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Research Article

ASSESSMENT OF SEROLOGICAL RESPONSE TO THE ANTI-RABIES VACCINATION IN PET DOGS: A HOSPITAL BASED STUDY

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ABSTRACT

The present study assessed prevalence of anti rabies antibodies in serum of dogs brought for vaccination in and around Jammu region, J&K, India using the I-ELISA. A total of 180 serum samples, {CVH (n=154), TVCC (n=11) and Private clinics (n=15)} were analyzed. Samples were categorized as Category I - <3 months of age group, Category II - 4 months to 1 year and Category III - >1 year. Sero-prevalence in different age groups was recorded as; Category I - 38.09%, Category II - 39.65% and Category III - 56.43% qualitatively. Quantitatively, out of total 43 positive samples, prevalence in category I was 60 %, in Category II- 41.66 % and in Category III- 61.53 %. Overall sero-prevalence of 49.44 % (89 samples out of 180 samples) and 79.06 % (35 samples out of positive 43 sera samples selected randomly from 89 positive sera samples) was recorded qualitatively and quantitatively, respectively. A total of 34 out of 43 samples (79.06%) had protective titre above 0.5 IU/µl. There was comparatively higher antibody titre in males (50.38%) as compared to females (46.93%). The study revealed that yearly booster dose (category III) provided better results as compared to the serum samples from first time brought for vaccination (category I) and first booster dose (category II). So, owners which go for only one time vaccination for their dogs during their life span must be educated to yearly vaccinate their dogs.

Key words: Antibody, Anti Rabies Vaccine, Canine, ELISA, Sero-prevalence

Rabies is a neurological disease of mammals caused by neurotropic virus of the genus *Lyssavirus* belonging to the family Rhabdoviridae that is almost invariably fatal, once the clinical signs develop. It is an acute, viral encephalomyelitis (inflammation of both brain and spinal cord) which affects all warm blooded animals (Vegad and Katiyar, 2008). Over 99 percent of human exposures to rabies result from the bite of domestic dog (*Canis familiaris)* (WHO, 2018). Because of the increasing dog population and poor dog ownership practices (Adaba *et al*., 2004), most dogs are not vaccinated against this vaccine preventable disease (Ahmed *et al*., 2000). Voluntary vaccination of dogs is declining and they are rarely tested for immune response. WHO recommends 70 per cent of dogs to be vaccinated to control rabies in a community (WHO, 2018). It is not enough just to vaccinate individual dogs, rather, the efficiency of the vaccines evidenced by sero-conversion needs to be monitored. It is possible to estimate the vaccine efficiency by various available serological tests for specific antibodies to Lyssa viruses (Trimarchi and Nadin-Davis, 2007). Keeping in view the fatal nature of disease, it becomes imperative to assess the immunological response to rabies vaccination *vis-a-vis* the protective titre. 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 (Cliquet *et al.,* 2003). This study was carried out to determine the prevalence of anti rabies antibodies in vaccinated pet dogs by indirect ELISA (a prescribed test for international trade) and to examine the level of protection achieved after various doses of vaccination (Ist time, Ist booster and yearly booster).

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MATERIALS AND METHODS

Sample collection

The blood samples were collected from 180 dogs from various places, *viz.*, Teaching Veterinary Clinical Complex, SKUAST-J (Jammu), Central Veterinary Hospital, Dept. of Animal Husbandry, Jammu and 3 private clinics in and around Jammu. The study period was from June, 2016 to April, 2017. Out of the 180 serum samples, 21 were from Category I-Ist time brought for vaccination, 58 were from Category II-Ist booster category and 101 were from Category III-yearly booster category.

Indirect Enzyme linked immunosorbant assay (ELISA):

I-ELISA was performed which was intended to identify antibodies (IgG) against epitopes of rabies virus, in serum samples.

Preparation and dilution of the sera samples

Qualitative ELISA: The sera samples were diluted using ELISA buffer (1:250) and added to the wells of the pre coated plate as per the format of test.

Quantitative ELISA: The serum sample for carrying out quantitative ELISA were titrated using a 3-times serial dilution, starting with a dilution of 1:50 up to1:1350. The plates were washed using the wash buffer by executing a cycle of 5 times. After washing, the bound antibodies were detected using HRP conjugated anti-species conjugate. The color reaction in the wells was directly related to the concentration of rabies virus antibodies in the serum sample.

Table 1
Age and vaccination wise sero-prevalence of rabies antibodies in dog serum samples (Qualitative)

Category (no.)	Age group	No.	Positive (%)	Negative (%)
First time (21)	<3 months	5	2	3
	3 months	7	2	5
	>3 months	9	4 (38.09%)	5 (61.90%)
First booster (58)	4-6 months	38	15	23
	7-9months	13	3	10
	10-12months	6	4	2
	>1yr	1	1 (39.65%)	0 (60.34%)
Yearly booster (101)	1.5-3 years	65	36	29
	3.5-5 years	18	10	8
	5.5-7 years	6	3	3
	7.5-above	12	8	4
		12	(56.43%)	(43.56%)
Total (180)			89 (49.44%)	91 (50.55%)

ELISA test protocol by qualitative and quantitative method: The test was performed by using the kit contents provided by the manufacturers. The absorbance values were read at 450 nm within 10 minutes by using ELISA reader with substrate control as blank. Serum titres were expressed in International units per microlitre (IU/µl). The quantity of antibodies was determined by standard curve method.

Test Protocol for Qualitative and Quantitative ELISA was used according to the instructions of the manufacturer and absorption values were recorded by ELISA reader at 450 nm.

Calculations:

Qualitative

The mean optical density for negative control (NC) and positive control (PC) were calculated. The ratio (S/P) of sample OD to mean OD of the positive control was calculated according to the following equation:

$$\frac{S}{P} = \frac{OD_{sample} - MOD_{NG}}{MOD_{PC} - MOD_{NG}}$$

Where, S/P=Samples/Positive, MOD=mean optical density, OD=optical density, PC=positive control, NC= negative control

Quantitative

The ELISA titre were calculated by constructing a curve and using cut-off line (dilution 1:50-1:150-1:450 and 1:1350) with OD on Y-axis and titres on X-axis. ELISA titres were calculated using a cut-off 2.5 times the OD value of negative control at 1:50.

The Fluorescent Antibody Virus Neutralisation (FAVN) titre of the positive control was 1.83 IU (as per the manufacturers). The K-factor was calculated by dividing the obtained ELISA positive titre by 1.83 to get K factor in IU. All the ELISA titres obtained in the constructed graph were divided by K factor so as to obtain FAVN titres in IU.

RESULTS AND DISCUSSION

Dogs (180) of different ages from and around Jammu were tested for rabies virus specific antibodies. Almost all of the dogs were immunized with different makes of rabies vaccine. Antibodies were detected in 89 (49.44%) serum samples out of the total 180 serum samples qualitatively and 34 (79.06%) serum samples out of the total 43 positive samples for anti-rabies antibodies quantitatively. Out of the total 180 serum samples, 21 dogs were from the age group of 0 to 3 months, 58 were between the age group of 4 months to 1 year and 101 were from the age group of 1.5 yrs and above. Males and females were 131 and 49, respectively in number.

Among 180 dogs, 21 dogs were from Category I (Ist time brought for vaccination) out of which 8 (38.09%) showed positive anti-rabies antibodies, 58 dogs were from Category II (Ist booster category) with 23 (39.65%) showing positive titre and 101 dogs were from Category III (yearly booster category) with 57 (56.43%) having positive titre against rabies (qualitatively) (Table 1).

Out of these 89 positive serum samples, 43 samples were used for quantitative analysis taking 0.5 IU/µl as standard as recommended by WHO. Amongst these, 5 dogs were from Ist time brought for vaccination in

Table 2
Age wise rabies antibody titre level in dogs based on an ELISA cut off level 0.5 IU/µl

AGE (Vaccination time)	No. of animals tested	Titre above 0.5 IU/µl		Titre below 0.5 IU/µl		Protectiion (%)	Total (43)
		No.of animals	Titre range in IU/µl	No. of animals	Titre range in IU/µl		
0 to 3 months (Ist time vaccination)	5	2	0.512-0.617	3	0.4155-0.452	40	
4 months to 1 year (Ist booster)	12	10	0.56-2.267	2	0.32-0.4095	83.33	79.06 %
1.5 years & above (Yearly booster)	26	22	0.502-3.54	4	0.397-0.483	84.61	

Table 3
Sex-wise sero-prevalence of anti-rabies antibodies

Species (No. of samples)	Sex (No. of samples)	Positive (%)
Dog (180)	Males (131)	66 (50.38)
	Females (49)	23 (46.93)

Table 4
Age-wise sero-prevalence of anti-rabies antibodies

Age - group	No. of animals	ELISA positive (%)
0 to 3 months	21	8 (38.09)
4 to1 year	58	23 (39.65)
1.5 years and above	101	24 (56.43)
Total	180	89 (49.44)

the age group of 0 to 3 months out of which 2 (40%) showed positive anti-rabies antibodies, 12 dogs were from 4 months to >1 year age group with 10 (83.33%) showing positive titre and 26 dogs were from 1.5 years and above age group with 22 (84.61%) having positive titre against rabies (Table 2)

On the basis of sex and age, males and dogs above 1.5 years of age revealed better sero-prevalence as compared to females and those pets which were having age less than 1.5 years, respectively (Table 3 and 4).

The present study was conducted to examine the level of protection achieved in dogs of various age groups after various doses of vaccination (Ist time, Ist booster and yearly booster) against rabies. On analysis of 180 serum samples of dogs brought for vacciantion, an overall prevalence (qualitative) of 49.44% was observed (Table 4). World Health Organization recommended 0.5 IU/µl as a protective titre (WHO, 2013). So, on quantitative analysis, out of the positive samples (43), rabies virus specific antibodies (0.5 IU/µl) were detected in 34 (79.06%) serum samples. Whereas, 20.94 percent vaccinated dogs did not reveal protective rabies titre (Table 2). The results are comparable to Mugale *et al.,* 2012 who found rabies virus specific antibodies (0.5 IU/µl) in 70.55% (115/163) cases. Whereas 29.44% vaccinated dogs did not reveal protective rabies titre.

The lack of proper protective antibody level after the vaccination of dogs against rabies was reported (Jaijaroensup *et al.,* 1999 and Sage *et al.,* 1993). In contrast, Chomel *et al.* (1988) found that 97% of the animals had titer 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). The low specificity through ELISA may also occur due to failure of maintenance of cold chain of vaccines which may further decrease the efficacy of the vaccines being administered. However, in the present study protective antibody titer was 79.06% (34/43) in vaccinated dogs.

The titre in dogs from 0-3 months of age was in range of 0.415-0.617 IU/µl (Table 2). The protective titre was observed in 2 pups out of 5 with a range of 0.512-0.617 IU/µl. Kasempimolporn *et al*. (1996) analyzed serum antibodies titer 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.

On the basis of age, overall prevalence of anti-rabies antibodies (qualitatively) is presented in Table 1.

A higher sero-prevalence was observed in males 66 (50.38%) as compared to females 23 (46.93%) (Table 3). The lower antibody titre in females may be due to the breeding season of bitches followed by immune suppression.

CONCLUSION

The study revealed that yearly booster dose provided better results as compared to the serum samples from first time brought for vaccination and first booster category. So, owners which go for only one time vaccination for their dogs during their life span must be educated to continue vaccination every year.

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