Virus

Research

Heterogeneity of the L2 gene of field isolates of bluetongue virus serotype 17 from the San Joaquin Valley of California

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Summary

Genome segment 2 (L2) from six field isolates of bluetongue virus (BTV) serotype 17 was sequenced by cycling sequencing after the amplification of the viral cDNA by the polymerase chain reaction. The viruses were isolated from sheep, cattle and a goat in the San Joaquin Valley of California during the years 1981 and 1990. These viruses exhibit divergent patterns of neutralization with BTV 17-specific monoclonal antibodies. The six L2 genes of the BTV 17 field isolates all encode a protein of 955 amino acids. Similarity of the nucleotide sequences of the L2 genes with respect to the prototype strain ranges between 93.8% and 95.1%, whereas the similarity between the field isolates ranges from 96.8% to 99.1%. Although very closely related, the L2 gene of each virus is distinct. Furthermore, mutations in the L2 gene of field isolates of BTV do not consistently follow a linear pattern of accumulation over time. Some amino acid changes in the VP2 protein of field strains were conserved over time, whereas others were not correlated with the year of isolation and some substitutions were unique to individual viruses. The predicted VP2s constitute a group of non-identical, but closely related proteins. Phylogenetic analyses suggest that the viral variants which co-circulate in the San Joaquin Valley could evolve by different evolutionary pathways.

Bluetongue virus; Sequencing; Evolution

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Introduction

A variety of RNA viruses such as influenza virus, foot and mouth disease virus, rhabdoviruses and some alphaviruses are characterized by extensive genetic polymorphism (Domingo et al., 1980; Nichol, 1988; Rico-Hesse et al., 1988; Webster et al., 1992). This genetic diversity is responsible for differences in antigenic properties, tissue tropism and virulence exhibited by individual members of the same virus group or serotype (Wege et al., 1981; Faragher et al., 1988). Even viruses isolated at the same time and from a common geographical region may be genetically heterogenous, as may RNA viruses isolated from a single outbreak of disease or even from a single infected host (Sobrino et al., 1986; Domingo, 1989; Kinnunen et al., 1990).

Bluetongue virus (BTV), the prototype member of the genus Orbivirus in the Reoviridae family, is also genetically heterogenous (Roy, 1989; Heidner et al., 1991). Infection of wild and domestic ruminants occurs throughout much of the tropical and temperate areas of the world, likely wherever competent vector gnats occur. Of the 24 known serotypes of BTV only five, serotypes 2, 10, 11, 13 and 17, have been isolated in the US since 1953. BTV has 10 viral dsRNA genome segments which encode at least 10 viral polypeptides, seven of which are structural and three nonstructural. The structural proteins form a two-layered particle which consists of a core constituted by VP1, VP3, VP4, VP6 and VP7, and an outer capsid formed by VP2 and VP5 which are encoded by genome segments 2 (L2) and 5 (M2) respectively (Roy, 1989). VP2 is responsible for neutralization and serotype specificity of BTV (Roy, 1989), although VP5 might affect neutralization by its conformational influence on VP2 (Cowley and Gorman, 1989; Mertens et al., 1989). The genetic diversity of the BTV serogroup is clearly established (Roy, 1989; Heidner et al., 1991; de Mattos et al., 1992) with gene segments L2 and M2 being the most divergent amongst the prototype viruses of different BTV serotypes (Roy, 1989; Heidner et al., 1991). The genetic diversity that characterizes this serogroup is the result of both genetic drift from individual point mutations, and reassortment of individual viral gene segments (Roy, 1989; de Mattos et al., 1991). The genetic variability of the BTV serogroup is reflected in its antigenic diversity. The antigenic diversity of US BTV prototype and field isolates has previously been characterized with neutralizing monoclonal antibodies (Appleton and Letchworth, 1983; Greider and Schultz, 1990; MacLachlan et al., 1992; Rossitto and MacLachlan, 1992). Phenotypic variation is specially pronounced amongst isolates of serotype 17 (Appleton and Letchworth, 1983; MacLachlan et al., 1992; Rossitto and MacLachlan, 1992).

Phylogenetic studies have helped to clarify the evolution and epidemiology of viral infections such as influenza and Venezuelan equine encephalitis (Yamashita et al., 1988; Weaver et al., 1992). There is less information available regarding the evolution of the BTV serogroup. Evolutionary relationships among prototype and some field strains of different BTV serotypes have been characterized using the complete or partial nucleotide sequences of conserved or variable genes (Gould and Pritchard, 1990, 1991; Kowalik and Li, 1991; Hwang et al., 1992a,b; Yang et

al., 1992). Field isolates of the same BTV serotype obtained from different host species in the same or different years and from the same geographical region have yet to be adequately compared by sequence analysis. The purpose of this study was to further characterize the L2 gene of phenotypically distinct field isolates of BTV serotype 17 isolated in the San Joaquin Valley of California in 1981 and 1990, and to evaluate the evolutionary relationship of these field isolates to each other and with the five US prototype strains of BTV serotypes 2, 10, 11, 13 and 17.

Materials and Methods

Viruses and RNA purification

The L2 genes of six independently isolated strains of BTV 17 obtained from ruminants in the San Joaquin Valley of California during the years 1981 and 1990 were characterized. The origin of each isolate is listed in Table 1. The isolates were processed as described (Heidner et al., 1991; MacLachlan et al., 1992). The viruses were propagated in BHK 21 cells (Heidner et al., 1991) and the viral dsRNA was phenol extracted, ethanol precipitated and lithium chloride purified (de Mattos et al., 1989).

PCR amplification and cycling sequencing

Fifteen synthetic 22-mer and one 19-mer oligonucleotide primers were constructed from the published sequence of the L2 gene of BTV 17 (Ghiashi et al., 1987). These correspond to nucleotide positions (Fig. 1): 1–19, 230–251, 338–359, 661–682, 921–942, 1000–1021, 1262–1283, 1519–1540, 1631–1652, 1922–1943, 1949–1970, 2148–2169, 2316–2337, 2494–2515, 2731–2752, 2902–2923. Different combinations of these primers were used to reverse transcribe the positive and negative strand of viral nucleic acid using mouse Moloney leukemia virus reverse transcriptase (M-MuLV) (Akita et al., 1992). cDNAs were amplified using the polymerase chain reaction as described by Akita et al. (1992). The annealing temperature for the PCR amplification was adapted according to the characteristics of each primer pair used, ranging from 55°C to 59°C. The PCR products were

TABLE 1

Bluetongue virus serotype 17 field isolates studied

Field isolate	Species of origin	Year of isolation	County of isolation	State
17B81	Bovine	1981	Fresno	CA
17C81	Caprine	1981	Kern [a]	CA
17O81	Ovine	1981	Kern [a]	CA
17B90Z	Bovine	1990	Fresno	CA
17O90X	Ovine	1990	Kern	CA
17O90Y	Ovine	1990	San Joaquin	CA

[a] Field isolates obtained from the same farm.

purified using a commercial kit (Gene-Clean, BIO 101). Both strands of the cDNAs were sequenced by cycle sequencing using a commercial kit (f-mol Kit, Promega). The sequencing reaction was done with 500 ng of cDNA, 20 pM of primer, an initial 2 min incubation at 95°C, followed by 30 cycles of 95°C for 1 min, 57°C for 1 min and 70°C for 1 min. A final extension step, using terminal deoxynucleotidyl transferase, was done at 37°C for 60 min. Each sequencing

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        1                                                                   70
BTV17   GTTAAAAGAGTGATCCACCATGGAGGAGTTCGTCATTCCAGTCTATTCAGAAGATGAGATTCCATATGCG
17B81   ...........................................................C...
17C81   ...............................................................
17O81   ...........................................................C...
17B90Z  .........................................................G..C...
17O90X  .........................................................G..C...
17O90Y  .........................................................G..C...

        71                      ▼                                  ▼      140
BTV17   TTACTAAGCAGATACCCTCTGGCGATACAGACAAATGTTAAAATAGAGGATGTTGAAGGAAAACATAATG
17B81   .....G.............T.............................G........
17C81   ...................A.............................G........
17O81   .....G.............T.............................G........
17B90Z  ..G..G.............T........................C.....G........
17O90X  ..G..G.............T........................C.....G........
17O90Y  ..G..G.............T........................C.....G........

        141                       ▼              ▼                         210
BTV17   TTGTAAAGATTCCTGAATCCGATATGATAGATATACCAAAATTAACGATTGTAGAGGCGATGAATTATAA
17B81   .....G.............................G.....GA...............
17C81   .................................G.......G......A.........
17O81   .................................G.......G......A.........
17B90Z  .........................................G......A.........
17O90X  .........................................G......A.........
17O90Y  .........................................G......A.........

        211                                                                280
BTV17   ACCAGCGAGAAACGATGGGATCGTTGTACCTAGATTACTTGATATAACATTACGCGCTTATGATGATAGA
17B81   ...................A..........................T..............
17C81   ...................A..........................T...........G..
17O81   C..................A..........................T..............
17B90Z  ...................A..................C.........T..............
17O90X  ...................A..................C.........T..............
17O90Y  C..................A..................C.........T..............

        281                                           ▼                    350
BTV17   AAATCGACGAAAAGCGCTCGAGGGATAGAGTTCATGACGAACGCTAGATGGATGAAATGGGCCATAGATG
17B81   ...........T.................................
17C81   ...A....A.....T..............................
17O81   ...A....A.....T..............................
17B90Z  ........A..............................T.....
17O90X  ........A..............................T..C..
17O90Y  ........A..............................T.....

        351                                              ▼                 420
BTV17   ATAGAATGGATATCCAACCTCTTAAAGTGACTTTAGATCATTATTGTTCCGTCAACCATCAACTCTTTAA
17B81   .................G......................C.............G........
17C81   .................G......................C.............G........
17O81   .................G......................C.............G........
17B90Z  .................G......................C........T........G........
17O90X  .................G......................C........T........G........
17O90Y  .................G......................C........T........G........
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Fig. 1. Nucleotide sequences of the plus-strand of the L2 gene of the US prototype and field isolates of BTV 17. The start and stop codons are underlined and bases identical to the prototype are represented by dots. The arrows indicate the examples described in the text.

reaction was repeated with PCR products from several different amplifications to control for artifacts.

Computer analyses

The nucleotide sequence of the L2 gene as well as the predicted amino acid sequences of VP2 of each of the six field isolates and the five US BTV prototype strains were analyzed using the Wisconsin Package (Genetic Computer Group Inc., 1991). Phylogenetic analyses were done with the PHYLIP package version 3.41

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        421                                                                    490
BTV17   CTGCGTTGTTAAAGCGAATGCTGCCAACGCTGATACGATCTATTATGATTATTTCCCGCTTGAGGACTAT
17B81   ......C..C.....................C............................A.....A...C..
17C81   ......C..C.....C...........................................A.....A..C..
17081   ......C..C.....C...........................................A.....A...C..
17B90Z  ......C..C.................................................A.....A...C..
17090X  ......C..C.................................................A....A...C..
17090Y  ......C..C.................................................A.....A..C..

        491                                                                    560
BTV17   AAAAAGAGATGTAACCATACAAATCTTGATTTATTGAGAAGTTTAACGAATATGGAGTTGTTCCACGCGT
17B81   ....................C.............................G..T.................
17C81   ....GA.............C.............................G..T.................
17081   ....GA.............C.............................G..T.................
17B90Z  ...................C.............................G..T.................
17090X  ...................C.............................G..T.................
17090Y  ...................C.............................G..T.................

        561                                                                    630
BTV17   TGCAAGGTGCTGCGTACAGTATCAAATCAAGCTACGAATTAGTGGCATACTCCGAAAGAGGAAGCCTGGA
17B81   ............A..............................A............A....T....
17C81   ............A..............................A............A....T....
17081   ............A..............................A............A....T....
17B90Z   ............A..................T...........A...........A...TT....
17090X  ............A..................T...........A...........A...TT....
17090Y  ............A..................T...........A...........A...TT....

        631                    ▼                                               700
BTV17   GGAGACTTATGTGGTAGGACAGCCAAAATGGATACACTTGACTAGAGGAACGCGAATAGGCAATAGTGGA
17B81   ...........C...G...........G..........................................
17C81   ...........C.A.............G..........................................
17081   ...........C.A.............G..........................................
17B90Z  ...........CA........................................................
17090X  ...........CA........................................................
17090Y  ...........CA........................................................

        701                                                                    770
BTV17   TTATCCTATGAACGGTTCATTTCGAGCATGGTCCAGGTAAGCGTAAATGGAAAGATTCCAGACGAGATAG
17B81   ...A..................................G.T....................AG..T.....
17C81   C..C.T...........T..C..A.............G.T....................AG........
17081   C..C.T...........T..C..A.............G.T....................AG........
17B90Z  ...C.T...........T..C..A.............G.TT...................AG........
17090X  ...C.T...........T..C..A.............G.TT...................AG........
17090Y  ...C.T...........T..C..A.............G.TT...................AG........

        771                                                                    840
BTV17   CGAACGAGATCGCGCAACTGAATAGAATAAGGGCAGAGTGGATAACAGCCACATACGATAGAGGCAGGAT
17B81   ........G............................................................
17C81   ........G.....................................G.G..T.................
17081   ........G.....................................G.G..T.................
17B90Z  ........G.....................................G.G..T.................
17090X  ....T..A......................................G.G..T.................
17090Y  ........G.....................................G.G..T.................
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Fig. 1 (continued).

(Felsenstein, 1991). The first 19 nucleotides of the 5' end and the last 22 of the 3' end were not included in the phylogenetic studies because primers were used to amplify the gene. The genetic distance between any two nucleotide sequences was calculated using the DNADIST program based in the Kimura two parameter model (Kimura, 1980). The phylogenetic analyses were carried out utilizing a distance matrix method, based on the least-square method (Fitch and Margoliash, 1967), and implemented in the FITCH program of the PHYLIP package. This procedure makes no assumptions about the relative rates of evolution in all

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        841                                                                   910
BTV17   TAGAGCGCTAGAGCTATGCAGTATCCTTTCCACGATTGGGCGTAAGATGCTAAATACGCATGAGGAGCCG
17B81   .....................A...............................A..G..C........A......
17C81   .....................AG..............................A..G.................
17O81   .....................AG..............................A..G..C........A......
17B90Z  .....................AG.................................G..C........A..A...
17O90X  .....................AG.................................G..C........A..A...
17O90Y  .....................AG.................................G..C........A..A...

        911                                      ▼                            980
BTV17   AAAGATGAAATGGACCTATCGACGAGATTTCAGTTTAAACTTGATGAGAAATTTAACAGAGCGGATTCAG
17B81   ..............T.....A...........C.........C..A..............A...C...
17C81   ..............T.....A..A........C.........A..A..............A...C...
17O81   ..............T.....A..A........C.........C..A..............A...C...
17B90Z  ..............T.....A..A...................C..A..............A...C...
17O90X  ..............T.....A..A........C.........C..A..............A...C...
17O90Y  ..............T.....A..A........C.........C..A..............A...C...

        981                                                                  1050
BTV17   AACATGTTAATATTTTTGGTGTAAGAGGTCCAGCAACAGATGAAGGAAGATTCTACGCTCTGATCGCAAT
17B81   .....................................................T..T........T.....
17C81   .....................................................T...........T.....
17O81   ...............................CC.....G..............T...........T.....
17B90Z  ...............................CC.....G..............T...........T.....
17O90X  ...............................CC.....G..............T...........T.....
17O90Y  .....................................................T...........T.....

        1051                                                                 1120
BTV17   CGCAGCGACAGATACACAAAAGGGCAGAGTGTGGAGAACGAATCCGTATCCATGCTTGCGGGGTGCTTTA
17B81   .........G..............T..............A......................A.........
17C81   .........G..............T..............A......................A.........
17O81   ........................T..............A......................A.........
17B90Z  .........G..............T..............A......................A.........
17O90X  .........G..........T...T..............A......................A.........
17O90Y  .........G..............T..............A......................A.........

        1121                                                                 1190
BTV17   GTTGCAGCTGAGTGTGAATTAGGTGATGTTTACAGTACACTTCGACGTGTGTATACATGGAGTCTAAGGC
17B81   ..........................C...............................G...........
17C81   ..........................C..........G..C...........C.G...............
17O81   ..........................C..........G..C...........C................
17B90Z  ..........................C..........C...........G...............
17O90X  ..........................C..........C...........A...G........T......
17O90Y  ..........................C..........C...........G...............

        1191                                                                 1260
BTV17   CAGAATATGGACAGCACGAGCGACAGTTAGAGAACAATAAATACGTCTTTAATCGTATAAATTTATTTGA
17B81   .G..G........A.............................T.....C......................
17C81   .G..G.........G........................................................
17O81   .G..G.........G........................................................
17B90Z  .G..G.............................................T.....................
17O90X  .G..G.................................................................
17O90Y  .G..G.............................................T.....................
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Fig. 1 (continued).

branches. The L2 gene from epizootic hemorrhagic disease virus (EHDV) serotype 1 was used as an outgroup for the phylogenetic analyses because it is distantly related to the homologous genome segment from the BTV serogroup and it is clearly recognized as a distinct orbivirus serogroup (Gould and Pritchard, 1991; Iwata et al., 1992). Previous studies on orbivirus phylogeny have placed EHDV-1 outside the BTV serogroup (Gould and Pritchard, 1991; Iwata et al., 1992; Roy,

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       1261                                                              1330
BTV17  TTCAAACTTAGCAGTTGGTGACCAGATTATTCATTGGCGTTATGAGGTTAAAGCATCGGCGGAGACGACC
17B81  ............G..C..C........A...................................T
17C81  ............G..C..C........A...................................AT
17081  ............G..C..C........A...................................AT
17B90Z ............G..C..C........A...................................T
17090X ............G..C..C........A...................................T
17090Y ............G..C..C........A...................................T

       1331                                                              1400
BTV17  TATGACAGCGGATATATGTGTCGGCATGAAGCTGAGGAGGATGAACTATTATGTAAAATTAATGAGGACA
17B81  .............C..........................C.........C..........
17C81  ......A......C........A.....G....G............C.........C..........
17081  ......A......C........A.....G....G............C.........C..........
17B90Z .....T.......C..............G................C.........C..........
17090X .....T.......C..............G................C.........C..........
17090Y .....T.......C..............G................C.........C..........

       1401                                                              1470
BTV17  AATATAAAGAAATGCTGGACAGAATGATTCAGGGTGGGTGGGATCAGGAAAGATTTAAACTTCATAACAT
17B81  ..........C.................................................
17C81  ..........C.................................................
17081  ..........C.................................................
17B90Z ..........G...........G.....................................
17090X ..........C.................................................
17090Y ..........C.................................................

       1471            ▼                                                 1540
BTV17  ACTGACGGACCCAAACTTATTGACGATTGATTTTGAAAAAGACGCGTATCTGAATTCACGGTCCGAGTTA
17B81  ............T......................T..............T..............
17C81  ............T......................T..............T..............
17081  ............T......................T..............T..............
17B90Z ............T..T..................T..............T..............
17090X ............T..T..................T..............T..............
17090Y ............T..T................C.......T..............T..............

       1541                                                   ▼          1610
BTV17  GTTTTGCCGGATTATTTCGACAAATGGATCAGTTCACCAATGTTTAACGCGCGCTTAAGAATTACTAAAG
17B81  ........................T...................................
17C81  ...........................................................
17081  ...........................................................
17B90Z ........................................................A...........
17090X ........................................................A...........
17090Y ...........................................................

       1611                                                              1680
BTV17  GGGAGATCGGAACATCAAAAAAGGATGACCCATGGAACAACCGCGCGGTACGTGGATACATTAAGCCCCT
17B81  ................G..........T.................A................T....
17C81  ................G..........T.................A................C...T....
17081  ................G..........T.................A................C...T...C
17B90Z ..........GT...G..........T.................A................C.G.T.T.C
17090X ..........GT...G..........T.................A................C...T...C
17090Y ..........GT...G..........T.................A................C...T.T.C
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Fig. 1 (continued).

1992). Although EHDV was first isolated in 1955, historical data indicate that the virus has been present in the US at least since 1890 (Shope et al., 1960). A bootstrap analysis with 100 replicates was done to assess confidence limits of the branch pattern. The results are presented as a majority rule consensus tree which shows the most frequent branching order (Felsenstein, 1985). A value > 95% is considered significant.

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         1681                                                       1750
BTV17   TGCGGAATCGTTAGATTTTGTTCTCGGGCCCTACTACGATCTGCGGCTTCTATTTTTTGACGAGACGTTG
17B81   ......G.....G...................T...................A.........G.........
17C81   ......G.....G...................T...................A.........G....G.....
17O81   ......G.....G...................T...................A.........G.........
17B90Z  ......G.....G...................T...................A.........G....G.....
17O90X  ......G.....G...................T...................A.........G....G.....
17O90Y  ......G.....G...................T...................A.........G....G.....

         1751                                                       1820
BTV17   AGCTTAAAACAGGAACAGTCCGCGGTTTTTCAATATTTGAGTCAGCTCGATGATTTTCCCGCGCTTACGC
17B81   ................A....................................................
17C81   ................A.........................C.........................
17O81   .........................................C.........................
17B90Z  ................A....................................................
17O90X  ................A....................................................
17O90Y  ................A....................................................

         1821                                                       1890
BTV17   AGCTAAGAGGAGATGCCGTATGCCCACATTCAGGCGGAGCGTTATATACGTTTAGGAAAGTCGCGCTATT
17B81   ..................................................C..................
17C81   ......C...........................................C..................
17O81   ......C...........................................C..................
17B90Z  ......C...........................................C..................
17O90X  ......C...........................................C....C............
17O90Y  ......C...........................................C..................

         1891                                                       1960
BTV17   TTTGATCGGGAATTATGAAAAGTTAAGTCCGGATCTACATGAAGGTATGGAACACCAGAGATATGTGCAT
17B81   ...A.........................................................T..AGC.........
17C81   ...A..................A......................................T..A.C.........
17O81   ...A..................A......................................T..A.C.........
17B90Z  ...A.........................................................T....C.........
17O90X  ...A.........................................................T....C.........
17O90Y  ............................................................T....C.........

         1961                                                       2030
BTV17   CCGTCGACTGGCGGGACGTATCAGAAACGCGTGCTAGAGATGAAGGATTCCTGCCAGCTTACGTGTTTTG
17B81   ..........T.............A...T.....................CC.T..T..A..C.T...C....
17C81   ..........T.................T.....................CC.T..T..A........C..C.
17O81   ..........T.................T.....................CC.T..T..A........C..C.
17B90Z  ..........T.................T.....................CC.T..T..A....T...C....
17O90X  ..........T.................T.....................CC.T..T..A....T...C....
17O90Y  ........C..T................T.....................CC.T..T..A....T...C....

         2031                                                       2100
BTV17   TGATTGATTACATCTTTGAAAAACGTGAACAGCTACGTGATACCAAAGAGGCGAGGTACATTGTGTATCT
17B81   .......................................................C.........
17C81   .......................................................C.........
17O81   .......................................................C.........
17B90Z  ..................G....G.........................A.........G.C......T.
17O90X  .A...............G.........................A...........C......T.
17O90Y  ................C.........G.........................A.........G.C......T.
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Fig. 1 (continued).

Results

L2 nucleotide sequence comparison of BTV 17 field isolates

The six L2 genes of the BTV 17 field isolates all are 2923 nucleotides in length, and each encodes a protein of 955 amino acids. The initiation codons are located at position 20–22 and the termination codons at position 2885–2887 (Fig. 1). Nucleotide sequences of the BTV 17 genomes were compared using the sequence of the prototype strain of BTV 17 as reference (Ghiasi et al., 1987). There are no

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        2101                                       ▼                      2170
BTV17   AATTCAAAGTCTTACTGGGACACAACGGCTGAGTGTTTTAAGGTCGACGTTTCCGAATTTCTTCCAACGA
17B81   ............C...................GA....C.G.AA........C........T.........
17C81   ............C...................GA....C.G.AA........C........T.........
17081   ............C...................GA....C.G.AA.............................
17B90Z  ............C...................GA....C.G.AA.............................
17090X  ............C...............T..GA....C.G.AA........C........T.........
17090Y  ............C...................GA....C.G.AA........C........T......G..

        2171                                                             2240
BTV17   TTGTTGATGCTAAAAGAGATCAAATTTGTGCGCGATCTGAATGTGATTAACTTCCTCCCTCTGATGTTCC
17B81   ..A..A.....G.....................T...............C..T.................
17C81   ..A..A.....G.....................T...T..........C.....................
17081   ..A..A.....G.....................T...T..........C.....................
17B90Z  ..A..A.C.T........G..............T..............C.....................
17090X  ..A..A.C.T........G..............T..............C.....................
17090Y  ..A..A.C.T........G..............T..............C.....................

        2241                                                             2310
BTV17   TTGTTCATGATAACATCTCGTATTGGCATAGACAGTGGTCGATTCCAATGGTACTGTTTGACGATACGAT
17B81   ...................................A...........G...................
17C81   ...C....................C..............A...........................
17081   ........................C..............A...........................
17B90Z  .............T.........C..............A...........................
17090X  .............T.........C..............A...........................
17090Y  .............T.........C..............A...........................

        2311                                                             2380
BTV17   CAAGTTAATACCTGTAGAGGTTGGTGCATATGCGAATAGATTTGGGTTCAAAAGTTTTATGAACTTTACA
17B81   ............C.................................A......................
17C81   ............................................A......................
17081   ............C...........C..G.................A......................
17B90Z  ..G........C...........C..G...CAA...........A...............T......
17090X  ..G........C...........C..G.....A...........A...............T......
17090Y  ..G........C...........C..G.....A...........A...............T......

        2381                                                             2450
BTV17   CGGTTTCACCCTGGTGAGTTGAAGAAAAAACAGATTGCCGAAGATATACATAAAGAGTTTGGTGTGGTCG
17B81   ..................CA.....................G..........G........A.......
17C81   ..................CA.....................G.....G.....G........G.......
17081   ..................CA.....................G.....G.....G........G.......
17B90Z  ..................CA.....................G.....G.....G........A.......
17090X  ..................CA.....................G.....G.....G........A.......
17090Y  ..................CA.....................G.....G.....G........C.......

        2451                                                             2520
BTV17   CTTTCGAATATTACACCAATACAAAAATTTCCCAGGGGAATGTGCATACACCAGTAATGACTACGAAAAT
17B81   ..........................................G...C......................
17C81   ..........................................G...C......................
17081   ..........................................G...C......................
17B90Z  .............T............................G...C.....G................
17090X  .............T............................G...C.....G................
17090Y  .............T............................G...C.....G................
```

Fig. 1 (continued).

deletions or insertions in any of the field isolates with respect to the prototype strain. Many of the mutations of the L2 gene of BTV 17 field isolates are silent. Sequence differences amongst the L2 genes most often are due to transitional mutations. Similarity of the nucleotide sequences of the L2 gene of BTV 17 viruses ranges between 93.8% and 95.1% (Table 2). The L2 genes of the 1981 viruses differ from one another in 25–71 nucleotides and thus are more closely related to each other than they are to the prototype from which they differ in 140–164

```
        2521                                                          2590
BTV17   GGATGTATTGAGAGTACATTTATCTTCTTTATGTGCAGGTCTGGCGGACTCAGTCGTATATACATTACCA
17B81   ...........A.A.......G................G............T..TA...............
17C81   ...........AGA......G.............................T..TA...............
17O81   ...........AGA......G.............................T..TA...............
17B90Z  ...........A.A......G.............................T..TA...............
17O90X  ...........A.A......G.............................T..TA...............
17O90Y  ...........A.A......G.............................T..TA...............

        2591                                                          2660
BTV17   GTTGCGCATCCTAAAAAATGCATCGTTCTAATAATTGTGGGAGACGACAAATTGGAACCGCATACGCGTT
17B81   ..............G...............................T......................
17C81   ..............G...............................T......................
17O81   ..............G...............................T......................
17B90Z  ..............G...............................T......................
17O90X  ..............G...............................T......................
17O90Y  ....T.......G..G..............................T......................

        2661                                                          2730
BTV17   CAGAACAGATAGTTAGTCGGTATAATTACTCACGTAAGCACATTTGTGGAATTGTATCCGTCACTATCGG
17B81   .......A...................................T.C.....................CG....
17C81   .......A...........................................................CG....
17O81   .......A...........................................................CG....
17B90Z  .......A...........................................................CG....
17O90X  .......A.....................................................A....CG....
17O90Y  .......A...........................................................CG....

        2731                                                          2800
BTV17   GCAGAACAGTCAGTTGAGAGTTCATACATCTGGAATTGTAAAACACCGTGTATGCGACAAGTTCATTCTA
17B81   ......T....................C..........T............................
17C81   ......T....................C..........T............................
17O81   ......T....................C..........T............................
17B90Z  ......T....................C..........T............................
17O90X  ......T....................C..........T............................
17O90Y  ......T.............................T............................

        2801                                                          2870
BTV17   AAACACAAGTGCAAGGTGATATTAGTGAGGATGCCGGGGTACGTTTTCGGAAATGATGAATTAATGACGA
17B81   .....................................................................
17C81   ...............................................T.....................
17O81   .............A.......................................................
17B90Z  .....................................................................
17O90X  .....................................................................
17O90Y  .....................................................................

        2871                                              2923
BTV17   AACTATTGAATGTCTAGGTCCTATGACACGGACCGATGGCCTCTTACACTTAC
17B81   ....................................................
17C81   ....................................................
17O81   ....................................................
17B90Z  ....................................................
17O90X  ....................................................
17O90Y   ....................................................
```

Fig. 1 (continued).

TABLE 2

Bluetongue virus 17 field isolates: nucleotide and amino acid sequence comparison

	BTV 17 [a]	17B81 [b]	17C81 [b]	17O81 [b]	17B90Z [b]	17O90X [b]	17O90Y [b]
BTV 17	–	140 [c] (95.1) [d]	159 (94.5)	164 (94.3)	178 (93.8)	181 (93.8)	179 (93.9)
17B81	33 [e] (96.44) [f]	–	69 (97.6)	71 (97.6)	91 (96.9)	90 (96.8)	89 (96.9)
17C81	45 (95.3)	25 (97.4)	–	25 (99.1)	88 (97)	89 (96.9)	85 (97.1)
17O81	42 (95.6)	25 (97.4)	9 (99.6)	–	74 (97.5)	78 (97.3)	76 (97.3)
17B90Z	43 (95.5)	26 (97.3)	28 (97.1)	25 (97.4)	–	28 (99)	26 (99.1)
17O90X	42 (95.6)	24 (97.5)	27 (97.1)	24 (97.5)	8 (99.2)	–	34 (98.8)
17O90Y	47 (95.1)	28 (97.1)	30 (96.9)	27 (97.2)	12 (98.7)	14 (98.5)	–

[a] BTV 17 prototype virus.
[b] BTV 17 field isolates.
Upper section: [c] number of nucleotide changes; [d] percentage of nucleotide sequence homology.
Lower section: [e] number of amino acid changes: [f] percentage of amino acid sequence identity.

nucleotides. Similarly, the L2 genes of 1990 viruses differ from one another in only 26–34 nucleotides whereas they differ from the prototype in 178–181 nucleotides (Table 2). The viruses isolated in 1981 and 1990 also are more closely related to each other than they are to the prototype, however they differ from one another in 74–91 nucleotides despite the fact they were isolated from the same geographical area at an interval of only 9 years (Table 2). Some mutations were unique to a single virus (e.g., nucleotide 91 in 17C81 and 325 in 17O90X), whereas others were common to all field isolates (e.g., positions 180, 958 and 2142). Mutations sometimes were common to all 1981 (e.g., positions 132 and 658) or all 1990 isolates (e.g., positions 403 and 1486), but were unique to the isolates of that year. Some mutations were present in two of the 1981 (e.g., position 167 in 17C81 and 17O81) or 1990 (e.g., position 1599 in 17B90Z and 17O90X) viruses, while the other field strains had the same nucleotide as the prototype. Mutations which are common to all the field isolates suggest an ancestor-descendant relationship but other mutations clearly are not consistent with this hypothesis. Specifically, mutations in the 1981 viruses were not consistently present in the 1990 isolates and some mutations were unique to individual viruses regardless of year of isolation, which indicates that these mutations are not cumulative. Comparison of the nucleotide sequences of the BTV 17 prototype and field isolates indicates that although very closely related, the L2 gene of each virus is distinct. Furthermore, mutations in the L2 gene of field isolates of BTV do not necessarily follow a linear pattern of accumulation over time.

Predicted VP2 amino acid sequence comparison
The deduced amino acid sequence of VP2 of the six field isolates and the BTV 17 prototype strain are shown in Fig. 2. The number of amino acid substitutions in the VP2 of the six field isolates ranges from 33 in 17B81 to 47 for 17O90Y (Table 2). Mutations were randomly distributed throughout VP2, although extensive regions of homology occur at amino acid 1–37, 90–156, 397–436, 604–643 and positions 903–955 where only VP2 from 17C81 has a tryptophan at position 940

instead of the glycine present in all other proteins (Fig. 2). These regions might be conserved because they are critical to maintain the function of VP2.

Some amino acids are unique to the prototype strain (e.g., position 54), or to a particular field isolate (e.g., 17B81: position 895; 17C81: 742; 17B90Z: 552; 17O90X: 351 and 17O90Y: 677). In contrast, specific amino acids were shared by the prototype and all the 1981 field isolates (e.g., positions 535 and 765), or by the

```
        1                                    ▼                  ▼            ▼  70
BTV17   MEEFVIPVYSEDEIPYALLSRYPLAIQTNVKIEDVEGKHNVVKIPESDMIDIPKLTIVEAMNYKPARNDG
17B81   ..........................................R....E..........R.R.............
17C81   ..........................................R..........V...R................
17O81   ..........................................R..........V...R.........N......
17B90Z  ..........................................R................................
17O90X  ..........................................R................................
17O90Y  ..........................................R...........N......

        71                                                                     140
BTV17   IVVPRLLDITLRAYDDRKSTKSARGIEFMTNARWMKWAIDDRMDIQPLKVTLDHYCSVNHQLFNCVVKAN
17B81   ......................................................................
17C81   ...............G.T....................................................
17O81   .................T....................................................
17B90Z  ......................................................................
17O90X  ......................................................................
17O90Y  ......................................................................

        141              ▼                                                    210
BTV17   AANADTIYYDYFPLEDYKKRCNHTNLDLLRSLTNMELFHALQGAAYSIKSSYELVAYSERGSLEETYVVG
17B81   ..............H..............................................N...E......A..
17C81   ..............H.R.............................................N..........AI.
17O81   ..............H.R.............................................N..........AI.
17B90Z  ..............H..............................................N...E......A..
17O90X  ..............H..............................................N...E......A..
17O90Y  ..............H..............................................N...E......A..

        211                                                                    280
BTV17   QPKWIHLTRGTRIGNSGLSYERFISSMVQVSVNGKIPDEIANEIAQLNRIRAEWITATYDRGRIRALELC
17B81   ....................T............I......SV....V.........A..............
17C81   ...................P............I......S.....V..........A..............
17O81   ...................P............I......S.....V..........A..............
17B90Z  ...................P............I......S.....V..........A..............
17O90X  ...................P............I......S...............A..............
17O90Y  ...................P............I......S.....V..........A..............

        281       ▼                                                            350
BTV17   SILSTIGRKMLNTHEEPKDEMDLSTRFQFKLDEKFNRADSEHVNIFGVRGPATDEGRFYALIAIAATDTQ
17B81   N..........I.................................P.........................
17C81   K..........I...........................E......P.........................
17O81   K..........I.................................P.........A...............
17B90Z  K............................................P.........A...............
17O90X  K............................................P.........A...............
17O90Y  K............................................P........................
        ▼
        351                                                                    420
BTV17   KGRVWRTNPYPCLRGALVAAECELGDVYSTLRRVYTWSLRPEYGQHERQLENNKYVFNRINLFDSNLAVG
17B81   .....................................R................................
17C81   .....................................R.........R......................
17O81   .....................................R.........R......................
17B90Z  .....................................R................................
17O90X  M....................................A................................
17O90Y  .....................................R................................
```

Fig. 2. Alignment of the deduced amino acid sequences of the VP2 proteins of the US prototype and field isolates of BTV 17. Amino acids identical to those of the prototype are denoted by a dot. Examples described in the text are indicated by arrows.

prototype and all the 1990 viruses (e.g., positions 38 and 290). Furthermore amino acid substitutions occur which were common to only two of the 1981 isolates (e.g., position 159 in 17C81 and 17O81) or the 1990 isolates (e.g., position 527 in 17B90Z and 17O90X). The same amino acid change was shared by some viruses isolated in different years, while the other isolates had the same amino acid as the prototype (e.g., position 64). The data indicate that some amino acid changes in VP2 of field strains were conserved through time, whereas others were not correlated with the year of isolation and some substitutions were unique to individual viruses. The

```
         421                                                                    490
BTV17    DQIIHWRYEVKASAETTYDSGYMCRHEAEEDELLCKINEDKYKEMLDRMIQGGWDQERFKLHNILTDPNL
17B81    ...............................................D.....................
17C81    ...............N..N........G...............D.....................
17O81    ...............N..N........G...............D.....................
17B90Z   ....................................................................
17O90X   ...........................................D.....................
17O90Y   ...........................................D.....................

         491                          ▼             ▼              ▼▼     560
BTV17    LTIDFEKDAYLNSRSELVLPDYFDKWISSPMFNARLRITKGEIGTSKKDDPWNNRAVRGYIKPLAESLDF
17B81    ...........L......................................................S.......
17C81    ...........L......................................................S.......
17O81    ...........L......................................................SP......
17B90Z   ...........L.......................K.......V................RSP......
17O90X   ...........L.......................K.......V................SP......
17O90Y   .....Q.....L.................................V................SP......

         561         ▼                                                          630
BTV17    VLGPYYDLRLLFFDETLSLKQEQSAVFQYLSQLDDFPALTQLRGDAVCPHSGGALYTFRKVALFLIGNYE
17B81    ............G.......................................................
17C81    ............G.A.........................T...........................
17O81    ............G..........................T...........................
17B90Z   ............G.A........................T...........................
17O90X   ............G.A........................T...........................
17O90Y   ............G.A........................T...........................

         631                          ▼         ▼              ▼           700
BTV17    KLSPDLHEGMEHQRYVHPSTGGTYQKRVLEMKDSCQLTCFVIDYIFEKREQLRDTKEARYIVYLIQSLTG
17B81    ..............A...........C.....P...M................................
17C81    ..............T...........C.....P...................................
17O81    ..............T...........C.....P...................................
17B90Z   ..............T...........C.....P...M...........G.........V.........
17O90X   ..............T...........C.....P..M...........G...................
17O90Y   ..............T...........C.....P..M........Q..G.........V.........

         701                                            ▼                  770
BTV17    TQRLSVLRSTFPNFFQRLLMLKEIKFVRDLNVINFLPLMFLVHDNISYWHRQWSIPMVLFDDTIKLIPVE
17B81    ....D..K............................................................
17C81    ....D..K............................A......S........................
17O81    ....D..K...................................S........................
17B90Z   ....D..K..........T...V....................S...............R.....
17O90X   ....D..K..........T...V....................S...............R.....
17O90Y   ....D..K........G..T...V....................S...............R.....

         771                                                                    840
BTV17    VGAYANRFGFKSFMNFTRFHPGELKKKQIAEDIHKEFGVVAFEYYTNTKISQGNVHTPVMTTKMDVLRVH
17B81    ........................S..............................S.............KI.
17C81    ........................S........M.....................S.............KI.
17O81    ........................S........M.....................S.............KI.
17B90Z   ....Q...................S........M.....................S.............KI.
17O90X   ........................S........M.....................S.............KI.
17O90Y   ........................S........M.....................S.............KI.
```

Fig. 2 (continued).

predicted VP2s constitute therefore a group of non-identical, but closely related proteins. The similar amino acid constitution of these proteins is reflected in the similarity of their predicted hydropathic profiles and secondary structure as predicted by the methods of Kyte and Doolittle (1982) and Chou and Fasman (1974) respectively. Some cysteine (position 657), proline (positions 320 and 664) and glycine (position 574) residues were conserved amongst all field isolates, but absent in the prototype strain. Conversely, a proline was present in the prototype (position 553) and absent in all field isolates. These amino acids can significantly influence secondary structure of the proteins, so their presence or absence could alter the local or overall conformation of VP2 and so induce important phenotypic changes. Even minor conformational changes can have significant effects on virus neutralization or virulence, as has been demonstrated for other RNA viruses (Tuffereau et al., 1989; Aldovini and Young, 1990; Wakefield et al., 1992; De-Maula et al., 1993).

Phylogenetic analyses of field and prototype strains of BTV 17

Phylogenetic relationships between the L2 genes of the five US prototype strains of BTV and the six BTV 17 field isolates were determined using a distance matrix method (Fig. 3) and bootstrap analysis (Fig. 4). Two distinct monophyletic groups are evident, one which includes BTV 2 and BTV 13, and the other which includes the prototype strains of BTV 10, BTV 11 and BTV 17 as well as all six field isolates of BTV 17. This topology is supported by the high confidence values (100%) obtained by bootstrap analyses (Fig. 4).

The monophyletic grouping of BTV 10, 11 and 17 is supported by the common deletions present in the nucleotide sequences of their L2 genes. These deletions are evident by alignment with the L2 gene of EHDV 1. The deletions occur in groups each of three nucleotides between nucleotides 301–302, 384–385, 499–500, 1327–1328, 1374–1375 and 1478–1479 of the prototype strains as compared to EHDV 1. The prototype and field isolates of BTV 17 also share a common deletion between position 1827–1828 which is not present in the other four US serotypes of BTV. Additional deletions are present between positions 1919–1920

```
        841                                                    ▼          910
BTV17   LSSLCAGLADSVVYTLPVAHPKKCIVLIIVGDDKLEPHTRSEQIVSRYNYSRKHICGIVSVTIGQNSQLR
17B81   ...........I.............................................T.......V.......
17C81   ...........I....................................................V.......
17O81   ...........I....................................................V.......
17B90Z  ...........I....................................................V.......
17O90X  ...........I..................................................I.V.......
17O90Y  ...........I......V..EE.........................................V.......

        911                                       955
BTV17   VHTSGIVKHRVCDKFILKHKCKVILVRMPGYVFGNDELMTKLLNV
17B81   .............................................
17C81   ...............................W.............
17O81   .............................................
17B90Z  .............................................
17O90X  .............................................
17O90Y  .............................................
```
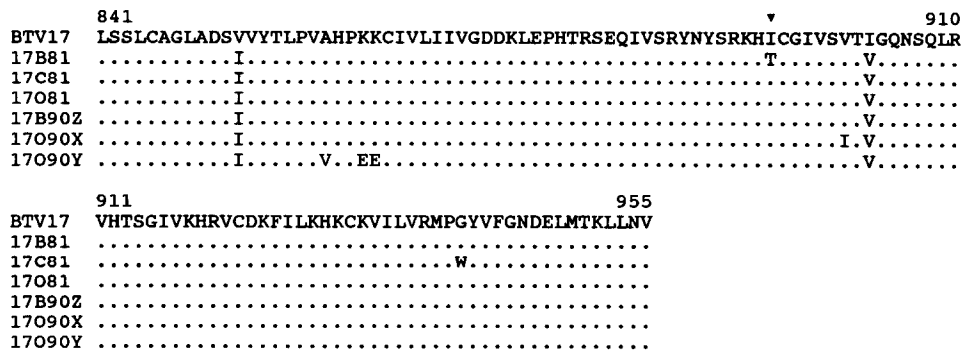
Fig. 2 (continued).
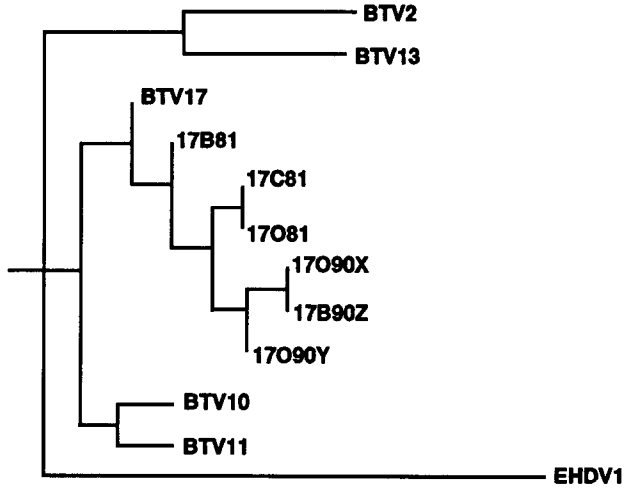
Fig. 3. Phylogenetic tree of the six BTV 17 isolates studied and the five US BTV prototype strains. EHDV 1 was used as an outgroup.

of the prototype strains of BTV 10 and BTV 11, and positions 1916–1917 of the prototype and field isolates of BTV 17. The common deletions in the L2 genes of BTV 10, 11 and 17 suggest that these deletions occurred in a common ancestor prior to the divergence of each serotype. BTV 10 and BTV 11 do cluster as a single group with a common origin, however this topology is not statistically significant (61%) by bootstrap analysis. This lack of defined relationship within the second
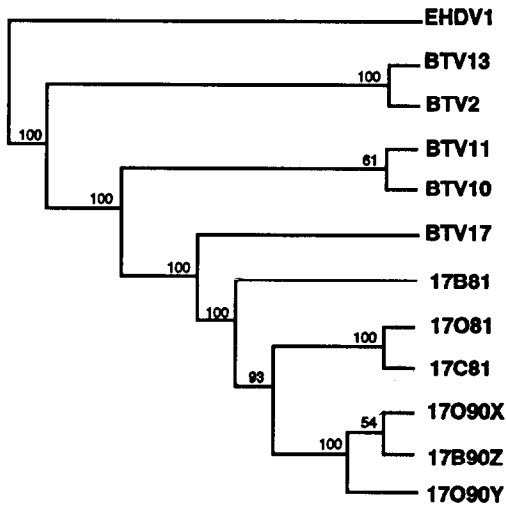
Fig. 4. Tree diagram showing the majority rule consensus tree resulting from 100 bootstrap replicates of the six L2 genes of the BTV 17 field isolates and the five US prototype strains. The number at each node indicates the number of times that the group above and to the right is found.

TABLE 3

Genetic distances of the BTV 17 field isolates and US BTV and EHDV1 prototype strains

	17B81[a]	17C81[a]	17O81[a]	17B90Z[a]	17O90X[a]	17O90Y[a]	BTV 17[b]	BTV 10[b]	BTV 11[b]	BTV 2[b]	BTV 13[b]	EHDV 1[c]
17B81	0.0000											1.6177
17C81	0.0250	0.0000										1.6188
17O81	0.0250	0.0091	0.0000									1.6138
17B90Z	0.0322	0.0311	0.0261	0.0000								1.5947
17O90X	0.0329	0.0318	0.0275	0.0098	0.0000							1.5984
17O90Y	0.0315	0.0300	0.0279	0.0091	0.0119	0.0000						1.6130
BTV 17	0.0523	0.0575	0.0594	0.0645	0.0657	0.0645	0.0000					1.6525
BTV 10	0.4381	0.4396	0.4411	0.4357	0.4329	0.4366	0.4263	0.0000				1.7577
BTV 11	0.4315	0.4412	0.4385	0.4385	0.4299	0.4368	0.4335	0.4547	0.0000			1.7952
BTV 2	1.0496	1.0547	1.0558	1.0469	1.0444	1.0573	1.0375	1.0676	1.0806	0.0000		2.1702
BTV 13	1.0343	1.0454	1.0395	1.0418	1.0440	1.0519	1.0492	1.0817	1.0484	1.8653	0.0000	2.1557
EHDV1	1.6177	1.6188	1.6138	1.5947	1.5984	1.6130	1.6525	1.7577	1.7952	2.1702	2.1557	0.0000

[a] BTV 17 field isolates.
[b] US BTV prototype strains.
[c] EHDV 1 prototype strain.

monophyletic group is further evidenced by comparison of the genetic distances of the BTV 17 field isolates to the prototype strains of BTV 10 and 11. The L2 genes of the prototype BTV 17 and field isolates 17C81 and 17B90Z are slightly more closely related to BTV 10 than they are to BTV 11, as determined by genetic distance matrices (Table 3). In contrast, the genetic distance values of 17O90X, 17B81 and 17O81 are slightly closer to BTV 11 than they are to BTV 10, whereas the field isolate 17O90Y is almost equidistant between the L2 genes of BTV 10 and BTV 11 (Table 3). The evolutionary relationships amongst viruses within the monophyletic group of BTV 10, 11 and 17, therefore, are undefined.

The phylogenetic analyses indicate that the prototype and field isolates of BTV 17 have descended from a common ancestor, as would be expected. Bootstrap analyses placed a confidence value of 100% on this monophyletic group. Isolate 17B81 is placed as a sister in an independent branch of the tree (confidence value of 100%). Although the tree topology indicates that all the other viruses (17O81, 17C81, 17O90X, 17B90Z and 17O90Y) could be descendants from a common immediate ancestor, the low bootstrap value of 93% does not support this concept. This suggests that these viruses could derive through different evolutionary pathways within the lineage. The two field viruses 17O81 and 17C81, although isolated from the same farm and sharing the same ancestor (100% confidence value), have diverged from one another perhaps to successfully replicate in the different host species from which they were isolated. The topology of the tree indicates that the three 1990 field isolates also were derived from a common origin (100% confidence value), however the branching pattern which has two viruses (17O90X and 17B90Z) emerging from the same node is not statistically significant (54% confidence value), indicating that these three viruses too might have arisen via different evolutionary pathways. The phylogenetic analysis of the L2 genes of prototype and field strains of BTV 17 suggests that the unique viral variants of a single BTV serotype, which co-circulate in the same geographical region, could evolve from different evolutionary pathways.

Discussion

The objective of this study was to further characterize the evolution and genetic variation of BTV 17 in a localized region of California by sequencing the L2 gene of field strains of BTV 17. The L2 gene was selected as it encodes outer capsid protein VP2 which expresses the neutralizing determinants of BTV, thus the L2 gene is the only serotype specific genome segment of BTV. The viruses compared in the study were selected from a panel of field isolates with different neutralization profiles (MacLachlan et al., 1992). In spite of their restricted origin, both geographically and spatially, the L2 genes of these viruses were heterogenous which is consistent with the phenotypic variability of these viruses.

The data indicate that the five US BTV serotypes segregate into two monophyletic groups with BTV 2 and 13 forming one group and BTV 10, 11 and 17 the other. This is in agreement with previous phylogenetic analyses based on the

sequence of the L2 and VP2 of prototype strains of BTV (Gould and Pritchard, 1990; Roy, 1992). Whereas it previously was concluded using L2 gene sequences that prototype strains of BTV 10 and BTV 17 are more closely related to one another than they are to BTV 11 (Gould and Pritchard, 1990; Kowalik and Li, 1991), our analyses with field isolates of BTV 17 demonstrate that the relationship between the viruses of this group is less certain. Sequencing of the L2 gene of field strains of BTV 10 and BTV 11 likely will be necessary to more accurately define the evolutionary relationships between BTV 10, 11 and 17. Earlier phylogenetic studies of the BTV serogroup used the nucleotide sequences from conserved BTV genes (Gould and Pritchard, 1991; Hwang et al., 1992a), and these analyses produced trees which suggest different evolutionary relationships between the BTV serotypes than those we obtained. These divergent results likely can be attributed to the evolutionarily conserved nature of the genes characterized (Hwang et al., 1992b), and to the likelihood of reassortment of viral genes amongst viruses which co-circulate. Reassortment of gene segments clearly occurs amongst strains of the four serotypes of BTV present in the western US (Heidner et al., 1991), thus the serotype-specific L2 gene must be used to characterize the evolutionary relationships amongst viruses isolated from a single region.

Analyses of genomes from field strains of other RNA viruses indicate that multiple variant genomes may occur which differ from the average consensus sequence that represents the prototype virus sequence. Different prototype consensus sequences exist for viruses corresponding to particular geographical regions (Domingo and Holland, 1988). Our data indicate that the L2 gene sequences of BTV 17 isolates made in California differed from each other at the nucleotide level by an average of 2.3%, whereas they differed from the prototype BTV 17 virus isolated in Wyoming in 1962 by an average of 5.8%. Our results also identified mutations common to all the California field isolates with respect to the prototype virus. These genetic differences might reflect the comparison of two different prototype consensus sequences of viruses which have evolved in different geographical areas. Hence, it would be interesting to evaluate the concept of geographical prototype by comparing the US BTV 17 prototype virus with field isolates obtained in Wyoming as well as those obtained in California.

Results of this study clearly demonstrate genetic heterogeneity amongst field isolates of the same BTV serotype which were obtained from the same geographical region at an interval of nine years. Heterogeneity was even demonstrated in the L2 genes of two isolates obtained from the same farm in the same year (17O81 and 17C81). The accumulation of mutations in the field isolates is not proportional to the interval between their years of isolation (Table 2), and some nucleotide changes present in the 1981 isolates were absent in those from 1990. The data are not, therefore, consistent in all instances with a direct ancestor-descendant relationship between the field isolates, rather individual viruses might evolve via different evolutionary pathways. This conclusion is further supported by the phylogenetic analyses which indicate that although all field isolates are included in the same monophyletic group, they do not share an immediate common ancestor.

The natural replication cycle of BTV, which includes an insect vector and

various ruminant hosts, offers significant opportunity for the generation of genetically novel viruses. First, reassortment of individual viral genome segments can occur in both the vector and the ruminant host (Roy, 1989; Heidner et al., 1991). Second, it is likely that only viruses best adapted to replication in either the vector or the ruminant host will be amplified. The lack of proof-reading capability of the BTV RNA polymerase also might contribute to genetic diversity during replication in the vector or ruminant host (Kowalik and Li, 1991). Finally, immune selection of variant viruses likely occurs in infected ruminants. All these phenomena might account for the different evolutionary pathways apparently followed by various field strains of BTV 17, and to the generation of the related but not identical viruses which co-circulate in the San Joaquin Valley.

Acknowledgements

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