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Assessment of immune responses to anti-rabies vaccination in dogs in Jos Metropolis, Plateau State, Nigeria

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Abstract

Rabies is a zoonotic disease of serious public health significance and has existed since 3000 BC. This study aimed to evaluate the immune responses to rabies vaccinations within the dog population in Jos metropolis, Plateau State, Nigeria. A total of 82 dogs with records of anti-rabies vaccination were purposively selected from the Veterinary Teaching Hospital, University of Jos, Plateau State, between October and December 2020. Blood samples were collected from the dog. Enzyme-Linked Immunosorbent Assay (ELISA) was utilised to determine the levels of Rabies virus neutralising antibodies in the serum samples. Results indicated that 79.27% (65/82) of the dog population had Rabies Virus Neutralising Antibody (RVNA) with a Percentage Inhibition (PI) of ≥ 40% and 67.07% (55/82) of the sampled dogs had RVNA with a PI of > 70%, equivalent to 0.5 IU/ml, while 20.73% (17/82) had no RVNA with a PI of < 40%. The analysis of immunity status based on the type of vaccines used showed no significant difference (P> 0.05; χ^2=0.6679). Additionally, the frequency of vaccine administration did not indicate a significant increase in immune response (P> 0.05; χ^2=0.12). The study identified a positive immune response in the vaccinated dogs. It is recommended that a booster dose of the anti-rabies vaccine be administered to dogs with immune response levels < 70% PI.

Keywords: Anti-rabies, Dog, Immune response, Plateau State, Vaccine

Introduction

Rabies is a deadly zoonotic disease of very serious public health importance and has existed since 3000 BC (Rupprecht, 2010). Rabies is caused by a rabies virus, which belongs to the family Rhabdoviridae and the genus Lyssavirus with 14 known genotypes (Coertse *et al.,* 2017). Only in few rabies cases where recovery of the victim has occurred were they mainly caused by the classical rabies virus (CDC, 2012). Rabies Virus Neutralising Antibodies (RVNA) can be found in both roaming and hunting dogs that have previously been exposed to the rabies virus, but the

rabies virus antibodies are usually at low levels (Oluwayelu *et al.,* 2015).

The dog is the principal host of rabies and globally accepted as the source of transmission of the disease to humans through bite (FAO, 2013). The WHO specifies a minimum of 0.5 IU/ml serum level of rabies antibodies titre for the conferment of protection against rabies (WHO, 2018).

The vaccination status of the suspected rabid dog alone should not be taken as a major factor for considering whether to initiate post exposure prophylaxis with anti-rabies vaccine or not, as human deaths have occurred even from dogs reported to have been adequately vaccinated against rabies (WHO, 2018). Lack of an adequate antibody titre surveillance system has made it quite difficult to state the level of vaccination of dogs against rabies in Nigeria (Kolawole *et al.,* 2018). A high number of human rabies deaths usually occur in developing countries, although effective and economical control measures are available; their application in some developing countries has been hindered by several economic and political factors (WHO, 2005).

Public health awareness on the dangers of rabies and annual dog anti-rabies vaccination toward the elimination of dog-mediated human rabies still needs to be intensified and sustained (Konzing *et al.,* 2021). This study aimed to determine the immune responses of owned dogs vaccinated against rabies in Jos, Plateau State, Nigeria.

Materials and Methods

Study area

The study was conducted in 2 Local Government Areas, namely Jos North Local Government Area (LGA) and Jos South Local Government Area, Plateau State.

Jos North LGA covers an area of 291 Km2, a postal code of 930001, and has a population of 729,300 at the 2006 national census (FGN, 2012). The major ethnic groups are the Anaguta and Berom, and it serves as the commercial nerve centre of Plateau State (Plateau, 2025). Jos South LGA has a total area of 510km2 serves as the de facto capital of Plateau State (Plateau, 2025). Berom is the major ethnic group and has Du, Kuru, Vwang, Gyel, and Zawan districts and a population of 506,716 (Plateau, 2025).

Sample collection

Blood samples were collected from 82 dogs at the Veterinary Teaching Hospital (VTH), University of Jos, Plateau State, from October to November 2020. Samples were collected from both exotic and indigenous dogs, and data on breed, sex, age, type of vaccine, and vaccination history were recorded. With the aid of a needle and syringe (21 G x 5 ml syringe), 3ml of blood was collected from the cephalic vein from each properly restrained dog into a labelled plain sample bottle and centrifuged at 1500g for 10 minutes (Olugasa *et al.,* 2011). The sera were carefully decanted into labelled screw-capped bottles and were stored at -200C until the samples were processed (Kolawole *et al.,* 2018; Konzing *et al.,* 2021).

Laboratory procedure

BioPro Rabies ELISA Ab kit, Czech Republic, was used according to the manufacturer's instructions. Briefly, the microtiter plate was coated with 100µl of positive control sera in A1 and B1 wells, 100µl of positive control sera in C1 and D1 wells, 100µl of control sera 1, 2, and 3 into E1, F1, and G1 wells, 100µl of serum samples into the remaining wells. The plate was covered with adhesive foil and was shaken using the vortex mixer and incubated overnight (18-24 hours) at 2-8℃. The foil was removed from the plate, and the contents of the plate were emptied and washed 6 times with washing solution. The plate was taped firmly on the adsorbent paper after the last washing step. One hundred microlitres of diluted biotinylated anti-rabies antibody was dispensed per well, it was covered with adhesive foil, and gently shaken using a vortex mixer, and was incubated for 30minutes at 37℃.

The foil was removed, and the content of the plate was emptied and washed 4 times with washing solution. The plate was then taped firmly on the absorbent paper after the last washing step. About 100µl of streptavidin peroxidase conjugate was dispensed per well and covered with adhesive foil, and gently shaken on a vortex mixer, it was incubated at 37℃ for 30 minutes. The foil was removed, and the contents of the plate were emptied and washed 4 times using the washing solution. The plate was taped firmly on the absorbent paper after the last washing step. One hundred microlitres of ready-to-use TMB substrate was dispensed per well and incubated for 15 to 30 minutes after shaking gently with the vortex mixer, and stored at 18-25℃ away from direct sunlight. About 50µl of stop solution was dispensed per well, and the result was read using the ELISA reader and the optical density (OD) at 450nm was read.

Percentage inhibition (PI)< 40% nosero-conversion

PI≥ 40%indication of sero-conversion.

PI> 70% correlate to a protective antibody titre equivalent to 0.5IU/ml.

Data analysis

Data were entered into Microsoft Excel 2016, and descriptive statistics were performed using OpenEpi version 2.3.1. Chi-square analysis was performed to test for association between the variables, like the association between local and foreign vaccines and the results were presented in tables, and a value of P< 0.05 was considered statistically significant.

Result

The findings in this study showed that 32.92% of the dogs sampled were males, while 67.07% were females (Table 1).

The result further revealed that 79.27% of the dog population had Rabies Virus Neutralising Antibody (RVNA) with a PI of ≥ 40%, and 67.07% of the sampled dogs had RVNA with a PI of > 70%, equivalent to 0.5 IU/ml, while 17 (20.73%) had no RVNA with a PI of < 40% (Table 1).

Differences in immune response based on vaccine type were not statistically significant (P = 0.216; χ^2=0.6679) (Table 2). The study showed that there was no significant difference in immune response based on frequency of vaccine administration (P = 0.369; χ^2=0.1289) (Table 3). Vaccination based on sex difference revealed that 81.4% of male dogs vaccinated showed detectable antibodies, while 78.1% of the female dogs showed detectable antibodies. However, the association in antibody titre between males and females was not statistically significant at P=0.376; χ^2=0.12 (Table 4).

Discussion

It is obvious from the findings of this study that one of the reasons for keeping dogs by some dog owners in Plateau State is for breeding, that is, raising puppies for sale (Konzing *et al.,* 2021). Considering that 32.9%

Table 1: Rabies antibody titres of owned dogs in Jos, Plateau State, Nigeria

Category of Animal	Total	Percent (%)
Male	27	32.93
Female	55	67.07
Animals having antibodies ≥ 40% but < 70%	10	12.19
Animals having antibodies ≥ 40%	65	79.27
Animals having antibodies < 40%	17	20.73
Animals having antibodies > 70%	55	67.07

Table 2: Immune response of owned dogs based on type of antirabies vaccine in Jos, Plateau State, Nigeria

Vaccine Type	Positive	Negative	Proportion at 95% Confidence Interval	χ^2	P-value
Local (NVRIVom)	31	10	75.6 (60.8-86.9)	0.6679	0.2156;
Foreign (Forte Dodge and Biocan)	34	7	82.9 (69.1-92.2)		
Total	75	17			

χ^2 = Chi-square

Table 3: Immune responses of owned dogs based on frequency of vaccine administration in Jos, Plateau State, Nigeria

Vaccine administration	Frequency	Positive	Negative	Odd ratio at 95% Confidence Interval	χ^2	P-value
1	36	30	6	83.3 (68.5-93.0)	0.1289	0.3690
> 1	56	45	11	80.4 (68.4-89.2)		
Total	92	75	17			

χ^2 = Chi-square

Table 4: Immune responses of owned dogs based on sex of sampled dogs in Jos, Plateau State, Nigeria

Animal	Male	Female	χ^2	P-value
Animals having antibodies ≥ 40%	22 (81.4%)	43 (78.1%)	0.12	< 0.376
Animals having antibodies < 40%	5 (18.6%)	12 (21.9%)		
Total	27	55		

χ^2 = Chi-square

male dogs and 67.1% female dogs were sampled for this study agrees with the study earlier carried out by Konzing *et al*. (2021) in Plateau State, who reported similar findings of more female dogs being sampled than male dogs.

The fact that 79.27% of the dog population had rabies virus neutralising antibody (RVNA) shows that the dog owners have been regular with the vaccination of their dogs against rabies and probably had good knowledge of the risk associated with rabies, considering that this study was carried out in the Jos metropolis (Kolawole *et al*., 2018). However, the findings of this study do not agree with the work of Aiyedun (2013) in Ilorin, Kwara State, who reported a lower prevalence (53.07%, 43.60% and 25% for Ilorin East, Ilorin West and Ilorin South, respectively).

Furthermore, 17 (20.73%) of the sampled dogs had no RVNA, which further confirms the call for yearly and sustained vaccination of dogs against rabies to ensure a possible global elimination of rabies by 2030, hence the need for health workers, veterinary professionals, and political leaders to be involved to help (WHO, 2017).

Interestingly, 67.07% of the sampled dogs had protective rabies antibodies at 0.5IU/ml, which was higher than 57.80% of sampled dogs having protective antibodies previously reported by Visa (2017) in Kano, Kano State and 42.60% of dogs having protective rabies antibodies earlier reported by Olugasa *et al*. (2011) in Ilorin, Kwara State. Looking at the time interval between the previous studies and the present study is three years and nine years respectively, there could have been posssible increase in the number of dogs being vaccinated in the location of the study and an increase awareness on rabies. But Kolawole *et al.* (2018) in the Federal Capital Territory (FCT), Abuja reported that 83% of sampled dogs having protective rabies antibodies higher than 67.07% reported in the present study. The fact that sampled dogs (83%) in the study of Kolawole *et al*. (2018) had more protective rabies antibodies than the sampled dogs (67.07%) in the present study could be probably be due to an increase awareness and consciousness of the dog owners concerning rabies in the FCT, Abuja, hence they had more dogs vaccinated against rabies. Furthermore, in this study it was revealed that there was no statistically significant difference between sex and antibodies titre level of the dogs, but this finding disagrees with the study of Kolawole *et al.* (2018) who reported a significant difference between sex and antibody titre level of the dog. This may also mean that antibodies titre level was not dependent on sex of the dogs. Also the immune response of the dogs did not depend on the type of vaccine and frequency of vaccination against rabies, since there was no significance between the immune response of the dogs and the type of anti-rabies vaccine administered to the dogs (Konzing *et al.,* 2015). Similarly, Visa (2017) in Kano also reported that there was no significant difference between the rabies antibody titre level of dogs and the type of anti-rabies vaccine used.

It can be concluded from this study that the majority (67.07%) of the dogs sampled had protective antibodies against rabies and that 79.26% sampled dogs sero-converted. We thus recommend annual vaccination of dogs against rabies to boost their rabies antibody titres and continuous public health awareness campaigns on the dangers of rabies.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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