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Bluetongue: an overview of recent trends in diagnostics

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Summary

Bluetongue (BT) virus (BTV) is a species in the genus *Orbivirus* of the family *Reoviridae*; it causes a viral disease of sheep and occasionally cattle, and is transmitted by biting midges of the genus *Culicoides*. BTV replicates in the haemopoeitic and endothelial cells of the blood vessels. Rarely, and only when a bull is viraemic, may BTV be recovered from semen. Serological techniques, most notably enzyme immunoassays, based on the detection of a group antigen, have been used where required in the certification of animals as 'bluetongue-free'. The various techniques used for diagnosis are viral isolation, the enzyme-linked immunosorbent assay and the polymerase chain reaction.

Keywords

Bluetongue – Diagnosis – Sheep – Viral disease.

Introduction

Bluetongue (BT) is a viral disease of sheep and occasionally cattle, and is transmitted by biting midges of the genus *Culicoides*. Bluetongue virus (BTV) is a species in the genus *Orbivirus* of the family *Reoviridae*. There is considerable genetic variability within the serogroup of BTV which has at least 24 serotypes worldwide. This arises by genetic drift of individual gene segments when ruminants or the vectors are infected with more than one strain. Until the 1940s, this disease was recognised only in Africa, then following a major epidemic in 1956-1957 in Portugal and Spain, the disease was recognised in the United States of America (USA), the Middle East, Asia and later in Australia (1).

BTV replicates in the haemopoeitic and endothelial cells of the blood vessels. Rarely, and only when a bull is viraemic, BTV may be recovered from semen.

BTV is often difficult to isolate in the laboratory. The success of virus isolation is enhanced if blood is collected from animals showing clinical signs and at an early stage of the disease. Viraemia is primarily associated with red blood cells and leucocytes and the virus can coexist in infected animals with high concentrations of neutralising antibody. Serological techniques, most notably enzyme immunoassays, based on the detection of a group antigen have been used where required to certify animals as 'bluetongue-free'. However, intermediate serological reactions have been a major problem. For accuracy in diagnosis, more sensitive and specific assays, such as those based on antigens produced by recombinant DNA technologies and the polymerase chain reaction (PCR) should prove useful (2)

Diagnostic techniques for bluetongue: identification of the agent

Virus isolation

The same diagnostic procedures are used for domestic and wild ruminants. A number of virus isolation systems are in common use, but two of the most efficient are embryonated chicken eggs (ECE) and sheep. Identification of BTV following inoculation of sheep may still be a useful approach if the titre of virus in the sample blood is very low, as may be the case several weeks after infection. Attempts to isolate virus in cultured cells *in vitro* may be more convenient.

Isolation in cell culture

Virus may also be isolated in mouse L, baby hamster kidney (BHK)-21, African green monkey kidney (Vero) or *Aedes albopictus* (AA) cells in culture. The efficiency of isolation is often significantly lower following direct addition to cultured cells compared with that achieved in ECE. Greatest efficiency of isolation in cell culture is achieved by first passaging ECE homogenates in AA cells, followed by either

antigen detection procedures or additional passages in mammalian cell lines, such as BHK-21 or Vero. A cytopathic effect (CPE) is not necessarily observed in AA cells. Cell monolayers are monitored for the appearance of a CPE for 5 days at 37°C in 5% CO_2 with humidity. If no CPE appears, a second passage is made in cell culture. The identity of BTV in the culture medium of cells manifesting a CPE may be confirmed by antigen-capture ELISA, immunofluorescence, immunoperoxidase or virus neutralisation (VN) tests.

Immunological methods

Serogrouping of BTV

Orbivirus isolates are typically serogrouped on the basis of their reactivity with specific standard antisera that detect proteins, such as VP 7, that are conserved within each serogroup. The cross-reactivity between BT and epizootic haemorrhagic disease (EHD) viruses raises the possibility that an isolate of EHD virus could be mistaken for BTV on the basis of a weak immunofluorescence reaction with a polyclonal anti-BTV antiserum. For this reason, a BT serogroup-specific monoclonal antibody (MAb) can be used. A number of laboratories have generated such serogroup-specific reagents (3). In contrast to serogrouping, the usual method of serotyping is by VN testing using methods described below. Commonly used methods for the identification of virus to serogroup level are as follows:

a) immonofluorescence
b) antigen-capture enzyme-linked immunosorbent assay (ELISA)
c) immunospot test
d) indirect peroxidase/antiperoxidase identification.

Serotyping by virus neutralisation

Neutralisation tests are type-specific for the 24 BTV serotypes currently recognised and can be used to serotype a virus isolate, or can be modified to determine serotype of antibody. In the case of an untyped isolate, the characteristic regional localisation of BTV scrotypes should generally obviate the need to attempt neutralisation by all 24 antisera, particularly when endemic serotypes have been identified.

There is a variety of tissue-culture-based methods available to detect the presence of neutralising anti-BTV antibody. Cell lines commonly used are BHK, Vero and L 929. BTV serotype-specific antisera generated in guinea-pigs or rabbits have been reported to have less serotype cross-reactivity than those made in cattle or sheep. It is important that antiserum controls be included to ensure that an effective level of standard antiserum is used against comparable and standardised titres of standard and untyped virus. Four methods to serotype BTV are outlined briefly below:

a) plaque reduction
b) plaque inhibition
c) microtitre neutralisation
d) fluorescene inhibition test.

Polymerase chain reaction

Primer-directed amplification of viral nucleic acid has revolutionised BT diagnosis. Results to date indicate that PCR techniques may be used, not only to detect the presence of viral nucleic acid, but also to 'serogroup' orbiviruses and provide information on the serotype and possible geographic source (topotype or genotype) of BTV isolates within a few days of receipt of a clinical sample, such as infected sheep blood. Traditional approaches, which rely on virus isolation followed by virus identification, may require at least three to four weeks to generate information on serogroup and serotype and yield no data on the possible geographic origin of the isolated virus.

Oligonucleotide primers used to date have been derived from RNA 7 (VP7 gene), RNA 6 (NS1 gene), RNA 3 (VP3 gene) and RNA 2 (VP2 gene). The size of the amplified transcripts is usually small (in the order of several hundred nucleotides) but can also be a full-length gene. In the procedure, a 101 nucleotide stretch of RNA 6 is amplified. Primers derived from the highly conserved genes, such as VP3, VP6, VP7, NS1 and NS3, may be used for serogrouping (i.e. they will react with all members of the BT serogroup) and topotyping (i.e. they will react with BTV isolates from the same geographic area), while primers whose sequence was determined from VP2 gene sequences provide information on virus serotype.

The capacity of PCR assays to detect single molecules of nucleic acid means that such tests are exquisitely sensitive to contamination by extraneous nucleic acids.

The PCR assay involves three separate procedures. In the first, BTV RNA is extracted from blood using a chaotropic agent such as guanidine thiocyanates (GuSCN) to denature protein and release viral RNA. A number of commercial kits are available for this purpose and the protocol describes the use of one such kit: IsoQuick (Orca Research, Bothell, Washington, USA). The reagents provided with the kit are numbered and their use is indicated in the protocol. Other kits are available and one, Trizol™ (Life Technologies, Grand Island, New York), is particularly useful for the extraction of viral nucleic

acid from spleen or blood clots. Operators should follow the procedures specified in each kit and use reagent solutions either provided or recommended for the kit of their choice. The second procedure is the denaturation of viral double-stranded RNA and reverse transcription (RT) to generate DNA, which is amplified by PCR. In the procedure, the Superscript™ Preamplification System (Life Technologies) is used to transcribe viral RNA and reagents from Perkin-Elmer are used for the PCR. Equivalent kits and reagents are available from other sources. The final step of the process is the analysis of the PCR product by electrophoresis (3).

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