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# Immunogenicity and Safety Study on Rabivac Vet, Rabies Veterinary Vaccine, Inactivated (Cell Culture) in Dogs

**Patil S.S.[*], Aher V.D., Sunil N.S.[*], Deshmukh V.V.[**], Reddy G.S.[*]. and Rao T.V.S.[*]**

College of Veterinary & Animal Sciences, MAFSU, Parbhani-431 402

*Corresponding Author E-mail: vivekdes@gmail.com

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**ABSTRACT**

*The present study was planned to study immunogenicity and safety of Rabivac Vet, an anti-rabies veterinary vaccine containing BHK-21 cell culture adapted rabies virus strain PV11 and inactivated with binary ethylenimine (BEI). A total of 100 dogs were divided into 3 groups, Group 'A' (24), Group 'B' (58) and Group 'C' (18). Rapid Fluorescent Focus Inhibition Test (RFFIT) was used for estimation of rabies virus neutralizing antibodies in pre and post vaccinated serum samples. In groups A, B and C the geometric mean antibody titre (RFFIT) values on the day of vaccination were 0.09 IU/ml, 1.51 IU/ml and 0.08 IU/ml respectively. There was a marked increase in geometric mean RFFIT values above protective level prescribed by WHO in all the groups at 28-30 days post vaccination. The RFFIT values of groups A, B and C on 28-30 days post vaccination were 3.22, 11.46 and 3.81 IU/ml respectively. The vaccine was found to be safe and efficacious in dogs against rabies.*

***Key words:*** *BHK-21, PV11, Binary ethylenimine, Rapid Fluorescent Focus Inhibition Test, Rabivac Vet*

## INTRODUCTION

Rabies is a zoonotic disease caused by Lyssavirus listed under the Family Rhabdoviridae[5]. In Asia and South Africa dogs and cats are mainly responsible for the spread of rabies in humans. About 60,000 human deaths occur worldwide due to rabies[9,10]. Thus, the control of rabies in dogs is the best way to reduce the number of cases of rabies infection in humans. In other words, the transmission of rabies to humans can be effectively controlled by mass vaccination of dogs against rabies.

World Health Organization (WHO) Expert Committee on Rabies described that the level of antibodies of at least 0.5 International Units, IU/ml in blood results in adequate protection by vaccine[8]. The antibody titre can be determined by using Rapid Fluorescent Focus Inhibition Test (RFFIT). This value of antibody titre was first mentioned by the Expert Committee on Rabies in the 8th WHO Report in 1992 and is recognized throughout the world[8].

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The RFFIT is a rabies virus neutralization test performed in cell culture to determine the rabies virus neutralizing antibody level in human or animal sera. In this test the immunofluorescent staining of infected cells is used as an indicator of rabies virus replication. This test is also recommended as the current gold standard serological assay by both the Advisory Committee on Immunization Practices (ACIP) and the World Health Organization[2].

After vaccination of dogs under field conditions, it is important to know whether the vaccinated subjects would show antibody titre above the protective level of 0.5 IU/ml as recommended by WHO preferably after first vaccination[9,12]. With reference to this, a brief study was carried out to measure rabies antibody levels estimated by RFFIT after a single dose of vaccination by using Rabivac Vet, Rabies Veterinary Vaccine, Inactivated (Cell Culture) manufactured by M/s Brilliant Bio Pharma Private Limited.

By and large, mass immunization of dogs could reduce the incidence of Rabies infection effectively in the society. Keeping this fact in view, the mass vaccination of dogs was attempted by using Rabivac Vet in a Rabies Vaccination Camp held on the occasion of "World Rabies Day" organized at College of Veterinary and Animal Sciences, Parbhani, Maharashtra State. Further to this, the sera samples collected before and after vaccination were checked for the development of virus neutralizing antibodies (humoral immunity) against rabies virus by RFFIT.

## MATERIAL AND METHODS

**Dogs for Vaccination:** A total of 100 dogs were registered for vaccination camp on the occasion of World Rabies Day 2017. The dogs were grouped into 3 groups based upon the vaccination history and age. The details are given in Table-1.

**Vaccine:** Rabivac Vet, Rabies Veterinary Vaccine, Inactivated (Cell Culture) manufactured by Brilliant Bio Pharma Private Limited, Hyderabad was used for vaccination. The vaccine contains rabies virus strain PV11 propagated on BHK-21 cells, inactivated with binary ethylenimine (BEI) and adjuvanted with aluminium hydroxide gel. The potency of the vaccine is more than 2.5 IU per dose of 1 ml.

**Table 1: Details of dogs used for Rabies vaccination**

| Group | Age group | Vaccination status | Number of dogs vaccinated |
|---|---|---|---|
| Group A | 3 months to 6 months | Non vaccinated | 24 |
| Group B | 6 months to 8 years | Vaccinated | 58 |
| Group C | 6 months to 8 years | Non vaccinated | 18 |
| | | Total | 100 |

**Vaccination:** All the 100 dogs were vaccinated with 1 ml of vaccine by subcutaneous route as per manufacturer's instructions by following standard procedure. Sterile syringes and needles were used for each and every dog. The details of dogs and vaccination were recorded in Vaccination Card.

**Serum sample collection:** Serum samples of all vaccinated dogs were collected before and after vaccination by using standard procedures and protocols. A total of 100 serum samples were collected before vaccination and a total of 100 serum samples after 28-30 days post vaccination.

All the serum samples were heat inactivated and stored at -20°C for further studies.

All the serum samples were submitted to Department of Research and Development, Brilliant Bio Pharma Private Limited, Pashamylaram, Sangareddy District, Telangana State for further *in vitro* testing to estimate antibody levels.

**Clinical observations for safety of vaccine:** The vaccinated dogs were observed clinically for one hour after vaccination for any local and systemic reactions. At the same time the physiological parameters were also recorded by measuring rectal temperature, heart rate, respiratory rate and observations for behavioural changes, if any.

**Serum antibody Assay:** All the 200 serum samples (i.e. 100 before vaccination and 100 post vaccination samples) were subjected to Rapid Fluorescent Focus Inhibition Test (RFFIT) for estimation of rabies virus neutralizing antibodies as per the technique described in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals published by OIE in 2008. The lowest serum dilution used in the test was 0.06 IU/ml and compared with 2nd WHO International Reference Standard Anti-rabies Immunoglobulin. The antibody titre was expressed as IU/ml.

**Statistical analysis:** Microsoft Excel Version 2007 was used to determine the mean values of RFFIT antibody titre on day 0 and 28-30 days after vaccination.

## RESULTS AND DISCUSSION

**Clinical observations:** All 100 dogs vaccinated against Rabies were clinically normal without any adverse reaction during observation period. The physiological parameters such as temperature, pulse rate and respiration rate were found within the normal range in all vaccinated dogs. After one hour of vaccination, the rectal temperature, heart rate and respiratory rate of all dogs were observed in the range of 100.5 to 102.8°F, 72 to 120 per minute and 12 to 34 per minute respectively.

Furthermore, none of the vaccinated dogs showed any local or systemic reaction after vaccination during the observation period indicating that the vaccine Rabivac Vet was found to be clinically safe. Earlier, Rabivac Vet vaccine was found to be clinically safe[5].

**Immunogenicity studies:** The results of RFFIT screening of pre- and post- vaccinated serum samples are shown in Table-2.

**Table 2: RFFIT values before and after vaccination with Rabivac Vet**

| Sr. No. | Group | No of serum samples tested | Geometric Mean of Antibody Titre (RFFIT) in IU/ml (Range in parenthesis) | |
|---|---|---|---|---|
| | | | Before vaccination | After vaccination |
| 1 | A | 24 | 0.09 (0.06 to 0.22) | 3.22 (2.07 to 5.42) |
| 2 | B | 58 | 1.51 (0.38 to 2.96) | 11.46 (5.48 to 18.15) |
| 3 | C | 18 | 0.08 (0.06 to 0.19) | 3.81 (2.07 to 6.46) |

It is interesting to note that in groups A and C which are non vaccinated groups the geometric mean RFFIT values were 0.09 IU/ml and 0.08 IU/ml respectively. However, the same in Group B which was vaccinated group the values were 1.51 IU/ml. The higher geometric mean RFFIT values in Group B compared to Groups A and C are due to preexisting vaccinal antibodies showing immunological competence of the vaccinated dogs. The age group of Group B prevaccinated dogs also contributed to higher mean RFFIT values than other groups (A and C).

It is evident from analysis of post vaccinated serum samples after 28-30 days that a marked increase in geometric mean RFFIT values indicating development of immunity against Rabivac Vet vaccine. The RFFIT values of groups A, B and C were 3.22, 11.46 and 3.81 IU/ml respectively. The higher value of 11.46 IU/ml in Group B dogs than other groups A and C were due to exaggerated secondary immune response to booster vaccination as the doge were prevaccinated. This was also due to superior immunogenicity of Rabivac

Vet vaccine. Persistence of rabies antibodies in vaccinated dogs was also reported earlier[4].

The data given in Table-2 revealed that the sera samples showed a marked increase in antibody levels after vaccination. In this study, all the dogs (100) showed antibody levels well above the protective threshold of 0.5 IU per ml after 28-30 days, which is in accordance with the previous studies indicating that the peak titre antibodies generally reach between 4-6 weeks after vaccination[9]. This indicates immunogenicity and specificity of Rabivac Vet vaccine.

In this study, all the dogs in Groups A and C showed the antibody titre within the range 0.06 to 0.22 IU/ml before vaccination indicating that they were under potential threat of rabies infection and similar findings were reported earlier[10].

As per the results of Group B, the dogs with previous vaccination history had antibody titre above the protective level (>0.5 IU/ml) on 0 day of vaccination and showed marked increase in antibody titre after 28-30 days (Table-2). Such anamnestic antibody response was earlier observed in the previously vaccinated dogs against rabies [11] with a single booster dose of vaccination[1].

Similarly, the results of Groups A and C in the dogs without previous vaccination history showed low antibody titre on 0 day and resulted in a satisfactory antibody titre above the protective level (>0.5 IU/ml) on 28-30 days of vaccination. It indicated that most of the primo-vaccinated dogs did respond to vaccination by using a single dose of Rabivac Vet. Similar observations of good protection levels of rabies antibody titre on 30 days of vaccination were noted earlier in dog sera [3,5] and some research workers found antibody titre development in adult dogs and puppies in their preliminary study conducted in Kotte, Sri Lanka[4]. However, some dogs in all the groups had shown lower antibody titre after vaccination. This deviation in the antibody titre in individual dogs may be due to various reasons such as health or nutrition status, age of the animal and sub-clinical infections. In order to overcome the problem of uncertain immunity noted in a few dogs following vaccination, it is recommended to adapt at least 2-dose regimen given with a gap of 3 to 4 weeks.

Although the dogs in Groups A and C were without previous vaccination history, the development and maintenance of antibody titre above the recommended protective level could be acquired by a primary vaccination in majority of dogs. This is an indirect evidence for the efficacy of Rabivac Vet, the rabies veterinary vaccine used in this study.

## CONCLUSION

As per recommendation of WHO the antibody titre of ≥0.5 IU/ml is acceptable protective value against rabies virus. In this study, anti-rabies vaccination done in dogs by using Rabivac Vet resulted in the mean protective antibody titre against rabies well above 3 IU/ml including in the primo-vaccinates meeting the acceptance criteria. None of the vaccinated subjects showed any local or systemic reaction. To summarize, the Rabivac Vet, Rabies Veterinary Vaccine, Inactivated (Cell Culture) manufactured by Brilliant Bio Pharma Private Limited, Hyderabad is found safe and efficacious for the immunization of the pets against rabies.

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