VBD Molecular Epidemiology Report Form (2012-11-30)

066 - ARB-RES-FOR-066

Date(s) samples received at IAH: **21/11/12** IAH -ISIS/STARS sample number: A160/12 number 05 Sender Ref: ID number: none Date collected: end of september Species: Bovine	Diagnostic Report date: 22/11/12 Diagnostic Report sent to: Jan F. Zmudzinski Date cleared for general release: 22/11/12 Follow up emails : 23/11/12
Orbivirus Ref Collection No: not yet assigned (isolation in progress) Date received for typing/sequencing: **22/11/2012** Material used: blood Genome segment sequenced: **Seg-2** Method used: **RT-PCR** Primers used: type specific primers for BTV Seg-2 Region and No. Nt determined: 1294-2132 **bp (840nt)** Gene length: **2922 bp** No. of ambiguities: **0**	Sequencing Report date: 30/11/12 Prepared by: Kiki Nomikou, Sushila Maan, Carrie Batten, **Peter Mertens** Checked by: **Peter Mertens** Serotype: **BTV-14** No. of nt compared: 840 nt

Comments / conclusions:

Five of seven samples were positive by BTV group specific RT-PCR (Shaw *et al* ISO17025 test) with Ct values of 24 – 33). Sample number 05 (blood) was chosen for sequence analysis as it had a high load of RNA (Ct value 27).

Type specific real-time RT-PCR assays for the European BTV serotypes (BTV-1, 6, 8, and 11) were performed in Pulawy Poland and all gave negative results indicating that the samples did not contain these serotypes.

The RNA from sample 05 was tested using type-specific real-time RT-PCR assay for BTV-1, 8 and 14 (provided by AMRG, targeting genome segment 2) in the reference laboratory. Sample 05 gave positive signal with Ct values of 28 for BTV-14. The remaining 4 positive samples were also confirmed as BTV-14 (Ct values 25-34). This indicates that the samples contain Seg-2 RNA of BTV-14. All samples were negative for BTV-1 and 8 RNA.

The RNA from sample 05 was tested by conventional RT-PCR assays, using three sets of BTV-14 specific primers and 2 pairs of experimental 'Nucleotype C' specific primers targeting BTV Seg-2.

A strong cDNA amplicon of the expected size was obtained for Seg-2 (the VP2 gene) with the Nucleotype C' specific primers, which was subsequently sequenced using the same PCR primers, as sequencing primers.

Out of the three BTV-14 Seg-2 specific primers only one pair generated a Seg-2 amplicon, which upon sequencing (with reverse PCR primers) generated VP2 sequence data.

Phylogenetic comparisons of the assembled sequence data confirmed that the Polish strain is BTV-14, with 100% nt identity with the South African reference strain and vaccine strains of BTV-14 (RSArrrr/14 and vaccine bottle A). The Polish strain has significantly lower identity levels with other BTV-14 isolates from Cameroon, USA and Belize (91.8%-88.3%). The Polish strain showed 99.9% nt identity with the Russian BTV strain RUS2011/01 (see earlier report 22/11/2012).

Full genome sequence analysis of the Polish strain (after isolation in cell culture to provide sufficient material) would be required to fully establish its relationship to other previously characterised BTV isolates in other genome segments (and if it represents a reassortant strain).

Discussion.

The similarity in Seg-2 to the reference and vaccine strains, demonstrates that Seg-2 of these strains and the Polish strain were derived from a common and very recent ancestor/source. This is also true for the Russian and Spanish strains (of 2011 – see earlier reports) which showed 99.8 nt sequence identity in a slightly shorter region of Seg-2, to the reference/vaccine strains

Sequence data was provided for Seg-2 of BTV from two viraemic Latvian bovines (by Dr. Ieva Rodze, Head of Animal Disease Diagnostic Laboratory , Institute of Food Safety, Animal Health and Environment BIOR, Latvia by email on 29/11/12. These data showed 99.3 and 100% similarity respectively, to the vaccine / reference strains of BTV-14 over the region 545 to 839 (294bp).

These data collectively suggest the release of the BTV-14 reference or vaccine strain (which are identical in the regions analysed) into the field, possibly indicating the use of a live BTV-14 vaccine in the field. The similarities of these different samples (Russia 2011, Spain 2011, Poland 2012 and Lativia 2012 (data obtained in Latvia)) indicate that they are derived from a common source and suggest significant spread of the virus in the field.

Unfortunately we do not have full genome sequences for the South African vaccine strains, (including BTV-14) so it is difficult to confirm exactly how closely the Polish, Russian and Spanish strains of BTV-14 are related to the vaccine or reference strains in the rest of the genome.

Report prepared by:
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