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Incidence of Rabies in animals of Kamrup Metro district of Assam, India

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Abstract

Rabies is a neglected fatal viral zoonosis caused by *Lyssavirus* genus of *Rhabdoviridae* family, mostly mediated by dog-bites. The present study specifies the diagnosis of Rabies in suspected clinical cases in animals of Kamrup metro district of Assam. During the study period (2019-2020), a total of 17 brain tissue samples were collected from clinically suspected animals using Foramen magnum method as per OIE and on spot screening for rabies virus was done using Lateral Flow Assay (LFA) kit. Further, the collected brain tissue samples were being sent to OIE referral lab for confirmatory diagnosis by Direct Fluorescent Antibody Test (DFAT), which is the gold standard test for confirmation of rabies. Out of 17 brain tissue samples, 13 were found to be positive for rabies virus and the incidence of rabies was 76.47 percent. Species-wise, canines (76.92%) had the highest incidence of rabies followed by bovine (15.38%) and caprine (7.69%).

Keywords: *Lyssavirus*, Foramen magnum, LFA, DFAT

Introduction

Rabies is a fatal zoonosis which continues to kill about 20,000 people every year in India, accounting for almost a third of the global human deaths due to rabies (WHO, 2013; Sudarshan *et al.*, 2007) [15, 12]. Rabies in animal acts as source of infection for human rabies and thus it is important to diagnose and control rabies in animal, in order to control rabies in human (Chandel *et al.*, 2016) [4]. Most cases of dog-bite commonly occurred in domestic animals like cattle, sheep and goats, including dogs and cats. Female and younger animals were the frequent victims of dog bite (Rajkumar *et al.*, 2018; Pradhan *et al.*, 2021) [9, 7]. Kayali *et al.* (2003) [6] reported 86% more rabies cases in free roaming dogs than in confined or partially confined. FAT is internationally considered as a gold standard test for diagnosis of rabies (Chandel *et al.*, 2016) [4]. There is paucity of quantitative information about the status of rabies in animal populations, especially in stray animal populations, which play an important role in endemicity of the disease (Brookes *et al.*, 2017) [3]. The burden of rabies in animal populations in India is largely unknown. Therefore, the present study was deliberated to estimate the annual incidence of rabies in animal populations and to compare inter-species incidence among the domestic animals in Kamrup metro district of Assam based on laboratory confirmations.

Materials and Methods

The present study was undertaken for a period of one year (2019-20) and the animals were suspected based on clinical signs exhibited by them *viz.* bellowing, lethargy (Fig 1), profuse salivation (Fig 2) and incoordination in most of the cases.



Fig 1: Lethargy in rabies suspected HF cow



Fig 2: Salivation in a rabies suspected calf with a dog-bite history

Collection of brain tissue samples

The brain tissue samples from suspected animals after death of the animals were collected by inserting AI sheath through the occipital foramen (Foramen-magnum method) (Fig 4) and then brain tissue is drawn using 10ml disposable syringe. While collection of brain sample personal protective equipments (PPE) (Fig 3) were used to avoid chances of infection as rabies virus is zoonotic in nature.



Fig 3: Adoption of Personal protective equipment (PPE) prior to collection of brain sample



Fig 4: Incision site for collection of brain sample

On spot screening of brain tissue samples by lateral flow assay (LFA) kit

The collected brain tissue samples were screened on spot for presence of rabies virus using lateral flow assay (LFA) kit. The LFA kit (Fig 5) used in the present study was Anigen Rapid Rabies Ag Test kit manufactured by BioNote, Inc. 22 Samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 445-170, Republic of Korea (TEL: 82-31-8003-0618).



Fig 5: Lateral Flow Assay (LFA) kit

The protocol given in the product manual of LFA kit was followed to perform Lateral Flow Assay (LFA). In case of positive, two bands (Fig 6) will be present both in Test (T) line and Control (C) line in the result window of LFA cassette, while only single band in the Control(C) line will indicate negative result.



Fig 6: LFA Kit showing two bands indicating positive result

Packaging and Dispatch of brain tissue samples

After collection, the brain tissue samples were packed in tertiary layered sample cups and then placed in a thermo-stable box provided with ice packs followed by dispatch of samples to KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, KVAFSU, Hebbal, Bangaluru- 560 024, Karnataka for laboratory confirmation through India-post courier.

Laboratory confirmation of rabies by direct fluorescent antibody test (DFAT)

All the brain tissue samples were shipped to the OIE referral laboratory for confirmation of rabies and were subjected to Direct Fluorescent Antibody Test (DFAT) as per standard OIE protocol.

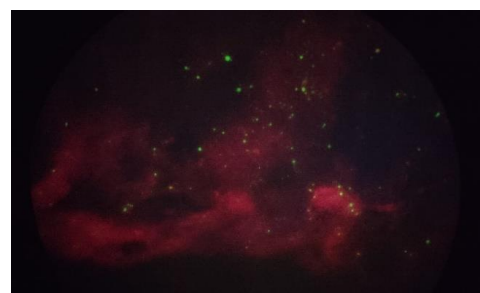


Fig 7: Apple green fluorescence indicating rabies viral inclusion bodies in the brain tissue (red part)

In case of positive result, apple green fluorescence due to the presence of Rabies viral inclusion bodies (Fig 7) will be seen under the Fluorescent Microscope.

Results and Discussion

In the present study (2019-2020), a total of 17 post-mortem brain samples (Table. 1) comprising 12 canines, 2 bovines, 2 felines and 1 caprine were collected from the Kamrup Metro District, Assam based on the history of dog bite and clinical signs including profuse salivation, bellowing, anorexia, dullness and depression, lethargy, etc.

Table 1: Result of laboratory confirmation by DFAT

Species	Total sample collected	Total positivity	Incidence % (Nos. of positivity/ Total positive sample)
Canine	12	10 (83.33%)	76.92 (10/13)
Bovine	2	2 (100%)	15.38 (2/13)
Feline	2	0	0
Caprine	1	1 (100%)	7.69 (1/13)
Total	17	13	76.47

Out of 17 samples, 13 were found to be positive for rabies virus by laboratory confirmation. Therefore, the incidence of rabies in animals was 76.47 percent. Similarly, Govindaiah *et al.* (2022) ^[5] reported 80.65% percent of incidence from six different states of India. Qureshi *et al.* (2003) ^[8] also reported 71 percent incidence of rabies in various domestic animals in Pakistan. In a five years study, Singh and Sandhu (2007) ^[11] reported 55.98 percent overall incidence of rabies in suspected animals.

Species-wise, incidence was highest in canines (76.92%) followed by bovine (15.38%) and caprine (7.69%). In agreement to the present finding, Govindaiah *et al.* (2022) ^[5] reported high number of rabies cases in canine and cattle from six different states of India. Waltner *et al.* (1993) ^[13] reported 76 percent incidence in canines which was in agreement with the present findings. As a major reservoir of rabies virus which is transmitted through bites, a higher incidence in canine was quite obvious. Besides, the furious behaviour of rabid dogs which leads to higher tendency of biting other dogs, higher incidence in canines might also be due to their territorial behaviour which resulted into conflicts amongst the dogs when their territory is invaded. Moreover, during mating season, competition for mating partners resulted in higher incidence of dog-bites besides increased libido in rabid dogs. While, Silva *et al.* (2004) ^[10] reported 17.7 percent incidence of canine rabies in Brazil during 1993-1997, the incidence was less in the developed countries like Canada (Wandeler and Casey, 1993) ^[14] attributable to proper vaccination and awareness about rabies. In bovine, Singh and Sandhu (2007) ^[11] from the Punjab reported similar incidence of 13.48 percent, which is in agreement to the present findings. Bharathy and Gunaseelan (2015) ^[1] reported 10.64 percent incidence in caprine which was slightly higher than the findings in the present study (7.69%), which might be due to high number of reported animals as compared to the present study.

However, the incidence reported from different areas of the world by different authors varied from 3.2 to 29.5 percent (Blancou and Barrat, 1989; Wandeler and Casey, 1993) ^[2, 14]. This might be due to a host of factors *viz.* number of samples tested, varied population, managemental practices, vicinity to forest of the animal population present in different regions of

the world.

Conclusion

This study suggests the diagnostic role of LFA kit in suspected field cases and the procedure for sample collection as well as packing of brain tissue samples for their confirmatory lab diagnosis through DFAT. Dogs being the major transmitter of rabies virus (RABV) to dogs and livestock which posed a risk to human, prompt and accurate diagnosis is of paramount importance to undertake post-exposure measures and thereby control economic loss of farmers. This study indicated that rabies monitoring and surveillance programs need to be strengthened in Assam as in other parts of the country and improve reporting of suspected rabies cases to veterinary authorities, followed by mandatory recording and reporting of rabies cases in the national veterinary database systems. Improving Syndromic surveillance and reporting of cases to appropriate animal and public health officials will be beneficial in evaluations of control programmes. The present study had some limitations and reporting of suspected rabies cases was dependent on contact between farmers and veterinary officials, leaving a higher likelihood of under-reporting of rabies. However, this report is expected to will help the stakeholders and policy makers to develop control programmes for the prevention of rabies in domestic animal populations to a great extent.

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