Available online at www.sciencedirect.com
SCIENCE DIRECT®
Virus Research 113 (2005) 116–122
Virus Research
www.elsevier.com/locate/virusres

Persistence of bluetongue virus serotype 2 (BTV-2) in the southeast United States

James O. Mecham[a,*], Donna J. Johnson[b]

[a] USDA, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Laboratory, Dept. 3354, 1000 E. University Ave., Laramie, WY 82071, USA
[b] USDA, APHIS, NVSL, Ames, IA 50010, USA

Received 6 January 2005; received in revised form 17 April 2005; accepted 17 April 2005
Available online 8 June 2005

Abstract

The prototype United States (US) strains of bluetongue virus serotype 2 [BTV-2 (OnaA) and BTV-2 (OnaB)] made in Florida in 1982 were compared to a recent BTV-2 (FL99) isolate made in Florida in 1999 to determine if the original strain(s) had persisted or if a new strain of BTV-2 had been re-introduced into the southeast US. Viral RNA and protein electropherotypes, and sequence analysis of five RNA genome segments for these early and later BTV-2 isolates were compared. These comparisons indicated that BTV-2 (OnaB) has persisted in the southeast US since its first isolation in 1982. Sequence analysis of concurrent isolates of BTV-13 (FL99) and BTV-17 (FL99) from the same location in Florida in 1999 provides evidence of genetic reassortment between BTV-2 and other co-circulating serotypes of BTV.
Published by Elsevier B.V.

Keywords: Bluetongue virus (BTV); Epidemiology; Sequence; Phylogeny

1. Introduction

Bluetongue virus (BTV) is an arthropod-borne *Orbivirus* that infects both domestic and wild ruminants (Borden et al., 1971). Bluetongue virus serotype 2 (BTV-2) is the most recent bluetongue serotype to be introduced into the United States (US). Originally described in South Africa, it was first isolated from sentinel cattle and *Culicoides insignis* at Ona, Florida in 1982 (Collisson and Barber, 1985; Gibbs et al., 1983; Greiner et al., 1985). The initial isolates represented two RNA electropherotypes that were designated BTV-2 (OnaA) and BTV-2 (OnaB). The OnaA electropherotype was identical to the South African prototype strain (BTV-2 SA). The OnaA strain was isolated in September and October of 1982 and then no further isolations were made. OnaB isolations were made in late October and November at the same site and at additional sites in Florida the following year (Collisson and Barber, 1985; Collisson et al., 1985). The OnaB strain was subsequently isolated in 1984 and 1985 from cattle in Alabama (Lauerman et al., 1986). A serological survey in the winter of 1986 suggested that BTV-2 was still circulating in cattle in Alabama (Lauerman et al., 1986). No isolations of BTV-2 were reported after 1985, suggesting that this serotype may have disappeared from the US. However, an isolate of BTV-2 from sheep in Florida in late 1999 suggests that this serotype was either reintroduced or has persisted since its first recognition in 1982. To investigate this question, the RNA and protein electropherotypes, and sequences of several genome segments of this isolate were compared to the BTV-2 SA strain, the original BTV-2 OnaA and OnaB strains, and to two 1999 isolates of BTV-13 and BTV-17 from the same area in Florida. Phylogenetic analysis provides evidence that the BTV-2 OnaB strain has persisted in the southeastern US for the past 20 years. It also suggests that mixed infections and reassortment have occurred between BTV-2 and other BTV serotypes in the area.

* Corresponding author. Tel.: +1 307 766 3620; fax: +1 307 766 3500.
E-mail address: jmecham@uwyo.edu (J.O. Mecham).

0168-1702/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.virusres.2005.04.022

2. Materials and methods

2.1. Viruses

The South African BTV-2 (557) SA, the US OnaA and OnaB prototype strains of BTV-2, and US prototype strains of BTV-10, BTV-11, BTV-13 and BTV-17 were obtained from the USDA, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Laboratory, Laramie, WY (Wilson et al., 2000). Virus isolates BTV-2 (FL99), BTV-13 (FL99) and BTV-17 (FL99) were made from blood samples collected from sheep in Lake Placid, Florida, US between October and December, 1999 by the USDA, APHIS, National Veterinary Services Laboratory, Ames, IA.

2.2. RNA and protein electropherotyping

Bluetongue viral proteins and RNA were produced and analyzed as previously described (Thompson et al., 1988). Viral proteins labeled with ^{35}S-methionine were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography; while RNA was analyzed by SDS-PAGE and silver staining.

2.3. RNA sequencing

Viral dsRNA was purified from infected BHK-21 cell cultures using the Qiagen RNA/DNA purification system (Qiagen Inc.). Full-length cDNA templates for sequencing selected genome segments were prepared by reverse transcription and polymerase chain reaction (PCR) using terminal primers based on published sequences for US BTV strains (Table 1). The PCR products were sequenced directly using an ABI 3100 automated sequencer (Applied Biosystems Inc.; Smith et al., 1986).

2.4. Sequence analysis

Sequence data were compiled with SeqMan software (DNA Star Inc., Madison, WI) and analyzed with Vector NTI version 8 software (InforMax Inc., Fredrick, MD). Phylogenetic and molecular evolutionary analyses were done using Molecular Evolutionary Genetics Analysis (MEGA) version 2.1 (Kumar et al., 2001).

3. Results

3.1. RNA and protein electropherotyping

The electrophoretic mobility of viral RNA segments and proteins were analyzed to compare gross relationships between virus strains and isolates. Analysis by electrophoresis of the RNA genome segments isolated from the BTV-2 SA strain, the original US OnaA and OnaB strains of BTV-2, and the 1999 Florida isolates of BTV-2, BTV-13 and BTV-17 is shown in Fig. 1, panels A and B. The South African and OnaA BTV-2 strains showed identical electrophoretic migration of all 10 RNA genome segments. The OnaA and OnaB strains showed major differences in the migration of numerous RNA segments, the most notable of which were S5, S6, S7 and S8. These results are similar to those previously reported (Collisson et al., 1985). Comparison of the prototype OnaA strain with the 1999 BTV-2 Florida isolate also showed major differences in electrophoretic migration of a number of RNA segments. Comparison of the prototype OnaB strain with the 1999 BTV-2 Florida isolate showed fewer differences, with major differences in migration noted for S4 and S5 and perhaps minor differences for S2/3 and S8/9. The electrophoretic migration patterns of RNA genome segments from the BTV-13 and BTV-17 isolates made in Florida at the same time and in the same area as the BTV-2 isolate are also shown in Fig. 1. The majority of RNA genome segments from the 1999 BTV-13 and BTV-17 Florida isolates showed differences in migration compared to the 1999 BTV-2 Florida isolate. However, RNA segments S4, S5 and S6 from the BTV-13 Florida isolate and S7 from the BTV-17 Florida isolate showed identical migration to the corresponding RNA genome segments from the BTV-2 Florida isolate.

Fig. 1, panel C shows electrophoretic migration of the proteins of the above virus strains and isolates. For comparison, the US prototype strains of BTV-10, BTV-11, BTV-13 and BTV-17 were also included in the analysis. Electrophoretic mobility was similar for all the proteins of the BTV-2 isolates, with the exception of NS2 and VP7, encoded by gene segments S8 and S7, respectively (Mertens et al., 1984). These results are similar to the initial report comparing the South African BTV-2 strain and the US prototype BTV-2 OnaA and OnaB strains (Collisson et al., 1985). The major shift to a faster migrating NS2 and a slightly slower migrating VP7 for the OnaB strain, compared to the South African and OnaA strains, was also observed with the 1999 Florida BTV-2 isolate. A noticeable shift in the electrophoretic mobility of the NS2 protein of the 1999 BTV-17 Florida isolate compared to the prototype BTV-17 isolate was also observed.

3.2. Sequence analysis

Since electrophoretic analysis provides only a crude estimation of genetic differences and similarity, RNA genome segments showing major shifts in electrophoretic mobility among the BTV-2 strains and isolates were sequenced for phylogenetic analyses. The S2 RNA segment, which encodes for VP2, was also sequenced to verify virus serotype. Virus strains/isolates, genome segments sequenced and GenBank accession numbers are listed in Table 1. These included BTV-2 (557) SA (South African strain); BTV-2 (OnaA), BTV-2 (OnaB) (prototype US strains); and BTV-2 (FL99), BTV-13 (FL99), BTV-17 (FL99) (1999 Florida isolates). Published sequences for US BTV-10, BTV-11, BTV-13 and BTV-17 strains were also included in the phylogenetic comparisons

Download English Version:

https://daneshyari.com/en/article/9289290

Download Persian Version:

https://daneshyari.com/article/9289290

Daneshyari.com