EPIDEMIOLOGY AND DIAGNOSIS OF BLUETONGUE IN EUROPE

Stéphan Zientara, Corinne Sailleau, Emmanuel Breard

Agence Française de Sécurité Sanitaire des Aliments, UMR 1161 Afssa/INRA/ENVA
Maisons-Alfort, France
s.zientara@afssa.fr

1. GENERAL INTRODUCTION

Bluetongue (BT) is an Office International Epizooties (OIE) list arthropod-borne viral disease caused by the BT virus (BTV). The virus is infectious but non-contagious, affecting domesticated and wild ruminants. Disease is usually limited to fine wool and mutton breeds of sheep and some species of deer. The BTVs are transmitted between ruminant hosts almost entirely by the bites of certain species of Culicoides biting midges. C. imicola is considered to be the most efficient vector in Africa, Asia and Europe. 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 favourable and adult insects are active (Mellor & Boorman, 1995). Globally, BTV is considered to be present between approximate latitudes 35°S and 40°N, although in parts of western North America and China BTV may extend up to almost 50°N (Dulac et al. 1989; Qin et al. 1996; Kirkland et al. 2002).

BT was first observed in imported Merino sheep in the temperate areas of South Africa in the 19th century and was first described as “malarial catarrhal fever of sheep” in 1902 (Spreull, 1902). Disease was proven to be caused by a filtrable agent in 1905 (Spreull, 1905) having a vertebrate host range that included both sheep and cattle. By 1918, BT had been described in West Africa and several other countries in sub-Saharan Africa (Tomori et al. 1992).

Excursions of BTV outside of Africa were first reported in Cyprus (1943) and in North America (1940), although the causal agent in America was not isolated until 1952 (McKercher et al. 1953). In 1950s, BTV was identified in Israel and the Indian subcontinent. Incursions of BTV into Europe are relatively infrequent; the Iberian Peninsula in 1956 Cyprus 1977 and the Greek island of Lesbos in 1979 but these epizootics have not persisted and have involved only a single BTV serotype. This situation now appears to be changing, in October 1998, BT was reported in sheep on the Greek islands of Rhodes, Kos, Leros and Samos. The causal agent, identified as BTV 9, has since spread northward and westward and been confirmed in European Turkey (1999), Bulgaria (1999, 2001 and 2002), mainland Greece and/or Greek islands (1999-2001), Kosovo, Serbia, Montenegro, Macedonia and Croatia (2001) and Italy (2001-2002). Hence this strain of virus (BTV 9) has persisted and been circulating in these regions for 5 years. During the same period three other BTV
serotypes were reported in some of these countries; BTV 4 and 16 in either mainland Greece and/or some of the Greek Islands in 1999; BTV 16 in Italy in 2002 and BTV 1 in the Greek Island of Lesbos in 2001 (Baylis & Mellor, 2001; Mellor & Wittmann, 2002).

Another unrelated wave of BTV caused by serotype 2, probably originating from sub-Saharan Africa, has also been circulating in North Africa and the western Mediterranean basin. Outbreaks of disease due to this BTV serotype have been reported in Tunisia (1999-2000), Algeria, the Balearic Islands and Sicily (2000), Corsica and Sardinia (2000-2001) and Italy 2001-2002 (Zientara et al. 2001; 2002; Baylis & Mellor, 2001; Mellor and Wittmann, 2002).

In 2003 and 2004, outbreaks caused by BTV serotypes 4 and 16 have been reported in Italy (4), Spain (4), Corsica (4 and 16), Morocco (4) and Portugal (4).

2. BLUETONGUE VIRUS

Bluetongue virus is the type species of the genus Orbivirus within the Reoviridae family. The virions are spherical, 60 to 80nm in diameter and exhibit icosahedral symmetry. Virions are made up of three shells that together make up the outer capsid and the inner core of the virus particle. The BTV genome consists of 10 dsRNA segments, which are located within the core particle. The inner core is composed of 3 minor proteins, VP1, VP4 and VP6 and two major proteins, VP3 and VP7 (Verwoed et al. 1970; 1972; Huismans et al. 1987). The VP7 protein is extremely hydrophobic and is the major bluetongue virus group reactive antigen (Roy, 1992a and b). The outer capsid surrounding the inner core proteins is made up of 2 virus specific proteins, VP2 and VP5, which determine the antigenic variability of the BTV. Each of the 10 dsRNA segments encodes at least one polypeptide, including the 3 non-structural proteins, NS1, NS2 and NS3, located at the surface of the virion (Mertens et al. 1984; Roy et al. 1990). To date, 24 internationally recognized and serologically distinct serotypes of the virus have been identified, namely BTV-1 to BTV-24 (Roy, 1992b).

3. BLUETONGUE DISEASE

BTV can cause severe disease in certain breeds of sheep but rarely in cattle, goats and wild ruminants (Lefèvre, 1991; Lefèvre & Desoutter, 1988). The severity is dependent on the virus serotype, certain non-quantifiable environmental factors and the breed of sheep (Lefevre, 1991; Lefevre & Dessouter, 1998). Mortality can be as high as 70% in individual flocks but generally it is much lower, 10 to 20%. However, the main consequences of BTV infection come from the indirect losses due to abortion, loss of condition and prolonged convalescence (McKercher et al. 1953). Sheep either die directly as a result of infection or from a secondary bacterial infection such as pneumonia.

Following the introduction of virus by insect bite, BTV is transported to the regional, draining lymph nodes where initial replication occurs. Virus is then disseminated via lymph and vascular systems to secondary sites of replication, particularly lymph nodes, spleen and lungs (Pini, 1976). The virus replicates in the vascular endothelium and endothelial cells lining the blood vessels, and is released into the circulation where it is primarily associated with erythrocytes (Sellers, 1981). Hassan & Roy (1999) have shown that VP2, the cell attachment protein, is responsible for attachment of virus to red blood cells. This is an immuno-privileged site and the virus is protected from humoral antibody. In some animals the viraemia persists for several weeks (Hamblin et al. 1998). BTV serotype specific antibody can be detected in serum as early as 6 days after infection. This humoral antibody provides solid immunity against homologous challenge.

BT is characterized by fever (42°C), hyperaemia, inflammation of the oral mucosa, tongue, coronary band and occasionally the nasal mucosa, erosions and ulceration of the dermis and
laminitis. Severe oedema of the tongue can result in restricted blood flow and cyanosis. The swollen tongue may protrude giving the appearance of a blue tongue. Sick animals may exhibit profuse salivation, depression, anorexia, weight loss due to muscle degeneration, stiffness of the limbs, lameness, and excessive nasal and ocular secretion. Death may occur in 8-10 days.

On necropsy, sheep show haemorrhages in the tunica media at the base of the pulmonary artery, on the epicardium, endocardium and in the myocardium, and widespread subcutaneous oedema. The most prominent lesion histopathologically is myocardial necrosis, which is more pronounced in the papillary muscles of the left ventricle (Lefevre, 1991). Cattle usually suffer inapparent infections, however they are important in the epidemiology of BT because they can remain viraemic for several months (MacLachlan et al. 1992) and therefore act as silent reservoirs of virus.

4. LABORATORY CONFIRMATION OF CLINICAL DIAGNOSES

Rapid and reliable confirmation of BTV and serotype differentiation is essential at the start of an outbreak to allow for the early selection of vaccine. Historically, laboratory confirmation of the BTV serotype depended on the isolation and amplification of virus by inoculation of washed and lysed sheep red blood cells (RBC) or homogenized tissue into embryonated chicken eggs and/or cell cultures (BHK21, Vero and insect cells), and the subsequent serotyping of the virus using the virus neutralization test (VNT) (Anonymous, 2002).

More recently, indirect ELISAs have been described and used for the serogroup confirmation of BTV directly in homogenized tissue. However, since many animals die following a chronic infection either most or all of the BTV virus and viral antigen have been eliminated. Similarly, the ELISA is not suitable for the detection of BTV directly in blood because of the relatively low concentration of BTV antigen associated with the RBC membrane, which is below the sensitivity threshold for the assay.

Various techniques have been used to detect antibodies against BTV. These include agar gel immunodiffusion (AGID), haemagglutination-inhibition, complement fixation and ELISA, which are serogroup-specific and serum neutralisation, which is serotype-specific. Although all these assays are available, only AGID and competitive-ELISA are recommended as prescribed tests for international trade in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Anonymous, 2002).

4.1 Interest of PCR for Bluetongue diagnosis

BTV routine diagnosis is based primarily on serological methods that detect virus-specific antibodies in serum. A number of other procedures are also currently used to detect BTV from blood or tissues of infected animals. These include direct inoculation of cultured mammalian or insect cells, or intravenous inoculation into 10-12 embryonated chicken eggs, followed by one passage in insect cell cultures and up to three passages in mammalian cell cultures. In particular, the inoculation of embryonated chicken eggs and passaging through cell culture is the generally accepted method for testing of animals for export and other regulatory purposes. This is, however, a laborious and time-consuming protocol that may take up to 5 weeks for completion. Consequently, alternative methods of virus detection have been sought. These include antigen capture enzyme linked immunosorbent assay (ELISA), dot immunobinding assay (DIA), immunoelectron microscopy and polymerase chain reaction (PCR). The use of antigen capture ELISA for the detection of bluetongue virus in the blood of infected ruminants has either been unsuccessful, has detected antigen only in animals with high viremias, or was not consistent enough to allow for the reliable diagnosis of BTV.
To avoid these problems, RT-PCR-based assays were developed and evaluated for the detection of BTV serotypes based on nucleotide sequences of different genome segments. So, by comparing the nucleotide sequences of the amplified products, molecular epidemiological studies can be performed which allow to better understand the genetic relationships between the circulating strains (Figure 1). PCRs have also been developed to differentiate between the vaccine and wild BTV strains (Bréard et al. 2003) (Figure 2).

Figure 1. Phylogenetic associations of the Corsican, vaccine and other bluetongue virus isolates

Complete VP2 proteins of these isolates were compared by using the DNASTar software.
Figure 2. **RT-PCR products on BTV RNA isolates from different biological or supernatant samples**

Lines M represent the molecular weight marker

Line 1: blood sample of vaccinated sheep (18 days after vaccination)

Lines 2 and 3: blood samples of vaccinated sheep (21 days after vaccination)

Lines 4 to 7: blood samples from Corsican sheep infected with the wild-type BTV 2 in 2000

Lines 8 to 10: blood samples from Corsican sheep infected in 2001 (vaccinated 6 month before the outbreak)

Line 11: supernatant fluids from cells culture infected with the BTV 2 Corsican strain

Line 12: water control

A: Group specific primers derived from BTV 2 segment 10
B: specific primers from segment 10 of the BTV 2 vaccine strain
C: specific primers from segment 10 of the BTV 2 corsican strain

The different RT-PCR assays are shown to be sensitive as they could detect quantities of BTV as low as about 10-100 TCID50. Mainly, the diagnostic sensitivity of group-specific RT-PCR is greater than the virus isolation in embryonated chicken eggs or assays of cytopathic effects on cultured cells.

Determination of the nucleic acid sequence of portions of genome segment 3 (VP3) gene may provide information on whether the virus came from Australia, North America or South Africa.
5. **BTV TRANSMISSION AND VECTOR DISTRIBUTION**

BTV is transmitted by certain biting *Culicoides* species and the distribution of these vectors is controlled by various environmental factors, particularly temperature (Mellor & Boorman, 1995). During an epizootic, the viraemic ruminant hosts serve as a source of virus for the vectors and act as a reservoir for maintenance of virus. Using standard laboratory procedures infectious BTV can be detected for approximately 50 days in sheep and 100 days in cattle. Because BTV is not transmitted transovarially, overwintering and persistence of virus is thought only to occur in areas where adult vectors are present and remain active throughout the year (Mellor & Boorman, 1995). However, a recent paper by Takamatsu hypothesised an overwintering mechanism whereby virus might persist in the ruminant host for longer periods and remain available to vector midges (Takamatsu *et al.* 2003).

*C. imicola*, the major old-world vector of BTV, is generally regarded as an Afro-Asiatic species although in recent years it has been identified and has become established in several European countries and Mediterranean Islands (Mellor & Wittmann, 2002). However, BTV 9 is currently circulating beyond the known *C. imicola* line in the Balkans and Yugoslavia where *C. imicola* is absent and *C. obsoletus* and *C. pulicaris* are abundant (Mellor & Wittmann, 2002). Both of these latter species have been implicated as potential vectors based on previous virus isolations of BTV in Cyprus (Mellor & Pitzolis, 1979) and African horse sickness virus in Spain (Mellor *et al.* 1990). Most recently, BTV 2 has been isolated from *C. obsoletus* in Italy (Conte *et al.* 2003).

6. **CONTROL**

Traditional control measures for bluetongue include the restriction of animal movements and slaughter of infected animals to prevent them acting as a source of virus for vector insects and housing susceptible stock during times of exophilic vector activity (crepuscular periods and during the night). Vector abatement could be considered with insecticide treatments. Local application of insecticides on animals and around animal holdings can be efficacious against *Culicoides* species (Braverman, 1989; Braverman *et al.* 1995). Repellents, larvicide and adulticide treatments have all been tested and they have significant but temporary effect.

Vaccination of susceptible species is the most efficient way of controlling disease. Vaccines with variable efficacy have been developed. These include attenuated, inactivated vaccines and more recently, DNA recombinant vaccines (Pearson & Roy, 1993; Roy, 1992a, 1996). Currently, attenuated and inactivated vaccines are commercially available. The South African bluetongue vaccine produced by Onderstepoort Biological Products, Onderstepoort, South Africa has been used in Europe (Hunter & Modumo, 2001 & Hammouni *et al.* 2003) until the recent development of an inactivated vaccine by Merial. This inactivated vaccine containing the BTV serotypes 2 and 4 is used in Spain, Corsica and Italy.

Although these vaccines protect sheep against disease, they do not prevent infection. It has been postulated that reassortments (exchange of genome segments) may occur between live vaccine viruses and wild-type viruses, resulting in "new" viruses which could possess enhanced virulence characteristics or express novel antigenic properties (Mellor & Wittmann, 2002). The preparation and use of new vaccines such as inactivated, whole virus or sub-unit vaccines are being investigated (Roy, 1996).

7. **CONCLUSION**

The presence of 5 BTV serotypes (1, 2, 4, 9 and 16) in the Mediterranean Basin and/or the Balkans in the last 5 years (1998-2002), and the persistence of at least two of these virus serotypes (BTV 2
and 9) should act as a cautionary note to Veterinary Authorities and Governments for continued vigilance. Further development of sensitive and specific diagnostic tools that can be used to monitor and rapidly diagnose BT throughout Europe and beyond should also be continued.

8. SUMMARY

Bluetongue (BT) is an Office International Epizooties (OIE) list arthropod-borne viral disease caused by the BT virus (BTV). The virus is infectious but non-contagious, affecting domesticated and wild ruminants. Disease is usually limited to fine wool and mutton breeds of sheep and some species of deer. The BTVs are transmitted between ruminant hosts almost entirely by the bites of certain species of Culicoides biting midges. Since its re-emergence in 1998 in the Mediterranean basin, five serotypes have been reported in different Mediterranean countries. In 2003 and 2004, outbreaks caused by BTV serotypes 4 and 16 have been reported in Italy (4), Spain (4), Corsica (4 and 16), Morocco (4) and Portugal (4). Vaccination of susceptible species is the most efficient way of controlling disease. Attenuated or inactivated vaccines with variable efficacy have been developed.

9. KEY WORDS

Bluetongue, epidemiology, vaccine.

10. RESUME


11. MOTS CLES

Fièvre catarrhale ovine, épidémiologie, diagnostic.

12. REFERENCES


