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Michael Okpara University of Agriculture

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Post Vaccination Vitamins` Treatments and PHA Antibody Testing

Maduike Chiehiura Onwubiko Ezeibe[α], Kingsley John Jaja[σ], Ijeoma Favour Ezeibe[ρ], Ijeoma Joy Ogbonna[Ↄ] & Clara Amaka Akpan[¥]

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Rabies antibody titres in dogs (aged ≥ 3 months) which had been vaccinated with the Ptovac® Anti-rabies vaccine brand and administered B-vitamins, post vaccination, in Nigeria, were assessed by passive haemagglutination (PHA) test. The groups were named: Treated with B-vitamins (Vetzyme®) for one day post vaccination; Treated with B-vitamins for two days post vaccination; Treated with B-vitamins for three days post vaccination and Control. Two weeks post vaccination, serum from each dog was tested for rabies antibodies by PHA. Antibody titres in the groups differed, significantly (P≥ 0.05), from 24.74±0.08 in the control to 64.08±0.03 in Treated with B-vitamins for one day post vaccination, 89. 29±0.17 in Treated with B-vitamins for two days post vaccination and 140.73±0.14 in Treated with B-vitamins for three days post vaccination. Adopting post vaccination B-Vitamins` treatment and routine assessment of immune response of dogs to anti-rabies vaccinations could improve the success of rabies control-efforts in the country.

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Author α σ ρ Ↄ ¥: College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike-Nigeria.

I. INTRODUCTION

Rabies is fatal encephalitis, caused by *Rabies virus* (Nandi and Kumar, 2011). It is a zoonosis that occurs in all parts of the world, accounting for more than 59,000 deaths per year (WHO, 2013). Of human deaths caused by rabies, 56 % occur in Asia while 44% is in Africa (Bourhy *et al.*, 2010, WHO, 2013; OIE, 2016). The disease affects all warm blooded animals (Ogunkoya *et al.*, 2003), both wild and domestic including man. This wide host range makes its control difficult.

Main method of rabies control in most countries is annual vaccination of dogs and cats. Such vaccinated animals need to be tested to ensure they have high levels of rabies antibodies but presently, the method of assessing effectiveness of anti-rabies vaccination processes is by challenging vaccinated laboratory animals with the virus (WHO, 2013).

Virus-neutralization tests to assess immune response in vaccinated animals also involves challenging laboratory animals with the live virus. By such methods antibody titre of 0.5 IU/ml has been suggested as protective (WHO, 2013) but it has also been reported that vaccinated animals with lower antibody levels survived RABV challenge. Other reports also have it that some animals with neutralizing antibody titres at time of challenge came down with the disease (Aubert, 1992) though such animals were better protected against RABV than those without detectable virus neutralizing antibodies (Aubert, 1992).

The type of vaccine used, number of vaccinations, intervals between vaccinations and blood sampling, age at vaccination, sex, reproductive status, size and breed can influence antibody response of animals to anti-rabies vaccines (Mansfield *et al.*, 2004). Also, it has been reported that in post-vaccination serological studies, the percentage of animals with inadequate titres range between 3.10 to 8.10 % for dogs (Minke *et al.*, 2008) and 2.85 % for cats (Mansfield *et al.*, 2004).

According to WHO recommendations, titre of 0.5 IU/ml or its equivalent is adequate vaccination-response and a booster vaccination is recommended once the level goes lower. Despite annual vaccination of Nigerian dogs, records still show that dog bites are still responsible for a very high percentage of cases of human rabies in Nigeria (Ogunkoya, 2008). Yet the immune status of Nigerian dogs (even when vaccinated) is not known.

There is therefore a need to adopt a simpler, cheaper and more rapid test so that anti-rabies vaccinations can be verified to ensure that vaccinated animals have protective antibody titres. Also, post vaccination Vitamins-treatment is known to enhance immune response in poultry.

It may also enhance rabies antibody titres in vaccinated dogs and so prolong the time vaccinated dogs would remain protected.

II. MATERIALS AND METHODS

Twenty Nigerian mongrel dogs, aged 3 months and above were randomly assigned to 4 groups (A, B, C and D) of 5 dogs each. They were vaccinated with a foreign anti-rabies vaccine (Provac®). Group A was given post vaccination vitamins` treatment (Vetzyme®) for one day.

Group B was given the post vaccination vitamin-treatment for 2 days, group C was treated for 3 days while group D served as control (no post vaccination vitamin- medication). Two weeks post vaccination, blood samples were collected from each dog for passive haemagglutination (PHA) test, to determine titres of antibodies against rabies.

Chicken RBCs were sensitized with *Rabies virus* (Gough and Dierks, 1971). To 30 ul of 0.25 % chicken RBC, equal volumes (30 ul) of *Rabies virus* and of 0.1% chromium chloride in 0.86 % NaCl were added. The mixture was kept at room temperature for 5 minutes. Then the RBC was washed again, 3 times with 0.86 % NaCl. To obtain a highly concentrated RBC, the entire washing fluid was decanted, leaving just sufficient for homogenization of the RBCs. Haematocrit concentration of the RBC was determined by inserting a capillary tube into the container and allowing the tube to fill up to one quarter. Then its other end was sealed before it was centrifuged in a microcentrifuge at 1176xg (3000rpm) for 5 minutes. The haematocrit value was read on a PCV reader.

For the PHA test, to a clean "V" bottomed microtiter plate, 0.05ml of 0.86 % NaCl was put in each well in a row. Equal volume of the dog-serum (after inactivation) was added to the first well in the row and serially double-diluted till the last well. Then, equal volume of the sensitized chicken RBCs was added to each well and the setup was incubated at 4 °C for 1 hour. Reciprocal of highest dilution of each dog-serum sample which gave complete agglutination of the *Rabies virus*- sensitized chicken RBCs was read as titre of Rabies antibody in it. Means of antibody titres of the 4 groups of dog sera were compared by one way analysis of variance (ANOVA).

III. RESULTS

Rabies antibody titres in sera of Nigerian dogs vaccinated with anti-rabies vaccine and given post vaccination Vitamin-B treatment increased from 24.74±0.08 in the controls to 64.08±0.03 in those treated with the vitamins for 1 day, 89.29 ± 0.17 in those treated with the vitamins for 2 days and 140.73±0.14 in those treated with the vitamins for 3 days (Table 1)

Table 1: Passive Haemagglutination Antibody Titres in Nigerian Mongrel Dogs Vaccinated With Anti-Rabies Vaccine and Given Different Courses (0-3 Days) of Post Vaccination Vitamin-B Treatment

Days	Control	A(1 day)	B (2 days)	C (3 days)
	32	64	64	64
	8	32	128	128
	16	32	128	0
	4	64	64	256
	64	128	0	128
Mean	24.74±0.08	64.08±0.03	89.29±0.17	140.73±0.14

IV. DISCUSSION

Rabies is the most fatal infectious disease that is known worldwide (WHO, 2018). Although it has been neglected for some time, international health organizations (WHO, OIE and FAO) are now working together to eradicate the disease by the year 2030. They have adopted different strategies to achieve this goal, mainly through intensive vaccination of domestic and wild carnivores. New generation anti-rabies vaccines that are more economical and more efficient than conventional vaccines are now being used. There are even oral vaccines against rabies (plant based) that are showing promising results (Lucka *et al.*, 2015; Laere *et al.*, 2016).

Existence of these prophylactic measures against rabies does not diminish the fact that rabies is still a significant cause of human and animal mortality (WHO, 2018). Success of post-exposure prophylaxis against rabies infection in endemic countries is usually hindered by obstacles such as cost of vaccine, availability of post infection care and lack of awareness about the disease in rural areas. Critical prophylactic regulations such as massive vaccination of dogs often fail to achieve their aims in rabies-endemic countries due to lack of awareness and vaccine-related issues such as inadequate transportation and storage facilities for vaccines (Ullas *et al.*, 2012).

Rabies and canine distemper are the most endemic viral diseases of dogs in Nigeria to which vaccination is usually applied as a control measure (Ezeibe *et al.*, 2008; Nwoha, 2015). In Nigeria, though rabies has been responsible for many human deaths and suffering, vaccination has been poorly implemented. Shortcomings in anti- rabies vaccination in Nigeria include low coverage and poor compliance rates as in most other developing countries (Fagbami *et al.*, 1981).

Challenges to use of vaccine in rabies control include, high cost of materials, lack of reagents, lack of biologics, lack of chemicals, lack of consumables and other supplies, lack of constant electric power and failure of governments to enforce legislations on annual vaccination of dogs and cats (Foggin and Swanepoel, 1985). Also, there are problems of lowered vaccine potency due to improper cold chain, failure to achieve sufficient herd immunity after vaccination and antigenic variations (Foggin and Swanepoel, 1985).

In this study, post vaccination treatment of the Nigerian dogs with Vitamins improved their antibody responses to the vaccine. Prolonging the post vaccination Vitamins` treatment for three days increased the antibody titres from 24.80±10.91 to 140.80±31.35. Stantic-Pavlinic *et al.* (2004) reported that vitamin C enhances interferon production in humans and could therefore be used for stimulation of interferon response to rabies vaccines. So, these results agree with their suggestion. It is also in agreement with Igado *et al* (2010) who reported that vitamin C has immune-potentiating effects.

Vetzyme is a tablet dosage form of Vitamins and antioxidants. Its use in this study was for convenience. With the tablet-vitamin medication, dog owners do not need to come back to the clinic for days after anti-rabies vaccination. This makes adoption of post vaccination Vitamins-treatment for control of rabies, convenient.

Failure to monitor antibody responses of vaccinated dogs is another hindrance for efforts

to control rabies in Nigeria. For Nigerian veterinarians to start post vaccination assessment of dogs for rabies antibodies, there is a need to adopt a simple, inexpensive and yet reliable diagnostic test.

Previous clinical studies have found that *Rabies virus* neutralizing antibody titres of between 8-16 IU/ml is protective (CDC, 2016) but the neutralization test is complex, such that most field veterinarians cannot run it. Results of Rabies passive haemagglutination (RPHA) gave correlation coefficient of 0.81 with results of Mouse Serum Neutralization test (Gough and Dierks, 1971) and RPHA test requires less sophisticated and less rigorous protocols, compared to other serological techniques including the Mouse neutralization test (MNT), ELISA test and rabies fluorescent antibody test (RFAT). RPHA is easy to use and affordable.

Its adoption would reduce limitations veterinarians in most African countries have in carrying out post vaccination assessment of antibody titres against rabies. It may be easier to convince field veterinarians to adopt post vaccination antibody tests with the RPHA test than with the other tests which are more difficult to perform.

Currently, most veterinarians in Nigeria do not practice post vaccination Vitamins medication for rabies control. Though dogs in the control group in this study had titres up to the protective titre, such titres may not remain at protective level for a long time. So, it would be good to ensure higher antibody levels in vaccinated dogs so that the titres may not fall below the protective level before the dogs are revaccinated. Administering multivitamins to dogs for three days post anti-rabies vaccination would ensure high levels of antibodies that would remain at protective level till next annual revaccination.

V. CONCLUSION

Post vaccination Vitamins treatment of dogs under the Nigerian environmental conditions is necessary to improve their antibody responses against rabies. Also, post vaccination assessment of dogs for rabies antibody titres can be adopted if veterinarians are taught to use the rapid and inexpensive passive haemagglutination test which does not require sophisticated equipment and results are got within 3 hours. These two strategies could enhance the success of rabies control efforts in Nigeria.

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