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Serological response to anti-rabies vaccination in animals: Failure to achieve a protective antibody level

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ABSTRACT

The present study was conducted to elucidate the serological response in 183 rabies suspected cases of small and large animals presented Veterinary Clinical Complex from July 2010 to June 2011. Immune status was assessed by ELISA. Out of 183 cases, 180 were dogs and 3 large animals. In vaccinated dogs, rabies virus specific antibodies (0.5IU/µl) were detected in 70.55% (115/163) cases. Whereas 29.44% vaccinated dogs did not reveal protective rabies titre. None of the unvaccinated dogs and large animals showed protective rabies specific antibody titre. The antibody level was directly proportional to the number of vaccine shots. Vaccination in dewormed animals revealed better protection and antibody titre (8.878 IU/µl) than non-dewormed animals (4.01 IU/µl). However, no significant difference in the rabies antibody titre among the sexes was observed.

Key words: Antibody, Anti rabies vaccine, Dogs, ELISA, Serosurveillance, Titre

Rabies is one of the important fatal zoonotic viral diseases afflicting humans and animals and till date the disease is under-reported in many developing countries. It is caused by highly neurotropic negative sense single stranded RNA (ssRNA) virus, belonging to family *Rhabdoviridae* of the Order Mononegavirales (Pringle 1991). The quantitation of anti-rabies antibodies is of great importance and is frequently employed to determine the immune status in case of animals and human beings at pre-exposure and post-exposure treatment. The detection of antibodies is also useful in rabies diagnosis, late in the course of disease (Crepin *et al*. 1998). The presence of neutralizing antibodies in serum of vaccinated animals is taken as a reliable indicator for the success of active immunization. Enzyme-linked immunosorbent assay (ELISA), based on the detection and titration of the anti-glycoprotein antibodies may be the method of choice in rabies diagnosis, as it is simple, safe, rapid and sensitive method of detecting rabies antibody (Cliquet *et al.* 2003). The present study was conducted to examine the level of protection achieved after primary and secondary booster vaccination against rabies.

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MATERIALS AND METHODS

Sample collection: The blood samples were collected from 183 cases submitted (July, 2010 to June, 2011) to Veterinary Clinical Complex of the University for vaccination. Out of these 183 animals, 163 samples were from vaccinated dogs, 17 were from unvaccinated dogs and 3 were from rabies suspected large animals. The data regarding species, breed, habitat, age, sex, purpose of pet, history of bite, vaccination status of animals, type of vaccine used (single or multiple), route of vaccination, and other vaccine used were recorded. The blood was collected in disposable vials and serum was separated carefully to avoid hemolysis and stored at –20 °C until further analysis.

Protocol of ELISA by qualitative and quantitative method: The ELISA test was performed by using kit as per the procedure of manufacturer. The optimal density was read at 450–620 nm by using ELISA reader within 30 min after stopping the reactions. Serum titres were expressed in equivalent unit per milliliter (EU/µl). The quantity of anti-rabies antibodies was determined by standard curve method.

RESULTS AND DISCUSSION

Dogs (183) of different age presented at Veterinary Clinical Complex, GADVASU, Ludhiana were tested for rabies virus specific antibodies. Out of these 180 dogs, 163 were immunized with different makes of rabies vaccine. World Health Organization (WHO 1992) recommended standard of ≥0.5 IU/µl. Antibodies were detected in 115 out

of the 163 dogs (70.55%) by ELISA. However, 48 cases were tested to have ≤0.5IU/µl antibody titre.

Among 180 dogs, 17 dogs were unvaccinated, in which 10 dogs were control and 7 dogs were of unvaccinated status, but suspected for rabies based on history and clinical signs. The antibody titres are shown in (Tables 1). All the large animals (1 cow and 2 buffaloes) were not vaccinated but suspected for rabies based on history and clinical signs. None of the unvaccinated control dogs (17/180) and no large animals showed protective rabies specific antibody titre i.e. above 0.5 IU/µl.

According to different makes of vaccine used, the present study revealed that 115 out of 163 (70.55%) vaccinated dogs showed rabies antibody above 0.5 IU/µl whereas, 48 out of 163 (29.54%) showed rabies specific antibodies below protective level (0.5 IU/µl) (Table 1). The lack of proper protective antibody level after the vaccination of dogs against rabies was reported (Tepsumathanon *et al.* 1999 and Sage *et al.*1993). In contrast, Chomel *et al.* (1988) found that 97% of the animals had titre above 0.5 IU/µl after vaccination. The lower levels of antibody titre in the present study may be attributable to genetics, nutrition, or parasitic infections that contribute to the poor immune response (Sage *et al.*1993). However, in the present study protective antibody titre was 70.55% (115/163) in vaccinated dogs. Feyssaguet *et al*. (2006) and Servat *et al*. (2006) found the sensitivity of ELISA 98.6% and specificity 99.4%. Whereas, Servat *et al*. (2006) found a kit highly specific (> 98%) using a cut off level 0.5 IU/µl.

Effect of number of doses of vaccine/age of the animal on antibody titre against rabies virus in different breeds of dogs: Out of total 173 serum samples of dogs, 10 dogs were tested for rabies virus specific antibodies before vaccination at the age of 2 to 6 months and their antibody titres ranged from 0.052 to 0.490 IU/µl (Table 1). All these dogs had antibody level less than protective level (0.5 IU/µl). Whereas, Kasempimolporn *et al*. (1996) analyzed serum antibodies titre to rabies virus in 32 puppies before primary vaccination and only five showed protective level of antibody. However, Koprowski *et al*. (1967) reported that low levels of neutralilizing antibodies (less than 0.5 IU/µl) did not protect the animals. Gangadhar and Raghvan (1996) and Kasempimolporn *et al*. (1996) reported that maternal antibodies were found in pups up to 2½ months of age and their level of passive maternal antibodies dropped below the detectable levels at 3½ months of age. Therefore, first vaccination should not be done in dogs younger than 3 months of age. Povey *et al*. (1997) and Vos *et al*. (2001) reported that presence of blocking levels of passive maternal antibodies might interfere with the active immunization.

In the present study, 38 out of 63 dogs (3–15 M) tested for rabies virus specific antibody 1 month after single vaccination, revealed that 60.31% cases had antibody titres ranging from 0.50 to 8.56 IU/µl. Similarly, 26 out of 39 dogs i.e. 66.66% (15–28 M) after single booster vaccination revealed protective antibody titre. Whereas, 19 out of 23 dogs i.e. 82.60% (30–42M) after 2 booster vaccinations revealed antibody titres of 0.581 to 8.31 IU/µl. In addition, 32 out of 38 dogs i.e. 84.21% (> 36 M) tested after 3 or more than 3 booster vaccinations revealed antibody titre that ranged from 0.748 to 8.878 IU/µl. The findings of this study were similar to those of Seghaier *et al*. (1999), who reported that one month after single vaccination, 65% of the dogs less than 1 year of age and 76–84% of the older dogs had significant antibody titres. One month after annual revaccination, these percentages ranged between 92 and 100%, respectively. Similarly, Adeyemi *et al*. (2004) compared single vaccination and booster coverage and noted that booster vaccination gave significantly higher level of antibodies. In contrast, Bunn (1991) and Sage *et al.* (1992) reported that single injection of pre-exposure rabies vaccine did not produce a long lasting

Table 1. Dose-wise/age-wise rabies antibody titre level in dogs based on an ELISA cut off level 0.5 IU/µl

Age (Number of booster (s))	Total No. of animals tested	Number of animals				Protective level %
		Titre above 0.5 IU/µl (protective level)		Titre below 0.5IU/µl		
		No. of animals	Titer range in IU/µl	No. of animals	Titer range in IU/µl	
2–6 months (non vaccinated)	10	0	0	10	0.052–0.490	0
3–15 months (with single vaccine/ without booster)	63	38	0.50 to 8.56	25	0.03to 0.498	60.31
15 to 28 months (1 booster)	39	26	0.720 to 6.58	13	0.07to 0.392	66.66
2.5 – 3.5 years (2 booster)	23	19	0.581 to 8.31	04	0.148to0.494	82.60
More than 3.5 years (3/ more than 3 booster)	38	32	0.748 to 8.878	06	0.097to 0.494	84.21
Total	163+10 (not vaccinated)	115		48		70.55

protective level of antibody titre in a significant number of animals. Moreover, Bota *et al*. (1987) reported that titre of neutralizing antibodies correlated with number of vaccinations and it did not correlate with age of the dogs or with the presence or absence of anaemia.

Effect of purpose on antibody level against rabies virus in vaccinated dogs: Out of vaccinated (163) dogs, all guard dogs (3/3) showed antibody titre above 0.5 IU/µl and their antibody titre ranged from 1.72 to 4.10 IU/µl whereas, pet dogs showed protected level of rabies antibodies in 67.29% (107/159) and their antibody titre ranged from 0.503 to 8.878 IU/µl. There was only one hunting dog that was tested for rabies antibodies and it did not show protective level. It indicated that antibody titre was better in guard dogs as compared to pets or hunting dogs, which may be due to the reason that guard dogs are given individual attention and properly vaccinated compared to pets and hunting dogs. In contrast, Silvia and Pedro (1996) reported that guard dogs had lower seroprevalence (40%) than used for hunting or as pets (62 and 56% respectively).

Effect of sex on antibody titre against rabies virus in vaccinated dogs: During this study period of the 163 vaccinated dogs, higher number of female (69.09%; 38/55) dogs showed protective levels of antibodies compared to male (66.66%; 72/108). It indicates that there was no significant effect of sex on the antibody titre in dogs. Ohore *et al*. (2007) and Silvia and Pedro (1996) reported similar observations. Total 180 cases were tested for rabies virus specific antibodies in various breeds of dogs on the basis of their habitat. No significant difference was observed in rabies antibodies of vaccinated dogs of both rural and urban habitat. Silvia and Pedro (1996) gave similar findings.

Effect of rabies antibody titre on ante-mortem diagnosis: Total 7 cases of rabies suspected dogs were tested for rabies antibodies. Out of these suspected dogs, 5 were of unknown vaccination status and did not show protective level of antibodies, whereas 2 non-vaccinated dogs had antibody titre below protective level. All these 7 dogs died within 2 days and were found to be positive for rabies by FAT, H and E staining, and immunohistochemistry. Similarly, 3 unvaccinated and rabies suspected large animals (1 cow and 2 buffaloes) were tested for rabies antibody status by ELISA. All these cases showed rabies antibody titre below protective level. Koprowski *et al*. (1967) also reported that low levels of neutralizing antibodies did not protect the animals.

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