Journal of Animal Research: v.9 n.5, p. 697-699. October 2019

DOI: 10.30954/2277-940X.05.2019.10

Status of Maternal Derived Antibody Against Rabies in Naive Puppies in Chennai

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Received: 24 July, 2019 **Revised:** 02 Sept., 2019 **Accepted:** 15 Sept., 2019

ABSTRACT

Thirty-six blood samples were collected randomly from puppies [18 puppies each from vaccinated (Group I) and unvaccinated (Group II) dam] brought to Immunization Unit, Madras Veterinary College Teaching Hospital, Chennai. The samples were subjected to functional antibody assay (RFFIT) to know the kinetics of maternal derived antibody (MDA) against rabies. The mean MDA titre in group I and II puppies were found as 1.07 ± 0.18 IU/mL and 0.30 ± 0.037 IU/mL respectively. The statistical analysis (Student "t" test) revealed a highly significant difference ($P<0.01$) between MDA of two groups. Thus, this study strongly suggests that the dogs less than three months of age in endemic regions needs to be immunized against rabies in view of maintaining the population immunity and to reduce the bioburden of rabies risk.

Keywords: MDA, Puppies immunization, Rabies, RFFIT

Rabies is a vaccine preventable disease which affects both warm blooded animals and mankind since ancient times. The disease is caused by *Lyssavirus*, Rhabdoviridae and it causes acute progressive viral encephalitis (Rupprecht *et al*., 2002). It is estimated to cause 60,000 human deaths globally, of which 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, TRS 1012, 2018). It is commonly transmitted to other animals and humans through close contact with saliva (primarily biting) from infected animals.

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). Mass vaccination of domestic and community owned dogs is a key for the successful control of canine rabies and a strong body of theoretical and empirical evidence indicates that vaccinating 70 per cent of the dog population during annual campaigns should be sufficient to control rabies (WHO, 2013).

Effective coverage could be achieved through vaccinating juveniles and adults dogs but puppies less than three months of age are often excluded from vaccination programme on the perception that immature immune systems and maternal antibodies inhibit sero-conversion to rabies vaccine. Unfortunately, few cases of rabies were recorded in less than three months old puppies at Rabies Surveillance Unit, Madras Veterinary College, Chennai, India.

Since India is being endemic for rabies, it is essential to know whether the puppies born to vaccinated dam have protective MDA levels until the time of priming against anti-rabies immunization. With these views, this study has been undertaken to know the present status of MDA in naive puppies.

How to cite this article: Sowmiya M., Asokkumar M., Vijaya Bharathi M., Rathnapraba S., Senthil Kumar R.L., Devi T. and Nagarajan B. (2019). Status of maternal derived antibody against rabies in naive puppies in Chennai. *J. Anim. Res.,* **9**(5): 697-699.

MATERIALS AND METHODS

A Mouse Neuro Blastoma (MNA) cell, Rabies Challenge Virus Standard (CVS-11) strain, Standard rabies immunoglobulin (WHO) obtained from Indian Immunologicals Ltd, Hyderabad was used. Dulbecco's Modified Eagle's medium (DMEM) (Invitrogen, Gibco, USA) and Anti-Rabies Nucleocapsid FITC Conjugate (Bio Rad, USA) were also used for this study.

A total of 36 blood samples were collected from puppies using a pro-coagulation tube at Immunization Unit, Madras Veterinary College Teaching Hospital, Chennai, Tamil Nadu and serum was obtained after centrifugation at 2000 rpm for 3 minutes and stored at -20 °C until tested. They were categorized into two groups (Group I: Puppies from vaccinated dam & Group II: Puppies from unvaccinated dam) with 18 puppies in each group and analyzed for the presence of MDA. The data regarding age, breed and vaccination history of dam were also recorded. The sera were subjected to complement inactivation at 56° C for 30 minutes and functional rabies virus neutralizing antibody (RVNA) was estimated by Rapid Fluorescent Foci Inhibition Test (RFFIT) according to Smith *et al.* (1996).

RESULTS AND DISCUSSION

The mean antibody titres of RVNA in puppies from vaccinated and unvaccinated dam were 1.07 ± 0.18 and 0.30 ± 0.037 IU/mL respectively (Fig. 1). Statistical analysis revealed that a significant difference ($P<0.01$) was noted between MDA in puppies of both vaccinated and unvaccinated dam (Table 1).

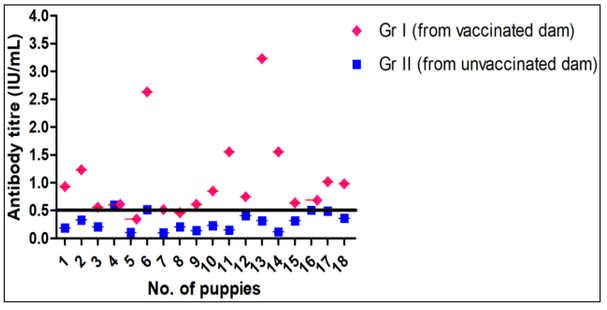

Fig. 1: MDA profile against Rabies in puppies

Animal is considered protective if the rabies virus neutralizing antibody titre is greater than or equal to 0.5 IU/mL (WHO, 2013). In our study, 88.8 per cent of puppies from vaccinated dam (Group I) were having protective titre above 0.5 IU/mL at the age of 2 months. This finding was in accordance with Chappuis (1998) who found protective antibody titre above 0.5 IU/mL in 10 out 12 beagle puppies of vaccinated dam. Similarly, Vos *et al*. (2003) also detected MDA in fox cubs which were born to orally immunized foxes. It implies that antibody may be transferred from dam to offspring via placenta and colostrum (Muller *et al.,* 2002). In the present study two puppies from vaccinated dam did not have protective titre. This was in agreement with findings of Pimburage *et al.* (2017) who reported very low antibody titre of 0.10 IU/mL in puppies of vaccinated dam. This might be due to negative influence of dog litter size as stated by Pollock and Carmichael (1982).

Only three out of 18 (16.66%) puppies from unvaccinated dam (Group II) showed presence of protective titre above the cut off value of 0.5 IU/mL. Amongst three, two were non-descriptive dogs and it could be due to the presence of natural immunity. The rest of the dogs (83.3%) showed antibody levels less than the protective level, indicated their vulnerability for getting the rabies infection and thus posing threat to surrounding human beings and animals. Pimburage *et al.* (2017) reported a very low antibody titre of 0.07 IU/mL in puppies of unvaccinated dam. This finding is in concurrence with our result.

Our study showed that puppies of vaccinated dam had protective antibody when compared to puppies of unvaccinated dam. The presence of maternal antibody depends upon many factors which include health status of dam, quality and quantity of colostrum secreted and quantity of colostrum ingested by pups and previous history of vaccination (Muller *et al.*, 2012). Assessment of maternal antibody in the offspring is not only important to know the immune status, but also it is helpful to decide the proper age for vaccination in an area.

Generally, Countries like India the puppies less than 3 months of age were excluded from rabies vaccination programme on assumption that they have immature immune system and MDA (Day, 2007). But Siegrist (2012) stated that MDA and immune function may not limit the immune response to inactivated vaccine which stimulates both T and B cell response. The interference between passive neutralizing antibodies of maternal origin and

Table 1: Comparison of mean ± SE rabies virus neutralizing antibody titres between group I & II

Variables	N	Group I			Group II			t Value	P value
		No. of dogs with antibody titre ≥ 0.5 IU/mL	% with antibody titre ≥0.5 IU/mL	Mean ± SE	No. of dogs with antibody titre ≥ 0.5 IU/mL	% with antibody titre ≥0.5 IU/mL	Mean ± SE		
Below 3m of age	18	16	88.88	1.07 ± 0.1807	3	16.66	0.30 ± 0.0373	4.17	0.0002**

** Significant at 1% level (P<0.01), N- Number of dogs.

active immunization has been studied by Precausta *et al*. (1985) and they found that when puppies vaccinated at the age of one-month responded with the same neutralizing antibody level as puppies vaccinated at seven months of age.

CONCLUSION

The present study advocates the importance of early vaccination especially in the endemic countries like India and also recommends that all dogs including puppies less than three months of age should be vaccinated and booster vaccine should be given at a suitable interval as per WHO, 2013 guidelines.

ACKNOWLEDGEMENTS

We would like to thank Director of Clinics, Tamil Nadu Veterinary and Animal Sciences University and Professor and Head, Department of Clinics, Madras Veterinary College for permitting to collect samples from Immunization Unit, Madras Veterinary College Teaching Hospital, Chennai, Tamil Nadu and also Dr. K. Anandhakumar, Managing Director, Indian Immunologicals, NDDB, Hyderabad for granting permission to carry out the partial work.

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