

Seroprevalence of infectious bovine rhinotracheitis in mithun (*Bos frontalis*) in India

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Summary

A preliminary study on the seroprevalence of infectious bovine rhinotracheitis (IBR) in different strains of mithun maintained at the National Research Centre on Mithun, Nagaland, India, revealed that the overall prevalence of IBR in these mithun was 19%. This paper examines the results of that preliminary study. To the knowledge of the authors, this is the first report on the seroprevalence of IBR in mithun in India.

The highest prevalence of IBR was observed in mithun found in Arunachal Pradesh (38.46%), followed by those found in Mizoram (18.18%) and Nagaland (15.15%). None of the animals from Manipur were found to test positive for antibodies against IBR. The sex of the animal had no influence on IBR prevalence. The prevalence was found to be highest (27.03%) in mithun above three years of age and lowest (7.69%) in mithun aged between six months and one year. The prevalence of IBR was found to be 88.9% in breeding mithun bulls. In this paper, the authors briefly discuss the possible roles of feral fauna and domestic livestock in the transmission of this disease to mithun and vice versa. Various measures that may help in the prevention and control of IBR in mithun are also surveyed. However, it should be emphasised that this study is a preliminary one, and the authors are currently engaged in further research.

Keywords

Age – Altitude – *Bos frontalis* – Gaur – Gayal – India – Infectious bovine rhinotracheitis – Mithun – Rainfall – Ruminant – Seroprevalence – Sex – Strain.

Introduction

The mithun (*Bos frontalis*) is a unique ruminant found in the hill regions of northeast India, Myanmar, Bhutan, Bangladesh, the People's Republic of China and Malaysia. The Indian gaur (*Bos gaurus*), also known as the Indian bison, is the wild ancestor of the mithun. In terms of chromosomes, the gaur and the mithun are identical. Like the gaur, the mithun has 27 pairs of acrocentric/telocentric autosomes and one pair of sub-metacentric autosomes, X being sub-metacentric and Y metacentric (5). 'Gayal' is another term used to describe the mithun (5).

There are four distinct strains of mithun:

- the Arunachal strain
- the Manipur strain
- the Mizoram strain
- the Nagaland strain.

These different strains are distinguished by their distinct physical and genetic features (15). The genetic distances among these strains are shown in Table 1.

Table I
Genetic distances among the four strains of mithun found in India

Strain	Arunachal	Manipur	Mizoram	Nagaland
Arunachal	–	0.164	0.181	0.154
Manipur	–	–	0.123	0.143
Mizoram	–	–	–	0.119
Nagaland	–	–	–	–

This prized hill animal of the North-Eastern Hill Region (NEHR) has an important role in the economic, social, cultural and religious life of the local tribal population who inhabit the area. The mithun is used for many purposes (2). As a potential source of meat (7), the mithun is considered to be an efficient converter of forest biomass into valuable beef, with a daily body weight gain of between 324 g and 497 g. The body weight of the adult mithun varies from 400 kg to 600 kg. The mithun also produces superior quality milk (14), with the percentage of fat ranging from 11% to 13%. The milk production potentiality of the mithun has also been reviewed (3). When mithun milk was evaluated for human consumption, it was observed that this milk was higher in total solids, protein and fat content than cow or buffalo milk, but lower in lactose content, in both whole milk and dry matter (13). The mithun is a useful draught and pack animal, due to its surefootedness on the steep hill slopes, and it is also used as a bridal gift (20).

Mithun hybrids with related species, i.e. gaur, taurine cattle and yak, have been reported from the NEHR. When yak are cross-bred with local Zebu cattle (*Bos indicus*), the resulting first hybrid (F_1) females may then be crossed with mithun bulls. The female progeny from such a cross, called jatsamin, yield more milk than Zebu cows, while the males (jatsa) make more powerful draught bullocks. Jatsamin cows may also be intentionally cross-bred with mithun bulls. The male progeny of such crosses are called 'nupsa' and the female are 'nupsamin' (16). Nupsamin are considered superior even to local cattle or yak cows for milk production.

Mithun prefer a moderate climate, dense forest and steep slopes, the general geographical features of this region of India. They are generally found at altitudes ranging from 300 to 3,000 metres (m) above mean sea level (AMSL). Mithun cannot withstand hot sun, particularly at midday, so they retire to the deep forest, near small ponds, water springs or streams. One can tame a mithun by offering common salt. This animal is still kept in a semi-wild condition in the forest under the free grazing system, as there is nobody to look after them. Harnessing the full potential of this animal is still a dream for the resource-poor farmers who are engaged in mithun husbandry. 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. The aim of the Centre is to domesticate this rare species of animal, and to examine their performance under a semi-intensive system of management.

Infectious bovine rhinotracheitis (IBR) is an important emerging viral disease of livestock caused by bovine herpes virus 1 (BHV-1). Serological evidence of IBR has been reported in various species of animals in different parts of the world, for example, the following:

- cattle
- buffalo
- sheep
- goats (12)
- dromedaries (12)
- deer (17)
- elephants (10)
- hippopotami (6).

The isolation of BHV-1 has also been reported (11, 21). In addition, a study has been conducted on the prevalence of antibodies against BHV-1 in European bison (8). However, reports on the prevalence of IBR in mithun or the isolation of BHV-1 from this species in India and elsewhere are largely lacking. The present study appears to be the first report on the seroprevalence of IBR in mithun. The authors examine the seroprevalence of IBR in the four different strains of mithun, as well as the influence of age, sex and climatic factors on the occurrence of the disease. However, it should be mentioned that this study is only a preliminary one. The literature on mithun, with particular reference to their health, is very scarce, and detailed information on mithun diseases is not available from any other country. Thus, the authors are in the process of furthering their research on mithun.

Materials and methods

The location of the study

This study was conducted on the two mithun farms of the National Research Centre on Mithun, in Nagaland, India. The state of Nagaland is located in the extreme northeast and lies between latitude 25°10' and 27°4'N and longitude 93°15' and 95°15'E. Nagaland comprises a narrow strip of hilly land running north-east to south-west, which is located in the northern extension of the Arakan Yona ranges of Myanmar. The altitude of the terrain varies from 194 m to 3,826 m AMSL. The average rainfall in the state is 2,000 mm. The mean temperature in summer varies from 15°C to 30°C, while the mean winter temperature ranges from 4°C to 25°C.

Animals and management practices

A total of 101 mithun, from the mithun farms (located at different altitudes) of the National Research Centre on Mithun, were selected for this study. These 101 animals consisted of 36 males and 65 females, from all four strains.

Farm 1 is located at an altitude of 305 m AMSL, whereas Farm 2 is 2,134 m AMSL. Meteorological data from these two areas are given in Figures 1 and 2. All the animals in the study, with the exception of breeding bulls, were kept under a semi-intensive system of management and allowed to graze in the forest areas of the farm specially developed for grazing mithun. The bulls, however, were kept in a single shed and not allowed to mix with the other animals, except when they were required for mating. There was also no opportunity for animals from the different strains to come into contact with one another, as the individual strains were separated by fencing. Nonetheless, animals from each strain would have come into contact with other domestic animals. There was no contact between the animals of Farm 1 and those of Farm 2. The distance between the two farms is 130 Km.

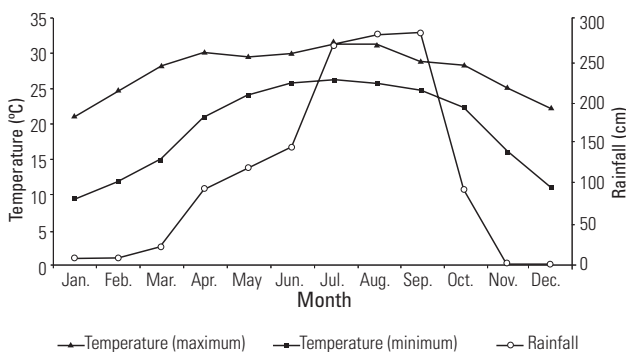


Fig. 1
Meteorological data (maximum and minimum temperature and rainfall) for Farm 1, National Research Centre on Mithun, in Nagaland, India

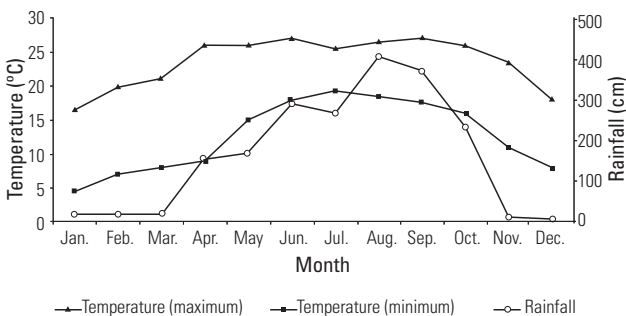


Fig. 2
Meteorological data (maximum and minimum temperature and rainfall) for Farm 2, National Research Centre on Mithun, in Nagaland, India

During the night, the mithun were tethered in their respective sheds. In addition to sufficient green fodder, their daily food rations normally included a concentrate mixture, according to the body weight of the animal. For sampling, the mithun were restrained in a controlling crate and blood was collected by puncturing the jugular vein. The sera were separated, numbered and stored at - 20°C until testing.

Serological test: avidin-biotin enzyme-linked immunosorbent assay

The serum samples from the mithun were subjected to avidin-biotin (A-B) enzyme-linked immunosorbent assay (ELISA), using the method described by Suresh *et al.* (23) to determine the presence of antibodies against BHV-1 in bovines. (The kits were obtained from the Project Directorate on Animal Disease Monitoring and Surveillance, Bangalore, India.) A large number of bovine serum samples were also collected and tested simultaneously with the mithun sera for comparison purposes. The detailed results are shown in Table II.

In the earlier studies of the authors, it was observed that the mithun serum immunologically cross-reacted with antibodies raised against bovine serum. Thus, the authors concluded that mithun immunoglobulin G (IgG) is highly similar to bovine IgG, if not identical. A cut-off value of 28% percentage positivity (PP) was determined for mithun serum and used in this study. All the relevant controls, including cell controls, serum control, conjugate controls, positive and negative controls were kept during the test.

Only the A-B ELISA kit for the detection of BHV-1 is available in India. Although blocking or competitive ELISAs and virus neutralisation tests are highly sensitive, these kits are not available. In addition, the Project Directorate on Animal Disease Monitoring and Surveillance, in Bangalore, India, is the only reference laboratory available to confirm findings on BHV-1 antibodies in mithun.

In brief, microtitre plates were coated with a BHV-1 antigen

Table II
A comparison of cattle and mithun samples testing positive for the presence of antibodies against infectious bovine rhinotracheitis in three northeastern states of India

State	Number of animals testing positive/animals tested		
	Cattle	Mithun	Total
Meghalaya	17/220 (8%)*	—	17/220 (8%)
Mizoram	40/77 (52%)	—	40/77 (52%)
Nagaland	—	19/101 (19%)	19/101 (19%)
Total	57/297 (19%)	19/101 (19%)	76/398 (19%)

*All percentages are rounded up to the nearest whole number

(50 µl per well, 1:50 dilution, i.e. 20 µl per ml of phosphate-buffered saline [pH 7.4]). The antigen-coated plates were incubated at 4°C overnight and then kept at room temperature for 30 min. The plates were subsequently washed five times with washing buffer (phosphate-buffered saline). Fifty microlitres of test and control sera (1:100 dilution in blocking buffer) were then added to the wells of the microtitre plates, in duplicate.

The microtitre plates were incubated at 37°C over a rotary shaker for 1 h and washed, as above. Then biotin-antibovine-IgG (1:25 dilution, i.e. 40 µl per ml of blocking buffer) was added and incubated at 37°C for 1 h. After washing the microtitre plates, avidin-horseradish peroxidase (1:25 dilution, i.e. 40 µl per ml of blocking buffer) was added and incubated at 37°C for 30 min. The plate was washed again and 50 µl of *o*-phenylenediamine dihydrochloride (4 µl of 30% H₂O₂ per ml of chromogen) were added and the plate was incubated at room temperature for 10 min. The reaction was stopped by adding 50 µl of 1 molar H₂SO₄. The plate was read at 492 nm and the optical density (OD) value (absorbance) was recorded.

The PP value was calculated using the following formula:

$$PP = \frac{\text{OD value of test well/control well}}{\text{median OD of strong control well}} \times 100$$

A value greater than 28% in the test well was considered positive.

The accepted OD range of strong positive lies between 0.600 and 1.200.

The PP value of control wells must fall within the ranges depicted in Table III.

Statistical analysis

The data were analysed using the chi square (χ^2) test, as described by Snedecor and Cochran (22).

Results

From 101 serum samples tested by A-B ELISA for the presence of antibodies against IBR, 19 were found to be positive, giving a prevalence rate of 19%.

The prevalence of IBR per strain is presented in Table IV. Statistical analysis showed a significant difference ($P < 0.01$) in the prevalence of IBR among the four strains of mithun. The highest prevalence was observed in Arunachal mithun (38.46%), followed by the Mizoram strain (18.18%) and Nagaland mithun (15.15%). None of

the Manipur mithun were found to be positive for the presence of antibodies against IBR.

Although the prevalence of IBR was higher in males (25%), than females (15.38%), this difference was not statistically significant (Table V).

The prevalence of IBR was highest (27.03%) in mithun above three years of age, followed by mithun between one and three years old (18.42%) and mithun aged six months to one year (7.69%). Statistical analysis showed a significant difference ($P < 0.05$) in the prevalence of IBR among these three age groups (Table VI). The prevalence of IBR was highest in mithun breeding bulls (in 88.9%, or eight out of nine bulls). The results of the nine breeding bulls are incorporated into Tables IV, V, VI and VII. However, these breeding bulls were kept in an individual

Table III
The percentage positivity values of control wells required in the avidin-biotin enzyme-linked immunosorbent assay for detecting infectious bovine rhinotracheitis

Range of values	Lower limit	Upper limit
Strong positive control	70%	120%
Moderate positive control	35%	55%
Negative control	0%	16%
Conjugate control	0%	10%

Table IV
The prevalence of infectious bovine rhinotracheitis in mithun in India, presented by strain

Strain	Number of animals tested	Number of animals found positive	Prevalence (%)	χ^2 value
Mizoram	22	4	18.18	11.50*
Nagaland	33	5	15.15	
Manipur	20	0	0	
Arunachal	26	10	38.46	

* $P < 0.01$
 χ^2 : chi square

Table V
The prevalence of infectious bovine rhinotracheitis in mithun in India, presented by sex

Sex	Number of animals tested	Number of animals found positive	Prevalence (%)	χ^2 value
Male	36	9	25.00	1.40 NS
Female	65	10	15.38	

χ^2 : chi square
NS: not significant

shed and not allowed to mix with the other animals, except when they were needed for mating.

Statistical analysis showed a significant difference ($P < 0.05$) in the prevalence of IBR between Farms 1 and 2 (Table VII). Farm 1 had 56 animals. However, six calves aged less than six months of age were not screened for IBR and thus were not included in the results given in Table VII. Farm 2 had 59 animals, with eight calves of less than six months. Similarly, these eight calves were not screened for IBR and thus are excluded from the results shown in Table VII.

The prevalence of IBR was found to be higher (28%) on Farm 1, which is located at a higher altitude (2,134 m AMSL) than Farm 2, and has high rainfall. Farm 2 is situated at 305 m AMSL, with low rainfall and a prevalence of 9.80%.

Discussion

The overall prevalence of IBR in mithun was 19%. Although these findings could not be compared with any others, due to the lack of other studies on IBR in mithun, it was possible to compare them with findings on other bovine species, such as cattle and buffaloes. When such comparisons were made, IBR prevalence was found to be lower in mithun (18). This may be due to resistance of this species to this disease. Therefore, further studies involving larger mithun populations should be conducted.

This study revealed that the Arunachal mithun were more susceptible to IBR than the other three strains. The variation in the prevalence of IBR among these four strains may be due to genetic differences. Characterisation of the mithun germ plasm through the randomly amplified polymorphic deoxyribonucleic acid technique, using different primers, has demonstrated the existence of different types of band sharing and band differences, indicating genetic differences among the different strains. Arunachal mithun showed the highest number of genetic differences, in comparison with the three other strains (15). The highest prevalence of IBR in the Arunachal strain might be due to the involvement of a particular gene (or genes) which is responsible for this trait. On the other hand, the lower rate of IBR prevalence found in the Mizoram and Nagaland mithun might also be due to the involvement of a particular gene or genes.

The study also revealed that none of the Manipur mithun tested positive for the presence of antibodies against IBR. Again, this may be due to a genetic factor. However, another reason could be that there were fewer Manipur mithun included in the study.

Table VI
The prevalence of infectious bovine rhinotracheitis in mithun in India, presented by age

Age group	Number of animals tested	Number of animals found positive	Prevalence (%)	χ^2 value
6 months to 1 year	26	2	7.69	8.53*
1 to 3 years	38	7	18.42	
> 3 years	37	10	27.03	

* $P < 0.05$
 χ^2 : chi square

Table VII
The prevalence of infectious bovine rhinotracheitis in mithun on Farms 1 and 2 of the National Research Centre on Mithun, Nagaland, India

Farm	Number of animals tested	Number of animals found positive	Prevalence (%)	χ^2 value
Farm 1	50	14	28.00	5.47*
Farm 2	51	5	9.80	

* $P < 0.05$
 χ^2 : chi square

What is certain is that the genetic factors which may be responsible for disease susceptibility or resistance in different strains of mithun must be investigated if the actual cause of the differences in IBR prevalence among the four strains is to be revealed. The authors recommend that a detailed study be undertaken, involving a large mithun population.

Sex, however, had no influence on the prevalence of IBR in mithun. This indicates that, under the same circumstances, both male and female mithun have an equal chance of acquiring infection with BHV-1.

It was also observed that the prevalence of IBR increased with age, and that the prevalence was highest in mithun more than three years old. Again, this finding could not be compared with those of any other study on mithun. However, the findings of the authors are in agreement with those of other workers studying cattle and buffaloes (1, 9, 24). The increase in the prevalence of IBR with age could be due to the fact that as animals grow older, they are more likely to be exposed to the virus since they are more likely to come into contact with other animals which have recovered from the disease but remain carriers (17). The prevalence of IBR was found to be higher (88.9%) in mithun breeding bulls, a finding which corroborates those of Vilain *et al.* (25) in cattle.

The study revealed that the prevalence of IBR was lower in areas with low rainfall and at low altitude. This finding was in agreement with that of Durham and Sillars (4), who also reported a significantly lower prevalence of IBR in areas which had a low annual rainfall. A higher prevalence of IBR was reported in cattle herds raised in mountainous regions (19).

As mithun live in a small herd of twenty to forty animals, and frequently come into contact with other livestock species while grazing and browsing, there is the potential for diseases to be transmitted from one species to another. Surveillance of IBR in all domestic and sylvatic animals in mithun-inhabited areas is thus an important exercise if one wishes to know the exact status of this disease in mithun. More detailed study, involving larger populations of mithun and other livestock, should be undertaken to reveal the role of mithun in transmitting IBR to other livestock species, and vice versa. Such a study would also aid in understanding the epidemiology of IBR in mithun.

Conclusion

This study was based on animals kept under a semi-intensive system of management. Thus it was not possible to determine the status of IBR in the natural habitat of mithun, which is generally inaccessible. Assessing the status of IBR in mithun in their home environment will be an important step towards preventing and controlling this disease in field conditions.

The size of the samples was based on the availability of the animals on the farms. Again, because of the inaccessibility of their natural habitat, it was not possible to collect serum samples from wild mithun. Despite this shortcoming, the authors feel that this study does provide an insight into the incidence of IBR in this rare species. It is their hope that such insights will ultimately contribute towards formulating successful strategies for the prevention and control of this disease in mithun. ■

No previous studies on IBR in any bovine species in the NEHR of India have thus far been conducted. Thus, there is a pressing need for future studies on the seroprevalence of IBR in other domestic animals, not least for comparison purposes. The authors intend to include this aspect in their subsequent studies, and hope that this first attempt at examining IBR in mithun will prompt other workers to undertake similar studies in other bovine species.

Since the study conducted was a preliminary one, and the principal aim of the authors was to find the influence of age, sex and strain on the seroprevalence of IBR in mithun, they did not examine whether BHV-1 infections cause severe disease in mithun or how often IBR infection occurs sub-clinically in this species. However, the authors do intend to address both these questions in their subsequent research. As research on mithun is in its infancy, an economic evaluation of mithun diseases was not attempted (even basic information on mithun health is lacking). However, work on this rare species progresses, and the authors hope that, in coming years, sufficient information will be generated on all the different aspects of mithun, including information on mithun health.

As this study has found IBR to be prevalent in mithun, there is a distinct possibility that the disease may also occur in clinical or sub-clinical form in this species. No definite conclusion may be drawn without further extensive study. However, it should perhaps be noted that possible preventive and control measures against IBR in mithun may prove a fruitful and economically important area for future study.

Séroprévalence de la rhinotrachéite infectieuse bovine chez le gayal (*Bos frontalis*) en Inde

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Résumé

Une étude préliminaire sur la séroprévalence de la rhinotrachéite infectieuse bovine (RIB) dans différentes lignées zootechniques de gayal élevées au Centre national de recherche sur le gayal, Nagaland, Inde, a révélé que la prévalence globale de la RIB chez le gayal était de 19%. Le présent article examine les résultats de cette étude préliminaire. A la connaissance des auteurs, il s'agit ici du premier rapport sur la séroprévalence de la RIB chez le gayal en Inde.

C'est dans la lignée Arunachal du gayal que l'on a observé la plus forte prévalence de la RIB (38,46%), suivie par la lignée Mizoram (18,18%) et la lignée Nagaland (15,15%). Aucun des animaux du groupe de Manipur ne s'est révélé donner un test positif pour les anticorps anti-RIB. Le sexe des animaux n'avait pas d'influence sur la prévalence de la RIB. La prévalence se trouvait être la plus élevée (27,03%) chez le gayal âgé de plus de trois ans, et la plus faible (7,69%) chez le gayal âgé de six mois à un an. On a trouvé que la prévalence de la RIB était de 88,9 % parmi les reproducteurs mâles.

Dans cet article, les auteurs examinent également brièvement les rôles possibles de la faune sauvage et des troupeaux domestiques dans la transmission de cette maladie au gayal et vice-versa. Diverses mesures susceptibles de favoriser la prévention et la prophylaxie de la RIB chez le gayal sont également passées en revue. Cependant, il faut souligner qu'il ne s'agit ici que d'une étude préliminaire, les auteurs ayant entrepris une recherche plus approfondie.

Mots clés

Age – Altitude – *Bos frontalis* – Gaur – Gayal – Inde – Pluviométrie – Rhinotrachéite infectieuse bovine – Ruminant – Séroprévalence – Sexe.



Seroprevalencia de la rinotraqueítis infecciosa bovina en el gayal (*Bos frontalis*) de la India

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Resumen

Los autores examinan los resultados de un estudio preliminar de la seroprevalencia de la rinotraqueítis infecciosa bovina (RIB) en distintas estirpes de gayal (*Bos frontalis*) presentes en el Centro Nacional de Investigaciones sobre el gayal de Nagaland (India), que puso de manifiesto una prevalencia global del 19%. Por lo que saben los autores, se trata del primer informe sobre la prevalencia de la RIB en el gayal de la India.

Los índices de prevalencia más altos se observaron en la estirpe Arunachal (38,46%), seguida por la Mizoram (18,18%) y la Nagaland (15,15%). Ningún

ejemplar de la estirpe Manipur dio resultado positivo a las pruebas de detección de anticuerpos. El sexo del animal no tenía efecto alguno sobre la prevalencia. Ésta era máxima (27,03%) en los animales de más de tres años y mínima (7,69%) en los de seis meses a un año. En los toros reproductores se observó una prevalencia del 88,9%.

Los autores reflexionan también brevemente sobre la posible intervención de la fauna salvaje y el ganado doméstico en la transmisión de la enfermedad al gayal, y viceversa. Pasan revista asimismo a una serie de medidas que pueden ser útiles para prevenir y controlar la RIB en el gayal. Conviene puntualizar, sin embargo que se trata de un estudio preliminar, que los autores ya han empezado a llevar más lejos.

Palabras clave

Altitud – *Bos frontalis* – Edad – Estirpe – Gaur – Gayal – India – Pluviosidad – Rinotraqueítis infecciosa bovina – Rumiante – Seroprevalencia – Sexo.



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