

Report concerning identification of the Northern European isolate of BTV (26th August 2006)

Peter Mertens, Carrie Batten, Simon Anthony, Lydia Kgosana, Karin Darpel, Kasia Bankowska, Eva Veronesi, Abid Bin Tarif, Natalie Ross Smith, Andrew Shaw, Mandy Corteyn, Sushila Maan, Chris Oura, Houssam Attoui, Philip Mellor

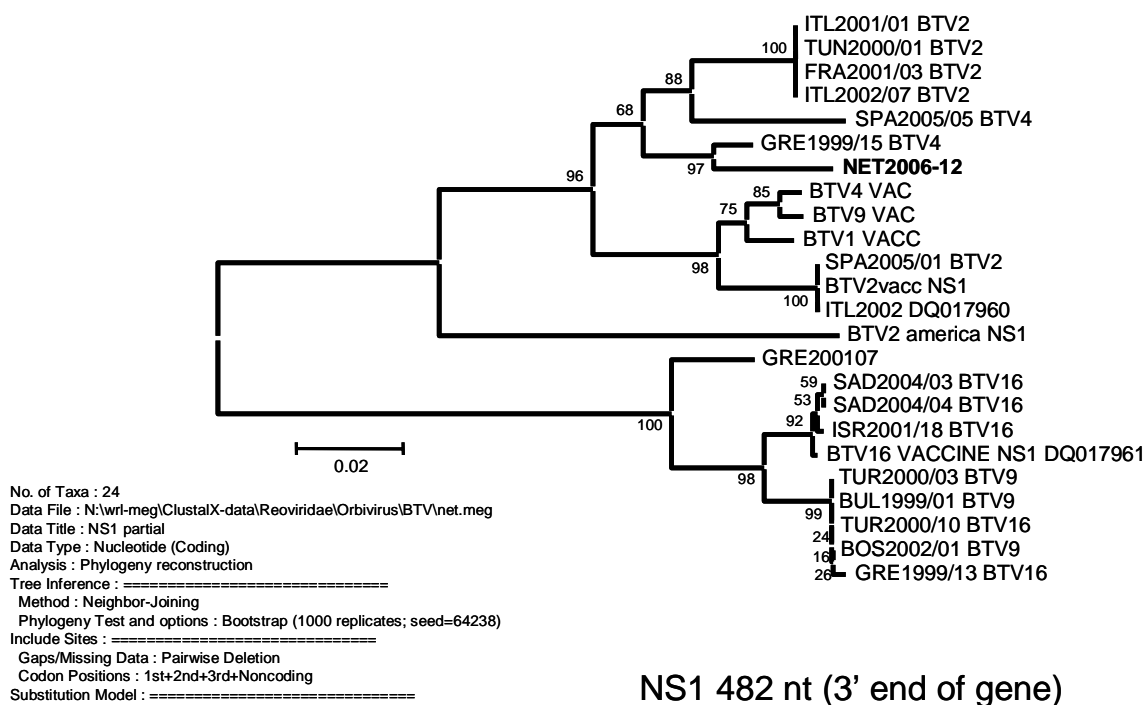
With 24 hours of samples arriving at Pirbright from Eugene van Rooij in the Netherlands, the initial identification of BTV specific antibodies (by the BTV Reference Centre) was +ve, confirming the observations by colleagues in the Netherlands.

At the same time by an rRT-PCR assay was carried out on EDTA treated blood samples, targeting genome segment 7 (Anthony et al 2007). This confirmed the Netherlands results (for segment 8) demonstrating the presence of viral RNA (and hence almost certainly virus) in the blood samples from at least two animals in the Netherlands. Since these were Dutch animals, this showed they must have acquired the infection *in situ* and confirmed virus transmission in the Netherlands

Within the Arbovirus Research Group at IAH, we initially demonstrated (by real time PCR targeting Seg-1 - Shaw et al 2007) that the Northern European virus strain is a western virus. That is to say it comes originally from Africa or America. This ruled out the European field strains of BTV-1, 9 and 16 which are all Eastern viruses.

Serotype specific RT-PCR assays (targeting genome segment 2 [which controls virus serotype] of the known BTV serotypes in Europe [1, 2, 4, 9 and 16]- www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/btv-S2-primers.htm) were set up within 24 hours of sample receipt. However, these failed to amplify cDNAs from any of the RNAs extracted from blood sample (even though they were segment 1 and segment 7 +ve – see above). These assays were repeated but were still negative.

RT-PCR assays for genome segment 5 (encoding NS 1) were +ve. The resulting product was sequenced and shown to be different from NS1 of any of the recent European field strains or the live vaccine strains widely used in Europe.

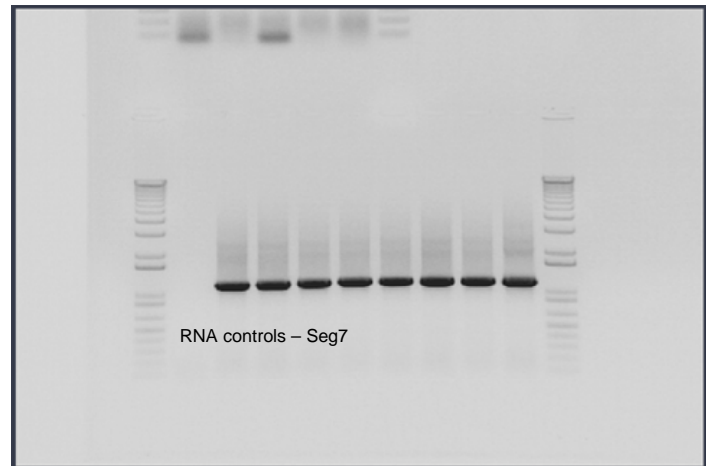
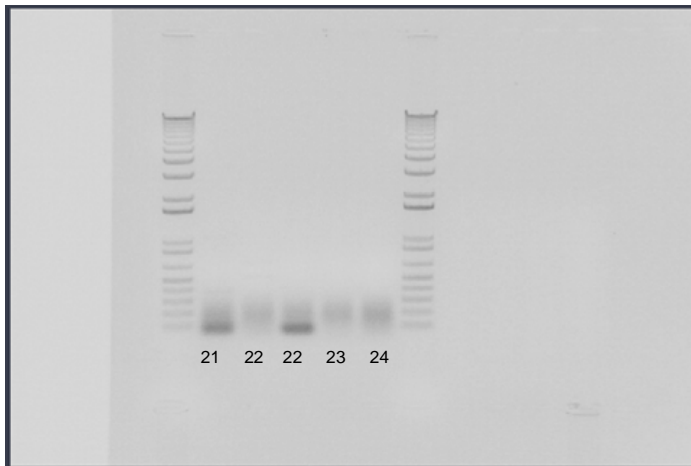
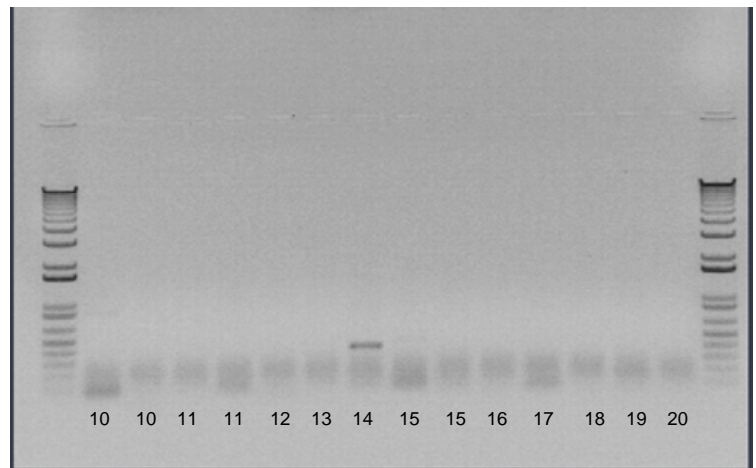
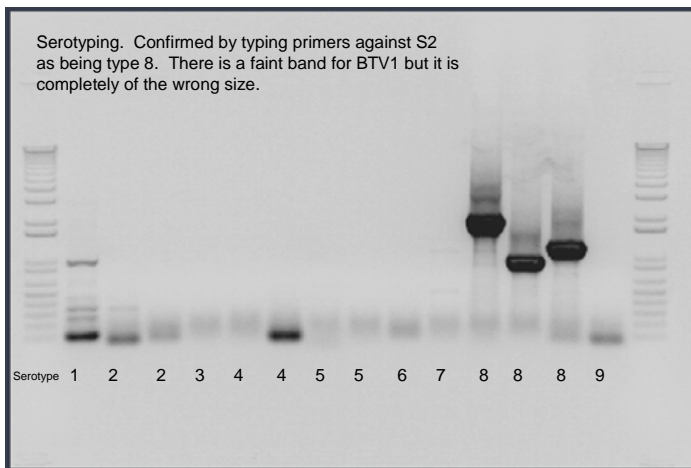


This indicates that the northern European strain is not simply derived from one of the previous European strains but is most likely a novel introduction to the region.

On this basis we went back to the sequence data which we have recently generated for Seg-2 of over 300 isolates of BTV, including reference strains of all 24 serotypes (Maan et al 2007) and identified regions as potential targets for serotype specific primers for each of the remaining 19 serotypes (Mertens et al 2007)

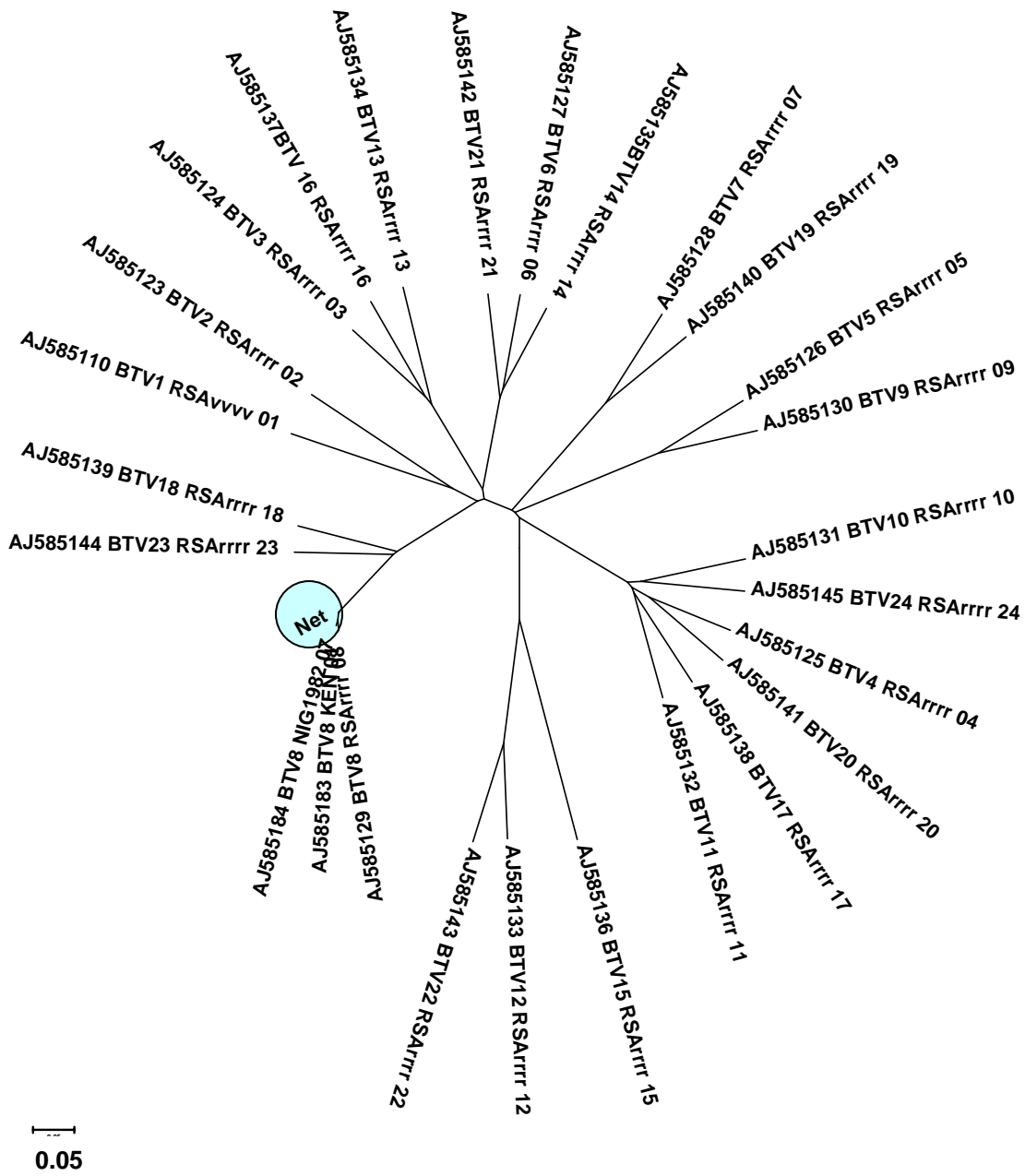
+ve blood samples were placed on a *Culicoides sonorensis* cell line (KC cells provided by colleagues at the USDA lab in Laramie, Wyoming) and this generated a virus isolate.

RNA from the infected KC cells was extracted and tested with the type specific primers for all 24 serotypes, generating cDNA products only with three sets of primers, all of which are derived from BTV-8 sequences (demonstrating that the virus is BTV-8).



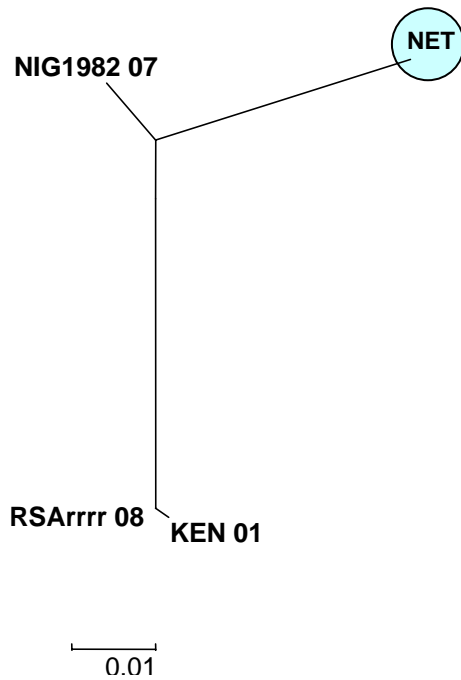
The antisera from initial blood samples that were strong +ves by cELISA, have been put into virus neutralisation assays initially for the European types and subsequently with all 24 serotypes (results not yet ready). Samples were also put into eggs for virus isolation (at the earliest possible date - depending on egg availability after 4 days). The virus isolate is not yet available from that process.

The +ve PCR products from the Seg-2 BTV- 8 specific RT-PCR were sequenced and the VP2 aa sequence was compared to other BTV VP2s for which data is available, confirming the identification of the Netherlands isolate as BTV-8.



A comparisons of the nucleotide sequences for segment 2 show differences with the other African strains of BTV-8 but confirmed its sub-Saharan lineage.

Segment 2 nucleotide tree



References

[Anthony S, Jones H, Darpel KE, Elliott H, Maan S, Samuel A, Mellor PS, Mertens PP.](#) (2007) A duplex RT-PCR assay for detection of genome segment 7 (VP7 gene) from 24 BTV serotypes. *Journal of Virological Methods* **141**: 188-197.

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[Sushila Maan, Shujing Rao Narender Singh Maan, Simon Anthony, Houssam Attoui, Alan Richard Samuel and Peter Paul Clement Mertens](#) (2007) Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. *Journal of Virological Methods* **143**:132-139.

Mertens, P.P.C. Maan N. S., Prasad, G., Samuel A.R., Shaw A., Potgieter, A.C., Anthony, S. J., and Maan S. (2007) The design of primers and use of RT-PCR assays for typing European BTV isolates: Differentiation of field and vaccine strains (*Journal of general Virology* – in press).

Amino acid sequence distance matrix for VP2

	5	9	4	20	17	11	10	24	1	2	8SA	8KEN	8NIG	NET	23	18	3	16	13	6	14	21	7	19	12	22	15	
[BTV5]																												
[BTV9]	0.245																											
[BTV4]	0.610	0.612																										
[BTV20]	0.628	0.624	0.224																									
[BTV17]	0.628	0.628	0.251	0.272																								
[BTV11]	0.624	0.628	0.283	0.289	0.263																							
[BTV10]	0.621	0.620	0.267	0.297	0.292	0.292																						
[BTV24]	0.631	0.622	0.276	0.295	0.301	0.289	0.257																					
[BTV1]	0.599	0.595	0.589	0.596	0.593	0.599	0.596	0.572																				
[BTV2]	0.586	0.587	0.582	0.596	0.584	0.595	0.591	0.596	0.411																			
[BTV8SA]	0.605	0.608	0.584	0.573	0.575	0.569	0.586	0.575	0.479	0.484																		
[BTV8KEN]	0.606	0.611	0.584	0.573	0.575	0.569	0.586	0.575	0.481	0.485	0.004																	
[BTV8NIG]	0.600	0.604	0.581	0.570	0.572	0.565	0.584	0.572	0.468	0.479	0.020	0.024																
[NET]	0.586	0.596	0.552	0.535	0.528	0.528	0.552	0.548	0.473	0.464	0.022	0.028	0.004															
[BTV23]	0.608	0.619	0.600	0.590	0.577	0.570	0.581	0.575	0.474	0.473	0.242	0.246	0.237	0.208														
[BTV18]	0.603	0.601	0.588	0.583	0.561	0.571	0.588	0.582	0.472	0.482	0.241	0.243	0.240	0.226	0.249													
[BTV3]	0.607	0.615	0.601	0.610	0.598	0.598	0.613	0.609	0.530	0.511	0.518	0.519	0.514	0.490	0.519	0.512												
[BTV16]	0.639	0.637	0.614	0.626	0.626	0.613	0.637	0.620	0.529	0.538	0.517	0.518	0.512	0.503	0.536	0.526	0.253											
[BTV13]	0.618	0.631	0.606	0.618	0.611	0.609	0.626	0.623	0.541	0.531	0.535	0.536	0.529	0.531	0.547	0.539	0.292	0.275										
[BTV6]	0.597	0.604	0.598	0.616	0.604	0.596	0.608	0.596	0.490	0.486	0.501	0.503	0.497	0.472	0.484	0.494	0.485	0.509	0.501									
[BTV14]	0.590	0.602	0.585	0.604	0.585	0.597	0.608	0.593	0.483	0.468	0.488	0.489	0.484	0.463	0.483	0.480	0.483	0.501	0.504	0.227								
[BTV21]	0.590	0.599	0.584	0.615	0.591	0.603	0.603	0.597	0.495	0.481	0.508	0.511	0.502	0.483	0.488	0.487	0.485	0.506	0.506	0.248	0.253							
[BTV7]	0.612	0.619	0.608	0.620	0.620	0.617	0.611	0.601	0.598	0.601	0.591	0.593	0.587	0.547	0.571	0.585	0.588	0.593	0.593	0.583	0.591	0.579						
[BTV19]	0.618	0.610	0.597	0.595	0.612	0.603	0.603	0.595	0.582	0.590	0.592	0.595	0.590	0.549	0.585	0.589	0.595	0.603	0.615	0.573	0.594	0.589	0.253					
[BTV12]	0.718	0.704	0.696	0.687	0.691	0.692	0.683	0.682	0.688	0.696	0.680	0.681	0.675	0.648	0.681	0.690	0.712	0.714	0.714	0.720	0.712	0.717	0.706	0.716				
[BTV22]	0.709	0.711	0.695	0.694	0.705	0.704	0.691	0.686	0.700	0.705	0.696	0.696	0.692	0.659	0.711	0.705	0.714	0.720	0.716	0.730	0.722	0.726	0.712	0.724	0.241			
[BTV15]	0.698	0.706	0.703	0.698	0.708	0.710	0.694	0.691	0.692	0.706	0.709	0.709	0.701	0.688	0.714	0.706	0.703	0.706	0.717	0.717	0.726	0.712	0.702	0.712	0.543	0.550		

Identities between BTV-8 isolates

NET / NIGERIA : 99.6%
NET / SA : 97.8%
NET/ KENYA : 97.2%