

BLUETONGUE IN EUROPE, THE PAST, THE PRESENT AND THE FUTURE WITH A FOCUS ON DIAGNOSTICS

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Bluetongue (BT) caused by the insect borne orbivirus BTV (*Orbivirus*, *Reoviridae*) is a notifiable virus disease of ruminants according to the World Organization for Animal Health (OIE). The BTV particle contains ten genome segments S1-10 of double stranded RNA encoding seven structural proteins VP1-7, present in the virus particle, and at least four non-structural proteins NS1-NS4, expressed in the infected cell. Historically, the BTV species consists of 24 BTV serotypes showing no or low cross protection. BTV transmission is almost completely dependent on competent biting *Culicoides* midges. Large parts of the world are endemic for BTVs associated with the presence of competent midge species. In the last decade, expansion of affected areas has been shown; northwards in Europe and the USA and southwards in Australia. In the same period, new serotypes have been discovered. Currently, >28 serotypes have been recognized based on genetic analyses of S2. More importantly, BTV and its competent midge vector seem to establish in formerly BTV-free areas, likely because of global warming and changed environmental conditions beneficial for the competent midges.

Since the end of the 20th century, several BTV serotypes have emerged in Europe (1,2,4,8,9,16) by expansion from northern Africa and the Middle East to southern European countries. In August 2006, North-West Europe was very surprised by incursion of BTV8, a serotype never recorded in or close to Europe before. Large parts of Europe were affected in two following years and has demonstrated re-circulation after the winter ('overwintering') which is considered as free of active midges in the moderate climate. In 2008, BTV6 and 11 closely related to conventional live attenuated vaccines (LAVs) were reported in NW Europe, although use of these LAVs was prohibited in Europe. Today, BTV is still present in southern Europe. Circulation of BTVs is very dynamic over the years and intends to expand northwards, like re-emerging of BTV8 in France, a new BTV4 reassortant in South East Europe, and expansion of BTV3 from Tunis to Sicily, Italy. Because of increased alertness and surveillance programs, new variants and atypical BTV serotypes 25 and 27 has been discovered in Europe. In summary, alertness and diagnostics have reduced losses due to Bluetongue. Safe, efficacious, and preferably broad protective or tailor-made DIVA vaccines but above all accepted and affordable vaccine will be needed to further minimize economic losses eventually aiming the eradication of Bluetongue. Compatible DIVA assays will be needed to survey vaccinated livestock for BTV circulation.

Laboratories and research institutions have developed and incorporated diagnostics for BTV and BTV antibodies. Proficiency testing well organized by the EURL for >10 years demonstrates that EU member states are well equipped to diagnose BT. Generally, serogroup specific real time RT-PCR tests and commercially available ELISAs are used for frontline diagnostics.

BTV detection by virus isolation (VI) as diagnostic test has been completely replaced by high throughput PCR diagnostics. VI is however still operational to culture virus from index cases aiming fundamental virological research. In general, panBTV PCR tests are used to confirm BTV infection followed by serotyping of the virus with serotype specific PCR assays or sequencing of S2. PanBTV PCR tests detect BTV as early as 1-2 days after experimental infection, irrespective of the serotype. Further, PCR positivity lasts much longer than the infectious period, e.g. up to 200 days post infection in cattle. Thus, PCR tests are suitable to diagnose BTV infected animals. BTV PCR tests target BTV specific regions in several highly conserved genome segments, like S1 and S7, but also in highly targets in variable S10. Obviously, serotype specific PCR tests target highly variable S2 encoding serotype dominant VP2 protein. Serotype identification is important for epidemiological studies aiming to trace the source of a BTV outbreak. In addition, the serotype must be determined to vaccinate with the appropriate vaccine. Further, to detect wild type BTV in vaccinated livestock, DIVA PCR tests could be used compatible with used BT DIVA vaccine. Finally, a tremendous progress in the field of next generation sequencing will shorten the time between detection through frontline panBTV PCR testing and detailed like full genome information. Full genome sequences of all kind of BTV variants will become massively available in the future. This genetic information is important for *in silico* validation of panBTV frontline diagnostics and serotype specific PCR tests.

Serogroup specific ELISAs detecting antibodies against immunodominant VP7 protein of BTV are commercially available. Several setups of VP7-based high throughput ELISAs such as competition - ,

indirect -, and sandwich ELISAs have been developed. All assays are sensitive and specific and detect animals as early as one week after experimental infection. VP7 antibodies can also be detected in milk samples. Milk samples of professional dairy cattle farms are often available as these are collected for surveillance programs of other bovine diseases. Detection of BTV-antibodies is very sensitive, and a very low percentage of seropositive lactating cows can be detected by testing one single sample of bulk milk. VP7-based ELISAs cannot be used as DIVA assays, since applied BT vaccines are traditionally virus-based BT vaccines such as LAV or inactivated BTV vaccine inducing high antibody titres against VP7. Neutralizing antibodies are serotype specific but are less sensitive and are only detected later after infection. Anyway, neutralization assays are also time consuming, laborious and therefore expensive. There is a need for serotype specific ELISAs, Elispot assays or other serological tools to determine the serotype specific immune response. At the moment, there is a strong preference for serotype specific PCR assays or next generation sequencing to determine the serotype of circulating BTV.

Expectedly, Bluetongue will maintain and re-emerge in Europe and other parts of the world in the coming decades. Quality controlled, high throughput, rapid BT diagnostics has significantly improved disease control but rapid serotype specific diagnosis and detailed identification of emerging BTVs should be improved. Further, safe, efficacious, affordable and generally accepted BT DIVA vaccines will reduce losses. For this, highly specific and sensitive DIVA assays that are compatible with the used vaccine must be developed and validated, primarily by comparison with frontline diagnostics for Bluetongue.