

REVIEW ARTICLE

Bluetongue virus: a known virus, a current threat

Ana María Acevedo, Yoandry Hinojosa, Damarys Relova, Carmen Laura Perera

Grupo de Virología Animal, Departamento de Microbiología, Centro Nacional de Sanidad Agropecuaria (CENSA),
Apartado 10, San José de las Lajas, Mayabeque, Cuba. E-mail: acevedo@censa.edu.cu.

ABSTRACT: Bluetongue is a non-contagious acute viral disease affecting ruminants. It is a disease of great clinical importance for the physical deterioration and the long convalescence that it causes and, from the economic point of view, for the colossal production losses and prevention and control expenses. It is highly feared in spite of being innocuous for man and of only medium mortality. Bluetongue virus spreads from animal to animal by biting insects of the genus *Culicoides* and this is the reason for the disease being more prevalent in the geographic areas where climate conditions are favorable for the insect development. The disease was described in detail in early 1900, and at present, is one of the main concerns in animal health, being studied in order to clarify its epidemiology and pathogenesis and to control its spreading. In this review, an attempt has been made to summarize some aspects of the disease related to its history, economic importance and etiological agent, the viral genome organization, the disease epidemiology and distribution, its clinical signs and lesions, as well as its diagnosis.

Key words: Bluetongue, bluetongue virus, epidemiology, clinical signs, diagnosis.

Virus de la Lengua azul: un virus conocido, una amenaza presente

RESUMEN: La Lengua azul es una enfermedad viral no contagiosa, aguda, que afecta a los rumiantes. Es una enfermedad de gran importancia clínica por el deterioro físico y la larga convalecencia que provoca y, desde el punto de vista económico, por las colosales pérdidas de producción y gastos de prevención y control. Es muy temida pese a ser inocua para el hombre y de solo mediana mortalidad. El virus se transmite entre los animales por picaduras de insectos del género *Culicoides*, lo que hace que su prevalencia sea mayor en las zonas geográficas donde el clima favorece el desarrollo de los mismos. Esta enfermedad se describió en detalle a principios del siglo XIX y aún hoy constituye una de las principales preocupaciones en la salud animal, por lo que se continúa estudiando para aclarar su epidemiología, patogenia y controlar así su diseminación. Esta revisión tiene como objetivo resumir algunos aspectos de la enfermedad relacionados con su historia, importancia económica, agente etiológico, organización del genoma viral, la epidemiología y la distribución, los signos clínicos y lesiones, así como el diagnóstico de la enfermedad.

Palabras clave: Lengua azul, virus de la lengua azul, epidemiología, signos clínicos, diagnóstico.

INTRODUCTION

Bluetongue (BT) is an insect-transmitted viral disease of ruminant species. It is listed as a "notifiable disease" by the Mundial Organisation for Animal Health. This disease is caused by Bluetongue virus (BTV) (1).

BTV infection of ruminants is not contagious; the virus is transmitted among hosts almost entirely by the bites of certain species of *Culicoides* biting midges. Thus, the distribution of BTV is restricted to areas where competent vector species are present, and

transmission is limited to those times of the year when climatic conditions are appropriate and adult insects are active (2). Major signs are high fever, excessive salivation, swelling of the face and tongue and cyanosis of the tongue. Nasal symptoms may be prominent, with nasal discharge and stertorous respiration. Some animals also develop foot lesions (1). Laboratory confirmation is based on virus isolation in embryonated chicken eggs or mammalian and insect cell cultures. For the identification of viruses to serogroup level, immunofluorescence, antigen capture enzyme-linked

immunosorbent assay or immunospot test are used. Reverse-transcription polymerase chain reaction (RT-PCR) and real time RT-PCR (rRT-PCR) have allowed the rapid identification of BT viral nucleic acid. Serologic techniques for the detection of BTV antibody include complement fixation, agar gel immuno-diffusion and competitive enzyme-linked immunosorbent assay, indirect ELISA (1, 3).

HISTORY OF DISEASE

The bluetongue disease was already recognized in South Africa in the early 19th century, where it was initially called Malarial Catarrhal Fever or Epizootic Catarrh of Sheep. The name of «bluetongue» was later adopted to describe the distinctive cyanotic tongue of some affected sheep. A comprehensive description of the disease was not published until the first decade of the 20th century (4, 5). In 1906, Arnold Theiler showed that bluetongue was caused by a filterable agent (6).

For many decades bluetongue was thought to be confined to Africa. It first achieved prominence outside Africa during the 1950s and 1960s, when it caused major outbreaks of the disease in Europe, killing over 179 000 sheep. Its identification at around the same time in the Americas and Asia confirmed in the minds of many farmers, scientists and legislators that it was an emerging disease that could devastate the livestock industries of the most advanced farming nations in Europe, the Americas, and Australia. For these reasons, it was allocated the status of a List 'A' disease by OIE.

The first confirmed outbreak outside Africa occurred in Cyprus in 1943 (7). Subsequent outbreaks happened in this region, for example, in Israel (1949) (8), Pakistan in 1959 (9) and in India in 1963 (10). BTV was isolated and identified from Californian sheep samples (11).

The first occurrence of BT in Europe, outside Cyprus, was in 1956 when the disease was diagnosed in southern Portugal (12) and shortly thereafter also in Spain (13). The BTV infection was extended into the Middle East and Asia and eventually into Australia and Oceania (14, 15, 16).

During the 1970s and 1980s, in the absence of major outbreaks of the disease, the perception that BTV had reached its potential to emerge and should be down-graded in importance and removed from the OIE List 'A' category grew. More recently, possibly as a result of climate change or other environmental changes, BT has once again captured the attention of

much of the developed world, with extensions of new serotypes into North America, Australia and Europe.

ECONOMIC IMPORTANCE

The economic losses due to BT are not expressed in exact numbers, but the estimate is of three thousand million dollars per year in the United States of America. The losses are direct (death, abortion, loss of weight, decreased milk production and efficiency of meat production) and mainly indirect as a consequence of the restrictions to the export of live animals, semen and some products like bovine fetal serum. The costs of the preventive measures and control should also be taken into account. In the cases of higher disease prevalence, the control measures may have a serious impact on the amount of meat and animal products available for the consumption market (17).

ETIOLOGY

Bluetongue is caused by the pathogenic virus of the genus *Orbivirus*, Reoviridae family, species *bluetongue virus* (18). Twenty-six serotypes are now recognized for this virus (19). The virus particle consists of ten strands of double-stranded RNA surrounded by two protein shells. Unlike other arboviruses, BTV lacks a lipid envelope. The particle has a diameter of 86 nm (18).

VIRAL ORGANIZATION OF GENOMA

VIRAL PROTEINS

Seven of the viral proteins (VP1-VP7) are structural and form the double-shelled virus particle. At least four non-structural proteins (NS1, NS2, NS3, NS3A and NS4) have been identified. The internal core is formed by two layers containing VP1, VP3, VP4, VP6 and VP7 [encoded by genome segment (Seg)-1, Seg-3, Seg-4, Seg-9 and Seg-7, respectively]. These core proteins and three of the non-structural proteins (NS1, NS2 and NS4, encoded by Seg-5, Seg-8 and Seg-9, respectively) are thought to be relatively conserved and are antigenically cross-reactive between different strains and serotypes of BTV (20).

Among the most studied proteins of the virus are VP2, VP5, and VP7. VP2 and VP5 are two major viral proteins that compose the outer capsid encoded by Seg-2 and Seg-6, respectively, which determine the antigenic variability of BTV (21). Seg-2 and Seg-6 show the highest levels of sequence variation in the BTV genome (3); VP2 correlates perfectly with virus

serotype, whereas VP5 shows partial correlation with virus serotype. However, these segments vary within each serotype and correlate with the geographical origin of the virus strain (Seg-2 and Seg-6 topotypes). NS3/NS3A proteins, encoded by Seg-10, have been associated with the release of virus particles from insect cells (22). The internal core protein VP7 can mediate surface attachment, penetration and infection of insect cells (3). Seg-7, encoding VP7, also shows significant variations (23), despite the role of VP7 as the main serogroup-specific antigen. It has been suggested that this variation could also be related to the insect populations that act as vectors for different virus strains in different geographical areas (23).

EPIDEMIOLOGY AND DISTRIBUTION

Bluetongue occurrence is seasonal in the affected Mediterranean countries, subsiding when temperatures drop and hard frosts kill the adult midge vectors (24) which may promote viral survival and vector longevity during milder winters. A significant contribution to the northward spread of Bluetongue disease has been the ability of *Culicoides obsoletus* and *C. pulicaris* to acquire and transmit the disease, both of which are spread widely throughout Europe. This is in contrast to the original *C. imicola* vector which is limited to North Africa and the Mediterranean. The relatively recent novel vector has facilitated a far more rapid spread than the simple expansion of habitats north through global warming.

During the period from late 2006 to 2007, BTV appears to have fulfilled or exceeded all of the concerns previously raised about its northerly extension in range due to climate change and the involvement of Palearctic vector species of *Culicoides* in the most northerly areas. During 2006 a series of incursions of BTV into various parts of the European region occurred; the most significant incursion by far was the introduction of BTV-8, a serotype new to Europe, into locations far beyond the usual range of any BTV and indeed further north than BTV had ever been recorded anywhere in the world (e.g., up to 53°N). This outbreak was first detected in the Maastricht area of the Netherlands in August 2006. Cases of bluetongue were found not only in the Netherlands, also in Belgium, Germany, and Luxembourg. In 2007, the first case of bluetongue in the Czech Republic was detected in one bull near Cheb at the Czech-German border. In September 2007, the UK reported its first ever suspected case of the disease, in a Highland cow on a rare breed farm near Ipswich, Suffolk. Since then the virus has spread from cattle to sheep in Britain. By October 2007, bluetongue had

become a serious threat in Scandinavia and Switzerland, and the first outbreak in Denmark was reported. In autumn 2008, several cases were reported in the southern Swedish provinces of Småland, Halland, and Skåne, as well as in areas of the Netherlands bordering Germany, which prompted veterinary authorities in Germany to intensify controls. Norway saw its first finding in February 2009, when cows at two farms in Vest-Agder in the south of Norway showed an immune response to bluetongue. Norway was declared free of the disease in 2011 (25).

There are substantial data about the presence of BTV in North America, Central America and the Caribbean. Serotypes 1, 2, 3, 5, 6, 10, 11, 13, 14, 17, 19, 22, and 24 have been detected in North America, whereas serotypes 1, 3, 4, 6, 8, 12, and 17 have been identified in Central America and the Caribbean. However, in South America, information regarding detection of BTV is limited to very few reports. Serological evidence for the presence of BTV has been reported in Peru (26), Argentina (27), Brazil (28), Ecuador (29), and Chile (30). The serotypes found by serological techniques to be probably present in South America are: 4, 6, 14, 17, 19, and 20 in Brazil (31); 12, 14, and 17 in Colombia (32); 14 and 17 in Guyana; and 6, 14, and 17 in Suriname (33). Brazil and Argentina are the only countries in South America where BTV has been isolated. Clavijo *et al.* (34) reported the first isolation of BTV in Brazil, and typed it as serotype 12 by the seroneutralization test (SNT). In Argentina the serotype 4 was determined by SNT and RT-PCR (35).

The disease is not a threat to humans. It affects domestic and wild ruminants and, although severe forms of the disease are most frequent in sheep and white-tailed deer, clinical signs can also occur in cattle, goats and camelids (36). Although infection is often unapparent in these other species, they can act as reservoirs, remaining viraemic for several months (particularly in cattle).

CLINICAL SIGNS AND LESIONS

Bluetongue occurs principally in sheep and some species of wild ruminants. BTV infection of cattle, goats and most wild ruminant species is typically asymptomatic or subclinical (37).

The clinical signs of BTV infection are also highly variable even in susceptible species such as sheep, reflecting inherent differences in the susceptibility of different sheep breeds, as well as of individual animals and external stressors such as solar irradiation that

can exacerbate the clinical signs of BT. The signs of BT in sheep are the result of virus-mediated vascular injury that produces oedema, hyperaemia and vascular congestion, haemorrhage and tissue infarction (38). Thus, sheep with acute BT have any combination of fever, anorexia and malaise, respiratory distress, excessive salivation, serous to bloody nasal and ocular discharge that becomes increasingly mucopurulent so that crusty exudates accumulate around the nostrils, petechial and ecchymotic haemorrhages in the mucous membranes of the oral and nasal cavities, oral erosions and ulcers, lameness and/or a stiff gait, hyperaemia and haemorrhage of the coronary band, oedema of the head and neck (including the ears) and congestion and focal haemorrhages in the conjunctiva and skin. The swollen and cyanotic tongue that gives the disease its name is uncommon. Mortality rates vary from 0% in mild outbreaks to 30% or even higher in outbreaks caused by virulent strains of BTV in highly susceptible breeds of sheep. Most animals that succumb to acute BT die within 14 days of infection

Sheep that survive the acute disease can have a prolonged convalescence and some of them will show substantial deterioration in body condition and become emaciated. BTV induced muscle injury and necrosis prevent normal locomotion in some animals and can lead to torticollis (wryneck). The wool of convalescent sheep is frequently shed (wool break), and some sheep shed their hooves. These chronically affected sheep may succumb to other diseases such as bacterial pneumonia.

The post-mortem lesions of severe acute BT in sheep include vascular congestion, haemorrhage, erosion and ulceration of the mucosa of the upper gastrointestinal tract (oral cavity, oesophagus, forestomachs) and nasal cavity; sub-intimal haemorrhages in the pulmonary artery; pulmonary oedema with abundant froth in the trachea; pleural and/or pericardial effusion; oedema within the fascial planes of the muscles of the abdominal wall and necrosis of skeletal and cardiac muscle with the papillary muscle of the left ventricle being an especially characteristic site (38).

Not all animals develop symptoms, but all those that do lose condition rapidly, and the sickest animals die within a week. For affected animals which do not die, recovery is very slow lasting several months.

The incubation period is 5-20 days, and all symptoms usually develop within a month. The mortality rate is normally low, but it is high in susceptible breeds of sheep. In Africa, local breeds of sheep may have no mortality, but in imported breeds it may be up to 90

percent. Despite high virus levels in blood, infection is usually asymptomatic in cattle, goats, and wild ruminants. Red deer are an exception, and in them the disease may be as acute as in sheep (39).

DIAGNOSIS

Diagnosis of BT is the identification of animals that are or have previously been infected with the BTV.

CLINICAL DIAGNOSIS

Clinical signs as an early indicator of BTV infection. Recognition of the clinical signs of BT can provide an early indication of infection and forms a basis for «passive surveillance», particularly in areas where BTV is exotic and not the subject of routine «active surveillance». Clinical diagnosis can therefore be of critical importance, as BTV like many other exotic diseases can spread rapidly and may quickly become established within a naive and susceptible host population. Early diagnosis leading to a rapid implementation of control measures is therefore vital. The infection may be widespread before the first clinical signs are detected. The incursion of BTV-8 in northern Europe was first detected in the Netherlands although retrospective investigations indicate that the disease had been present in the region for several weeks, with many animals already infected in the Netherlands, Belgium and Germany (40).

None of the typical clinical signs of BT in sheep and cattle are pathognomonic. Any disease that causes haemorrhage or oedema could be confused with BT, such as foot-and-mouth (cattle, sheep and goats, camelids, pigs), vesicular stomatitis (horses, pigs, cattle, sheep and goats) malignant catarrhal fever (cattle), mucosal disease and/or bovine viral diarrhoea (cattle), orf (sheep), sheep and goat pox (sheep and goats), peste des petite ruminants (sheep and goats), rinderpest (cattle), lumpy skin disease (cattle), infectious bovine rhinotracheitis (cattle), bovine papular stomatitis/pseudo-cowpox (cattle), bovine herpes mammillitis (cattle), contagious ovine digital dermatitis (sheep), foot rot (sheep), clostridial enterotoxaemia (sheep), non-infectious diseases: photosensitisation (cattle and sheep), cobalt deficiency (sheep), poisoning (e.g., bracken) (cattle and sheep) (41).

The severity and range of clinical signs caused by BTV in its hosts can also vary significantly and may be influenced by a number of factors such as:

- The species breed and even individual animal variations within a host species. BTV infection, particularly in cattle, is frequently asymptomatic.

- Age of the animal. There is some indication that older animals may be more severely affected.
- Differences among infecting virus strains can influence the severity of the clinical signs elicited, ranging from inapparent to fatal.
- The immune status of the host. Antibodies generated by previous infections with the same, or even different strain/serotypes of the virus, or maternal antibodies (colostrum), may have particular significance in determining the outcome of the infection.
- Environmental stress (e.g., high levels of solar radiation) can significantly influence the severity and outcome of the disease caused by BTV infection.

The clinical diagnosis of BT always requires confirmation by laboratory testing. Diagnostic samples must be sent to a laboratory where BTV can be detected and identified by appropriate diagnostic assay systems.

LABORATORY DIAGNOSIS

A positive diagnosis usually involves detection and identification of BTV-specific antigens, antibodies or RNA in diagnostic samples taken from animals potentially infected using virus isolation and serological or molecular assays to identify the virus serogroup and serotype.

The BT «serogroup» reflects the presence of antigens or RNAs that are both conserved and cross-reactive between the different members of the virus species this includes most of the BTV non-structural and structural proteins of the virus core (particularly VP7). Consequently, serogroup-specific assays can detect any BTV strain and can be used to distinguish it from the other orbiviruses.

BTV serotype is determined by the BTV outer capsid proteins VP2 and VP5, particularly VP2, which primarily controls the specificity of interactions with neutralising antibodies in serum neutralisation assays. In recent years, multiple strains of different BTV serotypes have been introduced into Europe and other areas, either by natural incursion or by the use of live attenuated vaccines. It is particularly important to know which serotype and strain of BTV is circulating in a region to ensure the use of an appropriate vaccine. These different BTVs can now also be detected and differentiated by RT-PCR and phylogenetic analyses targeting segment 2 (Seg-2) of the virus genome, which codes for outer capsid protein VP2 (42; 3). However, BTV also includes a number of distinct geographical variants (topotypes) and subtypes, which show distinctive nucleotide sequence variations in each of their genome segments (including segment 2) (42).

Any of these serotypes, topotypes, and subtypes has the potential to cause disease.

The identification of any individual «type» of BTV can be used to demonstrate conclusively that the virus belongs to the BTV serogroup/species and can therefore be used to confirm an initial diagnosis. Indeed, the identification of a specific BTV serotype is cited as one of the most reliable methods of BTV diagnosis (43).

Many of the different assays developed to identify BTV, or animals that have been infected with the virus, are commonly referred to as «group-specific». BTV group-specific assays include serological methods to detect BTV-specific antibodies generated during infection of the mammalian host, serological assays to detect and identify BTV-specific protein antigens, and molecular techniques such as RT-PCR and cDNA sequencing/phylogenetic analyses that can be used to detect and identify BTV RNA extracted from diagnostic samples (e.g., blood, spleen, and insects). These assays may also involve or depend on the isolation of the virus and its growth in cell culture.

DIAGNOSTIC TECHNIQUES

1- Identification of the BTV agent

a) Virus isolation. The same diagnostic procedures are used for domestic and wild ruminants. A number of virus isolation systems for BTV are in common use, but the most sensitive method is by inoculation of embryonated chicken eggs (ECE). Inoculation of sheep may also be a useful approach if the viral titer in the blood sample is very low, as may be the case several weeks after virus infection. Attempts to isolate virus in cultured cells *in vitro* may be more convenient, but the success rate is frequently much lower than that achieved with *in vivo* systems (44).

b) Immunological methods

• **Serogrouping of viruses.** Orbivirus isolates are typically serogrouped on the basis of their reactivity with specific standard antisera that detect proteins, such as VP7, that are conserved within each serogroup. A BT serogroup-specific MAb can be used. A number of laboratories have generated such serogroup-specific reagents. Commonly used methods for the identification of viruses to serogroup level are: immunofluorescence, antigen capture enzyme-linked immunosorbent assay, immunospot test (45).

• Serotyping by virus neutralization

Neutralization tests are type specific for the currently recognized 26 BTV serotypes and can be used to serotype a virus isolate or can be modified to determine

the specificity of antibody in sera. In the case of an untyped isolate, the characteristic regional localization of BTV serotypes can generally obviate the need to attempt neutralization by all 26 antisera, particularly when endemic serotypes have been identified.

c) RT-PCR, sequence analyses and phylogenetic comparisons, molecular probes and electrophoresis.

-RT-PCR techniques have allowed the rapid identification of BT viral nucleic acid in blood and other tissues of infected animals. The RT-PCR procedure will detect virus-specific nucleic acid, but this does not necessarily indicate the presence of infectious virus. RT-PCR can also be used to 'serogroup' Orbiviruses and may ultimately be possible to 'serotype' BTV within a few days of receipt of a clinical sample, such as infected sheep blood (3).

-rRT-PCR tests there are two published real-time assays that have been shown to detect all 26 serotypes (46). A further development is of triplex and duplex assay kits that detect a combination of groups, BTV-8, BTV-1, BTV-6, and internal control.

2- Serological tests

Complement fixation, agar gel immunodiffusion competitive enzyme-linked immunosorbent, indirect ELISA (1).

Anti-BTV antibodies generated in infected animals can be detected in a variety of ways that vary in sensitivity and specificity. Both serogroup-specific and serotype-specific antibodies are elicited and if the animal was not previously exposed to BTV, the neutralizing antibodies generated are specific for the serotype of the infecting virus. Multiple infections with different BTV serotypes lead to the production of antibodies capable of neutralizing serotypes to which the animal has not been exposed.

CONCLUSIONS

Despite the significant advances and developments achieved in BTV diagnosis, further efforts in this regard continue being required due to the expansion of this virus into new areas and the need for better and faster assays. The advent of real-time RT-PCR assays has already increased sensitivity and the speed with which BTV can be detected and identified, and these technologies are currently being applied to the typing of BTV in diagnostic samples. However, it is important to keep in mind that the segmented nature of the BTV genome and the ability of different virus strains to reassort their genome segments make genome segment 2 (and possibly segment 6) the only valid targets for

serotyping assays. Variations in the other genome segments do not reliably correlate with virus serotype (47). Real-time RT-PCR assays to detect and identify BTV serotypes 1 and 8 are commercially available. Assays for detecting a specific strain of BTV can be rapidly designed and produced; however, the design and validation of those assays that are reliable serotype-specific take much longer and are more difficult to undertake, but the development of such monoplex and multiplex real-time assays that can be used to detect and distinguish all 26 BTV serotypes is currently underway.

Robust and reliable 'pen-side' assays for BTV based either on antibody/antigen detection methods or on RT-PCR could offer significant and possible vital time savings in diagnosis. The further development of probe-based diagnostic systems, using libraries of oligonucleotides printed on chips, could enable detection of BTV and other orbiviruses even to serotype level. Printing of different peptide or protein antigens into ELISA plates also has the potential to allow testing of antibodies against a wide range of pathogens in a single plate, or even a single well. However, distinguishing between animals that have received live-attenuated or semi-purified inactivated BTV vaccines and naturally infected animals is likely to remain problematic. The development of the next generation of subunit vaccines will ease the problems of designing and producing an effective DIVA assay.

The scientific community must continue working for more sensible and robust diagnostic methods so that this viral disease be controlled in a more effective way to avoid or minimize the damages caused by it.

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