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Short Communication

A Note on Seroconversion of Prophylactic Anti-rabies Vaccination in Dogs

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ABSTRACT

The present study was conducted to assess the seroconversion following prophylactic anti-rabies vaccination in dogs and to compare the efficacy of intramuscular and subcutaneous route of administration of vaccine. Fifty one unvaccinated dogs, of either sex and above three months of age were selected and randomly divided into two groups. Two doses of inactivated tissue culture anti-rabies vaccine containing 2.5 IU /dose was given on day one and 21 by intramuscular route to group I and by subcutaneous route to group II. Serum samples collected on day 1, 21 and 28 days of vaccination and assessment of serum neutralizing antibodies was done by rapid fluorescent focus inhibition test. Intramuscular route of administration resulted in seroconversion to protective level in 31.6 percent dogs by 21st day with a mean titre of 0.63±0.22 IU/ml and in 90 per cent dogs by 28th day with a mean titre of 1.75±0.28IU/ml. Subcutaneous route resulted protective titre in 64.5 per cent with a mean titre of 1.45±0.46 IU/ml by 21st day and in 96.8 per cent of dogs by 28th day with a mean titre of 5.09±1.18 IU/ml. Highly Significant rise in the mean titre was observed between the intervals. Both routes of administration of vaccine were found to be providing protective titre in 90 per cent and above of the vaccinated dogs, but statistical analysis revealed significantly higher titre by subcutaneous route than intramuscular route on day 28, but no significant difference was observed on day 21.

Key words: Rabies, Vaccination, Seroconversion, RFFIT, Dogs

INTRODUCTION

Rabies is viral encephalitis of man and animals. Rabies remains as a potential public health problem all over India, especially in Kerala with an increasing incidence during recent years. Prophylactic vaccinations in dogs are considered to be the most important method for control of rabies. In India majority of rabies cases were attributed to the bite of dogs (Yadav, 2002). The probability of success of rabies vaccinations of dogs depends on type of vaccine used, number of rabies vaccinations, the breed size of the dog, age at vaccination, and number of days after vaccination when the antibody titres are tested (Berndtsson *et al*., 2011). Immune response to vaccination is quantified by using different techniques such as Virus neutralisation test (VNT), Rapid fluorescent focus inhibition test (RFFIT), Fluorescent antibody virus neutralisation test (FAVN) and ELISA (Ondrejkova *et al*., 2002; Kostense *et al*., 2012). Among these RFFIT has high sensitivity and is extensively used (Singathia *et al*., 2012). Even though prophylactic vaccinations are being done regularly in dogs, seroconversion studies are not being undertaken and the effects of these vaccinations are not being assessed. Hence the present study was conducted to assess the seroconversion following prophylactic anti-rabies vaccination in dogs and to compare the efficacy of intramuscular and subcutaneous route of administration of vaccine.

MATERIALS AND METHODS

Fifty one unvaccinated dogs, of either sex and above three months of age were selected and randomly divided into two groups. Group I consisting of 20 and group II with 31 dogs. Inactivated tissue culture anti-rabies vaccine containing 2.5 IU /dose was used for the study. Two doses of vaccine were given on day zero and 21 by intramuscular route to group I dogs and by subcutaneous route to group II. Serum samples collected on day 1, 21 and 28 days of vaccination and assessment of serum neutralizing antibodies was done by rapid fluorescent focus inhibition test (RFFIT) (Smith *et al*., 1996).

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The REFIT was performed by mixing 50µl of test serum with 100 µl of rabies virus (50 percent fluorescing foci doses, 50 FFD_{50}) in a multichambered glass slide. After allowing the mixture to react in a CO_2 incubator at 37^0C for 90 minutes, 150 µl mouse neuroblastoma cells in eagle's minimum essential medium with ten per cent foetal bovine serum was added to each serum-virus mixture. The serum - virus- cell cultures were incubated for 20 hours in a CO_2 incubator at 37^0C. The contents in the chamber slides were removed by inverting the slides so that only the monolayer remained at the bottom of the chamber slides. Added 10 µl phosphate buffered saline (PBS 0.01M, Ca_2+ and Mg_2+ free, pH 7.4) and removed by inverting the chamber. After drying, added 100 µl chilled acetone and kept at -20^0C for 15 minutes. Removed the acetone and washed with PBS until the trace of acetone was removed. After drying the slides, added 50 µl of diluted antinucleocapsid conjugate and incubated for 30 minutes at 37^0C. The slides were then screened under a fluorescent microscope for the presence of fluorescing cells .Twenty microscopic fields were read for each serum dilution and compared that against the virus control.

The mean antibody titres on day 1 and 21 and 28 days after intramuscular and subcutaneous routes were compared using paired t-test and comparison between the routes was done by independent t-test. Also the titre on different days of vaccination was compared with the protective titre of 0.5 IU/ml of serum using one sample t-test.

RESULTS AND DISCUSSION

Rapid fluorescent focus inhibition test showed serum antibody titres in dogs under study before and after vaccinations (Table1). The usefulness of RFFIT for detection of antirabies antibodies in nonvaccinated and vaccinated dogs was described by several workers (Pandit *et al*., 1991; Smith *et al*.,1996). Among the 51 dogs selected two dogs were having protective antibody titre on day zero itself prior to vaccination. Presence of maternal antibody might account for this protective titre. This finding is in agreement with that of Vos *et al*. (2003), who detected maternal antibodies in fox cubs born to orally immunised foxes and fed with colostrum. Among the 51 vaccinated dogs, only 26 dogs (52%) developed protective titre of >0.5IU/ml by 21st day and 48 dogs (94%) developed protective titre by 28th day. Failure of development of protective titre in 25 dogs (48 %) after first vaccination might be due to interference of maternal antibody at the time of vaccination. Morshedi and Aslani (2002) also observed failure in development of adequate protective antibody titre after a single dose of rabies vaccine in a group of dogs. Lower antibody titres in younger dogs may also be due to the fact that the vaccine has been administered before the dog has reached immunocompetence as suggested by Day (2007)

The mean antibody titre in group 1 and 2 were 0.07±0.06 and 0.07±0.04 respectively. Intramuscular route of administration in 20 dogs resulted in seroconversion by 21st day with a mean titre of 0.63±0.22 IU/ml and by 28th day with a mean titre of 1.75±0.28IU/ml. Statistical analysis using paired t-test showed highly significant rise in the virus neutralising titre from day 1 to day21 and day 28. The protective titre of 0.5 IU/ml of serum was achieved in 31.6 percent dogs by 21st day and in 90 per cent dogs by 28th day. Subcutaneous route of vaccination resulted in increase in mean antibody titre to 1.45±0.46 IU/ml by 21st day and to 5.09±1.18 IU/ml by 28th day. Highly Significant rise in the mean titre was observed between the intervals. The protective titre of 0.5 IU/ml of serum was achieved in 64.5 percent dogs by 21st day and in 96.8per cent dogs by 28th day. When the mean antibody titres were compared

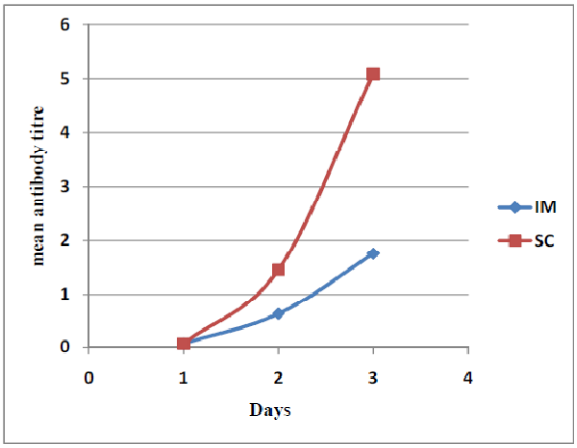

Fig.1: Comparison of mean rabies antibody titres after vaccination by intramuscular and subcutaneous routes (Days 1-day of first vaccination, 2-21st day and 3-28th day)

Table 1: Comparison of titre between days using paired t-test

Route	Mean	N	Mean±SE	t-value	P-value
Intramuscular	Pair 1	Day1	0.07±0.06	2.529**	0.02
		Day21	0.63±0.22		
	Pair 2	Day1	0.07±0.06	6.03**	< 0.001
		Day28	1.75±0.28		
	Pair 3	Day21	0.63±0.22	4.37**	< 0.001
		Day28	1.75±0.28		
Subcutaneous	Pair 1	Day1	0.07±0.04	2.956**	0.006
		Day21	1.45±0.46		
	Pair 2	Day1	0.07±0.04	4.243**	< 0.001
		Day28	5.09±1.18		
	Pair 3	Day21	1.45±0.46	3.458**	0.002
		Day28	5.09±1.18		

Table 2: Comparison of titre with Protective titre (0.5IU/ml) using one sample t-test

Route	Days	Mean	t-value	P-value
Intramuscular	Day1	0.07±0.06	6.903**	< 0.001
	Day21	0.63±0.22	0.592^{ns}	0.561
	Day28	1.75±0.28	4.453**	< 0.001
Subcutaneous	Day1	0.07±0.04	10.003**	< 0.001
	Day21	1.45±0.46	2.066*	0.048
	Day28	5.09±1.18	3.904**	< 0.001

ns - No significant variation; * -Significant variation (P<0.05); ** -Highly significant variation (P<0.01)

Table 3: Comparison between intramuscular and subcutaneous using independent t-test

Days	Intramuscular	Subcutaneous	t-value	p-value
Day1	0.07±0.06	0.07±0.04	0.058^{ns}	0.954
Day21	0.63±0.22	1.45±0.46	1.605^{ns}	0.116
Day28	1.75±0.28	5.09±1.18	2.759**	0.009

ns - No significant variation; ** - Highly significant variation (P<0.01)

with the level of protective titre using one sample test, significantly low titres were observed on day 1 prior to vaccination and significantly higher titre were observed on day 28 after vaccination in both groups. The mean titres on day 21 was higher than the protective titre, but was statistically non significant (Table 2).

Both routes of administration of vaccine were found to be providing protective titre in 90 per cent and above of the vaccinated dogs. Comparison of the mean antibody titres between groups using independent t-test revealed significantly higher titre by subcutaneous route than intramuscular route on day 28, but no significant difference was observed on day 21 (Table 3). Thus subcutaneous route of administration gives higher antibody titre and comparatively higher percent of protection than intramuscular route of administration (Fig.1). In contradictory to this several workers demonstrated higher antibody titres with intramuscular vaccinations than by subcutaneous route (Bunn, 1985; Soulebot *et al*., 1970). Merry (1970) found no clear advantage for IM route over SC route.

Poor immune response to the vaccine was noticed in some of the dogs in the present study which might be due to difference in the host factors such as breed, age, genetics, nutrition and presence of other infections as suggested by Kalanidhi *et al*. (1998) and Delgado and Carmenes (1997). Results of the present study stress the importance of booster dose after 3 to 4 weeks of the primary vaccination. The need for a booster vaccination regimen was also recommended by Berndtsson *et al*. (2011) especially for larger breeds of dogs

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