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A Preliminary Study on Bat Lyssavirus in Assam, India

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ABSTRACT

Background: Rabies is a zoonotic disease caused by Lyssavirus of the family Rhabdoviridae, affecting all warm-blooded animals. Although a vast majority of the cases are canine-mediated, many cases of bat-mediated rabies in humans have been documented since the early part of the 20th century. Different lineages of Lyssavirus have been detected in bat populations across Africa, Eurasia and Australia. Although there is no direct evidence of the prevalence of bat Lyssavirus in India thus far, indirect evidences are emerging of late. This has significant public health implications considering the not so infrequent human bat interactions in a country like India. In view of the absence of any systematic surveillance of bat rabies especially in the context of Assam state, a preliminary study was conducted to determine the prevalence of bat lyssavirus in Assam.

Methods: 34 brain samples belonging to nine species of bats were collected from fourteen locations in eight districts of Assam, for detection of bat *Lyssavirus* by Lateral Flow Assay (LFA), Direct Fluorescent Antibody Technique (DFA) and One-step PCR at KVAFSU-CVA Rabies Diagnostic Laboratory, WOAH Rabies Reference Laboratory, Veterinary College, Bengaluru, Karnataka-560 024.

Result: None of the samples were positive for lyssavirus indicating absence of an active lyssaviral infection in the representative bat population under study. However, considering the small sample size mere absence of the lyssavirus antigen among bats in the present study does not preclude the pathogen's presence in bat populations across the state or occurrence in the future, considering the rapidly dynamic state of ecology and environment. Continuous surveillance through One-Health collaboration is essential to monitor the status of bat rabies in India.

Key words: Bat lyssavirus, Rabies, Surveillance.

INTRODUCTION

Rabies is one of the oldest known diseases, having a history of more than 4000 years. A disease affecting the Central Nervous System, rabies is almost invariably fatal once manifested (Bhosale *et al*., 2022). It has been recorded in all the continents except Antarctica although the vast majority of the fatalities are from Asia and Africa (WHO, 2023). Considered as a neglected tropical disease (WHO, 2018), it is responsible for the death of more than 60,000 people worldwide annually (WHO, 2013).

India is endemic for rabies bearing an annual burden of 35 per cent of the world's human mortality most of which is canine-mediated (WHO, 2018; Bharathy and Gunaseelan, 2016). Except for RABV, no other lyssaviruses have been reported from India (WHO, 2013).

Rabies is caused by a bullet-shaped RNA virus called Lyssavirus belonging to the family Rhabdoviridae (Gr. Rhabdos-rod) and genus Lyssavirus (Gr. Lyssa-madness), affecting all warm-blooded vertebrates, including man. For almost two-millennia, rabies was considered to be transmitted almost exclusively by the bite of rabid dogs (McColl *et al*., 2000). However, today it is well established that a number of animals like wolves, foxes, coyotes, jackals, cats, bobcats, lions, mongooses, skunks, badgers, monkeys including bats are equally capable of transmitting the deadly disease (Menezes, 2008). Although rare, airborne and transplantation transmission of rabies has also been documented. About seventeen species of Lyssaviruses have been isolated from various species of mammals worldwide out of which fifteen species have been reported in bats (WHO, 2018). Except for rabies virus (RABV), no other lyssaviruses have been reported from India (WHO, 2013).

In India, atleast 131 species of bats have been documented so far (Saikia *et al*., 2022), out of which the North-eastern region of India boasts the presence of over 80 species. Among these, thirty-three species of bats have been recorded so far from Assam (Boro *et al*., 2018; Saikia, 2023).

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Bats are an indispensable component of ecology and human economics. They are an important agent of plant pollination especially in the tropical and arid regions. Bats may be the primary pollinators for over 1000 species of plants across the tropics many of which are of commercial significance lik mango, guava, banana etc (Raghuram *et al*., 2011; Tremlet *et al*., 2019). One of the most important ecosystem services of insectivorous bats is the control of herbivorous arthropods including pest insects (Kunz *et al*., 2011; Ghanem and Voigt, 2012).

Contrary to the positive roles played by bats in maintaining the health of ecosystem and human economics, they have their fair share of burdens too. Bats are recognized to be a natural reservoir of a large variety of zoonotic viruses, which can potentially cross species barriers to infect humans and other domestic or wild animals (Wang and Cowled, 2015). In numerous communites across Asia and Africa bats have also been frequently hunted and exploited for bush meat, traditional medicine and souvenir. This increased contact with wildlife also enhances the odds of interspecific transmission and bat associated spill over.

To date, more than 80 virus species of different groups have been isolated or detected (Callisher *et al*., 2006). Despite being a carrier with a very high load of various pathogens bats are rarely seen to suffer clinical diseases owing to a unique immune system namely pathogen sensing pattern recognition receptors (PRRs) by expressing anti-viral pro-inflammatory cytokines (Janeway and Medzhitov, 2022). Their cells have been found to contain a number of modifications that allow for effective antiviral immune responses. The evolution of flight in bats appears to have selected for a special set of antiviral immune responses by species-specific adaptor proteins expressing antiviral and pro-inflammatory cytokines that control virus spread, while limiting self-destructive inflammatory responses (Banerjee *et al*., 2020). Such modifications of their immune system possibly allow bats to serve as reservoirs of some of the hazardous viruses, including the *Lyssavirus.*

Modern diagnostic techniques like Direct Fluorescent Antibody Assay (DFA), Direct Rapid Immunohistochemical Test (dRIT), Lateral Flow Assay (LFA) and One-step PCR are efficient tests for the detection of Lyssavirus. The World Organisation for Animal Health (WOAH) recommends DFA as the gold standard test for diagnosis of rabies. One-step PCR is utilized to detect the partial N gene sequence which is common for all Lyssaviruses identified so far (WOAH, 2019).

Till date, fifteen of the seventeen recognised species of lyssaviruses have been isolated from different bat species worldwide (WHO, 2018). In the recent years, isolation of Lyssavirus or detection of rabies neutralising antibodies from bats have been occasionally reported from Asia suggesting that bats are regularly exposed to sylvatic lyssaviral infections. Circulation of Lyssaviruses in bats of Asia have been confirmed by isolation of Aravan, Khujand, Irkut and Gannoruwa bat lyssaviruses (Arguin *et al*., 2002; Jiang *et al*., 2010; Liu *et al*., 2012; Gunawardena *et al*., 2016). From South-east Asia, neutralising antibodies against lyssaviruses were detected in the bat samples from Philippines (Arguin *et al*., 2002), Cambodia (Reynes *et al*., 2004), Bangladesh (Kuzmin *et al*., 2006) and Northern Vietnam (Nguyen *et al*., 2014).

Mani *et al*., (2017) conducted surveillance for rabies virus (RABV) infection in bats in Nagaland, northeastern India, at sites with intense human-bat interfaces during traditional bat harvests. Brain tissues and sera from bats were tested for evidence of infection due to RABV. None of the bat brains tested (n = 164) were positive for viral antigen or viral RNA. However, they detected rabies neutralizing antibodies in 4/78 (5·1%) bat sera tested, suggesting prior exposure to RABV or related lyssaviruses.

For the first time in the South-east Asia Pal *et al.* (1980) reported an active infection of frugivorous flying fox (*Pteropus poliocephalus*) with a virus belonging to the Rhabdovirus group-a bat virus. Negri body-like structures were demonstrated by Sellar's stain and direct immunofluorescence in the brain and salivary gland of the dead bat. The virus was isolated after intracerebral inoculation of homogenate of bat brain, salivary gland or brown fat separately in new-born mice.

Although no bat lyssaviruses have been reported from India so far, this can also be attributed to a lack of systematic surveillance within its geographic boundary.

Assam, a north-eastern land-locked state of India, is well known for its rich biodiversity and unique cultural heritage. However, its fast dwindling forest cover is a cause of concern as it has resulted in more frequent man-animal conflicts (Das *et al*., 2012). In interiors of Assam certain tribal communities traditionally go bat hunting for consumption as a delicacy and folklore medicine. This has also resulted in habitat destruction, reduction of foraging and roosting grounds for bats and also facilitated increased human and domesticated animal contact with wildlife. This is an issue of One-health concern because increasing interactions between the two ecological components with result in spill-over events and flaring up new pandemics (Phelps *et al*., 2019). Moreover, rabies in livestock with no dog/animal bite history has been reported while bat exposure has yet to be investigated as mentioned by Dutta and Isloor, 2019. Under such circumstances it becomes increasingly important to carry out surveillance in areas with intense human-wildlife interactions, through hunting or clearing of forests.

Considering the rise in reports of bat lyssaviral infections from other parts of the globe and absence of proper surveillance on bat rabies in this part of the country, an attempt to study the prevalence of bat lyssavirus in Assam was undertaken for the first time.

MATERIALS AND METHODS

For our study bat samples were collected randomly from 14 different locations in Assam. The sampling protocol for

the present study was approved by Institutional Animal Ethics Committee (IAEC), AAU, Khanapara vide approval No.770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/20-21/847 dated 31.07.2021. The duration of the study was one year (January-December, 2021).

Bat samples of nine different species originating from fourteen different locations in eight districts of Assam were collected randomly. The sampled bats were identified using standard taxonomic protocol and keys (Bates and Harrison, 1997). The sources of bats varied from incidentally grounded, apparently sick/diseased, electrocuted or hunted for consumption by certain ethnic groups (Fig 1 and 2). After collection the entire sample was immediately packed in triple layers as per the OIE protocol and shipped to KVAFSU-CVA Rabies Diagnostic Laboratory, OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, KVAFSU, Hebbal, Bengaluru-560 024, Karnataka.

The samples were processed in a BSL-3 laboratory facility. All the bat brain samples (n=34) were subjected to Lateral Flow Assay (Anigen Rapid, Rabies Ag Test kit manufactured by BioNote, Inc. 22 Samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 445-170, Republic of Korea, TEL: 82-31-211-0516; FAX: 82-31-8003-06181; www.bionote.co.kr), Direct Fluorescent Antibody Assay (DFA) and One-step PCR for detection of lyssavirus as per WOAH (2019).

RESULTS AND DISCUSSION

A total of 34 bat brain samples belonging to nine (9) species (Table 1) were collected from fourteen random locations in eight (8) districts of Assam (Table 1). The distribution of locations in Assam is shown in Fig 3.

None of the bat brain samples (n=34) were positive for lyssavirus by Lateral Flow Assay (LFA), Direct Fluorescent Antibody Assay (DFA) and One-time PCR (Table 2) (Fig 4, 5 and 6).

Fig 1: Bat head (considered offal).

Fig 2: Bat which was unable to fly.

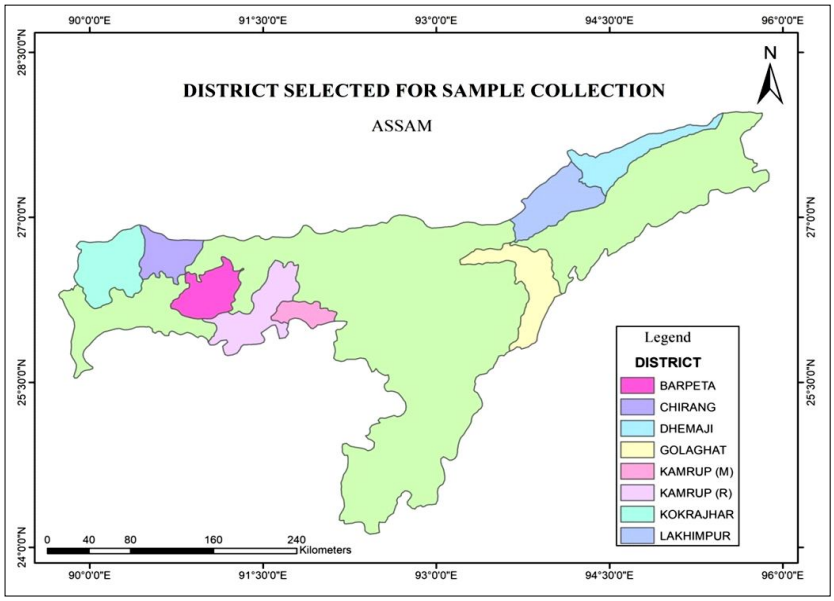


Fig 3: Bat samples collected from eight districts of Assam.

For the first time in Assam state in northeastern India, an attempt to study the prevalence of bat Lyssavirus was made. Our study was unable to detect the presence of bat Lyssavirus in bat populations of the state. However, considering the small sample size and limited geographical coverage, these results cannot be interpreted to signify the absence of bat Lyssaviruses circulating in the native bat population. This is more so considering the fact that serological evidences of past Lyssavirus infection among bats of neighbouring Nagaland has already been reported (Mani *et al*., 2017).

Fig 4: LFA of a bat brain sample.

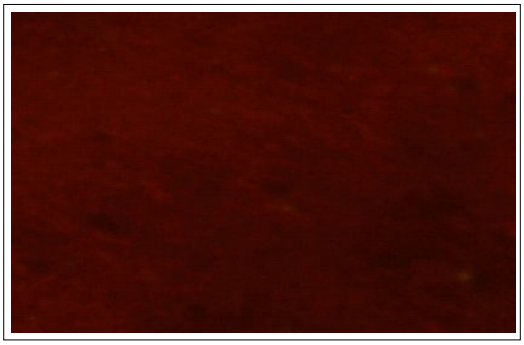
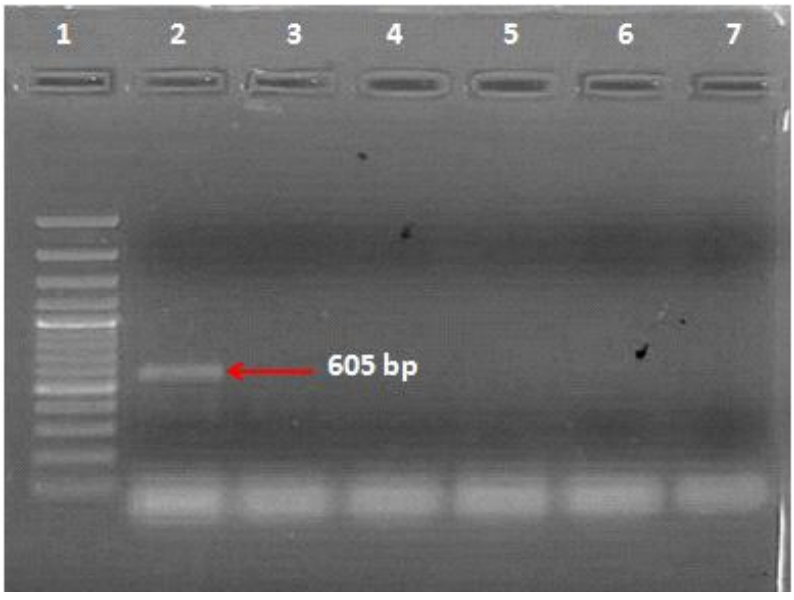


Fig 5: Photomicrograph of DFA of a bat brain sample.

Bats have a particularly strong immune system which allows them to be reservoirs of some of the deadliest viruses (Banerjee *et al*., 2020). Factors such as evolution of flight and co-evolution with their viruses might have shaped the unique immunological responses in bats. In response to invasion of any RNA virus, the immune system of bats mount an anti-viral response but limit the expression of inflammatory cytokines (Banerjee *et al*., 2020).

Hence, despite an exposure to lyssaviral infection, the immune system of bats quickly reacted and expelled the virus from the body. During this process, neutralising antibodies against lyssavirus developed in the exposed bats. Studies for detection of bat lyssaviruses in the samples from Nagaland, Philippines and Cambodia conducted by Mani *et al*. (2017); Arguin *et al*. (2002) and Reynes *et al*. (2004) respectively, detected rabies neutralising antibodies and not the pathogen in them. This is plausible that those bats had an exposure to some lyssaviral infections in the past, but could successfully survive the assault owing to their strong immune responses (Bowen *et al*., 2013).

Although lyssaviral infection was not detected in the present study, systematic surveillance and emergence of



Lane 1: 100 bp DNA ladder.
Lane 2: Positive control (VMC 1575).
Lane 3-7: Bat brain samples: VMC 1891, 1908, 1888, 1900 and 1889.

Fig 6: Amplification of lyssavirus partial N gene (605 bp) in One-step PCR.

Table 1: Bat sample collection from 14 randomly selected locations in eight districts of Assam with geocoordinates.

	Species	Districts											Total
		Baksa	Barpeta	Chirang	Dhemaji	Golaghat	Kamrup (M)	Kokrajhar	Lakhimpur				
Geo-coordinates		91.711998	91.108169	90.524710	94.316120	93.553469	91.719453	89.997095	94.040733				
		-91.720853	-91.108169			-93.555653	-91.826202	-89.974921	-94.315947				
		26.508509	26.301194	26.498099	27.428440	26.665356	26.121603	26.432181	27.216408				
		-26.535135	-26.373806			-26.671771	- 26.509680	-26.445664	-27.427775				
Locations		Goreswar	Barpeta	Theka	Chirang	Dhemaji	Bokakhat	Kohora	Khanapara	Rukminigaon	Sixmile	Gosaigaon	Azad	Joyhing	Koilamari	
	Cynopterus sphinx											1		1		2
	Hipposideros sp							1		1						2
	Lyroderma lyra											8				8
	Pipistrellus coromandra		1	1	1					3						6
	Pipistrellus tenuis	2								1	1	1		1		6
	Pteropus medius					1				1			2			4
	Saccolaimus saccolaimus	1					1									2
	Scotophilus heathii	1										1				2
	Taphozous longimanus	2														2
	Total															34

Table 2: Results of the bat brain samples screened for lyssavirus.

Total number of samples	Test conducted	Positive	Negative
34	LFA	0	34
	DFA	0	34
	One-step PCR	0	34

bat lyssavirus in India is strongly warranted considering the dynamics of epidemiological determinants leading to outbreak of bat-mediated rabies with a potential threat to human and other mammals.

To overcome the challenge of finding reliable information, collaborative partnerships were formed with local community organizations, village key person, field veterinarians, wildlife conservation groups and relevant government agencies. These partnerships facilitated access to valuable insights and existing data on bat-consuming communities in the state. Existing databases, scientific literatures and online resources were examined to gather information on bat-consuming communities. In light of the ongoing COVID-19 pandemic, strict adherence to biosafety protocols was paramount. Researchers followed guidelines established by health authorities and research ethics committees to ensure the safety of all individuals involved. This included wearing appropriate personal protective equipment (PPE), practicing proper hygiene measures and maintaining social distancing during fieldwork and sample collection.

Lack of awareness among the locals regarding zoonoses and infection spill-over compounded by negligence on the importance of systematic human and animal disease surveillance in India can result in catastrophes like bat-mediated rabies. In all the locations of the present study, it was noted that the locals handled the bats bare-handed. Such practices of collection, along with destruction of flora bring humans in close contact with wildlife resulting in spill-over events in no time.

CONCLUSION

A total of thirty-four bat brain samples belonging to nine bat species were collected from eight districts of Assam and were subjected to lateral flow assay (LFA), direct fluorescent antibody assay (DFA) and one-step polymerase chain reaction (one-step PCR) for the detection of lyssavirus in them. All the samples were found to be negative for the presence of lyssavirus, indicating absence of an active lyssaviral infection in any of the bat samples. However, mere absence of the lyssavirus antigen among bats of the present study does not rule out the possibility of such an occurrence in the future, considering the dynamics of ecology and environment. Hence continuous surveillance of the bats is the need of the hour, taking into consideration the importance of rabies as a matter of One health concern.

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Conflict of interest

The authors declare no conflict of interest.

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