

Cross-Sectional Study

Role of Cats as Intermediate Rabies Vector: A Cross-Sectional Retrospective Laboratory Surveillance in Kerala (India)

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A B S T R A C T

Introduction: Rabies in cats has been reported from all parts of the world where animal rabies is present. Today, cats are a popular and beloved pet.

Objective: A preliminary attempt to understand the role of cats in rabies epidemiology is made by undertaking a cross-sectional retrospective analysis of rabies cases in cats in the state of Kerala (India) for a period of 4 years from 2021 to 2024.

Methodology: The data on the rabies occurrence in cats brought for routine rabies diagnosis to the State Institute of Animal Diseases (SIAD), Palode, Kerala, are analyzed for test positivity, positivity in clinically suspected cases, the proportion of feline rabies in animal rabies, and the performance of different tests for cat rabies diagnosis, namely the Lateral Flow Immunochromatography Test, Direct Fluorescent Antibody Test (DFAT), and Microchip-based PCR (TrueNat PCR).

Result: The test positivity in cat samples was 6.5% from among all the cat samples received for rabies diagnosis. The test positivity was 24.6% for those samples from cats with clinical symptoms suggestive of rabies. DFAT detected 16 brain samples as positive out of the 69 tested. Out of the 16 cases detected as positive by DFAT, only 5 (31.3%) were positive in the lateral flow immunochromatography test. TrueNat PCR detected all 16 positive cases detected by the reference test as positive, demonstrating 100% sensitivity.

Conclusion: This document discusses the potential of feline rabies infection and the importance of considering rabies in cats along with dog rabies control in rabies-endemic areas.

Keywords: Epidemiology, State Institute of Animal Diseases (SIAD), Feline, Lateral Flow Immunochromatography Test, Direct Fluorescent Antibody Test (DFAT), Microchip-based PCR (TrueNat PCR)

Introduction

Domestic cats are an important part of humans' lives. Today, cats are a popular and beloved pet. However, feral cats living in the community remain problematic. They can cause considerable mortality to local wildlife, act as reservoirs of many pathogens and transmit zoonotic infections.

Rabies remains a global public and animal health issue of the 21st century. Globally, rabies is mainly circulated through domestic dogs and some wild animals. Dogs remain the largest reservoir of rabies worldwide and are responsible for 99% of human rabies transmission. The virus has a broad host range, with both domestic and wild animals acting as environmental reservoirs. Rabies in cats has been reported from all parts of the world where animal rabies is present. Infected cats shed RABV in saliva. Despite being able to transmit RABV effectively, cats (domestic or wild) are not usually maintenance hosts. Rabies in cats has got one health implication, as there is an increasing trend of keeping cats as pets, especially post-Covid. Unlike dogs, cats are usually maintained as indoor pets, and usually the pet owners have more risk of getting exposed to rabies if their pets are infected. Cats often groom themselves and scratch owners, which increases the risk of exposure to rabies. The role of cats in rabies transmission needs to be well studied.

A preliminary attempt to understand the role of cats in rabies epidemiology is made by undertaking a cross-sectional retrospective analysis of rabies cases in cats in the state of Kerala (India) for a period of 4 years from 2021 to 2024.

Materials and method

The data on the rabies occurrence in cats (cat carcasses) brought for routine rabies diagnosis to the State Institute of Animal Diseases (SIAD), Palode, Kerala, India, the referral laboratory for the diagnosis of animal diseases, for a period of 4 years from 2021 to 2024, are analysed for the test positivity, positivity in clinically suspected cases, the proportion of feline rabies in animal rabies and the performance of different tests for cat rabies diagnosis, namely the Lateral Flow Immunochromatography Test (Rapid Card Test), Direct Fluorescent Antibody Test (DFAT) and Microchip-based portable PCR (TrueNat PCR).

Lateral Flow Immunochromatography Test

A lateral flow immunochromatographic assay is a rapid antigen detection test which is simple to perform and helps faster diagnosis of rabies. Lateral flow devices are principally based on colloidal gold-conjugated monoclonal antibodies that capture the antigen within a sample. The antigen-antibody complex thereupon binds to a second

detection antibody that is fixed at the test zone "T" on a nitrocellulose membrane, showing a coloured line for a positive sample. The kit used was of Bionote, Korea. The test was performed as per manufacturers' directions. Brain homogenate was used as sample (Figure1).

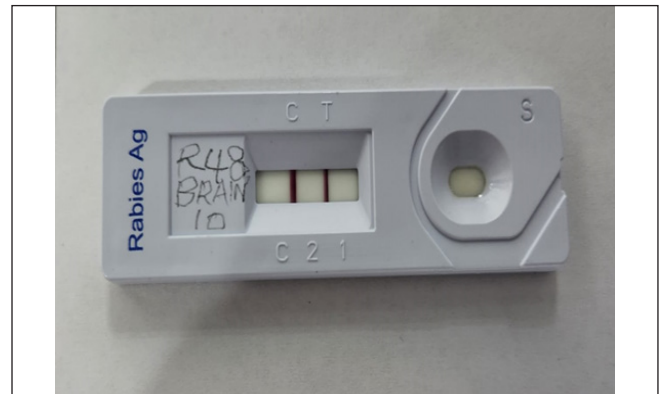


Figure 1. Lateral Flow Immunochromatography (LFT) Direct Fluorescent Antibody Test (DFAT)

High-grade chilled acetone-fixed brain impressions were subjected to the fluorescent antibody test for the detection of the rabies virus. The fixed slides were air-dried and incubated with anti-rabies nucleocapsid conjugate for 35 min at 37°C and further washed with phosphate-buffered saline (PBS) of pH 7.2 in two successive washes for 5-10 min, air-dried, and mounted with buffered glycerol and then visualized under a fluorescent microscope at 400x magnification. Results were interpreted as bright, dull, dim apple green, round to oval intracellular fluorescence accumulations, or no fluorescence. DFAT is the gold standard test recommended by the WHO and WOAHP for rabies testing (Figure 2).

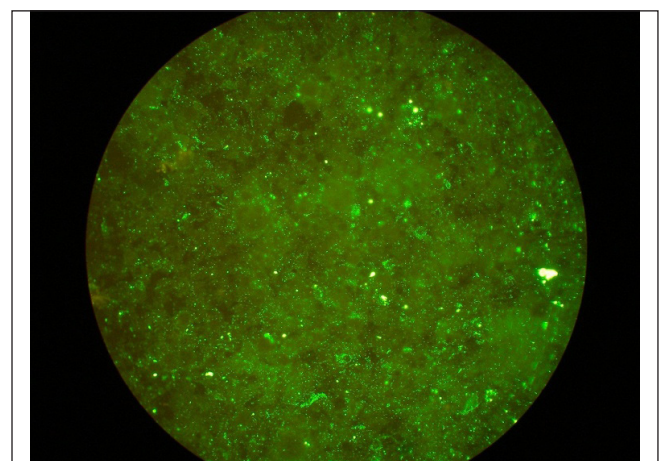


Figure 2. Direct Fluorescent Antibody Test (DFAT) Truenat Pcr

TrueNat PCR is a simple, reliable, and easy-to-conduct test for rabies diagnosis, and the test has been validated

for rabies diagnosis in animals and humans.^{4,6} The results were given either as detected (high, medium, low, or very low), not detected, or as test invalid. One can visualize the reaction in real time, and the CT value was also given.

Results

The test positivity in cat samples was 6.5% from among all the cat samples received for rabies diagnosis. The test positivity obtained for those samples from cats with clinical symptoms suggestive of rabies submitted for rabies diagnosis stood at 24.6%. The percentage proportion of cat rabies among all animal rabies cases studied was 4.9.

The efficacy of common diagnostic tests for rabies in cats was evaluated in the study. DFAT, the gold standard test for rabies diagnosis, detected 16 brain samples as positive out

of the 69 tested. The lateral flow immunochromatographic test, which demonstrated very good reliability for dog rabies diagnosis, displayed poor performance in cat rabies detection. Out of the 16 cases detected as positive by DFAT, only 5 (31.3%) were positive in LFT. TruNat PCR, a recently developed test for rabies, detected all 16 positive cases detected by the reference test as positive, demonstrating 100% sensitivity compared to DFAT.

The results obtained for all the cat samples submitted for rabies diagnosis for the period 2021 to 2024 are shown in Table 1.

The results obtained for those cat samples whose clinical symptoms are suggestive of rabies and were submitted for rabies diagnosis for the period 2021 to 2024 are shown in Table 2.

Table 1. Test positivity in cat samples

Year	Number of cat brain samples tested	Number of positives	Percentage positivity
2021	60	5	8.33
2022	77	3	3.80
2023	50	4	8.00
2024	71	5	7.04
TOTAL	258	17	6.5

Table 2. Positivity in clinically suspected cases

Year	Number of clinically suspected cat samples	Number of positives	Percentage positivity
2021	11	5	45.4
2022	16	3	18.75
2023	27	4	14.81
2024	15	5	33.3
Total	69	17	24.6

Table 3. Depicts the proportion of cat rabies among animal rabies

Year	Total animal samples tested	Total animal rabies cases	Total cat rabies cases	Percentage proportion
2021	235	84	5	5.9
2022	327	113	3	2.7
2023	233	82	4	4.9
2024	271	71	4	5.6
Total	1066	350	17	4.9

Table 4. Efficacy of different tests for diagnosing rabies in cats with DFAT as reference test

Sample no	Year	LFT	DFAT	TrueNat PCR
1	2021	Negative	Positive (1+)	Positive
2	2021	Negative	Positive (1+)	Positive
3	2021	Negative	Positive (2+)	Positive
4	2021	Negative	Positive (1+)	Positive
5	2021	Positive	Positive (3+)	Positive

6	2022	Positive	Positive (4+)	Positive
7	2022	Positive	Positive (4+)	Positive
8	2022	Positive	Positive (4+)	Positive
9	2023	Negative	Positive (1+)	Positive
10	2023	Negative	Positive (2+)	Positive
11	2023	Negative	Positive (1+)	Positive
12	2023	Negative	Positive(1+)	Positive
13	2024	Positive	Positive (2+)	Positive
14	2024	Negative	Positive (1+)	Positive
15	2024	Negative	Positive (1+)	Positive
16	2024	Negative	Positive (1+)	Positive
17	2024	Negative	Negative	Positive
Number of samples positive by the test		5	16	17

Discussion

In India, the country that reports the largest number of rabies deaths has got a smaller number of reported cat rabies cases and human deaths associated with cat rabies. However, as mammals, cats are susceptible to rabies, and being biting animals, they can readily transmit the infection to humans and other animals. It has been observed that stray cat populations grow in areas where stringent measures are implemented to control the population of dogs but food resources such as waste management are not addressed. This imminent threat should be foreseen by countries who are working towards eliminating dog-mediated rabies.

Documents on rabies in cats and human rabies cases from exposure to cats are scanty. Stray cats are not included in mass vaccination campaigns whose focus is mainly on dogs. Stray cats present significant challenges in urban areas across the world. They are more prolific than dogs; however, survival rates are estimated to be poor.² Their reproductive peculiarities, such as early sexual maturity, seasonally polyoestrous cycles, and multiparous pregnancies, contribute to their prolific nature. They are highly adaptable and thrive in harsh environmental conditions.¹ Free-roaming cats are more likely to be exposed to the rabies virus through potential encounters with infected wildlife or domestic dogs. Communities deciding to manage the overpopulation of feral cats are often torn between the competing interests of cats, wildlife, and public health. Only very few NGOs work for the welfare of cats in comparison to dogs. Group feeding of cats by the public is a common practice in India. This also increases risk for contracting rabies by enabling greater contact and is augmented by increased animal density. Cross-species contact also allows feral cat populations to spread

diseases to wildlife.⁵ Feeding sites can attract animals such as rodents, mongoose, foxes, and dogs; many of them are rabies virus reservoirs.

Our analysis demonstrates that rabies is prevalent in cats, and rates remain more or less steady over the years (Table 1). Around 5 percent of total animal rabies cases were in cats (Table 3). It was observed that all cases brought to the laboratory for disease confirmation had one or more human exposures, highlighting public health implications.

The efficacy of common diagnostic tests for rabies in cats was evaluated in the study (Table 4). DFAT, the gold standard test for rabies diagnosis, detected 16 brain samples as positive out of the 69 tested. The lateral flow test, which demonstrated very good reliability for dog rabies diagnosis,⁷ displayed poor performance in cat rabies detection. Out of the 16 cases detected as positive by DFAT, only 5 (31.3%) were positive in LFT. False negative observations were mainly in cases with low antigenic mass. TruNat PCR, a recently developed test for rabies, detected all 16 positive cases detected by the reference test as positive, demonstrating 100% sensitivity compared to DFAT. However, it gave a positive result in one sample, which was negative by DFAT. Although it can be labeled as a false positive, it is likely to be a true positive, as real-time PCR assays are more sensitive than DFAT to detect viral RNA in decomposed brain samples.³ However, the study could not confirm the hypothesis because further confirmatory testing was not performed. Previous studies evaluating TruNat PCR for rabies diagnosis in animals and humans have reported low-grade disagreements with DFAT and conventional real-time PCR.^{4,7}

With the general focus on dogs for both rabies control and population management, the possibility of cats emerging as a potential carrier of infection to humans and animals

in the future cannot be overtly excluded. It is necessary to initiate simultaneous intervention strategies in cats along with canine rabies control. Cats are the second most preferred companion animal after dogs in most places. A higher rate of cat bite exposure than dog bite in many medical institutions has been recorded in this region (IDSP data, Kerala) and is a matter of concern. The same could be true in other regions, if analyzed, as rabies epidemiology and stray animal ecology are almost the same throughout the country.

This preliminary local analysis highlights the importance of considering rabies in cats along with dog rabies control in canine rabies-endemic areas. Public education is important in strengthening responsible pet ownership, vaccination, sterilization, and keeping pet cats indoors to reduce contact with dogs and wildlife. Vaccination of cats must be promoted to reduce transmission risk to humans and other animals. It has been observed in this study that all positive cases were unvaccinated. Safe and potent vaccines are available for cat vaccination. The stray cat population also needs to be managed.

Conclusion

This document discusses the potential of feline rabies infection. A possible decline in dog rabies in the coming years can create a false sense of security hampering elimination possibilities unless rabies in other animals, such as cats, are also concomitantly addressed. It is important to develop strategies and approaches for rabies control in cats and to manage stray cat populations. With the year 2030 drawing nearer, and with the WHO goal of zero human rabies death, much work is still required.

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