

Bluetongue infection rate in mithun (*Bos frontalis*) in the north-eastern upland region of India

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Summary

Studies conducted on 106 mithun at the National Research Centre on Mithun and 66 free-ranging mithun in Nagaland, India, revealed an infection rate with bluetongue virus of 86%, using a commercially available competitive enzyme-linked immunosorbent assay. Animals were grouped according to their age: 36 of 1 to 2 years of age, 50 of 2 to 4 years of age and 86 aged 4 years and over. The highest infection rate (98%) was found in mithun > 4 years old and the lowest (58%) in those 1 to 2 years old. No statistically significant difference was observed between infection rates of males (89%) and females (85%). The infection rate was higher (95%) in free-ranging mithun than in mithun kept under a semi-intensive system (80%). This is the first report of serological evidence of antibodies to bluetongue virus in mithun. The possible role of vectors in the epidemiology of bluetongue virus infection in mithun is discussed briefly.

Keywords

Bluetongue virus – *Bos frontalis* – India – Infection rate – Mithun – Risk factor.

Introduction

The mithun (*Bos frontalis*) is a unique ruminant found in the hill regions of north-east India, Myanmar, Bhutan, Bangladesh, China and Malaysia. The Indian gaur (*Bos gaurus*), also known as the 'Indian bison' or 'gayal', is the wild ancestor of the mithun. The mithun is well adapted to steep jungles at an elevation that ranges from 300 m to 3,000 m above sea level (masl) and plays an important role in the economic, social, cultural and religious life of the tribal people inhabiting the region. The mithun is a semi-wild animal and its breeding selection is not controlled by humans. This is an under-utilised animal and it has great potential for production of quality meat, milk and leather.

The mithun is already being used on a limited basis for production of meat, and the meat obtained is of superior quality. The mithun produces milk with a fat percentage ranging from 11% to 13% (17) and the milk production potential of this animal has been reviewed (2, 16). The mithun prefers a moderate climate, dense forest and steep slopes, which are the general geographical features of the north-eastern region of India. Mithun cannot withstand warm and hot sun, and during the midday period they retire to the deep forest near small ponds, water springs or streams. Mithun can be tamed by offering common salt. The animals are still kept in the forest under a free-grazing system, with minimal human intervention. The National Research Centre on Mithun has been established in Nagaland, India, to develop technologies and techniques

for the scientific management of mithun and to maximise their economic contribution.

Bluetongue is an arthropod-transmitted viral disease that affects domestic and wild ruminants. It is included in the list of notifiable diseases of the World Organisation for Animal Health (OIE). India has significant populations of domestic and wild ruminants, which are known to be susceptible to infection with bluetongue virus (BTV) (24). Bluetongue is endemic in many parts of the country and clinical disease causing mortality in sheep has been reported from various regions (6, 10, 21). Of the 24 serotypes of BTV recognised internationally, 21 have been reported from India (24). Although many serosurveys (8, 19, 24) have indicated the presence of BTV antibodies in cattle and buffalo in India, the clinical disease has not been observed in these species. However, these animals may play an important role in the maintenance of virus. The absence of a vaccination programme against bluetongue in India implies that the occurrence of BTV antibodies in the sera of ruminants has arisen as a consequence of natural exposure to the virus. The distribution of bluetongue serotypes in India is shown in Table I. There is no information available on BTV infection in mithun, and as far as the authors are aware this is the first report on the infection rate of BTV in mithun. The main objectives of the study were:

- to determine the infection rate of BTV in mithun in India
- to analyse the associations between the infection rate and age, sex and system of management.

Materials and methods

Study location

This study was conducted in Nagaland (India) on mithun farms of the National Research Centre on Mithun, where mithuns from four mithun-rearing states are kept, and on mithun found in their natural habitat (free-ranging system). The mithun-rearing areas of India are shown in Figure 1. The state of Nagaland is located in the extreme north-east of the country and lies between latitudes 25° 10' and 27° 4' N and longitudes 93° 15' and 95° 15' E. The altitude varies from 194 masl to 3,826 masl. Average annual rainfall in the state is 2,000 mm. The mean temperature in summer varies from 15°C to 30°C and the winter temperature ranges from 4°C to 25°C.

Animals and management practices

One hundred and seventy-two mithun were used for this study (they are usually only found in the deep forest and are not readily available). Of these, 106 were from two farms that kept mithun in a semi-intensive system and 66 were free-ranging animals. The 172 animals consisted of 54 males and 118 females. The distribution of animals in both systems of management according to their age is shown in Table II. The criteria for inclusion of a particular age group of animals in the study was the same for both systems of management and was based on the availability of animals within that particular age group at the time of

Table I
Distribution of bluetongue virus serotypes in India

State	Species	Virus isolation	Serotype		Reference
				Serology	
Tamil Nadu	Sheep	3, 16, 23	1, 4 to 7, 11, 12, 14 to 17, 19, 20		Janakiraman <i>et al.</i> (9)
Andhra Pradesh	Sheep	2	4, 12 to 14, 17 to 19		Sreenivasulu and Subba Rao (22)
	Cattle	–	6, 12		
Karnataka	Sheep	23	1, 2, 12, 16, 17, 20		Mehrotra and Shukla (13)
	Cattle	–	1, 14, 16		
Maharashtra	Sheep	1, 2 to 4, 8, 9, 16, 18	–		Kulkarni and Kulkarni (11) Deshmukh and Gujar (4); Mehrotra <i>et al.</i> (15)
	Gujarat	Buffalo	–	1, 15, 17	Chauhan <i>et al.</i> (3)
	Cattle	–	2, 12, 20		
Madhya Pradesh	Sheep	18	–		Mehrotra <i>et al.</i> (14); Mehrotra <i>et al.</i> (15)
Uttar Pradesh	Sheep	9, 18, 23	–		Mehrotra <i>et al.</i> (14); Mehrotra <i>et al.</i> (15)
Haryana	Sheep	1, 4	14		Mahajan <i>et al.</i> (12)
	Cattle	–	1, 2, 8, 12, 16		Jain <i>et al.</i> (8)
Himachal Pradesh	Sheep	3, 9, 16, 17	4		Sreenivasulu <i>et al.</i> (24)
Jammu and Kashmir	Sheep	18	–		Mehrotra <i>et al.</i> (14); Mehrotra <i>et al.</i> (15)

Source: Sreenivasulu *et al.* (24)

Table II
Distribution of mithun in India according to age and management system

Management system	Number of animals	Age group	Number of animals	
			Tested	Positive
Semi-intensive system				
Farm 1	57	1 to 2 years	12	6
		2 to 4 years	18	13
		> 4 years	27	25
Farm 2	49	1 to 2 years	11	5
		2 to 4 years	12	10
		> 4 years	26	26
Free-ranging system	66	1 to 2 years	13	10
		2 to 4 years	20	20
		> 4 years	33	33
Total	172		172	148

sample collection. Animals were categorised into three age groups: 1 to 2 years old, 2 to 4 years old and 4 years old and above. Only animals of known age were included in the study. Each animal included in the study was identified by a unique identification number. The animals on the farms were kept under a semi-intensive system of management and were allowed to graze in the forest areas of the farms that had been specially developed for grazing mithun. During the night the mithun were tethered in sheds. In addition to sufficient green fodder, their daily food rations normally included a concentrate mixture, administered according to the body weight of the animal.

Given that mithun are very fond of common salt (sodium chloride) and that even a ferocious mithun can easily be tamed by offering common salt, mithun farmers routinely offer common salt to bring free-ranging mithuns under their control. Each individual mithun rearer keeps all relevant information about their mithun herd, including the total number of animals, identification numbers, number of pregnant animals and calving dates. Mithun farmers traditionally bring pregnant mithun to the vicinity of their house and provide preparturient care about one week before the expected date of calving, and the animals are sent back to the forests one week after calving. The food rations of free-ranging mithun mainly consist of forest leaves, young plants and grasses.

The sera collected from all animals were separated, numbered and stored at -20°C until testing.

Serological examination

Sera were analysed for antibodies to BTV by a commercially available competitive enzyme-linked immunosorbent assay (cELISA) (VMRD Inc., Pullman, USA) that uses monoclonal antibodies. The test is based on the detection of antibodies specific to the group-specific structural protein 7 (VP7) of BTV. The test sensitivity and specificity reported by VMRD for the BTV cELISA are 100% and 99%, respectively. The test was carried out according to the manufacturer’s instructions. The true infection rate was estimated from the apparent infection rate using the method of Noordhuizen *et al.* (18).

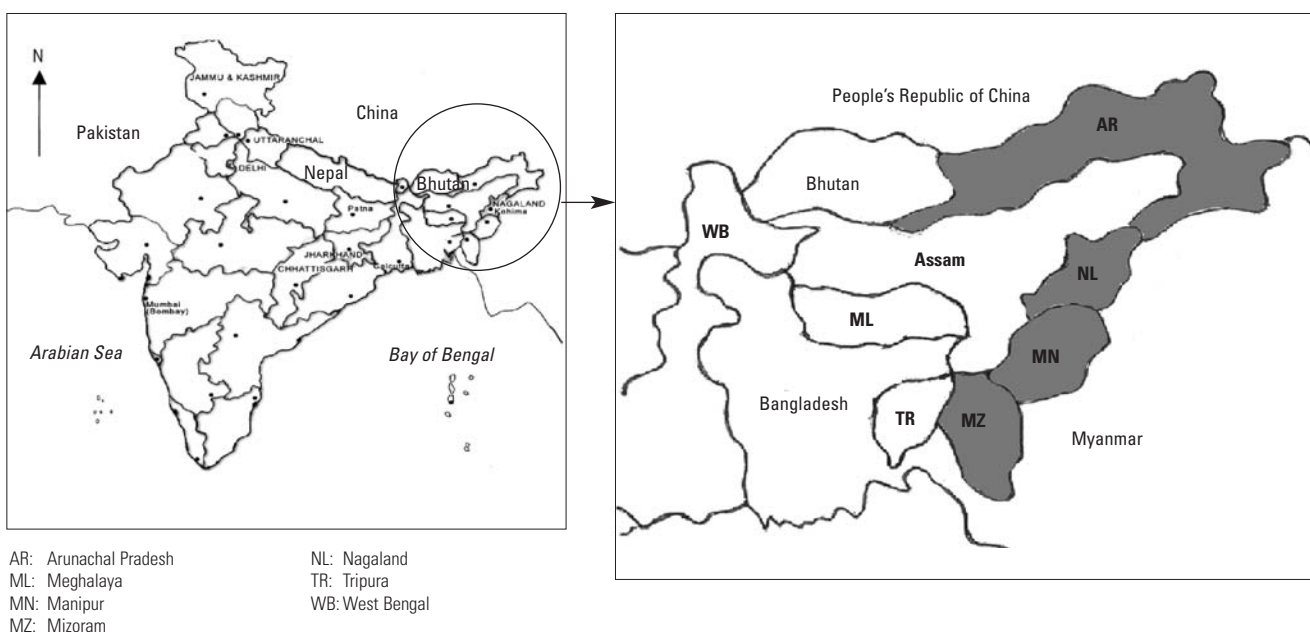


Fig. 1
Mithun-rearing areas of India

Grey shading indicates mithun-rearing areas; some neighbouring countries are also shown

Table III**Bluetongue virus infection rate in mithun in India according to age, gender and system of management**

Results of univariate analysis presented as odds ratio (OR) and 95% confidence interval (CI)

Factor	Number of animals		Percentage prevalence (95% CI)	OR	95% CI	P-value
	Tested	Positive				
Age						
1 to 2 years	36	21	58 (42.1 to 72.9)	1.00		< 0.001
2 to 4 years	50	43	86 (73.8 to 93.0)	4.38	1.5 to 12.3	
> 4 years	86	84	98 (91.9 to 99.3)	5.47	2.5 to 11.8	
Gender						
Male	54	48	89 (77.7 to 94.7)	1.00		0.47
Female	118	100	85 (77.1 to 90.1)	1.44	0.5 to 3.8	
Management system						
Semi-intensive	106	85	80 (71.6 to 86.6)	1.00		0.01
Free-ranging	66	63	95 (87.4 to 98.4)	5.18	1.4 to 18.1	
Total	172	148	86 (80.7 to 90.4)			

Data analysis

The chi-square (χ^2) test was used to analyse the influence of age, sex and system of management as independent categorical variables on the BTV infection rate. Only the variables that were found to be significant at the 5% ($P < 0.05$) level of significance in univariate analysis were included in the multivariable logistic regression model. The results are presented as adjusted odds ratios (OR) with 95% confidence intervals (CI). Analyses were done using SPSS 13.0 software for Windows with a probability (P) value < 0.05 taken to be statistically significant. Confidence limits for proportions were established by the exact binomial test.

Results

The age, gender and farm management characteristics of the examined mithun population are presented in Table III. The overall infection rate of BTV in mithun was found to be 86% (95% CI = 80.7% to 90.4%). According to the sensitivity (100%) and specificity (99%) of the test used the true infection rate was estimated to be 85.9% (95% CI = 80.5% to 90.3%). Univariate analysis of risk factors showed that age and type of management system are associated with BTV infection rate in mithun. There was no significant ($P = 0.47$) influence of gender on BTV infection rate. The infection rate increased significantly ($P < 0.001$) with the increase in age of the animals. The highest infection rate was observed in mithun > 4 years of age with the lowest infection rate recorded in mithun that were between 1 and 2 years old (Table III). A higher infection rate was observed in mithun from the free-ranging system in comparison with that of mithun kept under the semi-intensive system of management ($P = 0.01$)

(Table III). These factors (age and type of management system) were simultaneously analysed in a logistic regression model to determine their relative contributions to BTV infection rate, while adjusting for confounding effects (Table IV). Compared with the respective reference categories, the likelihood of BTV infection was found to be almost five times greater in mithun of 2 to 4 years ($P = 0.008$) and > 4 years ($P < 0.001$). Likewise, the BTV infection rate was found to be five times greater in mithun from the free-ranging system ($P = 0.01$) in comparison with mithun kept under the semi-intensive system of management. The distribution of the ELISA percentage of inhibition for the two subpopulations kept under semi-intensive and free-ranging conditions is shown in Figure 2.

Discussion

The serological results of the study indicate that mithun in India are exposed to BTV infection. Although these findings could not be verified, owing to the lack of other

Table IV**Risk factors for bluetongue virus infection in mithun in India**

Final logistic regression model

Factor	Adjusted odds ratio	95% confidence interval	P-value
Age			
1 to 2 years	1.00		
2 to 4 years	4.63	1.4 to 14.3	0.008
> 4 years	5.44	2.4 to 11.8	< 0.001
Management system			
Semi-intensive	1.00		
Free-ranging	5.43	1.4 to 20.6	0.01

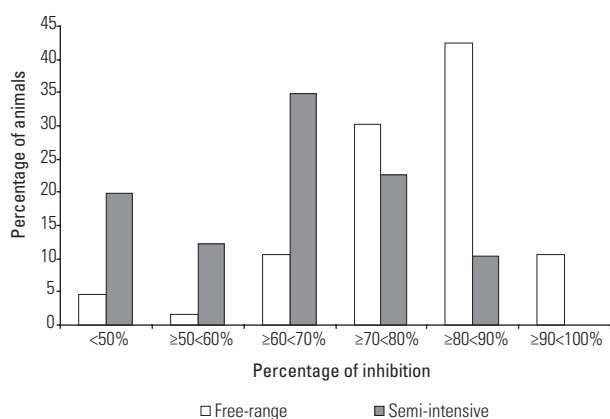


Fig. 2
Distribution of percentage of inhibition in sera from mithun kept under semi-intensive and free-ranging systems of management

studies on BTV infection in mithun, it is possible to compare them with findings from other bovine species such as cattle and buffalo. An increase in infection rate was evident in the older mithun. The present findings are in agreement with the findings of Ward *et al.* (25) and Bhalodiya and Jhala (1) in cattle and Formenty *et al.* (5) in wild herbivores. The increase in BTV infection rate with age could be due to the fact that as animals grow older they are more likely to be exposed to the virus, because they are more likely to come into contact with vectors that are responsible for the transmission of BTV. Detection of BTV antibodies in mithun sera indicates that this species comes into contact with BTV in nature. However, further studies are required to clarify the role of this species in the epidemiology of BTV infection in other domestic and wild ruminants. Sex had no influence on the infection rate of BTV in mithun. This indicates that under the same circumstances, both male and female mithun have an equal chance of acquiring BTV infection. From the present investigation, it was also observed that the infection rate of BTV was greater in mithun kept under free-ranging conditions than in mithun managed under a semi-intensive system. Likewise the percentage of sera with a higher degree of inhibition was found to be greater in samples from animals from the free-ranging system, which could be due to repeated exposure of those animals to BTV vectors, which are abundant in the environment. The high infection rate of BTV in free-ranging mithun could be a result of the abundance of the vector of BTV (*Culicoides* midges) in the deep forest areas, a habitat which is conducive to their propagation. *Culicoides* species are found in a wide variety of habitats, ranging from moist compost or leaf litter to mud at the margins of ponds, lakes, streams or flowing water (7). At least 39 species of *Culicoides* have been reported to occur in India, but only

very few species have been demonstrated to be vectors for BTV, with the principal vectors varying geographically (24). *Culicoides oxystoma* is thought to be a potential vector for BTV in India (20), but precise details of the vector species responsible for BTV transmission in India are lacking. Therefore further studies are required on the prevalence of *Culicoides* species in India, and their role in the transmission of BTV, to determine the status of BTV infection in domestic as well as other wild ruminants in India.

As intensive mithun farming is gradually adopted by the mithun rearers in the north-eastern upland regions of India, the disease status of these animals will greatly influence the strategies formulated for investigation and surveillance of diseases in large ruminants, because of the increased chance of intermingling of this species with other ruminants. The sample size used in the present study was based on the availability of animals in the semi-intensive system on the farms as well as the lower number of mithun available in the free-ranging system. An effort to collect a large number of samples from mithun in the wild is unlikely to succeed because of the inaccessibility of their natural habitat. Despite this shortcoming the authors feel that the study does provide an insight into the BTV infection rate in this rare species of animal.

As mithun live in small herds of 20 to 40 animals, and frequently come into contact with other livestock species while grazing and browsing, there is a possibility that mithun may play an important role in the epidemiology of BTV infection in other domestic ruminants. Surveillance of BTV infection in all domestic as well as wild ruminants in mithun-inhabited areas is thus an important exercise to determine the exact status of this disease in mithun. It is also suggested that attempts should be made to isolate the virus from both wild and domestic ruminants (including mithun) in mithun-inhabited areas and to identify the vector species responsible for the transmission of BTV among these animals.

Conclusion

The present study has revealed the presence of BTV antibodies in mithun in India. Under the prevailing agro-climatic conditions in India the highest BTV infection rate was observed in mithun of > 4 years of age and in those found under free-ranging conditions.



Taux d'infection par la fièvre catarrhale du mouton chez le gayal (*Bos frontalis*) dans la région des hauts plateaux en Inde nord-orientale

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Résumé

Une étude sérologique réalisée par dosage immunoenzymatique de compétition à l'aide d'une trousse commerciale, sur 106 gayals du Centre national de recherche sur le gayal ainsi que sur 66 gayals vivant en liberté à Nagaland, Inde, a révélé chez cette espèce un taux d'infection par le virus de la fièvre catarrhale du mouton de 86 %. Les animaux ont été regroupés par tranches d'âge : le premier groupe rassemblait 36 gayals âgés de 1 et de 2 ans, le deuxième, 50 gayals âgés de 2 à 4 ans, et le troisième, 86 gayals âgés de 4 ans ou plus. Le taux d'infection le plus élevé (98 %) a été observé dans le groupe des gayals de 4 ans ou plus, et le plus faible dans le groupe des animaux âgés de 1 et de 2 ans. Aucune différence significative au plan statistique n'a été constatée suivant le sexe des animaux, le taux d'infection étant de 89 % chez les mâles et de 85 % chez les femelles. Le taux d'infection était plus élevé chez les gayals vivant en liberté (95 %) que chez ceux élevés dans un système semi-intensif (80 %). Il s'agit de la première confirmation sérologique de la présence d'anticorps dirigés contre le virus de la fièvre catarrhale du mouton chez le gayal. Les auteurs examinent brièvement le rôle possible des vecteurs dans l'épidémiologie de l'infection par le virus de la fièvre catarrhale du mouton chez le gayal.

Mots-clés

Bos frontalis – Facteur de risque – Gayal – Inde – Taux d'infection – Virus de la fièvre catarrhale du mouton.



Tasa de infección por lengua azul del gayal (*Bos frontalis*) en las tierras altas del noreste de la India

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Resumen

Los autores describen los estudios realizados con 106 gayales del Centro Nacional de Investigación sobre el Gayal y 66 de esos animales en libertad en Nagaland (India), estudios que revelaron una tasa de infección por el virus de la lengua azul del 86%. Para ello se utilizó un ensayo inmunoenzimático (ELISA) de competición que está comercializado. Los animales fueron agrupados por edad: 36 de entre 1 y 2 años, 50 de entre 2 y 4 años y 86 de 4 o más años. La tasa de infección más alta (98%) correspondió a los gayales de más de 4 años, y la más baja (58%) a los de edad comprendida entre 1 y 2 años. No se observó una diferencia estadísticamente significativa entre los machos (89%) y las hembras

(85%). La tasa de infección en los animales que vivían en libertad (95%) resultó más alta que en los gayales mantenidos en régimen de cría semi-intensiva (80%). Esta es la primera vez que se da a conocer una demostración serológica de la presencia de anticuerpos contra la lengua azul en el gayal. Los autores examinan brevemente la posible intervención de vectores en la epidemiología de la infección en estos animales.

Palabras clave

Bos frontalis – Factor de riesgo – Gayal – India – Tasa de infección – Virus de la lengua azul.



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