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Full Length Research Paper

Genetic characterization of VP6 and NS4 genes of bluetongue viruses isolated in Israel during 2006 to 2011

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Twenty-nine (29) Israeli bluetongue virus isolates belonging to eight different serotypes (BTV-2, 4, 5, 8, 12, 15, 16, and 24) were selected to study the nucleotide sequences of segment 9 encoding VP6 protein and the non-structural protein, NS4. Phylogenetic analysis revealed that all Israeli BTV isolates were subdivided into three main groups, of which the 1st and 2nd groups each comprised at least three clusters. Each of the clusters A, B, C, D, and G contained only one serotype – BTV-24, 4, 8, 16, and 15, respectively – whereas clusters E and F each included several different serotypes. Cluster E contained viruses belonging to serotypes BTV-2, 5, and 16; cluster F contained viruses belonging to serotypes BTV-12, 8, and 24. Coding by segment 9 (VP6) proteins varied amongst Israeli isolates, whereas NS4 was highly conserved. The clinical manifestations of the disease did not correlate with the amino acid sequence of VP6 or NS4 proteins.

Key words: Bluetongue virus, Israel, sequence analysis, VP6, NS4.

INTRODUCTION

Bluetongue virus (BTV) belongs to the genus *Orbivirus* within the family Reoviridae. BTV is one of the main pathogens of sheep, goat and cattle, and is generally transmitted by biting midges. Recently, BTV invaded Europe, and consequently became endemic (Maclachlan, 2011; Wilson and Mellor, 2009; Dal Pozzo et al., 2009). European investigators documented novel BT clinical symptoms in addition to those of classical BT. Moreover, for the first time cattle showed clinical manifestations attributed to BTV infection (Darpel et al., 2007; Elbers et al., 2008a, b; Brenner et al., 2011; Santman-Berends et al., 2011). In Israel, eight different BTV serotypes have been identified since 2008: BTV-2, BTV-4, BTV-5, BTV-8, BTV-12, BTV-15, BTV-16 and BTV-24 (Brenner et al., 2010, 2011). A recent study identified three distinct serotypes on some farms. Moreover, the ruminant populations in Israel were being infected with multiple serotypes, either at the same time or over a relatively short period. Since BTV mass vaccination was not adopted, these novel viral strains have been able to spread freely in Israel, providing opportunities for the exchange or reassortment of genome segments (Brenner et al., 2011).

BTV viruses are composed of three protein layers, surrounding a genome consisting of 10 segments of linear double-stranded (ds) RNA. All BTVs are subdivided among 26 serotypes, according to the antigenic structure of the outer-layer (VP2 and VP5) proteins (Maan et al., 2012). The genome of BTV encodes seven structural (VP1-VP7) and three non-structural (NS1, NS2 and NS3/3a) proteins (Roy, 2008; Stuart and Grimes, 2006). Recently, it was shown that BTV expressed an additional non-structural protein which was designated as NS4 (Ratinier et al., 2011; Belhouchet et al., 2011). The NS4 is

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Table 1. List of BTV viruses used in present study.

Registration number	Date of sample collection	Host	Serotype	Designation	Accession number
2302/2006	Nov, 2006	Ovine	15	BTV15/2302/06	JQ970458
2228/2008	Oct, 2008	Goat	16	BTV16/2228/08	JQ970462
2305/2008	Nov, 2008	Ovine	24	BTV24/2305/08	JQ970465
2404/2008	Nov, 2008	Cattle	16	BTV16/2404/08	JQ970463
2425/2008	Nov, 2008	Ovine	24	BTV24/2425/08	JQ970466
1035/2010	Jan, 2010	Cattle	4	BTV4/1035/10	JQ970444
1047/2010	Jan, 2010	Cattle	4	BTV4/1047/10	JQ970445
1957/1/2010	Jul, 2010	Cattle	8	BTV8/1957/1/10	JQ970450
2089/2/2010	Aug, 2010	Ovine	8	BTV8/2089/2/10	JQ970451
2120/2010	Sep, 2010	Cattle	8	BTV8/2120/10	JQ970452
2196/2010	Sep, 2010	Ovine	4	BTV4/2196/10	JQ970446
2214/1/2010	Sep, 2010	Ovine	24	BTV24/2214/1/10	JQ970464
2646/3/2010	Sep, 2010	Ovine	8	BTV8/2646/3/10	JQ970453
2688/2010	Oct, 2010	Cattle	12	BTV12/2688/10	JQ970456
2714/2010	Oct, 2010	Cattle	15	BTV15/2714/10	JQ970459
2944/1/2010	Nov, 2010	Ovine	24	BTV24/2944/1/10	JQ970467
2944/2/2010	Nov, 2010	Ovine	4	BTV4/2944/2/10	JQ970447
2993/2/2010	Nov, 2010	Cattle	12	BTV12/2993/2/10	JQ970457
3027/1/2010	Nov, 2010	Ovine	24	BTV24/3027/1/10	JQ970468
3027/2/2010	Nov, 2010	Ovine	8	BTV8/3027/2/10	JQ970454
3027/3/2010	Nov, 2010	Ovine	4	BTV4/3027/3/10	JQ970448
3027/6/2010	Nov, 2010	Ovine	24	BTV24/3027/6/10	JQ970469
3258/1/2010	Dec, 2010	Cattle	24	BTV24/3258/1/10	JQ970441
870/2011	Jan, 2011	Cattle	2	BTV2/870/11	JQ970442
1822/2011	Aug, 2011	Cattle	2	BTV4/1035/10	JQ970443
1405/2011	May, 2011	Cattle	5	BTV5/1405/11	JQ970449
2017/2011	Nov, 2011	Cattle	16	BTV16/2017/11	JQ970460
2019/2011	Nov, 2011	Cattle	16	BTV16/2019/11	JQ970461
2078/2011	Nov, 2011	Cattle	12	BTV12/2078/1	JQ970455

encoded by an open reading frame in segment 9, overlapping the open reading frame encoding VP6. The VP6 of BTV is a multi-functional protein that exhibits several activities, such as nucleoside triphosphatase, RNA binding, and helicase (Stäuber et al., 1997; Kar and Roy, 2003). It is hypothesized that NS4 plays an important role in virus-host interactions, counteracting the antiviral response of the host and, probably, manifesting additional activities (Ratinier et al., 2011).

The present paper presents the data based on molecular and phylogenetic analysis of segment 9 (coding VP6 and NS4 proteins) of 29 Israeli BTVs. In addition, an attempt was made to link the nucleotide sequence to the clinical cases in which the samples were originated.

MATERIALS AND METHODS

Virus isolation and characterization

Twenty-nine (29) BT viruses isolated in Israel in 2006 to 2011 were selected from the Kimron Veterinary Institute collection and used in this study (Table 1). Amongst these viruses 13 were isolated from sheep, 15 from cattle, and 1 from a goat. Whole blood and tissue samples from animals exhibiting clinical signs were used for BTV isolation and serotype identification. The viruses were isolated in embryonated chicken eggs and in tissue culture as described earlier (Brenner et al., 2010). Briefly, after one to three passages in chicken eggs, the virus was passaged in BHK_{21} and Vero cells. The serotype identity was determined by virus neutralization test with in-hose serotype-specific hyperimmune sheep antisera serotype- and reverse transcriptase polymerase chain reaction (RT-PCR) with a set of serotype-specific primers for the VP2 gene. In most cases, the RT PCR results were confirmed by nucleotide sequencing.

RT-PCR

RNA was isolated from EDTA-treated blood, homogenized spleen tissue, tissue culture supernatant, and chicken embryo homogenates. RNA was extracted with commercial kits: Invisorb Spin Virus RN Mini Kit (STRATEC Molecular,Berlin, Germany), QIAmp Viral RNA Mini Kit and RNeasy Mini Kit (QIAGEN, Germany). Virus detection was performed by RT-PCR with the OneStep RT-PCR kit (QIAGEN, Germany) and real-time RT- PCR based on NS3 with the VIROTYPE BTV Plus test kit (Labor Diagnostik, Leipzig, Germany) or VP1 genes. To determine the

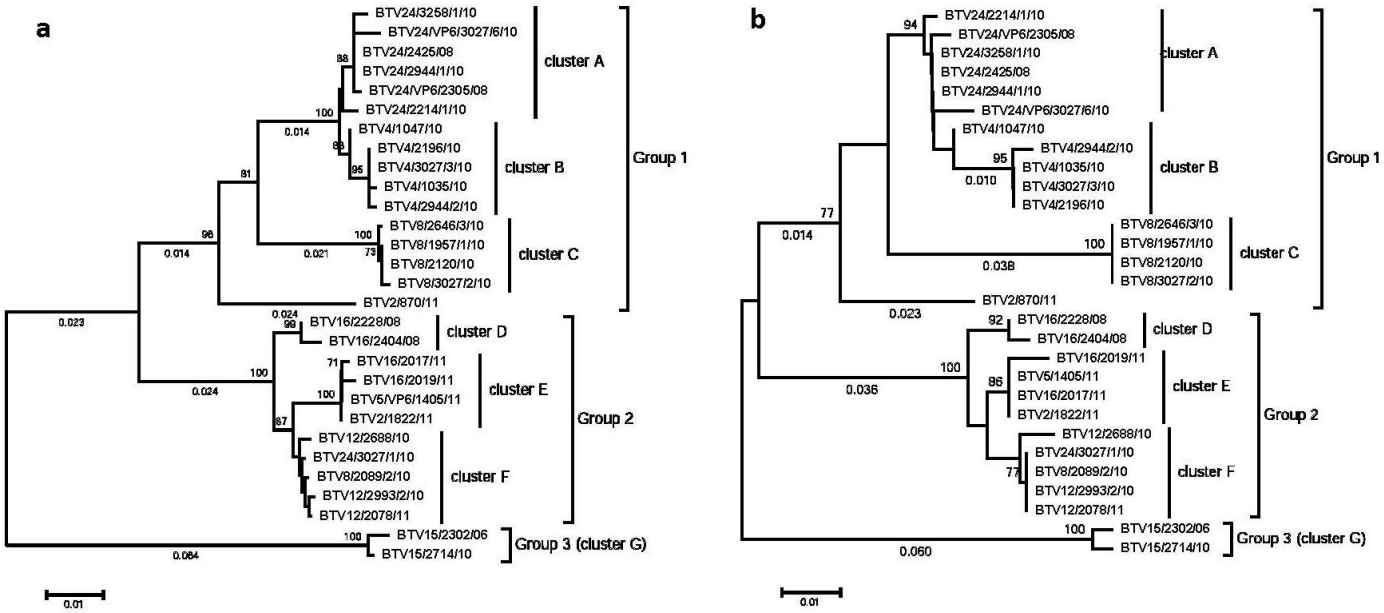

Figure 1. Phylogenetic relationships of VP6 genes from 29 bluetongue viruses isolated in Israel in 2008-2012. The phylogenetic trees based on nucleotide (a) and protein (b) sequences were generated by neighbor-joining analysis with the Maximum Composite Likelihood model, using MEGA 5.0 software. Numbers below branches indicate neighbor-joining bootstrap values; numbers above branches indicate branch lengths.

serotype of local BTV isolates, RT PCR with VP2 serotype-specific primer sets were used and were followed by nucleotide sequence analysis. The serotype-specific primers were available from the Kimron Veterinary Institute, Israel. To study VP6 and NS4 genes, RT PCR carried out to segment 9, followed by nucleotide sequence analysis.

Nucleotide sequence analysis

The partial (sub-total) segment 9 nucleotide sequences of Israeli BTV isolates were analyzed and compared by using the BLAST program (Altschul et al., 2009). Sequence assembly and analysis of segment 9 nucleotide, as well as multiple alignments of the deduced VP6 and NS4 amino acid sequences and phylogenetic comparisons were performed with the MEGA5 software Tamura et al. (2011).

RESULTS

The 9th genome segments of bluetongue viruses isolated in Israel during the five years from 2006 to 2011 were partially sequenced. The main data of the viruses used in the present study are presented in Table 1. Twenty-nine (29) virus isolates belonged to eight serotypes: seven to serotype BTV-24; two to BTV-15; four to BTV-16; five to BTV-4; five to BTV-8; three to BTV-12; two to BTV-2; and one to serotype BTV-5. Phylogenetic analysis of nucleotide sequences of segment 9 showed that all Israeli isolates could be divided among three main groups (Figure 1a): the first group comprised 1 non included in any cluster virus (BTV2/870/11), belonging to serotype BTV-2, and clusters A, B, and C, containing viruses belonging to serotypes 24, 4, and 8, respectively; in the second group cluster D contained serotype BTV-16, cluster E contained serotypes BTV-2, -5 and -16, and cluster F contained serotypes BTV-12, -8 and -24; the third group comprised cluster G, with only the two viruses belonging to serotype BTV-15. The phylogenetic trees based on amino acid sequences of VP6 (Figure 1b) or nucleotide sequences of NS4 (Figure 2a) were practically identical to the above-mentioned dendrogram. At the same time, the phylogenetic tree based on amino acid sequences of NS4 proteins showed this protein to be highly conservative (Figure 2b).

The studied part of the VP6 protein was represented by 301 amino acids (out of the total of 329 within the complete VP6 protein), located between amino acid residues positions 21 and 321. About one-fifth, that is, 63 from 301, or 21%, of the protein sites was variable (Figure 3). Only a few amino acid substitutions were specific to individual serotypes, as follows: R126 – for BTV4; G44, S45, S54, A111, V118, D137, I158, K205, R213, A214, R227, V235, and A280 – for BTV15. Among the Israeli isolates other serotypes contained heterogenic VP6, at the same time, the VP6 of some viruses belonging to different serotypes had identical amino acid sequences (Figure 3). Amino acid variability involved sites associated with three main activities of VP6: ATPase, Helicase, and RNA binding (Figure 4). The prevalence of T320 in VP6 among viruses isolated from sheep was noteworthy: 10 out of 13, compared with six

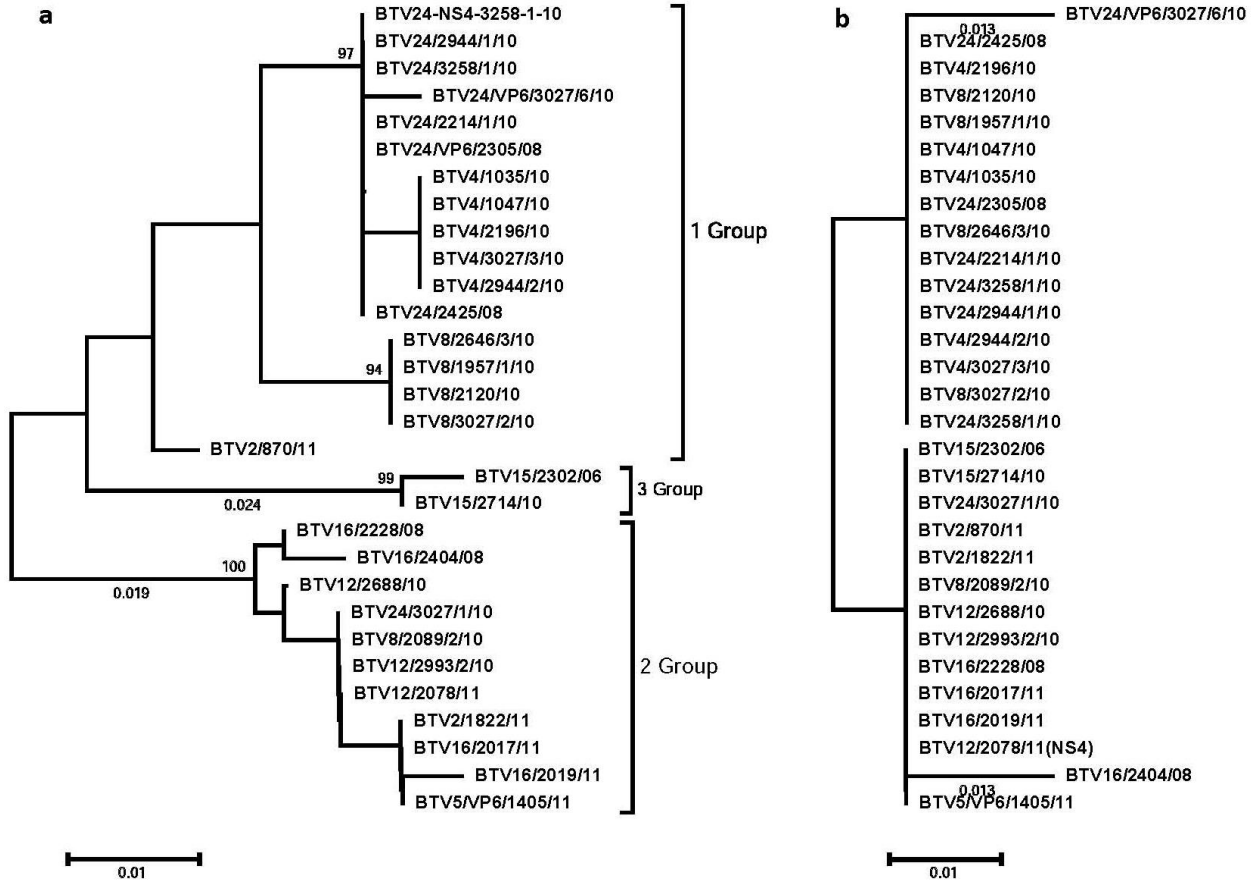

Figure 2. NS4 genes of representative bluetongue viruses isolated in Israel. Trees based on nucleotide (a) and amino acid (b) sequences were generated by the neighbor-joining method with the MEGA 5 software. Numbers below branches indicate neighbor-joining bootstrap values; numbers above branches indicate branch lengths.

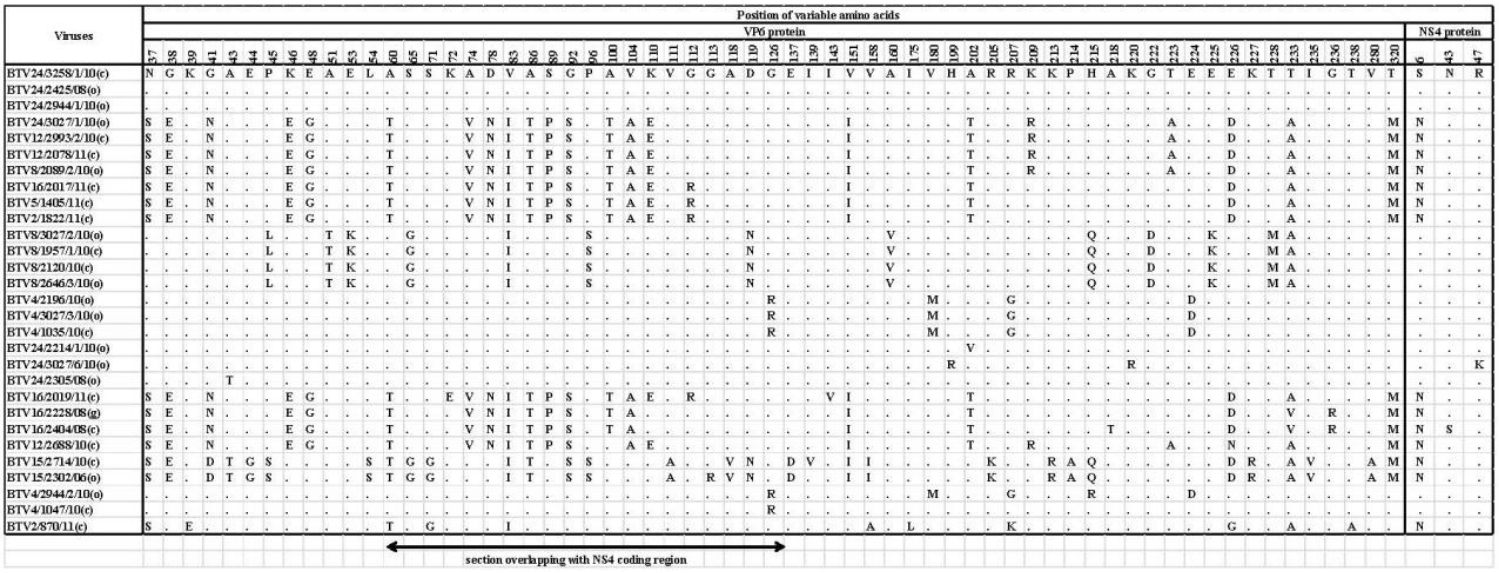

Figure 3. Amino acid substitutions in VP6 and NS4 proteins of bluetongue viruses isolated in Israel. (c), Virus isolated from cattle; (o), virus isolated from ovine; (g), virus isolated from goat.

Viruses	Sites with following functional activities																													
	ATPase							Helicase										RNA binding												
	107	108	109	110	111	112	113	153	154	155	156	157	158	159	160	161	162	201	202	203	204	205	206	207	208	209	210	211	212
BTV24/3258/1/10(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV24/2214/1/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	V	A	E	R	G	R	R	K	Q	G	K
BTV24/3027/1/10(o)	G	D	G	E	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	R	Q	G	K
BTV24/2305/08(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV24/3027/6/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV24/2425/08(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV24/2944/1/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV16/2228/08(g)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K
BTV16/2404/08(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K
BTV16/2017/11(c)	G	D	G	E	V	R	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K
BTV16/2019/11(c)	G	D	G	E	V	R	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K
BTV15/2714/10(c)	G	D	G	K	A	G	G	V	Y	R	D	E	I	P	A	Q	I	K	A	A	E	K	G	R	R	K	Q	G	K
BTV15/2302/06(o)	G	D	G	K	A	G	R	V	Y	R	D	E	I	P	A	Q	I	K	A	A	E	K	G	R	R	K	Q	G	K
BTV12/2688/10(c)	G	D	G	E	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	R	Q	G	K
BTV12/2993/2/10(c)	G	D	G	E	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	R	Q	G	K
BTV12/2078/11(c)	G	D	G	E	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	R	Q	G	K
BTV8/3027/2/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	V	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV8/1957/1/10(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	V	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV8/2089/2/10(o)	G	D	G	E	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	R	Q	G	K
BTV8/2120/10(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	V	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV8/2646/3/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	V	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV5/1405/11(c)	G	D	G	E	V	R	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K
BTV4/1035/10(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	G	R	K	Q	G	K
BTV4/1047/10(c)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	R	R	K	Q	G	K
BTV4/2196/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	G	R	K	Q	G	K
BTV4/2944/2/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	G	R	K	Q	G	K
BTV4/3027/3/10(o)	G	D	G	K	V	G	G	V	Y	R	D	E	V	P	A	Q	I	K	A	A	E	R	G	G	R	K	Q	G	K
BTV2/870/11(c)	G	D	G	K	V	G	G	V	Y	R	D	E	A	P	A	Q	I	K	A	A	E	R	G	K	R	K	Q	G	K
BTV2/1822/11(c)	G	D	G	E	V	R	G	V	Y	R	D	E	V	P	A	Q	I	K	T	A	E	R	G	R	R	K	Q	G	K

Figure 4. Amino acid substitutions in the ATPase, Helicase and RNA binding sites of VP6 protein of bluetongue virus strains isolated in Israel. The data presented by Kar and Roy (2003) were used to identify the sites associated with functional activities. (c) – virus isolated from cattle, (o) - virus isolated from ovine, (g) - virus isolated from goat.

out of 15 among cattle. In contrast with the relative variability of VP6, NS4 was highly conservative, with only three out of seventy seven sites, that is, 4%, containing amino acid substitutions (Figure 3). Only one case of substitution was found at each of positions 43 and 47. Two amino acid variants were found at position 6: asparagine, R6, at this position was found in all BTV-2, BTV-12, BTV-15, and BTV-16 serotypes; and serine, S6, was present in all BTV-4 and in the vast majority of viruses belonging to serotypes BTV-24 and BTV-8 (Figure 3).

In order to assess the variability of the NS4 proteins we studied the deduced amino acid sequences of those proteins available in the GenBank (Figure 5), and found 26 amino acid variants of NS4 among all BT viruses – comprising Israeli isolates and the sequences available in GenBank – whereas only four variants were present among Israeli isolates.

All the present clinical cases and the animal species from which the isolates were taken are summarized in Table 2. Nervous-system-like manifestations were observed in the serotype BTV-24 viruses 2214/2010, 3027/6/2010 (from cluster A) and 3027/1/2010 (from cluster F); BTV-8 virus 3027/2/2010; BTV-4 virus 3027/3/2010 (clusters C and B, respectively); that were isolated from two different flocks that exhibited a shaky-lambs like syndrome, resembling border disease (Cluster A), in the BTV-8 in Cluster C from sheep, and in the BTV-4 in Cluster B from sheep. A BTV12 serotype was isolated from a case that resembled sheep-associated malignant catarrhal fever, which is also considered to be a kind of central nervous system manifestation (Brenner et al., 2002). The classical BT manifestations in sheep were also observed (Batten et al., 2008); they were scattered among the all serotype groups which were present in sheep, including BTV-15 virus 2302/2006 - exhibiting classical sounds of "kissing and dancing" symptoms; BTV-24 viruses 2425/2008 and 2305/2008 -exhibiting, coronitis, hoof hemmorages and intramural lesions, mucosal ulcerations,

Number of viruses	Source	Amino acid sequences of NS4 protein
14	Israeli collection	MVRGRSRRAARRKRAAKRLKMQMWIDAYILQWDLDQAQKDLENARTRMLTEEMERLEEEVEMLMRELELLERMEEDG
4	Gene Bank	
1	Israeli collection	.....K.....
13	Israeli collection	.....N.....
48	Gene Bank	
1	Israeli collection	.....N.....S.....
12	Gene Bank	....HN..M.....K.....A....
6	Gene Bank	.....N.....WF
1	Gene Bank	.....QF
1	Gene Bank	.....N.....P.....
1	Gene Bank	.....N.....Y.....WF
1	Gene Bank	.....R.....
1	Gene Bank	.....R.....
1	Gene Bank	.....R.....
1	Gene Bank	.....MT.....
1	Gene Bank	.....V....
1	Gene Bank	.....N.....N.
1	Gene Bank	....HN..M.....K.....Q.....A....
1	Gene Bank	....HN..M.....K.....S.....A....
1	Gene Bank	....HN..M.....KP.I.....A....
1	Gene Bank	....HN..M.....T..K.....A....
1	Gene Bank	.....N.....G.RRR.....
1	Gene Bank	.....N.....FV..L.....
1	Gene Bank	...DAIGEQPEEREQP.....PDV....T.....
1	Gene Bank	....PX..M..TT....K.....A....
1	Gene Bank	....HN..M.....T..K.....X.....A....
1	Gene Bank	....PX..M.....MN.L.....PIA.....A....LY
1	Gene Bank	...RQN.....K.....M.....RE..E.Q...Q.....QD.I.L....M..M.Q.R...
1	Gene Bank	...RHN.....R..R....M.....RR...E.QA..Q.....D.I.L....M..M..IG...
1	Gene Bank	....HN..M.....K.....S...R*

Figure 5. Alignment between the NS4 protein sequences of bluetongue virus isolated in Israel and other BTV sequences available in GenBank.

swelling tongues and heads; BTV-8 viruses 2120/2010 and 2646/2010- exhibiting the same above mentioned symptoms and the characteristic BT-attributed acute severe pleuropneumonia; BTV-4 virus 2196/2010 – exhibiting severe mucosal disease (Table 2), in clusters G, A, C, and B, respectively. It also seems that, irrespective of the serotype or phylogenetic group, abortion might be induced by infection with any BTV serotype, as may happen also in case of any unspecified viral infection.

DISCUSSION

Phylogenetic analysis of Seg-9 showed some correlations of nucleotide and amino acid sequences with the BTV serotypes in the Israeli strains used in the present study. However, the Israeli strains identified as BTV-12 (BTV-12/2078/11, BTV-12/2993/2/10, and BTV-12/2688/10) clustered with BTV-24/3027/1/10 and BTV-8/2089/2/10 and, furthermore, the last two of these viruses were distantly clustered with other viruses belonging to the same serotypes.

Thus, the correlations between Segment 9 and the serotypes were mild amongst the Israeli isolates used in the present study. On the other hand, these findings indicate a possibility of reassortment among BT viruses circulating in Israel, as was hypothesized in a previous publication (Brenner et al., 2011).

In contrast to VP6, protein NS4 was found to be highly conservative: amino acid substitutions were revealed in three sites, where the part ofVP6 that overlapped the NS4 nucleotide sequence had 20 variable sites. Among 29 Israeli isolates only four variants of the amino acid sequence of protein NS4 were found, which is consistent with the concept of strong conservation of this protein (Ratinier et al., 2011). However, the presence of 26 variants of the protein among the 121 field isolates of BT viruses (29 Israeli and 92 from GenBank BTVs) suggests that the amino acid sequence of this protein can vary over a relatively wide range. The function of this protein

Table 2. The clinical cases and the species from which the various bluetongue virus serotypes were isolated.

BTV serotype	Virus	Collection date	Host	Clinical manifestations from which the BTV was isolated	Cluster[a]
BTV-15	2302/2006	Nov-06	Ovine	Classical sheep BT and high mortality	G
	2714/2010	Oct-10	Cattle	Fever and recumbency: BEF-like symptoms	G
BTV-16	2228/2008	Oct-08	Goat	Respiratory distress in young kids	D
	2404/2008	Nov-08	Cattle	Foot rot-like disease. 16% case mortality rate	D
	2017/2011	Nov-11	Cattle	Coronitis and lameness	E
	2019/2011	Nov-11	Cattle	BEF-like symptoms (recumbency and fever)	E
BTV-24	2305/2008	Nov-08	Ovine	Classical BT symptoms	A
	2425/2008	Nov-08	Ovine	Classical BT symptoms	A
	2214/1/2010	Sep-10	Ovine	Central nervous system symptoms Border disease-like	A
	2944/1/2010	Nov-10	Ovine	No specific symptoms were reported besides abortions	A
	3027/1/2010	Nov-10	Ovine	Central nervous system symptoms Border disease-like	F
	3027/6/2010	Nov-10	Ovine		A
	3258/1/2010	Dec-10	Cattle	Abortion and bloody diarrhea	A
BTV-8	1957/1/2010	Jul-10	Cattle	Fever	C
	2089/2/2010	Aug-10	Cattle	Subcutaneous emphysema	F
	2120/2010	Sep-10	Ovine	Classical BT symptoms & respiratory distress	C
	2646/3/2010	Sep-10	Ovine	Pneumonia	C
	3027/2/2010	Nov-10	Ovine	Central nervous system symptoms Border disease- like	C
BTV-2	870/2011	Jan-11	Cattle	Abortion	n/c
	1822/2011	Aug-11	Cattle	Abortion	E
BTV-5	1405/2011	May-11	Cattle	Fever and abortion	E
BTV-12	2993/2/2010	Nov-10	Cattle	Fever and storm and high mortality rate (16%)	F
	2078/2011	Nov-11	Cattle	Malignant catarrhal fever-like disease - head and eye form	F
	2688/2010	Oct-10	Cattle	Fever and abortion storm	F
BTV-4	1035/2010	Jan-10	Cattle	Subcutaneous emphysema	B
	1047/2010	Jan-10	Cattle	Subcutaneous emphysema	B
	2196/2010	Sep-10	Ovine	"mucosal disease" PPR like	B
	2944/2/2010	Nov-10	Ovine	No specific symptoms were reported besides abortions	B
	3027/3/2010	Nov-10	Ovine	Central nervous system symptoms Border disease-like	B

a - in accordance with the notation of the clusters in Figure 1.

remains unclear in many respects; its involvement in virus-host interactions as a counter measure to the antiviral response of the host has been confirmed only for BTV-8. Thus, there is a need for further study of the functions of NS4 in the context of the various serotypes and varied host specificity of the virus. As shown in Table 2, in the light of the clinical data from the previous and the present study, taken together, we suggest that the pathogenic mechanisms are probably not to be found in the amino acid sequence profile of VP6 and NS4.

However, we consider that it is necessary to continue studies of the correlations between clinical manifestations of BT disease and the characteristics of the amino acid composition of virion proteins. The phylogenetic analyses of genome segments will be available to clarify the molecular epidemiology of BTV in the region, and additional sequence analyses of other segments of the Israeli strains are currently being conducted. As our understanding of BTV epidemiology improves, we will be able to establish more effective diagnostic and preventive measures against BT.

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