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Immunological response of rabies vaccine in dogs in Jabalpur area of Madhya Pradesh central India

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Abstract
Rabies is endemic in India and kills 20000-30000 people every year. Vaccination in dogs and humans is one of the major tool to prevent and control the occurrence of rabies in India and abroad. The vaccine effectiveness and efficacy need to be monitored regularly so that its potential as lifesaving can be evaluated. Therefore, the present study was carried out to observe the antibody titre in vaccinated dogs and non-vaccinated dogs.

Methods: A total of 146 serum samples of dogs were collected from Jabalpur city (Veterinary College (n=82) and private pet owners (n=64) and the collected samples was divided into three categories of dogs-category I (before booster), category II (after booster) and category III (annual booster vaccination). The samples were subjected to qualitative and quantitative indirect ELISA test.

Results: The qualitative I-ELISA showed that out of 146 samples, only 14.38% including male-15.30% and females-12.5%, Veterinary college -8.54% and private places -21.87% were seropositive. The quantitative assay showed 23.80% (5/21) samples titre above 0.5 IU among seropositive dogs. All the non-descript dogs had antibody titre below 0.5IU.

Conclusions: The study revealed poor protective antibody titre in dogs. The possible reasons for lower level of protective antibody titre post vaccination against rabies are cold chain failure during transport of vaccines, parasitic infestation, and poor nutrition etc. There is need of regular monitoring of anti-rabies antibodies to protect animals and humans.

Keywords: Rabies vaccine, dogs, Jabalpur area, vaccinated dogs and non-vaccinated dogs

Introduction
Rabies is caused by neurotropic virus of the genus *Lyssavirus* in the family of Rhabdoviridae. It is an acute, viral encephalomyelitis which affects all warm-blooded animals with mortality rate being close to 100% once the clinical signs develop (Vegad and Katiyar, 2008) [34]. It is responsible for an estimated 59,000 human mortalities annually worldwide (Gold *et al.*, 2020) [8]. Most of the cases are mainly reported from the developing world, especially in Asia (56.0%) and Africa (44.0%). About 40% of people are bitten by suspected rabid animals are children under 15 years of age (WHO, 2020) [36]. Over 99 percent of human exposures to rabies results from the bite of domestic dog (*Canis familiaris*) due to higher dog population and prevailing poor dog ownership practices (Dzikwi *et al.*, 2011) [6]. The annual cost of rabies in Africa and Asia was estimated at US$ 583.5 million, most of which is due to the cost of post exposure prophylaxis (Knobel *et al.*, 2005) [12].

India is numerouno worldwide with death toll of 20000-30000 humans due to rabies. The higher chances of rabies may be due to proximity between animals and humans, lack of awareness about vaccination, lack of managemental practices of bite wound etc. (Rimal *et al.*, 2020) [24]. In India, most animal bites are by dogs, of which about 60.0% are stray and 40.0% pets and the incidence of animal bites is 17.4 per 1000 population. A person is bitten every 2 seconds, and someone dies from rabies every 30 seconds in India (Sudarshan, 2004) [31]. Majority of people who die of rabies are from poor or low socio-economic status (Ghosh, 2006) [7]. India spends about 15 billion rupees for rabies vaccines alone, exerting a sizeable economic burden on the government (Meslin, 2009) [16].

The rabies virus is readily transmitted through contact with infectious saliva, transplantation of organs particularly cornea and aerosol transmission in laboratories and bat caves, wherein viable viruses are in unusually high density. Rabies viruses have been transmitted by ingestion in experimentally infected animals (OIE, 2022) [21].

Rabies is maintained in two epidemiological cycles, urban and sylvatic. In the urban cycle,

dogs are the main reservoir host and the cycle predominates in areas of Africa, Asia and Central and South America. The sylvatic cycle is the predominant in Europe and North America.

WHO recommends that about 70% of dogs need to be vaccinated to control rabies in a community (WHO, 1989; Knobel and Lankshear, 2007) [35, 13] but most of the dog population is not vaccinated against preventable disease (Ahmed *et al.,* 2000) [1]. Besides vaccination, the efficiency of the vaccines should be evaluated regularly by serological test for specific antibodies to *Lyssaviruses*.

Materials and Methods

Study area and Sampling

The cross-sectional study was conducted from October, 2019 to August, 2020 in Jabalpur (the cultural capital of Madhya Pradesh), India. The population of the city is approximately 1,450,000 and the latitude and longitude position are 23.1815° N and 79.9864° E, respectively.

In the present study 146 serum samples were collected aseptically from Veterinary Clinical Complex of College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (n=82) and private places (private veterinary clinics and pet owner houses; n=64) in Jabalpur city. The collection of samples was divided into three categories of dogs-category I (before booster, n=25), category II (after booster, n=35) and category III (annual booster vaccination, n=86). The samples includes both male (n=98) and female (n=48) dogs and the descriptive (63) and non-descriptive breeds (83). Descriptive dogs include Labrador-36, German Shepherd-08, Pomerarian-13, Pug-02 Saint Bernard -2 and one each of Rottweiler and Golden-Retriever.Among non-descriptive breed, 14 were stray dogs.

The blood from dogs was collected aseptically from cephalic or saphenous vein puncture into BD vacutainer® and blood was allowed to clot for 2 to 4 h at room temperature. The serum was separated by centrifugation at 3000 rpm (4 ºC) for 7 min and stored at -20 ºC until further use.

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Indirect enzyme linked immunosorbent assay

All the samples were subjected to qualitative I-ELISA test and the seropositive samples were tested by quantitative I-ELISA. The ELISA kit was procured from B.V. European Veterinary Laboratory (EBL, Rabies virus antibody ELISA D1006-AB03 test kit). The test is based on the reaction of semi purified virus with polyclonal dog antibodies.

Qualitative I-ELISA

The Qualitative I-ELISA was performed as per manufacturer's instruction. Briefly, diluted serum samples, (100 µl) (1:250) along with both the controls (positive and negative) were transferred to the antigen coated wells and was incubated for 1h at 37 ºC. After washing, each well was dispensed with 100 µl of conjugated anti-species antibody followed by incubation for 1h and washing. The buffers A and B were added (100 µl each) and was incubated for 10-20 min in the dark at room temperature. The stop solution (50 µl) was added finally, and absorption values recorded using ELISA reader within 10 min at 450 nm.

The cut off mean value (MV) of the measured OD value of positive control, (PC) and negative control (NC) were ≥1.000 and ≤0.400, respectively. The ratio (S/P) of sample OD to mean OD of the positive control was calculated as

$$S/P = \frac{\text{OD sample} - \text{MV OD of NC}}{\text{MV OD} - \text{PC} - \text{MV OD of NC}}$$

Where S/P=Samples/Positive, MV=Mean values, OD=optical density, PC=positive control, NC= negative control

Quantitative I-ELISA

The Qualitative I-ELISA was performed as per manufacturer's instruction. In brief, a 3-step dilution of positive, negative control and samples was carried out *viz*. 1:50, 1:150, 1:450 and 1: 1350 and incubated at 37 ºC for 1h. After washing, conjugated anti- species antibody (100µl) was added followed by incubation. Substrate solution (100µl) was dispensed and finally stop solution was added in each well. The OD values of positive control was ≥1.0 OD units (450 nm) and gave an endpoint titre of ≥150 and the negative control was ≤ 0.40 OD (450 nm) units and gave an end point titre of ≤ 50. The ELISA titre was calculated by constructing a curve and using a cutoff line OD on Y and titre as on X axis. ELISA titres were calculated as cut off 2.5 times OD value of negative control at 1:50.

The FAVN titre of the positive control was 1.83IU. The K factor was calculated by dividing the obtained ELISA titre by 1.83. All ELISA titre obtained in the constructed graphic was divided by K to obtain FAVN titres in IU.

The p-value <0.05 was considered statistically significant (Snedecor and Cochran, 1994) [29].

Ethical approval

Ethical approval was taken by institutional animal ethical committee for conducting research [No.09/IAEC/Vety.2019; Dated: 16/9/2019].

Results and Discussion

The rabies incidence in tropical countries can be prevented by intensive vaccination and measuring their immune response. Inactivated tissue culture rabies vaccines are commonly used in India to vaccinate dogs and cats with the potency of at least 1 IU/ml. A serum titre of 0.5 IU/ml and above of rabies virus-specific antibodies is considered adequate protection against rabies and titre below this level is considered a vaccination failure, leaving the dog susceptible to rabies virus infection

(Ma *et al*., 2012 and OIE, 2022) [14, 21]. The standard methods of measuring whether a dog has adequate immunological protection is by virus neutralizing antibodies (VNA), fluorescent antibody virus neutralization test (FAVN), rapid fluorescent focus inhibition test (RFFIT) and also by enzyme-linked immunosorbent assay (ELISA) (Smith *et al*., 1973; Cliquet *et al*., 2003 and Ma *et al*., 2012) [28, 4, 14].

The qualitative seropositivity was observed in 14.38%, with 15.30% and 12.5% in males and females, respectively. The quantitative assay showed 19.04% (4/21) samples had titre above 0.5 IU among seropositive dogs. All the non-descript dogs had antibody titre below 0.5IU. The maternal antibodies against rabies weren't observed in 25 puppies upto 3 months age, while immune response after first booster vaccination (from >3 month-1 year) was found in 20.58% dogs. The protective antibody titre was reported in 16.09% in dogs came for annual booster vaccination (>1 year of age) (Table 3, 4 and 5).

Table 1: Details of serum samples from different collection points

Place of sample collection (Number of samples)	Category of dogs as per vaccination schedule		
	I (<3 months-3 months)	II (>3 months-<1 year)	III (1 year and above)
Veterinary Clinical Complex, Jabalpur (M.P.) (82)	04	10	68
Private places in Jabalpur (68)	21	25	18
Total	25	35	86

Table 2: Distribution of dog serum samples (Descriptive dog and non-descriptive)

Descriptive				
Breed (Number of serum samples)	Age group	Number	Sex	
			Male	Female
Labrador (36)	<3 months	09	07	02
	3-6 months	04	04	-
	>6-9 months	08	07	1
	>9-12 months	03	03	-
	1.5-3 years	05	02	03
	3.5-5 years	02	02	-
	5.5-7 years	02	-	02
	7.5-above	03	01	02
Pomeranian (13)	3 -6 months	01	01	-
	1.5-3 years	03	02	01
	3.5-5 years	01	01	-
	5.5-7years	02	-	02
	7.5-above	06	06	-
German Shepherd (8)	3-6 months	02	02	-
	4-6 month	01	01	-
	1.5-3 years	01	01	-
	3.5-5 years	02	01	01
	7.5-above	02	01	01
Pug (2)	5 years	01	01	-
	8.5 years	01	01	-
Golden retriever (1)	3.5 years	01	01	-
Rottweiler (1)	7 years	01	-	01
Saint Bernard (2)	1.5 year	01	-	01
	6 years	01	01	-
	Total	63	46	17
Non-descriptive				
	<3 months	13	09	04
	3-6 months	10	05	05
	>6-9 months	08	06	02
	>9-12 months	07	05	02
	>1-1.5 years	02	02	-
	>1.5-3 years	18	08	10
	>3-3.5 years	06	06	-
	>3.5-4 years	11	07	04
	5.5-7years	08	04	04
		83	52	31
Grand total		146	98(67.12%)	47(32.19%)

Table 3: Immunological response in dogs as per place, age, sex, breed and vaccine-wise

Place of collection	Number of samples	Number of positive samples	Chi square	p -value
Veterinary Clinical Complex, Jabalpur	82	07(8.53%)	4.6517 at p<0.05	0.31024
Private places in Jabalpur	64	14(21.87%)		
Category (age)				
I (upto 3 months)	25	00	0.3446 at p< .05	0.557206
II (>3 months-1 year)	34	07(20.58%)		
III (1 year and above)	87	14(16.09%)		
Sex				
Male	98	15(15.30%)	0.206 p<0.05	.649907
Female	48	06(12.5%)		
Breed				
Descriptive	63	17(26.98%)	14.288 p<0.05	.000157
Non-descriptive	83	04(4.81%)		
Vaccine				
A	133	17(12.78%)	3.1114 p<0.05	.077748
B	13	04(30.76%)		
Total	146	21(14.38%)		

Table 4: Immune response observed in descriptive dogs

Breed	Number	Number of positive samples (Qualitative)
Labrador	36	15(41.66%)
Pomeranian	13	1(7.69%)
German Shepherd	8	1(12.5%)
Pug	2	00
Golden Retriever	1	00
Rottweiler	1	00
Saint Bernard	1	00

Table 5: Category wise Immune response in dogs

Breed	Number of Male/Female	Category and Number	Immune response (Qualitative)	Quantitative I-ELISA titre
Labrador (36)	Male-26	I(9)	-	-
		II(14)	6(42.85%)	2(14.28%)
		III(5)	4(80.0%)	1(20.0%)
	Female-10	I(2)	-	-
		II(1)	-	-
		III(7)	5(71.42%)	1(14.28%)
Pomeranian (13)	Male-10	II(1)	-	-
		III(9)	1(11.11%)	-
	Female-3	III(3)	-	-
German Shepherd (08)	Male-	II(3)	1(33.33%)	
		III(3)	-	-
	Female-	III(2)	-	-
Non-descriptive (83)	Male-52	I(9)	-	-
		II(16)	-	-
		III(27)	3(11.11%)	
	Female-31	I(4)	-	-
		II(9)	-	-
		III(18)	3(16.66%)	-

Like present study, Gunatilake *et al*. (2003) [9], Pimburage *et al*. (2017) [22] and Arega *et al*. (2020) [2] reported that maternal antibodies weren't detected in the in puppies between 6

weeks-3 months, like present study. Some of the puppies from vaccinated dam didn't show antibody titre as observed by Pimburage *et al.* (2017) [22]; while Sowmiya *et al*. (2019) [30] observed the mean maternal derived antibodies titre in puppies from vaccinated and unvaccinated dam as 1.07 ± 0.18 IU/ml and 0.30 ± 0.037 IU/ml, respectively. The lack or absence of appreciable maternal antibody depends on 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*., 2002) [19]. In our study, many puppies did not show any protective antibody titre because, their–dam was also found negative even after vaccination. In a finding Pollock and Carmichael (1982) [23] reported that low immune response might be due to negative influence of dog litter size. In a study, Siegrist (2012) [26] stated that maternal derived antibodies and immune function may not limit the immune response to inactivated vaccine.

In India, puppies less than 3 months of age are not vaccinated due to the immature immune system and of belief that presence of maternal antibodies may limit the response (Day, 2007; Morters *et al*., 2015) [5, 17]. This may be the reason that vaccine manufacturer may not recommend the vaccination before 3 months. It has also been seen that antibody titre decreases slowly in young ones after primary vaccination, so it is recommended to provide second dose after few weeks followed by annual booster vaccination (Tasioudi *et al*., 2018) [33]. Even in our study, the antibodies were mainly observed after first booster or annual booster.

Differences in immune response against rabies vaccines have been reported in various studies *viz*. the protective antibody titre in dogs was observed by Ogawa *et al*. (2009) [20], Mugale *et. al*. (2012) [18], Savaliya *et al.* (2015) [25], Pimburage *et al.* (2017) [22] and Rimal *et al*. (2020) [24] as 27.7%, 70.6%, 62.37%, above 90.0% and 89.09%, respectively. Singh *et al.* (2011) [27] recorded only 1.0% and 16.0% protective antibody titre in street and pet dogs respectively. Tandon *et al.* (2018) [32] reported anti-rabies antibodies in dogs after booster vaccination was 39.65% and of that with quantitative I-ELISA testing protective titre was observed in 83.33%. Pimburage *et al.* (2017) [22] also found more than 78.0% of dogs had protective antibody titre within the age of 3 months to 1year.

In present study, non-descriptive had shown anti-rabies antibody lower than descriptive breeds as reported in previous study by Berndtsson *et al*. (2011) [3] and Savaliya *et al*. (2015) [25]. Berndtsson *et al*. (2011) [3] found that vaccinated dogs, 91.9% had an approved test result of ≥0.5 IU/ml. They concluded that larger breeds were at higher risk of having antibody titre of <0.5 IU/ml, but if vaccinated twice, the risk was reduced. Moreover, there were an increased risk for dogs of <6 months of age and >5 years of age to have antibody titer of <0.5 IU/ml, but this was affected by number of days from vaccination till testing. Singh *et al*. (2011) [27] in a study in Chandigarh (India) revealed antibody titre in 1.0% of stray dogs. In a similar study, Savaliya *et al*. (2015) [25] reported (Gujrat, India) that only 1.88% stray dogs showed anti-rabies antibody titre above 0.5 IU/ml and suggested that there is high susceptibility to rabies infection and possible threat to surrounding human and animal populations. They also reported anti rabies antibody level were maximum during >1 to < 3 months after vaccination and in decreasing trend thereafter.

The low titre of anti-rabies antibodies might be due to serum samples taken from dogs coming to Veterinary Clinical Complex, which might be in stress or had subclinical disease or high parasitic load. Also, non-descriptive dogs are mainly reared by poor or lower medium income class people who can't effort proper nutrition to the dogs and the dogs are not dewormed regularly. Besides, lack of maintenance of cold chain during transportation or storage may be significant reason in developing countries. Failure of vaccination in descriptive and non-descriptive dogs makes them more vulnerable to transmit rabies. The protective efficacy of vaccine can be influenced by various associated factors *viz*. age, sex, breed, vaccine brand (Mansfield *et al*., 2004; Kennedy *et al*., 2007; Jakel *et al*., 2008 and Berndtsson *et al*., 2011) [15, 11, 10, 3]. Other scientist showed higher risk of lower antibody titre with increasing age as well as dogs <1 year of age compared to adults (Mansfield *et al*., 2004 and Kennedy *et al*., 2007) [15, 11]. Savaliya *et al*. (2015) [25] found that persistence of anti-rabies antibody was higher within 1-3 month after vaccination as found in our study.

The finding in the present study on the level of antibody titer in male and female was similar to the findings by Tandon *et al.* (2017) [32], who observed that anti-rabies antibodies were higher in males (50.38%) than in females (46.93%). They concluded that the lower antibody titre in females may be due to breeding season stress of bitches followed by immune suppression.

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