The Pharma Innovation Journal 2022; SP-11(2): 883-887

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(2): 883-887

www.thepharmajournal.com
Received: 20-12-2021
Accepted: 24-01-2022

Authors Details Given Below

Comparative evaluation of lateral flow assay with direct fluorescent antibody assay for surveillance of rabies in animals in India

Kavitha Govindaiah, Dilip Lakshman, Isloor Shrikrishna, Rathnamma Doddamane, Sharada Ramakrishnaiah, Narayanaswamy H Doddappaiah, Byregowda S Munivenkatappa, Venkatesha M Dasappa Gupta, Gyanendra Gongal, Avinash S Bhat, Hridya S Varughese, Tilak Chandan Shivakumar and Vinay C Prakash Rao

Abstract
Rabies is an infectious viral disease that is invariably fatal following the onset of clinical symptoms. In India, the incidence of rabies in animals is increasing. Although final laboratory diagnosis using OIE approved techniques such as Direct Fluorescent Antibody (DFA) assay is always necessary, there are several potential benefits to the use of an initial screening test that can be performed at the field level. The recent development of immunochromatographic assays, also referred to as lateral flow assays (LFAs), enables immediate testing with limited equipment, infrastructure and expertise. The present study was aimed to comparatively evaluate Lateral Flow Assay (LFA) with DFA assay for surveillance of rabies in animals by testing the brain samples resourced from different parts of India (Karnataka, Assam, Manipur, Andhra Pradesh, Chhattisgarh and Haryana) to the KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, KVAFSU, Bengaluru. The samples were subjected to both Lateral Flow Assay (LFA) and DFA for the detection of rabies antigen and viral inclusions respectively. The study revealed that 458/561 dogs, 0/4 cats, 33/43 cattle, 1/2 goats, 3/4 horses and 1/1 pigs were positive by both the tests, in all amounting to 496/615 (80.65%) to be positive for rabies which is alarming and an emerging threat in India. The high occurrence of rabies in the state of Karnataka (71.87%) could be attributed to a greater number of samples from Karnataka. Further, there was a 100 per cent concordance between both the tests. So, the present study supports the application of this LFA as simple test that can be employed at the field conditions as a preliminary rapid screening test and contribute to the epizootiology of rabies in India.

Keywords: Rabies, India, brain, DFA, LFA

Introduction
Rabies is an infectious viral disease that is almost always fatal following the onset of clinical symptoms. The disease causes about 59,000 human deaths annually worldwide, most of them being in Asia and Africa, particularly in resource-constrained countries. Dog bites account for almost the entire incidence of human rabies, whereas, rabies in animals could be attributed to a sylvatic cycle between wild as well as feral canines and other carnivores. The first laboratory confirmation of rabies in a wolf in India was reported by Isloor and his co-workers [1].

Laboratory based confirmatory diagnosis of rabies constitutes the most important component of rabies control programs. Since the recognition in the early 20th century of Negri bodies as being the pathognomonic histopathological lesion in brain or spinal-cord sections, an array of immunoassays and molecular techniques have been developed for the laboratory diagnosis of rabies [2, 3].

Antigen can be detected by various immunoassays such as the Direct Fluorescent Antibody assay (DFA), enzyme-linked immunosorbent assay (ELISA), direct rapid immunohistochemical test (dRIT) and immunoblots (immunochromatography, dot-blot). Among them, the DFA test has been recommended as the gold standard of rabies diagnosis by the WHO [4, 5]. However, the higher cost involved in fluorescent microscopy, the requirement for specialized training, and its unsuitability for decomposed samples limits the wide usage of DFA in resource-limited countries.

Corresponding Author
Kavitha Govindaiah
Ph.D. Scholar, Department of Veterinary Microbiology and Analyst, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Although final laboratory diagnosis using OIE approved techniques is always necessary, there are several potential benefits to the use of an initial screening test that can be performed at the field level. The recent development of immunochromatographic assays, also referred to as lateral flow assays (LFAs), enables immediate testing with limited equipment, infrastructure and expertise. The present study aims to comparatively evaluate LFA with DFA assay for surveillance of rabies in animals in India. The brain samples in animals like dog, cat, cattle, goat, horse and pig were resourced from different states of India. Also, this is one of the large scale studies in India to investigate and evaluate the utility of LFA in the immunodiagnosis of rabies in animals.

Materials and Methods

The brain samples (cerebellum and brain stem) were resourced from six different states of India *i.e.* Karnataka, Assam, Manipur, Andhra Pradesh, Chhattisgarh and Haryana, to the KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, Bengaluru, India. The samples shipped were from dog, cat, cattle, goat, horse and pig origin. The brain samples resourced during December 2019 to September 2021 were used in the present study. Initially, the samples were tested by LFA as a preliminary screening test (some of the samples were tested in the field and most of the samples were tested in the laboratory) and then DFA was employed as a confirmatory immunodiagnostic test for rabies in animals.

Lateral Flow Assay (LFA)

The kit used for this study was Anigen, Rapid Rabies Ag Test Kit by produced Bionote, Inc. (Hwaseong-si, Korea). The kit comprises of an LFA device, a plastic pipette / dropper, a sterile swab and a 1 mL vial with diluent (Fig. 1). The procedure followed was according to instructions described in the product manual of the kit.

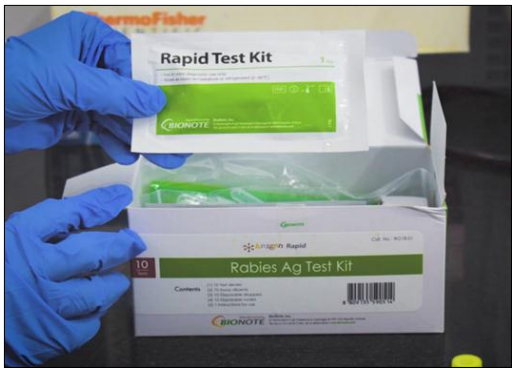

Fig 1: The contents of Lateral Flow Assay (LFA) kit

Direct Fluorescent Antibody (DFA)

Brain tissue samples were subjected to DFA for detection of rabies viral inclusions. The technique is based on microscopic examination of impressions of brain tissue (chilled acetone fixed for 1 hour at -20 °C) after incubation with Rabies DFA III anti-nucleocapsid IgG-FITC conjugate (Light Diagnostics, Merck Millipore, Temecula, CA, USA) in phosphate-buffered saline (PBS) pH 7.2 ± 0.2 containing 0.0125% Evans blue for 30-45 min at 37 °C in a humidified chamber. The stained impressions were visualized under a fluorescent microscope.

Results and Discussion

Lateral Flow Assay: Test (T) line and control (C) line in the result window indicates the presence of rabies antigen. Depending on the colour intensity of the Test (T) line, the positive results were read as strong, moderate and weak positive. If only control (C) line appears in the result window, it was read as negative (Fig. 2).

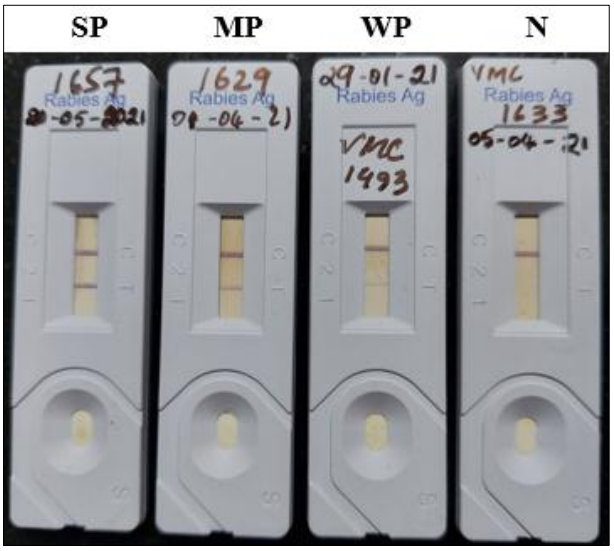

Fig 2: The results of LFA (Dogs from Karnataka): (from left to right) strong positive (SP), moderate positive (MP), weak positive (WP) and negative (N)

Direct Fluorescent Antibody test: The presence or absence of typical granular intra-cytoplasmic apple green fluorescence of aggregated viral nucleocapsids against the red background of brain tissue was used as the criterion in declaring positive and negative samples respectively (Fig. 3 and 4).

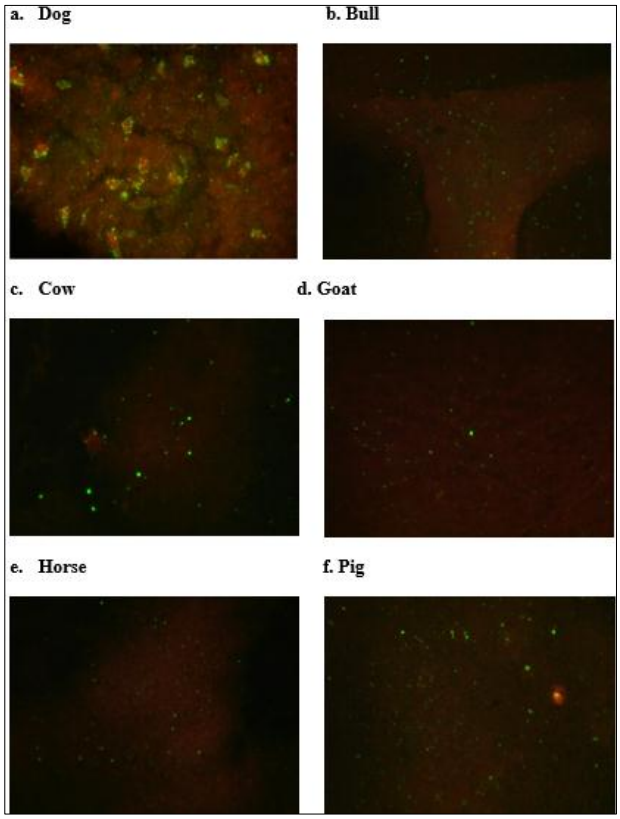

Fig 3: The presence of a bright apple green fluorescent foci against red colored brain tissue indicating the positivity by DFA (400X magnification)

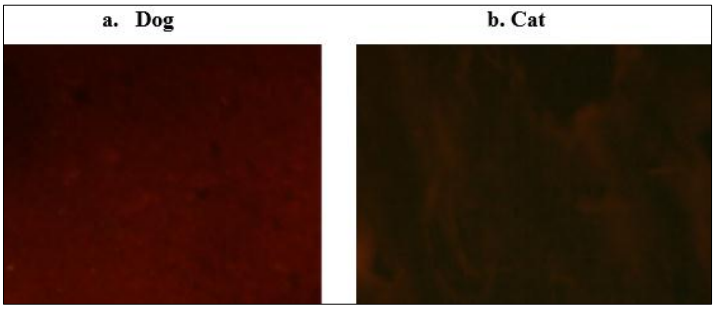

Fig 4: The absence of bright apple green fluorescent foci against red colored brain tissue indicating the negativity by DFA (400X magnification)

A total of 615 samples including 561 dogs, 4 cats, 43 cattle, 2 goats, 4 horses and 1 pig were tested for rabies by both LFA and DFA test (Table 1). Out of 615 samples tested, 496 were tested positive for rabies by both LFA and DFA which is accounting to 80.65 per cent is alarming and an emerging threat in India. The number of samples received from dogs and cattle origin was high.

Table 1: The results of LFA and DFA

Tests	**Both LFA and DFA**											
Species	**D**		**CT**	**CL**		**G**		**H**		**P**	**Total**	
States	+	-	-	+	-	+	-	+	-	+	**+ (%)**	**- (%)**
KA	431	97	3	9	-	-	-	1	1	1	442 (71.87)	101 (16.42)
AS	12	-	1	21	9	1	1	2	-	-	36 (5.86)	11 (1.79)
MN	15	4	-	1	-	-	-	-	-	-	16 (2.60)	4 (0.65)
AP	-	2	-	1	-	-	-	-	-	-	1 (0.16)	2 (0.33)
CG	-	-	-	1	-	-	-	-	-	-	1 (0.16)	-
HR	-	-	-	-	1	-	-	-	-	-	-	1 (0.16)
Total	458	103	4	33	10	1	1	3	1	1	496 (80.65)	119 (19.35)
%	74.47	16.75	0.65	5.37	1.63	0.16	0.16	0.49	0.16	0.16	80.65	19.35

(D – Dog, CT – Cat, CL – Cattle, G – Goat, H – Horse and P – Pig)

The data revealed that 431/528 (dog, fig. 3a - positive, 4a - negative), 0/3 (cat, fig. 4b - negative), 9/9 (cattle – bull (3b - positive) and cow (3c - positive)), 1/2 (horse, fig. 3e - positive) and 1/1 (pig, fig. 3f - positive) from Karnataka (KA); 12/12 (dog), 0/1 (cat), 21/30 (cattle), 1/2 (goat, Fig. 3d) and 2/2 (horse) from Assam (AS); 15/19 (dog) and 1/1 (cattle) from Manipur (MN); 0/2 (dog) and 1/1 (cattle) from Andhra Pradesh (AP); 1/1 (cattle) from Chhattisgarh (CG) and 0/1 (cattle) from Haryana (HR) were positive by both the tests (Table 1). As per the above data, the highest number of rabies cases are prevalent in Karnataka, followed by Assam and Manipur; least in Chhattisgarh and none in Haryana.

In the present study, the statistical analysis (Pearson's Chi-squared test with Yates' continuity correction [6]) was performed using R software. The two categorical variables, the two test methods, DFA and LFA tested as pairs for the detection of rabies were compared. Since the study consisted of only two categories, degrees of freedom (df) became 1. The analysis resulted in huge Chi (c) -squared value (608.6491) and a very small p value (<2.2e-16) which is very significantly less than 0.05 will disprove the null hypothesis indicating the correlation between the two test results are not because of chance factor and favours for alternative hypothesis that the both analytical methods have a very strong correlation.

The animals from which the brain samples collected and tested had a clinical history of biting tendency, anorexia, paralysis, recumbency, hypersalivation, aggressive behaviour, shivering and behavioural changes in dogs; the history of dog bite, hypersalivation in cows, head butting and attacking tendency in bulls; hyperexcitement, aggressive behaviour in horses and neurological signs in goats.

Historically, DFA on brain tissue was the only 'gold standard' test for the confirmation of rabies both in animals and humans. The DFA has been extensively used to confirm the cases of rabies in wide range of animal species and from different states and Union Territory of India at Veterinary College, Bengaluru [7-11]. But, there are few limitations of DFA include requirements of fresh brain tissue, expensive fluorescent microscope and observer expertise to distinguish specific fluorescence in addition to the need of a deep freezer for chilled acetone based fixation of brain tissue and incubator.

In view of these limitations of employing DFA, there is need of a rapid, cost effective, user friendly pen side test for post mortem diagnosis of rabies. Such test has the potential to encourage the retrieval and testing of samples from suspect cases of rabies in animals in developing countries at the filed level. In this context, availability of a quality LFA for rapid diagnosis of rabies in animals especially in the rural areas where laboratory facilities are not available is need of the hour. It is possible that the use of LFA would provide additional motivation for testing samples in parts of the country where laboratory infrastructure and transportation are limited. Alongside the introduction of LFA, additional focus could be placed on overcoming the challenges of sample transportation and regional laboratory capacity with OIE approved tests. Recently, the WHO published the third Technical Report Series of Rabies Expert Consultation, where LFA is recommended as a surveillance tool for developing countries lacking adequate laboratory facilities [12].

On the other hand, recent studies have shown inconsistencies in the specificity and sensitivity of some of the currently available LFAs, with unacceptable sensitivity variation leading to claims that they are not suitable as a component of rabies surveillance activities [13, 14]. But, Yale and her co-

workers in the year 2019, evaluated the utility of LFA (Anigen Rapid Rabies Ag Test Kit, Bionote, Hwaseong-si, Korea) for rapid post mortem diagnosis of rabies in animals. The study found that the Bionote LFA has potential as a screening tool in rabies endemic countries [15]. Similar observation was made by other researchers [16-20] wherein they reported that the preliminary screening of brain samples of dogs suspected for rabies using the LFA of Bionote, Korea was handy, most user friendly and faster.

With the year 2030 being target for elimination of dog-mediated rabies, momentum is building towards canine rabies control in the country [21-23], it would be highly recommended to increase rabies laboratory testing facilities in endemic regions. It would be beneficial to include reliable LFAs as a surveillance tool in the national rabies control plan, to support the expansion of surveillance and testing systems to areas with limited rabies diagnostic capacity.

In the present study, a 100 per cent concordance between LFA and DFA was observed. The results support the application of LFA as a simple test that can be adapted to field conditions as a preliminary rapid test and contribute to the epidemiology of rabies in India. As per the study, percent positivity (80.65%) of rabies in animals is very high which is highly alarming. Along with canine rabies, the incidence of rabies in other livestock species is increasing and is a threat in several countries where Pre-Exposure Prophylaxis (PrEP) is not carried out in other species including the livestock except the pet animals like dogs and cats.

Conclusion

The present investigation is a large scale study of LFA usage (615 brain samples in six species of animals from six states of India) for evaluation of its immunodiagnostic performance in India. Based on our results, the preliminary screening of brain samples of animals suspected for rabies using the monoclonal antibody based rapid diagnostic test (RDT) like immunochromatography tool or LFA was the most user friendly and faster. This enabled the documentation of cases of rabies at the field level and also at laboratory level (before performing DFA as a confirmatory test) helped to understand the epizootiological aspects related to species and geographical distribution. The majority of the cases were reported from dog species and in Karnataka state as the laboratory where this work was carried out itself is located in Karnataka and hence the ease of shipping large number of samples from field to the laboratory. The study supports the application of LFA as a simple test that can be employed at the field conditions as a preliminary rapid screening test and contributes to the epizootiology of rabies in India.

Acknowledgement

The authors are thankful to the KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bangalore for providing the necessary facilities to conduct the research work smoothly.

References

1. Isloor S, Marissen WE, Veeresh BH, Nithin Prabhu K, Kuzmin IV, *et al*. First case report of rabies in a wolf (*Canis lupus pallipes*) from India. Journal of Veterinary Medicine and Research. 2014;1(3):1012.
2. Dacheux L, Wacharapluesadee S, Hemachudha T, Meslin FX, Buchy P, Reynes JM, *et al*. More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. PLOS Neglected Tropical Diseases. 2010;4:e765.
3. Duong V, Tarantola A, Ong S, Mey C, Choeung R, Ly S, *et al*. Laboratory diagnostics in dog-mediated rabies: An overview of performance and a proposed strategy for various settings. International Journal of Infectious Diseases. 2016;46:107-114.
4. Dean DJ, Abelseth MK, Atanasiu P. The Fluorescent Antibody Test, 4th ed.; World Health Organization: Geneva, Switzerland. 1996.
5. World Health Organization. WHO Expert Consultation on rabies. WHO Press, Geneva. 2005. Available at www.who.int/rabies/931/en/index.html.
6. Yates F. Contingency table involving small numbers and the $\chi 2$ test". Supplement to the Journal of the Royal Statistical Society. 1934;1(2):217-235. DOI: 10.2307/2983604. JSTOR 2983604.
7. Isloor S, Yathiraj S, Satyanarayana ML, Leena G, Veeresh BH, Nithinprabhu K. Rabies research in India: Our initiatives In: Proceedings of APCRI conference. 2013, 52.
8. Veeresh BH, Nithin Prabhu K, Chandrasekhar N, Rathnamma D, Satyanarayana ML, Yathiraj S, *et al*. Complete nucleoprotein gene based phylogenetic analysis of rabies virus in and around Bangalore. India. *In*: Proceedings of APCRI conference, 2013, 52.
9. Nithin Prabhu K. Nucleoprotein gene - based molecular epidemiology of rabies virus and development of isothermal nucleic acid amplification assay for its detection. Ph.D. thesis, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India. 2014.
10. Nithin Prabhu K, Isloor S, Veeresh BH, Rathnamma D, Sharada R, Das LJ, *et al*. Application and Comparative Evaluation of Fluorescent Antibody, Immunohistochemistry and Reverse Transcription Polymerase Chain Reaction Tests for the Detection of Rabies Virus Antigen or Nucleic Acid in Brain Samples of Animals Suspected of Rabies in India. Veterinary Sciences. 2018;5(1):24.
11. Chandrashekhara N. Molecular Characterization of Rabies Virus and Diagnosis of Rabies by RT-PCR in Animals. Ph.D Thesis. Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India. 2013.
12. World Health Organization. WHO Expert Consultation on Rabies: Second Report. 2018.
13. Lechenne M, Naïssengar K, Lepelletier A, Alfaroukh IO, Bourhy H, Zinsstag J, *et al*. Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. PLOS Neglected Tropical Diseases. 2016;10(10):e0005010. DOI: 10.1371/journal.pntd.0005010.
14. Eggerbauer E, de Benedictis P, Hoffmann B, Mettenleiter TC, Schlottau K, Ngoepe EC, *et al.* Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. PLOS Neglected Tropical Diseases. 2016;10(6):e0004776. DOI: 10.1371/journal.pntd.0004776.
15. Yale G, Gibson AD, Mani RS, Harsha P K, Costa NC, Corfmat J, *et al.* Evaluation of an Immunochromatographic Assay as a Canine Rabies Surveillance Tool in Goa, India. Viruses. 2019;11(7):649.

16. Tajunnisa M, Isloor S, Gongal G, Rathnamma D, Sujith SN, Santosh AK, *et al*. Comparative evaluation of Rapid immunochromatographic test and Monoclonal antibody based Direct Fluorescent Antibody assay for detection of Rabies virus. In: XIX National conference of Association for Prevention and Control of Rabies in India: Towards elimination of human rabies by 2030. 2017, 53.
17. Mohamed Ghouse H, Isloor S, Satyanarayana ML Gongal G. Evaluation of brain sample collection through foramen magnum vis-a-vis conventional skull open methods for diagnosis of rabies in suspected animals. APCRI Journal. 2020;22(1):40-47.
18. Udupa KG, Tajunnisa M, Malatesh DS, Patel S, Chaitra GR, Ravikumar BP, *et al*. Epidemiological aspects of canine rabies based on user friendly monoclonal antibody based immunochromatography in and around Shivamogga of Karnataka state. APCRI Journal. 2021;22(2):45-51.
19. Sunil Kumar, Isloor S. Diagnosing rabies cases in animals through collection of brain sample through foramen magnum in Shivamogga dist. APCRI Journal. 2021;22(2):36-44.
20. Samrudh MC. Evaluation of the Pan-lyssa virus by LN34 real time PCR assay for detection of rabies virus and other lyssa viruses in animals. M.V.Sc. Thesis, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India. 2021.
21. Totton SC, Wandeler AI, Zinsstag J, Bauch, CT, Ribble CS, Rosatte RC, *et al*. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. Preventive Veterinary Medicine. 2010;97:51-57.
22. Abbas SS, Venkataramanan V, Pathak G Kakkar M. Rabies control initiative in Tamil Nadu, India: A test case for the 'One Health' approach. International Health. 2011;3:231-239.
23. Kakkar M, Venkataramanan V, Krishnan S, Chauhan RS, Abbas SS. Roadmap to Combat Zoonoses in India (RCZI) Initiative. Moving from Rabies Research to Rabies Control: Lessons from India. PLOS Neglected Tropical Diseases. 2012;6:e1748.

Authors Details

Kavitha Govindaiah
Ph.D. Scholar, Department of Veterinary Microbiology and Analyst, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Dilip Lakshman
Technical Manager, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Isloor Shrikrishna
Professor, Department of Veterinary Microbiology and Laboratory Director, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Rathnamma Doddamane
Professor and Head, Department of Veterinary Microbiology and Biosafety Officer, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Sharada Ramakrishnaiah
Assistant Professor, Department of Veterinary Microbiology and Quality Manager, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Narayanaswamy H Doddappaiah
Hon'ble Vice Chancellor, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Bidar, Karnataka, India

Byregowda S Munivenkatappa
Director, Institute of Animal Health and Veterinary Biologicals (IAH & VB), KVAFSU, Bengaluru, Karnataka, India

Venkatesha M Dasappa Gupta
Professor and Head, Department of Biological Production, IAH & VB, KVAFSU, Bengaluru, Karnataka, India

Gyanendra Gongal
World Health Organization South-East Asia Region (WHO SEARO), New Delhi, India

Avinash S Bhat
Trouw Nutrition India Pvt. Ltd., Hyderabad, Telangana, India

Hridya S Varughese
Ph.D. Scholar, Department of Veterinary Microbiology and Analyst, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Tilak Chandan Shivakumar
M.V.Sc, Department of Veterinary Microbiology and Analyst, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India

Vinay C Prakash Rao
M.V.Sc, Department of Veterinary Microbiology and Analyst, KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Veterinary College, KVAFSU, Bengaluru, Karnataka, India