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SERO-PREVALENCE OF RABIES VIRUS NEUTRALISING ANTIBODIES IN OWNED VACCINATED AND UNVACCINATED DOGS

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Abstract: Rabies is a zoonotic disease of serious public health concern worldwide, especially in rabies endemic region including Sabon Gari Local Government Area (LGA) in Kaduna State, Nigeria. Understanding the seroprevalence of rabies antibodies in dogs is critical for assessing the effectiveness of vaccination programs. This study aimed to evaluate the seroprevalence of Rabies Virus Neutralising Antibodies (RVNA) in dogs in Sabon Gari LGA, Kaduna State Nigeria from December 2022 to March 2023. Sera from 37 dogs including vaccinated (14) and unvaccinated (23) were tested RVNA using Rabies ELISA Ab kit from BioPro (Prague, Czech Republic). Result from the serological investigation revealed that only 28% of the vaccinated dogs had RVNA above the protective level. Statistical analysis revealed a significant difference ($p = 0.00$) in the RVNA titre values of vaccinated and unvaccinated owned dogs. The study revealed a significant gap in the prevalence of protective RVNA in dogs as only 28.6% of the dogs tested had RVNA above the protective threshold. This raises concern about the adequacy of rabies vaccination coverage in Sabon Gari LGA. It is critically necessary to improve and increase rabies vaccination campaigns in Sabon Gari LGA. This should include efforts to improve vaccination coverage and effectiveness in eliciting protective RVNA titers.

• Introduction

Rabies is a deadly viral disease that affects the nervous system of mammals and man with almost 100% case fatality rate (Rupprecht *et al.*, 2002). It is characterized by central nervous system irritation, followed by paralysis and death (Beeler and Ehnert, 2020). The disease is caused by a bullet shaped single-stranded RNA virus that belongs to the genus *Lyssavirus* of the family *Rhabdoviridae*. Rabies is usually transmitted by bites and to a lesser extent by contamination of open wounds and mucous membranes with saliva from rabid animals (Barecha *et al.*, 2017). Several authors have declared rabies as endemic in Nigeria (Umoh and Belino, 1979; Fagbami *et al.*, 1981; Dzikwi *et al.*, 2011a; Kia *et al.*, 2018; Al-Mustapha *et al.*, 2021). In Nigeria, many dogs are left unvaccinated against the rabies virus, due possibly to increased dog population and poor ownership practices (Ahmed *et al.*, 2000; Adaba *et al.*, 2004; Okeme *et al.*, 2020; Al-Mustapha *et al.*, 2021). Research reports have emphasized that in order to attain herd immunity in a community, up to 70% of dog population must be immunized (WHO, 2018; Knobel *et al.*, 2007). In Nigeria, vaccinated dogs' antibody titre to the rabies virus are not usually checked but are assumed to be protected (Kolawole *et al.*, 2018). Therefore, it is expedient to determine the level of titre coverage achieved through immunization in the owned dogs that have been previously vaccinated against rabies. This has become necessary as rabies in vaccinated dogs is being documented in Nigeria and other countries (Murray *et al.*, 2009). Many canine rabies endemic countries with poor dog management issues have documented that a good proportion of dogs do not seroconvert following vaccination (Albas *et al.*, 2013; Babboni *et al.*, 2014; Pimburage *et al.*, 2017). This study aimed to investigate the presence of rabies neutralising antibodies in vaccinated and unvaccinated owned adult dogs in Sabon Gari LGA, Kaduna State, Nigeria.

• Material and method

Experimental design:

The study was conducted in Sabon Gari LGA, Kaduna State, Nigeria. A cross-sectional study was used to collect blood samples from vaccinated dogs (14) and unvaccinated (23) owned dogs from December 2022 to February 2023.

Ethical Approval

Ethical approval for this study was obtained from Ahmadu Bello University Ethical Committee on Animal Use and Care (ABUCAUC) with approval number: ABUCAUC/2023/028.

Sample collection:

A total of 37 blood samples were collected randomly from vaccinated and unvaccinated dogs in Sabon Gari LGA. Current vaccine status of the dogs was determined. With the consent of the dog owners, the dogs were restrained using a leash and muzzle with the assistance of their owners. The dogs were subjected to general physical examination and basic parameters on sex and breed were duly noted and recorded. Using sterile needles and syringes, 4ml of blood was collected from each dog by venepuncture through the cephalic vein and deposited into sterile sample bottles without anticoagulants. The blood was allowed to clot, then sera were obtained by centrifugation at 3000 rpm and decanted into bijoux bottles which were stored at -20°C until analysed.

Serological analysis

Rabies ELISA Ab kit from BioPro (Prague, Czech Republic) was used to conduct the Enzyme linked immunosorbent assay. The test was carried out according to the manufacturer's instructions. The conditions of validation described by the manufacturer was implemented to interpret the results obtained for the different samples. The percentage of blocking (%PB) was calculated for each sample according to the manufacturer's specifications. For interpretation of this ELISA kit, a positive titre for rabies neutralising antibodies is indicated by a signal equal to or greater than 40% PB and negative titre is indicated by neutralising antibody levels of less than 40% PB. The manufacturer's specification indicates that serum samples with PB equal to or higher than 70% are considered as serum samples with antibody levels equal to or higher than 0.5 IU/mL based on FAVN test.

Results and discussion

Result from this study showed that only 4 (28.6%) out of the 14 vaccinated dogs had protective levels of RVNA while no dog from the unvaccinated group had RVNA above the protective level. Statistical analysis showed a significant difference ($P = 0.015$) in the titre values from RVNA of the vaccinated and unvaccinated groups of the dogs (Table 1). All the positive dogs were between the age of 1-5 years. Out of the 4 dogs that were positive, 1 (33.3%) was always confined, 2 (8.3%) were partially confined and 1 (10.0%) was never confined and roamed freely (Table 2).

Table 1: Prevalence of Rabies Virus Neutralising Antibodies in Vaccinated and Unvaccinated Owned Adult Dogs

Vaccination status	Total sampled	No. (%) Positive
Not vaccinated	23	0(0.0)
Vaccinated	14	4(28.6)
Total	37	4(10.8)

Fisher's exact test = 7.368; df = 1; p value = 0.015

Table 2: Prevalence of Rabies Virus Neutralising Antibodies Based on Different Age Groups and Confinement Status of Owned Adult Dogs in Sabon Gari LGA Kaduna State

Variables	Total sampled	Positive (%)	χ^2	df	p value
Age					
1-5 years	30	4(13.3)	1.000*	2	0.631
6-10 years	6	0(0.00)			
>10 years	1	0(0.00)			
Confinement					
Always	3	1(33.3)	1.738	2	0.419
Partial	24	2(8.3)			
Never	9	1(10.0)			
Total	37	4(10.8)			

* Fisher's exact test

An animal is considered to have protective immunity if its rabies virus neutralising antibody (RVNA) level is equal to or greater than 0.5 IU/mL, as stated by the World Health Organization in 2013. The presence of RVNA above the protective level indicates that the vaccinated dogs have developed an immune response capable of neutralising the rabies virus. This agrees with previous research on the efficacy of rabies vaccination in dogs. Previous studies (Cliquet *et al.*, 2003; Wallace *et al.*, 2017) have shown that vaccination stimulates the production of neutralising antibodies and provides immunity against rabies. The lack of detectable RVNA titres in the unvaccinated dogs could possibly be due to the fact that they have not received the rabies vaccine. The absence of detectable RVNA titres in the unvaccinated group emphasizes the vulnerability of unvaccinated dogs to rabies. This shows the importance of vaccination in conferring protection against rabies in dogs. Previous research by (Wosu and Anyanwu, 1990) have demonstrated that unvaccinated dogs are at a higher risk of acquiring and transmitting rabies, emphasizing the crucial role of vaccination in preventing the spread of the disease. Most of the dogs belonged to the age range of below 5 years (mostly 1-2 years) and had received only one shot of vaccination previously. Many authors (Seghaier C., 1999; Cliquet *et al.*, 2003; Pimburage *et al.*, 2017) have shown that administering a single dose of anti-rabies vaccination is not adequate for sustaining antibody titres for a duration of one year. A booster dose is recommended before one year to maintain the antibody titre. In this study, it was observed that the prevalence of RVNA was higher in confined dogs (33.3%) compared to those that were partially confined (8.3%) and never confined (free roaming) (10%). This finding agrees with previous research (Olugasa *et al.*, 2011) that also found higher prevalence of rabies antibody in confined dogs in Ilorin city Nigeria which was attributed to multiple potent booster vaccination received in some instances.

• Conclusion

The study revealed a significant gap in the prevalence of protective RVNA in dogs as only 10.8% of the tested dogs had RVNA above the protective threshold. This raises concern about the adequacy of rabies vaccination coverage in Sabon Gari LGA. It is critically necessary to improve and increase rabies vaccination campaigns in Sabon Gari LGA, Kaduna State. Further studies should be conducted with larger sample sizes to validate findings and get a broader picture of the proportion of dogs with protective RVNA in the study area.

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