ORIGINAL ARTICLE

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

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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.

Keywords: 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

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Breed</th>
<th>Age</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puppy A</td>
<td>Female</td>
<td>German Shepherd</td>
<td>3 Months</td>
<td>Black and Tan</td>
</tr>
<tr>
<td>Puppy B</td>
<td>Male</td>
<td>German Shepherd</td>
<td>3 Months</td>
<td>White (Albino)</td>
</tr>
</tbody>
</table>

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 1.5 IU/ml respectively for both Puppy A and Puppy B (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Primary Vaccination Titre (IU/ml)</th>
<th>Booster Vaccination Titre (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Puppy A</strong></td>
<td>0.2</td>
<td>24.0</td>
</tr>
<tr>
<td><strong>Puppy B</strong></td>
<td>0.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**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 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.

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