

Report from Bluetongue Community Reference lab, Pirbright, UK.

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21st November 2006

Bluetongue serotype analysis for Bulgarian samples:

First set of samples:

The first set of samples received from Bulgaria arrived at the CRL on the 27/10/04. They included five serum samples from the Bourgas district of Bulgaria taken from two calves, two goats and one sheep that were exhibiting no clinical signs. These serum samples were taken from the diagnostic samples originally sent to the Bulgarian reference laboratory. The volumes supplied to the CRL were low in each case. The samples were also haemolysed and bacterially contaminated, making diagnosis difficult.

Results:

All 5 serum samples were tested by group specific cELISA (for antibodies), and in each case gave positive results for BTV specific antibodies (result obtained within 24 hours of receipt). Percentage inhibition values with the cELISA, in duplicate, were as follows: Sheep – 83/83, Goat – 73/71, Goat 80/78, Calf – 77/74, Calf – 91/93 (cut off of test - 50).

A Serum Neutralisation Test (SNT) against all 24 BTV serotypes was also set up within 24 hours of receipt of samples. However, due to the poor state of the samples (bacterial contamination) and the low volumes of sera that were available, the results were inconclusive. No blood samples were received in the first set of samples.

The second set of Bulgarian samples:

A second set of samples were sent from the Bulgarian Reference Laboratory, which arrived at the CRL on the 6/11/06. The samples included matched pairs of serum and EDTA treated blood samples, from 4 calves in Rezovo village in the Bourgas district of Bulgaria. The samples were in a good condition when they arrived and there was sufficient volume to carry out the required tests.

Results:

A group specific cELISA, which was carried out within 24 hours, confirmed the presence of BTV specific antibodies in three of the four samples. Percentage inhibition values with the cELISA, in duplicate were as follows: Calf 1: 89/91, Calf 2: 90/90, Calf 3: 95/97, Calf 4: 44/54 (cut off of test- 50).

RNA was extracted from the blood samples and tested by BTV genome segment 1 specific, real time RT-PCR within 24 hours of receipt. There was no BTV RNA detected in the samples.

The samples were subsequently also tested using a segment 7 specific, conventional RT-PCR assay. Again no BTV RNA was detected in the samples.

The blood samples were inoculated into embryonated chicken eggs. There was no sign of viral infection and the eggs were still alive after 8 days.

The serum samples were used to set up a SNT against all 24 serotypes within 24 hours of receipt of samples. After 7 days, the test results showed that antibodies against BTV serotype 8 were present

(neutralising 100 TCID₅₀) at a dilution of 1:120 for calf 3, 1:30 for calves 1 and 2 and 1:10 for calf 4. No significant neutralisation was detected against most of the other BTV serotypes, although the results using type 11 and 12 were inconclusive (the virus titre was either too high or too low respectively, in control tests).

A second SNT assay was therefore set up to retest for antibodies to type 8, 9 and 11. In this assay neutralisation was only detected against BTV-8, at a highest dilution of 1/160 in calf 3 and at a dilution of 1/40 and 1/30 in calves 1 and 2 respectively.

Discussions and Conclusions:

After the first set of samples from the Bourgas region of Bulgaria were identified as BTV antibody positive in Bulgaria on the 10th of October, additional samples were taken from 4 sentinel calves in Rezoro village (second set of samples). The CRL was informed that these four calves were previously tested by cELISA in June, July, August and September, in each case with negative results, suggesting that the seroconversion happened in late September/early October.

The observation that these sentinel calves were cELISA positive for BTV specific antibodies, but RT-PCR negative for BTV specific RNA, or virus isolation, is unexpected and difficult to explain. If the calves were infected in the time period of about 1 month (since they were previously sampled and found negative) they would be expected to be PCR positive.

In previous studies (including recent animal experiments with the northern European strain of BTV 8) infected animals have become positive for viral RNA in blood samples (by RT-PCR) after 3 to 5 days post infection. They only become positive for BTV specific antibodies (by cELISA), after ~ 7-10 days. It has also been reported that cattle tested by RT-PCR can return to being negative for BTV RNA, after 80 to 180 days post infection, but remain +ve by cELISA for longer periods (years).

A possible explanation of the results obtained at the CRL is that the calves might have been infected >100 days earlier, or may have acquired maternal antibodies, although neither hypothesis fits the reported results from previous testing of these sentinel animals in Bulgaria.

Neutralising antibodies to BTV serotype 8 were identified in 4 of the calves from the Bourgas district of Bulgaria. This was unexpected, as BTV-8 has not previously been detected in Bulgaria. However traditionally, confirmation of the identification of a virus serotype usually requires virus isolation, followed by testing in virus neutralisation assays. More recently virus BTV serotype has been identified and confirmed by segment 2 specific RT-PCR and sequence analysis of the amplicon and comparisons to Seg-2 databases. However, so far all virus isolation attempts and RT-PCR results for the Bulgarian samples have been negative.

In conclusion, the CRL can confirm the presence of BTV specific antibodies, as well as BTV-8 specific neutralising antibodies, in animals in Bulgaria but cannot yet confirm the presence of BTV serotype 8.

The CRL is in discussion with the Bulgarian colleagues and has requested more samples from affected animals in the region.