Evaluation and Optimization of Vaccine Priming in Dogs against Rabies

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ABSTRACT

Vaccination of dogs against rabies is fundamental for rabies control since the dog is the main reservoir and transmitter of rabies in India. Though the young puppies are generally primed with rabies vaccine when they attain three months of age, a few cases of puppies aged below 2 months old were presented to the Rabies Surveillance Unit, Madras Veterinary College Teaching Hospital, which showed positive for rabies by FAT. This study was designed to assess the maternal-derived antibody status of puppies from vaccinated and unvaccinated bitches (Group I and II), and the dynamic status of rabies virus neutralizing antibody in adult dogs (Group III: one year after primary vaccination, Group IV: Regularly vaccinated adults and Group V: Irregularly vaccinated adults). Longitudinal vaccine efficacy was also studied to elucidate the frequency and magnitude of Rabies Virus Neutralization Antibody (RVNA) in pre-exposure prophylaxis (PrEP; Groups VI and VII). Each group comprised 18 healthy dogs, vaccinated with inactivated vaccine and blood samples were collected on 0, 21, 90 and 180 days post vaccination (dpv0, 21, 90 and 180) for PrEP, and the serum neutralizing rabies antibody titres were assessed by Rapid Fluorescent Foci Inhibition Test (RFFIT). In the pre-exposure study, all puppies in both groups had insignificant antibody titre on day 0, which evidenced the waned status of maternal-derived antibody at 12 weeks of age. The dogs that received the prime-boost dose resulted in maintaining the protective titre (≥0.5 IU/mL) in all immunized dogs on dpv180, with a mean titre of 7.09±0.83, whereas primovaccinated dogs resulted in having protective titre only in 88.88 per cent of immunized dogs with a mean titre of 1.94±0.34. Since the level of maternal-derived antibodies, even in puppies born to vaccinated bitches are insignificant at 10 to 12 weeks of age, the immunization strategy against rabies in puppies warrants the advancement of priming age with one booster vaccine at a suitable interval of 3 to 4 weeks to achieve the population immunity in view of effective containment of rabies spread.

Keywords: Rabies vaccine, Pre-exposure priming, Population Immunity

INTRODUCTION

Rabies is an alarming infectious zoonotic disease. Out of 60,000 human rabies victims reported globally, 20,000 rabies deaths are from India, with 59.9% in Asia and 35% globally (WHO, 2018). The disease is still under-reported due to a lack of awareness and integrated surveillance. Although several carnivores are natural reservoirs, dogs are the main source of human infections and pose a threat to more than 3.3 billion people worldwide (WHO, 2010). In India, the risk of exposure is high because of the massive stray and pet dog population of about 70 million, with an estimated dog: human ratio of 1:20 in 2025 (20th Livestock Census of the Government of India). It was estimated that 97% of human rabies cases are due to dogs, followed by cats (2%) and 1% by jackals, mongoose and others (Cliquet *et al.,* 2007).

Developed countries like North America and Europe have successfully eliminated canine rabies through mass immunization and dog population control, whereas it is widespread in over 80 developing countries due to free-roaming dogs. Effective vaccination in dogs is a crucial strategy for rabies. WHO and its global partners aim to end human deaths from dog-mediated rabies through a

comprehensive One Health approach. Periodic vaccination of domestic dogs against rabies would result in a significant reduction of human rabies incidence.

A few cases of rabies were recorded in less than three-month-old puppies at the Rabies Surveillance Unit, Madras Veterinary College, Chennai. It was imperative to know whether dogs in a representative population have antibody titres above the protective level (≥0.5 IU/ml) after the prime vaccination, and to know whether the antibody titres are maintained above the protective level until the time of annual revaccination. Hence, this study was undertaken to determine the dynamics of immunogenicity and the suitable time for booster vaccination in dogs.

MATERIALS AND METHODS

The study was carried out in dogs presented at the Immunisation Unit, Madras Veterinary College Teaching Hospital, Chennai, to study the efficacy of the anti-rabies vaccine in dogs following pre-exposure prophylactic immunization. Based on the age and rabies vaccination history, they were divided into five groups (Group I to V) with 18 dogs in each group, through stratified random sampling. The longitudinal vaccine efficacy study was conducted to elucidate the frequency and magnitude of Rabies Virus Neutralization Antibody (RVNA) in pre-exposure prophylaxis (PrEP; Group VI and VII) as detailed in Table I. The approval from the IEAC and the owner's consents were obtained to conduct this study.

Vaccine and Sera Samples: The anti-rabies vaccine produced with BHK-21 cell substrate, inactivated by β-propiolactone and adsorbed on aluminium phosphate with the potency of greater than or equal to 2 IU per dose, was used for immunization in the study. A total of 36 blood samples were collected randomly from puppies at the age of 45 to 60 days (18 puppies each from vaccinated (Group I) and unvaccinated (Group II) groups to know the dynamic status of the maternal-derived antibody against rabies, and 54 blood samples were collected from adult dogs already primed prophylactically a year before, regularly or irregularly. The dogs were then immunized with inactivated anti-rabies vaccine (1 mL) subcutaneously. The blood samples were collected on 0, 21, 90 and 180 days post-vaccination (dpv0, 21, 90 and 180) for PrEP and sera were obtained after centrifugation at 2000 rpm for 30 mins and stored at -20° C until used. The sample size was determined by executing power analysis.

Virus and Cells: MNA cells and Rabies challenge virus (CVS-11 MNA adopted) obtained from Indian Immunologicals Ltd, NDDB, Hyderabad grown in 96 well plastic microtitre plates (Nunc) in DMEM medium supplemented with 10% fetal calf serum were used.

Media and Reagents: Dulbecco's Modified Eagle's Medium (DMEM from Safc Bioscience, USA) supplemented with 10% fetal calf serum (FCS from Southerncross Biotech, NZ), 1% L-glutamine and Anti-rabies nucleocapsid FITC conjugate procured from Bio-Rad, France were used for RFFIT.

Procedure for Rabies Virus Neutralizing Antibody Estimation by RFFIT: The serum samples were subjected to Rapid Fluorescent Focus Inhibition Test (RFFIT) as per Smith *et al.* (1996) to estimate the functional virus-neutralising antibodies against rabies in the test serum used with the 5th International Standard Reference Serum available at Indian Immunologicals, Hyderabad to calibrate this test.

Statistical Analysis: Statistical comparison between groups was done by Student's independent t-test, and the comparison between different days within treatment groups was done by repeated measures one-way ANOVA (IBM SPSS Statistics 20.0).

Table I: Categorisation of the Study Group

Categorisation		Groups	Number of sera samples collected
Pre-exposure group (PrEP)	Puppies between 45 and 60 days of age	**Group I:** Puppies from vaccinated bitch	18
		Group II: Puppies from unvaccinated bitch	18
	Adult dogs (Between one and five years of age)	**Group III:** One year after primary vaccine	18
		Group IV: Regularly vaccinated dogs above two years of age	18
		Group V: Irregularly vaccinated dogs above two years of age	18
	Puppies at three months of age	**Group VI:** Single-dose anti-rabies vaccination	72
		Group VII: Prime-boost dose anti-rabies vaccination	72
		Total	**234**

RESULTS

The mean RFFIT titres of RVNA in puppies of both vaccinated (Group I) and unvaccinated bitches (Group II) were 1.07 ± 0.18 and 0.30 ± 0.037 IU/mL, respectively. Of 18 dogs in Group I, all puppies except two (88.81%) were having required protective titre above 0.5 IU/mL, whereas 15 (83.3%) puppies of Group II were having sub-protective level of neutralizing antibody at the age of 45 to 60 days. Statistical analysis using Student's t-test revealed that a significant difference was noted in MDA from puppies of both vaccinated and unvaccinated bitches (Fig. 1). Adult dogs were categorized into three groups (Group III, IV and V) based on the time interval after most recent vaccination in view of assessing the persistence of humoral antibodies against rabies. The mean antibody titres of Group III, IV & V were 0.899 ± 0.13, 30.78 ± 12.77 and 1.38 ± 0.44IU/mL respectively. The persistence of protective titre of 0.5 IU/ mL was observed in 12 (66.66%), 16 (88.88%) and 8 (44.4%) adult dogs in Group III, IV & V respectively (Fig.2).

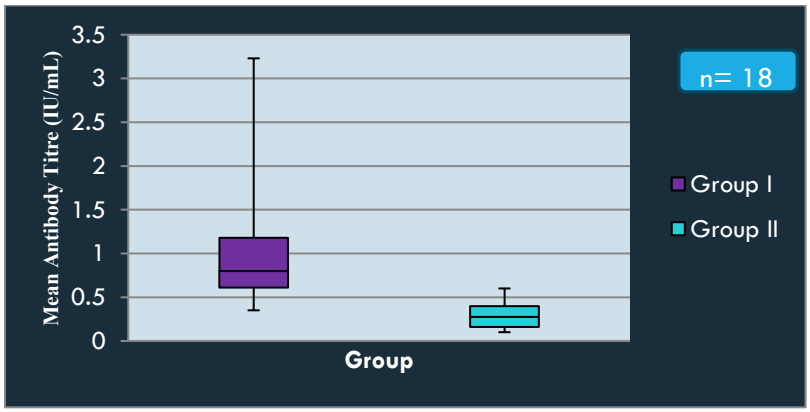

Fig.1: Comparison of MDA against Rabies in Groups I and II

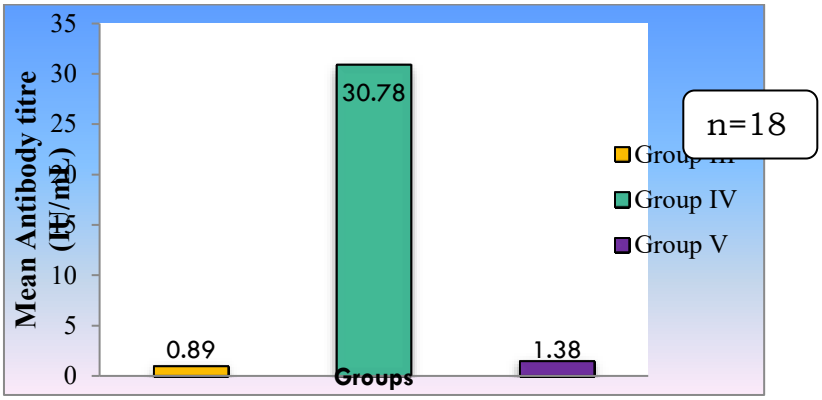

Fig. 2: Comparison of Longitudinal Antibody against Rabies in Groups III, IV and V

RVNA titres of Group VI had shown that the mean titre on dpv 0 was 0.18 ± 0.023 IU/mL, and 100 per cent seroconversion was observed on dpv 21 with a mean titre of 12.21±3.20 IU/mL. Statistical analysis showed a significant (P< 0.01) rise in the RVNA titre between dpv 0 and dpv 21 from 0.18 ± 0.023 IU/mL to 12.21 ± 3.20 IU/mL. The highest titre was observed at dpv 21 post vaccinations, and thereafter the titre was observed to be declining. No significant difference was observed on dpv 90 (3.35 ± 0.65 IU/mL), whereas the statistical reduction in mean titre from dpv 21 (12.21 ± 3.20 IU/mL) to dpv 180 (1.94 ± 0.33 IU/mL) was noticed. On dpv 180, all vaccinated dogs except two showed antibody titres above the protective level of 0.5IU/mL (WHO mandated minimum titre). The mean RVNA of Group VII was found as 0.24 ± 0.027 IU/mL on dpv 0, and all dogs had the titre level below 0.5 IU/mL before vaccination. There was a steady rise in antibody titre (P< 0.01) from dpv 0 (0.24 ± 0.027 IU/mL), dpv 21 (14.99 ± 2.43 IU/mL), to dpv 90 (27.29 ± 3.91 IU/mL) compared to dpv 180. But a significant (P<0.01) reduction of mean rabies antibody titre of 7.09 ± 0.83 IU/mL was observed on dpv 180 (Table II). The highest mean antibody titre of 27.29 IU/mL was noticed on dpv 90, and the trend analysis of RVNA was presented in Fig 3.

Group	Days	Mean	SE	F value
Group VI (Single dose)	dpv0	0.18[a]	0.12	10.57**
	dpv21	12.21[b]	5.44	
	dpv90	3.35[a]	1.97	
	dpv180	1.942[a]	1.23	
Group VII (Prime with booster dose)	dpv0	0.24[a]	0.18	24.57**
	dpv21	14.99[b]	9.84	
	dpv90	27.29[c]	19.03	
	dpv180	7.09[d]	5.32	

Table II: Comparison of RVNA against PrEP in Groups VI and VII

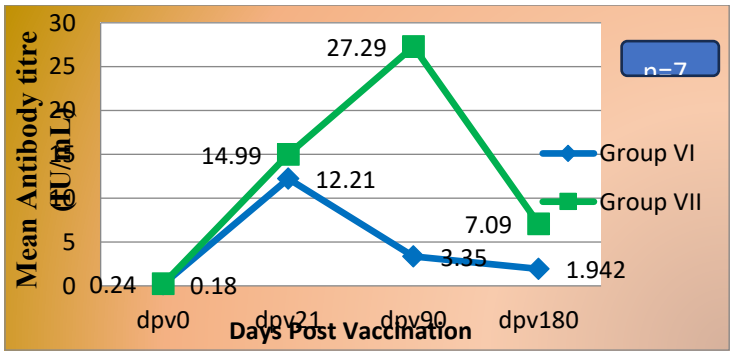

Fig. 3: Trend Analysis of RVNA against Rabies in Groups VI and VII

Compared to only primo (single dose) vaccinated dogs, the dogs that received prime-boost (two doses at three weeks interval) showed significantly high titre (P<0.01) on dpv 90 and dpv 180, and difference was also observed by the student "t" test, though no significant difference was noticed on dpv 0 and dpv 21.

DISCUSSION

The means ± SE titres of RVNA in puppies of vaccinated and unvaccinated bitches were 1.07 ± 0.18 and 0.30 ± 0.037 IU/mL, respectively, revealing a significant (P<0.01) difference of MDA between puppies in both vaccinated and unvaccinated bitches. Out of 18 puppies from vaccinated bitches, only two puppies did not have a protective antibody titre and was in agreement with the findings of Pimburage *et al.* (2017), which might be due to the negative influence of litter size (Pollock and Carmichael, 1982). Puppies of vaccinated bitches had protective antibody titres, compared to puppies of unvaccinated bitches.

A few cases of rabies were recorded in puppies less than three months at the Rabies Surveillance Unit, Madras Veterinary College, Chennai. The dogs with regular vaccination (Group IV) history (88.88%) had higher mean protective neutralizing antibody titres (30.78 ± 12.77 IU/mL) compared to dogs (44.44%) with irregular vaccination (Group V) (1.38±0.44 IU/mL) and young dogs (66.6%) that had been primo vaccinated one year before (Group III; 0.89±0.13 IU/mL). In Group III (dogs which were vaccinated one year before), 33.3 per cent had an inadequate titre (<0.5 IU/mL) after one-year post vaccination. In this study, adult dogs with a previous regular vaccination history had a higher chance of maintaining an RVNA for a period of one year compared to the dogs with an irregular immunization history. In Group V, 55.55 per cent of dogs were seronegative with low humoral response to rabies, which is in agreement with Fernandes *et al.* (2017). An increasing proportion of dogs failing to reach the antibody response cut-off with increasing days from vaccination to sampling was also shown by Kennedy *et al*. (2007).

In primovaccinated dogs, the mean anti-rabies antibody level was found to be maximum during dpv21 in 100 per cent of dogs (12.21 ± 3.20 IU/mL) and thereafter a declining trend was observed with a lower mean antibody titre of 1.94 ± 0.33 IU/mL on dpv180. This result was well correlated with Santosh *et al.* (2017), who found that 85.45 and 100 per cent of dogs and cats, respectively, had protective neutralizing antibody titre on dpv20. The booster vaccination was given at dpv21 for prime-boost dogs and resulted in a peak antibody titre of 27.29±3.91 IU/mL at dpv90, whereas primovaccinated dogs showed a reduction of titre on dpv90. Persistence of RVNA was found more within 1–2 months post vaccination, and waning of titre was noticed in primovaccinated dogs. Oliveira *et al*. (2000) also demonstrated a better immune response in the animals with booster administration, whereas the serum neutralizing antibody titre of the dogs with single vaccination was reported to fall below a protective level.

The results also demonstrated a rapid drop of antibody titre on dpv90 in Group VI dogs (primo-vaccinated dogs) compared to Group VII (dogs vaccinated with prime-boost dose). The importance of booster vaccination and antibody testing was also highlighted by Cliquet *et al*. (2003). Thus, the results of this study underscore the use of two doses of vaccine for dogs to obtain higher and sustained antibody titre till the next annual booster dose.

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