Bluetongue: pathogenesis and duration of viraemia

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Summary

Bluetongue (BT) is a non-contagious, insect-transmitted disease of domestic and wild ruminants caused by bluetongue virus (BTV). Whereas BTV infection of the haematophagous *Culicoides* insect vector is persistent (life-long), BTV infection of ruminants is transient. The prolonged viraemia that occurs in many BTV-infected ruminants occurs through a novel interaction of the virus with erythrocytes and, initially, other blood cell types. The presence of BTV in ruminant blood can readily be detected by polymerase chain reaction which provides a very conservative assay for the screening of ruminants prior to movement to BTV-free regions as animals remain positive by PCR assay long after all infectious virus is cleared from their blood. BT disease occurs in sheep and some wild ruminant species and is characterised by vascular injury with haemorrhage, oedema 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 a global emerging disease that was spread by animal movement and trade, it now is clear that BTV exists throughout tropical, subtropical and some temperate regions of the world in distinct, relatively stable, ecosystems where different strains of the virus have co-evolved over long periods of time with different species of insect vector.

Keywords

Bluetongue – Cattle – Epidemiology – Pathogenesis – Sheep – Virus.

Introduction

Bluetongue (BT) virus (BTV) is the aetiological agent of BT, a non-contagious, insect-transmitted disease of sheep and some species of wild ruminants (18, 19, 24, 30, 33, 41). BT was first recognised and comprehensively described in southern Africa, and BTV has subsequently been isolated from ruminants and/or vector insects from all continents except Antarctica (reviewed in 21). As 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 Épizooties (OIE). As a direct consequence of its inclusion in OIE List A, BT continues to have an impact on the global trade of ruminants and their germplasm (2). Concerns over the potential impact of BT on animal production have been heightened by the recent re-emergence of BT in Mediterranean and south-eastern Europe (6).

History

The disease of BT was first described as 'malarial catarrhal fever' and 'epizootic catarrh of sheep' in the original written descriptions 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 centuries, although farmers in South Africa recognised the disease soon after the introduction of fine-wooled European breeds of sheep to that region of the world (18, 41). Prior to the 1940s, BT was thought to be confined to southern Africa. The first well-documented epizootic of BT outside Africa occurred amongst sheep on Cyprus in 1943. The disease was recognised in Texas soon thereafter, and an extensive epizootic occurred on the Iberian Peninsula in 1956-1957. Subsequently, the disease was recognised in the Middle East, Asia and 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 is now 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 in 4, 21, 43).

The pathogenesis of bluetongue virus infection of ruminants

The pathogenesis of BTV infection is similar in sheep and cattle, and most probably, all species of ruminants (5, 24, 28, 35). 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. Viraemia in BTV-infected ruminants is highly cell associated, and viraemia is prolonged but not persistent (reviewed in 5, 9, 24, 40). The virus promiscuously associates with all blood cells, thus titres 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 haematophagous insect vectors that feed on viraemic ruminants (10, 11). 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 by either intrathoracic inoculation or oral feeding (9, 24, 26, 44). Ruminant blood that is positive by PCR assay but not by virus isolation is also not infectious to other ruminants (25, 26).

Ruminants infected with BTV develop a prompt and high titred antibody response to a variety of viral proteins. Serotype-specific neutralising antibodies are directed against VP2, and these can be detected by the serum neutralisation test (12). Antibodies directed against core protein VP7, as well as other structural and non-structural proteins, may be detected with serogroup-reactive assays such as the agar gel immunodiffusion and competitive enzyme-linked immunosorbent assay (c-ELISA). A positive serological result confirms only that an animal was previously infected with BTV. Thus, although BTV infection of cattle and sheep often is prolonged, there is no credible evidence of truly persistent BTV infection of ruminants (5, 9, 21, 26, 40, 44) and the vast majority of seropositive cattle and sheep from BTV-endemic regions are not infected with the virus.

Clinical signs and lesions in BTV-infected sheep possibly reflect virus-mediated endothelial injury, as BTV replicates in endothelial cells causing cell injury and necrosis (28, 35). Similarly, white-tailed deer, which are highly susceptible to BT, develop consumptive coagulopathy as a consequence of BTV-induced damage to endothelial cells (22). Consumptive coagulopathy (disseminated intravascular coagulation) in BTV-infected sheep and deer predisposes to the bleeding tendency that characterises fulminant BT. Endothelial injury is also probably responsible for increased vascular permeability leading to oedema in tissues, such as the lung (pulmonary oedema), and vascular thrombosis leads to tissue infarction.

Foetal infection was once proposed to lead to persistent, immunotolerant infection of cattle. However, this theory has been thoroughly and repeatedly discredited (reviewed in 25). It now is increasingly clear that only strains of BTV that have been modified by growth in cell culture, such as modified live virus (MLV) vaccine strains, have the capacity to cross the ruminant placenta. Once MLV BTV strains cross the placenta they cause embryonic or foetal death, and cerebral malformations after infection of older foetuses that survive congenital infection (reviewed in 27).

Bluetongue disease of ruminants

Although BTV infection of both wild and domestic ruminants occurs throughout tropical, subtropical and temperate regions of the world, BT disease is uncommon or not recognised in many areas where BTV is endemic. For example, BTV infection occurs throughout extensive regions of northern and eastern Australia yet outbreaks of BT disease have

not yet been described. Similarly, there are very few descriptions of BT amongst ruminants in the Caribbean islands or in Central and South America, despite endemic BTV infection in much of this region.

BT occurs principally in sheep and some species of wild ruminants, whereas BTV infection of cattle, goats and most wild ruminant species is typically asymptomatic or subclinical. The signs of BT in sheep reflect congestion, oedema and haemorrhage 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 hyperaemia of the coronary band, and weakness secondary to muscle necrosis. Lesions present at post-mortem of affected sheep can include hyperemia, haemorrhages, erosion and ulceration of the mucosa of the upper gastrointestinal tract (oral cavity, oesophagus, forestomachs); subintimal haemorrhages in the pulmonary artery; pulmonary oedema; pleural and/or pericardial effusion; oedema 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 in 18, 19, 24, 28, 30, 33, 35, 41).

It is to be emphasised 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 naive sheep populations at the interface of BTV-endemic and non-endemic regions. Expression of BT disease reflects a variety of virus, host 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 (4, 8, 16, 17, 34). This genetic variation amongst field strains of BTV has arisen as a consequence of both genetic 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 (37, 38). 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 that 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 have established that genetic drift of BTV genes occurs by selective acquisition and amplification in vector insects of specific viral variants from the quasispecies virus population that arises in the blood of infected ruminants through the process of founder effect (7, 9). 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

Selected breeds of sheep and wild ruminant species (e.g. white-tailed deer) are most susceptible to BT disease. 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 stress 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 in cattle (5, 36). The fact that the pathogenesis of BTV infection of sheep and cattle is similar further emphasises this obvious paradox. Fundamental differences have recently been identified in the inherent susceptibility of endothelial cells from cattle and sheep to BTV infection (13, 14, 15). 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 oedema 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 (20, 42). In an extensive field study in California, virtually all (approximately 98%) isolations of BTV from ruminants were made between August and the end of November (32, 42).

Climatic conditions also have a significant impact on the transmission of BTV. For instance, insect survival is inversely related to temperature so that *Culicoides* insects survive for longer periods in cool temperatures. In contrast, higher ambient temperatures stimulate insect feeding and promote virogenesis of BTV in insects, both of which enhance virus transmission (31). Lastly, it is to be stressed that the environmental conditions that produce the highest numbers of vector insects are likely to optimise the transmission of BTV amongst ruminants. The species of *Culicoides* that transmit BTV in different regions of the world are clearly very different, as may be the environmental factors that promote population expansions of each.

The role of ruminants in the epidemiology of BTV infection

Both insects and ruminants are essential to the life-cycle of BTV. Vector *Culicoides* 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 (24, 43). Climatic conditions also dictate the global distribution 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°N and 35°S. Exceptions are areas of North America and Eurasia, where BTV infection of ruminants can occur as far as 50°N. The species of vector insects that transmit BTV differ between regions, and are especially poorly characterised in much of both Europe and Asia (23, 39).

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 in 29). Vector insects can only transmit BTV to another susceptible ruminant after an extrinsic incubation period of approximately 10 to 14 days, during which time the virus is disseminated from the gut of the insect to its salivary glands. The external incubation period is shorter when insects are held at high ambient temperatures. Ambient temperature has a profound effect on the survival of vector insects, their feeding activity and the replication of BTV in the insect vector (31). Thus, temperature-dependent control of virogenesis and other similar insect-dependent factors possibly limit the expansion of BTV into regions outside 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 (21), as has recently been the case in southern Europe (6).

It is increasingly evident that BTV has not recently been spread globally through international trade and movement of ruminants. Rather, the virus exists in distinct, relatively stable ecosystems in different regions of the world where specific strains of the virus have probably co-evolved with different species of insect vector (4, 21, 43). 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 and the movement of enormous numbers of cattle between the two regions without any testing for BTV. The essential difference lies in the different species of vector insects in the two 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, 3, 4, 6, 8, 12, 14 and 17 in the Caribbean and Central/South America.

A variety of other hosts have been implicated in the life-cycle 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 (3). 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.

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