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Review

Bluetongue virus: Past, present, and future scope

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Abstract

Bluetongue (BT), once considered a disease of sheep confined to the southern African region, has spread all over the world. BT is a viral disease caused by the bluetongue virus (BTV). BT is regarded as an economically important disease in ruminants of compulsory notification to OIE. BTV is transmitted by the bite of *Culicoides* species. Research over the years has led to a better understanding of the disease, the nature of the virus life cycle between ruminants and *Culicoides* species, and its distribution in different geographical regions. Advances have also been made in understanding the molecular structure and function of the virus, the biology of the *Culicoides* species, its ability to transmit the disease, and the persistence of the virus inside the *Culicoides* and the mammalian hosts. Global climate change has enabled the colonization of new habitats and the spread of the virus into additional species of the *Culicoides* vector. This review highlights some of the current findings on the status of BT in the world based on the latest research on disease aspects, virus-host-vector interactions, and the different diagnostic approaches and control strategies available for BTV.

Key words: Bluetongue virus; *Culicoides*; arthropod disease; ruminants.

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Introduction

Bluetongue (BT) disease or catarrhal fever is a non-contagious, insect-borne, viral disease of ruminants, mainly sheep and less frequently cattle. BT disease results in both direct and indirect economic losses. High morbidity and mortality, abortions, stillbirths, abnormalities in the fetus, low birth weight in young ones, reduced milk yield, lowered fertility rate, early culling, meat and fleece losses, etc. contribute to direct losses whereas trade restrictions imposed on animal movement, germplasm, and animal products, expenditure for vaccination, diagnosis, vector control and treatment of clinically pretentious animals are some of the indirect losses encountered due to BT [1]. Due to its economic impact on animal livestock, BT is listed as a multispecies disease by World Organization for Animal Health (OIE) [2].

BTV is the type of species of the genus *Orbivirus* within the family *Sedoreoviridae* [3]. BTV genome is similar to that of *Reoviruses* [4] but there are distinct differences between *Reoviruses* and BTV. Cryo-electron microscopy showed a well-ordered morphology with a unique icosahedral organization of the virus [5]. The virion is spherical in shape and its size varies from 60-80 nm in diameter [6]. It has two icosahedral protein shells termed inner and outer capsids (Figure 1A). The core particle is composed of 32 distinct capsomeres arranged as hexameric rings. This characteristic ring-like configuration leads to the name of this genus – Orbivirus [7].

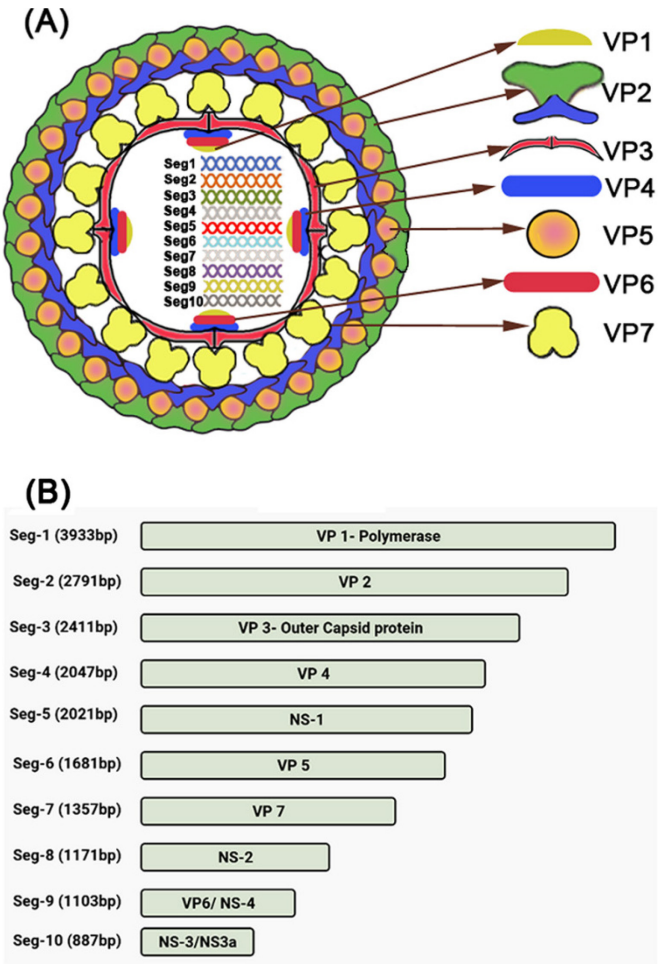
Bluetongue virus causes serious diseases in livestock (sheep, goats, cattle, and deer). Partly due to this BTV has been at the forefront of molecular studies for the last three decades and now represents one of the best-understood viruses at the molecular and structural levels. Data obtained from studies over several years have defined the key players in BTV entry, replication, assembly, and exit and have increasingly found roles for host proteins at each stage. These areas are important for BTV replication but they also indicate the pathways that may be used by related viruses, which include viruses that are pathogenic to man and animals, thus providing the basis for developing strategies for intervention or prevention. The recent findings in molecular biology, diagnosis, and pathogenesis are reviewed here.

Molecular biology

The genome of the Bluetongue virus (BTV), comprises a double-stranded ribonucleic acid (RNA)

that has ten segments, each of which codes for a different protein [8] (Figure 1B). BTV sub-core encloses 10 linear segments of ds RNA, which are designated as large segments L1, L2, and L3; medium segments M4, M5, M6 and small segments S7, S8, S9, and S10. The length of RNA segments varies from 3954 to 822 bp. The molecular weight of RNA varies from 0.5-2.7 × 10^6 Da [9]. The different RNA segments are classified as highly variable, moderately conserved, or highly conserved. Oligonucleotide fingerprint analysis of individual gene segments among different BTV serotypes revealed that except for L2 and M5, the fingerprint is identical for all gene segments [10]. These 10 gene segments code for 7 structural proteins, i.e. VP 1-7, and four non-structural proteins, i.e. NS 1-3 and 3A [11]. Gene segments 2 and 5 code for outer coat proteins, while segments 1, 3, 4, 7, and 9 code for core proteins, whereas gene segments 6, 8, and 10 code for non-structural proteins [12]. All genes coding for non-structural proteins of BTV (segments 6, 8, and 10) along with a majority of genes coding for inner capsid polypeptides are highly conserved. *In vitro* translation studies have shown that segment 10 (S10) of the BTV is the smallest BTV RNA segment encoding for NS3 and NS3A *proteins* (previously referred to as P8 and P8a respectively) [13]. The S10 gene segment is conserved among different BTV serotypes [14]. Genes that encode the NS3 protein of different orbiviruses are also compared [15]. The purified virus is composed of 80% protein and 20% ds RNA [6]. The G + C content is 42.4 % [7].

Figure 1. (**A**)Virion structure of Bluetongue virus; (**B**) Genomic organization of Bluetongue virus. The diagram depicts the BTV genome (~19.2 kb) organization.



BTV is an RNA virus with 10 segments encoding for 4 non-structural and 7 structural proteins. Segment 1 encodes for the RNA polymerase VP1. Segment 2 encodes for the highly variable VP2. Segment 3 encodes for the inner core protein VP3. Segment 4 encodes for the methyl transferase and RNA capping enzyme VP4. Segment 5 encodes for NS1, which forms cytoplasmic tubules. Segment 6 encodes for the outer capsid protein VP5. Segment 7 encodes for the inner core protein VP7. Segment 8 encodes for NS2, an RNA-binding non-structural protein. Segment 9 encodes for the helicase VP6 and for NS4, a non-structural protein involved in immune evasion. Segment 10 encodes for NS3 and its isoform NS3a, which are polyfunctional non-structural proteins involved in viral particle exit from the cell and also in interference with the mammalian IFN system. Segment 10 may also encode for a fifth non-structural protein (NS5), which could be playing a role in the cellular shutdown.

Incidence and seroprevalence of BTV

The first official report of BTV infection was from the Cape Province of South Africa in the late 18th century, following the import of fine-wool Merino sheep from Europe [16]. The term 'Bluetongue' was derived from the Afrikaans word 'bloutong' or 'Blaauwtong', which was used by Afrikaans farmers after observing the cyanosis of the tongue in clinically affected sheep [17]. Various studies have revealed that BTV infection is recognized as an enzootic disease in areas between latitude 40 ºS and 53 ºN in almost all continents like America, Africa, Australia, and Asia [18]. A severe outbreak of BT was reported in Europe involving four Greek islands in 1998 and which over the next seven years, spread to at least 16 Mediterranean countries. By 2005, this outbreak has already killed over a million sheep and was registered as the largest epidemic of BT ever seen [19]. Further during 2006, BTV exhibited a massive northwards leap, into northern Europe and invaded a series of countries in the northwest of the continent (Northern France, Belgium, Netherlands, Luxembourg, and Germany [20,21]. Later in 2007, BTV outbreaks re-emerged in these affected countries affecting more than 45,000 farms and spreading into the UK, Denmark, and the Czech Republic, etc except Antarctica. [22]. In the Asiatic sub-continent, Bluetongue was first reported in Pakistan, as early as 1958 [23] followed by the state of

Maharashtra in India [24]. Since then outbreaks of BT have been reported from different parts of India [25-27]. BTV is now considered to be endemic in both India and Pakistan. However, there have been few reports on the incidence and prevalence of BT from other countries in the region, like Afghanistan, Bhutan, and Sri Lanka [28]. BTV antibodies have been observed in animals, however, the disease has not been reported in Bangladesh, Myanmar, and Sri Lanka [29]. A study in Nepal reported a BTV seroprevalence rate of about 45.20% among domestic ruminants [30]. While another study conducted on dairy cattle in Nepal, reported about 29.3% to be positive for BTV antibodies [31]. A study from the Tibetan plateau reported an overall seroprevalence of BTV of about 20.3% in Tibetan sheep and 13.3% in yaks [32].

Based on virus isolation from clinical specimens or on the detection of virus-neutralizing antibodies in the sera of recovered animals, various BTV serotypes have been recorded worldwide. Based on serum neutralization tests and virus neutralization assays, 24 serotypes of BTV have been recognized worldwide [28]. As the specificity of these serum and virus neutralization tests shows a close correlation with the amino acid variations of the VP2 gene, this was used to identify the existence of the 25th serotype in Switzerland [20], 26th from Kuwait [33], and 27th from France [34]. Bluetongue virus serotypes have shown geographic localization and at times, multiple serotypes have been reported from a single country (Table 1). Although BTV-17 was originally isolated in the United States, it has now been reported from other places. Similarly, BTV-18 and BTV-19 were isolated from clinical cases from South Africa in 1976 (Erasmus, Unpublished). BTV-20 and BTV-21 were first isolated in Australia [35]. BTV-22 and BTV-23 were first isolated from wild-caught Culicoides in South Africa [36]. Later BTV-23 was also isolated from sentinel cattle in Australia [37] whereas BTV-24 was isolated from sick sheep (Erasmus, Unpublished) as well as from wild-caught Culicoides in South Africa [36]. Of these 27 serotypes of BTV, 23 have been reported from India [38]. Antibodies that will neutralize a wide range of BTV types can be generated only after sequential infection with different BTV strains [39]. This can make the serological identification of BTV serotypes very difficult, particularly in endemic areas, like India, where multiple serotypes are circulating. BTV serotypes 1, 3, 4, 9, 16, and 17 were reported in two outbreaks in Himachal Pradesh [25]. From BT outbreaks in Maharashtra, serotypes 1, 2, 3, 4, 7, 8, 10, 16, 17, and 18 were reported [40,41]. Serological surveys conducted in Tamil Nadu showed neutralizing antibodies against BTV serotypes 1, 4, 5, 6, 7, 11, 12, 13, 14, 15, 16, 17, 19, and 20 [26,42]. There are reports of concurrent outbreaks of BT and PPR in Dehradun, Uttarakhand, and BTV-23 was isolated [43]. Later, BTV-2 and BTV-9 were isolated from outbreaks in native sheep of Andhra Pradesh [44,45].

Host range

Blue Tongue has been recorded in most ruminants like sheep, cattle, buffalo, goats, camels, wild ruminants like sambar, white-tailed deers, blue bulls, lamas, antelopes, etc. [46]. Among the ruminants, sheep are the most susceptible animal and suffer from variable clinical manifestation symptoms and sometimes a hemorrhagic disease [47]. Indigenous African breeds generally show subclinical infections. Indigenous sheep are comparatively less susceptible to BT than the exotic breeds of sheep like Merino [16,48]. The European mutton breeds such as the Dorset Horn [16] are regarded as highly susceptible compared to Merino sheep. However, there is also a marked variation in susceptibility among individuals within a specific breed. This, together with the marked variation in the

Table 1. Prevalence of various bluetongue virus (BTV) serotypes worldwide.

Region	Serotypes detected
African continent (South Africa, Egypt, Algeria, Libya, Morocco, Tunisia and Nigeria	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24
European continent (France, the Netherlands, Germany, Belgium, Spain, Portugal, Switzerland, Ireland, Luxembourg)	1, 2, 4, 6, 8, 9, 10, 11, 14, 16, 25, 27
North American continent (USA, Mexico, Canada)	1, 2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 17, 18, 19, 22, 24
South American continent (Brazil, French Guiana, Argentina, Colombia, Suriname, Guyana, and Ecuador)	1, 2, 3, 4, 6, 8, 9, 10, 12, 13, 14, 17, 18, 19, 20, 21, 22, 24, 26
Central America (Guatemala) and Caribbean region (Jamaica and Caribbean islands)	1, 3, 4, 6, 8, 10, 11, 12, 13, 14, 17, 19, 22
Australian continent	1, 2, 3, 4, 5, 7, 9, 12, 15, 16, 20, 21, 23, 24
South Asia (India, Pakistan, Sri Lanka, Bangladesh, Afghanistan)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24
East Asia (China, Japan and Taiwan)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 20, 21, 23, 24
Southeast Asia (Indonesia and Malaysia)	1, 2, 3, 5, 6, 7, 9, 12, 15, 16, 20, 21, 23
Western Asia (Turkey, Cyprus, Syria, Lebanon, Israel, Jordan, Oman, Kuwait, Saudi Arabia)	1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 16, 24, 26

pathogenicity of different BTV strains, is responsible for unpredictable and variable mortality in both natural and experimental infections. The severity of BT in sheep is greatly influenced by environmental conditions like solar rations which aggravate the disease [49]. Hyperaemia of the skin is usually very severe in those areas not covered by wool but often involves the entire body [48]. This dermatitis contributes to the 'break' of wool fibers with the occasional casting of the entire fleece 3–6 weeks later [50]. The mortality rate can be very high if infected sheep are exposed to cold, wet conditions which often happens in late autumn [51]. Older sheep are more susceptible than lambs [52]. Sheep of 6-12 months of age were more susceptible when compared to the 3-6 months of age group and suckling lambs of 0-3 months of age without visible clinical signs [27].

Other domestic ruminants have also been shown to be susceptible. Susceptibility of calves to experimental BTV infection has been demonstrated [16] and natural outbreaks in cattle have also been reported [53]. BTV from naturally exposed cattle was also recovered but any clinical signs or mouth or foot lesions in such cases or any clinical diseases in any experimental cases were not noticed [54,55]. Cattle serve as the reservoir source of BTV and the infection in cattle is usually asymptomatic or sub-clinical [56]. The recent outbreak of BTV-8 in Europe has shown remarkable differences in the clinical expression of disease, with clinical illness and reproductive disorders such as abortion, stillbirth, and fetal abnormalities [57]. BTV-8 infection has been reported as the cause of infertility in dairy cattle and was transmitted vertically to offspring [58].

The susceptibility of goats to BTV infection was first recorded in the year 1905 [16]. Clinical signs in goats were regarded as characteristic of BT and some goats even succumbed to the infection [24]. A mild febrile reaction and slight hyperaemia of the conjunctival and nasal mucous membranes in artificially infected goats have also been recorded [59]. Viraemia was demonstrated in some goats for 19 days but the level of viremia was about a hundred-fold lower than in sheep. Experimental infection of Saanen goats with American strains of BTV resulted in all goats being infected as evidenced by leucopenia, viremia, and the development of precipitating antibodies [52]. Febrile responses were variable and viremia was about 10-fold lower than in sheep. Susceptibility of Poll Dorset sheep to artificial infection with BTV-14 showed mild clinical symptoms with a peak viremia between days 6 and 7 post-infection [47].

Virological and serological evidence of BTV infection has been recorded in many wild ruminants [46]. The African antelope do not develop clinical disease, whereas, in the USA, the white-tailed deer (*Odocoileus virginianus*), the pronghorn (*Antilocapra americana*), and the desert bighorn sheep (Ovis canadensis) may develop severe clinical disease. Sheep seem to be involved in a secondary epizootiological cycle [51]. Camels may act as reservoir hosts for BTV and play an important role in its transmission [60]. Fatal BT was also described in dogs immunized with a vaccine contaminated with BTV. The disease was also re-produced in experimentally infected dogs [61]. Incidences of abortion by pregnant bitches and deaths due to severe pulmonary edema have also been reported [62]. Sero-positivity of 21% in dogs was reported in Morocco [63].

Transmission

BT was most prevalent during the late summer months, especially following wet seasons; the disease being more common in low-lying pastures and valleys than on the high-lying ground. There is very strong evidence to suggest that BT was an insect-transmitted disease [16]. Amongst these were *Culicoides* midges, a group of insects that were not viewed as a potential vector of any pathogen. BTV was successfully transmitted either by injecting emulsions of wild-caught Culicoides into sheep or by allowing *Culicoides pallidipennis* (imicola), previously fed on infected sheep, to bite susceptible sheep 10 days later [64]. The biological transmission of BTV by *Culicoides* was proven beyond any doubt with colonized *C. variipennis* [65].

The vectorial role of *Culicoides* has since been corroborated in all areas in which BTV occurs as well as for many other *Orbiviruses* (Figure 2). Although there are more than a thousand *Culicoides* species reported in the world only a few were found as a potent vector to transmit BTV [66]. *C. bolitinos* was identified as an additional vector of BTV [67] and *C. bolitinos* had a significantly higher transmission potential for BTV-1 than did *C. imicola* [68]. The predominant species in Tamil Nadu were *Culicoides imicola* and *C. peregrinus*, while in the Marathwada region and Kolkata, *Culicoides schultzei* was reported to be the predominant species [69]. The presence of several species of *Culicoides* (*C. actoni, C. anophelis, C. inoxius, C. majorinus, C. peregrinus,* and *C. oxystoma) from* Chittoor and Prakasam districts of Andhra Pradesh as a prominent source of transmission of BT was reported [70]. The multiplication of *Culicoides* is more during

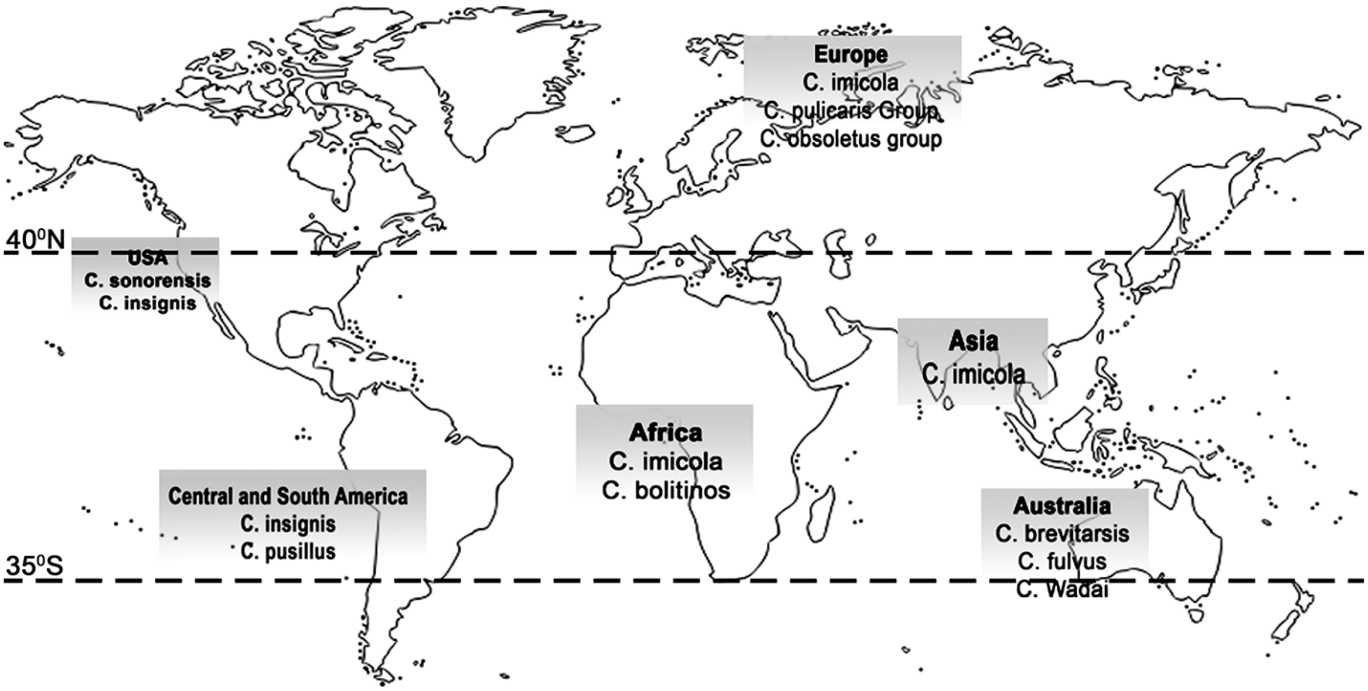
the rainy season resulting in more outbreaks as compared to other seasons [27]. Continuous climate change also favors the multiplication and extension of *Culicoides* species in new geographical areas, resulting in the occurrence of severe bluetongue outbreaks in sheep in Greece, Italy, and hitherto BT-free zones [19]. Ticks and sheep keds act as mechanical vectors but are probably of minor importance in disease transmission. Venereal transmission from infected bulls is also a possible method of disease transmission. BTV can occasionally be transmitted via seminal fluid and across the placenta [71]. The virus can also spread mechanically through surgical equipment and needles.

Pathogenesis and Clinical manifestations

Following the bite of the insect vector, the virus is transported to the lymph nodes where initial replication occurs. From the skin, the virus is then disseminated to lymph nodes via dendritic cells. The virus replicates in the vascular endothelium and endothelial cells lining the blood vessels. Subsequently, the virus is released into the circulation where it associates with the erythrocytes resulting in tissue infarction, necrosis, and vascular thrombosis [46]. During this period, the viremia persists for 30 days or more in sheep and up to 100 days or more in cattle [72]. BTV is mostly associated with erythrocytes and platelets. Virus is also disseminated via the lymph and vascular system to the secondary site of replication, particularly lymph nodes, spleen, and lungs [73]. The spleen is the principal site of secondary viral replication in BT-infected animals [74]. The release of BTV from the spleen was followed by a secondary viremia in which the virus was highly cell-associated [75]. BT infection leads to cell apoptosis and necrosis by the activation of p38MAP kinase causing vascular permeability and enhanced production of thromboxane and prostacyclin in the blood leading to the excessive inflammatory response [76].

Bluetongue outbreaks are recorded in an acute or sub-acute form among ruminants with characteristic symptoms of congestion, edema, and hemorrhages [17]. The incubation period of BT is 6 – 9 days [77]. The clinical signs recorded are a rise in body temperature (104-108 °F), respiration rate, hyperemia, and swelling of buccal, nasal, and ocular mucosa [78]. These above symptoms are followed by nasal discharge and frothing at the mouth. The nasal discharge may be serous, mucopurulent, or bloody. Frothy salivation is also noticed in affected animals. Swelling of lips and licking of lips and nostrils is followed by cracking of epidermis at the commissures of lips along with edema of the face, sub-maxillary space and occasionally ears and eyelids. The mucosa of gums, cheeks, and tongue may ulcerate. The tongue becomes swollen, markedly congested, and may protrude from the mouth. Because of the cyanotic appearance of the tongue [16,53], the name bluetongue has been assigned to the disease. Later there is an excoriation of the oral epithelium, which leads to infection and necrosis.

Figure 2. Geographical distribution of Culicoides midges.



The other related clinical signs in acute cases are arching of the back-muscle stiffness, lethargy and anorexia. Later the affected animals develop marked lameness due to coronitis with petechial hemorrhage around the coronary band and periople [79]. There is a breaking of wool fibers in affected sheep. Degeneration of skeletal muscle leads to extreme weakness, prostration and terminal torticollis occurs. In some affected animals vomition is recorded due to smooth muscle lesions on the pharyngeal region and esophagus causing aspiration pneumonia, leading to death. Pregnant ewes may abort or give birth to lambs with congenital abnormalities [77]. BT-infected sheep show gross pathological changes like generalized hyperemia, widespread sub-cutaneous edema, and hemorrhages. Hemorrhages are seen on the pulmonary and aorta on the cardiac end. Hemorrhages on the upper gastrointestinal tract (GIT), mostly in the oral cavity, esophagus, ruminal pillars, and omasum are found. Erosions and ulceration of the oral cavity and GIT are also seen along with necrosis of skeletal and cardiac muscle, particularly in the myocardium of the papillary muscle of the left ventricle [37]. BTV replicates in endothelial cells and produces coagulation abnormalities such as consumptive coagulopathy, which ultimately pre-disposes to hemorrhagic diathesis, a typical characteristic of fulminant BT [80]. Cattle naturally infected with BTV generally show no clinical signs but, viremic bulls might shed BTV in the semen [75]. Recent reports on clinical symptoms in cattle with reproductive disorders and fatal abnormalities have been recorded [57,58].

Diagnosis

Characteristic clinical signs and post-mortem lesions help in the tentative diagnosis of BT. Differential diagnosis is of critical importance, as BTV like many other exotic diseases can spread rapidly and may quickly become established within a naive and susceptible host population. Clinical diagnosis of BT always requires confirmation by laboratory testing. Confirmative diagnosis of bluetongue is done by virus isolation and identification, detection of bluetongue viral proteins and nucleic acids in clinical samples, and/or by demonstration of antibodies against bluetongue virus [81].

The isolation of BTV from clinical samples is the most reliable and classical way of bluetongue diagnosis. BTV can be isolated from blood or semen collected from animals during febrile response and high viremia [82]. BTV can also be isolated from tissue samples including spleen and lymph nodes, liver, brain, and mucosal epithelium from affected animals. The virus is very stable at neutral pH, in solutions with a high protein content [13], and has been isolated successfully from blood samples stored in the presence of EDTA at 4 °C for more than a year [83]. Primary isolation of BTV is done by inoculation of clinical material (mostly blood) into sheep and suckling mice [41].

BTV can also be propagated in eight days old embryonated chicken eggs (ECE) [41,44]. Nowadays in-vitro cell culture system is used regularly for the isolation and propagation of BTV [84]. Various cell lines can support the growth of BTV, which includes vertebrate cell lines like baby hamster kidney, African green monkey kidney (Vero), rabbit kidney, bovine kidney, canine kidney, bovine turbinate, bovine endothelium (CPAE), bighorn sheep tongue, equine dermis, gekko lung, rainbow trout gonad, mouse fibroblast (L929); the invertebrate cell lines from mosquito and biting midge [44].

Isolation and propagation of BT virus is very cumbersome and time-consuming. Due to this reason, direct detection of viruses, their antigens, and antibodies in suspected clinical samples is commonly used for rapid diagnosis. Different techniques like fluorescent antibody staining and virus neutralization, immuno-histochemical, immuno-enzymatic and immuno-electron microscopic techniques, using monoclonal antibodies (MAb), offer more rapid, specific and sensitive approaches for BTV identification and antigen detection [72,85]. Few researchers have also reported the development of indirect and sandwich ELISA using purified or recombinant BTV protein for the detection of antibodies [86].

Due to the advances made in nucleic acid-based testing (NAT) methods, various molecular assays like reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR using molecular beacons and probes have been developed for identification, characterization, and quantification of BT virus [87,88]. These assays are rapid, sensitive, and more reliable as they don't rely on the isolation and identification of a live virus, but depend on the detection of highly specific molecular subunits of the virus in infected cells and clinical specimens.

Economic impact and control

Recurring outbreaks of clinical BT in sheep and other domestic ruminants have caused a great economic loss in developing and developed countries. As per a global estimate, BT can cause a loss of about US$ 3 billion in these countries [2]. The majority of these

losses are classified as direct losses in production due to mortality, abortions, reduced fertility rate, congenital abnormalities, and reduced meat production. It is difficult to assess the effect of BTV infection in cattle and buffalo, as they are largely asymptomatic, but can result in a significant drop in milk yield and productivity [89]. Indirect losses include costs of vaccines and lost revenue due to trade restrictions. As per estimates, it is believed that the Indian sheep industry suffered an annual economic loss accounting for approximately 231 million rupees due to the outbreak of BT in 2005 [90]. In 2007, overall estimates of the financial impact in France and the Netherlands, due to BTV, were US$ 1.4 billion and US$ 85 million, respectively.

Mass vaccination seems to be a good approach to controlling the disease. With the improvements in culturing the virus in cell lines, it is now possible to facilitate the production of large volumes of vaccines. Despite the major improvements in BTV vaccine production, problems were still encountered with its efficacy, which can be attributed to various factors. The most significant of these was undoubtedly the multiplicity of BTV serotypes. As more serotypes were identified in the field, a polyvalent vaccine was needed. The vaccine contained all 14 serotypes, which is ludicrous if one considers that these are all live attenuated vaccine strains, and good immunity is dependent upon the replication of each of these vaccine strains in the recipient host. It soon became clear that administration of a polyvalent live attenuated vaccine does not automatically lead to complete polyvalent immunity. Due to this reason, some of the poorly immunogenic strains were omitted. Between 1969 and 1975, the vaccine contained only nine serotypes. An inactivated adjuvanted pentavalent vaccine containing BTV-1, -2, -10, -16, and -23 was developed and is currently used in India for the control and prevention of the disease [46].

Along with effective vaccination, interruption of the BTV transmission cycle is considered to be the most effective method of prevention and control of BT disease. Most of the vector control approaches involve systematic and repeated application of insecticide and are expensive to implement. Several approaches have been used to control *Culicoides* vectors, including the use of chemical insecticide sprays in livestock housing and 'pour-on' insecticides applied to animals during the vector-abundant season. The treatment of sheep in insecticide dips is an effective method for the control of ectoparasites and has been used against *Culicoides* on several of the more 'organized' sheep farms.

Similarly, parenteral administration of certain insecticides (e.g. Ivermectin) has been reported to reduce the abundance of ectoparasites and the incidence of BT. Although synthetic chemical insecticides are very effective in destroying midges and their larvae, these also pose a significant risk to human and animal health and the environment. Consequently, efforts are being made to develop alternative pest management strategies that are non-polluting and environmentally benign. One such strategy that has attracted much interest involves the use of naturally occurring bio-insecticides such as products from the Neem tree (*Azadirachta indica*) [91].

Future scope

Bluetongue is a non-contagious arthropod-borne viral disease of livestock, causing severe economic losses. A highly concerted effort is needed to control this disease in endemic areas. This is further hampered by the existence of 27 BTV serotypes, which largely fail to cross-protect (most of which are circulating in India). This has made the goal of protective immunization against the disease particularly difficult to achieve. Live attenuated or inactivated vaccines, based on all circulating local Indian strains of the virus, are not available. However, recent developments of inactivated or sub-unit vaccines may help to control the disease in the subcontinent. This may be the only alternative for the coming days. Continuous monitoring of the different strains of bluetongue virus circulating in a particular area is necessary for developing relevant diagnostics and vaccines. Surveillance and epidemiology in endemic areas are of great significance to control the entry of viruses into newer regions.

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