NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., March 2023 Vol 44 (1): 65 - 72.

https://dx.doi.org/10.4314/nvj.v44i1.6 **ORIGINAL ARTICLE**

INADEQUATE ANTIBODY RESPONSE TO PRIMARY RABIES VACCINATION IN PUPPIES UNDER FOUR MONTHS OF AGE IN IBADAN, NIGERIA: A CASE REPORT

Sopeju, E. A[1].; Okpara, G[1].; Abiodun, V. T[1].; and Sopeju, S. O[1].

[1]Research and Development unit, Emmsar Veterinary Medical Diagnostic Services (EVMEDS). - www.evmeds.com

*Corresponding author: Email: adeemmanuels@gmail.com; Tel No: + 234 813 545 0706.

INTRODUCTION

Rabies, a disease caused by a virus of the family *Rhabdoviridae*, is recognized globally as a disease with zoonotic implications as it is present in all continents except Antarctica (WHO, 2021). Domestic dogs have been said to be responsible for up to 99% of rabies transmission to humans (WHO, 2021). The disease, though preventable, is incriminated to be the cause of 40,000 – 70,000 deaths annually across the globe, with almost all the deaths occurring in developing countries (Zinsstag *et al.*, 2017). In Nigeria, where the disease is endemic (Bello *et al.*, 2007; Chieloka, 2022), reports on human death due to rabies infection in Nigeria are low and have been associated with factors like under-reporting, cultural beliefs, inadequate rabies diagnostic units and poor knowledge on the mode of transmission and prevention of the disease (Oginni *et al.*, 2002). Considering the public health implications of rabies globally, a crucial decision was made during the Global Strategic Plan to End Human Death from Dog-Mediated Rabies by 2030 to develop evidence-based tools and strategies for effective dog vaccination (OIE, 2018). Vaccinating dogs against rabies virus has been the primary method of controlling the disease (Dong-Kun, *et al.*, 2013). According to the World Health Organization (WHO) and World Organization for Animal Health (WOAH) standard, a rabies neutralising antibody titre ≥ 0.5IU/ml is the post-vaccination level considered to be protective against field infection (WHO, 2012; Fooks *et al.*, 2012). A popularly recommended vaccination schedule in dogs is first done at 3 months of age to stimulate primary immune response; followed by an annual booster which is believed to sustain threshold immunity. This is largely adopted by Nigerian small animal practitioners (Adeyemi *et al*., 2005; Ohore *et al.*, 2007; Olugasa *et al*., 2011; *et al.*, 2020). This protocol also aligns with the anti-rabies manufacturers' Arega recommendation (Arega *et al.*, 2020). However, some studies have reported that about 10% of rabies vaccine-naïve dogs fail to mount protective immunity after

primary vaccination (Aubert, 1992; Moore and Hanlon, 2010). This failure has been associated with some factors like pet health, vaccine type, vaccination timing, blood sampling, maternal vaccination history, maternal antibody interference, and age. A study submitted that dogs vaccinated at an age greater than 16 weeks had a significantly higher antibody titre when compared to dogs vaccinated at a younger age (Wallace *et al.*, 2017). The study also reported that there was no statistically significant difference in the level of rabies antibody titre in dogs vaccinated at less than 12 weeks of age and those vaccinated between 12-16 weeks of age. Furthermore, more than 90% of dogs that failed at primary vaccination were able to develop protective levels of immunity when given a booster dose of the anti-rabies vaccine (ARV) (Wallace *et al.*, 2017). Following primary vaccination, previous studies have shown that the titre of antibodies against ARV generally peaks between 4 to 6 weeks post-vaccination (Kasempimolporn *et al.*, 2018). Also, eliciting immune response (Berndtsson *et al.,* 2011) and the duration of immunity (Mansfield et al. 2004, Jakel et al., 2008) have been suggested to vary based on vaccine brands and quality respectively. In line with this, for international trade, a study reported that the choice of vaccine and the time of blood sampling significantly affect the antibody titre after primary ARV vaccination (Minke, *et al.*,2009).

It then becomes important that the type of vaccine used should satisfy certain criteria in terms of immunogenicity, potency and safety to meet the market authorisation for rabies vaccines used in veterinary practice (European Pharmacopoeia, 2007). Unfortunately, most of the ARV used in Nigeria are imported and their potency locally is hardly known to properly guide the recommendations regarding vaccination protocols (Chieloka, 2022). In this same vein, a previous study in Nigeria pointed out a significant difference in antibody response to 3 different imported anti-rabies vaccines across the study groups under the same experimental conditions, particularly the absence of an immune response in all members of one group during the study (Awoyomi and Ogundipe, 2019). In Nigeria, there is a paucity of data on the post-vaccination antibody levels in puppies vaccinated with ARV. Therefore, we are writing this paper based on a case study, presenting another view of the popularly adopted vaccination regimen in Nigeria with an assumption of production of a protective antibody titre 4-6 weeks post-vaccination in anti-rabies vaccine-naive three-month-old puppies until about 15 months old (1 year later) when a booster vaccine is given. This report also emphasises the relevance of post-vaccination screening particularly for primary rabies vaccination both for international trade and non-commercial pet travel. We are also presenting this paper as a contribution to improving strategies for effective dog vaccination as part of the Global Strategic Plan to End Human Death from Dog-Mediated Rabies by 2030.

K**eywords:** Rabies, Antibody response, Vaccination, Booster, Age, Titre.

CASE REPORT

History: Two puppies (A and B) were booked in for their first ARV shots in preparation for exportation to a European country. The two dogs were of the German Shepherd breed, one male and the other female.

Patient profile:

Table 1: Patients' profiles

	Sex	Breed	Age	Colour
Puppy A	Female	German Shepherd	3 Months	Black and Tan
Puppy B	Male	German Shepherd	3 Months	White (Albino)

Primary Vaccination:

The puppies were examined; physiologic parameters were within normal ranges, both intact with no abnormalities detected on physical examination. They were both up to date with their antiparasitic prophylaxis and DHLPP vaccination for their age. They were vaccinated parenterally (intramuscularly) using Biocan® ARV primarily at 3 months of age.

Sample Collection and Transportation: Puppy A and B were bled on day 34 post-vaccination. But Puppy B had to be re-bled on day 47 post-vaccination due to sample loss in transit. 5 ml of blood was collected aseptically from both puppies using a sterile needle and syringe from the cephalic vein. The blood was carefully transferred into sample tubes containing Ethylenediaminetetraacetic acid (EDTA). Using a sterile Pasteur pipette, 2mls of plasma was then pipetted out from both blood samples into Eppendorf tubes and clearly labelled. The Eppendorf tubes were placed in separate Styrofoam boxes containing ice packs and packed into another box then transported carefully to an OIE-accredited laboratory (ARC-Onderstepoort Veterinary Institute) in South Africa for analysis. This was the only known accredited laboratory in Africa for anti-rabies antibody titre analysis.

Laboratory Analysis: In South Africa, the OIE Fluorescent Antibody Virus Neutralization (FAVN) test was used for the laboratory analysis to determine the anti-rabies antibody titre quantitatively.

Booster Vaccination: Puppies A and B were re-vaccinated at 6 months of age following the same vaccination procedures above.

Sample Collection and Transportation: Puppy A and B were bled 32 days post-booster vaccination and transported following a similar procedure above except that there was no sample loss this time and samples were tested after reaching the laboratory in South Africa.

RESULT

The result after Primary Vaccination: After analysis using the Fluorescent Antibody Virus Neutralization (FAVN) test, Puppy A and Puppy B had similar anti-rabies antibody titre (0.2 IU/ml) which were lower than the minimum protective titre (0.5 IU/ml) recommended by OIE.

The result after Booster Vaccination: Booster vaccination gave a titre value of 24.0 IU/ml and 1.5IU/ml respectively for both Puppy A and Puppy B (Table 2).

TABLE II: Dog's immune response to primary and booster anti-rabies vaccination.

	Primary Vaccination Titre (IU/ml)	**Booster Vaccination Titre (IU/ml)**
Puppy A	0.2	24.0
Puppy B	0.2	1.5

DISCUSSION AND CONCLUSION

This study is a case report following the vaccination of two 3-month-old dogs in preparation for overseas travel. The inadequate immune response to primary vaccination prompted this report. Following the common rabies vaccination protocol, it is expected that puppies vaccinated at 3 months old should have immunity against rabies (antibody titre >0.5IU/ml) until they are about 15 months old when a booster dose is given. However, we found out that Puppy A and B did not have adequate antibody titre for an average of 40 days (34 and 47 days for puppies A and B respectively) post-vaccination (Table II), hence, unfit for travel/export. This observation agrees with Wallace *et al.*, (2017) who reported that dogs vaccinated at ages greater than 16 weeks (4 months) had significantly higher antibody titre values when compared to dogs vaccinated at younger ages and that more than 90% of dogs that failed at primary vaccination would develop a protective antirabies antibody titre when given a booster vaccination. With a laboratory result of low antibody titre, and after scientific recommendations, puppy A and Puppy B were re-vaccinated at 6 months old which gave titres >0.5IU/ml precisely: 1.5IU/ml (Puppy B) and 24.0IU/ml (Puppy A) (Table II), which aligns with WHO and WAOH recommended antibody titre of ≥0.5IU/ml (WHO, 2012; Fooks *et al.*, 2012). In line with other researchers' findings and recommendations (Chieloka, 2022; Awoyomi and Ogundipe, 2019), our findings also question the current ARV vaccination protocol in Nigeria. Does the current ARV vaccination protocol need to be revisited to ensure successful efforts among relevant stakeholders towards eradicating rabies by 2030 according to the WHO global plan (OIE; 2018). In addition, Berndtsson *et al.*, 2011; Mansfield *et al.*, 2004 and Minke *et al.*, 2009 have implicated the vaccine used, in response to risk factors associated with rabies vaccine failure and inadequate immune response following primary ARV vaccination. According to Chieloka, 2022, commercially available ARVs in circulation within the country need to be reevaluated to ensure they are potent enough to elicit protective immune responses by following the manufacturer's recommendations. Furthermore, according to Wallace *et al.*, 2017, underlying health conditions are another possible reason for

vaccine failure. To answer any query that may arise from this, puppies A and B were fully examined before each vaccination. With neither overt physical clinical signs observed, nor abnormal physiological parameters recorded, the puppies were certified healthy each time. It is therefore important to note that a patient (dog) could be infected with rabies especially if the antibody titre is not protective, despite being vaccinated; as our first result clearly showed. Another interesting finding was the difference in value between the male (Puppy B) and female (Puppy A) titre (Table II). This result provides a possible justification for the reports that males are more susceptible to infections of several pathogens and are more likely to develop severe diseases than females due to the high influence of sex hormones on the immune system in infants and adolescents. (Muenchhoff and Goulder, 2014; vom Steeg and Klein, 2016; Roberts *et al.*, 2001; Snider *et al.*, 2009; Bernin *et al.*, 2014). On the other hand, Jakel *et al.*, 2008, did not find sex as a significant risk factor affecting the antibody response to anti-rabies vaccination. The study rather, supported the discourse of age as a vital factor for the development of protective immunity. While we cannot make a conclusive suggestion based on our findings, due to sample size, we would recommend a further study on the effect of sex on antibody response post rabies vaccination in Nigeria. Considering the gravity of the danger associated with assumed protection against rabies in vaccinated dogs, and its implication on public health (WHO, 2021), a bespoke local, regional and national approach should be adopted to solve the problems associated with anti-rabies vaccination compliance and immune response assurance post ARV-vaccination, particularly after primary rabies vaccination in Nigeria. Also, this work is to present a gap in the services of animal diagnostic organisations in Nigeria as lack of proper rabies testing and screening equipment or kits may have allowed for the strong assumptions that maternal immunity is sufficient for puppies until 3 months and that vaccination at 3 months can protect them for the next twelve (12) months against field infection. This study therefore recommends that policies around post-vaccination testing should be put in place and implemented across the country both for local pets and imported puppies from foreign countries into Nigeria.

REFERENCES:

ADEYEMI, I. G., ADETUNJI, V. O., JAMES, V. O. and ALONGE, D. O. (2005): Ten Year (1993-2002): Retrospective Evaluation of Vaccination of Dogs Against Rabies at The University of Ibadan, Nigeria. *African Journal of Biomedical Research*, 8: 71-77

AREGA, S., CONAN, A., SABETA C. T., CRAFFORD, J. E., WENTZEL, J., REININGHAUS, B., BIGGS L., LEISEWITZ, A. L., QUAN, M., TOKA, F. and KNOBEL, D. L. (2020): Rabies Vaccination of 6-Week-Old Puppies Born to Immunized Mothers: A Randomized Controlled Trial in a High-Mortality Population of Owned, Free-Roaming Dogs. *Tropical Medicine* and *Infectious Disease*, 5(1): 45.

AUBERT, M. F. Practical Significance of Rabies Antibodies in Cats and Dogs. *Revue scientifique et technique*, 11(3):735-760.

AWOYOMI, O. J. and OGUNDIPE, G. A. (2019): Evaluation of Antibody Responses of Dogs Vaccinated with Three

Imported Inactivated Rabies Vaccines in Nigeria. *Alexandria Journal for Veterinary Sciences*, *60*(2).

BELLO, M. L. B. and USMAN, B. (2007): A Fifteen-year Retrospective Study of the Prevalence of Rabies in Bauchi State, Nigeria. *Nigerian Veterinary Journal*, 28(2):18–23.

BERNDTSSON, L. T., NYMAN, A. K. J., RIVERA, E. and KLINGEBORN, B. (2011): Factors Associated with The Success of Rabies Vaccination of Dogs in Sweden. *Acta Veterinaria Scandinavica*, 53(1):1-7.

CHIELOKA, O. S. (2022). An outbreak investigation of a case of canine rabies in Ebonyi State Nigeria, from the 9th to 14th of April, 2022. *PAMJ-One Health*, *8*(6).

CHIELOKA, O. S. (2022). Elimination of canine rabies in Nigeria: existing gaps and the way forward? *PAMJ-One Health*, *9*(23).

DONG-KUN, Y., HA-HYUN, K., KYUNG-WOO, L. and JAE-YOUNG, S. (2013): The Present and Future of Rabies Vaccines in Animals. *Clinical and Experimental Vaccine Research*, 2:19-25.

FOOKS, A., HORTON, D., MULLER, T., FREULING, C. and RUPPRECHT, C. (2012): Rabies Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds, bees). 7th ed. Paris: Office International Des Epizooties: 263-282.

Global Alliance for Rabies Control. Zero by 2030: The Global Strategic Plan to End online: https://apps.who.int/iris/bitstream/handle/10665/272756/9789241513838-eng.pdf?ua=1 (accessed on 28 November 2019).

Global Strategic Plan to End Human Deaths from Dog-Mediated Rabies by 2030 [Internet]. OIE; 2018. Available from: https://oiebulletin.com/?p=4466

HANNAH, B. and HANNA, L. (2014): Sex Bias in the Outcome of Human Tropical Infectious Diseases: Influence of Steroid Hormones, *The Journal of Infectious Diseases*, 209(3): S107–S113

JAKEL, V., KÖNIG, M., CUSSLER, K., HANSCHMANN, K. and THIEL, H. J. (2008): Factors Influencing the Antibody Response to Vaccination Against Rabies. *Journal of Developmental Biology (Basel)*, 8(131): 431-437.

JENNIFER, C. N., RACHEL, C. L. B., LORA, B., SHANSHAN, Z., JAMES, B., JAMES, G. D., SIMON, J. H., STEVEN, J. J., NICOLA, P. K., ALLISON, L. N., KENNETH, M. Z. and LISA, A. J. (2009): Compliance with Multiple-Dose Vaccine Schedules among Older Children, Adolescents and Adults: Results from a Vaccine Safety Datalink Study. *American Journal of Public Health*, 99: S389-S397

KASEMPIMOLPORN, S., JITAPUNKUL, S. and SITPRIJA, V. (2008): Moving towards the elimination of rabies in Thailand. *Journal of the Medical*

Association of *Thailand*, 91: 433–7.

KLEVAR, S., HOGASEN, H. R., DAVIDSON, R. K., HAMNES, I. S., BERNDTSSON, L. T. and LUND, A. (2015): Cross-Border Transport of Rescue Dogs May Spread Rabies in Europe. *Veterinary Record*, 176(26): 672. doi:10.1136/vr.102909

MINKE, J. M., BOUVET, J., CLIQUET, F., WASNIEWSKI, M., GUIOT, A. L., LEMAITRE, L. and GUIGAL, P. M. (2009): Comparison of antibody responses after vaccination with two inactivated rabies vaccines. *Veterinary microbiology*, *133*(3): 283-286.

MOORE, S. M. and HANLON, C. A. (2010): Rabies-Specific Antibodies: Measuring Surrogates of Protection against a Fatal Disease. *PLOS Neglected Tropical Diseases*, 4(3): e595.

MUENCHHOFF, M. and GOULDER, P. J. R. (2014): Sex Differences in Pediatric Infectious Diseases, *The Journal of Infectious Diseases*, 209(3): S120–S126

OGINNI, F. O., AKINWANDE, J. A., FAGADE, O. O., AROLE, G. F. and ODUSANYA, S. A. (2002). Facial dog bites in Southwestern Nigerian children: an analysis of eight cases. *Tropical Doctor*, *32*(4): 239–240. https://doi.org/10.1177/004947550203200423

OHORE, O. G., EMIKPE, B. O., OKE, O. O. and OLUWAYELU, D. O. (2007): The Seroprofile of Rabies Antibodies in Companion Urban Dogs in Ibadan, Nigeria. *Journal of Animal and Veterinary Advances* 6(1): 53-56.

OLUGASA, B. O., AIYEDUN, J. O. and EMIKPE, B. O. (2011): Prevalence of Antibodies Against Rabies Among Confined, Free-Roaming and Stray Dogs in A Transit City of Nigeria. *Veterinaria Italiana*, 47(4): 453-460.

Organization of World Health. WHO Expert Consultation Rabies. TRS 982 ed. Geneva: World Health Organization; 2012. Rabies. Key Facts. Published 17 May 2021 (Accessed 15 August 2022). Available from: https://www.who.int/news-room/fact-sheets/detail/rabies

ROBERTS, C. W., WALKER, W. and ALEXANDER, J (2001): Sex-associated Hormones and Immunity to Protozoan Parasites. *Clinical Microbiology Reviews*, 14: 476–488.

SNIDER, H., LEZAMA-DAVILA, C., ALEXANDER, J. and SATOSKAR, A.R. (2009): Sex Hormones and Modulation of Immunity against Leishmaniasis. *Neuroimmunomodulation*, 16 (2): 106-113.

VOM STEEG, L. G. and KLEIN, S. L. (2016): SeXX Matters in Infectious Disease Pathogenesis. *PLoS Pathogens*, 12(2): e1005374.

WALLACE, R. M., PEES, A., BLANTON, J. B. and 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.

WHO Africa. Nigeria Joins the World in Raising Awareness on Rabies. Published 27 September 2019 (Accessed 15 August, 2022). Available from: https://www.afro.who.int/news/nigeria-joinsworld-raising-awareness-rabies

ZINSSTAG, J., FRANZISKA, A., MONIQUE, L., ROLANDE, M., ASSANDI, O., SERVICE, N., IDRISS, O. A., CELINE, M., DAUGLA, D. M., PAUL, G. C. and NICOLE, P. (2017): CostEstimate and Proposal for a Development Impact Bond for Canine Rabies Elimination by Mass Vaccination in Chad. *Acta Tropica*, 175: 112-120

ZINSSTAG, J., LECHENNE, M., LAAGER, M., MINDEKEM, R., NAÏSSENGAR, S., OUSSIGUÉRÉ, A., BIDJEH, K., RIVES, G., TESSIER, J., MADJANINAN, S., OUAGAL, M., MOTO, D. D., ALFAROUKH, I. O., MUTHIANI, Y., TRAORÉ, A., HATTENDORF, J., LEPELLETIER, A., KERGOAT, L., BOURHY, H., DACHEUX, L., STADLER, T. and CHITNIS, N. (2017): Vaccination of Dogs in an Africa City Interrupts Rabies Transmission and Reduces Human Exposure. *Science Translational Medicine*, 9 (421).