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# Potential mechanisms underlying bluetongue virus emergence and spread

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Bluetongue is a non-zoonotic arboviral disease transmitted by biting midges of the genus *Culicoides*. Among 1,347 *Culicoides* species, 30 are known to be vectors of the disease. Bluetongue affects domestic and wild ruminants across all continents, except the Antarctica, causing growing economic losses in livestock production and international trade. In this work, we focused on potential mechanisms underlying the bluetongue virus emergence and spread. Of these mechanisms, the emergence of novel serotypes makes vaccination controversial despite its effectiveness, along with the vector's ability to acquire the virus and successfully transmit it, spatio-temporal adaptation, and identification of new transmission routes. We also discussed the predisposition of the vertebrate and invertebrate hosts to contract the virus, as well as factors hijacking vector population activity and abundance. Indeed, the ecological dynamics of biting midges strongly influence the ability of the virus to be transmitted and to cause epizootic into parts of the world never before affected, helped by interactions between the pathogen and its host in addition to multiple routes deployed to escape defense barriers in the target infected cells. The points raised in this review can help inform aspects of interest for future studies that may elucidate factors to consider in monitoring and controlling the expansion of *Culicoides*-transmitted arboviruses.

## KEYWORDS

bluetongue virus, serotypes, transmission routes, *Culicoides*, ecological dynamics, bluetongue monitoring

## 1 Introduction

Bluetongue virus (BTV) is an arthropod-vectorized emerging pathogen of wild ruminants and livestock endemic in many parts of the world. BTV causes notifiable severe hemorrhagic disease mainly in sheep, some species of deer, and pronghorn antelope (1–3) as reported by the zoo-sanitary code of the World Organisation for Animal Health (WOAH) (4–7). In fact, bluetongue (BT) is considered potentially debilitating, infectious

but a noncontagious disease in ruminants (8) with clinical signs that differ widely between species. Infected cattle rarely exhibit clinical manifestations but constitute an important reservoir for epidemiological studies because they have longer viremia than observed in sheep (9). In goat and camelid, the infection usually goes unnoticed with subclinical manifestation. Recovery is spontaneous and rapid in most cases. Whereas, in sheep, the disease produces severe physical and cellular damage ranging from fever, edema, excessive salivation, anorexia, nasal discharge, lameness, coronitis, and swelling of the head to death in extreme cases. Since the 2000s, high morbidity has been recorded with the incursion of several virulent viral strains in sheep and cattle leading to growing economic impact in livestock production (10). Mortality in sheep ranges from 5% to 30% and sometimes reaches 70%, which can cause serious socio-economic and trade restrictions at local and global levels (11–13).

BTV is the prototype member of genus *Orbivirus* within the family *Sedoreoviridae* (5, 10, 14, 15) transmitted by competent adult female *Culicoides* midges. The virus particle is icosahedral and is made of an outer capsid (consisting of VP2 and VP5 proteins) and a double-layered core (VP3 forms the inner layer of the core and VP7 forms the outer layer of the core). Five additional non-structural proteins, NS1, 2, 3/3a, 4, and 5 (16–18) are produced in virus-infected cells (19) and are involved in BTV release from mammalian and insect cells via interactions between viral and host cell proteins (20).

BTV genome is known to undergo high frequency of genetic polymorphism and reassortment events responsible for the emergence of new serotypes or antigenic variants of the same serotype (20). BTV as a species is identified by the group-specific antigen, that is the VP7 protein, while the serotype is determined by the VP2 protein (19). Reassortment involving genome segment 2 will cause an antigenic shift thus changing the serotype of the particle. However, vaccination remains the most adequate response to protect the animals and control virus spread, yet a vaccine-matching strategy should be implemented. BT continues to be debilitating with wide virus propagation in both ruminant and insect cells inducing considerable socioeconomic concern. In addition, incursion of novel serotypes in enzootic regions as well as combination of genetic drift and genomic reassortment offer increasing pathways for the virus to bypass and evade the defense barriers of the host (19). Hence, a broad-spectrum vaccination strategy based on the use of cross-protective vaccine subunits such as the NS1 would confer a cross-serotype protection.

The aim of this paper is to discuss potential mechanisms underlying bluetongue virus emergence and epidemiology with an overview of disease outbreaks prediction criteria.

## 2 Control and prevention of BTV infection

### 2.1 Sanitary preventive measures

Sanitary protection measures (21) are based on two axes:

- The control of animal movements from infected countries by isolating or even slaughtering sick and infected animals and destroying cadavers in endemic areas.
- Vector control through regular insecticide treatments.

These measures can be reinforced by serological surveys in sentinel farms to monitor the possible spread of the disease. Trapping of *Culicoides* helps define areas of vector activity.

### 2.2 Medical prophylaxis

As a medical approach, vaccination remains the best way to protect against bluetongue, although controversial due to the emergence of new serotypes, given the difficulty of protecting oneself from insect bites. Different types of vaccines are available, live attenuated vaccines, inactivated virus vaccines, and new generation vaccines. In non-African breeds of sheep, live attenuated vaccines cause severe clinical signs and hence, are not licensed for use in several countries.

## 3 BTV serotypes, a number likely to increase

BT was considered for a long time as an exotic disease limited to tropical and temperate sub-tropical regions (5, 12). It is believed to be that until the 1950s. The disease was first recognized in South Africa, the most probable origin of BT, in Merino sheep in the late 18th century (22). Alkhamis et al. (23) proposed that goats are the ancestral hosts for BTV. It is even thought to be endemic in wild ruminants in sub-Saharan Africa since antiquity. In 1906, Thelier et al. (24) demonstrated that BT was caused by a filterable pathogen. Serotype 4 is the first BTV serotype identified in South Africa. They also introduced the first attenuated vaccine against BTV-4 that has been used in farming sheep for over 40 years and that provides cross-protection to other serotypes (25). Outside of Africa, infectious outbreaks were reported in Cyprus in 1943 (26, 27). After that, BTV has spread subsequently to regions between the latitudes of 35°S and 40°N (1, 3, 25, 28–31). In addition to the African continent, these areas encompass Asia including the Indian subcontinent and China, Central and South America, Middle East, Israel, and Australia where BTV was isolated for the first time from *Culicoides* in the Northern Territory in 1975 (29–32). In the autumn of 1977, serotype 4 was isolated from female *Culicoides obsoletus* during a second epizootic of BT in Cyprus (27, 29). The European continent was considered safe from the disease with just a few sporadic incursions in the Iberian Peninsula (5, 33) until 1998. However, since the virus incursion into several Mediterranean basin countries in 1998 with the arrival of BTV-9 in Greece, the emergence of new serotypes in disease-free areas as naïve populations, as well as in endemic regions has modified the epizootic situation generating massive losses for the livestock industry through reduced productivity and death affecting herds particularly in farmed sheep. Since then and for a few years, at least

six BTV serotypes (BTV-1, BTV-2, BTV-4, BTV-8, BTV-9, and BTV-16) have been continuously present in parts of the Mediterranean Basin (34).

Explosive unprecedented outbreak of BT disease has been reported in 2006 in Northern and Central Europe with the arrival of BTV-8 in the Netherlands and the disease spread to neighboring countries. During the hot seasons of 2006 and 2007, the situation was alarming with the outbreak of a devastating epidemic (5), which triggered Europe-wide monitoring projects on the dynamics of biting midges of the genus *Culicoides* (35) as well as intensive vaccination campaigns against BTV-8. The propagation of BTV-8 involved two previously unknown to be effective BTV vectors, *Culicoides obsoletus* and *C. pulicaris* (12, 36).

The first occurrence of BT in Tunisia dates to 1999. BTV-2, BTV-1, and BTV-4 have been reported in 2000, 2006, and 2009 respectively (37). Since then, active circulation of Mediterranean BTV strains has been noted.

Since the onset of the disease until 2008, 24 classical serotypes of BTV (BTV-1, BTV-24), replicating in vertebrates and *Culicoides*, were officially recognized according to cross neutralization assays (38, 39). Live attenuated vaccine strains, originally developed in South Africa were the origin of BTV-6 and BTV-11 strain outbreaks in Northern Europe between late 2008 and early 2009 (40). Other serotypes have been proven to come from reassortments; a field reassortant between BTV-1 and BTV-8 named FRA2008/24 had been described in France (41). Reassortment was also detected in *Culicoides sonorensis* being co-infected with BTV-10 and BTV17 (42).

Due to the movement of infected ruminants, the abundance and wind dispersal of infected midges and the changes in climate, novel BTV strains have appeared with the possibility of reassortment between different serotypes (32). Genome comparison, conventional serotyping completed by evolutionary divergence data, allows determining the source of the infection and its spread in different areas. At present, the disease is present all over the inhabited world except Antarctica (43). BT is a notifiable disease and while the WOAHP manual cites BTV-1 to BTV-27, at least 12 'atypical' serotypes have been recognized so far, bringing the total number of published BTV-serotypes to 36 (44, 45). The serotype identification relies on the sequence of Seg-2/VP2. (3, 19, 46–48). Unlike classical serotypes, atypical ones appear to be unable to replicate in the arthropod vector suggesting direct transmission between vertebrate hosts (between infected and susceptible animals). Contamination occurs possibly via aerosols expelled into the air by hosts carrying the virus (49). To date, this mode of transmission is suspected for certain serotypes such as BTV-25 (50, 51), BTV-26 (12, 52, 53), BTV-27 (47), and BTV-28 (54). Although the recognized atypical serotypes have not caused severe bluetongue epidemics or economic losses to date, some of them have been shown to cause mild to moderate clinical signs in small ruminants exclusively (by experimentation or direct observation in the field) (55).

Ongoing epidemiological studies based on sequencing of complete or partial nucleotide genomic regions complemented by several phylogenetic analyses allow statistical estimation of genetic variability generated by RNA segments reassortment,

recombination events, genetic drift, and mutation within serotypes. BTV genome replication is highly error-prone due to the lack of proof-reading mechanisms (56, 57). These biological events could lead to the emergence of more or less virulent strains by driving phenotypic change resulting in a fitness advantage for the virus (19, 41, 58). Intrasegment recombination is likely to occur between BTV strains either within vertebrate host (ruminant) or insect host (*Culicoides*) (19). Such information helps in the appreciation of the geographical spread, and subsequently, the emergence of novel putative BTV serotypes into enzootic or non-enzootic regions (59).

Apart from the presence of different serotypes, two major geographic groups of BTVs are described and designated as eastern (e) or western (w) topotypes even within the same serotype. The eastern topotype includes viruses from Australia and the Middle/Far East; the western viruses originate from Africa and the Americas (38, 39).

## 4 Pathogenic index depending on infecting serotype

An interesting aspect of BTV infection is the variability of the host susceptibility. This is attributed to several factors including species of the infected subject, its age, and its immune system, as well as the infecting strain (30, 60). Indeed, different degrees of virulence have been described depending on the infecting serotype and resulting in different clinical manifestations. Variable severity of BTV infection comes from phenotypic and genotypic polymorphism related to high mutation rates in viral genome inducing the expression of more or less pathogenic character. For the same serotype, different pathogenicity levels would be observed according to its geographical origin. For example, the serotype 1 is reputed to be very aggressive in Spain and France displaying intense clinical signs and severe pathological lesions in sheep and goat flocks (61–63). Whereas, in Africa, Asia, and Indian subcontinent countries, the same serotype 1 is commonly associated with high pathogenic potential in sheep but no evidence for severity in goat has been reported. Neither clinical signs nor mortality were observed in cattle herds. It has also been reported that serotypes 1 and 3 from South Africa are more virulent than those from Australia. In Tunisia, infection with BTV-3 has resulted in high mortality rates in sheep (64). The European serotype 8 that triggered BT outbreak in 2006 has shown to be highly virulent with high mortality rates in naive sheep and many cases of severe clinical manifestations in cattle (25, 29, 63, 65). Other BTV strains (BTV-6, BTV-11, BTV-14) detected in Europe exhibited very little pathogenicity in farm animals. In enzootic areas, BTV serotypes 1, 2, 3, 4, 6, 8, 10, 11, and 24 are known to have a high epizootic potential mainly in sheep (22, 66) unlike serotypes 20 and 21 that would be clinically the most moderate forms. BTV-25 was initially identified in goats following cases of still births and BTV-26 was isolated from sheep showing BT clinical signs. BTV-28 caused clinical signs in experimentally infected sheep. Care should be taken not to bestow the non-pathogenic character onto all atypical BTV serotypes. Co-infection with two or more serotypes

was reported in fields as well in experimental context implying biological consequences during viral replication within co-infected host cells (61). Moreover, Westrich et al. (45) established a model of BTV-17 infection in sheep including a longitudinal vertebrate host's immunological response and viral infection progression using a combination of conventional methods and cutting-edge technology.

## 5 Differential mechanism of virus release in host cells and attachment parameters

Upon feeding, the virus contained in the blood meals from infected vertebrate hosts is deposited in the mid-gut diverticulum. Viral particles attach to the surface of the gut cells and enter them by direct penetration or endocytosis (2). *Culicoides* cells are readily infected by core particles (usually non-infectious to mammalian cells). The core particle of an orbivirus is composed of an outer layer (VP7) and an inner layer (VP3) and this structure encloses the transcriptase complex (VP1, VP4, and VP6) together with the dsRNA genome (10). This core particle can bind to *Culicoides* cell surface and this is believed to be mediated by the interaction of VP7 with the RGD tripeptide of integrins (67, 68). Following replication within the midgut cells, virions are released from the basolateral membrane of the gut cells and migrate to sites of secondary replication. These include the salivary glands in which the virus replicates and is subsequently released into the salivary ducts. During subsequent blood meals, the infected female *Culicoides* injects the virus into the vertebrate host, by depositing it in the mammal's skin (to avoid recurrence). The viral particles travel through lymph to draining lymph nodes where the first replication in the ruminant takes place. The virus is then disseminated, via efferent lymphatic circulation, to secondary replication sites including lymphoid organs (lymph nodes and spleen) and lungs. Replication in these sites is followed by the release of BTV into the bloodstream. The VP2 is the cell attachment protein which mediates binding to the cell surface. Following endocytosis, acidification of the early endosome leads to the stepwise release of the VP2 and conformational changes of the VP5 (69). The VP5 then perforates the endosome, releasing the transcriptionally active core into the cell cytoplasm. Viral inclusion bodies (VIBs) are formed in the infected cell cytoplasm. The NS2 is the major component of VIBs and is the first viral protein that is detected in infected cells. It is a phosphoprotein able to bind ssRNA. These properties suggest that NS2 plays a significant role in genome assembly and packaging. BTV replication is accompanied by the synthesis of structural and non-structural viral proteins which are involved in the replication cycle, packaging, and assembly of novel viral particles but also play important roles in attenuating cellular responses (18, 70–72). In mammalian cells, the virus is released by budding during the early stages of infection (73), although virus egress progresses toward lytic release during the later stages of infection. In insect cells, the virus is released by budding, involving the NS3 viral protein (6, 10, 31). Studies suggested that NS3 forms viroporins, thus facilitating virus release (74).

## 6 BTV transmission routes

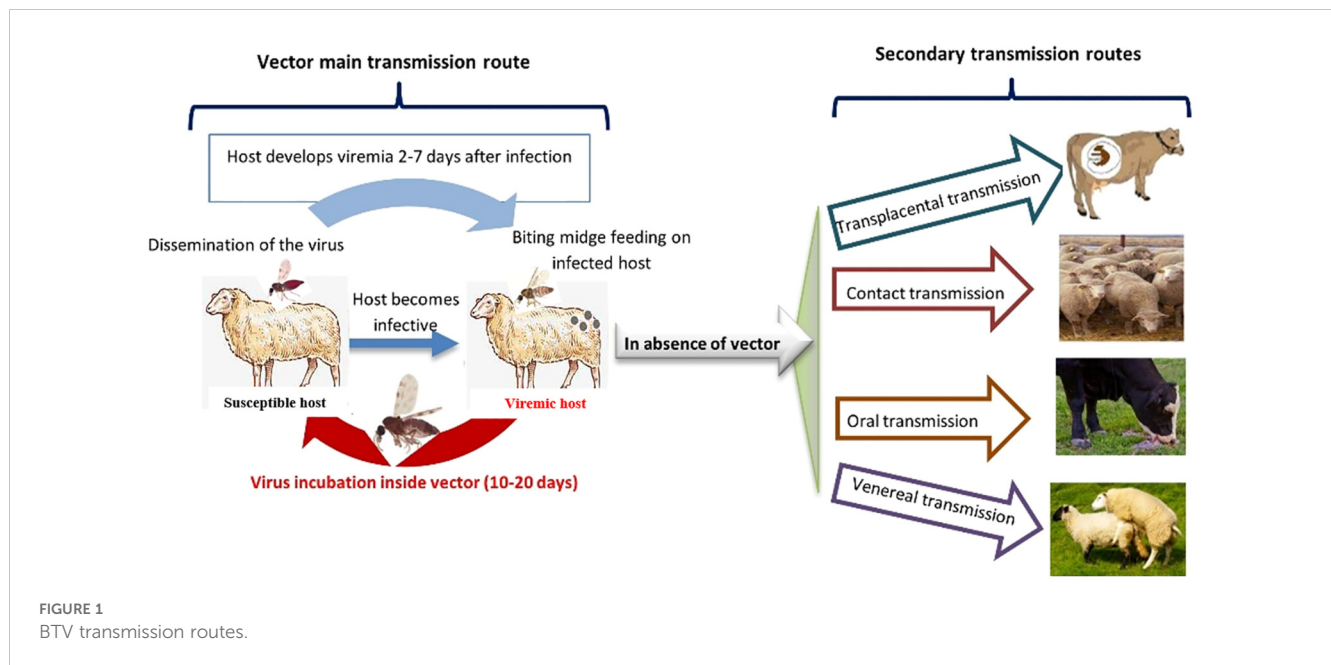
### 6.1 Vector transmission

The main source of BTV transmission is infected blood carried by *Culicoides* species. Only adult and sexually mature females, 28–48 h post-hatching, feed on blood. Their longevity is estimated to be at 10–20 days with a maximum of 50. They become infected during a blood meal on a viremic animal and transmit the virus to other susceptible hosts during subsequent blood meals using their piercing mouthparts (75). The virus is not shed, so it is not found in saliva, waste, oral lesions. The level of exposure to infectious bites is the most influential parameter in BTV transmission depending on the period of infection. However, other modes of transmission are evoked such as via seminal fluid, oral, and iatrogenic routes, and vertically across the placenta in both cattle and sheep (7, 40) (Figure 1).

### 6.2 Alternative transmission routes

#### 6.2.1 Bluetongue vertical transmission

BTV transplacental transmission has been described firstly in sheep and later in cattle mainly with live attenuated BTV, European BTV-8, and other vaccine strains (3). Live attenuated vaccines used in the USA in the early 1950s caused severe degenerative central nervous system lesions, an increase in stillbirths, blind live lambs, and other congenital malformations in the areas where vaccines were used. Such notifications suggested causative relationship between BTV and signs seen in newborn lambs resulting from vertical transmission from vaccinated pregnant females to fetuses at different gestational stages (12, 19, 76). Vertical transmission of BTV from infected gestating heifers to their calves had been reported in various studies for several BTV serotypes (BTV-1, BTV-2, BTV-4, BTV-8, BTV-9, BTV-10, BTV-11, BTV-13, BTV-16, and BTV-23) in cattle, sheep, goat, and elk (77). The ability of the BTV-8 strain to cross the placental barrier in cattle, sheep, and goats had been proven and vertical transmission could be quantified. Moreover, infection in early stage of fetal development may result in abortion or severe brain defects, while fetal infection in late stage of gestation could more likely result in the birth of viable RT-PCR positive calves (7). The BTV-8 epizootic in Europe was followed by an outbreak of hydranencephaly in aborted calves; spleen tissues of several of them were positive for BTV RNA (12). Nevertheless, estimates of vertical transmission may vary depending on the epidemiological context. Although vertical transmission has been proven, there is no evidence yet that infected calves could transmit BTV to *Culicoides* vectors nor how long they can keep antibodies. Moreover, vertical transmission is still considered a secondary route for BTV infection compared to transmission through vector bites. In the absence of an active vector transmission during the overwintering of the infection, BTV could be maintained in the host population through vertical transmission from an infected adult to their offspring resulting in the birth of healthy but BT-positive progeny. The virus could survive despite the absence of the vector due to adverse weather conditions (78).



### 6.2.2 Unusual contact horizontal transmission

Unusual features of BTV infection have been reported in the literature via horizontal transmission in the absence of the insect vector. Infection in a vector-free environment has been reported in cattle, goats, and sheep in experimental context as well as in field conditions for several serotypes such as BTV-1, BTV-2, BTV-8, and BTV-26 provided there is close contact between animals. However, it has been discussed that vector-independent transmission of BTV-1, BTV-2, or BTV-8 was only observed in gestating animals. Therefore, this observation is likely linked to the stage of gestation because no vector-independent transmission has been observed in non-gestating animals at least for BTV-8 or even BTV-4 (79).

Newly emerged atypical serotypes (BTV-25 - BTV-36) may be transmitted horizontally by direct contact between vertebrate hosts without the involvement of biting midges (19). In fact, over the past decade, atypical BTV strains have been shown to replicate efficiently only in mammalian cells, whereas their replication is severely restricted in *Culicoides* cells. BTV transmission is suggested to occur via aerosols expelled into the air by hosts carrying the virus (49), which needs to be explored more. To date, horizontal transmission is experimentally confirmed for some serotypes such as serotype 26 (52, 53), serotype 27 (47) and serotype 28 (54). For other serotypes, such as serotype 25, direct transmission is only suspected (50, 51). Although being an infrequent event, vector-independent transmission can induce an increase in morbidity rates, especially within farms (12, 80).

### 6.2.3 Venereal transmission

Transmission of BTV-8 by artificial insemination of contaminated frozen semen has been described (81). The infecting semen came from naturally infected bulls and was received by BT negative heifers causing their seroconversion with detectable BTV viremia, in most cases, displaying mild clinical signs of BT. The viral load transmitted may have significant impact on the

infection rate in inseminated females and abortion may occur at early stages of gestation although other infected heifers gave birth at term to healthy calves.

BTV-8 infection of breeding females previously negative for BT was supported by the hypothesis of Pascall et al. (82) assuming that the re-emergence of BTV-8 in France in 2015 may be the result of the use of contaminated BTV bovine semen collected during the 2006-2010 epizootic (82). This feature of BTV-8 should not be extrapolated and the potential for other BTV serotypes to occur by artificial insemination needs to be more investigated.

### 6.2.4 Oral transmission

Oral BTV transmission has been described in many cases in both domestic and wild ruminants. Infectious colostrum has been shown to be a source of infection for calves (19). Transmission of BTV-8 by ingestion of infected placenta has also been reported (76).

### 6.2.5 Mechanical transmission

In the veterinary sector, sharing blood-infected needles between animals could result in mechanical transmission. In 2010, a study showed that some tick species experimentally fed with BTV-spiked blood became infected, suggesting that they may have 'vectoring capacities'. However, the capacity of ticks to transmit the virus should be more deeply studied to clearly classify their role in the transmission cycle. Furthermore, BTV transmission by some mosquitoes and ticks has been discussed by Saminathan et al. (3).

## 7 Abundance of *Culicoides* species in the inhabited world

*Culicoides* are among hematophagous insects that occur throughout most of the inhabited world (29) (Table 1). Species of the *Culicoides* genus, which act as vectors for BT are recognizable

based on their morphological features (83). They are small diptera belonging to the order Diptera of the family Ceratopogonidae. The genus *Culicoides* includes 1,347 species of which 30 are known to be potential vectors of bluetongue. Adult *Culicoides* (imago) are 2–3-mm long. They have slender, elongated bodies with antennae consisting of 14 to 16 articles and short, often spotted, sparsely hairy wings (Figure 2) (83). As mentioned above, only adult females are hematophagous (Figure 3) (84), and therefore, able to transmit the virus to domestic and wild ruminants. Cyclically, viral particles adsorbed to the erythrocyte membrane are infectious and can thus infect any competent vector feeding on viremic animals.

In Africa, most countries have reported the presence of *C. imicola*, as the main vector for BTV transmission (85). An Afro-Asiatic species, *C. imicola*, is recognizable by its size and patterns on its wings. The most severe BT outbreaks are recorded when vector activity is optimal during the late summer and autumn. The virus has been isolated several times in South Africa, Tunisia, Kenya, and in other countries (2, 40, 83, 86). Populations of *C. Imicola* have also been detected in areas close to the African continent such as Greece, Israel, and Turkey (2), coinciding with BTV occurrence. In addition to *C. Imicola*, other species have been proven to be BTV invertebrate host vectors, *C. tororoensis*, *C. milnei*, and *C. obsoletus* in Kenya and Cyprus, respectively, without significant effect on the epidemiology of BT. *C. bolitinos*, an African blood-sucking insect closely related to *C. imicola*, is considered a possible vector for African horse sickness, a viral disease closely related to BT, that breeds in the dung of the African buffalo and cattle.

Toward the northern part of the Mediterranean basin, incursions of the virus into Europe, for a long time free from the infection, are due to the circulation of *C. imicola*, as predominant vector in the Mediterranean region. BTV outbreaks have also been reported in the European part of Turkey and Greece where *C. obsoletus* of the sub-genus *Avaritia* was common, and *C. imicola* was absent (30). In Northern and Western Europe, the main *Culicoides* species involved in BTV transmission are *C. obsoletus* and *C. scoticus* (87).

In North America, *C. variipennis* is the major vector with the implication of at least three subspecies: *C. v. occidentalis*, *C. v. sonorensis*, and *C. v. variipennis*, which are genetically different with variable susceptibility to viral infection (2). Works on genetic control and polymorphism provided evidence for the involvement of gene expression in controlling the susceptibility of *C. variipennis* species to viral transmission. In Central and South America and the Caribbean Region, *C. insignis* is potentially the most predominant vector of BTV. It has been shown capable of transmitting the virus to susceptible sheep (88).

In Asia, the presence of several species of *Culicoides* has been confirmed. Nevertheless, *C. imicola* seems to be the most documented vector in Middle East countries, India, China, and other tropical and subtropical regions. Works carried out in the southern and eastern parts of the Asian continent have described

TABLE 1 Most predominant efficient vectors of the bluetongue virus in the inhabited world.

Geographical location		Main vector species	Other vectors
Sub-Saharan Africa		<i>C. imicola</i> <i>C. bolitinos</i>	<i>C. obsoletus</i> , <i>C. sonorensis</i> , <i>C. milnei</i> , <i>C. tororoensis</i>
African Mediterranean countries		<i>C. imicola</i>	<i>C. obsoletus</i> , <i>C. newsteadi</i> , <i>C. kingi</i> , <i>C. puncticollis</i>
European Mediterranean countries and Portugal		<i>C. imicola</i> (abundant along coasts)	<i>C. obsoletus</i> , <i>C. scoticus</i> , <i>C. newsteadi</i> , <i>C. pulicaris</i> , <i>C. punctatus</i>
Northern Europe		<i>C. obsoletus</i> <i>C. scoticus</i>	<i>C. pulicaris</i> , <i>C. dewulfi</i> , <i>C. chiopterus</i> , <i>C. punctatus</i> , <i>C. sonorensis</i>
UK		<i>C. nubeculosus</i> <i>C. impunctatus</i>	<i>C. obsoletus</i> (potential)
Greece, Israel, Turkey, Cyprus, Western, and the Middle East		<i>C. imicola</i>	<i>C. obsoletus</i> <i>C. newsteadi</i>
Iran		<i>C. imicola</i>	
Tropical and subtropical regions, Western China, Eastern Middle East		<i>C. imicola</i>	<i>C. fulvus</i>
India		<i>C. imicola</i> <i>C. fulvus</i> <i>C. actoni</i> <i>C. brevitarsis</i> <i>C. dumdum</i> <i>C. oxystoma</i> <i>C. peregrines</i>	
China	Eastern and southern China	<i>C. actoni</i> , <i>C. fulvus</i> <i>C. brevitarsis</i> <i>C. obsoletus</i> <i>C. wadai</i>	
	Tropical area: Yunnan Province	<i>C. imicola</i>	
	High altitude		<i>C. tainanus</i> <i>C. obsoletus</i>
	East part		<i>C. jacobsoni</i> (potential) <i>C. tainanus</i>
North America (USA and southern Canada)		<i>C. variipennis</i> <i>C. sonorensis</i>	
Central America, South America, Caribbean Region, Brazil to Argentina		<i>C. insignis</i>	<i>C. pusillus</i>
Australia		<i>C. brevitarsis</i> <i>C. fulvus</i>	<i>C. actoni</i> <i>C. wadai</i>



FIGURE 2  
Female *Culicoides sp imicola*.

other species of *Culicoides* (*C. schultzei*, *C. gemellus*, *C. peregrinus*, *C. actoni*, *C. fulvus*, and others) as being able to harbor and transmit BTV. Along with these species, *C. wadai* and *C. brevitarsis* are considered efficient vectors in Australia (89).

## 8 Vector competence for transmission

Vector competence is measured by its ability to contract the virus, amplify it, and successfully transmit it to a susceptible host species. It depends on several factors. Firstly, the number of inoculations per host is based on insect population dynamics, abundance, trophic preferences, and survival rates. Secondly, vector capacity depends largely on extrinsic factors such as temperature, barometric pressure, and wind speed. *Culicoides* survive at temperatures ranging from 15°



FIGURE 3  
Engorged *Culicoides sp obsoletus*.

C to 40°C. At temperatures below 17°C, their ability to survive and replicate the virus is diminished (19). When there is little or no wind, midges can be carried over distances of up to hundreds of kilometers (up to 700 km). However, strong winds prevent vector from flying, which hinders virus dissemination (90).

## 9 Ecological dynamics of *Culicoides* species

The interaction between climatic changes, the midges' population dynamic, and ecology has been reported, and BTV transmission seems to be closely correlated to *Culicoides* vector's seasonal behavior. The assessment of the role of *Culicoides* species as a vector requires in-depth knowledge of their spatio-temporal occurrence. In fact, understanding their distribution is crucial to enable the identification of high-risk transmission areas (91). They are specifically present in agricultural areas with varying abundance. Most species are characterized by twilight and nocturnal activities with peaks around sunset and sunrise (92). Response to climatic conditions raises real challenge and has been used as a topographical predictive factor in modeling conception aided by the seasonal abundance of *Culicoides*.

### 9.1 Spatio-temporal monitoring of pathogens

Worldwide trade of animals can spread the disease to livestock herds across vast distances by introducing infectious animals. Such importations involve transport of midges, which by feeding on infected animals, the real BTV reservoir, will increase transmission rates (93). This is the reason why livestock movement restrictions are applied during an outbreak. Otherwise, BTV may persist in the ruminant host during the winter (5), through prolonged viremia, which can last up to 45 days in sheep and 60 days in cattle, the lifespan of infected red blood cells (75). Under these conditions, the virus can overwinter for months without infecting new hosts in the absence of the vector (5).

### 9.2 Spatio-temporal dynamics of *Culicoides*

In temperate to subtropical enzootic areas, the insect vector, the virus, and the mammalian host have the ability to coexist in climatic conditions suitable for BTV replication and transmission. Most infections occur between late summer and late autumn, which partly reverts to the seasonal nature of the lifestyle of *Culicoides* populations. The population density of midges is extremely reduced in over-wintering periods. Although infected adult midges can survive for a long time during cold periods, a study has shown that eggs are the most tolerant life stage of these insects in the cold season (94). Monitoring of *Culicoides* biting midges has been conducted at 12 locations in Switzerland over 3 years, using overnight trapping once a week (35). Results showed a statistically significant annual variation in *Culicoides* abundance

and activity. High activity was observed between April and November and strongly reduced in winter. More than 80% of the annual midge collection was counted from June to September.

### 9.3 Climate change/temperature

Among a panoply of environmental factors influencing the propagation and activity of *Culicoides* species, temperature remains one of the most influential variables by affecting the survival time and seasonal dynamic of adult biting midges. These insects are carried by hot and humid winds. Their survival would require temperatures above 12.5°C. A four-degree increase in temperature (13°C–17°C) was associated with a 5-day decrease in egg development time. In addition, fertility in females maintained at 13°C was significantly lower than in females maintained at temperatures of 17°C or above (19). Low vector competence for BTV-4 has been shown compared to other serotypes (such as BTV-1) after incubation at 23°C–25°C (85). A temperature of 37°C has been described as the optimal temperature for infectious blood during the experimental feeding of *Culicoides*.

Little is known regarding how or whether environmental factors such as temperature can affect the frequency of genetic reassortment within segmented arboviruses (95, 96). Therefore, the recent study conducted by Carpenter et al. (42) represents one of the first attempts to better characterize the impact of temperature on rates of reassortment in arthropod vectors. They suggested that reassortment frequency increased with higher temperature in *Culicoides sonorensis* coinfecting with two BTV serotypes, BTV-10 and BTV-17.

### 9.4 Over-wintering vector-free period

In the winter, low *Culicoides* biting midges activity is observed depending on unfavorable weather conditions and low temperatures. Populations are strongly reduced (35).

In northern and central Europe, *Culicoides* activity appears to reach its lowest level during the winter. Their endophagic behavior allows them to shelter from cold conditions inside farm buildings. Thus, the vector's life cycle can be maintained during the cold season, which may impact both the virus survival and the probability of infection. Unlike in Afro-tropical and Mediterranean basin countries, a complete cessation of the vector activity of *C. imicola* as an exophagic species, is observed (5).

In cool conditions, the persistence of orbiviruses in vectors for a long time have been reported. Replication of these latent viruses resumes when subsequent exposure to warm temperatures occurs, resulting in pathogen transmission.

### 9.5 *Culicoides* abundance and altitudes

Some groups of biting midges are dominant in altitudes up to 1,200 m. Others are more frequent above 1,500 m (35). Small populations of *Culicoides* species remain active during winter, which could explain the persistence of the transmission cycle. At northern latitudes, most of them survive the winter as larvae (5).

Northward expansion of *C. imicola* to Europe is suggested to be aided by climate change given that 1°C rise in temperature, corresponding to 90 km of latitude and 150 m of altitude (2).

## 10 Conclusion

Interactions between climatic changes, midges' population dynamic, and ecology are found to be likely risk factors for BTV infection and virogenesis rates. *Culicoides* species are hematophagous insects that occur throughout most of the inhabited world, and understanding their distribution is crucial to enable the identification of high-risk transmission areas. Moreover, there is evidence now of BTV infection through routes other than vector transmission, although still rarely considered as factors of the spread of the disease. Vector-independent transmission is considered limited in terms of epizootiology. However, it may be associated with the spread of novel serotypes and can have a substantial economic impact due to the birth of malformed calves (congenital malformations) or the loss of progeny.

Further investigation into predictive factors is required to better evaluate the risks associated with BT spread in endemic regions and outbreaks of new BTV serotypes to make appropriate assumptions for global control strategies and disease monitoring.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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