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Research Article

Evaluation of Anti-Rabies Antibody Titers in Vaccinated Dogs from Mumbai and Navi Mumbai Using Indirect ELISA

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A B S T R A C T

Introduction: Rabies is a lethal, zoonotic, and neurological disease which is practically 100% fatal but vaccine preventable. The vaccination program will be successful only after maintaining the protective level of rabies antibodies in vaccinated animals and continuous estimation of titers of anti-rabies antibodies in ARV-vaccinated animals.

Method: The present study investigates the status of anti-rabies antibody titers and estimates the humoral responses towards the rabies vaccination in rabies-vaccinated stray and pet dogs from the Mumbai and Navi-Mumbai regions using indirect ELISA.

Results: Out of 184 serum samples obtained from vaccinated stray and pet dogs, 149 (80.97%) serum samples showed rabies antibody titer ≥ 0.5 IU/mL, consisting of 70/98 (71.42%) from vaccinated stray dogs and 79/86 (91.86%) from vaccinated pet dogs. In vaccinated stray dogs, the sex-wise analysis revealed that 33/46 (71.73%) male dogs and 37/52 (71.15 %) female dogs had protective levels of rabies antibody titer. In pet dogs, 54/56 (96.42%) male dogs and 25/30 (83.33%) female dogs had protective anti-rabies antibody titers of ≥ 0.5 IU/mL. Vaccinated pet dogs show a high prevalence (91.86%) and p value of 0.969 of anti-rabies antibodies compared to vaccinated stray dogs (71.42%) and p value of 0.335.

Conclusion: The high prevalence in stray dogs in the present study indicates that the NGOs are vaccinating stray dogs regularly in their areas. Screening a large number of serum samples in stray dogs revealed the true status of antirabies antibody titers.

Keywords: Rabies Antibody Titer, Anti-Rabies Vaccine, Dogs, ELISA, Serosurveillance

Introduction

Rabies is a lethal, zoonotic, and neurological disease caused by Lyssavirus, belonging to the family Rhabdoviridae. The rabies virus can infect any warm-blooded animal, except humans.[1] Rabies is distributed throughout the world and is endemic in many countries except continents like Australia and Antarctica.[2] An estimated 20,000 human deaths occur from dog-mediated rabies in India, with the annual incidence of animal bites at a rate of 6.6 per 1000 people.[3] This is estimated to account for one-third of all rabies-related deaths worldwide.[4-8] As per a report, dog bites constitute the pioneering cause of human rabies in India, with an annual estimate of almost 17 million animal bites.[9] In India, 59.9% of rabies deaths annually have been registered, and roughly 35% of human rabies deaths have also been reported worldwide.[10,11] According to the World Health Organization (WHO), US$ 8.6 billion is the overall global cost involved in treating and preventing dog-mediated rabies.[12]

Rabies prevalence in domestic animals has increased from 20% to 50%, with 48% in canines, 21.9% in felines, 61.4% in cattle, 48.7% in caprine, and 45% in equines.[13] Rabies is spread unevenly within infected countries. Within cities, there are low and high endemic regions and zones with epizootic occurrences. Rabies is ubiquitous across Africa and Asia (except for Japan and Singapore). North and South America and Europe record disease outbreaks in disproportionately confined or present in areas of those countries.[14] For the prevention and control of rabies virus transmission, vaccination is essential.[15] As per the WHO, all endemic rabies countries should implement preventive vaccination programs for dogs (National Action Plan for Dog Mediated Rabies Elimination from India by 2030). The vaccination program will be successful only after maintaining the protective level of antibodies in vaccinated animals and continuous sero-monitoring.[16] Under the "One Health" policy, rabies eradication can be achieved by educating the community regularly and vaccinating the local dog population[9] It is essential for disease control to maintain a protective level of anti-rabies antibodies in vaccinated animals.[17-19] An anti-rabies antibody with a serum titer of 0.5 IU/mL or above is considered protective and animals must be vaccinated at least once a year.[20] As a result, it is recommended that following vaccination, ongoing sero-monitoring be performed to assess the efficiency of the immunization programs and herd immunity.[21-23]

Previous Sero-surveillance investigations conducted at Mumbai Veterinary College indicated that the a protective antibody titre of above 0.5 IU/ml. stray dogs is 39.2% and 62.66%, respectively.[24,25] A high seroprevalence of 97.87% of anti-rabies antibodies in pet dogs was reported from Mumbai.[25] The ideal way to control the rabies virus is to conduct vaccination programs for stray and pet animals along with wild carnivores, and it is highly important to determine the rabies antibody titer levels from time to time in vaccinated animals. Anti-rabies antibody levels in animals must be checked to determine the efficacy of control measures.[26] Seroprevalence studies can be conducted by collecting the animal's serum and using methods like Fluorescent Antibody Virus Neutralization (FAVN), Rapid Fluorescent Focus Inhibition Test (RFFIT), and quantitative ELISA. As per WHO, for cases with rabies antibody titer values less than 0.5 IU/mL, a supplemental dose of the vaccine needs to be administered.[27] Quantitative ELISA Biorad PATELIA™ RABIES II ASSAY is an in vitro diagnostic ELISA test that helps in identifying the rabies antibodies in post-vaccination serum samples of dogs and cats.[28, 29] The WHO recommends a neutralizing antibody titer of ≥ 0.5 IU/mL as a protective titer level.[30] This study is aimed at testing the presence of anti-rabies antibodies in serum samples of stray as well as pet dogs to assess the immune status of the vaccinated dog population against rabies.

Materials and Methods

Ethical Statement

The Institutional Biosafety Committee (IBSC) of Mumbai Veterinary College, Mumbai, provided the approval for conducting this study vide Resolution No 3.1.6. (Lr. No. MVC/Dean/VPH/IBSC/103 of 2021 dated 15/09/2021).

Sample Collection

In this study at the Mumbai Veterinary College, India, sterile disposable needles and syringes were used to collect blood samples from stray and pet dogs. Birthmarks, colour, and ear notches were used to identify the stray dogs. Separation and centrifugation of the serum were done and then it was stored at -20 °C in sterile microcentrifuge tubes. During the processing of the samples, the required precautions (biosafety measures) were taken. At the time of blood collection, information was gathered on the last date of vaccination, neuter status, breed, gender and age.

A total of 184 serum samples were obtained for sero-surveillance from both pet and stray dogs having a history of anti-rabies vaccination from May 2021 to January 2022. The samples were collected and received from different sources, including the Department of Veterinary Microbiology, Mumbai Veterinary College, Mumbai, NGOs like AHIMSA and In Defense of Animal (IDA-Mumbai and IDA-Turbhe, Navi Mumbai) and Animal Birth Control Program (ABC) units like BSPCA Veterinary Hospital, Mumbai Veterinary College, Mumbai. A total of 98 samples were obtained from vaccinated stray dogs from NGOs near Mumbai and Navi Mumbai region, and 86 serum samples were received from pet dogs vaccinated with the Anti-Rabies Vaccine (ARV) in

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and around the Mumbai region. The selection of stray dogs was based on their vaccination history, eliminating those without available records. Pet dogs were required to have received at least two doses of the rabies vaccine. These 98 serum samples collected from vaccinated stray dogs were categorized into 3 groups based on the time interval after the last anti-rabies vaccination. Group I represented the early phase of immune response development. During this stage, antibody titers may be rising but have not yet reached peak levels. Group II indicated the peak phase of the immune response, where the body has mounted a strong antibody reaction. Finally, Group III reflected the waning phase of immunity. Among these groups, Group I consisted of serum samples collected from both sexes less than one month after pre-exposure anti-rabies vaccination, Group II consisted of serum samples between one and six months after pre-exposure anti-rabies vaccination, whereas Group III consisted of samples between six months and one year after pre-exposure anti-rabies vaccination. The 86 serum samples obtained from pet dogs were split up into three age-based groups. In pet dogs, Group A consisted of young dogs that generally have a more active immune system due to immunological priming from early vaccinations. Group B included adult dogs, which typically exhibit a more stable immune response and Group C was made up of older dogs who may experience immunosenescence, a gradual decline in immune function. Serum samples were obtained from dogs aged 6 months to 2 years in Group A, from dogs aged 2–5 years in Group B, and from dogs aged 5–10 years in Group C.

Use of ELISA in the Sero-surveillance of Rabies Antibodies in Dogs

Samples were obtained for sero-surveillance from both pet and stray dogs having a history of anti-rabies vaccination. PLATELIA™ RABIES II ASSAY Ad Veterinarian (Ref: 3550180) BIORAD ELISA kit was used to analyze the samples obtained for the antibody titers. The provided kit included negative and positive controls. An ELISA test kit works on an indirect ELISA technique, which makes use of a solid-phase enzyme immunoassay. Rabies glycoprotein, taken from the inactivated and purified viral membrane, was put on a microplate. The manufacturer's instructions were adhered to while performing the ELISA test. Once the reactions were stopped, within 30 minutes, an ELISA reader was used to measure the optical density at 450–620 nm. The standard curve approach was used to calculate the level of anti-rabies antibodies. The Platelia Rabies II test mentioned here is a strong candidate for the routine detection of rabies antibodies in domestic carnivores as part of international trade for monitoring rabies vaccination programs.[31]

Statistical Analysis

Web Access Server Protection version (WASP) 2.0 software was used for statistical analysis.[32] Calculation of metrics such as mean, standard deviation, variance, maximum and minimum values, and standard error was done for stray dog sera samples. The stray dogs' anti-rabies antibody status after pre-exposure vaccination and the variation in the dogs' mean titer (IU/mL) values were analyzed using the chi-square test.

Results

Sero-surveillance of Rabies Antibodies in Vaccinated Stray and Pet Dogs Using ELISA

An overall seropositive percentage observed in the present study was 80.97% (149/184), which revealed that the rabies antibody titers for these 149 samples were equal to or above the cut-off value of 0.5 IU/mL, including 70/98 (71.42%) from stray dogs and 79/86 (91.86%) from pet dogs of both sexes (Table 1). All the stray and pet dogs were vaccinated with ARV from different companies.

Table 1.Group-Wise Percentage of Dogs Showing Positive/ Protective Titer Level

S. No.	Group	Species	Sex	Samples Examined (n)	Positive Samples (n)	Positive Samples (%)
1	Stray	Dog	Male	46	33	71.73
			Female	52	37	71.15
Total A				98	70	71.42
2	Pet	Dog	Male	56	54	96.42
			Female	30	25	83.33
Total B				86	79	91.86
Grand Total (A + B)				184	149	80.97

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Table 2.Percentage and Statistical Analysis of Anti-Rabies Antibody Titers at Different Time Intervals after Vaccination in Stray Dogs

S. No.	Groups	Duration from Last ARV	Samples Examined (n)	Positive Samples (n)	% Positive	Chi-Square Test p Value
1	I	< 1 month	44	32	72.72	0.335
2	II	> 1 to ≤ 6 months	36	27	75.00	
3	III	> 6 months to ≤ 1 year	18	11	61.11	
			98	70	71.42	

*Non-significant at the level of 5% ($p < 0.05$)

Table 3.Percentage and Statistical Analysis of Anti-Rabies Antibody Titers in Pet Dogs Categorized as per Their Ages

S. No.	Groups	Age	Samples Examined (n)	Samples Showing Antibody Titer Equal To/ Above 0.5 IU/mL (n)	% Positive	Mean Antibody Titer (IU/mL)/ Standard Error	p Value
1	A	6 months to 2 years	26	23	88.46	0.647 ± 0.086	0.969*
2	B	> 2 to 5 years	30	29	96.66	0.66 ± 0.047	
3	C	> 5 to 10 years	30	27	90.00	0.698 ± 0.079	
			86	79	91.86		

*Non-significant at the level of 5% ($p < 0.05$)

The status of anti-rabies antibodies after pre-exposure anti-rabies vaccination in stray dogs of Mumbai and Navi Mumbai region was analyzed, and out of 98 stray dogs' serum samples, 70/98 (71.42%) serum samples showed rabies antibody titer values of 0.5 IU/mL (cut-off value) or more. Similar values of rabies antibody titers were seen in 33/46 (71.73%) male dogs and 37/52 (71.15%) bitches (Table 2). Statistical analysis revealed a non-significant ($p \leq 0.05$) association between the anti-rabies antibody titer of vaccinated stray dogs and duration from the last ARV

The status of anti-rabies antibodies after pre-exposure anti-rabies vaccination in pet dogs of Mumbai and Navi Mumbai region was analyzed and among samples obtained from vaccinated pet dogs, 91.86% (79/86) exhibited rabies antibody titer values of 0.5 IU/mL (cut-off value) or more. Sex-wise distribution in vaccinated pet dogs indicated that 96.42% (54/56) male dogs and 83.33% (25/30) female dogs showed similar values of rabies antibody titers (Table 1). Statistical analysis was performed using chi-square test to investigate the relationship between anti-rabies antibody titers and the age at which pet dogs were vaccinated. The analysis revealed that the anti-rabies antibody titers of vaccinated pet dogs showed no significant differences ($p \leq 0.05$) after statistical analysis.

In the present study, the overall prevalence of protective levels of rabies antibody titer percentage in stray dogs was observed to be 71.42%, protective anti-rabies antibody titer. A non-significant difference was observed between the anti-rabies antibody titer of vaccinated stray dogs based on duration from the last ARV. According to their age group, 86 pet dog serum samples were divided into three groups (A, B and C). The highest mean antibody titer of 0.698 IU/mL in pet dogs was observed in Group C (age: > 5 years to 10 years). The mean antibody titer values observed in Groups A and B in pet dogs were 0.647 IU/mL and 0.66IU/mL, respectively (Figure 1). No significant difference was observed in the anti-rabies antibody titer values of vaccinated pet dogs belonging to varying age groups.

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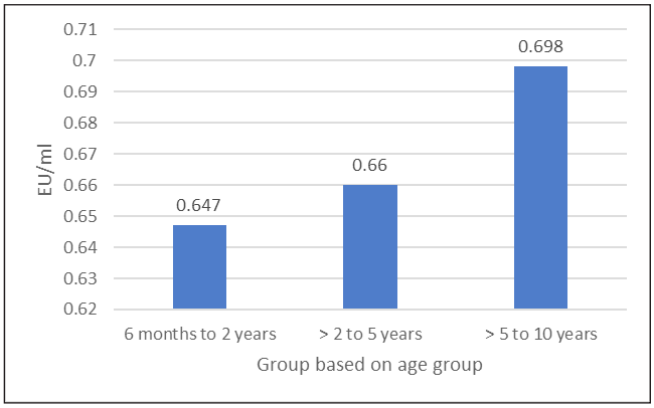

Figure 1.Age-Wise Distribution of Anti-Rabies Antibody Titers in Serum Samples of Pet Dogs

Discussion

Rabies is still a major public health concern in India. Rabies is an endemic disease that has been reported in almost every part of the country.[33,4,34] Brihanmumbai Municipal Corporation (BMC) has an estimated dog population of 2.75 lakhs. Rabies is a fatal disease for which immune prophylaxis is perhaps the only preventable measure.[23]

An overall seropositive percentage observed in the present study was 80.97% (149/184), which revealed that the rabies antibody titers for these 149 samples were equal to or above the cut-off value of 0.5 IU/mL, including 70/98 (71.42%) from stray dogs and 79/86 (91.86%) from pet dogs of both sexes. The seropositive percentage was higher in the pet dog population compared to stray dogs, likely due to regular vaccinations in pets. In this study, the sample size of pet dogs was greater than that of stray dogs. Factors such as age, sex, breed, and vaccine brand can all influence the protective levels of rabies antibody titer .[35] 71.42% of stray dogs had protective anti-rabies antibody levels above 0.5 IU/mL. A sero-surveillance study of anti-rabies antibodies in stray dogs was previously reported from the same region by Nale et al.[24] who documented 39.2% (47/120) and Kevin[25] who reported 61.01% (36/59) protective antibody titer in stray dogs. The present study showed that an anti-rabies antibody titer of 71.42% was seen in stray dogs. Correspondingly, Mugale et al.[36] reported 70.55% (115/163) protective antibody titer from stray dogs using ELISA. Rimal et al.[37] revealed that out of 50 serum samples screened from street dogs, 80% (40) showed values ≥ 0.5 IU/mL, while 10 (20%) were below the range (< 0.5 IU/mL). In Bangkok, Thailand, Kasempimolporn et al.[38] investigated 3314 serum samples obtained from stray dogs and found the antibody prevalence to be between 49% and 86% in stray dogs. Most of the serum samples included in the studies were collected from areas with a history of regular rabies vaccination by NGOs [AHIMSA and In Defense of Animal (IDA-Mumbai and IDA-Turbhe, Navi Mumbai)].

Low anti-rabies antibody prevalence in stray dogs was reported by Jeong et al.[39] when they screened 2408 serum samples and discovered that 36.8% (886) of the dogs had protective values. From India, Nale et al.[24], Kevin[25] from Mumbai, Singh et al.[40] from Chandigarh, and Savaliya et al.[35] from Gujarat reported that 39.2%, 61.01%, 1%, and 1.88%, respectively, of stray dogs had protective levels of anti-rabies antibodies.

In the present study, the sex-wise distribution of rabies antibody titer was 71.73% and 71.15% in male and female stray dogs, respectively. Nale et al.[24] reported that the sex-wise distribution of rabies antibody titer was 38% and 40.32% in male and female dogs, respectively, whereas Kevin[25] observed 63.33% in male and 58.62% in female dogs.

A non-significant ($p \leq 0.05$) association was observed between vaccinated stray dogs' anti-rabies antibody titer and duration from the last ARV. In the current study, stray dogs were divided into three groups according to the time since their last ARV and the highest mean antibody titer value was observed in Group I. In the case of vaccinated stray dogs, the positive percentage was 72.72% in Group I, 75.00% in Group II, and 61.11% in Group III. Nale et al.[24] reported 23/40 (57.5%) anti-rabies antibody titer in stray dogs in Group I, 11/40 (27.4%) in Group II and 13/40 (32.5%) in Group III. Kevin[25] revealed 6/13 (46.15%) anti-rabies antibody titer in stray dogs in Group II, 25/31 (67.74%) in Group I and 5/15 (33.33%) in Group III. Nodari et al.[41] revealed that when examining serological data, the vaccine choice was also relevant. When serum was obtained after 75 days of vaccination, they discovered a substantial difference between the two vaccine-type groups. It was found by Albas et al.[42] that the percentage of dogs with antibodies was not substantially different between reactive (51%) and non-reactive serum (49%). Santosh et al.[43] analyzed the serum samples of dogs taken between 20 and 50 days post-vaccination and found that a larger proportion of dogs had neutralizing antibody titer values of 0.5 IU/mL. Yale et al.[44] reported that 60% (108/180) of vaccinated young prima dogs and vaccinated adult dogs failed to show a protective level of antibodies from the time when the year of last ARV.despite having a history of prior rabies vaccinations,A total of 86 sera samples from vaccinated pet dogs were screened, 91.86% (79/86) showed rabies antibody titer values of 0.5 IU/mL (cut-off value) or more. The sex-wise distribution in vaccinated pet dogs indicated that 96.42% (54/56) of male dogs and 83.33% (25/30) of female dogs had rabies antibody titer values of 0.5 IU/mL or more. High percentages of rabies antibody titers in pet dogs [91.86% (79/86)] were observed in the present study indicating the awareness of pet owners towards the importance of rabies vaccination. The high percentage of protective titer observed in the present study (91.86%) was consistent with the findings of Kevin,[25] who collected

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107 serum samples from vaccinated dogs over a year. Out of 107 serum samples, 97.19% (104/107) of samples from pet dogs showed titer values ≥ 0.5 IU/mL. Santosh et al.[43] investigated 149 serum samples from vaccinated pet dogs over a year and revealed that 81.87% (122/149) of the serum samples showed protective titer values of more than 0.5 IU/mL. Under the German pet travel plan, Knoop et al.[45] examined 284 serum samples from vaccinated pet dogs; 78.1% (222/284) of the dog sera had protective titers equal to or above 0.5 IU/mL. Mugale et al.[36] documented 70.55% (115/163) rabies virus-specific antibodies (0.5I U/mL) in vaccinated dogs. From Seoul, South Korea, Jeong et al.[39] screened 990 sera samples from pet dogs; 55.3% (547/990) of the samples had protective antibody levels. Low percentages of protective rabies antibody titer from pet dogs were reported by Singh et al.[40] and Yale et al.[44] (16% and 40%, respectively).

The serum samples obtained from pet dogs were divided into three groups based on their age, to investigate the anti-rabies antibodies status following their usual anti-rabies vaccination schedule. Group A consisted of 26 serum samples collected from pet dogs, having ages between six months and two years. A cut-off value of 0.5 IU/mL was found in 88.46% (23/26) of serum samples. Higher mean values were observed in Group C (0.698) as compared to the other groups (Figure 1 and Table 3). In vaccinated pet dogs, the value of the mean antibody titer seen in Group A was 0.647 IU/mL and in Group B, it was 0.66 IU/mL. A statistical study employing chi-square test was used to examine the link between anti-rabies antibody titer and vaccination age in pet dogs (Table 3). Anti-rabies antibody titers of vaccinated pet dogs were shown to be non-significantly ($p \leq 0.05$) different after statistical analysis. The results of the present study regarding age has no effect on vaccinated pet dogs are in agreement with those of a study conducted by Nale et al.[24] who examined 15 serum samples from routinely vaccinated pet dogs and found that 86.66% (13/15) showed anti-rabies antibody levels of 0.5 IU/mL or more, whereas 13.33% (2/15) of samples showed values less than 0.5 IU/mL, higher mean antibody titer levels in Groups C. Kevin[25] reported higher mean antibody titer levels in pet dogs having age group between 2 to 5 years Olugasa et al.[46] examined 116 samples from vaccinated pet dogs and divided them into five groups based on how long it had been since the last ARV vaccination. According to the findings, anti-rabies antibody levels were highest in the third group of dogs whose serum was obtained after 3 to 6 months. Mansfield et al.[47] evaluated 14,035 serum samples from dogs for the UK Pet Travel Scheme and examined the impact of numerous characteristics on pet dogs, such as vaccine choice, interval between vaccinations, sex, and place of origin. In a few vaccinated pet dogs, low antibody titers may occur due to variability in immune response influenced by factors such as age, sex, health status, and previous exposure to the virus or vaccine. Therefore, when implementing control strategies for rabies, it is crucial to consider individual variability. Some dogs may need booster vaccinations or alternative methods to ensure they have adequate immunity. Research indicated that young animals under one year of age had a higher likelihood of failing to produce anti-rabies antibodies compared to those over one year of age. The antibody titers in six-month-old dogs were notably lower than in the older groups.

Conclusion

The study showed a higher prevalence of anti-rabies antibodies in vaccinated pet dogs compared to vaccinated stray dogs. In stray dogs, 71.42% were protected, and 28.58% showed non-protective anti-rabies antibody titer indicating that the NGOs were vaccinating stray dogs regularly in their areas. In stray dogs, low antibody titers may be due to booster shots, nutritional status, stress levels, and environmental exposure. Anti-Rabies Vaccination program showed encouraging trends, however, stray dogs still pose a high risk of rabies in the dog population. A non-significant association was seen between the gender of vaccinated stray dogs and their anti-rabies antibody titer. Similarly, a non-significant association was seen between the age of pet dogs and their anti-rabies antibody titer. Pet dogs showed 91.86% protective titer. This indicated that pet owners were regularly vaccinating their pets and were aware of the importance of rabies vaccination. More studies, preferably with a larger sample size, should be conducted to analyze true herd immunity in pet and stray dogs.

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References

1. Kang B, Oh J, Lee C, Park BK, Park Y, Hong K, Lee K, Cho B, Song D. Evaluation of a rapid immunodiagnostic test kit for rabies virus. J Virol Methods. 2007;145(1):30-6. [PubMed] [Google Scholar]
2. Singh R, Singh KP, Cherian S, Saminathan M, Kapoor S, Reddy GB, Panda S, Dhama K. Rabies - epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review. Vet Q. 2017;37(1):212-51. [PubMed] [Google Scholar]
3. Thangaraj JW, Krishna NS, Devika S, Egambaram S, Dhanapal SR, Khan SA, Srivastava AK, Mishra A,

Shrinivasa B, Gour D, Madhukar M, Verma N, Sharma P, Soni RK, Ramasamy S, Mohandas S, Baidya S, Rehman T, Yeldandi VV, Singh A, Sreedevi A, Tandale BV, Purakayastha DR, Reddy MM, Toppo M, Solanki NV, Ghosh P, Jaiswal P, Vyas S, Das S, Palo SK, Prasanth V, Rozario AG, Durairajan C, Delli A, Sasi A, Pandiyan C, Ashwathnarayana DH, Joy S, Isloor S, Sudarshan MK, Rahi M, Murhekar MV; Human Rabies Study Collaborators. Estimates of the burden of human rabies deaths and animal bites in India, 2022–23: a community-based cross-sectional survey and probability decision-tree modelling study. Lancet Infect Dis. 2025 Jan;25(1):126-34. [PubMed] [Google Scholar]

4. Pharande RR, Majee SB, Bannalikar AS, Gandge RS, Dighe RV, Moregoankar SS, Doiphode AY, Charan P, Mukherjee S. Comparative assessment of seller's staining test (SST), direct fluorescent antibody test and real time PCR for diagnosis of rabies in dogs. Int J Curr Microbiol Appl Sci. 2017;6(6):1222-8. [Google Scholar]
5. Pimburage RM, Gunatilake M, Wimalaratne O, Balasuriya A, Perera KA. Sero-prevalence of virus neutralizing antibodies for rabies in different groups of dogs following vaccination. BMC Vet Res. 2017;13(1):133. [PubMed] [Google Scholar]
6. Sudarshan MK. Vision 2030: dog-mediated human rabies-free India: action must begin now. Indian J Public Health. 2017;61(1):1-2. [PubMed] [Google Scholar]
7. Sudarshan MK, Madhusudana SN, Mahendra BJ, Rao NS, Narayana DH, Rahman SA, Meslin FX, Lobo D, Ravikumar K, Gangaboraiah. Assessing the burden of human rabies in India: results of a national multi-center epidemiological survey. Int J Infect Dis. 2007 Jan;11(1):29-35. [PubMed] [Google Scholar]
8. Sudarshan MK, Mahendra BJ, Madhusudana SN, Narayana DH, Rahman A, Rao NS, Meslin FX, Lobo D, Ravikumar K, Gangaboraiah. An epidemiological study of animal bites in India: results of a WHO sponsored national multi-centric rabies survey. J Commun Dis. 2006 Mar;38(1):32. [PubMed] [Google Scholar]
9. World Health Organization [Internet]. Rabies; 2024 Jun 5 [cited 2024 Jul 11]. Available from: https://www.who.int/news-room/fact-sheets/detail/rabies
10. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, Costa P, Freuling CM, Hiby E, Knopf L, Leanes F, Meslin FX, Metlin A, Miranda ME, Muller T, Nel LH, Recuenco S, Rupprecht CE, Schumacher C, Taylor L, Vigilato MA, Zinsstag J, Dushoff J; Global Alliance for Rabies Control Partners for Rabies Prevention. Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis. 2015 Apr;9(4):e0003709. [PubMed] [Google Scholar]
11. World Organization for Animal Health (OIE) 2018. – Rabies (infection with rabies virus and other lyssaviruses). Chapter 2.1.17. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE, Paris, France, 35 pp.
12. World Health Organization [Internet]. Control of neglected tropical diseases; [cited 2022 Jan 10]. Available from: http://www.who.int/neglected_diseases/diseases/en/
13. Rajendra Singh, Singh K. P. , Saminathan M. , Vineetha S. , Reddy G. B. M. , Madhulina M. Susan C., and Dhama K. . "Rabies, a vaccine preventable disease: current status, epidemiology, pathogenesis, prevention and control with special reference to India." (2018): 62-86.
14. World Health Organization 2007 Rabies; General Aspect and Laboratory Diagnostic Techniquies.1-469 (http://www.who.int/rabies/trs931_%2006_05.pdf)
15. World Health Organization. WHO expert consultation on rabies. Second Report. WHO Technical Report Series 982. Geneva: World Health Organization; 2013. p. 1-139. [Google Scholar]
16. NRCP, 2018 National Rabies Control Programme; [cited 2022 Jan 22].
17. Kim NH, Lee SH, Seo MG, Kim DH, Lee JH, Kwak D. Serologic detection of antibodies against rabies virus in dogs from animal shelters in Seoul, South Korea. J Anim Vet Adv. 2013;12(6):721-5. [Google Scholar]
18. Klingeborn B, Krogsrud J. Vaccination and antibody testing replacing quarantine as rabies-safety measure for transfer of dogs and cats into Sweden and Norway from EU/EFTA-countries. Rabies Bull Eur. 1993;17(4):13. [Google Scholar]
19. Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda ME, Shaw A, Zinsstag J, Meslin FX. Re-evaluating the burden of rabies in Africa and Asia. Bull World Health Organ. 2005;83(5):360-8. [PubMed] [Google Scholar]
20. World Health Organization. WHO expert committee on rabies. Eighth Report. WHO Technical Report Series 824. Geneva: World Health Organization; 1992. PP-1-72
21. Aiyedun JO. Community-based investigation of rabies antibody profile of dogs and control in Ilorin, Kwara State, Nigeria. J Environ Issues Agric Dev Cries. 2013;5(2):51-5. [Google Scholar]
22. Cox JH, Dietzschold B, Schneider LG. Rabies virus glycoprotein. II. Biological and serological characterization. Infect Immun. 1977 Jun;16(3):754-9. [PubMed] [Google Scholar]
23. Debnath A, Pathak DC, Ramamurthy N, Mohd G, Pandey AB, Upmanyu V, Tiwari AK, Saravanan R, Chellappa MM, Dey S. Serological profiling of rabies antibodies by enzyme-linked immunosorbent assay and its comparative analysis with rapid fluorescent focus inhibition test in mouse model. Vet World. 2019;12(1):126. [PubMed] [Google Scholar]

ISSN: 0973-5038
DOI: https://doi.org/10.24321/0973.5038.202411

24. Nale JM, Pharande RR, Majee SB, Gandge RS, Sawane MP, Ingle SA. Serosurveillance of rabies antibodies in dogs in Mumbai region by using indirect ELISA. Comp Immunol Microbiol Infect Dis. 2021 Jun;76:101655. [PubMed] [Google Scholar]
25. Kevin J. Molecular characterization of the nucleoprotein (N) gene of rabies virus and serosurveillance of rabies in dogs and cats [dissertation]. Nagpur: Maharashtra Animal and Fishery Sciences University; 2021. PP 61-93 [Google Scholar]
26. Clark KA, Wilson PJ. Postexposure rabies prophylaxis and preexposure rabies vaccination failure in domestic animals. J Am Vet Med Assoc. 1996;208(11):1827-30. [PubMed] [Google Scholar]
27. Feyssaguet M, Dacheux L, Audry L, Compoint A, Morize JL, Blanchard I, Bourhy H. Multicenter comparative study of a new ELISA, PLATELIA™ RABIES II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people. Vaccine. 2007;25(12):2244-51. [PubMed] [Google Scholar]
28. International Office of Epizootics. Biological Standards Commission, International Office of Epizootics. International Committee. Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds, and bees. Vol. 2. Office International des Épizooties; 2008. PP 1-38.
29. Rupprecht CE, Fooks AR, Abela-Ridder B. Laboratory techniques in rabies. 5th ed. Vol. 1. World Health Organization; 2018. 289 p. [Google Scholar]
30. World Health Organization. WHO expert consultation on rabies. First Report. WHO Technical Report Series 931. Geneva: World Health Organization; 2005. p. 1-121.
31. Servat A, Feyssaguet M, Blanchard I, Morize JL, Schereffer JL, Boue F, Cliquet F. A quantitative indirect ELISA to monitor the effectiveness of rabies vaccination in domestic and wild carnivores. J Immunol Methods. 2007;318(1–2):1-10. [PubMed] [Google Scholar]
32. Snedecor GW, Cochran WG. Statistical methods. 8th ed. Ames: Iowa State University Press; 1989. p. 71-82.
33. AravindhBabu RP, Manoharan S, Ramadass P, Chandran ND. Rabies in South Indian cows: an evidence of Sri Lankan Rabies virus variant infection based on the analysis of partial nucleoprotein gene. Indian J Virol. 2011 Dec;22(2):138-41. [PubMed] [Google Scholar]
34. Reddy GB, Singh R, Singh RP, Singh KP, Gupta PK, Desai A, Shankar SK, Ramakrishnan MA, Verma R. Molecular characterization of Indian rabies virus isolates by partial sequencing of nucleoprotein (N) and phosphoprotein (P) genes. Virus Genes. 2011 Aug;43(1):13-7. [PubMed] [Google Scholar]
35. Savaliya BF, Mathakiya RA, Bhanderi BB, Jhala MK. Evaluation of phenotypic factors for anti-rabies antibody in vaccinated pet dogs. Virusdisease. 2015 Dec;26(4):282-7. [PubMed] [Google Scholar]
36. Mugale M, Sandhu BS, Rai TS, Singh CK, Sood NK. Serological response to anti-rabies vaccination in animals: failure to achieve a protective antibody level. Indian J Anim Sci. 2013 Mar;83(3):10-3. [Google Scholar]
37. Rimal S, Ojha KC, Chaisowwong W, Shah Y, Pant DK, Sirimalaisuwan A. Detection of virus-neutralising antibodies and associated factors against rabies in the vaccinated household dogs of Kathmandu Valley, Nepal. PLoS One. 2020;15(4):e0231967. [PubMed] [Google Scholar]
38. Kasempimolporn S, Saengseesom W, Huadsakul S, Boonchang S, Sitprija V. Evaluation of a rapid immunochromatographic test strip for detection of Rabies virus in dog saliva samples. J Vet Diagn Invest. 2011;23(6):1197. [PubMed] [Google Scholar]
39. Jeong YK, Sung HK, Lee BJ. Seroprevalence of rabies virus antibody for dogs and cats in Seoul during 2017–2019. J Biomed Transl Res. 2021;22(1):19-25. [Google Scholar]
40. Singh MP, Goyal K, Majumdar M, Ratho RK. Prevalence of rabies antibodies in street and household dogs in Chandigarh, India. Trop Anim Health Prod. 2011 Jan;43(1):111-4. [PubMed] [Google Scholar]
41. Nodari ER, Alonso S, Mancin M, De Nardi M, Hudson-Cooke S, Veggiato C, Cattoli G, De Benedictis P. Rabies vaccination: higher failure rates in imported dogs than in those vaccinated in Italy. Zoonoses Public Health. 2017 Mar;64(2):146-55. [PubMed] [Google Scholar]
42. Albas A, Picolo MR, Soares CN, Bachega HV, Tarumoto MH. Humoral immune response in dogs and cats vaccinated against rabies in southeastern Brazil. J Venom Anim Toxins Incl Trop Dis. 2013;19(1):17. [PubMed] [Google Scholar]
43. Santosh AK, Isloor S, Rathnamma D, Sharada R, Sunilkumar KM, Balamurugan V, Yathiraj S, Satyanarayana ML. Assessment of humoral immune response in vaccinated domestic dogs and cats intended for pet-travel from India by Rapid Florescent Focus Inhibition Test (RFFIT). J Exp Biol Agric Sci. 2017;5(5):606-13. [Google Scholar]
44. Yale G, Sudarshan S, Taj S, Patchimuthu GI, Mangalanathan BV, Belludi AY, Shampur MN, Krishnaswamy TG, Mazeri S. Investigation of protective level of rabies antibodies in vaccinated dogs in Chennai, India. Vet Rec Open. 2021;8(1):e8. [PubMed] [Google Scholar]
45. Knoop EV, Freuling CM, Kliemt J, Selhorst T, Conraths FJ, Müller T. Evaluation of a commercial rabies ELISA

ISSN: 0973-5038
DOI: https://doi.org/10.24321/0973.5038.202411

as a replacement for serum neutralization assays as part of the pet travel scheme and oral vaccination campaigns of foxes. Berl Munch Tierarztl Wochenschr. 2010;123(7-8):278-85. [PubMed] [Google Scholar]

46. Olugasa BO, Aiyedun JO, Emikpe BO. Prevalence of antibody against rabies among confined, free-roaming and stray dogs in a transit city of Nigeria. Vet Ital. 2011;47(4):453-60. [PubMed] [Google Scholar]
47. Mansfield KL, Sayers R, Fooks AR, Burr PD, Snodgrass D. Factors affecting the serological response of dogs and cats to rabies vaccination. Vet Rec. 2004;154(14):423-6. [PubMed] [Google Scholar].

ISSN: 0973-5038
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