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Exploring transmission risk and challenges in the diagnosis of bovine rabies: A case report

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ABSTRACT

Rabies is a devastating zoonotic disease affecting all warm-blooded animals and humans. Although dogs are commonly recognized as primary transmitters of rabies in India, ruminants also significantly contribute as hosts within livestock populations. This study aimed to highlight the critical issues surrounding bovine rabies and propose practical strategies for documenting and managing it in resource-limited settings. A 40-day-old female Kangeyam calf presented with symptoms including aggressive behaviour, refusal to eat or drink and frequent bellowing. Clinical examination revealed hypersalivation, erected ears, extended neck, heightened alertness, hyperesthesia to sound, diarrhoea and straining. No external wounds were observed and there were no recent reports of dog bites. However, an epidemiological investigation indicated a semi-intensive rearing system and noted a previous history of dog bites on the farm. Based on history, clinical observation and epidemiological investigation, rabies was suspected. Saliva samples were collected from the suspected calf and its dam and subjected to real-time RT-PCR based on SYBR Green chemistry, confirming the presence of viral RNA in the calf and its absence in the cow. This farm investigation underscores the importance of timely bovine rabies diagnosis for safeguarding veterinarians and animal owners. It underscores the vital role of clinical diagnosis in resource-limited settings, where advanced diagnostic tools are often lacking, emphasizing the need for timely identification of rabies cases through clinical observations.

Keywords: Antemortem clinical diagnosis, Bovine rabies, Calf, Public health significance, SYBR Green real-time RT-PCR

Rabies is one of the most dangerous and deadliest zoonotic disease of all warm-blooded animals and humans with a 100% case fatality rate. Globally, approximately 59,000 human deaths are reported each year, with India contributing one third of this burden; 99% of these cases are due to dog-mediated rabies (Radhakrishnan *et al.* 2020). Though, the WHO recommended, the Fluorescent Antibody Test (FAT) on brain samples as a gold standard test in definitive diagnosis, molecular approaches were highly fruitful in the decision-making process (Manjunathareddy *et al.* 2016). In resource-limited settings, due to the lack of availability of standard laboratories and issues in conducting post-mortem, clinical diagnosis based on antemortem clinical signs index was considered an effective approach against this deadly disease (Tepsumethanon *et al.* 2005, Madhusudana and Sukumaran 2008, Naveenkumar *et al.* 2022).

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Though dogs were considered at the top in rabies transmission and epidemiology, ruminants are also an important host among livestock species. Ruminants are considered "Dead end host" in the transmission of rabies and still, the possibility of risk of transmission through the viral load in the saliva has been studied and revealed the significance of ruminants being a risk for rabies transmission to veterinarians, animal handlers and butchers (Dauda *et al.* 2020, Khalafalla and Ali 2021). While data on canine rabies incidences are reported frequently, very limited studies on the incidence of bovine rabies have been reported in India (Sudarshan 2004, Dandale *et al.* 2012, Bharathy and Gunaseelan 2015, Gill *et al.* 2019). Despite the scarce data available on the status of bovine rabies in India, the original burden is quite high. It may be due to poor reporting and availability of standard laboratories that the laboratory-confirmed data is very less, as suspected food animals are mostly sold rather than proper disposal (Dauda *et al.* 2020, Khalafalla and Ali 2021). In the epidemiological iceberg concept of rabies transmission, the underlying factors and risk accessibility of bovine rabies have to be studied briefly.

With this background discussion, the present study aimed to signify the importance of timely diagnosis of

bovine rabies in ensuring the safety of physicians and animal owners.

MATERIALS AND METHODS

Clinical presentation and medical history: A 40-day old female Kangeyam calf from a farm was presented with the illness of aggressive (Frenzy) behaviour, refusal to eat or drink and frequent loud bellowing over the past 1 day. Based on the history, a clinical and epidemiological farm investigation was performed. The day-wise clinical presentation and medical history of the farm investigation details are mentioned in Table 1. Making decision with farm investigation reports, the calf was suspected for rabies. Following the proper personal protective equipment, intermittent saliva samples were collected from the suspected calf at 1 h intervals (three samples in total) and from its dam, in a tightly closed sterile container. As per protocol (World Health Organisation 2013), the samples were labelled and sent to Rabies Diagnostic Laboratory (ISO/IEC 17025:2017 accredited laboratory for rabies testing in animals) at the Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, at 4°C. In the purview of isolation procedures, the animal was kept isolated under a rabies observation ward to receive the definitive diagnosis. Before receiving the results, the animal handlers and owners were advised to take post-prophylaxis doses and the dam animal was given post-exposure prophylaxis shot at day "0".

In laboratory, the total RNA was extracted from the pooled saliva sample using QIAamp Viral RNA Mini Kit (Qiagen, Germany) and cDNA synthesis was carried out using QuantiTect® Reverse Transcription Kit (Qiagen, Germany). Real-time PCR against the N gene of RABV with 5'-ATGTAACACCYCTACAATG-3' as forward and, 5'-GCAGGGTAYTTRTACTCATA-3'as reverse primers was performed as per earlier published methods (Rupprecht *et al.* 2018). The next morning, the isolated calf died with progressive paralytic signs. Since the owner was not interested in further post-mortem examination, the dead animal was disposed off according to standard protocol (World Health Organisation 2013). The dam was given all the doses of post-exposure prophylaxis (0, 3, 7, 14, 28 and 90 days) and the owner was briefed about the chances of risk, with the dam and the animal being periodically monitored.

RESULTS AND DISCUSSION

Despite concerns about the spread of rabies, the lack of awareness and the presence of abundant non-vaccinated host populations, particularly dogs, pose significant challenges for health officials in curtailing rabies epidemics globally (Naveenkumar *et al.* 2022). In developing countries like India, the policy and rules against rabies are though designed, the implementation steps are being underrated (Radhakrishnan *et al.* 2020). This leads to huge menace in achieving the elimination of dog mediated human rabies death by 2030, a call set by WHO and other global health agencies (Naveenkumar *et al.* 2022). While the role of dog-mediated rabies transmission is well-described, the importance of other hosts, especially ruminant rabies, requires further study, particularly given the limited data available in countries like India (Sudarshan 2004, Dandale *et al.* 2012, Bharathy and Gunaseelan 2015, Gill *et al.* 2019). The underlying factors in association with the transmission of rabies in other livestock species and the occupational risk of veterinarians in ruminant rabies have to be studied to combat this deadly disease (Dauda *et al.* 2020, Khalafalla and Ali 2021). In this context, the farm investigation was conducted and the findings of the risk factors in the ruminant rabies epidemiology were described for better understanding.

During the farm investigation, the affected calf exhibited symptoms without any visible external wounds or history of dog bites. The farm practiced a semi-intensive rearing system with a single cow and calf, along with sheep. Approximately 20 days prior, a sheep had died following a stray dog attack. The sheep had exhibited rabies-like symptoms after being bitten but was treated with local

Table 1. Date-wise clinical presentation and medical history of rabies cases in farm animals

29th September 2023	Sheep on the farm had multiple dog bite wounds, which were treated using local herbal medicines and lime. No efforts were made to seek medical attention for post-dog bite wound management and prophylaxis.
5th October 2023	Sheep exhibited hypersalivation, refused to eat and drink and were found dead on the farm.
17th October 2023	A 40-day-old female Kangeyam calf on the farm showed aggressive behaviour, reluctance to drink or eat and frequent loud bellowing.
18th October 2023	Clinical suspicion of rabies was raised. Appropriate preventive strategies and standard protocols for isolation were implemented. Dam received post-exposure prophylaxis dose on day 0. Intermittent saliva samples from the suspected calf and its dam were sent for intravitam rabies diagnosis.
19 October 2023	Calf tested positive for rabies in intravitam diagnosis; dam tested negative for rabies. The isolated calf died exhibiting progressive paralytic signs.
21st October 2023	Dam received post-exposure prophylaxis on day 3.
25th October 2023	Dam received post-exposure prophylaxis on day 7.
01st November 2023	Dam received post-exposure prophylaxis on day 14.
15th November 2023	Dam received post-exposure prophylaxis on day 28.
16th January 2024	Dam received post-exposure prophylaxis on day 90.

remedies instead of seeking medical attention. This history provided crucial epidemiological context for the suspected rabies case in the calf. Based on history, clinical observation and epidemiological farm investigation, the present calf was suspected to be a rabies case. Since, it was a 40-day old calf, the dam also might have been exposed and in this context, intermittent saliva samples were collected for molecular analysis. Real-time PCR against the N gene results showed positive amplification of with a Ct value of 14 which confirmed the presence of rabies virus in the suspected calf's saliva samples and negative for its dam.

The findings were supported by Suluku *et al*. (2017), Dauda *et al*. (2020), Sharif *et al*. (2021), Bhosale *et al*. (2022) and Naveenkumar *et al.* (2024) who documented the clinical spectrum of various bovine rabies outbreaks. The clinical presumptive diagnosis was by the physician using the antemortem clinical signs as strongly correlated with the earlier findings of similar signs.

The authors had experience in investigating numerous rabies outbreaks and gathering personal information from veterinarians across the state in various clinical settings. This experience revealed that many practitioners rely on antemortem clinical signs for decision-making (presumptive diagnosis) in field situations due to the limited availability of standard laboratories in the country (Naveenkumar *et al.* 2022, 2024). Further, post-mortem of dead animals is warranted to collect the brain samples for definitive diagnosis. During these situations, i.e. from antemortem clinical presumptive diagnosis to death followed by post-mortem and laboratory confirmation takes time for emotional understanding between the physician and animal owners.

Notwithstanding the fact, in countries like India, the increased temperature would be the most favourable for the brain samples being autolyzed quickly and chances of cold chain breaching, thus leading to failure in getting results with FAT and or other tests (Manjunathareddy *et al.* 2016).

In addition to this, frequent salivation is one of the most important clinical presentation in rabies cases irrespective of species, except dumb form (Slathia *et al.* 2023). Generally, in choke condition, animals unable to swallow their saliva experienced a hypersalivation. Veterinarians initiate an emergency protocol by opening the mouth cavity to examine and potentially save the animal, where rabies can also be misdiagnosed and veterinarian will be the most vulnerable for rabies transmission (Delpietro *et al.* 2001, Simani *et al.* 2012, Udupa *et al.* 2021). Despite ruminants being considered dead-end hosts in rabies transmission, saliva contact with eroded mucous membranes remains crucial for inter-animal or animal-to-human transmission of rabies (Wen *et al.* 2006, Blanton *et al.* 2011, Brito *et al.* 2011, Simani *et al.* 2012, Mshelbwala *et al.* 2013, Ramirez-Romero *et al.* 2014, Tekki *et al.* 2014, Ahmad *et al.* 2018, Chao *et al.* 2021). In farm investigation of earlier rabies outbreaks with increased chances of a ruminant-to-ruminant transmission through common feed and water trough, social licking behaviour and close tethering of animals emphasized the need for strict isolation of suspected ruminants (Wen *et al.* 2006, Chao *et al.* 2021). In this report, the suspected calf was isolated immediately to rule out further transmission.

From infected animals, the excretory pattern of rabies was expected before the onset of clinical signs (Bhosale *et al.* 2022). In consideration of the possible risk of being positive for rabies in the calf's dam during milking and other circumstances, the clinical sample from the dam was also collected to get a definitive diagnosis, though the dam was not evincing any signs.

Intravitam diagnosis using saliva was conducted and RT-PCR was found to be a highly sensitive test for antemortem diagnosis (Saengseesom *et al.* 2007, Madhusudana and Sukumaran 2008, Manjunathareddy *et al.* 2016). In this study, viral material was detected in the calf's saliva sample, while the dam's sample tested negative. A clinical presumptive diagnosis with confirmation of molecular test that viral RNA presence in the saliva would serve to arrive at a definitive diagnosis for rabies of suspected animals. However, the absence of viral RNA in the taken samples need not be necessary to exclude rabies, as rabies has intermittent excretion through saliva, a load of virus and maintaining the cold chain for transport would be difficult in countries like India (Saengseesom *et al.* 2007, Manjunathareddy *et al.* 2016). Although WHO recommends FAT as the gold standard for definitive diagnosis, intravitam diagnosis with situation-based decision-making would be appropriate in field investigations. Based on these results, the case was confirmed for rabies and negative for dam, suggesting adoption of proper post-exposure prophylaxis as recommended by the WHO (World Health Organization 2013). In this study, the dam was properly immunized and the possible risk of future occurrence was also explained. The study on possible reasons for being positive for rabies in cattle even after administration of post exposure prophylaxis was supported to decide in the present case scenario (Bhosale *et al.* 2022). Further, they explained the failure of post exposure prophylaxis with the animals that dog bites seen above the neck level. The animal collapsed on day two from the observed clinical signs. The findings were in agreement with earlier workers (Hudson *et al.* 1996, LojkiÄ *et al.* 2013, Bhosale *et al.* 2022), who reported that the death of infected bovines within 48 to 72 h from the rabid clinical signs evinced. Despite the specialist's recommendation to conduct a post-mortem examination, the necropsy was not performed due to the owner's unwillingness. This resulted in the failure to confirm the disease through FAT using brain samples. However, conducting post-mortem examinations of dead animals, especially for rabies, in the field is a cumbersome process, as psychological obstacles from the owner may hinder the process (Nguyen *et al.* 2018).

Due to lack of awareness, the owner did not follow post exposure prophylaxis after a previous dog bite incident on the farm. Effective community-based awareness campaigns are essential to curtail rabies transmission

(Naveenkumar *et al.* 2016, Naveenkumar *et al.* 2022). From an epidemiological perspective, the stray dog attack that happened 27 days back, would be the possible reason for the rabies outbreak and in the suspected calf, as there were no external wounds/ bite marks noticed. Similarly, without bite marks, many of the studies reported rabies transmission (Wen *et al.* 2006, Simani *et al.* 2012, Bawaskar *et al.* 2017, Sharif *et al.* 2021). This could be explained by, either ruminant-to-ruminant transmission via licking, tethering and other managemental factors and or dog bite/ scratches may take part in this case and the healing phase might be faster due to the young age of the calf.

In this study, strict monitoring advice led to the proper administration of post-exposure shots to the owners and dam animals. Due to the social ignorance or lack of awareness of the public, the common man may not be able to understand the magnitude of rabies and they consider that such incidents will affect their prestige and social isolation will be higher in village areas. Despite the awareness provided by the expert, the owners were not able to accept the facts and were ready to sell their suspected animals to another farmer or butcher (Dauda *et al.* 2020, Khalafalla and Ali 2021). Considering the current situation, we must acknowledge the challenges in raising awareness among owners and the potential risk of selling animals for food/meat purposes. Since the risk of slaughtering the rabies suspected animals is quite high, the necessary steps have to be taken for effective awareness creation and to ensure the quality of foods.

The rabies incidence in ruminants, is much higher than suspected and reported, it's necessary to take the initiative in understanding the epidemiology of ruminant rabies (Gill *et al.* 2019). Though the case was a single confirmed case, it does not mean having a single case in a population where the reservoir host or diseased host especially dogs, would serve to have many infected in the region, mostly under-reported (Franco-Molina *et al.* 2021).

Rabies is one of the important zoonotic diseases of animals and humans. Despite the 100% case fatality rates, its a vaccine preventable disease and dogs are the crucial component in countries like India, which account for 99% of transmission of rabies to other animals and humans. Similarly, ruminants are considered to be the next accountable species in livestock, the epidemiological significance has to be studied. In this study, the ruminant rabies case was confirmed by using molecular diagnosis and signifies the need for antemortem clinical diagnosis in limited resource settings to generate more data on ruminant rabies. The occupational risk of rabies to veterinarians, butchers, animal handlers and owners was explained and chances of selling rabies suspected food animals were also documented. In brief, this farm investigation of bovine rabies undermines the importance of bovine rabies and the challenges faced by the physician to document or creation of awareness among the public, thus helping us in devising suitable preventive strategies and the necessity to take appropriate pre-exposure prophylaxis to vulnerable human population.

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