Introduction
Bluetongue virus (BTV) is the etiologic agent of bluetongue (BT), a non-contagious, insect-transmitted disease of sheep and some species of wild ruminants [4,19,24,32]. BT disease was first recognized and comprehensively described in southern Africa [32], and BTV has subsequently been isolated from ruminants and/or vector insects from all continents except Antarctica [16]. Because BTV infection of ruminants is not contagious, the global distribution of BTV coincides with the distribution of competent Culicoides insect vectors. 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), which is the world organization for animal health. As a direct consequence of its inclusion in the OIE List A, BT continues to impact the global trade of ruminants and their germplasm [2]. Furthermore, BTV recently emerged throughout much of the Mediterranean Basin to precipitate the largest and most economically devastating epidemic of BT ever described in Europe, and the virus currently is endemic in substantial portions of Italy, Greece, the Balkans, and adjacent countries. The incursion of BTV into southern Europe has had a devastating impact on livestock production in the region (especially in Italy) because of severe disease and remarkably high mortality in sheep, and restrictions of the movement of domestic livestock [5]. To address the growing international impact of BT, the OIE and the European Union recently sponsored the Third International Symposium on Bluetongue that was held in October, 2003, in Taormina, Sicily (www.bluetonguesymposium.it/index.htm).

History
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 first 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 European breeds of sheep to that region of the world [32]. Prior to the 1940s, BT was thought to be confined to southern Africa and 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 [16,33].

Bluetongue Virus
BTV is the prototype virus of the genus Orbivirus in the family Reoviridae [17]. The BTV genome includes 10 segments of double-stranded RNA, each of which encodes at least 1 viral protein (7 structural and 4 non-structural). The 24 distinct
serotypes of BTV are distinguished by epitopes on the outer capsid protein VP2, although VP5 also can influence neutralization through its conformational influence on VP2 [11]. 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 [15,25]. 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 [29,30]. Individual BTV gene segments evolve and reassort independently of serotype in the field. Genetic drift of individual BTV genes occurs by the selective acquisition and amplification in vector insects of specific variants from the quasispecies virus population that arises in the blood of infected ruminants (founder effect; 6,7).

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 [16,33]. It has been repeatedly and comprehensively shown that BTV infection of fetal cattle is unimportant to the natural epidemiology of BTV infection. 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 (where infection periodically occurs as far north as the Okanagan Valley of British Columbia) and Asia (Kazakhstan and Mongolia), where BTV infection of ruminants can occur 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 [23]. 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.

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 [17,33]. 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 [2]. 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 [1]. There is no evidence, however, that dogs or other carnivores are important to the natural cycle of BTV infection.

Bluetongue Disease of Ruminants
BT occurs 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 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 [24,32].

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 exhibit remarkable genetic heterogeneity, even amongst strains that co-circulate [7,15,25]. 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 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 [4,28]. The similar or identical pathogenesis of BTV infection of cattle and sheep further emphasizes this obvious paradox but recent studies suggest that inherent differences in the susceptibility of endothelial cells from cattle and sheep to BTV infection may be responsible [12-14,28]. 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. 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 [23]. 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 each region of the world differ, as may the environmental factors that promote population expansions of each.

**The Pathogenesis of Bluetongue Virus Infection of Ruminants and Insect Vector**

The pathogenesis of BTV infection is similar in sheep and cattle, and most probably, all species of ruminants [4,19,21,26]. 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 [4,8,31].

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 [9,10]. 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 [8,20,34].

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 [21,26]. Similarly, white-tailed deer, which are highly susceptible to BT,
develop consumptive coagulopathy as a consequence of BTV-induced damage to endothelial cells [18]. Consumptive coagulopathy 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 [22]. 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 [23].

**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). 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 [4,16]. 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 BT 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 [4,19,24]. New generation vaccines, such as the virus-like particles produced by baculovirus expression of different viral proteins [27], 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. Recognition of this reality, along with the fact that ruminants are not persistently infected with BTV, has stimulated long-overdue rationalization of international trade policies pertaining to BT.

**References**


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