Veterinary Microbiology 176 (2015) 358–364

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Segment-2 sequencing and cross-neutralization studies confirm existence of a neutralization resistant VP2 phenotypic variant of bluetongue virus serotype 1 in India

Sanchay K. Biswas [a], Karam Chand [a], Waseem Rehman [a], Yella Narasimha Reddy [b], Bimalendu Mondal [a,*]

[a] Division of Virology, Indian Veterinary Research Institute, Mukteswar Campus, District Nainital-263 138, Uttarakhand, India
[b] Department of Veterinary Microbiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad 500030, India

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 29 January 2015
Accepted 30 January 2015

Keywords:
Antigenic similarity
Bluetongue virus serotype 1
Segment-2
Neutralization resistance
VP2 phenotypic variant

ABSTRACT

Segment-2 (seg-2) of a bluetongue virus seropype-1 (BTV-1) isolate WGV104/08/Ind of Indian origin was sequenced and its neutralization behavior was studied to understand the antigenic similarity and relationship with other BTV-1 isolates. Multiple alignments of the coding region of seg-2 of WGV104/08/Ind revealed 97.6–99.0% and 97.2–98.4% similarity with other Indian BTV-1 isolates at nucleotide and deduced amino acid sequence level respectively. Several conservative and non-conservative substitutions were observed on the deduced VP2 amino acid sequence of WGV104/08/Ind. Non-conservative substitution of Lys119Glu on the B-cell epitope and Arg330Gly on the neutralizing epitope of VP2 of this isolate was observed. Using isolate-specific heterologous hyperimmune serum (HIS) the phenotypic antigenic relationship (*r*) was determined between WGV104/08/Ind and other Indian BTV-1 isolates which ranged from 0.092 to 0.208. The relationship score ranged from 0.203 to 0.295 when neutralization behavior of other Indian BTV-1 isolates was studied with the HIS of WGV104/08/Ind. Antigenic similarity (*R*) between WGV104/08/Ind and other Indian BTV-1 isolates was estimated from a reciprocal cross-neutralization study and ranged from 14.70% to 24.80% indicating existence of major subtype antigenic divergence and neutralization resistant behavior of WGV104/08/Ind.

1. Introduction

Bluetongue virus (BTV), the type species of the genus *Orbivirus* within the family *Reoviridae*, is the causative agent of bluetongue, a major disease of ruminants. So far, 26 distinct serotypes of BTV have been identified worldwide (Maan et al., 2011), but a recent paper describes a possible 27th serotype of the virus which is circulating among goats on Corsica (Zientara et al., 2014). BTV particles consist of three concentric protein layers surrounding the 10 segments of linear double-stranded (ds) RNA genome. The dsRNA segments encode seven structural proteins (VP1 to VP7), with an additional four non-structural (NS1 to NS4) proteins observed during infection and replication (Belhouchet et al., 2011). Segment 2 (seg-2; encoding VP2) is the most variable of

* Corresponding author. Present address: Eastern Regional Station, Indian Veterinary Research Institute, 37 Belgachia Road, Kolkata 700037, West Bengal, India. Tel.: +91 33 2558 2965; fax: +91 33 2556 5725.
E-mail address: bimalendu.m@gmail.com (B. Mondal).

http://dx.doi.org/10.1016/j.vetmic.2015.01.023
0378-1135/

the BTV segments and VP2 is solely responsible for serotype determination and it is also the key determinant of neutralizing antibody specificity (Shaw et al., 2013). Genetic diversity among the BTV serogroup is clearly established on the VP2 gene, being the most divergent among different BTV serotypes and nucleotypes (Maan et al., 2007). BTV evolves through a combination of genetic drift and shift and therefore, quasispecies evolution coupled with founder effect and evolutionary selective pressure lead to the development of genetic and phenotypic variants of the virus (Bonneau et al., 2001). Phenotypic variations of BTV are constantly generated in the field and neutralization behavior may vary amongst the field strains of the same serotype.

Bluetongue is enzootic in India and a wide prevalence of the virus has been reported in domestic ruminants by sero-epidemiological studies. Amongst the BTV serotypes circulating in India, BTV-1 is one of the most predominant serotypes as evident by serological surveillance and isolation of a large number of viruses from different parts of the country (Jain et al., 1986; Prasad et al., 1994; Biswas et al., 2010). The present study was conducted to understand seg-2 sequence variability and neutralization behavior of a novel BTV-1 isolate recovered from the southern part of India.

2. Materials and methods

2.1. Virus isolate

The virus used in this study was isolated from a Nellore breed of sheep affected with bluetongue in the West Godavari district of Andhra Pradesh in 2008. Blood samples were collected from sheep showing symptoms of necrotic stomatitis, laminitis, facial edema, nasal discharge and pyrexia. The virus was initially isolated on embryonated chicken egg and subsequently adapted in BHK-21 cells. The isolate (WGV104/08/Ind) was confirmed as BTV-1 and submitted to the Bluetongue virus Repository under ICAR All India Network Program on Bluetongue (AINP-BT). The virus was revived using BHK-21 cultures, seg-2 was sequenced and hyperimmune serum (HIS) was produced. Ten other BTV-1 isolates (Table 1) and their corresponding HIS were also used in neutralization studies.

2.2. RNA isolation, cDNA synthesis and PCR amplification of VP2 gene

Total RNA was extracted from BTV-1 (WGV104/08/Ind)-infected BHK-21 cells (75 cm^2 culture flask) using TRI®-Reagent (Sigma) according to manufacturer's instructions and viral dsRNA was purified by lithium chloride precipitation (Attoui et al., 2000). Purified dsRNA segments were separated by electrophoresis on 1% agarose gel and seg-2 was excised from the gel and extracted using the QIAquick® Gel extraction kit (Qiagen) according to the manufacturer's instructions. Gel extracted seg-2 was used for synthesis of cDNA and PCR amplification of complete coding region in multiple overlapping fragments with different oligonucleotide primer pairs as described by Biswas et al. (2010).

2.3. DNA sequencing, comparison of sequence data and phylogenetic analysis

The amplified overlapping fragments of DNA were sequenced with Big dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., CA, USA) on an automated sequencer following the manufacturer's instructions. The generated nucleotide sequences were confirmed by BLASTN (http://www.ncbi.nlm.nih.gov/) analysis and the overlapping fragments were assembled with the help of the EditSeq program implemented in the DNASTAR program package (DNASTAR Inc., Madison, USA) to generate a complete seg-2 coding sequence of the virus. Nucleotide sequences were aligned with sequences of other BTV VP2 encoding genes (Table 1) using the CLUSTAL W algorithm (Thompson et al., 1994) implemented in the MegAlign of DNASTAR program package and aligned sequence data was used for phylogenetic analyses. The evolutionary history was inferred using the Maximum Likelihood (ML) method from 1000 replicates (Felsenstein, 1985), taken to represent the evolutionary history of the taxa analyzed. The best suitable model for analysis of evolutionary distances used to infer the phylogenetic tree was selected by the model selection program implemented in MEGA version 6.0 software (Tamura et al., 2013).

2.4. Production of hyperimmune serum and neutralization assay

The WGV104/08/Ind isolate was purified from a bulk culture of infected BHK-21 cells by ultracentrifugation through discontinuous sucrose gradients (Mertens et al., 1987) and HIS was produced in rabbits against purified virion following a procedure used earlier for hyperimmunization with a different BTV serotype (Pathak et al., 2008). The HIS was clarified, inactivated (at 56 °C for 30 min), filtered through 0.45 μm membrane filters and stored in multiple aliquots at −80 °C to avoid repeated freezing and thawing. WGV104/08/Ind was neutralized with homologous HIS and also with ten heterologous isolate-specific HIS (prepared earlier in our laboratory) against Indian BTV-1 strains (Table 1). Serum neutralization test (SNT) was performed on BHK-21 cells according to the method described by MacLachlan et al. (1992) in 96-well tissue culture plate using 200 $TCID_{50}$ (fixed) of each of 18 viruses (Table 1) and two-fold serial dilution of individual HIS. Serum neutralization titer was calculated by the method of Reed and Muench (1938) and the mean of anti-log values of three individual runs was considered as mean neutralization titer of the HIS against a particular virus isolate.

2.5. Determination of phenotypic antigenic relationship and antigenic similarity

Phenotypic antigenic relationship (r) was measured as the ratio of the mean heterologous anti-log titer to the mean of the thomologous anti-log titer as described by Archetti and Horsfall (1950). Reciprocal cross-neutralization data of the isolates was used to determine the antigenic similarity of WGV104/08/Ind with other Indian BTV-1 isolates and calculated in terms of 'R' value