Introduction

Bluetongue (BT) is an infectious, noncontagious, insect-borne viral disease of sheep and other domestic and wild ruminants. In many regions of the world the clinical signs of disease are uncommon in BT virus-infected domestic animals, and generally are observed only in sheep and some wild ruminants. Signs vary from sub-clinical to an acute, febrile response with targeting of blood vessels followed by hyperemia, inflammation and ischemia leading to facial edema and hemorrhages, with necrosis and erosion and ulceration of the mucous membranes. The tongue may be intensely hyperemic and become swollen and edematous and protrude from the mouth, so-called “bluetongue,” although this is unusual. Necrosis of cardiac and skeletal muscle is common, and severe, fatal cases of BT are often characterized by pericardial effusion and marked pulmonary edema. Wool break and loss occur in convalescent animals. Sheep may become lame as a result of coronitis and/or skeletal myopathy. Abortion and congenital fetal abnormalities can occur as a consequence of vaccination of pregnant animals with modified live virus vaccines.

Classification

Bluetongue is caused by BT virus, the prototype member of the Orbivirus genus in the family Reoviridae. The bluetongue serogroup contains 24 serotypes. Closely related viruses include epizootic hemorrhagic disease of deer (EHDV) and Chuzan viruses. Ibaraki virus, which is a member of the EHDV serogroup, causes a BT-like disease in cattle in Asia.

Virus structure and replication

Complete BT virus particles are double-shelled and contain seven major structural proteins. Proteins VP2 and VP5 form a diffuse outer layer, with VP2 being the major determinant of serotype specificity. The inner icosahedral core contains two major structural proteins, VP7 and VP3, and three minor ones VP1, VP4, and VP6. Three non-structural proteins also are produced in virus-infected cells, specifically NS1, NS2 and...
NS3. VP1: RNA dependent RNA polymerase. VP2: serotype-specific antigen, cell attachment protein, forms part of outer layer of outer capsid. VP3: a major component of the viral core. VP4: capping enzyme. VP5: inner layer of outer capsid, and conformationally influences neutralization determinants on VP2. VP6: ssRNA and dsRNA binding, helicase NTPase. VP7: forms outer core surface, and is responsible for attachment of core particles to insect cells. Group-specific antigen NS1 forms macrotubules in infected cells, although their function is largely unknown. NS2: viral inclusion body matrix protein. NS3, NS3a: glycoproteins, membrane proteins involved in virus egress from infected cells. The core consists of 32 capsomeres clustered as pentamer–hexamer morphological units. The genome consists of 10 segments of double-stranded RNA, and each gene segment encodes at least 1 viral protein; gene segment 10 encodes both NS3 and NS3A.

BT virus is extremely stable at 4° C if stored properly (as in anticoagulated blood) and is labile at low pH. Whole intact virus particles have an intact outer capsid of VP2 and VP5. Infectious sub-viral particles (ISVP) are produced by treatment of whole particles with proteolytic enzymes that digest VP2 from the particle. Core particles are produced by further removal of VP5 from the IVSP. Intact BT virus particles are equally infectious for insect and mammalian cells whereas core particles are at least 1,000-fold more infectious for insect cells than they are for mammalian cells.

**Brief history**

BT was first described in 1881 as an epizootic catarrhal fever in Merino sheep. The disease was first identified as bluetongue in a classic publication by Spreull in 1905, as an anglicized version of the name of “tongue-sickness” that was used by the Afrikaans farmers of southern Africa. In 1906 Theiler demonstrated that the agent was filterable and postulated that it was a virus. The seasonality of the outbreaks suggested insect transmission but transmission by *Culicoides* spp. was not reported until 1944 and was confirmed in 1963. Sheep attenuated vaccines were developed by Theiler in 1908 and used for 40 years. These were subsequently replaced by egg-attenuated tissue culture grown vaccines that are used until the present. These days severe clinical BT is rarely seen except perhaps in India, and certainly during the recent outbreak (1999-present in Mediterranean Europe). BT virus recently has extended its northern range in Europe to approximately 44° N, although there is serological evidence that the virus is present in adjacent regions of Asia as far north as 50° N. It now is clear that BT virus exists worldwide in a band between approximately 35° S and as far as 50° N, including extensive regions of North and South America, Africa, southern Europe, Asia and northern Australia.

**Epidemiology**

Midges of the genus *Culicoides* transmit BT virus between susceptible animals, after becoming infected by feeding on viremic vertebrates. After an extrinsic incubation period of approximately 10 days when virus travels from the insect midgut to salivary glands, the virus can be transmitted to a vertebrate host during a blood meal. Infected midges remain infective for life. The central role of the insect vector in BT epidemiology
ensures that prevalence of the disease is ruled by ecological events such as high rainfall, temperature and humidity, which favor both insect survival and replication of the virus within the vector. Thus, BT virus infection is highly seasonal in many areas of the world, as occurs with a number of other arbovirus diseases. BT virus viremia in ruminants is initially associated with all blood cell types, but later in infection virus principally is associated with erythrocytes. The intimate association of BT virus with the erythrocyte cell membrane facilitates prolonged infection of ruminants. Virus co-circulates with neutralizing antibodies for weeks. *Viremia is prolonged in both sheep and cattle, but not persistent, and sheep and cattle have no role in long-term maintenance of BT virus.* Low level cycles of infection of BT virus between vector insects and ruminants may explain the “overwintering” of BT virus in some regions, but the insect vectors likely provide the critical true long-term virus reservoir. The persistently infected bull is a failed scenario to explain overwintering of BT virus.

BT virus exists in distinct ecosystems, thus North America has different serotypes of BT virus than adjacent countries in Central and South America. Distribution of these distinct virus “topotypes” reflects the different vector species in each area. BT virus has minimal disease impact in much of the world despite endemic infection. International concerns regarding are based in part the long discredited concept of persistent BT virus infection of cattle following in utero infection.

**Clinical signs in sheep**

Signs may include all or part of the followings. Pyrexia up to 39–41°C over a period of 6–8 days; edema of lips with hyperemia and superficial ulceration of oral mucosa; edema of larynx with mild salivation; watery rhinorrhea with edema and cyanosis of lips and tongue and edema of face and ventral surface of neck; loss of condition and progressive muscle hypotrophy; torticollis; hyperemia and hemorrhage of coronary band and lameness. Various forms have been recognized: *Peracute form.* Fever with mucosal hyperemia and petechiae with death or recovery. *Acute form.* In addition to fever with mucosal hyperemia and petechiae, erosions appear on lips, oral cavity and mouth and tongue. This form may terminate in death or recovery. *Subacute form.* Characterized by coronitis and progressive loss of condition from which affected animals may eventually die. *Abortigenic form.* Abortion with mild mucosal hyperemia and mild fever and animals recover.

**Clinical signs in cattle**

In cattle the disease is almost always asymptomatic. Occasional reports have described a mild disease with oral erosions and dermatitis, although Koch’s Postulates remain to be adequately verified.

**Signs in Goats**

Clinical signs in goats are very mild, and disease is rarely if ever seen.
Lesions

Lesions in sheep include mucosal hyperemia, cyanosis of the tongue (occasional), erosive and ulcerative stomatitis and glossitis, rhinitis, conjunctivitis, pulmonary edema, coronary hemorrhage with dermatitis and laminitis, possible hoof detachment, erythematous multifocal dermatitis, pulmonary artery hemorrhage, and necrosis of cardiac (the papillary muscle of the left ventricle is a predilection site) and skeletal muscle. Vaccination of sheep with modified live virus vaccines during gestation, between 40 and 100 days of pregnancy can result in either fetal death or teratogenesis. Around 50-80 days of pregnancy porencephaly, hydranencephaly and cerebellar dysplasia can occur. Similarly, exposure of pregnant cattle to modified live virus vaccine strains of BT virus at critical stages of gestation (70–150 days) also can cause fetal death or central nervous system teratogenesis (hydranencephaly or porencephaly), although this is rare. Histological lesions are subtle but include is vascular alteration with endothelial hypertrophy, necrosis, vascular leakage of serum and blood with edema and hemorrhage, and ischemic necrosis of skeletal and cardiac muscle fibers.

Diagnostic procedures

Physical exam

Clinical signs are nonspecific and may be confused with numerous others infectious and noninfectious illnesses. See above for clinical presentation.

Pathology

Complete necropsy and histopathological examination of all tissues of aborted fetuses, stillborn animals, youngsters and adults must be performed to achieve a diagnosis and to differentiate BT from numerous other infectious and noninfectious diseases.

Virus isolation

The following isolation techniques may be used: sheep inoculation, inoculation of embryonated chicken eggs, intracerebral inoculation of newborn mice, tissue cultures such as baby hamster kidney cells (BHK-21) and African green monkey kidney (Vero cells), Aedes albopictus cells.

Virus identification

Serotyping by virus neutralization

Neutralization tests are type-specific for the currently recognized 24 BT virus 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 BT virus serotypes should generally obviate the need to attempt
neutralization by all 24 antisera, particularly when endemic serotypes have been identified. Microtiter neutralization, plaque reduction and plaque inhibition can all be used. Microtiter neutralization is the most widely used and requires specific antisera for all BT virus serotypes.

**Serogrouping of viruses**

This is based on the reactivity of group-specific antigens such as VP7. *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. Commonly used methods for the identification of viruses to serogroup level include immunocapture ELISA, immunofluorescence and immunospot, as well as indirect peroxidase-antiperoxidase test.

**Molecular virology**

The presence of BT virus in animal tissues is very readily detected by reverse transcriptase polymerase chain reaction (PCR) assay. Use of nested PCR procedures increases sensitivity far beyond that of conventional virus isolation, although contamination risks also increase.

**Differential diagnoses**

BT clinical signs and lesions can resemble several other infectious and noninfectious diseases, and for this reason experienced and trained diagnosticians and in particular pathologists need to be included in the diagnostic process. In particular BT can look like some other economically important diseases of ruminants.

Differential diagnoses in sheep include foot and mouth disease aphthovirus, vesicular stomatitis rhabdovirus, sheep poxvirus, peste des petites ruminants paramyxovirus, Rift valley fever flebovirus, contagious ecthyma (orf) parapoxvirus, pneumonia caused by *Pasteurella multocida*, *Mannheimia haemolytica* and *Mycoplasma* spp., Akabane bunyavirus, photosensitization, copper deficiency, *Oestrus ovis* larvae infestation, anticoagulant rodenticide poisoning.

Differential diagnoses in cattle perhaps can include foot and mouth disease aphthovirus, vesicular stomatitis rhabdovirus, the mucosal disease form of bovine pestivirus infection, stomatitis, infectious bovine tracheitis bovine herpesvirus 1, bovine popular stomatitis parapoxvirus, bovine herpesvirus mamillitis herpesvirus 3, malignant catarrhal fever herpesviruses, and Akabane bunyavirus.

**Prevention**

There is no treatment for bluetongue disease. The recovery of affected animals will be aided by the provision of shade, water, feed and shelter. The cycle of BT virus infection is best interrupted by the immunization of vertebrate hosts, as removal of vectors or prevention of vector attack is difficult. The control of midges by the
application of insecticides and larvicides to insect resting and breeding sites, or systemically to sheep and cattle has not been fully investigated but is likely to have local success only and is practically difficult or impossible. Mixing cattle with sheep will draw vectors with a host preference for cattle from sheep, but may raise the virus infection level of the midge population.

There is a very legitimate need for improved vaccines. Available vaccines include modified live, killed and, recently, recombinant vaccines. Subunit vaccines may contain baculovirus, yeast or bacterial-expressed VP2; others are based on various alphavirus-replicon systems; DNA vaccines are also being developed.

Killed vaccines have the advantages of being safe and efficacious if properly manufactured. Their potential disadvantages may include either over- or under-inactivation and their relatively high cost.

Modified live BTV vaccines are easy to manufacture and inexpensive. They are effective in reducing the disease and in producing a long term immunity. Their disadvantages include the possibility of containing non-contemporary strains of BTV, teratogenic effects, the potential for transmission and reassortment of genes with field strains, reversion to virulence (virulence determinants of BTV are poorly characterized). Modified live polyvalent BTV vaccines also result in interference, whereby individual animals may not respond serologically to all of the serotypes with which they were immunized.

References are available upon request.
Bluetongue: A Review and Contemporary Perspective

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Summary

Bluetongue (BT) is a non-contagious disease of domestic and wild ruminants caused by bluetongue virus (BTV). Hematophagous insects of the genus *Culicoides* transmit BTV from infected to susceptible ruminants. Whereas BTV infection of *Culicoides* insects is life-long, BTV infection of ruminants is transient. The prolonged viremia that occurs in BTV-infected ruminants occurs through a novel interaction of the virus with erythrocytes. BT disease occurs in sheep and some wild ruminant species, and is characterized by vascular injury with hemorrhage, edema and tissue necrosis. Inherent, species-specific differences in the susceptibility and responses of endothelial cells may be responsible for the occurrence of BT disease in BTV-infected sheep but not cattle. Although BT was once considered to be an emerging disease, it now is clear that BTV exists throughout tropical, subtropical and some temperate regions of the world in distinct ecosystems where different strains of the virus have co-evolved with different species of insect vector. Genetic drift of BTV genes likely occurs through a combination of quasispecies evolution of the virus in ruminant hosts that amplify the virus, combined with the "founder effect" that occurs when individual insects selectively amplify and transmit specific genetic viral variants from the blood of infected ruminants. The presence of BTV in ruminant blood readily can be detected by polymerase chain reaction, which provides a very conservative assay for the screening of ruminants prior to movement to BTV-free regions. Although existing modified live BTV vaccines can prevent disease in vaccinates, there is a considerable need to develop cost-effective, efficacious non-replicating BTV vaccines.
Introduction

Bluetongue virus (BTV) is the etiologic agent of bluetongue (BT), a noncontagious, insect-transmitted disease of sheep and some species of wild ruminants (Erasmus, 1975; Gard, 1984; MacLachlan, 1994; Moulton, 1961; Parsonson, 1990; Spreull, 1905). BT disease was first recognized and comprehensively described in southern Africa, and BTV has subsequently been isolated from ruminants and/or vector insects from all continents except Antarctica (reviewed Gibbs and Greiner, 1994). Because BTV infection of ruminants is not contagious, the global distribution of BTV coincides with the distribution of competent Culicoides insect vectors and hot or warm climatic conditions. Although BTV infection of domestic and wild ruminants occurs throughout much of the world with minimal occurrence of disease, BT is just one of 16 diseases classified in List A by the Office International des Epizooties (OIE). As a direct consequence of its inclusion in the OIE List A, BT continues to impact the global trade of ruminants and their germplasm (Alexander et al., 1996). The recent re-emergence of BT in Mediterranean and southeastern Europe has also caused considerable consternation (Bayliss and Mellor, 2001).

History

The disease of BT was first described as "Malarial Catarrhal Fever" and "Epizootic Catarrh of Sheep" in the original written descriptions of the disease by investigators in South Africa. The name of "bluetongue" was later used to describe the distinctive cyanotic tongue of
some severely affected sheep. The original written descriptions of BT were published in the late 19th and early 20th century, although farmers in South Africa recognized the disease soon after the introduction of fine-wooled European breeds of sheep to that region of the world (Spreull, 1905; Erasmus, 1975). Prior to the 1940s, BT was thought to be confined to southern Africa. The first well-documented epizootic of BT outside of Africa occurred amongst sheep on Cyprus in 1943. The disease was recognized in Texas soon thereafter, and an extensive epizootic occurred on the Iberian Peninsula in 1956-57. The disease subsequently was recognized in the Middle East, Asia, and in southern Europe. These epizootics were interpreted in the middle of the 20th century to reflect the emergence of BT disease from its presumed ancestral origin in Africa, leading to "doomsday" scenarios regarding putative global spread of BT that justified its inclusion in OIE List A. It now is clearly evident that BTV infection occurs throughout tropical and subtropical regions of the world, extending also into many temperate regions as well. BT disease, however, is either rare or non-existent in many regions with endemic BTV infection. Furthermore, it is clearly apparent that the global spread of BTV was not a recent event, and that different serotypes and strains of BTV have evolved in different regions of the world, coincident with the presence of distinct species of Culicoides insect vectors (reviewed Gibbs and Greiner, 1994; Tabachnick et al, 1992).

**Bluetongue Virus**

BTV is the prototype virus of the genus Orbivirus in the family Reoviridae. The BTV genome includes 10 segments of double-stranded RNA, each of which encodes at least 1 viral
protein. The BTV particle has icosahedral symmetry, is approximately 90 nm in diameter, and includes 7 structural proteins identified as virus proteins (VP) 1 - 7. Two viral proteins, VP2 and VP5, form the outer capsid that contains the neutralization determinants of BTV. The viral core consists of predominantly VP3 and VP7, with lesser amounts of VP1 (polymerase), VP4 (5' capping and methylation), and VP6 (helicase). VP7 mediates attachment of BTV core particles to insect cells. Four non-structural (NS) viral proteins, NS1, NS2, NS3 and NS3A, are also produced in BTV-infected cells (reviewed Gould and Hyatt, 1994; Roy, 1992).

At least 24 distinct serotypes of BTV are recognized. Serotype is determined only by epitopes that reside on the outer capsid protein VP2, although VP5 also can influence neutralization through its conformational influence on VP2 (reviewed DeMaula et al., 2000). The L2 gene, which encodes VP2, is the only serotype-specific BTV gene and there is considerable variation amongst all 10 genome segments of field strains of BTV within endemic areas such as California (de Mattos et al., 1994; de Mattos et al., 1996; Pierce et al, 1998). This variation of BTV genes in field strains of the virus has arisen as a consequence of both drift and reassortment of individual viral genes. Reassortment of BTV genes has been demonstrated after infection of either the ruminant host or insect vector with different strains or serotypes of BTV (Samal et al., 1987a; Samal et al, 1987b). It is clear in endemic areas that gene segments other than the L2 gene evolve and reassort independently of serotype amongst field strains of BTV, and individual genes also evolve and reassort independently of one another. Accumulation of nucleotide substitutions within each BTV gene leads to genetic drift of each. We recently demonstrated that the process of genetic drift of BTV genes is accomplished through selective acquisition and amplification in
vector insects of specific variants from the quasispecies virus population that arises in the blood of infected ruminants through the process of founder effect (Bonneau et al., 2001; Bonneau et al., 2002a).

**The epidemiology of BTV infection**

Biting insects of the genus *Culicoides* transmit BTV. Vector insects become persistently infected with BTV for their entire lifespan after acquiring infection through feeding on a BTV-infected ruminant. Although venereal and vertical transmission of BTV can occur in ruminants, these routes are unimportant to the maintenance of BTV and the distribution of BTV in the world coincides only with that of competent vector insects (Barratt-Boytes and MacLachlan, 1995; Gibbs and Greiner, 1994; MacLachlan, 1994; Tabachnick et al, 1992). Appropriate climatic conditions are also important in the maintenance of BTV, thus the virus exists in an extensive band that includes tropical, subtropical and temperate regions of the world between latitudes of approximately 40° North and 35° South. Exceptions are western North America and, very likely, Asia, where BTV infection of ruminants can occur perhaps as far as 50° North. The species of vector insects that transmit BTV differ between regions, and are especially poorly characterized in Europe and Asia. Recent studies have shown that ambient temperature has a profound effect on the survival of vector insects, their feeding activity, and the replication of BTV in the insect vector (Mullens et al., 1995). Specifically, insect lifespan is inversely related to temperature, and the replication of BTV in its insect vector increases with temperature. Thus, temperature-dependent control of BTV virogenesis potentially might limit the expansion of BTV.
into regions outside of its current range, even into areas where apparently competent vector insects occur. Global warming, however, would be predicted to expand the northern and southern extremes of global BTV distribution (Gibbs and Greiner, 1994), as recently has occurred in southern Europe (Bayliss and Mellor, 2001).

BTV infection of ruminants, both wild and domestic, occurs throughout tropical, subtropical and temperate regions of the world, however BT disease is uncommon or not recognized in many areas where BTV is endemic. For example, BTV infection occurs throughout extensive regions of northern Australia yet BT disease has not yet been described in Australia. Similarly, there are very few descriptions of BT amongst ruminants in the Caribbean islands as well as Central and South America, despite endemic BTV infection in much of this region.

It is increasingly evident that BTV has not recently been spread globally through international trade. Rather, the virus exists in distinct, relatively stable ecosystems in different regions of the world where specific strains of the virus likely have co-evolved with different species of insect vector (Tabachnick et al, 1992; Gibbs and Greiner, 1994). Thus, in the Americas, the serotypes of BTV that circulate in the United States are different from those in adjacent regions of the Caribbean and Central America, despite the lack of any substantial geographic barrier between the regions. The essential difference lies in the different species of vector insects in the 2 regions: *Culicoides sonorensis* is the vector of BTV serotypes 10, 11, 13 and 17 in the United States, whereas *Culicoides insignis* is the vector of BTV serotypes 1, 2, 3, 4, 6, 8, 11, 12, 13, 14 and 17 in the Caribbean and Central/South America. Movement of animals between the 2 regions has not altered the very different constellations of BTV serotypes that
occur in each.

A variety of other hosts have been implicated in the lifecycle of BTV infection. Serological evidence indicates that large African carnivores are infected with BTV, whereas smaller predators that co-habit with them are not, suggesting that large carnivores are infected through feeding on BTV-infected ruminants (Alexander et al, 1994). Inadvertent contamination of a canine vaccine with BTV confirmed that dogs are susceptible to BTV infection, indeed pregnant bitches that received this contaminated vaccine typically aborted and died (Akita et al, 1994). There is no evidence, however, that dogs or other carnivores are important to the natural cycle of BTV infection.

**The pathogenesis of bluetongue virus infection**

The pathogenesis of BTV infection is similar in sheep and cattle, and most probably, all species of ruminants (Barratt-Boyes and MacLachlan, 1995; Maclachlan, 1994; Mahrt and Osburn, 1986; Pini, 1976). There are marked differences in the severity of disease that occurs in different ruminant species after BTV infection, however, with cattle being especially resistant to expression of BT disease. After cutaneous instillation of virus through the bite of a BTV-infected *Culicoides* vector the virus travels to the regional lymph node where initial replication occurs. The virus then is disseminated to a variety of tissues throughout the body where replication occurs principally in mononuclear phagocytes and endothelial cells. Viremia in BTV-infected ruminants is highly cell associated, and viremia is prolonged but not persistent especially in cattle (reviewed Barratt-Boyes and MacLachlan, 1995; Singer et al, 2001; Bonneau et al,
2002b). The virus promiscuously associates with all blood cells, thus titers of virus in each cell fraction are proportionate to the numbers of each cell type; specifically, BTV is quantitatively associated most with platelets and erythrocytes and, because of the short lifespan of platelets, virus is most associated with erythrocytes late in the course of BTV infection of ruminants. BTV infection of erythrocytes facilitates both prolonged infection of ruminants and infection of hematophagous insect vectors that feed on viremic ruminants (Brewer and MacLachlan, 1992; Brewer and MacLachlan, 1994). Interestingly, BTV nucleic acid may be detected by polymerase chain reaction (PCR) in the blood of infected cattle and sheep for many months after it no longer can be detected by virus isolation in cell culture or inoculation of susceptible sheep. Furthermore, ruminant blood that contains BTV nucleic acid as determined by PCR assay, but not infectious BTV as determined by virus isolation, is not infectious to vector insects even by intrathoracic inoculation (Bonneau et al, 2002b; MacLachlan et al, 1994; Tabachnick et al, 1996).

Clinical signs and lesions in BTV-infected sheep likely reflect virus-mediated endothelial injury, as BTV replicates in endothelial cells causing cell injury and necrosis (Mahrt and Osburn, 1986; Pini, 1976). Similarly, white-tailed deer, which are highly susceptible to BT, develop consumptive coagulopathy as a consequence of BTV-induced damage to endothelial cells (Howerth et al, 1988). Consumptive coagulopathy (also known as disseminated intravascular coagulation) in BTV-infected sheep and deer predisposes to the bleeding tendency that characterizes fulminant BT. Endothelial injury also is likely responsible for increased vascular permeability leading to edema in tissues such as the lung (pulmonary edema), and vascular thrombosis leads to tissue infarction.
Culicoides insects are biological vectors of BTV, thus the virus replicates within the tissues of each insect after infection from feeding on the blood of a BTV-infected ruminant (reviewed Mellor, 2000). Vector insects can only transmit BTV to another susceptible ruminant after an extrinsic incubation period of some 10-14 days, during which time the virus is disseminated from the insect's gut to its salivary glands. The external incubation period is shorter when insects are held at high ambient temperatures. Vertical transmission with transovarial transfer of BTV has not been demonstrated in Culicoides insects, however, infection of adult insects is lifelong. Furthermore, individual insects can survive for relatively long periods of time, particularly in cooler ambient temperatures (Mullens et al, 1995).

Bluetongue Disease of Ruminants

BT 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. The signs of BT in sheep reflect congestion, edema and hemorrhage as a consequence of virus-mediated vascular injury. Thus, sheep with BT have any combination of fever, serous to bloody nasal discharge, oral erosions and ulcers, lameness with hyperemia of the coronary band, and weakness secondary to muscle necrosis. Lesions present at postmortem of affected sheep can include hyperemia, hemorrhages, erosion and ulceration of the mucosa of the upper gastrointestinal tract (oral cavity, esophagus, forestomachs); subintimal hemorrhages in the pulmonary artery; pulmonary edema; pleural and/or pericardial effusion; edema within the fascial planes of the muscles of the abdominal wall; necrosis of skeletal and cardiac muscle, with the papillary muscle
of the left ventricle being an especially characteristic site (reviewed Erasmus, 1975; Moulton, 1961; Spreull, 1905).

It is to be emphasized that most BTV-infected sheep develop mild or no obvious disease, especially in BTV-endemic areas. Outbreaks of BT typically occur either when susceptible sheep are introduced into BTV-endemic regions, or when the virus spreads into immunologically naïve sheep populations at the interface of BTV-endemic and non-endemic regions. Expression of BT disease likely reflects a variety of host, virus, and vector factors:

*Virus factors:* field strains of BTV in endemic areas such as California exhibit remarkable genetic heterogeneity, even amongst strains that co-circulate (Bonneau et al, 2002a; de Mattos et al, 1994; de Mattos et al, 1996; Pierce et al, 1998). This variation occurs through both genetic drift and gene segment reassortment, as described above. Similarly, we have shown with exhaustive cloning studies that genetic variants of BTV occur during the course of infection of a single cow or sheep, so-called quasispecies evolution that reflects the inherent infidelity of the BTV polymerase (Bonneau et al, 2001). It is logical that this considerable genetic variability of BTV is reflected by differences in phenotypic properties of each virus strain, including their virulence to susceptible ruminants.

*Ruminant factors:* It is clear that sheep and wild ruminants such as white-tailed deer are the species that are most susceptible to BT disease. Furthermore, sheep that are native to tropical and subtropical regions of the world where BTV is endemic are usually resistant to BT, whereas fine-wooled European breeds such as the Merino are highly susceptible. Nutritional status, immune status, and age also influence the severity of BT in individual sheep, as can
environmental stresses such as high temperature and ultraviolet radiation.

A fundamental question that has vexed scientists for many years is why virulent strains of BTV produce disease in sheep but not cattle (Barratt-Boytes and MacLachlan, 1995; Russell et al, 1996). The similar or identical pathogenesis of BTV infection of cattle and sheep further emphasizes this obvious paradox. We recently have identified fundamental differences in the susceptibility of endothelial cells from cattle and sheep to BTV infection (DeMaula et al, 2001; DeMaula et al, 2002a; DeMaula et al, 2002b). To facilitate these studies, we isolated and propagated pure cultures of endothelial cells from the microvasculature of sheep and cattle, and then evaluated their responses to infection with BTV. Lung microvascular endothelial cells were selected because pulmonary edema and microvascular injury are both highly characteristic of BT disease. Interestingly, whereas BTV infection of the bovine endothelial cells resulted in endothelial activation, with the increased transcription of genes encoding a variety of vasoactive and inflammatory mediators and increased expression of cell surface adhesion molecules, similar infection of sheep endothelial cells resulted in minimal activation of endothelial cells. Furthermore, the ratio of thromboxane to prostacyclin, which is indicative of enhanced coagulation and possible consumptive coagulopathy, was significantly greater in sheep than in cattle that were experimentally infected with BTV.

*Vector factors:* *Culicoides* vectors are critical to the survival and transmission of BTV as infection is not contagious and there is no credible evidence of long-term maintenance of BTV in ruminants. Thus, BTV infection occurs only where competent vectors are present. Furthermore, both BTV infection and BT disease usually occur during late summer and early autumn when numbers of
insect vectors are highest in BTV-endemic areas such as California (Gerry et al., 2001). While insect survival is inversely related to temperature, higher ambient temperatures stimulate insect feeding and promote virogenesis of BTV in insects, both of which enhance virus transmission (Mullens et al., 1995). Lastly, it is to be stressed that the environmental conditions that produce the highest numbers of vector insects are likely to optimize the transmission of BTV amongst ruminants, and thus the expression of BT in susceptible sheep. The species of *Culicoides* that transmit BTV in different regions of the world clearly are very different, as may be the environmental factors that promote population expansions of each.

**Diagnostics**

Ruminants infected with BTV develop a prompt and high titered antibody response to a variety of viral proteins. Serotype-specific neutralizing antibodies are directed against VP2, and these readily can be detected by serum neutralization test. Antibodies directed against core protein VP7, as well as other structural and nonstructural proteins, may be detected with serogroup-reactive assays such as the agar gel immunodiffusion and competitive enzyme-linked immunosorbent assay (cELISA). The cELISA is increasingly used for detection of BTV serogroup-specific antibodies in the blood of ruminants.

A positive serological result confirms only that an animal previously was infected with BTV. Furthermore, although BTV infection of cattle and sheep often is prolonged, there is no credible evidence of long-term persistent BTV infection of ruminants (Barrat-Boyes and MacLachlan, 1995; Bonneau et al., 2002b; Gibbs and Greiner, 1994). Thus, the vast majority of
seropositive cattle and sheep from BTV-endemic regions are not infected with the virus and pose no threat for movement. The presence of BTV in the blood of ruminants can be determined by isolation in embryonated chicken eggs, cell culture, or by inoculation of susceptible sheep. Nested PCR (nPCR) assay increasingly is used for screening of ruminants for the presence of BTV nucleic acids because it is highly sensitive and specific if performed properly, and it is an extremely conservative assay in that BTV nucleic acid may be detected in the blood of sheep and cattle long after infectious virus has been cleared. Ruminants whose blood is negative by nPCR assay pose no threat for inadvertent movement of BTV by trade.

**Vaccines**

Vaccination typically is used to prevent outbreaks of BT, and has also been used in an effort to control incursions of BTV. Only modified live BTV vaccines are in widespread use, particularly in Africa, the United States and, most recently, southern Europe. These vaccines have proven very useful in preventing losses attributable to BT, but they also suffer from a number of serious potential deficiencies that include: the introduction of novel virus strains into the environment, perhaps leading to infection of vector insects; quasispecies evolution with possible reversion to virulence or creation of new strains of BTV; reassortment of gene segments with indigenous viruses to generate potentially novel recombinants; fetal infection and teratogenesis. In fact, it is increasingly clear that only strains of BTV that have been modified by growth in cell culture, such as MLV vaccine strains, have the capacity to cross the ruminant placenta. Once MLV BTV strains cross the placenta they cause embryonic or fetal death, and
cerebral malformations after infection of older fetuses that survive congenital infection (reviewed MacLachlan et al, 2000). New generation vaccines, such as the virus-like particles produced by baculovirus expression of different viral proteins (Roy et al, 1990), have not yet been used extensively perhaps because of their cost.

Summary

BT is an important disease of sheep and some wildlife species in several areas of the world. Fears that BT was being spread through the world by international trade have not been confirmed, rather the virus exists in distinct ecosystems throughout tropical, subtropical and some temperate regions of the world. There is a considerable need to better define the environmental and epidemiologic factors that lead to expansion of the virus' range, as recently has occurred in Europe. Similarly, cost-effective and efficacious non-replicating vaccines are needed to control outbreaks of BT and incursions of BTV.

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