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Comparative Study of Postvaccinal Heamatological and Immunological Responses to Three Brands of Rabies Vaccines in Nigerian Local Dogs (Canis lupus familiaris)

Adejumobi O.A.,[1] Omobowale T.O.,[1] Alaba B.A.,[1] Ohore O.G.,[2] Adebayo A.K.,[1] Fagbohun O.A.,[3] and Nottidge H.O.[1]

[1]Department of Veterinary Medicine, University of Ibadan, Nigeria.
[2]Department of Veterinary Pathology, University of Ibadan, Nigeria.
[3]Department of Veterinary Microbiology, University of Ibadan, Nigeria.

Corresponding author: muyenko@yahoo.com
Telephone: +2348033728003

Abstract

Rabies is still a public health burden in Nigeria despite vaccination efforts. This may be due to impotent vaccines being administered to dogs. Therefore, this study was conducted to test the immunogenicity of some commonly-used anti-rabies vaccines (ARV) in Nigeria. Twenty four certified healthy local breed puppies were divided into 3 groups of 8 dogs per group, randomly selected equally based on sex. The groups were tagged groups A, B and C. Three brands of ARV coded A, B and C were administered to their respective dog groups. Blood samples of dogs were collected pre-vaccination (Day 0), Days 7, 14, 28 and 90 post vaccination. The blood samples were evaluated for haematological parameters and the serum samples were analysed using indirect ELISA to evaluate seroconversion. Statistical analyses were carried out using ANOVA and Student's t-test. The three brands of vaccines elicited seroconversion by day 7 post-vaccination as there were significant ($p<0.05$) increases in the antibody titres (IU/ml) A (0.45±0.06), B (0.64±0.14), C (0.78±.06) following the administration of the vaccines compared to the titers found in A (3.18±0.16), B (2.78±0.38) and C (4.20±0.17) on day 0. There were also significant increases ($P<0.05$) in the antibody titres on days 14, 21, 28, 90 compared to day 0. Comparatively, all the vaccines were immunogenic but vaccine C induced the highest level of antibody titre. There was no significant difference in antibody responses on specific days based on gender. All the three brands of evaluated in this study are immunogenicity and suitable for routine vaccination of dogs.

Keywords: Anti-rabies vaccines, Local dogs, ELISA, Antibody, Vaccination

Introduction

Rabies is a worldwide threat of which the domestic dog (Canis familiaris) is the main source of exposure and primary agent of the human disease (Ojo et al., 2016). Global estimates indicate that approximately ten million persons are bitten by animals around the world yearly and considered for prophylaxis and treatment against rabies (WHO, 2005). The disease causes 55,000 annual mortalities with 56% (30,800/55,000) and 44% (24,200/55,000) occurring in Asia and Africa respectively (WHO, 2005). Rabies is endemic in most parts of Africa, Asia, the Americas and Europe, where the virus is maintained in certain species of mammals (Acharya et al., 2012). In Africa and Asia (with the exception of Japan and Singapore), rabies is prevalent in almost the entire territory with a stable pattern (WHO, 2015).

Among the diseases of viral aetiology, rabies is unique in its distribution and range of susceptible hosts, since the virus is infectious to all warm-blooded animals (Consales et al., 2007).

Rabies is a highly infectious fatal zoonotic viral disease which is highly prevalent in developed and developing countries of the world (Singathia et al., 2012). Despite numerous efforts that have been made to control the outbreak of the disease through various public health campaigns and preventive strategies, the disease still accounts for a high number of human deaths every year with the highest number of cases reported in Asia and Africa (Wyatt, 2007). The disease leads to central nervous dysfunction and the majority of cases terminate fatal (Yousaf et al., 2012).

Dogs are the most important reservoirs of rabies (Gongal and Wright, 2011). Human cases have been reported due to exposure to rabid dogs and wildlife. Mongoose (Herpestes spp), jackals (Canis aureus), foxes (Vulpes bengalensis) and wolves (Canis lupus) have been incriminated as wildlife reservoirs of rabies.

Rabies is associated with death, central nervous dysfunction, respiratory collapse and paralysis and it affects all warm-blooded animals (Constable et al., (2017). The disease is majorly transmitted by dogs and less commonly cats. It is also transmitted by skunks and vampire bats in the urban areas (Binepal et al., 1991). It was also reported that in parts of the world where domestic animal control and vaccination programs are poorly done, dogs still remain the most important reservoir of rabies (WHO, 2014).

Rabies is a neglected disease in most countries in Africa and Asia (Meslin et al., 1994, Knobel et al., 2005) despite affecting tens of thousands of people annually (Warrel and Warrel, 2004) Rabies is associated with huge economic loss, the cost include direct medical cost from post exposure treatment including

the use of biological, (vaccines and immunoglobulin), bite wound dressing, cost of antibiotics, tetanus toxoid and materials. In addition, the indirect cost include patients' out of pocket expenditure for transportation and loss of income during the period of visiting rabies treatment centres, cost of controlling rabies among dogs, livestock losses and surveillance cost. Moreover, the cost of post-exposure treatment excluding the cost of accompanying the patient to the clinic was reported to be US$ 39.57 in Africa and US$ 49.41 in Asia (Knobel et al., 2005).

Fortunately, this disease is vaccine preventable as Meslin et al. (1994) reported that vaccination of dogs against rabies has considerably reduced human infection through rabid dogs and that it is the most effective control measure in medium to long term. Binepal et al. (1991) also reported that control of rabies can be achieved by vaccination, control of stray dogs and wildlife carrier of the virus. It was also reported that there is no other effective measure to control rabies in man and animal other than vaccination, control of stray dogs and cat and wild life reservoir of rabies virus (Ohore et al., 2007). Anti-rabies vaccines available for dogs and wild animals include modified live rabies vaccines, inactivated rabies vaccines, Oral modified live rabies vaccines, oral and parenteral recombinant rabies vaccines, Nucleic acid vaccines. The parenteral vaccines are available for dog and cats while the oral vaccines are for feral and wild animals (Greene and Rupprecht, 2012).

Anti-rabies vaccine elicit the production of neutralising antibodies, a form of humoral immune response which is protect vaccinated animals including dog and cat from rabies (Ohore et al., 2007, Overduin et al., 2019). Inactivated vaccines have been found to be effective in neonatal puppies and kittens but the effectiveness can be affected by the Maternal Derived Antibody (MDA). Generally, anti-rabies vaccination is done at the age of 2 months and repeated 1 year later (Greene and Rupprecht, 2012). Variation in duration, onset and peak periods of immunity development has been debated by many researchers. Moore et al. (2006) reported humoral response 3 days after a primary vaccination in there study. Some researchers also reported that thy found plasma cells, an evidence of immune response to rabies vaccination on day 7 to day 14 post vaccination while the found memory B-cells from day10 up to day 28in their own study (Overduin et al. (2019). The major factors that cause variation in the outcomes of vaccinations in Africa are inconsistencies in vaccination programmes, storage and handling of vaccines and other factors that cause vaccine failure (Ohore et al., 2007). Rabies have been reported in vaccinated dogs (Murray et al., 2009) hence the curiosity about the efficacy

of vaccines in preventing the disease especially in Nigeria where factors like cold chain break as a result of irregular power supply and other related factors affect the quality of vaccines.

This study was therefore undertaken to evaluate post-vaccinal antibody response and immunogenicity of three brands of canine anti-rabies vaccines commonly used in Nigeria.

Materials and Methods

Experimental Dogs

Twenty-four Nigerian local breed of dogs, 8 to 14 weeks old, comprising 12 males and 12 females were purchased from local dog breeders in Ibadan, Oyo-State, Nigeria. The dogs were kept at the Department of Veterinary Medicine Research kennel and separated into 3 groups (A-C) of 8 dogs (4 males and 4 females in each group). Dogs were acclimatized for a period of three weeks. They were kept in a facility that allowed good ventilation and 12 hours of day and night and were fed home cooked meals. Animal handling and research procedures were conducted according to the international guidelines for animal care and ethics written by Wolfenson and Lloyd (2013).

Anti-Rabies Vaccines

Three different commercial brands of anti-rabies vaccines were randomly selected from those commonly used in Nigeria. These selected brands of ARV include, BiomedR ARV (PVT Ltd, India), BiocamR ARV (Biovcta, Czechia) and LocalR ARV (NVRI, Vom, Nigeria) designated as A, B and C, respectively, were administered to each of the groups of dogs tagged A, B, and C, respectively.

Collection of Sample

Blood samples were collected via the jugular vein pre-vaccination on Day 0 and subsequently at weekly interval (days 7, 14, 21 and 28); and thereafter on Day 90 post vaccination. About 5ml of blood collected by jugular venepuncture from each dog was divided into EDTA and plain bottles for haematology and serology respectively. The serum samples harvested were stored at -20oC until they were analysed for rabies antibody in the Clinical Pathology Laboratory, Department of Veterinary Pathology, University of Ibadan Nigeria.

Laboratory Analysis

Indirect Enzyme-Linked Immunosorbent Assay Technique for Rabies Antibody Detection

The indirect ELISA technique used was described by Ohore et al. (2007) Optimal working dilutions obtained following checkerboard titration were antigen 1:500, sera 1:100 and rabbit anti-dog horse radish peroxidase IgG (Sigma, USA) 1:1000. The cut-off sample to positive (SP) ratio was calculated to be 0.25, which corresponded to twice the optical density (OD) value of the

negative control serum. The results were read using the Top-Read Micro plate ELISA reader (Axiom, Germany) and were considered valid when the difference between the mean OD of the positive and negative control sera was greater than 0.2 and the mean OD of the negative control serum was less than or equal to 0.25. Samples with SP ratio greater than the cut-off value of 0.25 were considered to have optimal rabies virus antibody levels (positive), those with SP ratio lower than the cut-off had suboptimal antibody levels while serologically negative samples were those with zero SP ratio. SP ratios were calculated and interpreted as follows: The titer of the sample = Sample mean OD – Mean of Negative Control / Mean of Positive Control – Mean of Negative Control.

Haematological Analysis

The haematological analysis was carried out in the Clinical Pathology Laboratory, Department of Veterinary Pathology, University of Ibadan, Nigeria. PCV was determined by the micro-haematocrit method, haemoglobin concentration was estimated by cyano-methaemoglobin method. Red and white blood cells were enumerated using the improved Neubauer slide method (Abayingin and Ekun 2019).

Statistical Analysis

Mean values of haematological parameters and ELISA antibody titers were calculated using descriptive statistics and the values obtained were compared for significant differences using ANOVA and Student t-test (Amelia 1998).

Results

Indirect Enzyme Linked Immunosorbent Assay (ELISA) Technique Results

The mean pre-vaccination antibody titer values of dogs in groups A, B and C were 0.45±0.06, 0.64±0.14 and 0.78±.06 (IU/mL) respectively. In group A, statistically significant increase ($P<0.0001$) was observed in the mean antibody titers (IU/mL) on Day 7 (3.18±0.16), day14 (2.68±0.18), day 21 (4.04± 0.26), Day 28 (2.99±0.30) and Day 90 (3.53±0.09) when compared to the basal mean antibody titers (Fig. 1).

There were no significant differences in the antibody titres from Day 7 to Day 90. Similarly, a significant increase was observed in the mean antibody titers in group B as the mean titers increased steadily from Day 7 (2.78±0.38) to Day 14 (3.26±0.37) and 21 (3.26±0.37) with respective antibody titre when compared with the titer value on Day 0. The mean titer values (IU/mL) of group B on Days 28 (3.08±0.32) and 90 (3.56±0.34) were also significantly higher when compared with that of Day 0 (Fig. 1). There was a significant difference in the mean titer values (IU/mL) of group C dogs given another brand of vaccine tagged vaccine C on Day 7 (4.20±0.17), Day 14 (3.68±0.40), Day 21 (4.02±0.49), Day 28 (3.76±0.27) and Day 90 (3.41±0.23) (Fig.1). The antibody titers fluctuated generally across the groups and over the periods of this study. In all the groups the antibody titre increased steadily on day 7 but decreased through Day 14 in group A (2.68±0.18) and C (3.68±0.40), group B antibody titer, however, increased (3.26±0.37). On Day 21, an increase was observed in the three groups A, B and C with their respective values (4.04±0.26, 3.88±0.66 and 4.02±0.49). On Day 28, a slight non-statistically significant decrease was observed in each of groups A (2.99±0.30), B (3.08±0.32), C (3.76±0.27) when compared to their respective titre on day 21. The antibody titer values rose again on day 90 in groups A (3.53±0.09) and B (3.56±0.34) when compared to the values of Day 28. The value of the antibody titer for group C however decreased on Day 90 (3.11±0.23) when compared to that of Day 28 (3.76±0.27) (Fig. 1).

The difference in the mean antibody titres (IU/ml) along the column with the same superscript a and b statistically significant ($P<0.05$). Means with the same letter along the column are not significantly different ($P>0.05$), while those with b are significantly different ($P>0.05$) when compared to the titre on day 0.

Packed Cell Volume (PCV)

The mean PCV values for all groups from Day 0 to Day 28 and Day 90 post-vaccination were given in Table 2. The PCV values were within normal range. There was no statistical difference in the PCV values of dogs across the groups and during the 90-Day period of sampling.

Table 2: Mean PCV (%) of Nigerian local breed of dogs vaccinated with three brands of anti-rabies vaccine

Sampling Days	Vaccine A	Vaccine B	Vaccine C
0	31.20 ± 6.61	30.60 ± 5.18	34.40 ± 2.41
7	29.60 ± 4.04	29.20 ± 6.87	33.80 ± 4.60
14	41.25 ± 5.06	32.20 ± 6.38	32.00 ± 7.42
21	33.20 ± 4.92	31.80 ± 6.80	33.20 ± 6.98
28	30.60 ± 6.84	28.60 ± 7.70	29.20 ± 5.63
90	33.20 ± 5.07	32.00 ± 3.16	31.80 ± 1.92

Haemoglobin Concentration (Hb)

The mean Hb concentrations for all the groups from Day 0 to Day 28 and Day 90 post-vaccination were given in Table 3. There was no statistical difference in the Haemoglobin concentration of dogs across the groups and during the 90-day period of sampling.

Table 3: Mean Haemoglobin (g/dl) of Nigerian local breed of dogs administered with three brands of Anti-rabies vaccine

Sampling Day	Vaccine A	Vaccine B	Vaccine C
0	10.16 ± 2.27	10.18 ± 1.68	11.44 ± 0.84
7	10.02 ± 1.35	9.62 ± 2.37	11.12 ± 1.52
14	12.50 ± 3.16	10.58 ± 2.21	10.84 ± 2.53
21	11.20 ± 1.45	10.86 ± 2.30	11.28 ± 2.16
28	10.32 ± 2.27	9.50 ± 2.71	9.88 ± 2.09
90	11.04 ± 1.90	10.58 ± 0.99	10.42 ± 0.86

Red Blood Cells (RBC)

The mean RBC counts for all the groups from Day 0 to Day 28 and Day 90 post-vaccination were given in Table 4. There was no statistical difference in the RBC counts of dogs across the groups and during the 90-day period of sampling.

Table 4: Mean RBC count (106 cells/µl) of Nigerian local breed of dogs administered with three brands of Anti-rabies vaccine

Sampling Day	Vaccine A	Vaccine B	Vaccine C
0	5.08 ± 1.30	4.94 ± 0.57	5.64 ± 0.49
7	4.43 ± 0.79	4.76 ± 1.28	5.44 ± 0.80
14	6.01 ± 1.61	5.32 ± 0.90	5.35 ± 1.35
21	5.47 ± 0.97	5.33 ± 1.12	5.61 ± 1.31
28	5.18 ± 1.17	4.84 ± 1.29	4.75 ± 0.96
90	5.36 ± 0.94	5.17 ± 0.47	5.14 ± 0.41

Monocytes

There was no statistical difference in the monocyte values of dogs across the groups from Day 0 to Day 28 and Day 90 post-vaccination (Table 5)

Table 5: Mean Monocyte counts (103cells/µL) of Nigerian local breed of dogs administered with three brands of Anti-rabies vaccine.

Sampling Days	Vaccine A	Vaccine B	Vaccine C
0	2.20 ± 0.84	2.60 ± 1.67	2.00 ± 0.71
7	2.20 ± 0.84	2.00 ± 1.00	2.40 ± 1.52
14	2.00 ± 1.00	1.60 ± 0.55	2.20 ± 0.84
21	3.20 ± 1.10	2.60 ± 1.14	3.60 ± 0.55
28	2.80 ± 1.30	3.20 ± 0.84	2.60 ± 0.89
90	1.80 ± 0.84	2.00 ± 1.00	2.20 ± 0.84

Eosinophil

There was no statistical difference in the eosinophil values of dogs across the groups from Day 0 to Day 28 and Day 90 post-vaccination (Table 6).

Table 6: Mean Eosinophil counts (103cells/μL) of Nigerian local breed of dogs administered with three brands of Anti-rabies vaccine.

Sampling Days	Vaccine A	Vaccine B	Vaccine C
0	1.60 ± 1.14	2.60 ± 0.55	2.00 ± 1.00
7	1.00 ± 0.71	2.40 ± 1.52	1.60 ± 0.89
14	1.60 ± 1.14	2.80 ± 0.45	1.20 ± 1.30
21	1.00 ± 0.71	1.00 ± 1.00	1.60 ± 0.55
28	1.60 ± 1.14	2.40 ± 0.55	2.20 ± 0.84
90	1.80 ± 1.10	2.60 ± 1.14	2.00 ± 1.00

White Blood Cells.

There was a rise in the total white blood cell count on Day 7 post vaccination which was sustained in most groups until Day 21. However, the WBC count decreased significantly ($p<0.05$) in group A on Day 28 when compared to the WBC count of groups A (Day 7) and B (Day 21) and in the mean value of group C on Day 28 when compared to that of group A (Day 7) and group B (Day 21). There was no significant difference in the WBC counts across the groups on Day 90. (Table 7)

Table7: Mean total WBC count (103cells/μL) of Nigerian local breed of dogs administered with three brands of Anti-rabies vaccine

Sampling Days	Vaccine A	Vaccine B	Vaccine C
0	8.57 ± 3.41	9.18 ± 2.28	7.53 ± 1.22
7	13.50 ± 2.80	11.68 ± 3.38	11.48 ± 4.90
14	6.83 ± 1.10	8.52 ± 3.45	11.34 ± 3.43
21	9.61 ± 4.31	13.32 ± 4.71	10.44 ± 2.67
28	6.05 ± 2.04a1b3	8.07 ± 2.95	5.02 ± 1.35a1b3
90	7.91 ± 1.43	7.56 = 1.44	8.10 ± 1.37

Platelet Count

There was no statistical difference in the platelet values of dogs across the groups and during the 90-day period of sampling (Table 8).

There was no statistically significant (P>0.05) difference in the mean PCV values of dogs across the groups and during the 90-day period of sampling. Normal PCV range in Local dogs is 24-48 % (Atata et al., 2018)

Table 8: Mean Platelet count (106cells/µL) of Nigerian local dogs administered with three brands of anti-rabies vaccine

Sampling Days	Vaccine A	Vaccine B	Vaccine C
0	260.0 ± 33.9	253.0 ± 92.1	203.2 ± 49.0
7	244.6 ± 31.0	254.2 ± 48.0	279.0 ± 58.8
14	171.2 ± 31.8	234.2 ± 55.7	226.0 ± 71.7
21	177.0 ± 58.3	188.8 ± 35.0	161.4 ± 20.5
28	238.8 ± 47.1	192.6 ± 85.0	185.8 ± 58.0
90	220.8 ± 73.3	195.4 ± 26.9	205.6 ± 40.5

There was no statistically significant (P>0.05) difference in the mean Haemoglobin values of dogs across the groups and during the 90-day period of sampling. Normal range of Hb concentration in Local dogs is 8.6-17 g/dl (Atata et al., 2018).

There was no statistically significant (P>0.05) difference in the mean red blood cell counts of dogs across the groups and during the 90-day period of sampling. Normal range of RBC count in Local dogs is 3.1-6.8 106 (cells/µl) (Atata et al., 2018).

There was no statistically significant (P>0.05) difference in the mean Monocyte count of dogs across the groups throughout the 90-day period of sampling. Normal range of monocyte count in Local dogs is 0.00-1.4 103 (cells/µl) (Atata et al., 2018).

There was no statistically significant (P>0.05) difference in the mean eosinophil counts of dogs across the groups throughout the 90-day period of sampling. Normal range of Eosinophil concentration in Local dogs is 0.00-1.7 103 (cells/µl) (Atata et al., 2018)

Means with superscripts a1 and b3 are showed statistically significant (P<0.05) difference in white blood cell count when compared with the mean values of Groups A on Day 7 and B Day 21 respectively. Normal range of WBC

count in Local dogs is 8-17 (103cells/µl) (Atata et al., 2018)

There was no statistically significant (P>0.05) difference in the mean platelet counts of dogs across the groups and during the 90-day period of sampling. Normal range of platelets count in Local dogs is 175-500X103 (cells/µl) (Atata et al., 2018)

Discussion

Endemicity of rabies in dogs in spite of routine vaccinations is a serious concern in Nigeria and this warranted testing the immunogenicity of commonly-used anti-rabies vaccines in dogs in Nigeria. Immunogenicity of anti-rabies vaccines has been evaluated by assaying neutralising antibody using fluorescent antibody virus neutralisation test (FAVN) which measures both IgG and IgM (Cliquet 1998). Enzyme Linked Immunosorbent Assay (ELISA) has also been used for this purpose by Ohore et al. (2007). In this study, ELISA was employed to quantify the antibody titres of the dogs immunised with the three brands of Anti-rabies vaccine as described by Ohore et al. (2007). The three brands of vaccine elicited protective levels of antibody by day seven of administration. In the earlier study, Ohore et al. (2007) reported uniform potency of the ARVs available in the Nigerian market. It was reported that a serum titre of 0.5 IU/ml and above of rabies specific antibody is considered protective while the titre below that is considered vaccine failure which can make such dog vulnerable to the rabies virus (Acharya et al., 2012). In this study, the antibody titre recorded as at Day 7 was high enough to protect dogs from rabies infection. This was similar to report of Overduin et al. (2019) who found humoral response and proliferation of plasma cells 7 days after a primary vaccination with rabies virus vaccine in people used for their study.

The pattern of the immune response varied slightly with vaccines at different days of sampling. On Day 7, vaccine C gave the highest response, followed by vaccine A while the vaccine B gave the least antibody titre. On Day 14, the antibody response was maintained by vaccine C, antibody titre increased more than the day seven titre and the value was higher than that of groups B and A. The antibody response to the three brands of vaccines reached their peaks on Day 21 with vaccine A producing a mean titre slightly higher than that of group C. The difference was however not statistically significant. Group B produced the least immune response on Day 21. The antibody titre declined slightly on Day 28 but the antibody titre produced by C was still the highest followed by that of group B while that of group A was the least. The last sampling was on Day 90, all the vaccine brands maintained their antibody titres with dogs in group A having the highest titre of the three groups, although the differences across the groups were not statistically significant. The result shows that the

may be attributed to other related factors that are related factors such as poor handling and storage conditions, incompetence of the health care personnel and quackery, poor knowledge about rabies, and rabies control or deliberate negligence and nonchalant attitude of dog owners in the rural and urban areas and animal related factors (Adejumobi et al., 2016). Similar findings have been reported by researchers from their studies (Adejumobi et al., 2016, Adejumobi and Omobowale, 2017). A report stated that vaccine failure may be caused by vaccine brands, sampling interval, age and origin of animals (Mansfield et al., 2004). Animal factors like sickness, immunosuppression, malnutrition and presence of maternally derived antibody (MDA) may make some dogs to fail to develop immunity (WHO, 2014). It was also reported that dogs respond differently to vaccination. Some for one reason or the other fail to develop protective immune response leaving them susceptible to the disease they were vaccinated against (Ek-kommonen et al., 1997).

The persistence of rabies can also be as a result of dog owners not having adequate knowledge about rabies control through vaccination of their pet (Adejumobi et al., 2016) or deliberately failing to immunise their dogs (Oluwayelu et al., 2015). Another probable reason for the reported persistence of rabies and inadequate vaccination coverage in Nigeria is lack of regulation and laws guiding dog ownership responsibility, registration of dogs and compulsory vaccination of dogs in the interest of the public, quackery, dog trade, dog movement and importation and lack of enforcement of the existing ones (Ohore et al., 2007, Adejumobi et al., 2016 and Adeyemi and Zezzin, 2000).

Some other researchers recommended public enlightenment, enforcement of law by relevant authority, community participation, disease reporting, (Adejumobi et al., 2016, Adejumobi and Omobowale, 2017). They further suggested that such trainings should be done in simple language that can be easily understood by the target audience (Adejumobi et al., 2016). In conclusion, it was found out from this study that the three brands of vaccines evaluated for immunogenicity were potent as they all elicited protective immune response. Therefore, to completely eradicate rabies in Nigeria, there is need to create awareness about the importance of anti-rabies vaccination. Comprehensive control measures are to be embarked upon by the government and all the stakeholders to ensure adequate education about anti-rabies vaccines and importance of vaccination in both man and animal. Control of urban and sylvatic rabies through vaccination is hereby recommended. Animals should be kept indoors in a hygienic environment and given adequate health care to keep them safe from rabies.

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Conflict of Interest
There is no conflicting interest among the authors.

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