Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.46
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

QUALITATIVE ASSESSMENT OF RABIES ANTIBODY IN DOGS PRE AND POST VACCINATION IN SOME COMMUNITIES IN PLATEAU STATE

[1,2]Nzenwata Emeka, *[1]Tekki Ishaya Sini, [1]Meseko Clement Adebajo

[1]National Veterinary Research Institute, PMB 01, Vom, Plateau State, Nigeria
[2]Formerly Pan African University, Life and Earth Sciences Institute (Including Health and Agriculture), University of Ibadan, Ibadan, Nigeria

*Corresponding Author Email Address: ishaya.sinitekki@yahoo.com

ABSTRACT
This study aimed to conduct a qualitative assessment of rabies virus antibody among domesticated dogs in some communities in Plateau State, Nigeria. A total of 45 domesticated dogs from eight (8) communities within Jos South and East local government areas (LGAs) were sampled. Two serum samples were collected from each dog, 1 pre-vaccination and the other 2 weeks post-vaccination, totaling 90. A serological assay was performed using a commercial IgG (Dog) ELISA kit acquired from Demeditec® Diagnostics GmbH, Germany, for the determination of IgG antibodies against rabies virus in the samples. The results revealed that 36 (80 %) of the dogs were positive for rabies immunoglobulin G, and 9 (20 %) were negative in pre-vaccination assessment across the communities. It also revealed a significant increase in the positive rabies immunoglobulin G post-vaccination by 96 %. Of the 8 LGAs studied, the dogs in 7 of the LGAs (90%) showed a significant ($p \leq 0.05$) increase in positive (≥ 0.34) rabies antibody titer. Only 1 LGA (10 %) showed a level of antibody titer below < 0.34, which was considered significant.

Keywords: Dogs, Rabies, Antibody, Pre-vaccination, post-vaccination, Seroconversion.

INTRODUCTION

Rabies is a vaccine-preventable viral zoonosis that is almost 100% fatal once clinical manifestations occur. Rabies mortality in humans can be very high, therefore making it the seventh leading cause of death on the globe (Wyatt, 2007). The disease is caused by neurotropic viruses of the genus *Lyssavirus* and family *Rhabdoviridae*, which include Rabies lyssavirus among 16 member species. Lyssaviruses are bullet-shaped enveloped viruses with a size of 75 nm in diameter and 100-300 nm in length. They are single-stranded, negative-sense RNA viruses (Fishbein & Robinson, 1993). Rabies virus infects almost all warm-blooded animals, including humans, wild dogs, wild carnivores (raccoons, skunks, and foxes), and bats are the known natural reservoirs (Dietzschold *et al*., 2008; Singh *et al*., 2017). Transmission occurs to humans and other susceptible mammals mainly through the bite of infected animals, especially domestic dogs in Africa and Asia.

Effective control and prevention of rabies is achieved by routine vaccination of domestic dogs with a potent vaccine as well as administration of post-exposure prophylaxis (PEP) to exposed persons. In many African countries however, the proportion of dogs vaccinated against rabies is far below the 70 % recommended by the WHO for herd immunity (Cleaveland *et al*., 2023). Sustainable long-term vaccination programs have remained a challenge for most developing countries. Consequently, more than 95 % of global deaths caused by rabies occur in Asia and Africa, where canine rabies is endemic irrespective of the implementation of extensive control schemes and public health awareness programs (World Health Organisation, 2013). Rabies in humans always occurs as a fatal disease despite the availability of advanced modern medicine, and Africa alone contributes 43 % of the human deaths due to rabies (Jibat *et al*., 2015).

Routine laboratory diagnosis of rabies is carried out by detection of the viral antigen in the fresh brain tissues using Direct Fluorescent Antibody Testing (DFAT), where specific viral anti-nucleocapsid monoclonal or polyclonal antibody-tagged fluorochrome is applied (Dean *et al*., 1996; World Organization for Animal Health, 2018). For the detection of the virus-neutralizing antibody in the serum of vaccinated or infected individuals, the Fluorescent Antibody Virus Neutralization Test (FAVNT) and the Rapid Focus Fluorescent Inhibition Test (RFFIT) (Smith *et al*., 1973; Cliquet *et al*., 1998) are the gold-standard assays approved by the World Health Organization (World Health Organization, 2018) and the World Organization for Animal Health (World Organization for Animal Health, 2018). Although the virus neutralization techniques are known to be the most reliable for successful vaccination evaluation, they are time-consuming, expensive, and demand so much in terms of cell culture facilities and skilled personnel (Fitria *et al*., 2023). It also requires the use of live virus and a containment facility, which may not be available in some laboratories. Sometimes, the results of the neutralization tests are difficult to read due to cytotoxic effects on the cells (Bedekovi´c *et al*., 2016; Wasniewski *et al*., 2016).

Consequently, Enzyme-Linked Immunoassays (ELISA) could be employed for research purposes, especially for epidemiological studies where a large number of samples are screened or where cell culture technology is not readily available (Wasniewski & Cliquet, 2012; Ukamaka *et al*., 2018; World Organization for Animal Health, 2018). In Nigeria, serological surveys have been employed in canine population studies to understand the pattern of rabies-neutralizing antibodies in apparently healthy unvaccinated dogs (Wosu & Anyanwu, 1990; Ohore *et al*., 2007; Olugasa *et al*., 2011) and dogs meant for slaughter for human consumption (Eze *et al*., 2018). Also, a study was carried out to evaluate the vaccination coverage of the canine population in retrospect at the University of Ibadan, Nigeria (Adeyemi & Zessin, 2000). However, there is limited information on Pre- and Post-Vaccination studies of dog populations in Nigeria. Consequently, this study was conducted to determine the Pre- and Post-Vaccination (PV) qualitative assessment of rabies virus-neutralizing antibodies among domestic dogs vaccinated with the Nobivac® Rabies vaccine in some communities in Plateau State, Nigeria, using the ELISA Kit.

Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.46
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

MATERIALS AND METHODS

Study Area

This study was conducted in seven communities in Jos South Local Government Areas (LGAs), which included: K-Vom (KV), Chugwi (CHG), Chuni Vom (CHV), Kwata-Zawan (KWZ), Vwang Chugwi (VCH), Dashwan Kuru (DASH), Anuguldi (ANG), and Zarazon (ZJE) communities in Jos East LGA, Plateau State, Nigeria. Plateau State (Fig. 1) is situated in the North-central region of the country, and lies within latitude 9°47'59.99" N and longitude 8°51'59.99" E. It occupies a land mass area of 11,936 square miles (30,913 square km) with a human population of 3,178,712 as of the 2006 population census. Administratively, it constitutes 17 LGAs and is bounded by the states of Kaduna and Bauchi on the north, Taraba on the east, and Nasarawa on the south and west. The Jos Plateau rises to about 5,250 feet (1,600M) above sea level in the north-central subregion, and the Benue River valley stretches along the southwestern border. It is mainly an agrarian state and is popularly known for the mining and export of Tin and Columbite (The Editors of Encyclopedia Britannica, 2025). The immunoassay was carried out in the National Reference Laboratory for Rabies, National Veterinary Research Institute (NVRI), Vom, Jos South LGA. It is the Centre of Excellence for the manufacture of animal vaccines and is equipped with modern laboratories for disease diagnosis in Nigeria.

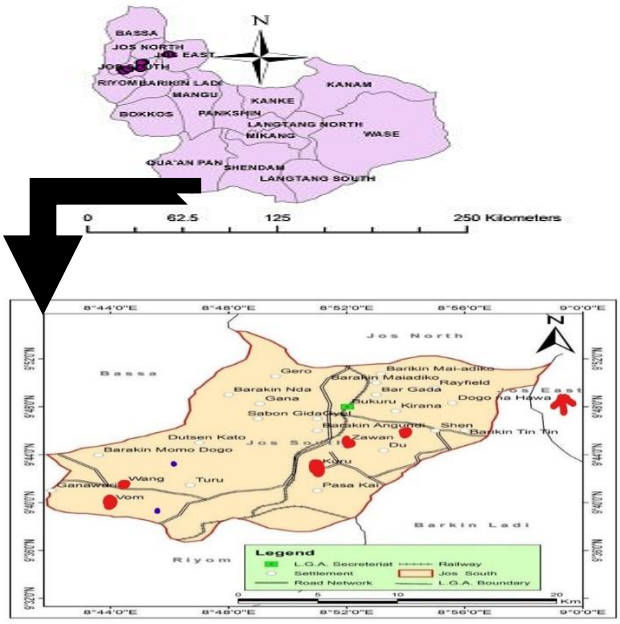

Figure 1: Map of Plateau State showing Jos South and Jos East (purple dots), indicating the communities from which samples were collected (red dots and arrow).

STUDY DESIGN

This study was both cross-sectional and longitudinal, involving 45 domestic dogs (*Canis familiaris*) recruited from the eight communities mentioned in the study area above. The dogs represented various breeds, and their sex, body weights, and ages ranged from 2 to 18 months. Inclusion criteria included naive/unvaccinated dogs and consent from dog owners, while exclusion criteria consisted of vaccinated dogs and lack of consent from dog owners. A convenience sampling technique was employed to sample the participants.

All relevant National and/or Institutional guidelines for the care and use of animals were duly followed, and Ethical Clearance (Ref. No. AEC/02/180/25) was obtained from the Institutional Animal Use and Care Committee of NVRI, Vom.

Sample Collection and Immunisation

Blood sample was collected from the cephalic vein of each of the 45 unvaccinated dogs prior to and on day 14 post-vaccination using sterile 5 ml syringes and needles and into an appropriately labeled plain sample bottle. The blood was allowed to stand at ambient temperature for 30 minutes to clot. The samples were carefully transported to the laboratory and centrifuged at 1000 rpm for 5 minutes to separate the sera. Each of the 90 sera was decanted into another suitably labeled cryovial and stored in a freezer at -20 °C till the time of use for detection of antibodies to rabies.

Serum Assay

Enzyme-Linked Immunosorbent Assay

Serology was done using a commercially available Rabies virus IgG Ab (Dog) ELISA kit from Demeditec® Diagnostics GmbH, Germany, for the determination of IgG antibodies against Rabies virus. This assay was carried out in the National Reference Laboratory for Rabies (NVRI), Vom, Plateau State.

Assay Procedure;

The positive (+ve) control serum was reconstituted with 0.5 ml of ultra-pure water, while the negative (-ve) control was reconstituted with 1.0 ml of the water. The predilution of +ve control was 1:50, 1:150, 1:450, 1:1350.

For positive control at 1:50

For Well1A: 80 µl of the diluent + 20 µl of the positive control were added; In Well 2A:180 µl of the diluent + 20 µl of the predilution from 1A were added; while in Well 2B:120 µl of the diluent + 60 µl from Well 2A were added. For Well 2C, 120 µl of the diluent + 60 µl from Well 2B were added. Similar treatment was given to Well 2D, where 120 µl of the diluent + 60 µl from Well 2C were added. Finally, 60 µl was pipetted from Well 2D and discarded.

For negative control, 1:50

For Well 2E, 147 µl of the diluent buffer + 3 µl of the negative control were added, while for Well 2F, 120 µl of the diluent buffer only, was added.

Sample dilution:

The carrier plate was divided into two, i.e., 1F and 2F: For 1F, 90 µl of the buffer + 10 µl of each of the 45 samples were added to separate wells; while for 2F, 144 µl of the buffer + 6 µl from 1F were added. Then, 100 µl from 2F was added into the coated plate and sealed it with the available sealant. I then incubated for 60 minutes. The coated plate was washed ushing ELISA washer 5 times and dabbed in adsorbent towel paper. Then 100 µl of conjugate was added into the plate, the plate was sealed and incubated again for another for 60 minutes. The 5 times washing was repeated as described in the protocol, then 10 µl of equal volume of substrate was added to each well. The plate was wrapped with a thick paper and incubated in the dark for 15 minutes. Following incubation, 50 µl of stop solution was added to

Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.46
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

each well and the reading was taken at 450 nM in by spectrophotometry, using an ELISA reader.

Test Validation

The qualitative result was considered valid if;

1. The mean value (MV) of the measured OD value for the positive control (PC), diluted at 1:50, must be >1.00.
2. The MV of the measured OD value for the negative control (NC) diluted at 1:50 must be ≤ 0.400.

The ratio (S/P) of sample OD to the mean OD of the positive control is calculated as:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

A sample with an S/P ratio of < 0.34 was considered negative, indicating that antibodies were not detected, while an S/P ratio of ≥0.34 was considered positive.

Residual Rabies Antibodies Evaluation

A structured questionnaire was administered to the owners of the 45 dogs sampled in the 8 Local communities studied. The questionnaire was modified according to the variables encountered during pre-testing. The questionnaire variable covered information on the age, the sex, the breed, the vaccination status, the purpose of keeping the dog, and the management system.

Data Analyses

The pre-vaccination and PV ELISA antibody titer data were subjected to a paired t-test. The questionnaire was subjected to descriptive statistics using tables, figures, and presented in percentages. Statistical packages used were GRAPHPAD Prism version 9.0 and SPSS version 26. Values of P ≤ 0.05 were considered significant.

RESULTS

Pre-Vaccination and Post-Vaccination Rabies Antibodies Serosurvey

Among the 45 pre-vaccination samples collected from the 8 communities in the two LGAs, the IgG ELISA test showed that 36 (80%) dogs were positive, while 9 (20%) were negative (Fig. 2). All 5 dogs, each tested in CHG, DASH, ANG, VCH, and CHU, were positive. Five of 10 dogs in KV, four of five in KJS, and two of five in ZJE were positive. The post-vaccination survey showed that 43 (96 %) were positive while 2 (4%) were negative (Fig. 3). All the dogs in each community except KV tested negative, while only two of 10 in KV were positive in the PV study (Fig. 3).

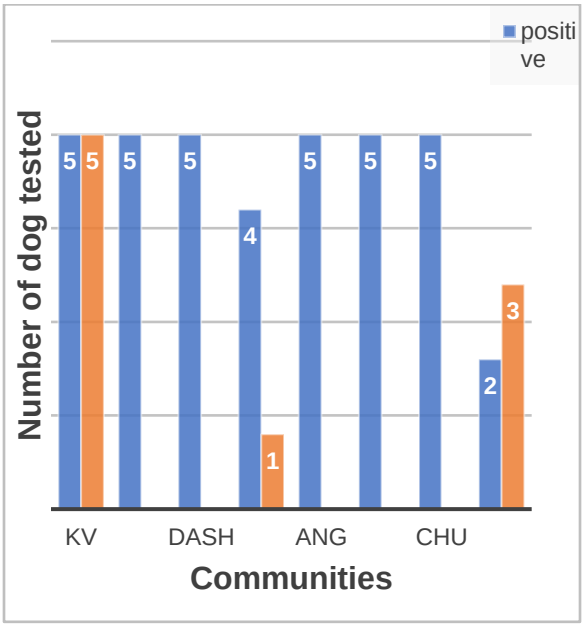

Figure 2: Pre-vaccination rabies antibodies serosurvey in eight communities of Jos South and Jos East LGA, Plateau State, Nigeria

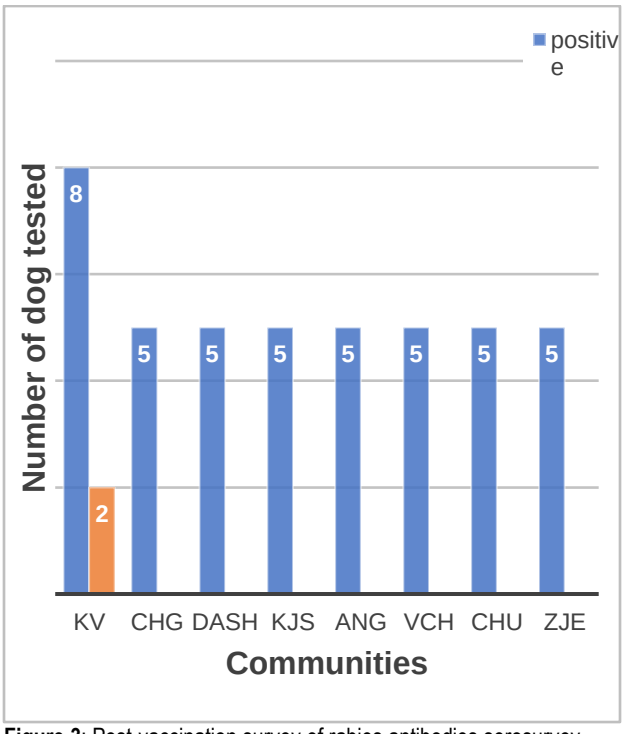

Figure 3: Post-vaccination survey of rabies antibodies serosurvey in eight communities of Jos South and Jos North LGA, Plateau State, Nigeria.

Validation of IgG Antibody Titer.

In the 8 communities, there was a significant 100% (P≤ 0.05) increase in positivity (≥ 0.34) of rabies antibody titer,

Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.46
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Table I: Test validation of samples indicating positive and negative antibody titers in eight Communities of Jos South and Jos East LGAs, Plateau State, Nigeria

Community	S/P	P-value
CHUNI VOM	1.044	0.0215
K-VOM	0.5320	0.0035
CHUGWI	1.732	0.4033
DASHWAN KURU	0.7860	0.0274
KWATA ZAWAN	0.5760	0.0552
ANUGULDI	0.7320	0.0406
VWANG CHUGWI	0.9840	0.0074
ZARAZON	1.134	0.0232

Hint; P Value ≤0.05 = positive, ≥ 0.34 = positive

Residual Rabies Antibody Evaluation

Of the 5 dogs sampled in the Chuni Vom community, 4 (80 %) were less than one year old, while one (20 %) was between 1 and 2 years old. All 5 dogs were on the free-range management system and non-vaccinated.

Female dogs constituted 80 % of the population, while 20 % were male (Table II). The difference in the frequency and percentage of dogs sampled in other LGAs in terms of age, sex, breed, vaccination status, and management system were further shown in Tables II to IX. Breeds of dogs sampled were 42 (93.3 %) indigenous, 9 (4%) mixed, and 2 (1 %) exotics. All indigenous breeds were allowed to roam freely. The age range, sex, management system, and breeds of dogs sampled are shown in Figs. 3 to 6, respectively (Table II).

In Kwata Zawan, Community, 3 (60 %) of the 5 dogs were < one year, one (20 %) was 1-years and one (20 %) was > 4 years. One (20 %) was male and 4 (80 %) were females. The breed distribution was 3 (60 %) indigenous and 2 (40 %) mixed breeds, while all 5 were on a free-range management system and non-vaccinated (Table III). In Table IV, the demography of the dogs sampled in Chugwi community, Jos South, comprised 2 (40 %) dogs of < one year and 3 (60 %) of 1- 2 years. Two (40 %) were males and 3 (60 %) were females. All 5 dogs were indigenous breeds, on a free-range management system, and non-vaccinated.

Table II: Demography of dogs sampled in Chuni Vom Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY (F)	PERCENTAGE (%)
Age	<1YR	4	80
	1-2YRS	1	20
	3-4YRS	0	0
	>4YRS	0	0
Sex	Male	1	20
	Female	4	80
Breed	Indigenous	5	100
	Mixed bred	0	0
	Exotic	0	0
Management System	Free range	5	100
	Confined	0	0
Vaccination status	Non-Vaccinated	5	100
	Vaccinated	0	0

Table III: Demography of Dogs Sampled in Kwata Zawan Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY (F)	PERCENTAGE (%)
Age	< 1YR	3	60
	1-2YRS	1	20
	3-4YRS	-	0
	>4 YRS	1	20
Sex	Male	1	20
	Female	4	80
Breed	Indigenous	3	60
	Mixed breed	2	40
	Exotic	0	0
Management System	Free range	5	100
	Confined	0	0
Vaccination status	Non-Vaccinated	5	100
	Vaccinated	0	0

Table IV: Demography of Dogs Sampled in Chugwi Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR	2	40
	1-2YRS	3	60
	3-4YRS	0	0
	>4YRS	0	0
Sex	Male	2	40
	Female	3	60
Breed	Indigenous	5	100
	Mixed breed	0	0
	Exotic	0	0

Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.46
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Management System	Free range Confined	5 0	100 0
Vaccination status	Non-Vaccinated Vaccinated	5 0	100 0

Table V: Demography of Dogs Sampled in Zarazon Community, Jos East LGA, , Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR 1-2YRS 3-4YRS >4YRS	5 0 0 0	100 0 0 0
Sex	Male Female	1 4	20 80
Breed	Indigenous Mixed breed Exotic	5 0 0	100 0 0
Management System	Free range Confined	5 0	100 0
Vaccination status	Non-Vaccinated Vaccinated	3 2	60 40

In the Zarazon Community of Jos East LGA, all 5 (100 %) dogs were < one year, one (20 %) was male, and 4 (80 %) were females. All 5 were indigenous breeds and on a free-range system of management. The vaccination status showed that 3 (60 %) were non-vaccinated, while 2 (40 %) were vaccinated (Table V).

Table VI: Demography of Dogs Sampled in Vwang Chugwi Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR 1-2YRS 3-4YRS >4YRS	5 0 0 0	100 0 0 0
Sex	Male Female	4 1	80 20
Breed	Indigenous Mixed breed Exotic	5 0 0	100 0 0
Management System	Free range Confined	5 0	100 0
Vaccination status	Non-Vaccinated Vaccinated	5 0	100 0

In Vwang Chugwi Community, Jos South LGA, all the 5 (100 %) 5 dogs were < one year, 4 (80 %) were males, and one (20 %) was a female. All 5 (100 %) were indigenous, one free range management system, and non-vaccinated (Table VI).

Table VII: Demography of Dogs Sampled in Dashwan Kuru Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR 1-2YRS 3-4YRS >4YRS	4 1 0 0	80 20 0 0
Sex	Male Female	0 5	100 0
Breed	Indigenous Mixed breed Exotic	5 0 0	100 0 0
Management System	Free range Confined	5 0	100 0
Vaccination status	Non-Vaccinated Vaccinated	5 0	100 0

Table VII shows that in the Dashwan Community of Jos South LGA, 4 (80 %) of the 5 dogs were < one year, and one (20 %) was 1- 2 years. All the dogs sampled in this community were females, indigenous, on a free-range management system, and non-vaccinated.

Science World Journal Vol. 20(No 2) 2025
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University
https://dx.doi.org/10.4314/swj.v20i2.46

Table VIII: Demography of Dogs Sampled in Anguldi Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR	2	40
	1-2YRS	2	40
	3-4YRS	1	20
	>4YRS	0	0
Sex	Male	1	20
	Female	4	80
Breed	Indigenous	4	80
	Mixed breed	0	0
	Exotic	1	20
Management System	Free range	4	80
	Confined	1	20
Vaccination status	Non-Vaccinated	5	100
	Vaccinated	0	0

In Anguldi Community of Jos South LGA, 2 (40 %) of the 5 dogs were < one year, 2 (40 %) were 1-2 years and one (20 %) was 3-4 years. One (20 %) was male and 4 (80 %) were females. The breed distribution was 4 (80 %) indigenous and one 2 (40 %) exotic breeds. The management system was 4 (80 %) free range and one (20 %) confined, and none of the dogs were vaccinated against rabies (Table VIII).

Table IX: Demography of Dogs Sampled in K-Vom Community, Jos South LGA, Plateau State, Nigeria

VARIABLE	CATEGORY	FREQUENCY(F)	PERCENTAGE (%)
Age	< 1YR	5	50
	1-2YRS	2	20
	3-4YRS	2	20
	>4 YRS	1	10
Sex	Male	1	10
	Female	9	90
Breed	Indigenous	10	100
	Mixed breed	0	0
	Exotic	0	0
Management System	Free range	9	90
	Confined	1	10
Vaccination status	Non-Vaccinated	9	90
	Vaccinated	1	10

Further analysis of the age range of dogs in the 8 Communities (fig. 4) shows that 30 (66.7 %) of the dogs were less than one year of age. The highest numbers (5 each) were found in Zarazon, Chuni Vom and K-Vom, and the list (2 each) in Chugwi and Anguldi communities. Only 10 (22.2 %) were one to 2 years of age, with the highest number (3) in Chugwi and the list (one each) in Chuni Vom, Kwata Zawan and Dashuwan Kuru communities. None in this age grade was sampled in Zarazon and Vwang Chugwi Communities. Only 3 (6.7 %) of dogs aged 3 to 4 years were only samples in Anguldi (one) and K-Vom (2), while 2 (4.4 %) those that were older than 4 years of age were sampled only in Kwata Zawan and K-Vom (fig. 4).

Of the total dogs sampled, only 10 (22.2 %) dogs were males while 35 (77.8 %) were females. The highest numbers (4) of male dogs were sampled in Vwang Chugwi Community while the least (1 each) were sampled in Chuni, Zarazon, Anguldi and K-Vom. For the female dogs, the highest number (9) was in K-Vom, and the lowest (1) in Vom Chugwi (Fig. 5). The dogs on free-range system of management were 42 (93.3 %) while 3 (6.7 %) were confined. The highest number of dogs on free-range system of management 9 (21.4 %) was in K-Vom with the least 3 (7.1 %) at Kwata Zawan. The 3 confined dogs encountered in the study were in Kwata-Zawan while the other one was in Anguldi (Fig. 6). The indigenous breed of dogs constituted 42 (93.3 %), 2 (4.4 %) were mixed breeds while only one (2.2 %) was exotic. Both mixed breeds and the exotic breeds were from Zarazon and Anguldi communities, respectively (Fig. 7).

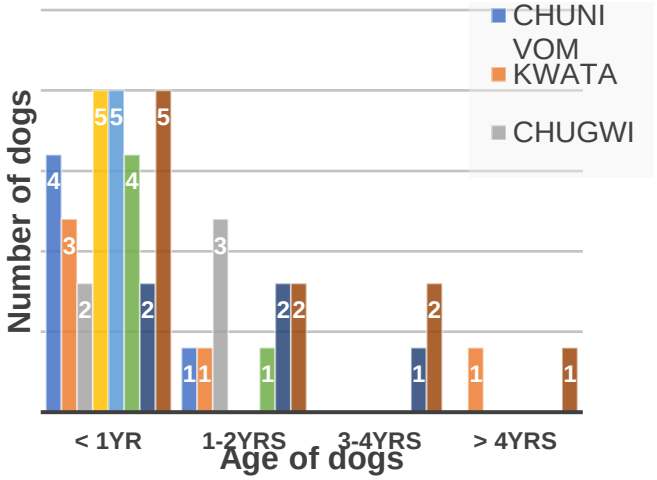

Figure 4: Age range of dogs in 8 Communities of Jos South and Jos East LGAs, Plateau State, Nigeria

Science World Journal Vol. 20(No 2) 2025
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University
https://dx.doi.org/10.4314/swj.v20i2.46

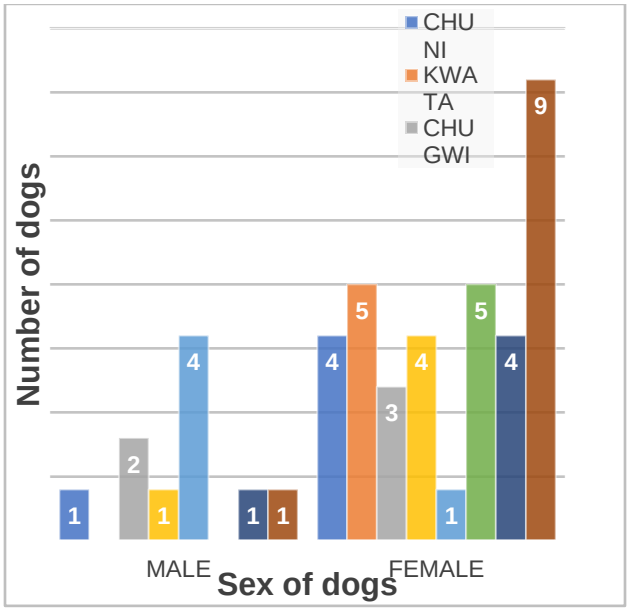

Figure 5: Sex of dogs in 8 Communities of Jos South and Jos East LGAs, Plateau State, Nigeria

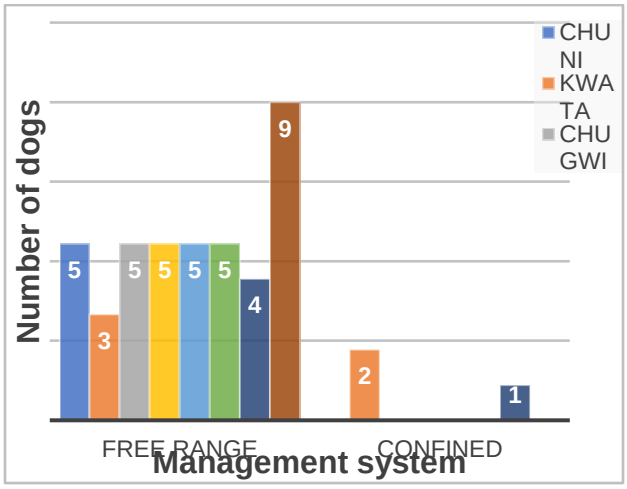

Figure 6: Management system of dogs sampled in 8 Communities of Jos South and Jos East LGAs, Plateau State, Nigeria

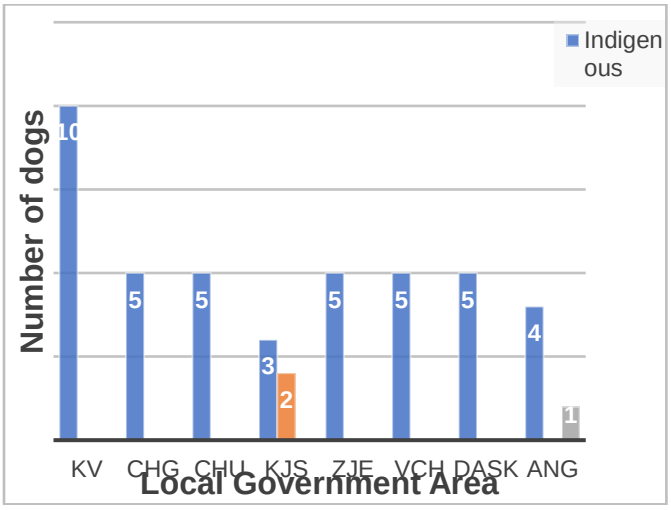

Figure 7: Breeds of dogs sampled in Communities of Jos South and Jos East LGAs, Plateau State, Nigeria

DISCUSSION

The high level of positives (80 %) IgG ELISA test prior to vaccination in this study (Fig. 2) can be attributed to various factors such as the presence of maternally-derived antibodies (MDA), subclinical infection, and previous vaccination. Innate and adaptive cellular immunity contribute to rabies protection (Gold *et al*., 2020; Moore, 2021). However, methods to measure these immune effectors are less developed and not commonly available; thus, ELISA rabies virus antibody (RVNA) measurement remains the primary means of verifying rabies immunity. This finding is similar to the work of Ogunkoya *et al*. (1990), where ELISA rabies virus antibody (RVNA) was used to detect rabies immunity in vaccinated animals (Ukamaka *et al*., 2018). Detectable rabies virus antibodies (RABVAbs) in sera were reported in populations of domestic dogs and humans in Nigeria, where rabies is considered endemic. Similar findings have also been found in wildlife in regions where rabies is also endemic (Wosu & Anyanwu, 1990; Ohore *et al*., 2007).

It was also observed that the majority of dogs vaccinated produced a significant increase (96 %) in the antibody titer post-vaccination (Fig. 3), which agrees with the results of a previous study where the sensitivity of ELISA was 96.77 % in detecting rabies antibodies (Olugasa *et al*., 2011). Some other studies (Adeyemi & Zessin, 2020) have also reported the use of ELISA to determine the post-exposure anti-rabies antibody titer in vaccinated dogs. The significance of detecting rabies virus-neutralizing antibodies cannot be overemphasized, as it plays a major role in determining the immune status and herd immunity in vaccinated animals and previous exposure of unvaccinated animals during routine diagnosis of rabies, surveillance, vaccine immunogenicity, and similar research studies. In a challenge study, the circulating MDAs in unvaccinated young animals have been shown to neutralize the pathogen following active vaccination of the dam (Moore, 2021).

Notwithstanding, some of the dogs sampled in our study did not have an adequate response to the vaccination, or seroconversion, hence the observation of negative values (4 %) post-vaccination antibody tire in this study. In a similar study conducted previously, an outrageously 16-24-fold inadequacy in seroconversion of 65.45

% - 95 % in comparison with our study has been recorded (Gold *et al*., 2020).

The results shows that 88.0 % of the dogs vaccinated in this study produced sufficient neutralising antibodies ≥ 0.34, within 2 weeks. The peak in immune response in this study at 2 weeks is similar to the findings of previous studies (Jorge *et al*., 2010; Moore, 2021). The decline in immune response in some dogs observed in this study may be attributed to the poor development of the immune system in younger dogs compared to older ones. Influencing factors like age, breeds, type of vaccines, poor administration, out-of-date and poor storage, etc., can contribute to insufficient and undetectable antibody titer levels (Ogunkoya *et al*., 1990). Some animals have been proven to produce normal but insufficient levels of immunoglobulins but not specific immunoglobulin G (IgG) antibodies, while others lack the ability to produce protective IgG antibodies against specific diseases due to abnormalities in their genetic makeup (Jorge *et al*., 2010). This can also be attributed to the low or negative level of IgG in some dogs in this study.

In addition, the method used to interpret results can lead to an erroneous conclusion of an absence of rabies antibody response if the assay only detects IgG while the sample contains primarily IgM rabies antibodies when collected early in the immune response before immunoglobulin class switching has occurred. Oftentimes, the expectation of a 'normal range' of rabies antibody levels arising from rabies vaccination or exposure often does not take into consideration the Ig class, the kinetics of response, individual variation in immune genes, and species differences (Bellan *et al*., 2012). There was no statistical significance in seroconversion in the sex of dogs sampled in this study, indicating that the primary immune response is not associated with sex. This finding also agrees with the work of Wallace *et al*. (2017).

The immune response based on the breed of dog in this study was not statistically different. Previous studies, though, have shown that exotic breeds' immune response to the anti-rabies vaccine is higher compared to Indigenous breeds of dogs (Morters *et al*., 2014; Realegeno *et al*., 2018). Previous studies have also shown that mixed-breed dogs had an improved titer response and failure rate compared to pure-breed dogs.

The vast majority of dogs sampled in this study were free-roaming, and there was no significant difference in seroconversion. This study is similar to the work of Morters *et al.* (2014), who reported that free-roaming dogs, in two regions of Africa and Asia where rabies is endemic, seroconverted to rabies vaccine regardless of health status, producing titers that exceeded the level considered necessary to protect against rabies. Other factors that can affect seroconversion in dogs are genetics and stress, which are harder to assess in real-time, especially in free-roaming dogs (Welch *et al*., 2009; Moore & Hanlon, 2010; Ma *et al*., 2012; Trujillo-Rojas *et al*., 2018; Rimal *et al*., 2020). Differences found in the level of seroconversion among animals suggest that anthropogenic variables can also influence the immune response of vaccinated animals (Jakel *et al*., 2008; Trujillo-Rojas *et al*., 2018; Moore, 2021).

The majority of dogs sampled in most of the 8 communities studied had antibodies against rabies pre-vaccination (Fig. 2). This may be as a result of residual immunity following previous vaccinations or exposure to rabies virus. Two weeks after vaccination, sero-conversion was almost 100% in all the communities, with the exception of K-Vom Community, where 2 (20 %) were yet to seroconvert (Fig. 3). It is expected that, K-Vom, which is the closest to NVRI, Vom, where dog rabies vaccine is produced for the country, and where a Veterinary hospital which provides Veterinary Services, including vaccination of dogs to the community, should have the highest pre-vaccination seroconversion. However, the K-Vom community comparatively has a higher human population, which may translate to having a higher dog population based on the human to dog population ratio of 1:16.3 as reported by Abubakar *et al.* (2023). Given the above, the medium rate of antibody (50 %) pre-vaccination and relatively higher rate of post-vaccination antibody level among dogs (80 %) in K-Vom may be an indication of non-compliance with vaccination by dog owners in the community.

The choice of the 8 communities for the study was based on convenience in terms of proximity the Jos, the city Centre, where the investigators resided. The age, gender, and breeds of dogs sampled depended on the types presented for vaccination. Every dog at the age of three months and above presented in each community was vaccinated and included in the study.

The majority of dogs vaccinated produced a significant increase (96 %) in the antibody titer post-vaccination. This shows that ELISA has proven to be effective in the determination of the post-vaccination anti-rabies antibody titer in vaccinated dogs. The study also reveals that rabies virus is still endemic and circulates within the Jos South LGA using the Enzyme-Linked Immunosorbent Assay. This further indicates that there is a potential risk of spread of rabies to the human population and to rabies-free dogs because of the high percentage of roaming dogs. However, if over 70% dog population is vaccinated within a locality, it meets the OIE recommendation for the protection of rabies within that locality. Thus, rabies remains a major concern and requires urgent attention to periodic vaccination programs, especially within rural communities.

Competing Interest

The authors declare no conflict of interest.

Limitation of The Study

The Antibody response pre- and post-vaccination was assessed using IgG ELISA, as the IgM ELISA Kit for the assessment of IgM was not available. Similarly, the virus neutralization test, the gold-standard technique for antibody assessment, was not used to determine the protective antibody level, especially in the post-vaccination period, for the unavailability of the required facility. Consequently, the protective antibody level could not be expressed in international units of neutralizing antibody per mL of blood (≥0.5 IU/mL).

Acknowlwdgement

We thank the management and staff of Pan African University, Life and Earth Sciences Institute (Including Health and Agriculture) - PAULESI of the University of Ibadan, for the sponsorship, and the National Veterinary Research Institute, Vom, for granting us access to its laboratory facilities.

REFERENCE

Abubakar, A. T, Al-Mustapha, A. I, Oyewo, M, Ibrahim, A, Abdulrahim, A, Yakub, J. M, Elelu, N, Nguku, P, Balogun, M. S, Awosanya, E. J, Kia G. S. N, Kwaga J. K. P, Okoli I, Bolajoko M. B, Alimi Y, Mbilo C, and Dacheux L (2023). Prospects for dog rabies elimination in Nigeria by 2030: 06 November 2023. https://doi.org/10.1111/zph.13084

Adeyemi, I, Zessin, K (2020). Retrospective dog rabies vaccination evaluation at the University of Ibadan, Nigeria (1988–1992). Veterinarski ARHIV.; 70:223-230. https://intranet.vef.hr/vetarhiv/papers/70-5/adeyemi.pdf

Bedekovi´c T, Simi´c I, Kristi's N; Lojkić I, Mihaljević Z, Sučec I, Lohman I J. and Hostnik P (2016). "Evaluation of ELISA for the detection of rabies virus antibodies from the thoracic liquid and muscle extract samples in the monitoring of fox oral vaccination campaigns," BMC Veterinary Research, vol. 12, no. 1, p. 76, 2016.

Bellan, S. E, Cizauskas, C. A, Miyen, J, Ebersohn, K, Ku¨sters, M, Prager, K., C, Van, V, Moritz, S, Claude, M. G, and Wayne, M (2012). Black-backed jackal exposure to rabies virus, canine distemper virus, and Bacillus anthracis in Etosha National Park, Namibia. *J Wildl Dis*.; 48(2):371–81. https://doi.org/10.7589/0090-3558-48.2.371.

Cleaveland, S., Kaare, M., Tiringa, P., Mlengeya, T., and Barrat, J. (2003). A dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine*, *21*(17-18): 1965-1973. DOI:10.1016/S0264-410X(02)00778-8 Corpus ID: 34235826

Cliquet F, Aubert M, and Sagne L (1998). Development of a fluorescent antibody virus neutralization test (FAVN test) for the quantitation of rabies-neutralizing antibody. *J Immunol Methods*.; **212**:79–87. doi: 10.1016/S0022-1759(97)00212-3.

Dean, D. J, Abelseth, M. K, and Atanasiu, P (1996). The fluorescent antibody test. In: Meslin F-X, Kaplan MM, Koprowski H, editors. *Laboratory techniques in rabies*. Geneva: World Health Organization; 1996.

Dietzschold, B., Li, J., Faber, M., and Schnell, M. (2008). Concepts in the pathogenesis of rabies. *Future Virology*, 3(5): 481–490. doi: 10.2217/17460794.3.5.481.

Eze, U. U, Ngoepe E. C, Anene, B. M, Ezeokonkwo, R. C, Nwosuh, C, and Claude T. Sabeta, C. T (2018). Detection of lyssavirus antigen and antibody levels among apparently healthy and suspected rabid dogs in South-Eastern Nigeria. BMC Res Notes; 11: 920. doi: 10.1186/s13104-018-4024-z

Fishbein, D. B., and Robinson, L. E. (1993). Rabies. *New England Journal of Medicine*, *329*(22): 1632-1638. https://www.nejm.org/doi/full/10.1056/NEJM199311253292208

Fitria Y, Febrianto N, Putri R. E, Rahmadani I, and Subekti D. T (2023). Evaluation of In-House ELISA for Antirabies Antibodies Detection in Domestic Canine. Hindawi Veterinary Medicine International Volume 2023, Article ID 4096258, 10 pages *https://doi.org/10.1155/2023/4096258*

Gold, S., Donnelly, C. A., Nouvellet, P., and Woodroffe, R (2020). Rabies virus-neutralizing antibodies in healthy, unvaccinated individuals: What do they mean for rabies epidemiology? *PLoS Neglected Tropical Diseases*, *14*(2): doi: 10.1371/journal.pntd.0007933.

Jakel, V, Ko¨nig M, Cussler K, Hanschmann, K, and Thiel, H. J (2008). Factors influencing the antibody response to vaccination against rabies. *Dev Biol (Basel)*. 131:431–437. https://pubmed.ncbi.nlm.nih.gov/18634505/

Jibat, T., Hogeveen, H., and Mourits, M. C. (2015). Review on dog rabies vaccination coverage in Africa: a question of dog accessibility or cost recovery? *PLoS Neglected Tropical Diseases*, *9*(2): doi: 10.1371/journal.pntd.0003447e0003447.

Jorge, R. S. P, Pereira, M. S, Morato, R. G, Scheffer, K. C, Carnieli, P Jr, Ferreira F, Furtado, M. M, Kashivakura, C. K, Silveira, L, Jacomo, A. T. A, Lima, E. S, de Paula, R. C, and May-Junior, J. A (2010). Detection of rabies virus antibodies in Brazilian free-ranging wild carnivores. J Wildl Dis. 46(4):1310–1315. https://doi.org/10.7589/0090-3558-46.4.1310 PMID: 20966286.

Ma, X, Niezgoda, M, Blanton, J.,D, Recuenco, S, Rupprecht, C. E (2012). Evaluation of a new serological technique for detecting rabies virus antibodies following vaccination. *Vaccine*, 30(36):5358–62. https://doi.org/10.1016/j.vaccine.2012.06.037.

Moore S.M., and Hanlon CA (2010) Rabies-Specific Antibodies: Measuring Surrogates of Protection against a Fatal Disease. PLoS Negl Trop Dis. 4(3): e595. https://doi.org/10.1371/journal.pntd.0000595.

Moore, S. M (2021). Challenges of Rabies Serology: Defining Context of Interpretation. Viruses, 13, 1516. https://doi.org/10.3390/v130815.

Moore, S. M. (2021). Challenges of Rabies Serology: Defining Context of Interpretation. Viruses, 13, 1516. https://doi.org/10.3390/v130815.

Morters, M. K, Bharadwaj, S, Whay, H. R, Cleaveland, S, Damriyasa, I, and Wood, J. L (2014). N., Participatory methods for the assessment of the ownership status of free-roaming dogs in Bali, Indonesia, for disease control and animal welfare, Preventive Veterinary Medicine, Volume 116, Issues 1–2,: 203-208, ISSN 0167-5877, https://doi.org/10.1016/j.prevetmed. 2014.04.012.

Ogunkoya, A. B, Beran, G. W, Umoh, J. U, Gomwalk, N. E, and Abdulkadir, I. A (1990). Serological evidence of infection of dogs and man in Nigeria by lyssaviruses (family Rhabdoviridae). Trans. R Soc Trop Med Hyg.; 84 (6): 842–5. https://doi.org/10.1016/0035-9203(90)90103

Ohore, O. G, Emikpe, B. O, Oke, O. O, Oluwayelu, D. O (2007). The Sero-profile of rabies antibodies in companion urban dogs in Ibadan, Nigeria. *J Anim Vet Adv*.;**6**(1):53–56. https://scienceworldjournal.org/article/view/23833

Olugasa, B. O, Aiyedun, J. O, Emikpe, B. O (2011). Prevalence of antibody against rabies among confined, free-roaming, and stray dogs in a transit city of Nigeria. Veterinaria Italiana.; 47:453–60. https://www.ncbi.nlm.nih.gov/pubmed/22194227.

Realegeno, S., Niezgoda, M., Yager, P. A., Kumar, A., Hoque L., Orciari L., Sambhara S., Olson V. A., and Satheshkumar, P. S (2018). An ELISA-based method for detection of rabies virus nucleoprotein-specific antibodies in human antemortem samples. *PLoS ONE,* 13(11): e0207009. https://doi.org/10.1371/journal.

Rimal, S, Ojha, K. C, Chaisowwong, W, Shah, Y, Pant, D. K, and Sirimalaisuwan A (2020). Detection of virus-neutralising antibodies and associated factors against rabies in the vaccinated household dogs of Kathmandu Valley, Nepal. *PLoS ONE* 15(4): e0231967. https://doi.org/10.1371/journal.

Singh, R, Singh, K. P., Cherian, S., Saminathan, M., Kapoor, S., Manjunatha Reddy, G. B., Panda, S., and Dhama, K. (2017). Rabies – epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review, *Veterinary Quarterly*, 37:1212-251, doi: 10.1080/01652176.2017.1343516.

Smith, J. S, Yager, P. A, and Baer, G. M (1973). A rapid, reproducible test for determining rabies-neutralizing antibody. *Bull World Health Org.* 1973; **48**:535 541. https://pmc.ncbi.nlm.nih.gov/articles/PMC2482941/pdf/bullwho00178-0027.pdf The Editors of Encyclopedia Britannica. (2025). https://www.britannica.com/place/Plateau-state-Nigeria

Trujillo-Rojas L.M., Gutierrez-Gutiérrez M. and Ruiz-Saenz J (2018). Low level of the immune response against rabies virus in dogs and cats, a cross-sectional study in sheltered animals, Santander, Colombia. Pesquisa Veterinária Brasileira 38(11):2109-2116.https://doi.org/10.1590/1678-5150-PVB-5997

Wallace, R. M., Pees, A, Blanton, J. B, Moore, S. M (2017). Risk factors for inadequate antibody response to primary rabies vaccination in dogs under one year of age. *PLoS Neglected Tropical Diseases*, 11(7): e0005761. https://doi.org/10.1371/journal.

Wasniewski M, Almeida I, Baur A, Bedekovic T, Boncea D, Chaves LB, David D, De Benedictis P, Dobrostana M, Giraud P, Hostnik P, Jaceviciene I, Kenklies S, König M, Mähar K, Mojzis M, Moore S, Mrenoski S, Müller T, Ngoepe E, Nishimura M, Nokireki T, Pejovic N, Smreczak M, Strandbygaard B, Wodak E and Cliquet F (2016). First international collaborative study to evaluate rabies antibody detection method for use in monitoring the effectiveness of oral vaccination programmes in fox and raccoon dog in Europe. *J Virol Methods.*; **238**:77–85. doi: 10.1016/j.jviromet.2016.10.006.

Wasniewski, M, and Cliquet, F (2012). "Evaluation of ELISA for detection of rabies antibodies in domestic carnivores," Journal of Virological Methods, vol. 179, no. 1, pp. 166–175, [11] T. DOI: 10.1016/j.jviromet.2011.10.019

Welch, R. J, Anderson, B. L, Litwin, C. M (2009). An evaluation of two commercially available ELISAs and one in-house reference laboratory ELISA for the determination of human anti-rabies virus antibodies. *J Med Microbiol*., 58(Pt 6):806–10. https://doi.org/10.1099/jmm.0.006064-0.

World Health Organisation (2013). Expert consultation on rabies. Second report. WHO Tech. Rep. Ser.., 982, p. 1–139.

World Health Organization (2018). WHO expert consultation on rabies: third report. http://apps.who.int/iris/bitstream/handle/10665/272364/9789241210218-eng.pdf. Accessed 6 June 2018.

World Organization for Animal Health (2018). Rabies (infection with rabies virus) and other lyssaviruses. OIE Terrestrial Manual. 2018; 2.1.17.

Wosu, L. O, and Anyanwu, H. N. (1990). Sero-epidemiological survey of rabies virus antibodies in nonvaccinated dogs in Nsukka Environs, Nigeria. *J Commun Dis.* ; **22**:124 - 128. https://doi.org/10.1111/j.1439-0450.1990.tb01025.x PMID: 2346070

Wyatt, J. (2007). Rabies—update on a global disease. *The Pediatric Infectious Disease Journal*, *26*(4): 351-352. doi: 10.1097/01. inf.0000258776.47697.97