of wound by saliva or secretion of rabid dog can be source of infection.

2.8 Epidemiology of rabies

Rabies is a fatal zoonosis characterized by encephalomyelitis. It is a disease of public health concern because the human fatality is almost 100% [6]. The disease exists in two epidemiological patterns; urban rabies involving dogs and cats and sylvatic rabies involving wild carnivores and bats[71]. Sylvatic rabies occurs mostly in developed countries like in North America where canine rabies has been eradicated[72]. Urban rabies occurs mostly in less developed countries where the dog is the principal host and vector giving rise to a greater opportunity for spillover infections into human populations. This pattern of rabies is by far more dangerous to humans accounting for an estimated 99% of all recorded human cases and 92% of all human post exposure related treatments[73].

The epidemiology of rabies in Nigeria can be compared to that of many developing countries where the urban rabies rather than the sylvatic rabies predominates [74, 75, 76]. The disease has been reported from all parts of the country by different researchers[4, 10, 77], but current status is not known because of poor surveillance and reporting system[75].

2.9 Diagnosis of rabies

Diagnosis can be determined by rapid neutralizing antibody detection test[78], PCR assay testing of oral or hair swabs[79] and formalin-fixed tissue[80] but definite confirmation requires histopathological confirmation of characteristic brain changes using histopathology and immunohistochemistry by a government reference laboratory such as the National Veterinary Research Institute in Vom, Nigeria.

Samples of cerebellum and medulla oblongata are required from necropsied dogs for the confirmatory testing.

2.10 Rabies control

Control of rabies should mostly be directed at the animals that serve both as the vector and transmitters of the infection. Animal rabies control consists of the vaccination of dogs and cats, the elimination of stray animals, health education for the public, etc. In some countries, mass vaccination of dogs is not implemented, and the effective coverage rate is not exactly known. The elimination of stray dogs and other animals by shooting and poisoning is still in certain countries; however, this has a minimal effect on rabies transmission[81].

Rabies has survived over 50 years inspite of concerted effort to control it[82]. The control and prevention strategies of rabies in human beings, livestock other non reservoir (dead-end) hosts are based on breaking the cycle of transmission in reservoir hosts and secondly on protection of these alternate hosts from exposure or infection[83].

According to World Health Organization, control measures should be applied to canine rabies irrespective of the presence or absence of rabies reservoir in wildlife[84]. The basic principle for the control of animal rabies as recommended by the World Health Organization Expert Committee on

rabies include, elimination of stray dogs, canine vaccination and control of wildlife[84]. In addition, the committee recommended the following control measures in areas affected with rabies:

1. Registration, licensing and taxation of dogs
2. Elimination of stray dogs
3. Restraining dogs while the control campaign is under way
4. Vaccination of dogs free of charge
5. Provision of adequate diagnostic facilities
6. Reduction of wildlife species where these are a reservoir of the disease
7. Continual and energetic publicity campaign

However, it is very difficult to implement these basic principles and specific measures in most countries and these are largely responsible for the continued occurrence of rabies in these countries[85].

2.6.1 Control of rabies in Nigeria

Rabies is a notifiable disease, and strict quarantine protocols are required for monitoring at-risk animals. In most countries including Nigeria, if rabies is suspected especially in dogs, wide spread culling is required of animals in the vicinity to minimize spread of the disease. Control measures for canine rabies include vaccination and reducing population density through culling or sterilization. Despite the evidence that culling fails to control canine rabies, efforts to reduce canine population density as a measure for rabies control continue in many parts of the world[86].

Attempts to control canine rabies in Nigeria have been directed towards controlling the disease in dogs and cats[87]; by stray dogs control and by vaccination with modified live virus (MLV) chick

embryo origin vaccine (LEP and HEP) The flury strain of the vaccine is manufactured domestically by the National Veterinary Research Institute (NVRI) Vom.

CHAPTER 3

3.0 METHODOLOGY

3.1 The study area

The study was carried out in Kaduna metropolis, which occupies an area of approximately 3,080 km^2 and has a population of 760,084 [88]. Kaduna lies on the intersection of latitude 10^o 31" N and longitude 7^o 26" E. It is the capital city of Kaduna state (appendix 3 and 4), located in North Western Nigeria on the Kaduna River. It is a trade center and a major transportation hub for the surrounding agricultural areas with its rail and road junctions. The state shares boundary with Katsina and Zamfara states in the north, Plateau and Bauchi states in the east, Nassarawa state and the federal capital territory in the south, Niger state in the west and Kano state in the north east. The state covers an area of 45,567 km^2, it comprises of 23 Local Government Areas (LGAs) and 3 senatorial districts. The area enjoys a tropical climate, falling within guinea savannah vegetation zone. Dog keeping is popular in the area, and dogs are mainly kept as pet or for security.

3.2 Study design

Study design is a cross sectional study using semi-structured questionnaire and indirect ELISA for serological analysis.

3.3 Study population

The study population comprises of dogs in Kaduna metropolis that were vaccinated against rabies at the Government veterinary clinics in the state capital.

Inclusion criteria: Dogs that were 4 months old or above, vaccinated against rabies within the last 1 to 11 months and recorded in the state veterinary clinic immunization register.

Exclusion criteria: Apparently sick dogs.

3.4 Sample size

Sample size was calculated using the formula:

$n = z^2pq/d^2$

Where:

n = sample size

p = prevalence (using 92% from a study) [89]

q = complementary probability (1 - p)

z = desired confidence 1.96 (95% Confidence level)

d = allowed error 5%

This implies:

n=?

p= 92% (0.92)

q= 1-p (1- 0.92) = 0.08

z= 1.96

d= 5% CI = 0.05

n= 3.8416 x 0.92 x 0.0.08/0.0025 = 113 samples + 10% = 125 (Minimum Sample Size).

3.5 Sampling method

Using simple random sampling, 300 apparently healthy vaccinated dogs of both sexes (males and females) were selected for this study from the vaccination registers of the 4 government veterinary clinics in Kaduna metropolis. Addresses of all the owners of the selected dogs were obtained from the register and used to trace the dogs and the owners. After obtaining consent from the owners, dogs were examined to clinically ascertain their health status and then questionnaire were administered to the dog owners to obtain information on demographic characteristics and dogs' vaccination record. Those interviewed were asked to produce vaccination certificates issued at the clinics as proof of vaccination.

With the aid of sterile needles and syringes, 2.5 ml of blood was collected from each dog, through the cephalic vein into plain sample bottles (without anticoagulants) and allowed to clot. Sera were obtained by centrifugation at 3000 rpm and these samples collected were temporarily stored at -4^0C until tests were conducted. The sera were collected between March and April 2015, while serological analysis was carried out on the 14th of May 2015.

A participant information leaflet (appendix 5) and a consent form (appendix 6) were given to each participant before embarking on the study and filling of the questionnaire (appendix 7) and blood (sera) sample collection.

3.6 Detection of rabies anti-glycoprotein antibodies

A quantitative indirect ELISA (i-ELISA), using the platelia™ Rabies II kit (BioRad, Mames-la-coqnette) [80] was used to detect rabies anti glycoprotein antibodies. The kit included a microplate that was pre-coated with rabies glycoprotein extracted from the inactivated and purified virus membrane. Steps was followed in accordance with the instruction of the manufacturer[80], and

results was read using an ELISA reader (IRE 96, Saint Jean d'Illac) at a wave length of 450-620mm. Dogs were considered to be immunized against rabies virus infection if they produced ELISA titres of ≥ 0.5 IU/ml[9].

3.7 Data Analysis

The data from the questionnaires were entered into Microsoft excel spread sheet and analyzed for variables using EpiCalc 2000, version 1.02. The function calculates the odds ratio with a 95% confidence interval. Proportion of dogs that sero-convert was calculated by dividing the number of tested positive samples by the total number of samples subjected to the test, expressed as percentage. This was also done for different variables such as types of vaccine, dogs' breed, age and sex.

CHAPTER 4

4.0 Results

All the dogs owners contacted for the study consented, giving a response rate of 100%. The respondents are made up of 215 (94.7%) male and 12 (5.3%) female. Their age ranges are; 13 (5.7%) are under 20 years old, 129 (56.8%) are between 20 to 39 years old, 52 (22.9%) are 40 to 59 years, 25 (11.0%) are 60 to 79 years old and 6 (2.6%) are 80 years old and above (Table 4.1).

A total of 300 dogs were sampled from 227 households. Of this figure, 182 (60.7%) were males and 118 (39.3%) were females. There were 145 (48.3%) exotic breeds comprising mainly Alsatians, Rottweiler, Doberman, Caucasians and Mastiffs; Local and Cross breeds were 72 (24.0%) and 83 (27.7%) respectively. All the dogs were divided into three different age groups: young (<1 year), adult (1-7 years) and old (>7 years) of which there were 46 (15.3%) young, 186 (62.0%) adults and 68 (22.7%) old dogs (Table 4.2).

Table 4.1: Socio-demographic data of dog owners interviewed in Kaduna metropolis, Nigeria

Age group	Male	Female	Total
< 20 years	13 (5.7%)	0 (0%)	13 (5.7%)
20 – 39 years	122 (53.7%)	7 (3.1%)	129 (56.8%)
40– 59 years	50 (22.0%)	2 (0.9%)	52 (22.9%)
60 – 79 years	25 (11.0%)	2 (0.9%)	27 (11.9%)
≥ 80 years	5 (2.2%)	1 (0.4%)	6 (2.6%)
Total	215 (94.7%)	12 (5.3%)	227 (100%)

Table 4.2: Demographic data of vaccinated dogs sampled in Kaduna metropolis, Nigeria.

Age groups	Local breed (%)	Cross breed (%)	Exotic breed (%)	Total
<1 year (young)	8 (2.6)	12 (4.0)	26 (8.6)	46 (15.3)
1-7 years (adult)	41 (13.6)	61 (20.3)	84 (28.0)	186 (62.0)
>7 years (old)	23 (7.6)	10 (3.3)	35 (11.6)	68 (22.7)
Total	72 (24.0)	83 (27.7)	145 (48.3)	300 (100)

Table 4.3 shows serum rabies anti-glycoprotein antibody levels equal to or greater than 0.5 IU/ml with respect to age, breed and sex of the dogs sampled, as well as type of rabies vaccine used. Out of the 300 sera samples examined, 276 (92.0%) have achieved the minimal threshold level.

The highest antibody level (96%) was seen among the adult dogs of 1 – 7 years, followed by those categorized as old (> 7years) with 92% and the least was in the young dogs (< 1 year) with 75%. There was statistical significance among the old dogs and antibody level (OR= 4.9636; 95% CI on OR: 1.6291 < OR < 15.1233). There was also statistical significance among antibody titre and the adult dogs (OR= 11.8182; 95% CI on OR: 4.1935 < OR < 33.3065).

All the 3 breeds' categories: Local, cross and exotic, shows a significantly high rate of sero-conversion. The cross breeds have a higher rate of about 95.2% with OR= 2.1269, but not statistically significant (95% CI on OR = 0.5964 < OR < 7.5857). While the exotic breeds have 91.0% rate with OR equals 1.0937 (95% CI on OR = 0.4163 < OR < 2.8721). The local breeds have a lower sero-conversion rate of about 90%.

The male dogs appear to have a slightly higher sero-conversion rate of 95.0% than the 87% by the female dogs. OR= 2.7994 but not statistically significant (95% CI on OR = 1.1826 < OR < 6.6263).

On anti-rabies vaccine, both local and foreign vaccines show high sero-conversion rates of 92.4% and 91.0% respectively with the local vaccine having sero-conversion OR of 1.2250 and 95% CI on OR = 0.5042< OR < 2.9760, also not statistically significant.

Multivariate analysis shows adult dogs (1 – 7 years old) to have adjusted OR of 3.8028 and P-value of 0.0321 which is statistically significant.

Table 4.3: Rabies antibody titres among vaccinated dogs in Kaduna metropolis, Nigeria

Variables	Number examined	Titre >0.5IU/ml (%)	Crude OR	95% CI on OR	p value
Age Group					
Young	46	33 (71.7%)	(Ref. group)		
Adult	186	180 (96.8%)	11.8182	4.1935-33.3065	<0.001
Old	68	63 (92.6%)	4.9636	1.6291-15.1233	0.0026
Breed					
Local	72	65 (90.3%)	(Ref. group)		
Cross	83	79 (95.2%)	2.1269	0.5964-7.5857	0.2357
Exotic	145	132 (91.0%)	1.0935	0.4163-2.8721	0.8560
Sex					
Female	118	103 (87.3%)	(Ref. group)		
Male	182	173 (95.0%)	2.7994	1.1826-6.6263	0.0154
Vaccine Type					
Foreign	88	80 (71.7%)	(Ref. group)		
Local	212	196 (92.4%)	1.2250	0.5042-2.9760	0.6536

CI: Confidence Interval, OR: Odd Ratio

Table 4.4: Multivariate Logistic Regression Analysis

Variables	Crude OR	Adjusted OR (95% CI)	P. Values
Age group			
Young	Reference		
Adult	4.1935	3.8028	0.0321
Old	1.6291	1.4785	0. 0473
Sex			
Male	1.1826	1.0977	0.0554
Female	Reference		

CHAPTER 5

5.0 Discussion

The most convenient method to assess the immune status induced by vaccination is to assay rabies antibodies. This study provides the rabies antibody profile of dogs vaccinated in a metropolitan city of Nigeria using ELISA technique which was considered to be a suitable alternative to the neutralization test [90].

The study demonstrates that the vast majority of dogs vaccinated against rabies in the State veterinary clinics sero-converted. The analysis raises a number of important issues relating to canine rabies vaccination. Proportion of dogs that achieved sero-conversion rate of greater or equal to 0.5IU/ml is higher than the rate reported in the study conducted in Zaria[91]. This is presumably due the fact that most of the dogs sampled in the study were given a booster dose during the state government organized rabies vaccination campaign less than 12 months and in some cases, even not more than 6 months after their last vaccination and also, the study in Zaria was not a clinic based study. The curve of neutralizing antibodies follows the pattern generally observed with other antigens: Seroconversion and rapid rise of the level of neutralizing antibodies after first vaccination, followed by a slow decrease, a new rise after a booster to reach a higher level than previously observed, then a decrease leading to a stabilized higher level. The proportion is also higher than the 73% found from the study in Tunisia[24]. The rate is again much higher than the 58% found in a study conducted in Bolivia[92]. It is however lower than the 93% and 97% found in a study in Thailand and Peru respectively [22]. Data from Thailand show that the neutralizing antibody titre decreases very rapidly after 60 – 120 days to levels 5 to 25 folds less than the highest point reached during the kinetics. The seroconversion rate is also less than the 97% found in the study conducted in Bali, Indonesia[89].

The study demonstrates that both vaccines produced locally and those imported into the country show no significant difference in their immunogenicity rates, so also is the breed of the dogs. With regards to the production of neutralizing antibodies and the type of vaccine used; the local flurry strain (LEP) anti-rabies vaccine produced by the National Veterinary Research Institute in Vom, Nigeria and the imported foreign vaccines both confer immunity when used for vaccinating dogs against rabies.

A general relationship between antibody response and age clearly exist. The observation that young animals (<1 year) make a poorer immune response to rabies vaccination than adults could be due to their immune systems being less mature. Also the presence of specific neutralizing antibodies transmitted to the puppies via colostrums impedes development of immunity: This corresponds with the conclusion of another study on dogs in Zaria, Kaduna State, Nigeria[93]. It also support the finding from the study in Thailand which shows that whenever the mean level of neutralizing antibodies was elevated after vaccination, the older dogs had the highest levels of response. In general, dogs in the age range of 1-7 years were seropositve and most frequently found with high antibody titre. This is most likely due the fact that they might have received more vaccinations than the dogs below 1 year of age. Also a reduction in immune regulation is thought to occur with age, this may explain why the adult dogs have better response to rabies vaccination than the old dogs.

Most cases of vaccination failure reported may therefore be due to either cold chain abuse, which can affect the efficacy of the vaccine or wrong administration of the vaccine.

Variation in antibody response relating to length of interval of sampling following vaccination is also not unexpected and presumably relates to the response kinetics for primary vaccination. Dogs iso-type shift from an IgM response to an IgG as an immune response develops. Measurement at

too early a stage will predominantly only capture IgM response developing but not be able to confirm whether an IgG response progresses. Measurement only at a later time point may reveal lower antibody levels, but this may not relate to a lack of immune protection as the total immunoglobulin measure may be proportionately more accounted for by IgG.

CHAPTER 6

6.0 Conclusion and recommendation

6.1 Conclusion

Dog rabies control relies principally on the mass immunization of dogs in order to achieve population immunity levels sufficient to inhibit rabies transmission.

Based on this study, we can conclude that:

1. Dogs vaccinated against rabies at the state veterinary clinics in Kaduna metropolis of Kaduna State Nigeria, achieved significantly high rate of sero-conversion (92%); over the 70% – 80% required to acquire herd's immunity and hence prevent an outbreak.
2. Rabies vaccine imported into the country to augment the inadequate supply of the local rabies vaccine produced by the National Veterinary Research Institute in Vom, compared favorably in terms of success rates.
3. Adult dogs between the ages of 1 – 7 years achieved greater sero-conversion rate following rabies vaccination, than dogs older than 7 years, or younger dogs that are less than 1 year old.
4. Also, the breed of the dog is not an important factor in achieving adequate sero-conversion following rabies vaccination. Local breeds, exotic breed as well as the cross breed dogs, all have good chances of developing immunity when vaccinated against rabies.

6.2 Recommendations

Mass vaccination campaigns rather than depopulation of dogs should be a long term goal for rabies control. Vaccine shortages can result from higher-than-expected demand, interruptions in production /supply, or lack of resources to purchase vaccines. In developing countries, the major cause of vaccine shortages is lack of resources to purchase them.

Based on the result from this study, the following are been recommended:

1. The Kaduna State government should sustain and intensify the annual rabies vaccination campaign since dogs vaccinated during this exercise achieved high sero-conversion rate.
2. The annual rabies vaccination campaign should be expanded to reach all the 23 Local Government Areas of the state to ensure adequate coverage of all dogs in the state so as to achieve the desired 70 – 80% immunization coverage required to prevent an outbreak.
3. The State Ministry of Agriculture Veterinary Public Health Department should try and incorporate both private and other veterinary clinics in the state to participate in the annual vaccination campaign.
4. The Kaduna State Government should also make policies to encourage private veterinary pharmaceutical companies in the state to import more foreign rabies vaccine to ensure continuous and adequate supply of the vaccine in the state.
5. The Kaduna State Ministry of Agriculture should expand the provision of dog license for vaccinated dogs to include all the dogs vaccinated at other private veterinary clinics. This will greatly assist in identifying the likely rabies immunity status of a dog following case of dog bite.

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APPENDIX 1

Figure 1: Rabid dog with the 'furious form', showing increased aggression

Source: CDC_INFO

APPENDIX 2

Figure 2: Characteristic appearance of the ‘dumb form’ of canine rabies

Source: CDC_INFO

APPENDIX 3

Figure 5: Map of Nigeria showing the location of Kaduna state

APPENDIX 4

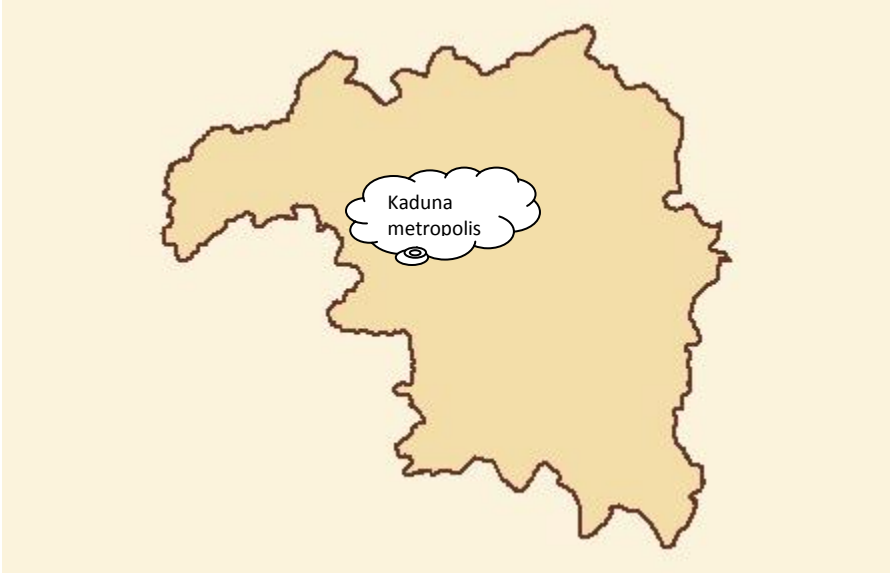


Figure 6: Map of Kaduna State showing the location of Kaduna metropolis

APPENDIX 5

DEPARTMENT OF COMMUNITY MEDICINE, FACULTY OF MEDICINE,

AHMADU BELLO UNIVERSITY, ZARIA

PARTICIPANT INFORMATION LEAFLET

Rabies is a zoonotic viral disease of great public health significance. It is caused by a rabies virus which is transmitted mostly through bite of a rabies infected animal principally dogs, cats, bats, etc. Published reports and surveys have implicated dog as the principal reservoir host for rabies in Nigeria and the transmitter of the infection to humans and other dogs/animals through bite. Some dogs show real furious rabies by their menial expression of madness, while others became dumb without noticing rabies in them.

Annual anti-rabies vaccination (ARV) of dogs has been advocated in Nigeria. However, due to inadequate supply of the vaccines by the National Veterinary Research Institute (NVRI),

Vom – Nigeria, people import and use foreign ARV. Despite this, rabies in some vaccinated dogs has been reported suggesting some dogs do not develop immunity even after vaccination. This could be due to many factors including type of vaccines used, storage and handling before use, route of administration, dog immune competent/response etc.

It is on this note that you are invited to participate in this research as volunteer to answer the questionnaire and donate 2ml of your vaccinated dog's blood.

Thanking you in anticipation of your honest participation, please.

Dr. Tukur Mustapha 08023580333, 08034619701

APPENDIX 6

DEPARTMENT OF COMMUNITY MEDICINE, FACULTY OF MEDICINE,

AHMADU BELLO UNIVERSITY, ZARIA

DOG BLOOD COLLECTION CONSENT FORM [No. -----]

I…… (Dog owner) of ……. Town, do voluntarily and willingly agree and accept that the blood sample of my dog be collected and for rabies research purposes by Dr. Tukur Mustapha of the above named address

This day……of…….20….

Sign……. Sign…….

(Dr. Tukur Mustapha) (Dog owner)

08023580333, 08034619701 Phone no…….

APPENDIX 7

DEPARTMENT OF COMMUNITY MEDICINE, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

[No _ _ _ _] QUESTIONNAIRE SURVEY ON DOG OWNERS IN KADUNA STATE, NIGERIA

INSTRUCTION: Kindly read and answer the following questions. The obtained information will be used strictly for academic purpose only and absolute confidentiality will be ensured. Thank you for the anticipated cooperation. Fill or tick the space or box provided accordingly.

1. Age _____years: < 20 [ ] , 20 – 39 [ ] , 40 – 59 [ ] , ≥ 60 [ ]

2. Sex a. Male [ ] b. Female [ ]

3. Ethnic group a. Hausa [ ], b. Yoruba [ ], c. Ibo [ ], d. Others (specify) _____

4. Marital status: a. single [ ], b. married [ ], c. divorced [ ], d. widow [ ]

5. What is your religion: a. Islam [ ], b. Christianity [ ], c. Others (specify) _____

6. What is your occupation? a. civil servant [ ], b. private practitioner [ ],

   c. businessman/woman [ ], d. farmer [ ], e. others (specify) _____

7. Duration of service/practice/business/farming/others? ____years

8. What is the size/number of your households'? _____ people

9. What is your education status? a. Primary [ ], b. Secondary [ ], c. Tertiary [ ], d. Religious [ ], e. None [ ]

10. How many dogs do you have? : a. [1], b. [2], c. [3], d. >3 [ ]

11. Have you been vaccinating your dog? :a. Yes [ ], b. No [ ]

12. When last did you vaccinate your dog(s)? : a. Within last 1 month ago [ ], b. Within last 3 months ago [ ], c. Within last 6 months ago [ ], d. Within last 12 months ago [ ]

13. Where was the vaccine administered to the dog? : a. At home [ ], b. In a Gov't Veterinary Clinic/Hospital [ ], c. Private Veterinary Clinic [ ], d. At Veterinary drug store [ ]

14. What is the type of vaccine given? : a. Local NVRI, Vom vaccine [ ], b. Foreign vaccine [ ]

15. Who administered the vaccine? : a. Veterinary Doctor [ ], b. Para-veterinary staff [ ], c. By self [ ],

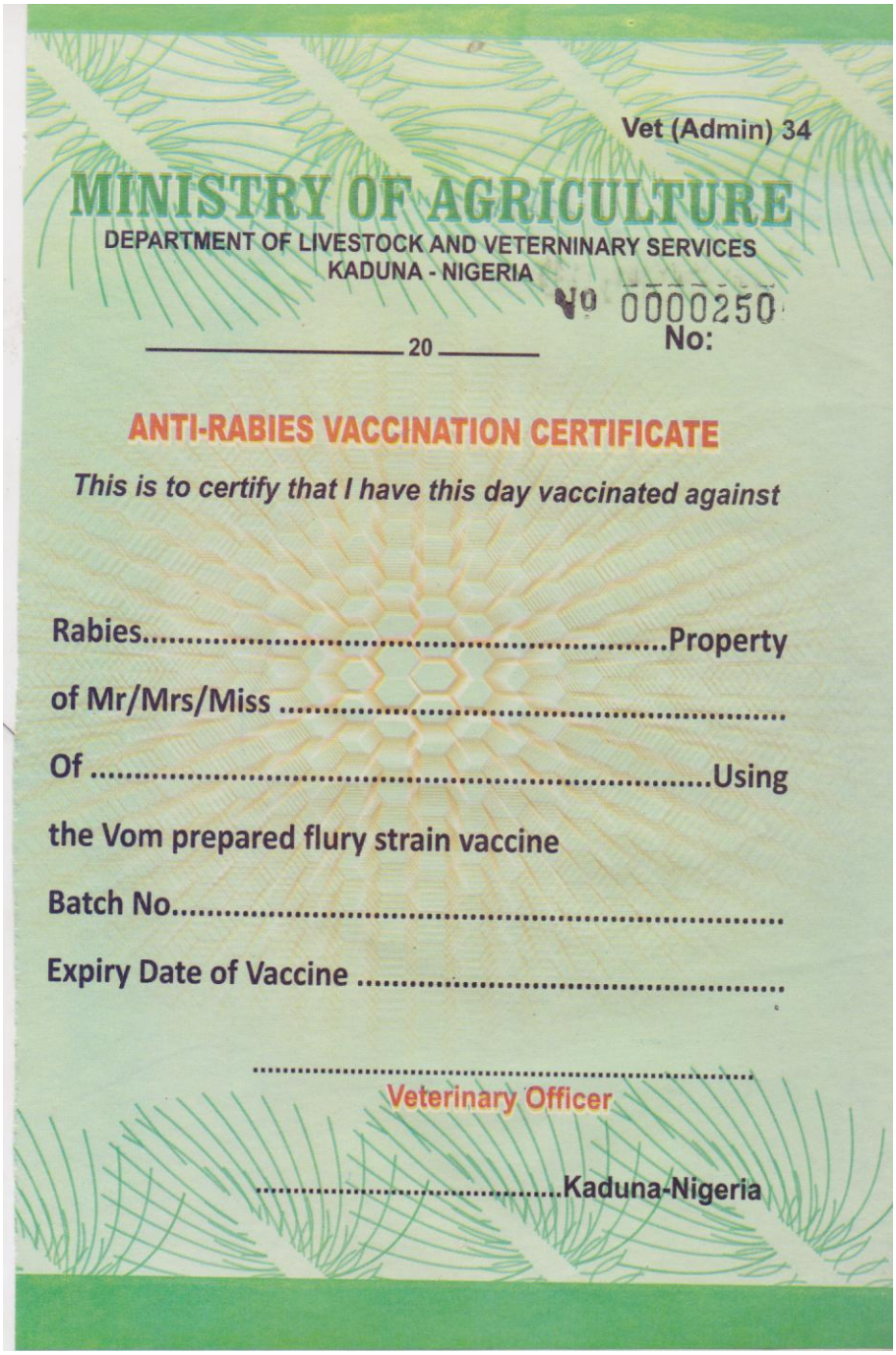
16. Has your dog been bitten by another dog(s) recently? : a. Yes [ ], b. No [ ]

17. If yes, when did it occur? : a. within last 1 month ago [ ], b. within last 3 months ago [ ], c. within last 6 months ago [ ], d. within last 9 months ago [ ]

18. If yes, have you observe any sudden change in behavior of your dog? : a. Yes b. No [ ]

APPENDIX 8

A COPY OF THE ANTI-RABIES VACCINATION CERTIFICATE, ISSUE AFTER DOG VACCINATION AT THE STATE VETERINARY CLINICS IN KADUNA STATE.



Vet (Admin) 34

MINISTRY OF AGRICULTURE

DEPARTMENT OF LIVESTOCK AND VETERNINARY SERVICES
KADUNA - NIGERIA

No 0000250

No:

_____ 20 _____

ANTI-RABIES VACCINATION CERTIFICATE

This is to certify that I have this day vaccinated against

Rabies.....Property

of Mr/Mrs/Miss .....

Of .....Using

the Vom prepared flury strain vaccine

Batch No.....

Expiry Date of Vaccine .....

.....

Veterinary Officer

.....Kaduna-Nigeria