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Short communication

Isolation of novel variants of infectious bursal disease virus from different outbreaks in Northeast India

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ABSTRACT

Infectious bursal disease virus (IBDV) is a highly infectious disease of young chicken that predominantly affects the immune system. In the present study, we are reporting first comprehensive study of IBDV outbreaks from the Northeastern part of India. Northeast India shares a porous border with four different countries; and as a rule any outbreak in the neighboring countries substantially affects the poultry population in the adjoining states. Nucleotide sequence analysis of the VP2 gene of the IBDV isolates from the Northeastern part of India suggested the extreme virulent nature of the virus. The virulent marker amino acids (A222, I242, Q253, I256 and S299) in the hypervariable region of the Northeastern isolates were found identical with the reported very virulent strains of IBDV. A unique insertion of I/L294V was recorded in all the isolates of the Northeastern India. The study will be useful in understanding the circulating pathotypes of IBDV in India.

1. Short communication

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease of young chicken. IBDV replicates in developing B lymphocytes in the bursa of Fabricius leading to its destruction and bursal inflammation [1]. The IBDV infection can cause immunosuppression and decrease in vaccine response, making chicken susceptible to secondary bacterial infection [2,3]. Variant and classical forms are the two subtypes of IBDV [4]. The classical subtypes have been divided into three pathotypes: attenuated, virulent and very virulent (vv). Two distinct serotypes of IBDV are reported till date. Serotype I contains virulent viruses while serotype 2 are avirulent to chicken [5]. Both the serotypes are reported from domestic and wild birds. IBDV infection in chickens is mostly asymptomatic but the disease is more pronounced in birds of age group of three weeks [6,7]. Moreover, the maternal antibody response has limited role in protecting the birds against very virulent IBDV (vvIBDV) infection [4].

IBDV belongs to the genus *Avibirnavirus* under the family *Birnaviridae*. The genome of IBDV consists of dsRNA, which is divided into segments A and B [8]. The segment A consists of two partially overlapping open reading frames (ORF), the first encodes for VP5 (17kd) which plays a role in viral release while the other encodes for polyprotein (VP243) [9]. The polyprotein VP243 later self cleaved by viral protease VP4 into VP2 (48 kDa), VP3 (33–35 kDa) and VP4 (24 kDa) [3]. The segment B encodes for VP1 (90 kDa), an RNA-dependent RNA polymerase [10]. The VP2 and VP3 form the viral capsid, out of which VP2 is the major contributor of viral neutralizing antibodies and elicits a protective immune response in the host [11]. IBDV has a hyper variable region (HVR) between 206 and 350 amino acid of VP2 protein. This region is known as the major region of IBDV to elucidate neutralizing antibodies response [12]. Due to its antigenicity, HVR region of IBDV strains are used for its characterization. The modulation of amino acid sequence in the HVR region of IBDV leads to emergence of its pathogenic variant that can evade the host immune response [13,14].

Low virulent, intermediate plus strains are used as vaccines against IBDV. The low virulent strains may sometime cause bursal inflammation because of the reversion to the virulent pathotype. In addition, the low virulent strains failed to protect birds against vvIBDV infections [15,16]. In India, IBDV was first reported in 1971, after which a series of outbreaks were reported from different parts of India [17–20]. Currently, IBDV is endemic and a serious problem for the poultry industry in India. Northeastern part of India shares a porous border with China, Bangladesh, Bhutan and Myanmar. The new strains of IBDV are regularly reported from these parts of the

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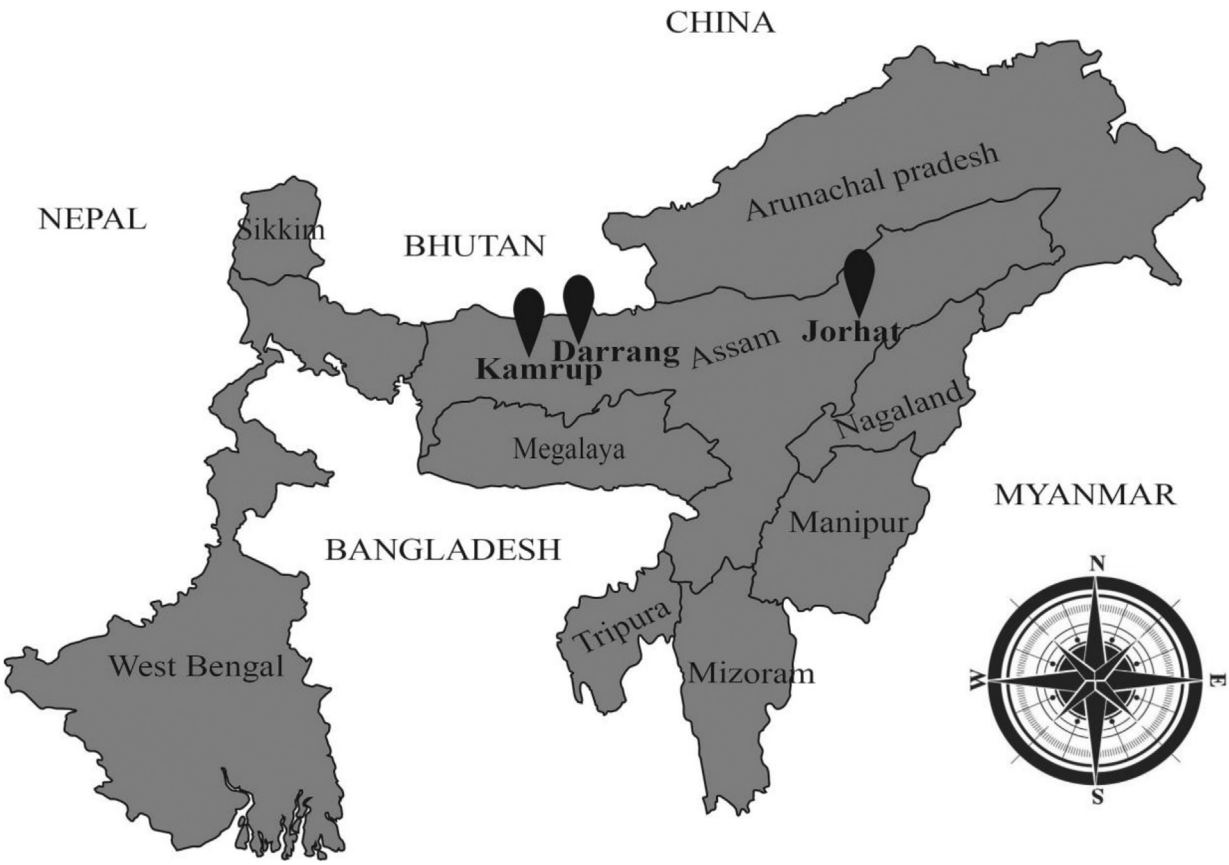

Fig. 1. Geographical location of the infectious bursal disease outbreaks in Northeast India.

world [21–23].

Four different IBDV outbreaks have been reported during 2014–15 in the Northeastern part of India (Fig. 1). The details of the outbreaks are summarized in Table 1. The outbreaks were recorded mainly from broiler chicken of age group 2–3 weeks. Bursal samples were collected both from ailing and dead birds. The tissue samples were fixed with 10% formalin and sectioned 4 μm thick for its staining with hematoxylin and eosin using standard procedure [24]. Total RNA was isolated from the atrophied bursal tissue using TRIzol® reagent (Invitrogen, Grand Island, NY, USA). cDNA was synthesized using PrimeScript™ RT reagent (Clontech Laboratories, CA, USA) using IBDV specific primer (5′CTAGCTAGCATGACA-AACCTGCAAGATC3′). The cDNA was further used for the amplification of VP2 gene of IBDV by gene specific forward and reverse primers (VP2F 5′CTAGCTAGCATGACA-AACCTGCAAGATC3′ and VP2R 5′GGGAATTCTTACCTTAGGGCCCGGATTATG3′) using Phusion® high-fidelity DNA polymerase (New England Biolabs, USA). The PCR products were purified and sequenced by BigDye terminator v 3.1 kit (Applied Biosystems, USA). The sequence data was analyzed using SeqMan software from DNASTAR 5.0 version (DNASTAR Inc., Madison, WI). The nucleotide sequences of all the isolates were submitted to GenBank. Phylogenic analysis was done based on the comparison of the HVR region of the VP2 gene of the isolates with the reference strains from GenBank [25–27]. The sequences were aligned and assembled by MEGA6 software using the neighbor-joining method [28].

Table 1
Details of infectious bursal disease outbreaks form the Northeast India.

Particulars	Sample number			
	51	54	PD4	PD5
Location of outbreak	Sonapur, Kamrup	Mangaldoi, Darrang	Teok, Jorhat	Majuli, Jorhat
Year of collection	2015	2015	2014	2014
Host	Broiler chicken			
Clinical sign	Off fed, Whitish diarrhoea, swollen feces-stained vent			
Post mortem lesion	Nephrosis, haemorrhages on the leg and breast muscle and haemorrhagic atrophied bursa			
Vaccination status	Vaccinated in between 12 and 14 days of age except PD5[a]			
Age of bird at the time disease condition	22–28 days of age in all except PD5[b]			
Flock size	1500	750	1000	500
Mortality	9.33%	8.66%	12.5%	19.20%
System of rearing	Deep litter			

All the samples were collected from Commercial Broiler Farm.
[a] PD5: The sample was collected from Majuli, the river island. The flock was unvaccinated.
[b] Disease recorded at early age i.e. on 18th day onwards.