

Final Report from Bluetongue European Union Reference Laboratory (EURL) on the annual meeting of the EU National Bluetongue Reference Laboratories:

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Joint BTV/AHSV meeting held in Brussels, Belgium: 2nd December 2013

Background:

In July to September 2013, test panels of antisera and EDTA blood for the inter-laboratory comparison test were sent to 48 participating laboratories including the national reference laboratories (NRLs) from 27 EU member-state countries plus 12 non EU countries including Canada, Switzerland, Montenegro, Morocco x 3, Norway, Russia x 2, Serbia, Turkey x 2, Brazil, Algeria, Tunisia and South Africa.

The principal aim of this ring-trial was to assess the ability of laboratories to detect antibodies to various BTV serotypes by ELISA and to assess the diagnostic capabilities of EU NRLs to detect BTV RNA in blood samples.

Participants were asked to test the samples using their 'in-house' ELISA and PCR assays to detect antibodies and viral RNA respectively.

Origin and description of samples sent out in ring-trial:

10 EDTA blood samples:

- 1 BTV14 – SH01/13 POL2012/01 28 dpi ovine blood
- 2 BTV4 GRE2012/01 spiked bovine blood
- 3 Negative bovine blood
- 4 as # 01
- 5 as # 03
- 6 as # 02
- 7 BTV2 ISR2011/02 spiked bovine blood
- 8 BTV14 – SH03/13 28 dpi ovine blood
- 9 as # 07
- 10 as # 08

8 Antisera samples:

- 11 BTV14 – SH03/13 28 dpi ovine serum
- 12 BTV14 – SH06/13 10 dpi ovine serum
- 13 Negative VM07 ovine serum
- 14 BTV2 UN05 ovine serum
- 15 Negative VM07 ovine serum
- 16 EHDV2 PG40 bovine serum
- 17 EHDV6 ME4 37 dpi ovine serum
- 18 BTV4 PF32 (Ex Oman) ovine serum

Serology component of 2013 ring trial:

- All 27 EU member-states participating plus an additional 15 participants took part in the serology component of the 2013 ring trial.

Conclusions: Serology component of 2013 ring trial:

- All commercial assays are ‘fit for purpose’ for the detection of antibodies for BTV and all 27 EU national BTV reference laboratories participating and additional laboratories outside the EU are using the assays effectively.
- EHDV antibody detection ELISA (*LSIVetTM Ruminant EHDV*) used by 12 participants to detect EHDV antibodies in samples 16 and 17. As four of the 12 participants gave a non-negative result for sample 18 there is a the possibility that there are low levels of EHDV antibodies present in the sample or that there was a low level cross-reactivity of the BTV antibodies with the EHDV ELISA. .

Recommendations: Serology component of 2013 ring trial:

- If only ELISA is available in the lab please send samples immediately to the EURL for confirmation of BTV serogroup and serotype.

Virology component of ring trial:

- 27 out of the 28 EU member states plus 16 other participants took part in the PCR component of the 2013 ring-trial.
- 27 EU NRLs used real-time RT-PCR technology. One laboratory (non EU NRL) used gel-based technology as its front line assay, two laboratories (EU NRL) used gel based RT-PCR in conjunction with real time RT-PCR.
- Of the real-time RT-PCR assays performed the Hoffmann *et al*, (2008) assay was performed by 6 laboratories, the Shaw *et al*, (2007) assay was carried out in 13 laboratories, the Toussaint *et al*, (2007) assay was carried out in 5 laboratories and other laboratories used a variety of commercial, in house and other published assays.

Conclusions –Conventional gel based RT-PCR assay technology:

- The conventional RT-PCR described in the OIE manual failed to detect the BTV-4 strain used in this ring trial (GRE2012/01) due to nucleotide differences in the primer regions.

Conclusions – Real-time RT-PCR assay technology (group-specific):

- Real-time RT-PCR assays were used by 27 EU NRLs, with only one laboratory experiencing problems. All NRLs will be provided feedback and advice.

Conclusions – Serotype-specific PCR technology.

- 20 participants (14 non EU) returned optional serotyping results. The majority of laboratories tested samples for BTV-1, 2, 4, 6, 8, 9, 11 and 16.
- The samples in this ring trial only contained BTV-2, 4 and 14.
- Commercial serotyping assays for the identification of the BTV serotypes in these samples are fit for purpose.

- Published assays detected the serotypes in these samples.
- It should be noted that conventional gel based serotyping assays are not always sensitive enough for serotyping weak positive samples.

Recommendations from ring trial (2013)

- If only ELISA is available in the laboratory please send blood samples immediately to the EURL for confirmation of BTV serogroup and serotype.
- Laboratories should send samples from new incursions of BTV in Europe to the EURL for confirmation of both serogroup and serotype and to add to the reference collection.
- The EURL recommends all serotyping of new incursions be confirmed by virus isolation and sequencing and where possible SNT. The EURL is happy to help.

Recommendations for 2014

- Samples of EHDV should be included if possible to allow differential diagnosis.
- Blood from naturally infected animals if possible
- Cell culture for sensitivity testing

Discussions:

Session 1:

BTV occurrence and work of the EURL

Lorraine Frost presented information on the overall BTV situation in Europe and gave data on the BTV-14 animal experiment conducted at the Pirbright Institute. Questions were asked regarding the clinical symptoms of the sheep. Carrie Batten provided information concerning a parasitic infection which may have contributed in the mild symptoms, but stressed the animals were viraemic.

Ring trial

Carrie Batten presented the ring trial data for 2013. Giovanni Savini offered to provide some infectious blood for ring trial 2014.

Session 2:

Piet Van Rijn

Piet presented data suggesting the use of reverse genetics to generate vaccine strains for BTV. He showed results illustrating the exchange of Seg-2 (VP2) and incorporation of mutated Seg-10 with different BTV backbones and described the principle of a Disabled Infectious, single animal (DISA), allowing differentiation of

infected and vaccinated animals. Discussions centred around vaccine development and whether all 26 BTV serotype vaccines would have to be developed separately, and if these would be combined as subgroups in one bottle similar to the South African live attenuated vaccines or produced separately.

It was discussed that the decision of which serotypes were available for each vaccine combination would be the decision of the country that would use them. Piet has sent serum samples from animal experiments using these strains to Ingenasa to show that an NS3 ELISA is working and is developing his own ELISA.

Giovanni Savini

Giovanni reported the recent BTV-1 outbreak in Sardinia which spread to mainland Italy and also to Corsica, this BTV-1 strain was 100% identical to the BTV-1 strain in 2012 which did not spread beyond the island. The reassortment of BTV-1 and BTV-4 was also discussed. Questions arose concerning vaccine availability in Sardinia. Giovanni reported that there was not enough vaccine and that the small number of vaccines they had, were set aside for ensuring animal movement could continue. The vaccine ordering system was discussed as vaccine is only manufactured on a need by basis, so orders have to be made by the end of the year for the following year and outbreaks cannot be predicted.

Stephan Zientara

Stephan reported on the current situation in Corsica. He described the recent BTV-1 outbreak which began in the south of the island affecting 99 herds with 9% morbidity and 2% mortality rate. Vaccination was discussed with compulsory vaccination of all ruminants with Merial BTV 1+8. The map of the affected areas showed gaps between the north, middle and south. Stephan explained that the young animals are in the middle of the island and these are disseminated out to the north and south where every three years there is a naïve population due to 30% animals changed per year. Clinical signs were noted in cattle, but majority of signs seen in sheep. Movements of animals from elsewhere were discussed but this is unknown, however there are some movements between Corsica and mainland France for slaughter. Giovanni discussed that the pattern for the BTV-1 outbreak has mimicked that of the BTV-2 outbreak in 2000 in Italy. Stephan agreed as the first farm affected this year was also the first farm affected in 2000.