



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(9): 177-179

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www.veterinarypaper.com

Received: 12-07-2025

Accepted: 15-08-2025

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Dynamic study to evaluate post exposure prophylaxis against rabies in canines

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i9c.2551>

Abstract

Vaccination of dogs against rabies is considered as fundamental for National rabies control programmes since the dog is the main reservoir and transmitter of the disease in India, where more than 20000 human deaths are reported only due to rabies. Though rabid bites cases are reported mostly in human beings, there are considerable rabid bite cases reported amongst dogs especially between stray and/or community owned and pet dogs, which results in dissemination of rabies pathogen from infected to other susceptible animals. By considering this threatening fact, the study was designed to elucidate the frequency and magnitude of rabies virus neutralization antibody (RVNA) in post-exposure prophylaxis. Each group comprises of 18 healthy dogs which were vaccinated with inactivated anti-rabies vaccine and blood samples were collected on days 0, 3, 7, 14, 28 and 90 and the serum neutralizing rabies antibody titres were assessed by Rapid fluorescent antibody test (RFFIT). All dogs which received post exposure prophylaxis were observed for 120 days post immunization and none of the animals showed any pathognomonic clinical signs of rabies during the observation period. PEP vaccination of dogs followed with Essen regime (0, 3, 7, 14 and 28 day) was found to be protective in all vaccinated animals.

Keywords: RFFIT, canine, rabies, post exposure prophylaxis, vaccinated animals

Introduction

Rabies is an alarming infectious as well as zoonotic disease because of its high incidence, world-wide distribution, treatment costs, mortality rate and high disability-adjusted life years (DALY) score. Out of 60,000 human rabies victims reported globally, 20,000 rabies deaths are only from India and it accounts for 59.9 percent of deaths in Asia and 35 per cent of deaths globally with over 3.7 million DALY score (WHO, 2018) ^[9]. The disease is still under-reported and neglected due to lack of awareness, resource and integrated surveillance. It is almost invariably fatal once clinical signs occur, as a result of acute progressive encephalitis. Although a number of carnivores serve as natural reservoirs, dogs are the main source of human infections and pose a potential threat to more than 3.3 billion people worldwide (WHO, 2010) ^[8]. In India, risk of exposure was high because of the massive dog population of about 11.67 million with an estimated dog: human ratio of 1:36 in the year 2012 as evidenced by Department of Animal Husbandry and Veterinary Services (19th Livestock census by Government of India) and it was estimated that 97 per cent of human rabies are due to dog, followed by cats (2%) and 1% by jackals, mongoose and others (Cliquet *et al.*, 2007) ^[1]. The probability of success of rabies vaccination in dogs depends on the type of vaccine used, number of boosters and age of vaccination. Even though prophylactic vaccinations are being done regularly by mass vaccination of domestic dog, the effects of these vaccinations are not being assessed. So, it is 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 also to know whether the antibody titres are maintained above the protective level until the time of annual revaccination. Considering these views and overall significance of rabies in India, the current study has been undertaken to ascertain antibody titre against rabies in dogs complying with standard post exposure prophylaxis schedule.

Materials and Methods

The present study was carried out in dogs brought at Immunization Unit, Madras Veterinary College Teaching Hospital, Chennai between September 2017 and November 2018. The longitudinal rabies vaccine efficacy study was also conducted to elucidate the frequency and magnitude of rabies virus neutralization antibody (RVNA) in post-exposure prophylaxis (PEP). The rabies vaccine (Rahsharab, Indian Immunologicals) produced with BHK₂₁ cell substrate, inactivated by β -propiolactone and adsorbed on aluminium phosphate with the potency of greater than or equal to 2.5 IU per dose was used for immunization in this study.

In this study, the dogs were immunized with inactivated rabies vaccine subcutaneously and the blood samples were collected on days 0, 3, 7, 14, 28 and 90 post vaccination (dpv) for PEP and sera were obtained after centrifugation at 2000 rpm for 30 mins and stored at -20° C until tested for rabies antibody quantification. FITC-labelled monoclonal rabies antibodies (M/s Merck Millipore, Germany)-Concentrated blend (5 mL) and Anti-Rabies Nucleocapsid Conjugate (M/s Bio rad, USA)-lyophilized (3 mL) were used. The serum

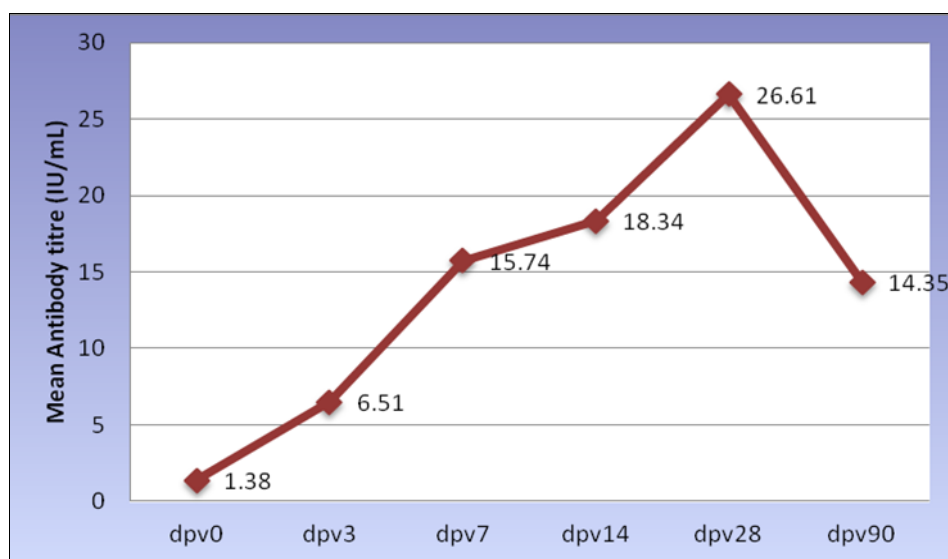
samples were subjected to Rapid Fluorescent Focus Inhibition Test (RFFIT) to estimate the functional virus neutralizing antibodies against rabies in the test serum and the procedure was carried out as per Smith *et al.* (1996) [7].

Results and Discussion:

The mean titre values ranging between 1.38 and 14.35 IU/mL was found from dpv 0 to dpv 90 (Figure 1) of 18 dogs, only four dogs had a protective titre on dpv 0 of post exposure prophylaxis study. The percentage of dogs with adequate antibody concentration (more than 0.5 IU/mL) was 94.44 per cent at dpv 3 and 100 per cent at dpv 7 with mean antibody titre of 6.51±1.98 and 15.74±4.50 IU/mL, respectively. At dpv 28, dogs elicited peak immune response with mean titre of 26.61±6.30 IU/mL of 18 dogs recruited, four dogs were not return on dpv 90. The statistical analysis revealed a decrease ($p<0.01$) in mean antibody titre (14.35±2.36 IU/mL) on dpv90 (Table 1) but antibody persistence and the percentage of dogs with adequate antibody concentration was remained higher than the protective level.

Table 1: Comparison of rabies virus neutralizing antibody (Mean $\pm$ SE) titre (IU/mL) to post exposure prophylaxis in dogs

Group	Days	N	No of dogs with antibody titre $\geq$ 0.5 IU/mL	Mean	SE	F-Value
PEP Group	dpv 0	18	4 (22.22%)	1.38 ^a	0.67	4.48**
	dpv 3	18	17 (94.44%)	6.51 ^{ab}	1.98	
	dpv 7	18	18 (100%)	15.74 ^{bc}	4.50	
	dpv 14	18	18 (100%)	18.34 ^{bc}	5.84	
	dpv 28	18	18 (100%)	26.61 ^c	6.30	
	dpv 90	14	14 (100%)	14.35 ^{bc}	2.36	



N-Number of dogs

** Significant at 1% level ($p<0.01$)

Mean bearing different superscripts differ significantly

Mean bearing same superscripts in the same column don't differ significantly

Fig 1: Rabies virus neutralizing antibody titres in dogs under PEP vaccination schedule.

All animals which received PEP were observed for 120 days and none of the animals showed clinical signs during the observation period. Out of 18 dogs, four dogs (22.22%) showed a protective titre on dpv 0 of post-exposure prophylaxis. This pre vaccination borderline protection of dogs might be due to earlier exposure to very mild antigen or regular prophylactic vaccination as reported by Fekadu (1988) [4]. The rest of 14 (77.7%) dogs showed titre values less than 0.5 IU/mL ranging from 0.07 to 0.4 IU/mL.

All post vaccinal sera except one showed protective titre on dpv 3. This was correlated with Manickam *et al.* (2008) [6] who challenged dogs intramuscularly with virulent rabies virus brain suspension and injected anti-rabies vaccine and found RVNA after 3 days of post vaccination with peak titre on dpv28. The percentage of dog with adequate antibody concentration were 100 per cent at dpv7, with peak antibody concentration on dpv28 (26.61±6.30 IU/mL) and waning of same on dpv90 (14.35±2.36 IU/mL). Similar observations were documented earlier by Sunilkumar *et al.* (2016) [10] and

Minke *et al.* (2009) ^[11]. Whereas, Shi *et al.* (2017) ^[12] observed peak immune response at dpv42 with mean of 12.29±9.751 IU/mL. Irrespective of bite site, age, sex, previous vaccination history, all dogs showed protective titre by dpv 7 which support the use of potent cell culture inactivated rabies vaccine for PEP. But, the initial elevation of antibody response in vaccinated animals was important to neutralize the virus.

Our findings were also supported by Manickam *et al.* (2008) ^[6] who showed that a high and sufficiently protective titer was produced after 5 vaccinations up to 28 days. But some studies conducted earlier by Clark & Wilson (1996) ^[3] included a booster vaccination on the day 90. Though neutralizing titre appeared to be waning in our study, it was likely that the immunity thus stimulated will last for a much longer period. Also, the increased potency of ≥ 2.5 IU/dose veterinary rabies vaccines was expected to contribute to better PEP results.

PEP vaccination of dog by employing Essen regime (0, 3, 7, 14 and 28 day) was found to be protective in all vaccinated animals which was accordance with the observation of Basheer *et al.* (1997) ^[2]. But, Hanlon *et al.* (2002) ^[5] documented that rabies post-exposure treatment (PET) with vaccine alone protocol without immunoglobulin was unable to provide protection against disease following severe exposure to rabies virus.

Although many PEP regimens have been proposed in human, it is still a controversial topic in animals. But, the Essen regime of PEP schedule of human beings is followed for animals also in veterinary clinical practice with a hypothetical idea of dumping antibody in the central nervous system by crossing the blood brain barrier through a series of vaccination regimen.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Sowmiya M, Asokkumar M, Ratnaprabha S, Devi T. Dynamic study to evaluate post exposure prophylaxis against rabies in canines. *International Journal of Veterinary Sciences and Animal Husbandry*. 2025;10(9):177-179.

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