

Analysis and typing of BTV isolates from Israel, 2006

Report Prepared by:

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Samples of three BTV isolates originally made in Israel from Sheep showing clinical signs of infection, were sent to the BTV Reference laboratory at IAH Pirbright on Sunday, December 10th 2006 and arrived 15th December 2006.

	Ins Nr.	Date	Material	Village in Israel	Comment	IAH Reference collection number (& link)
1	530659	01/11/2006	Chick embryo	Alon Hagalil	Eggs 100% mort.	ISR2006/10
2	532452	17/11/2006	Chick embryo	Yachini	Eggs 100% mort.	ISR2006/11
3	532390	17/11/2006	Chick embryo	Elyakim	Eggs 100% mort.	ISR2006/12

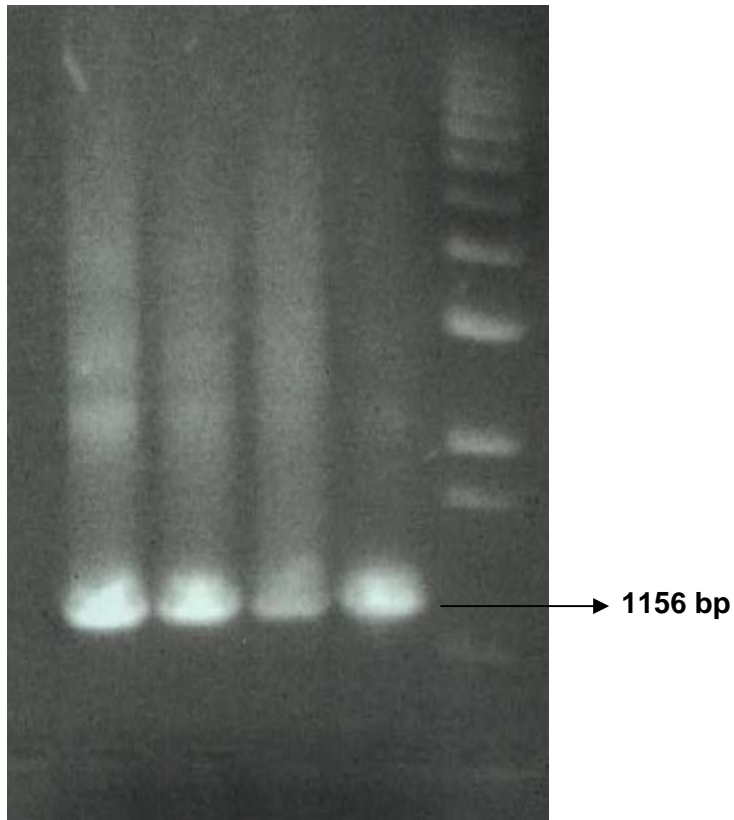
The viruses were grown in BHK cell culture at IAH and added to the IAH reference collection. Viral RNA was extracted from monolayers showing 90% CPE. The RNA was tested by BTV serogroup specific RT-PCR (on 10th January 2007) targeting BTV genome segment 7 (Anthony et al 2007), generating a strong band of the expected size (Figure 1). These data provide an initial demonstration that all three samples contain BTV (although this does not conclusively rule out the presence of other viruses (e.g. EHDV) which are also circulating in the region).

The RNA from the infected cell were tested by serotype specific RT-PCR using primers targeting BTV genome segment 2 of all 24 BTV serotypes (Maan et al 2007; Maan et al manuscript in preparation). The results are shown in figure 2. Two of the virus isolates ([ISR2006/10](#) & [ISR2006/12](#)) gave +ve reactions and products of the expected sizes with two sets of primers, those against BTV-4, and a weaker reaction with primers against BTV-24 (see figure 2). However a second set of BTV-24 specific primers gave no amplification (data not shown). The amplicons from the BTV-4 RT-PCR primers were sequenced, confirming the identity of the viruses as type 4. A Phylogenetic tree is being constructed for available BTV-4 Seg-2 sequences but is not yet available. The amplicons obtained with the first set of BTV-24 primers failed to generate readable sequence data.

Similar assays using serotype specific primers with the RNA sample from the third virus ([ISR2006/11](#)) gave +ve reactions and products of the expected sizes with primers against BTV-15 (see figure 2). Since this serotype has not previously been detected in the region, several further BTV-15 primer sets were tested against the RNA sample of this virus. All of the primer sets designed for BTV-15 gave strong bands of the expected sizes (see figure3), confirming the identity of this virus ([ISR2006/11](#)) as BTV-15. The cDNA amplicons were sequenced to generate data for ~ 1000 base pairs. These data were compared to other Seg-2 sequences for the 24 BTV serotypes, confirming the identity of ISR2006/11 as BTV-15 (figure 4).

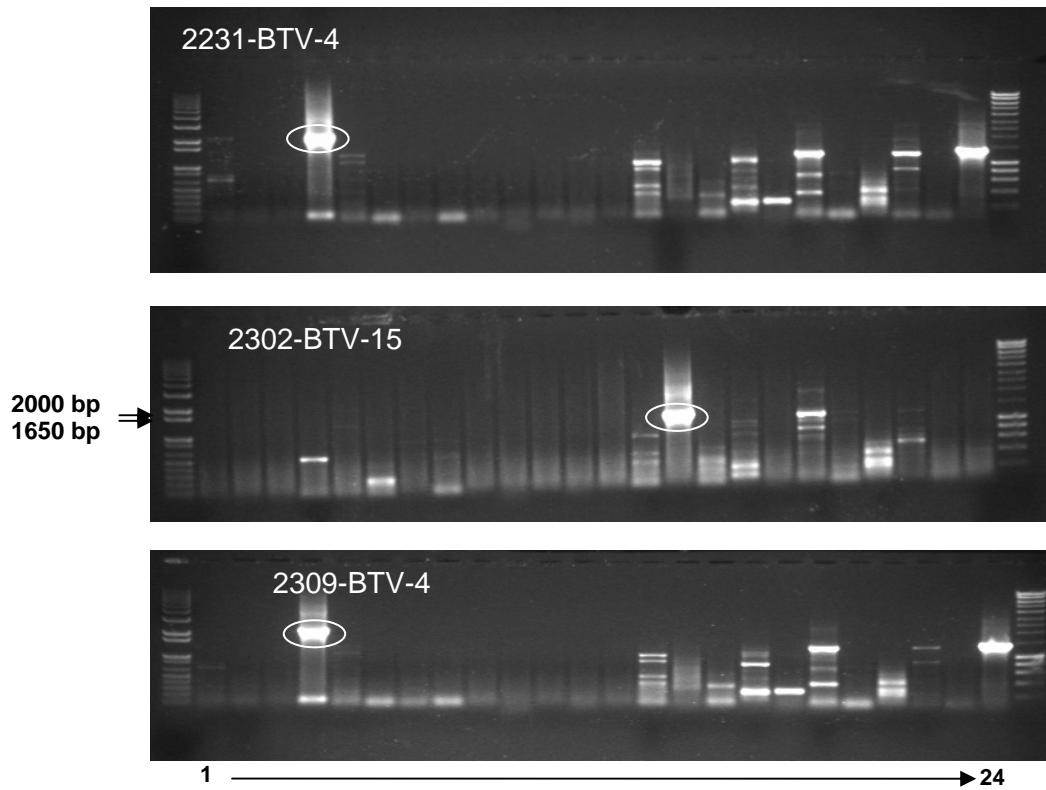
The results obtained with the BTV-24 primers, confirms previous sequencing and cross-hybridisation studies, which show that BTV4 and 24 are closely related. (Maan et al 2007). Indeed an analysis of the primer footprints, shows a high level of similarity between the BTV-24 primers and the isolates of BTV-4 from Israel 2006. This will require some further redesign of the first BTV-24 primer set to improve type specificity.

Figure 1: amplification of Seg-7 sequences by RT-PCR (BTV virus species specific assay (Anthony et al 2007))



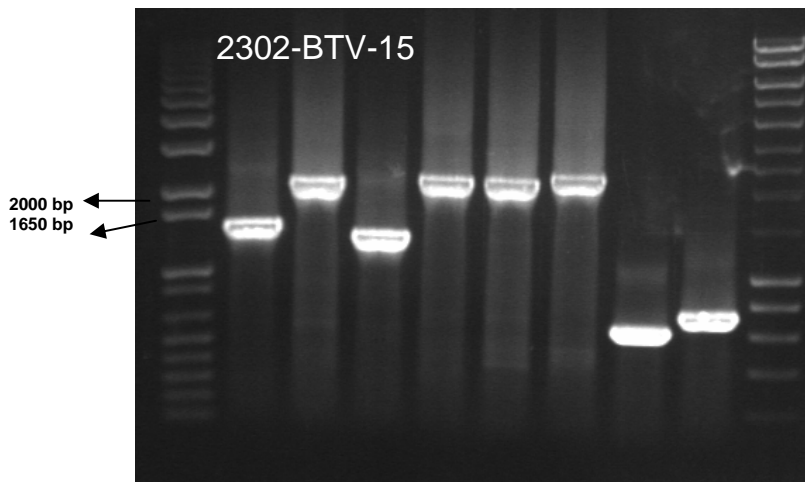
Lane 1 sample 2302 ([ISR2006/11](#)), Lane 2 sample 2309 ([ISR2006/12](#)), Lane 3 sample 2231([ISR2006/10](#)), Lane 4 Positive control (BTV-4 Spain)

Figure 2: RT-PCR based typing of 3 Israeli isolates using 24 serotype specific primers



The correct size products are circled, indicating virus type

Figure 3: Confirmation of Israel isolate as BTV-15 (no 2302) using multiple BTV-15 specific primer pairs in RT-PCR assay



Products of the correct size in all lanes, confirmed ISR2006/11 as BTV type 15

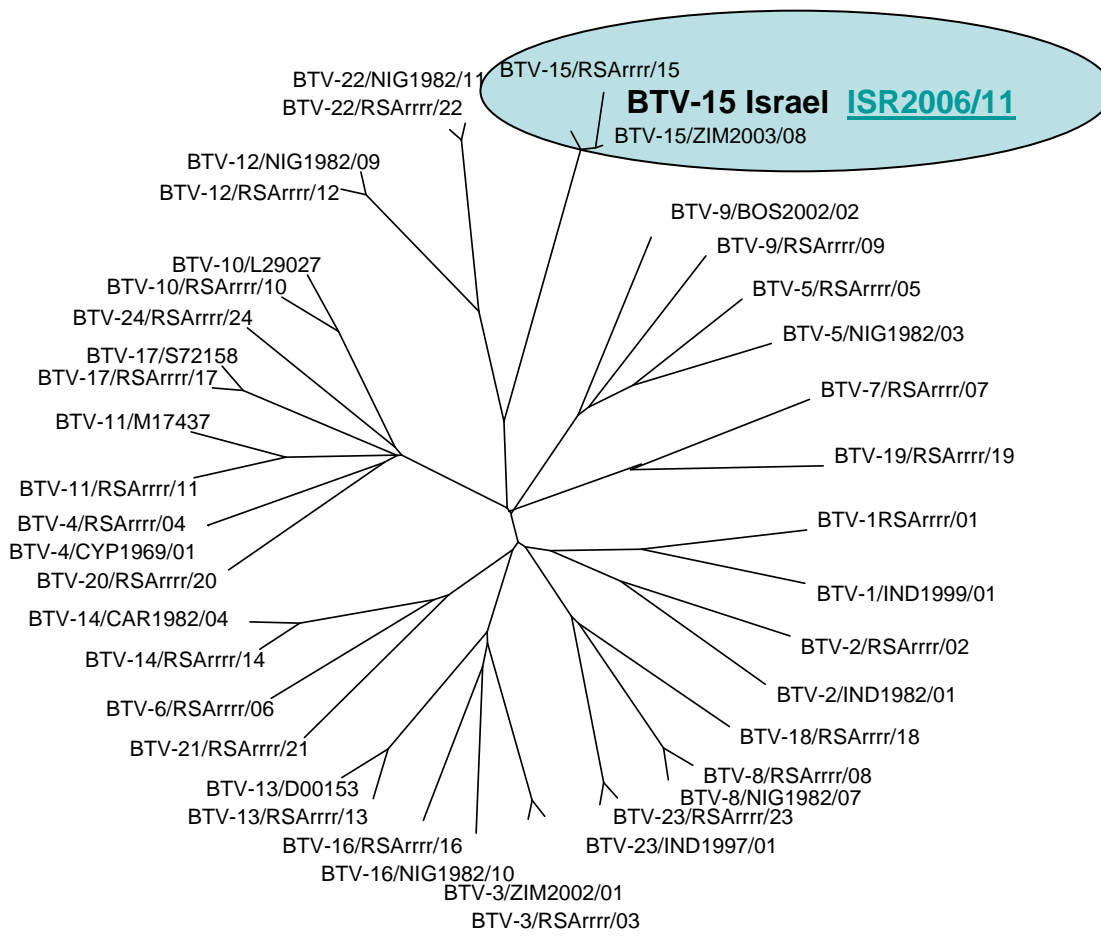


Figure 4: Radial tree comparing Seg-2 of BTV Israel (ISR2006/11) to reference strains of all 24 BTV serotypes, confirming its identity as BTV-15.

References

Maan et al (manuscript in preparation). The design and initial evaluation of BTV seg-2 specific primers for identification of BTV serotype by RT-PCR.

Maan S., Maan N.S, Samuel A.R., Rao S, Attoui, H., & Mertens P.P.C (2007) Analysis and Phylogenetic Comparisons of Full-Length VP2 Genes of the Twenty-Four Bluetongue Virus Serotypes. *Journal of General Virology* (in Press).

S. Anthony, H. Jones, K.E Darpel, H. Elliott, S. Maan, A. Samuel, P. S. Mellor, P. P. C. Mertens. A duplex RT-PCR assay for detection of genome segment 7 (VP7 gene) from 24 BTV serotypes. *Journal of Virological Methods* (in Press).