

# EVALUATION OF ANTIBODY RESPONSE BY DOGS VACCINATED WITH LOW EGG PASSAGE, FLURRY STRAIN ANTI-RABIES VACCINE USING SINGLE AND MULTIPLE SITES

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## Abstract

Twelve dogs were immunized with live attenuated flurry strain antirabies vaccine using single, double and triple inoculation sites of the recommended dose, but in divided doses for the double and triple sites. All the dogs were screened for neutralizing antibodies against rabies before immunization, while sera were collected on day 1, 3, 7, 10, 14, and 28 post immunization. These sera were analyzed for viral neutralization and protection using mouse neutralization test (MNT). All the sites induced sero-conversion in all subjects from day one post vaccination, but the levels of neutralizing antibody production with triple site regime showed a four-fold increase in serum titre over the two-site regime. A 100% sero-conversion was achieved by day 28 post vaccination for the triple-site regime.

**Key words:** Antibody, Response, Dogs, LEP Flury

## Introduction

Rabies, an acute infectious encephalomyelitis, which is distributed globally among a variety of warm-blooded animals, primarily affects dogs and cats, and on close association with man leads to transmission (Andrews & Walton, 1977). For centuries, the mode of transmission has been associated with bites (Okoh, 1981), and licks by rabid animals (Nawathe, 1980), or by aerosol (Fagbami *et al.*, 1981).

Canine rabies is endemic and occurs throughout the year in all parts of Nigeria; with dogs forming the major sources of infection to man, and about 10,000 persons exposed to rabies annually in the country (Umoh *et al.*, 1988).

Vaccination against rabies as been shown to be an efficient way for its control (Tuner *et al.*, 1976), and statistics have shown that successful immunization of 70% - 80% of dog population in a country places rabies under control (Beran, 1971).

In Nigeria, it has not been possible to successfully control and eradicate rabies, instead evidences show that the disease is on the increase (Ogunkoya, 1997). Furthermore there have been several reports of rabies occurring in dogs vaccinated with low egg passage (LEP) – flurry strain anti-rabies vaccine (Okoh, 1981; Ogunkoya, 1989), which is the official vaccine produced by National Veterinary Research Institute (NVRI) Vom – Nigeria and used in Nigeria.

A field serological survey of 140 dogs vaccinated with LEP – Flury strain vaccine revealed only 45.7% immuno response success, with younger dogs of 4 months or less having lower antibody titer than the older age group (Ogunkoya *et al.*, 1992). A significant improvement in the dynamics of antibody response was reported when human diploid cell rabies vaccine doses were split and given in several sites of the body in humans as compared to just one site (Umoh & Belino 1979). Other researchers (Bakaliac *et al.*, 1985; Warrell *et al.*, 1985) confirmed that multiple sites is superior to a single site administration of vaccine and this lead to World Health Organisation (WHO) approval of multiple site immunization system in humans (WHO, 1992). In view of the success of multiple site administration of vaccine in human beings, the idea is being

borrowed for application in dogs in order to observe whether the same level of success will be achieved.

## Materials and methods

### Animals

Twelve dogs of 7 to 8 weeks of age were purchased and housed for an eight, week acclimatization period. During this period, the dogs were subjected to detailed clinical and laboratory evaluations, endo and ectoparasitic control, and were vaccinated against infectious and contagious diseases of dogs using polyvalent vaccine of canine distemper, Hepatitis, Leptospirosis, Parvovirus enteritis and para-influenza at 8 weeks, 12 weeks and 16 weeks of age. Collars and tags were used to identify the dogs.

### Immunization, Sample Collection and Handling

Each dog was analyzed for possible viral serum neutralization using mice (Atanasiu, 1973). None of the dog's serum sample neutralized the virus as shown by the mouse neutralization test.

### Antirabies vaccine for dogs with official name of live attenuated Flury strain rabies virus

Chick embryo origin, low egg passage, was used. The vaccines were stored at 4°C until used. The dogs were grouped into 3 (A, B, and C). Of 4 dogs each. Group A was subdivided into 2 groups AV- having 2 dogs and AC – with two dogs, groups B and C were also subdivided into 2 groups (BV and BC) and (CV and CC) respectively, all having 2 dogs in each group. Groups AV, BV and CV served as single, double and triple sites respectively while groups AC, BC and CC served as the control.

Each vaccine was reconstituted before use, using 2.5mls of distilled water according to manufacturer's specification. Group AV dogs were vaccinated by injecting the entire vaccine into one vastus lateralis muscle and group AC dogs were injected in the same manner using 2.5mls distilled water.

Two vastus lateralis muscles (one on each limb) were used for the double sites injection (BV and BC) by splitting the

vaccine dose into 1.25mls, and injected at each site for BV dogs, while 1.25mls of distilled water was used at each site for the BC dogs. For the triple sites, both vastus lateralis muscle and one biceps muscle were used by injecting approximately 0.83ml of vaccine and 0.83ml of distilled water into CV and CC dogs respectively. Serum samples were collected from all the dogs on 1, 3, 7, 10, 14 and 28 days post immunization. Each serum was stored in a separate clean bijou bottle at 20°C until analyzed. The serum samples were analyzed for serum antibody titre and protection using mouse neutralization test (MNT) (Atanasiu, 1973).

#### Mouse Neutralization Test (MNT)

A standardized amount of virus 64LD50/0.2ml, which was prepared from brain tissue of a dog positive for rabies antigen in accordance with the Fluorescent Antibody Technique (FAT), was combined with 0.2ml of various dilutions of each serum in pre-labelled test tubes. The sera had been inactivated at 56°C for 30 minutes before the test tube were incubated at 37°C for 90 minutes after which they were kept at 4°C (Atanasiu, 1973). From each serum virus mixture, 0.03ml was inoculated intracerebrally into batch of six three-week-old mice placed in separate cages. The mice were observed daily for 28 days for any signs of rabies infection and death. The geometric mean titres (GMT) were calculated by computing the mean of the common logarithms to the base 10 (log<sub>10</sub>) of the titres and then reading the antilogarithms of the mean.

Results were expressed in terms of serum titre, which is defined as the dilution factor of the highest dilution of

serum which neutralizes a standard amount of virus (Roitt, 1984). The serum titres were calculated using the Reed and Muench (1938) method (Reed & Muench, 1938) for estimating 50% end point.

#### Results

The serum titres varied significantly depending on the serum collection days post vaccination. The results obtained are summarized in the table. The sera from the immunized dogs started showing some level of viral neutralization from day 1 post vaccination. It was observed that the triple site injection sera protected most mice about five times more than the sera from single and double regimens a day after vaccination, while the sera from the two site regimen protected more mice than those of the single site regimen on the same days post vaccination. By day 3 post vaccination, the recorded geometric mean titre (GMT) values dropped for single site regimen from 8.13 to 7.85, and from 9.02 to 8.41 for the two site regimen, that of the triple site regimen were virtually the same i.e. 49.55 and 50.12 for days 1 and 3 post vaccination respectively.

A 100% sero-conversion was noticed on day 28 post vaccination for the triple site regimen, while single and double sites had 12.88 and 25.41, respectively. From day 3 post vaccination the GMT values increased gradually for all the different sites and peak GMT value were attained on day 28 post vaccination for all these different sites considered.

All the controls for the different sites injected with sterile distilled water did not show any sero-conversion.

**Table 1:** Rabies serum neutralization of dogs vaccinated with live attenuated Flurry strain anti-rabies vaccine according to the number of injection sites using mouse neutralization test (MNT)

Group	Geometric Mean Titer					
	Day 1	Day 3	Day 7	Day 10	Day 14	Day 28
AV	8.1	7.9	8.4	9.0	11.2	2.9
BV	9.0	8.4	11.2	12.9	15.1	25.4
CV	49.6	50.1	65.3	70.8	>80	100

AV, BV and CV = 1, 2 and 3 sites, respectively

Day = Day post immunization serum collection from dogs

#### Discussion

It can be observed from the results that the significant level of protection was directly proportional to the number of the injection site regimen. The serum samples from the triple site had a higher protection level than those from the double site regimen, which in turn offered a higher protection than the single site regimen. The reason for these might be due to the fact that the more the sites that were used for inoculation the more the number of cells that were stimulated to form neutralizing antibodies.

It was also seen that the more number of days post vaccination the greater the level of antibody titre. This might be due to the fact that the clonal expansion of the neutralizing antibody producing cells was proportional to the number of days post vaccination during the period of study (Roitt, 1984).

The drop in mice protection level observed for serum samples collected 3 days post vaccination from single and double sites is quite unusual and does not conform with the already established trend in this study. The reason for this is not immediately known; probably it could be that the sera contained very small quantities of neutralizing antibodies and large quantities of unneutralized virus particles, which

killed more mice. However, reports have shown that by the third day post vaccination, the interferon stimulated by virus inoculation on day 1 would have reached its peak by day 2 and start dropping by day 3 (Thraenhart, 1988). This period coincides theoretically with the beginning of the appearance of specific antiviral antibodies in the serum. Hence the serum samples on day 3 must have contained low titres of neutralizing antibodies.

From the results it was observed that by day 7 post vaccination, the neutralizing antibodies had increased to appreciable levels and hence the increase continued steadily as the days also increased irrespective of the number of sites used for administering.

#### Conclusion

Multiple site vaccination is superior to single site using live attenuated flurry strain anti-rabies vaccine.

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