

## Persistence of antibody titres in adult dogs and puppies following anti-rabies immunization

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### Abstract

In Sri Lanka, the first dose of pre-exposure anti-rabies vaccine is given to puppies at the age of 3 months with annual boosters. There have been many instances where puppies of less than 3 months have been affected with rabies. Anti-rabies vaccination failures have also been reported. Therefore, it is important to know the duration of protection in dogs following anti-rabies immunization.

Animals for this study were divided into two main groups (adult dogs and puppies) and further subdivided into 4 groups (adult dogs with a past immunization history against rabies, adult dogs without a past immunization history, puppies of immunized bitches and puppies of unimmunized bitches). In this study, neutralizing antibody titres in puppies and adult dogs following pre and post anti-rabies immunization were determined by the rapid fluorescent focus inhibition test.

All animals were bled on days 0 ( $D_0$ , pre-immunization), 30 ( $D_{30}$ ), 180 ( $D_{180}$ ) and 360 ( $D_{360}$ ). Titres were less than 0.5 IU/mL (protective antibody titre against rabies) in  $D_0$  samples of all puppies recruited for this study, in 26.7% of  $D_{30}$  samples of puppies of unimmunized bitches, in 89.5% of  $D_{180}$  samples of puppies and in all  $D_{360}$  samples of puppies. Antibody titres in 50% of  $D_{360}$  samples of adult dogs without a previous immunization history were also less than 0.5 IU/mL. Antibody titres of all dogs with a past immunization history against rabies, were maintained above the protective level 1 year after immunization.

Based on the results of this study, it is recommended that puppies should be immunized against rabies before the age of 3 months with a

booster at a suitable interval. Thereafter, annual revaccination should be done. Adult dogs without a past immunization history also should be given two anti-rabies vaccines in the first year at a suitable interval in order to maintain a protective antibody titre until the annual booster.

**Key words :** rabies, canines, anti-rabies immunization, antibody titres, Sri Lanka

### Introduction

Rabies is considered as a significant public health problem in Sri Lanka (1). In year 2001, 100 human deaths due to rabies have been reported in Sri Lanka. The rabies antigen was detected in 477 dogs compared to 22 cats, 5 domestic ruminants and 7 wild animals in year 2001 and 74% of human rabies cases were due to dog bites (2, 3). Hence domestic and stray dogs are considered as the principal reservoirs and transmitters of human rabies in Sri Lanka. Therefore, immunization of dogs plays an important role in controlling this zoonotic disease. Although the national rabies control programme carries out immunization programmes at its maximum, it has not been able to achieve the required vaccination coverage recommended by the WHO in order to eliminate outbreaks of rabies (1, 4).

In Sri Lanka, dogs are immunized against rabies at the age of 3 months with annual boosters, taking into account the presence of maternal antibodies. Unfortunately there have been many instances where puppies of less than 3 months of age have been affected with rabies. Anti-rabies vaccine failures have also been reported (Omala Wimalaratne, unpublished data). Previous studies done in Tunisia, Thailand and North America have shown that inoculation with one dose of anti-rabies vaccine does not produce a long

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lasting protective antibody response in a significant group of animals (5, 6).

As rabies is endemic in Sri Lanka, it is important to know whether the puppies born to vaccinated bitches have protective antibody levels until the time of first pre-exposure anti-rabies immunization and the duration of protection in canines following immunization.

### Material and Methods

#### *Experimental time period*

This study was carried out during the period from June 1999 to June 2002 after obtaining approval from the Ethical Review Committee of the Faculty of Medicine, University of Colombo.

#### *Study area*

Healthy canines of mixed breeds brought for anti-rabies vaccination from Colombo City and its suburbs (Borella, Rajagiriya, Battaramulla, Malambe, Kotte and Nugegoda) to a veterinary clinic at Kotte were recruited for this study.

#### *Inclusion of animals to groups*

After obtaining the owner's consent these animals were divided to different groups considering their ages, past immunization history against rabies and immunization status of the mother. Adult dogs aged 1 to 5 years with a past immunization history against rabies were in Group A and adult dogs aged 1 to 5 years without a past immunization history were in Group B. Three months old puppies of previously immunized bitches were in Group C and 3 months old puppies of unimmunized bitches were in Group D. Numbers of animals included for each group were 21 adult dogs for Group A, 16 adult dogs for Group B, 4 puppies for group C and 15 puppies for Group D. All these animals were found to be well nourished and relatively free of parasites.

#### *Anti-rabies vaccine used in this study*

The anti-rabies vaccine used in this study was an inactivated vaccine containing Pasteur RIV strain rabies virus grown in cell cultures and it is said that it eliminates the risk of neuroallergic reactions. Antigenic content of this vaccine is greater than 2.00 IU per dose, which is higher than the antigenic content per dose recommended

by the WHO (1.01 IU per dose) (4). This vaccine has been approved by the National Rabies Control Programme. It is mentioned by the manufacturer, that this vaccine induces higher titres and better cell mediated immunity in animals and provides protection for 3 years in dogs and cats against rabies infection.

#### *Assessment of immunity*

The canines were vaccinated by injecting a single dose of anti-rabies vaccine (1 mL) intramuscularly in the thigh. Pre-immunization blood samples were drawn on day 0 ( $D_0$ ) and post immunization blood samples were drawn on days 30 ( $D_{30}$ ), 180 ( $D_{180}$ ) and 360 ( $D_{360}$ ) for antibody determination. Rabies virus neutralizing antibody titres were determined using rapid fluorescent focus inhibition test (RFFIT) at the Rabies Research Laboratory, Medical Research Institute, Colombo 8, Sri Lanka (7).

### Results

The mean antibody titres in  $D_0$ ,  $D_{30}$ ,  $D_{180}$  and  $D_{360}$  of different groups are given in Table 1. Details of number of samples analyzed, number of samples which had antibody titres less than 0.5 IU/mL and their percentages are given in Table 2. Antibody titre 0.5 IU/mL has been determined by the WHO as the minimum protective antibody titre following anti-rabies immunization (4).

Adult dogs in Group A developed high level of antibody titres 1 month after immunization. Although there was a reduction in titres compared to the level 1 month after immunization, antibody titres were maintained above the protective level even at 1 year after immunization.

Antibody titres in  $D_0$  samples of all dogs in Group B were below 0.5 IU/mL. However, all developed antibody titres greater than 0.5 IU/mL 30 days after immunization. Antibody development in adult dogs in Group B following immunization was poor compared to adult dogs in Group A. Only 87.5% of dogs in Group B have maintained the titres greater than 0.5 IU/mL 6 months after immunization. The mean antibody titre in  $D_{360}$  samples of these dogs was greater than 0.5 IU/mL. However, 50% of animals didn't have

protective antibody titres 1 year after immunization.

Antibody titres were below 0.5 IU/mL in D<sub>0</sub> samples of puppies in Group C and D. Antibody development, 1 month after immunization with a single anti-rabies vaccine was poor in puppies compared to adult dogs in Group B. It is important to mention that 26.7% of puppies in Group D did not develop antibody titres above the protective level even 1 month after immunization. 75% of puppies in Group C and 93.3% of puppies in Group D did not have protective antibody titres in D<sub>180</sub> samples. Antibody titres in all D<sub>360</sub> samples of puppies in Groups C and D were below 0.5 IU/mL.

#### **Discussion**

A wide variation was observed in the antibody titres in all the groups following anti-rabies immunization. Data shows that except in adult dogs with a past immunization history, a single dose of anti-rabies vaccine was unable to maintain a good protective antibody titre until one year in 50% of adult dogs in Group B and in all the puppies in Groups C and D.

Based on the results of 4 puppies recruited to Group C, we observed that the level of maternal antibodies present in puppies at the age of 3 months was very low. It is also observed by Winters that, on average maternal antibodies in puppies against rabies, parvo and infectious canine hepatitis viruses could be detected only up to 6-7 weeks post partum (8).

It should also be noted that generally recommended immunization schedules by various groups are as follows.

Manufacturer's recommended vaccination schedule for dogs and cats is to do primary vaccination at an age of 3 months and revaccination in every 3 years. They also say that primary vaccination for dogs and cats can be given at earlier age with a booster at the age of 3 months.

WHO recommendation is to give primary vaccination for dogs at the age of 3 months with annual boosters. However, the situation is slightly different with mass vaccination

campaigns. If inactivated vaccines are used, their recommendation is that puppies less than 3 months old should be given a vaccine and a booster at 6 months of age (4).

Recommendation of the International Veterinary Information Service is that puppies should be given the first injection at the age of 3 months and second injection one year later with revaccinations in every 2 to 3 years (9).

Recommendation of Professor M K Sudarshan (Head of the Rabies Epidemiology Unit at Kempegowda Institute of Medical Sciences, Bangalore, India) is that primary vaccination for puppies in India should be done at the age of 2 months and a booster 1 month later. Thereafter, annual revaccination is recommended (10).

In India 60% of rabid dogs are less than 1 year old, many are puppies below 3 months of age (11). Therefore, our recommendation is that all puppies irrespective of maternal immunity, should be given the anti-rabies vaccine before the age of 3 months, as the protective antibodies were absent at that age. Thereafter a booster vaccine should be given at a suitable interval, as protective antibody titres were not maintained until 6 months in the majority of puppies (75% of puppies in Group C and 93.3% of puppies in Group D). We cannot comment on the exact time interval for booster immunization on the findings of this study.

Dogs with a previous immunization history (Group A) have a higher chance of maintaining protective antibody titres for a period of 1 year compared to that of dogs in Group B who had no previous immunization history. Therefore, after the first anti-rabies vaccine given to adult dogs without a past immunization history, a booster vaccine should be administered at a suitable interval during the first year. Thereafter, annual boosters should be given to all dogs, in order to maintain the antibody titres above the protective level, as rabies is endemic in Sri Lanka.

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**Table 1. The mean antibody titres of adult dogs and puppies before and after immunization with anti-rabies vaccine**

Group	Mean antibody titres (IU/mL)			
	D <sub>0</sub>	D <sub>30</sub>	D <sub>180</sub>	D <sub>360</sub>
A <sup>a</sup>	5.73	26.69	10.43	4.95
B	0.23	9.37	2.78	1.38
C	0.20	0.84	0.40	0.12
D	0.15	0.74	0.21	0.15

<sup>a</sup>All 4 samples of 2 dogs were contaminated, a sample obtained from one dog on D<sub>0</sub> was haemolysed and one dog was not available for sample collection on D<sub>360</sub>. Readings of these 4 animals recruited for this study were not considered for the calculation of mean antibody titres.

A – adult dogs aged 1 to 5 years with a past immunization history against rabies

B - adult dogs aged 1 to 5 years without a past immunization history against rabies

C – three month old puppies of previously immunized bitches

D – three month old puppies of unimmunized bitches

D<sub>0</sub> – day of immunization with anti-rabies vaccine

D<sub>30</sub> – 30 days after immunization with anti-rabies vaccine

D<sub>180</sub> – 180 days after immunization with anti-rabies vaccine

D<sub>360</sub> – 360 days after immunization with anti-rabies vaccine

**Table 2. Number of canines recruited for each group, number of animals considered for analysis, number of samples which had antibody titres less than 0.5 IU/mL and their percentages**

Group	Number of animals recruited for each group	Number of animals considered for analysis	Number (percentage) of samples which had antibody titres < 0.5 IU/mL			
			D <sub>0</sub>	D <sub>30</sub>	D <sub>180</sub>	D <sub>360</sub>
A	21	17 <sup>a</sup>	2(11.8)	0(0)	0(0)	0(0)
B	16	16	16(100)	0(0)	2(12.5)	8(50)
C	4 <sup>b</sup>	4	4(100)	0(0)	3(75)	4(100)
D	15	15	15(100)	4(26.7)	14(93.3)	15(100)

<sup>a</sup> All 4 samples of 2 dogs were contaminated, a sample obtained from one dog on D<sub>0</sub> was haemolysed and one dog was not available for sample collection on D<sub>360</sub>. Readings of these 4 animals recruited for this study were not considered in the analysis.

<sup>b</sup> At the time of designing this investigation it was decided to include 15 animals for each group. The targeted number was achieved for Groups A, B and D. Puppies of vaccinated bitches (Group C) brought for ARV belonged to good breeds such as German Shepherd, Japanese Spitz, Doberman and Cocker Spaniel. The owners were not willing to give their consent for drawing of blood samples four times from their puppies. Therefore, we were unable to obtain the target number of samples for that group. The 4 puppies recruited to Group C were from 3 different mothers. Their immunizations was begun with Parvo at 6 weeks and anti-rabies at 12 weeks after completing Parvo I & II and DHL I & II immunizations. Bitch of C1 was given anti-rabies vaccine for the first time during the pregnancy period (4 – 5 weeks pregnant). She was 2 years at the time of first anti-rabies immunization.