

MedReoNet: WP3 Molecular Epidemiology An update and future perspective

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MedReoNet Coordination Meeting: Lisbon, 2nd - 5th December 2009



Discussion topics (for Day 3, WP3)

WP-3: Molecular epidemiology

The general objectives of this WP consist in the distribution, origins and movement of different BTV and EHDV strains,

by characterisation of well documented isolates of different serotypes.

The Specific objectives are

-to expand existing sequence databases for genome segments 2 and 6 of BTV and related orbiviruses, including high quality data for

-representative and well documented isolates of different virus serotypes from different geographical locations

- to expand the sequence databases for other genome segments of BTV and related orbiviruses, for representative and well documented isolates of different virus serotypes from different geographical locations

- to characterise novel and existing BTV/EHDV strains from Europe and the Mediterranean Basin, to help determine their geographical distribution, movement, potential for reassortment and their original sources (topotypes).

- to identify molecular probes / primers for the rapid/ early identification of new strains-serotypes



2.2.3. WP 3: Molecular Epidemiology : Objectives:

► Identification of the distribution, origins and movement of individual BTV and EHDV strains, by characterisation of well documented isolates of different serotypes.

• This will allow <u>individual virus lineages</u> and therefore virus isolates to be identified more accurately than is possible by serological methods.

• These studies will help to <u>correlate variations and changes</u> in the virus genome with variations in the virus properties (e.g. Serological properties, Geographic origins, Host range, Virulence, Vector range and Primer design etc).

D3.1	Collect relevant sequence and isolation data (Orbivirus sequence database)	M36	0	PU
D3.2	Generate and display phylogenetic trees of BTV isolates and serotypes	M36	R	PU
D3.3	Development of primers for identification of BTV/AHSV types (segment 2)	M36	0	PU
D3.4	Development of primers for identification of BTV/AHSV types (others segment)	M36	0	PU
D3.5	Database of sequences for specific virus isolates (reference collection)	M36	0	PU

Database / reference collection at IAH

BTV isolates	= 780
EHDV isolates	=127
AHSV isolates	= 29
EEV isolates	= 17
Other orbiviruses	~30







Virus Isolates



Reference collection of virus isolates from different geographic regions

 <u>Well documented</u> origins (including location, species, date, passage history, clinical signs, vaccination history, etc), historical and from recent outbreaks.



The reference strains of the 24 different BTV serotypes were supplied by the Onderstepoort Veterinary Research Institute (OVI) S. Africa. However, some of these strains have different geographical origins

Satellite virus ref collections and Isolate data

Family: Reoviridae, Genus: Orbivirus, Species: Bluetongue virus, serotype 1 (BTV-1)

*IAH dsRNA virus collection number (ICTVdb isolate <u>Accession</u> <u>Number)</u>	Virus Species & Serotype (identification method)	Place of original sample eg country town [grid reference] (date original sample was taken)	Isolated from which host species e.g cow, sheep, etc (Date of virus isolation, where, by whom)	Position of storage in reference freezers (Date added to collection) <u>Reference a</u>	Suppliers Isolate Designation	Original sample material, Adapted to which cell types (Passage history of sample) [IAH passage history]	* Original sample supplied to the collection by person / institution [IAH, ISIS sample number]	Comments	References & accession numbers
<u>RSArrrr/01</u> (41010B4F)	BTV Type 1 (Type confirmed by Seg-2 RT-PCR and sequencing)	Supplied by OVI S. Africa*	NK	Shelf B2, boxB2β, rows E1-F10 (added to collection on 17th March 1982)	(Ref. Strain)	Vero, C6/36, KC cells (E2/BHK7) [IAH-BHK2]		Duplicate samples, box B2β- position E2(p) supplied to Oscar R. González Llamazares (Avenida Párroco Pablo Díez 49-57, 24010 - Leon - Spain, 22nd Nov 2007)	[S2: <u>AJ585122</u> , S3: <u>DQ186792</u> , S6: <u>AJ586695</u>] <u>Maan et al 2007</u>

Database / reference collections Space for additional comments and discussion





Orbivirus sequences generated

Current Target: 100 full genomes from selected BTV isolates as geographic reference strains for different lineages from around the world.













Generation of sequence data for other orbiviruses

		Genome segment →	1	2	3	4	5	6	7	8	9	10	Comments
SI. No.	Serogroup Name 🗸	Virus Name 🗸											
1	Warrego (WARV)	Mitchell River virus (MRV)	٧	۷	٧	۷	۷	۷	۷	۷	7	٧	sequenced in our lab
		Warrego virus (WARV)			v/p		v/p		v/ p	v/p			
2	Eubenangee (EUBV)	Tilligerry virus (TILV)	۷	۷	۷	۷	۷	۷	۷	۷	۷	۷	sequenced in our lab
3	Palyam virus (PALV)	Chuzan/Kasba virus (KASV)	٧	۷	٧	۷	۷	۷	۷	٧	۷	۷	
4	Saint Croix River virus	SCRV	٧	۷	V	۷	۷	۷	۷	٧	۷	V	
5	Yunnan Orbivirus (YUOV)	Υυον	۷	۷	۷	۷	v	۷	۷	۷	V	v	
		Middle Point Orbivirus (MPOV)	√/p	7	۷		v/p						
6	Equine encephalosis virus	EEV	۷	>	۷	7	v	۷	۷	7	7	V	
7	Great Island virus (GIV)	Broadhaven virus (BRDV)		۷			۷	7	۷			۷	
8	Pervian horse sickness	PHSV	۷	۷	۷	۷	v	۷	۷	۷	۷	v	
9	Wallal virus (WALV)	Wallal virus (WALV)			v/p								
		Mudjinbarry virus (MUDV)						٧	۷		٧	٧	sequencing of other seqments is in progress
10	Wongorr (WGRV)	Wongorr (WGRV)			v/p								
11	Umatilla virus (UMAV)	Umatilla virus (UMAV)						۷	٧		٧		sequencing of other seqments is in progress
12	Corriparta virus				v/p								
	Tentative species												
	Stretch Lagoo	on Orbivirus (SLOV)	۷	۷									

Sequences

The creation of satellite collections which use the same data storage system for virus isolates and collect the same data, will help to create a virtual reference collection that will support molecular epidemiology studies of BTV and related viruses on a global scale





Diagnostic assays







Currently available diagnostic assay













BBSRC bioscience for the future

Diagnostic assays

Commercialisation / availability





Commercialisation of BTV detection assays with Qiagen







BTV typing kit - LSI



ReoID



Phylogenetic sequence analysis and improved diagnostic assay systems for viruses of the family *Reoviridae*



Edited by Peter. P. C. Mertens and Houssam Attoui

Back to dsRNA virus Homepage

[<u>Home</u>][<u>Up</u>][<u>BTV-2-seg-2-primers</u>][BTV-S2-Primers-Eurotypes][<u>BTV-S2-Field&vaccine</u>]

PCR primers designed to detect and identify Serotypes of BTV found in Europe since 1998

<u>BTV-1</u>	<u>BTV-4</u>	BTV-7	BTV-10	BTV-13	<u>BTV-16</u>	BTV-19	BTV-22
<u>BTV-2</u>	BTV-5	<u>BTV-8</u>	BTV-11	BTV-14	BTV-17	BTV-20	BTV-23
BTV-3	BTV-6	BTV-9	BTV-12	BTV-15	BTV-18	BTV-21	BTV-24

Primers have been designed for amplification and sequencing of Seg-2 from all 25 serotypes. These are under evaluation and will be published soon. They will also be presented on this web-site

http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/BTV-S2-Primers-Eurotypes.htm

Diagnostic assays

Need to maintain and update assays RT PCR assays for typing have a shelf life, so perhaps chips

Elisa Type specific

DIVA





Molecular epidemiology

- 15 BTV strains /lineages of 9 serotypes identified in Europe since 1998.
- New introductions / viruses detected every year.
- Major topotypes identified (Easter and Western).
- Additional strains detected in neighbouring regions (BTV-5, -15, -24, -9w).
- Work in progress with AHSV, EHDV, EEV & other orbiviruses.





15 different Introductions of BTV into Europe since 1998







Molecular epidemiology

More reference strains More isolates (Satellite collection) more sequencing Whole genome Direct from blood Other orbiviruses Other Arboviruses?





Risks to Europe

New BTV serotypes (maintain assays) Role of full genome sequencing? Evidence of Reassortants Role of Israel (Middle East?) as a focus for reassortment (vaccine strains + types 2, 4, 8, 15, 16, 24)

Toggenburg virus / BTV-25 Distinct Topotype /Genotype in the region?

The list of viruses that potentially threaten Europe is open ended New vaccines BTV EHDV AHSV







New BTV strains in 2008/9

BTV-6 (vaccine strain) The Netherlands BTV-11 (vaccine strain) Belgium BTV-25 (Novel Type) Switzerland



Serotyping of BTV from blood samples by conventional RT-PCR (Mertens et al 2007)

Identification of BTV-6 from Holland 2008

2226 1630 1 KB DNA M 723 4: 35F/ 439R – 1211 A182/08/1 2: 301F/790R 3: 153F/853R :155F/710R 3 4 3000 → 2000 1650 → 1000 → **500** → 300 → NET2008/04

Sequence comparison showed 100% sequence similarity to BTV-6 and 11 Vaccine strains

Identification of **BTV-11** from Belgium

Phylogenetic analysis of full length <u>Seg-2 of BTVs: highlighed is type 6 in the</u> <u>Netherlands and type 11 in the Belgium</u>



Sequence comparison showed 100% sequence similarity to BTV-6 and 11 Vaccine strains

Phylogenetic analysis of full length Seg-2 of BTV serotypes But will TOV reassort with other BTVs ?







Hofmann et al (2008) Genetic Characterization of Toggenburg Orbivirus, A New Bluetongue Virus, from Goats, Switzerland.

Emerg Infect Dis, doi 10.3201/eid1412.080818; in press.





Examples of reassortant bluetongue viruses detected in the Mediterranean region

Between field eastern and western strain and vaccine strains	• e.g.Seg-5 of 2SPA2005/01; 2RSAvvvv/02; 16-ITL2002
Between field eastern and western strains	• e.g.Seg-10 of 4 TUR/TR24, 9TUR2000/04 and 9 GRE1998/01
Between western field strains	• e.g. several segments of type 2 and 4 circulating in the western Mediterranean – see Poster by Maan et al, this meeting.
Between eastern field strains	 e.g. several segments of type 9 and 16 circulating in the eastern Mediterranean – Nomikou et al talk this meeting





New reporting system (from reference lab)





New reporting system: Standard format

Bluetongue Molecular Epidemiology Report Form (2008-12-01)

	066 - AR8-RES-FOR-066
Date sample received at IAH: 18 th Nov 2008 IAH -ISIS/STARS sample number: A169/08 no 1 Sender Ref: Animal ID: EL833063440340 Date collected: virus isolated at IAH on 01-12-06 Species: from sheep blood	Diagnostic Report date: 19/11/08 Diagnostic Report sent to: Olga Mangana, Greece. Date cleared for general release: 25 th Nov 2009
Orbivirus Ref Collection No: GRE2008/01 Date received for sequencing: 15-07-2009 Material used: KC coll culture virus Genome segment sequenced: Seg-2 Method used: Anchor ligation Primers used: phased and GS primers Region and No. Nt determined: full length (2939) Gene length: 2939 No. of ambiguities: none	Sequencing Report date: 19/11/09 Prepared by: Narender S.Maan/ Kiki Nomikou Checked by: Sushila Maan/Poter Mertens Serotype: BTV-8 Topotype of Segment analysed: 'Western' Nucleotype: D No. of nt compared: 2939

Comments / conclusions:

The virus isolate (GRE2008/01) was initially identified as BTV-8 by real-time RT-PCR targeting Seg-2 (Assay supplied by Laboratoire Service International (LSI)].

Sequence analyses also confirmed the identification of this virus as BTV-8 (figure 1). This analysis showed that it is most closely related to strains from the European outbreak caused by BTV-8, including the index case from the Netherlands (from 2006 - NET2006/04) and a later strain from Israel (from 2008) (figure 2). GRE2008/01 is distinct from the western strains of type 8 from Nigeria, Oman, Kenva and later strains from South Africa.

	Mo	st Closely Related Vir	uses	
In order of similarity to target sequence	Virus type / name	Reference collection number / [Accession number]	No. int compared / segment length	% Identity
1	8TV-8 Netherlands	NET2006/04 [AM498052]	2939/2939	99%
2	STV-8 Netherlands	[FJ183375]	2939/2939	99%
3	BTV-S Nigeria	NIG1982/07 (AJ585184)	2939/2939	96%
4	BTV-8 South Africa	RSA1992/01	788/2939	94%
5	BTV-8 South Africa	R5A1987/01	708/2939	93.6%
5	BTV-8 South Africa	R5A1998/01	675/2939	93.3%
7	BTV-8 South Africa (reference strain)	RSArmr/08 (AJ585129)	2939/2939	93%
8	ВТУ-8 Келуа	KEN/01 [AJ383183]	2939/2939	93%
8	Relations	hips to Reference Vi	rus Strains	
In order of similarity to target sequence	Virus type / name	Reference collection number / [Accession	No. nt Compared / segment length	35 Identity

We therefore conclude that this isolate/outpreak/incursion, represent an extension of the European outbreak of BTV-8.

	Second to a	number]		
1	BTV-8 South Africa (reference strain)	RSArm7/08 [AJ585129]	2939/2939	93%

* nt nucleotides:

Figure 1: Comparison of Seg-2 nucleotide sequence of BTV-8 from Greece in 2008 (GRE2008/01): comparison to 25 BTV reference strains



No Of Bootstrap Reps = 500

Phylogeny Test and options : Bootstrap (500 Include Sites : ==================== Gaps/Missing Data : Pairwise Deletion

Codon Positions : 1st+2nd+3rd+Noncoding

replicates: seed=64238)

4

23





New reporting system

Designed to give more information and greater precision...will eventually be archived via the dsRNA webpages





Reverse Genetics

Working for BTV and other reoviruses Generic methods for any reovirus dissemination and collaboration (e.g. by Emida)

This is the Future!!!!!





Publications

During Year 3

- ~ 50 refereed papers,
- ~ 20 book chapters
- ~ 50 Accession numbers

Space for additional comments and discussion





2009

- De Clercq, et al (2009). Emerge of bluetongue serotypes in Europe, Part 2: the occurrence of a BTV-11 strain in Belgium. Transboundary and Emerging Diseases. Accepted.
- Brenner, et al (2009). Orbiviral diseases of ruminants in Israel 2006 to 2009. Israel Journal of Veterinary Medicine. 64 (3):90.
- Anthony, et al (2009b). Genetic and phylogenetic analysis of the core proteins VP1, VP3, VP4, VP6 and VP7 of epizootic haemorrhagic disease virus (EHDV). Virus Research 145: 187–199.
- Anthony, et al (2009c). Genetic and phylogenetic analysis of the outer-coat proteins VP2 and VP5 of epizootic haemorrhagic disease virus (EHDV): Comparison of genetic and serological data to characterise the EHDV serogroup. Virus Research 145: 200–210.
- Anthony, et al. (2009d). Genetic and phylogenetic analysis of the non-structural proteins NS1, NS2 and NS3 of epizootic haemorrhagic disease virus (EHDV). Virus Research 145: 211–219.
- Nomikou, et al (2009). Evolution and phylogenetic analysis of full-length VP3 genes of eastern Mediterranean bluetongue virus isolates. PLoS ONE 4(7): e6437. doi: 10.1371/journal.pone.0006437.
- Maan, et al (2009). Molecular epidemiology studies of bluetongue virus. In: "Bluetongue Virus" from the Series: Biology of Animal Infections" (eds. Mellor, P.S., Baylis, M., and Mertens, P.P.C.), Elsevier, London.
- Mertens, et al (2009). Bluetongue virus diagnosis. In: "Bluetongue Virus"from the Series: Biology of Animal Infections" (eds. Mellor, P.S., Baylis, M., and Mertens, P.P.C.), Elsevier, London.
- Temizel, et al (2009). Epizootic haemorrhagic disease in cattle in western Turkey. Emerging Infectious Diseases 15, 317-319.

2008

- Maan, et al (2008). Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. Virology 377, 308–318.
- Batten, et al (2008). A European field strain of bluetongue virus derived from two parental vaccine strains by genome segment reassorment. Virus Research 137, 56–63.
- Yadin, et al (2008). Epizootic haemorrhagic disease virus type 7 infection in cattle in Israel. Veterinary Record. 162, 53-56.

2007

- Mertens, et al (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* 88, 2811–2823.
- Maan, et al (2007a) Analysis and Phylogenetic Comparisons of Full-Length VP2 Genes of the Twenty-Four Bluetongue Virus Serotypes. *Journal of General Virology*, 88, 621–630.
- Maan, et al (2007b), Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. *Journal of Virological Methods* 143(2):132-139.
- Anthony, et al (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.

The Future

- **SNPS** (coverage of BTv-8 across the whole of Europe)
- In depth sequencing
- Reverse Genetics
- Emida and other grants
- Reference collections (other continents (India))
- Space for additional comments and discussion





EMIDA: OrbiNet (Pre-Proposal submitted November 2009)

Combating orbivirus infections of livestock: understanding of the molecular basis for protein function / virus phenotype, molecular epidemiology and improving diagnostic assays.:

Summary

A research network (OrbiNet) will be established, involving partner laboratories in 8 European countries and Israel. OrbiNet will enhance orbivirus research capability, provide training in advanced molecular and diagnostic techniques (e.g. reverse genetics (RG) techniques and reagents currently in use by partners 1 and 7), investigate key aspects of BTV epidemiology, and pathogenesis and develop new reagents and diagnostic techniques, during exchange visits between laboratories. This will facilitate molecular studies of RNA / protein function, virus phenotype, identification / modification of viral epitopes and (potentially) development of novel DIVA / 'marked' vaccine strains.

Partners Prof. Peter Mertens (IAH – UK-England - Coordinator) Dr. Stephan Zientara (AFSSA - France) Dr Martin Beer (FLI –Germany) Dr. Dr. Piet van Rijn (CVI - The Netherlands)

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ANYTHING ELSE???

Four years ago (late 2006) BTV typing for diagnosis and as a basis for vaccination, would take approximately 4 to 6 weeks by conventional serology

Now typing can be achieved in < 4 hours.

Topotyping, lineage identification, full genome analysis and identification of reassortants are all possible

....Life and Bluetongue diagnosis have moved on MedreoNet and Ep+izone are clearly great successes!

There needs to be life after MedReoNet! Plans for further networks ? Framework 8?



