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Evaluation of Antibody Responses of Dogs Vaccinated with Three Imported Inactivated Rabies Vaccines in Nigeria

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ABSTRACT

Dog rabies vaccines are imported into Nigeria to complement the quantity being produced by the National Veterinary Research Institute, Vom, Plateau State, Nigeria. Routinely these imported vaccines are expected to be tested for safety, potency and efficacy; however this is hardly done. This study was carried out to determine the immunogenicity of three imported rabies vaccines available in Nigeria.

Twenty indigenous breeds of dogs aged between 3 and 4 months were randomly assigned to 4 groups (A,B,C and D), groups A,B and C were subsequently vaccinated with a single dose of three different brands of commercially available inactivated rabies vaccines while D was the control. Rabies antibodies were measured fortnightly until it started waning in all dogs, using indirect Enzyme linked immunosorobent assay.

Responses of dogs' immune system to the three rabies vaccines varied greatly. Groups B mean antibody titres peaked on day 14 post vaccination while groups A and C mean antibody titres peaked on day 28 (A=5.74±0.643 EU/Ml, B=0.184±0.091 EU/Ml and C=0.237±0.080 EU/Ml). All dogs in group A, two in group C and none in group B had protective antibody titres in the course of this study. The mean antibody titres against rabies on days 14, 28 and 56 were significantly higher in group A than in groups B and C.

There is need for regular evaluation of all batches of commercially available vaccines in the country for efficacy at port of entry before release into the market; in order to win the battle against human rabies by 2030.

1. INTRODUCTION

In Nigeria, the National Veterinary Research Institute (NVRI), Vom has been producing low and high egg passage flurry rabies vaccines for dog and cat respectively since 1956 (Ogunkoya, 2006). The vaccines are modified or attenuated live virus vaccines. Apart from the rabies vaccines produced by NVRI, rabies vaccines are imported from other countries of the world since the local production by the NVRI cannot meet up with the demand for the rabies vaccine in Nigeria. Annual rabies vaccine production for dog was less than 30,000 doses in 2011 (NVRI, 2012).With the occurrences of rabies in vaccinated animals and the influx of imported rabies vaccine in Nigeria, it is expedient to periodically monitor the potency and safety of rabies vaccines stored for use at veterinary clinics. Manufacturers are required to test the potency and safety of each batch of rabies vaccine before and after release into the market although this is seldom done in Nigeria. A formal surveillance system to determine the potency of commercially available rabies vaccine in Nigeria does not exist. It is unclear how well the vaccines perform in the field. Therefore, these studies aimed at evaluating the responses of local dogs to three commercially available rabies vaccines in Nigeria and determine vaccination coverage of dogs in some communities in Ogun State

2. MATERIALS AND METHODS

Three types of vaccines available for sale in the study area were purchased for the study with the aim of determining their efficacy. Twenty dogs within the ages of 3-4 months, weighing 5-7kg were purchased. They were obtained from bitches that were never vaccinated against rabies. The dogs were conventionally housed and fed with a high quality food and unlimited access to water. In addition, prior to vaccination with the rabies vaccines, the dogs were treated against bacterial and helminth infection. The dogs were randomly assigned into four groups of five dogs each and then tagged to aid identification.

Two weeks post prophylactic treatment; the dogs were vaccinated by injecting a single dose of inactivated rabies vaccines (1ml) intramuscularly in the thigh. Pre-vaccination blood samples were drawn on day zero (Day 0) and post vaccination blood samples were taken on days 14, 28 and 56 for serological study. Rabies virus neutralizing antibody titres were determined using platelia™ rabies kit ®. The blood samples were drawn from the cephalic or saphenous vein of each dog into tubes and allowed to clot at room temperature. The samples were centrifuged in the laboratory in the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. The separated serum was stored frozen at -20^{o}C until analyzed.

Sera were analyzed using a commercial indirect enzyme linked immunosorbent assay (ELISA) that employed the glycoprotein extracted from inactivated and purified virus membrane as antigen (Platelia rabies II kit, Bio Rad laboratory, France). Positive and negative controls were included for each series of samples analyzed. Absorbency was read on an ELISA reader at 450-620nm. For qualitative assay, Optical Density of each sample was compared to the high sero-conversion and threshold values obtained whereas for quantitative assay, a standard curve was established with rabies standard serum titrated in international units per millilitre (IU/ml). The test serum was read off the standard curve using manual data reduction. Antibody levels were expressed as equivalent units per millilitre (EU/ml) corresponding to IU/ml. Sera with a titre equal to or greater than 0.5 EU/ml were considered protective. The sensitivity and specificity values for the ELISA test used were 88.4% and 98.8% respectively (Feyssagnet et al., 2005). All steps were conducted in accordance with the instructions of the manufacturer.

Five non-urban communities were purposively selected in Ogun state to determine the rabies vaccination coverage of dogs. These communities included Odeda, Isiwo, Ososa, Itamapako and Ilaro III. Information on vaccination history and purpose of keeping the dogs were obtained from their owners. Blood was collected from dogs whose owners gave consent. The samples were handled as described for experimental dogs above. A one way between groups analysis of variance was conducted to explore the immunogenicity of three brands of canine vaccines used for the study and Chi square test was used to ascertain association between sero-conversion and purpose of keeping dogs among owned dogs. p- value was set at 0.05. Ethical approval for these studies was obtained from the University of Ibadan Animal Care and Use Research Ethical committee with reference number UI-ACUREC/App/2015/046.

3. RESULTS

The mean antibody titre in days 0, 14, 28 and 56 in groups A, B, C and D dogs are given in Table 1. Details of number of samples analyzed, number of samples which had antibody titres equal or great than 0.5 EU/ml and their percentages are presented in Table 2. Fig. 1 presents the comparison of the mean rabies antibody titres (log Equivalent units per ml) per group after one-dose vaccination on day 0 with the WHO threshold of positivity**.**

Group A. The dogs in this group were vaccinated with vaccine type A. They all developed high level of antibody titre 14 days post vaccination. The level of antibody titre rose to 5.744 EU/ml at Day 28, but dropped to 4.892 EU/ml at day 56.

Group B: The dogs were vaccinated with vaccine type B. Antibody titres of the dogs in this group on Day 14 were below 0.5EU/ml; mean antibody titre of all dogs in this group was 0.184EU/ml. By day 28 and 56 it dropped to 0.144 EU/ml and 0.07 EU/ml respectively.

Group C: The dogs were vaccinated with vaccine type C. At day 14, one of the dogs developed antibody titre of approximately 0.5EU/ml and another achieved the same titre at day 28. However, by day 56 both titres have dropped below 0.5EU/ml (0.18EU/ml and 0.32 EU/ml respectively). The group mean antibody titre reached the highest level at day 28 (0.237EU/ml) and dropped to 0.135 EU/ml at day 56.

Group D: The dogs in this group were given nothing and therefore served as control. Antibody titres of the dogs in this group were below 0.5EU/ml throughout the period of the experiment.

Table 1: The mean antibody titres of dogs before and after vaccination with rabies vaccines

Group number	Vaccine type	Mean EU/ml (std. Error)			
		Day 0	Day 14	Day28	Day 56
A	A	0.0450 (0.009)	2.74 (0.498)	5.744 (0.643)	4.139 (0.975)
B	B	0.036 (0.009)	0.184 (0.091)	0.144 (0.031)	0.069 (0.016)
C	C	0.047 (0.013)	0.188 (0.080)	0.237 (0.080)	0.135 (0..053)
D	NIL	0.068 (0.009)	0.043 (0.009)	0.038 (0.009)	0.037 (0.008)

Table 2: Number of animals considered for analysis and number of samples which had antibody titres ≥ 0.5 EU/ml and their percentages

Group	Number of dogs analyzed	Number (percentage) of samples which had antibody titres ≥ O.5 EU/mL			
		Day 0	Day 14	Day 28	Day 56
A	5	0(0)	5(100)	5(100)	5 (100)
B	5	0(0)	0(0)	0(0)	0 (0)
C	5	0(0)	1(20)	1(20)	0(0)
D	5	0(0)	0(0)	0(0)	0(0)

Day 0: There was no statistically significant difference (P>0.05) in antibodies titre for the four groups: F(3, 20) = 2.06, p = 0.16.

Day 14: There was a statistically significant difference (P<0.05) level in antibody titres for the four groups: F (3, 20) = 26.71, p = 0.003. The actual difference in mean antibody titres was quite large. The effect size, calculated using eta squared was 0.83. Post hoc comparisons using the Tukey HSD test indicated that the mean antibody titres for group A (M=2.77 EU/ml, SD = 1.11) was significantly different from group B (M=0.19 EU/ml, SD = 0.15) group C (M=0.18 EU/ml SD = 0.16) and group D (M=0.05 EU/ml, SD = 0.01), whereas groups B and C did not differ significantly from group D which was the control.

Day 28: There was a statistically significant difference (P<0.05) in antibody titres for the four groups: F (3, 20) = 74.64, p = 0.000. The actual difference in mean antibody titres was larger than what was obtained in day 14. The effect size, calculated using eta squared was 0.93. Post hoc comparisons using the Tukey HSD test indicated that the mean antibody titres for group A (M=5.74 EU/ml, SD = 1.44) was significantly different from group B(M=0.23 EU/ml, SD = 0.18) group C(M=0.14 EU/ml SD = 0.05) and group D (M=0.04 EU/ml, SD = 0.01), again groups B and C did not differ significantly from group D which was the control.

Day 58: There was a statistically significant difference (P<0.05) in antibody titres for the four groups: F(3, 20) = 24.25, p = 0.005. The actual difference in mean antibody titres was large, due to waning, when compared with day 28. The effect size, calculated using eta squared was 0.82. Post hoc comparisons using the Tukey HSD test indicated that the mean antibody titres for group A (M=4.89 EU/ml, SD = 2.18) was significantly different from group B (M=0.14 EU/ml, SD = 0.12) group C (M=0.07 EU/ml SD = 0.05) and group D (M=0.03 EU/ml, SD = 0.01), however, groups B and C did not differ significantly from group D which was the control.

There was no apparent adverse reaction observed among dogs in groups B and C, however by day 42, one of the dogs in group A developed neurological disorder which kept recurring till the end of the study.

Only ninety sera (comprising 40 sera of hunting dogs and 50 sera of guard and pet dogs) were obtained and analyzed from the total of 185 dogs identified in the selected communities. From the information gathered from the dog owners on hunting dogs, there was zero vaccination while eight of the non-hunting dogs were reported vaccinated with no evidence of rabies vaccination certificate. The result of sera analysis showed that only two (25%) of the vaccinated non-hunting dogs had titres considered to indicate sero-conversion (>0.5 EU/ml). One (2.5%) of the hunting dogs was however sero positive to rabies antibody with antibody titre of >0.05EU/ml (Table 3).

Table 3. Rabies antibody sero- prevalence in dogs in relation to uses

	Hunting dogs (%)	Non hunting dog (%)	p-value
Sero-positive	1(2.5)	2 (4.0)	0.69
Sero-negative	39(97.5)	48(96.0)	
Total	40	50	

4. DISCUSSION

There was a wide variation in responses of puppies to vaccination against rabies with the three types of rabies vaccines used in this study. The result revealed the production of protective antibody in only type A of vaccine because all vaccinated dogs with this vaccine developed rabies antibody titres above 0.50 IU/mL after vaccination. Also the vaccine induced an early sero-conversion with antibody titres peaking as early as 14 days post-vaccination.

From this study, dogs that have positive response had titre of ≥0.5 IU/ml at 28 days post vaccination. This is in agreement with WHO (2005) which stated that dogs can be assumed immunized at 28 days post vaccination. Studies have shown that interval between vaccination and blood screening for antibody response is one of the most significant factors in determining the host's apparent responses. Cliquet et. al., (2000) showed that antibody levels peak at about four weeks after vaccination, although the period may be slightly different with different vaccines. In this study, it was further noted that after four weeks post vaccination, there was a steady decrease in the levels of circulating antibody.

Many studies have shown that puppies' response to rabies vaccine is always poor. Gunatilake et al., (2003) noted that puppies generally had lower immune response to rabies vaccine. Study by Mansfield et al., (2004) in UK, revealed that dogs less than a year old had a slightly higher probability of responding poorly than ones more than one year old. Although, Aghomo and Rupprecht (1990) showed that young dogs can produce rabies antibodies from four weeks of age, after the maternal antibodies must have waned. However, if the dam was vaccinated, the puppies do not begin to respond to vaccination until 10 weeks of age, and they are less efficient at producing immunoglobulin.

All the puppies in this study were of local breed between three and four months, with no maternal antibody and were well fed. Therefore it was worrisome that only type A vaccine had 100% immune response in dogs in group A while the dogs in groups B and C had 0% and 50% responses respectively.

Many factors have been adduced for failure of vaccinated dogs to achieve protective immunity (antibody titre of 0.51U/ml. Some of these factors could be associated with age, route of vaccination, breed of the dog, haplotype of specific breeds of dogs, sex hormones (Coyne et al., 2001; Mansfield et al., 2004; Kennedy et al., 2007; Verthely and Kilinman, 2000; Schuurs and Verheul, 1990).

However, in this study all dogs used were of the same breed, of same country of origin and of the same age range, therefore all the aforementioned factors were not likely to have affected the immune responses of the dogs used in this study.

Apart from the dog and time factors affecting antibody titres in vaccinated dogs, other factors such as quality of the vaccine, may have been responsible for failure of some of the vaccines to induce protective antibody titre. Hu et al., (2007) also found that some of the vaccines available for veterinary use in China generated less than 0.5 IU of rabies neutralizing antibodies after administration. Another suspected factor that could have affected the quality of the vaccines and response to vaccination could be vaccine handling by distributors. In order to keep vaccines potent, it is expected that cold chain be maintained. In Nigeria, power supply is erratic, it is almost impossible to have continuous supply of power for 24 hours thus leading to break in cold chain. It is therefore recommended that the quantity of vaccines imported or purchased should be what could be managed or utilized within a short period of time in each company or veterinary vaccine retail outlets. Quality of vaccines sold or imported in Nigeria should be randomly inspected and evaluated by an independent government institution in order to check mate sales of impotent vaccines, in spite of the vaccine quality control undertaken by the manufacturer.

Consequences of failure of vaccine to induce protective immunity are grave. To the pet owners, who assume that all vaccinated dog are well protected. There is reason for him/her to worry when ever such vaccinated dog is bitten by rabid dog or the vaccinated dog bites human being. It is therefore of great importance that vaccination of dogs against rabies virus be followed by evaluation of antibody titre levels in order to ensure that vaccines used achieved protective antibody titre against rabies virus for the safety of the public.

According to the serological analysis, only 3.3% of the owned dogs have adequate rabies antibody titre that

can prevent spread of rabies virus in case of exposure and therefore 96.7% remained reservoirs of rabies infection to man and other animals. One unvaccinated dog among the hunting dogs was strongly sero positive for rabies. This situation could be due to the fact that the dog had been exposed or had long incubation period following rabies infection; recovered from clinical rabies; had abortive infections or was clinically unaffected carrier and shedder of rabies virus. The occurrence of dogs that are clinically normal but are carriers of rabies deserves utmost attention in order to assess their importance in epidemiology of rabies especially in Ogun State where majority of the dogs are not vaccinated against rabies.

In conclusion, the use of rabies vaccines in dogs is the bedrock of human rabies control and elimination, therefore it is expedient to monitor the quality of anti rabies vaccines supplied and used in Nigeria and carry out aggressive free mass vaccination of dogs against rabies to meet up with 2030 human rabies eradication target.

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