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AN ATTEMPT AT ORAL VACCINATION OF NIGERIAN LOCAL DOGS WITH DIFFERENT ANTI-RABIES VACCINES

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ABSTRACT

The immunogenicity and suitability of three commercially available anti-rabies vaccines (Flury –Lep, Rab Vac-3 and Dura Rab-3) were investigated in three different groups of Nigerian local dogs divided into 4 equal groups (A, B, C and D) of 4 dogs each. They were vaccinated through the oral or intramuscular route. Four other dogs (group D) served as unvaccinated control. The responses of the dogs were analyzed using hematology as well as complement fixation test. All the vaccines stimulated significant antibody response (≥ 4 fold) by day 28 post vaccination. However, the Flury-Lep vaccine group had relatively higher geometric mean antibody titre of complement fixing antibodies than the other two vaccine groups. The Flury-Lep followed by Rab Vac-3 vaccine groups also stimulated relatively higher percentage lymphocyte and total leucocyte counts than Dura Rab-3 vaccine by both the intramuscular and oral routes. As a result of the danger of releasing live viruses into the environment, the Flury-Lep live vaccine was not considered suitable for oral vaccination. It was, therefore, concluded that the Rab Vac-3, a killed rabies vaccine, was more suitable for oral vaccination of dogs against rabies because of its high immunogenicity and safety.

***Keywords*:** Rabies, Oral Vaccination, Indigenous Dogs, Nigeria

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INTRODUCTION

Rabies, a disease of antiquity, has continued to persist as a major public health problem especially in developing countries [1,2,3]. It is perhaps the most gruesome and dreadful of all communicable diseases afflicting human beings [4]. In most developing countries the dog remains the major transmission vector of rabies to man, despite the widespread use of parenteral vaccination. In developing countries, canine rabies control has achieved only limited success despite extensive effort and expense. One often suggested reason for this failure is that dog vaccination has not reached sufficient enough levels to break the dog-to-dog transmission cycles [5,6] or cannot be maintained, so that transmission is inevitably re-established [7]. It was suggested that problems such as inadequate logistics, insufficient community participation or inaccessibility to dogs were responsible for this failure and it was hypothesized that these

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could be overcome with oral vaccination for dogs. Rabies control by oral immunization using baits is possible and has been clearly demonstrated with respect to fox rabies in Europe [8,9].
In this study, an attempt was made to determine the suitability and immunogenicity of commonly available anti-rabies vaccines in dogs when administered orally as compared to the usual intramuscular route. It is envisaged that the study will help in addressing the possibility of oral vaccination of stray/ownerless dogs in Nigeria as was done for wild animals in Europe and North America.

MATERIALS AND METHODS

Experimental dogs

Sixteen puppies, aged 4 - 6 months, were obtained from dog owners within Maiduguri and kept in kennels at the Veterinary Teaching Hospital University of Maiduguri. The puppies had no previous record of anti-rabies immunizatoion, were dewormed with albendazole and allowed to acclimatize to their new environment for four weeks before they were vaccinated.

Source of vaccines

Three commercially available anti-rabies vaccines were purchased from reputable veterinary products distributors in Kaduna Nigeria. The vaccines included: the Flury low egg passage (Flury lep) live vaccine from the National Veterinary Research Institute, Vom, Nigeria; Rab Vac-3 killed vaccine from Solvay Animal Health Inc., USA and Dura Rab-3 killed vaccine from Immunovet Inc., USA.

Experimental design

The puppies were divided into four experimental groups (A, B, C and D) and were identified by tagging. Each of the groups were further divided into sub groups of 2 dogs each (A1, A2, B1, B2, C1, C2, D1 and D2). Puppies in subgroups A1 and A2 were given 2.5 ml of the Flury-lep orally and deep intramuscularly, respectively; 1 ml of Rab Vac-3 was given orally and intramuscularly to puppies in subgroups B1 and B2 respectively; 1 ml of Dura Rab-3 vaccine was also given orally and intramuscularly to puppies in subgroups C1 and C2 respectively while sub groups D1 and D2 served as unvaccinated control groups and were given phosphate buffered saline (PBS) orally and intramuscularly, respectively.

Sample collection

All the experimental puppies were bled on the day of vaccination (day 0) and every seven days for five weeks. Five milliliters (5 ml) of blood were collected from each of the experimental puppies by cephalic venipuncture and 3 ml were dispensed into plain vacutainer tube for serum and the remaining 2 ml for haematology were dispensed into vacutainer tubes containing ethylene diamine tetraacetic acid as anticoagulant.

Serology

Rabies virus antigen and antisera

The rabies virus antigen used was a Vero cell adapted inactivated rabies virus type 1 vaccine prepared at the Institute Pasteur, France and obtained from the Animal Virus Research Laboratory, University of Maiduguri, Nigeria.

Complement fixation test

The serum samples from the experimental puppies were tested for rabies virus antibodies using the protocol of Baba *et al*. [10].

Hematology

The total white cell count was carried using the Neubauer counting chamber [11], while the Battlement method as described by Hewitt [12] was used for the differential leucocyte count (DLC).

Statistical analysis

The Geometric mean titers (GMT) of antibodies were calculated using the formula described in CDC [13].

RESULTS

None of the experimental puppies exhibited any signs of rabies or other disease conditions throughout the period of the study. All the vaccinated dogs seroconverted to varying rabies virus antibody titres. The Flury-lep vaccine stimulated the production of relatively higher antibody response than any of the other two vaccines by both the oral and intramuscular routes (Table 1). There was a relative difference in antibody response between the two routes in all the three groups with the intramuscular route having higher titres. With the exception of the Flury-lep given intramuscularly that showed significant titre on day 14, all the other groups irrespective of route of vaccination did not show significant rise in antibody titres until day 21 post vaccination (PV).

All the three vaccines did not show any significant difference in the neutrophils values between the vaccinated and the control dogs (Fig. 1). Significant difference was noted in the percentage lymphocytes stimulated by the different vaccines given intramuscularly, with the Flury-lep vaccinated group having the highest (Fig 2). Dogs vaccinated orally with the Flury-lep and Rab-Vac3 exhibited higher percentage lymphocytes than the Dura-Rab and control groups (Fig. 2). A relative difference was observed in the total lucocyte response of the vaccinees between the Flury-lep vaccine and the other vaccines through the intramuscular route (Fig. 3). The orally vaccinated groups showed significant difference among all the different groups with the Flury-lep group recording the highest (Fig. 3).

DISCUSSION

Determination of rabies antibodies and cellular responses after immunization against rabies is an acceptable index of the efficacy of a vaccine [16]. All the three vaccines in this study induced very good antibody and cellular response when administered through either the intramuscular or oral route. This finding is at variance with the report of WHO [14] which suggested that only attenuated or recombinant vaccines were efficacious by the oral route in dogs.

Both cellular and humoral antibody responses are necessary in protecting dogs against rabies [15]. Antibodies help in controlling the spread of rabies virus infection as they are capable of effectively neutralizing the virus that is present in either intercellular spaces or body fluids, and they may bind to virus expressed on the cell surface, allowing complement or antibody dependent cellular cytotoxicity to mediate the killing of infected cells. Induction of cellular response following infection with rabies virus or vaccination is consistent with the observation that T lymphocytes are essential for protection against a lethal dose of rabies virus [16].

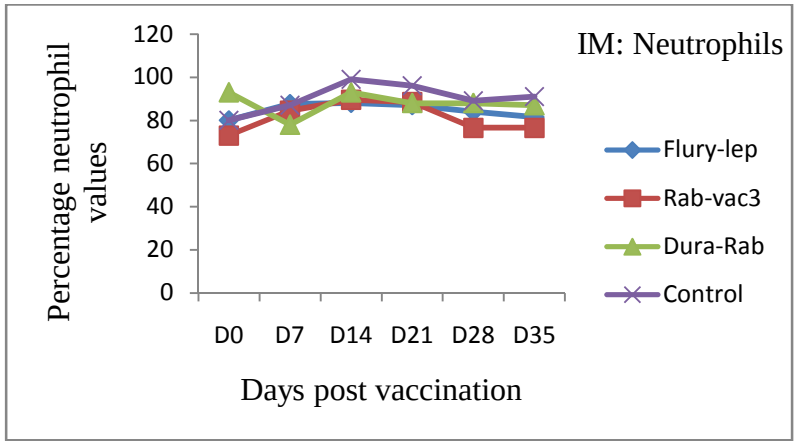
The Flury-lep live attenuated rabies vaccine appeared to have stimulated earlier and more protective antibodies and cellular response than the killed vaccines. This is similar to the report of Osinubi *et al*. [16]. Whereas the Flury-lep vaccine stimulated antibody production from day 7 post vaccination, the two other killed vaccines started on day 14 post vaccination.

All the three vaccines stimulated lymphocyte production by day 14 PV. This is contradictory to what was reported by Osinubi *et al*.[16], who observed antibody production as early as day 1 and day 7 after vaccination with live and killed Flury vaccines respectively. Although the Flury-Lep, a live attenuated vaccine has shown a better humoral and cellular stimulation when administered to dogs through both intramuscular and oral routes, it is not considered suitable for oral vaccination because of the danger of releasing live vaccines into the environment. It was therefore, concluded that Rab Vac-3, a killed rabies vaccine is more suitable for oral vaccination of dogs against rabies because of its immunogenicity and safety.

Table 1. Antibody response (titres) of dogs vaccinated with three different antirabies vaccines through oral and intramuscular routes

Group/Vaccine type	Route of vaccination	Days post vaccination 0	7	14	21	28	35
A/ Flury-lep	intramuscular	0	2.7	32.0	48.3	181.0	181.0
	Per os	0	0	8.0	23.9	45.3	64.0
B/Rab-Vac3	intramuscular	0	1.8	11.3	23.9	128.0	90.5
	Per os	0	0	2.0	11.3	23.9	45.3
C/Dura-rab	intramuscular	0	1.1	8.0	23.9	45.3	64.0
	Per os	0	0	5.7	16.0	23.9	32.0
D/Control	intramuscular	0	0	0	0	0	0
	Per os	0	0	0	0	0	0

Fig. 1. Neutrophil responses of dogs vaccinated with different anti-rabies vaccines through oral and intramuscular routes



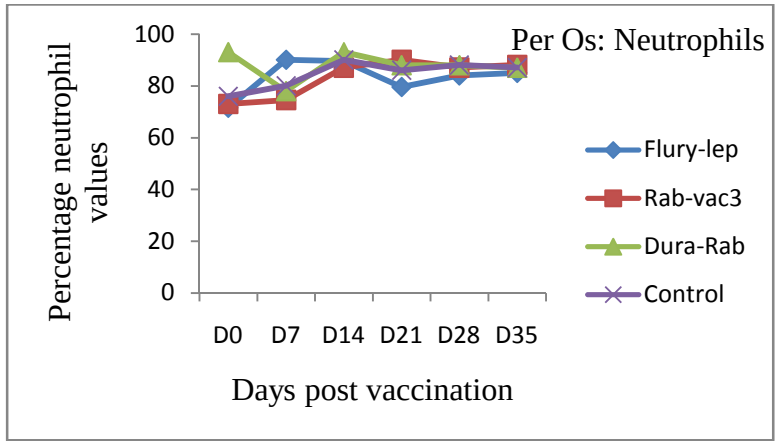
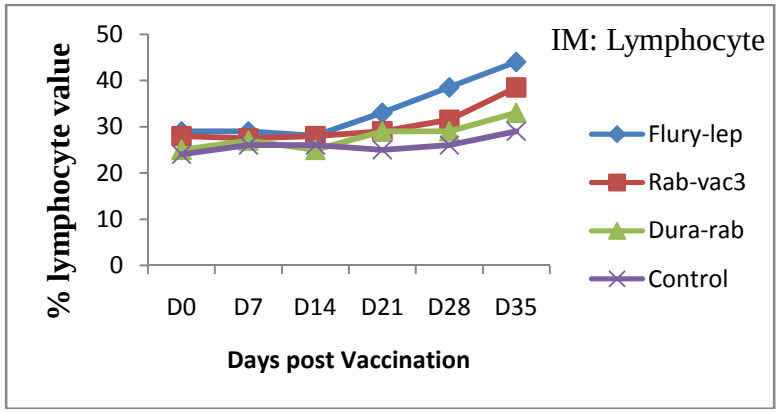


Fig. 2. Lymphocyte responses of dogs vaccinated with different anti-rabies vaccines through oral and intramuscular routes



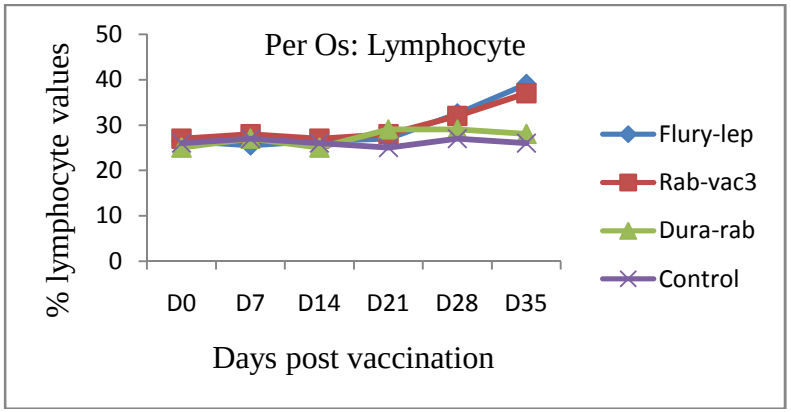
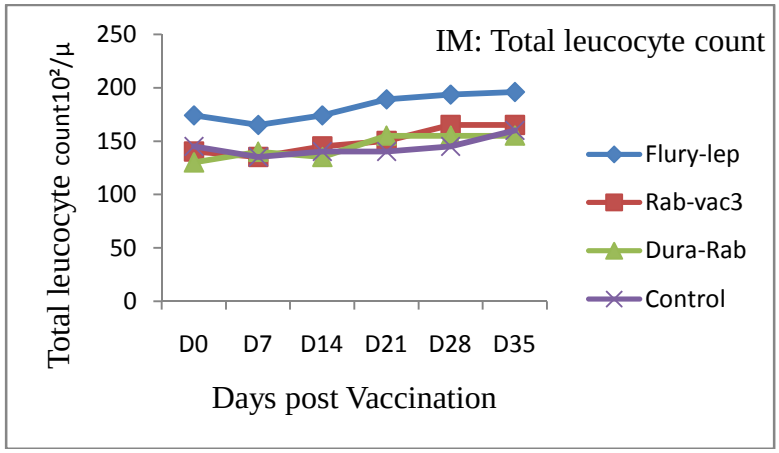
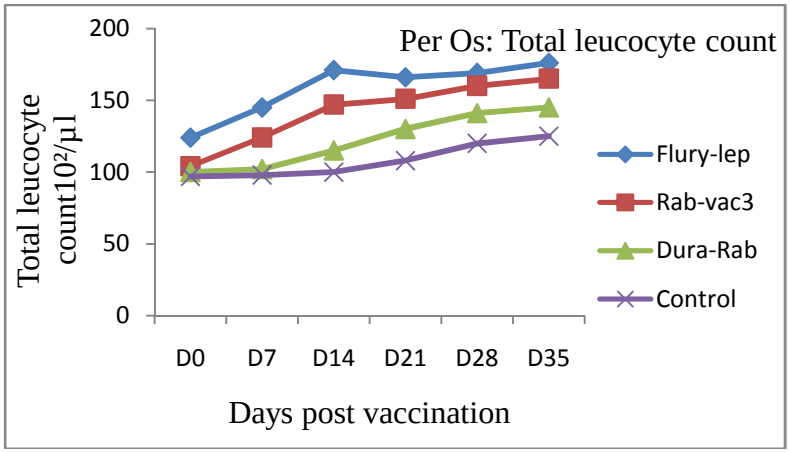


Fig. 3. Total leucocyte values of dogs vaccinated with different anti-rabies vaccines through oral and intramuscular routes.





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