



Bluetongue in small ruminants: An opinionated review, with a brief appraisal of the 2014 outbreak of the disease in Greece and the south-east Europe



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ABSTRACT

Bluetongue is an arthropod-borne viral disease of ruminants, especially of sheep, caused by *Bluetongue virus*, which belongs to the genus *Orbivirus* of the family Reoviridae and is classified into 26 antigenically distinct serotypes. Once thought to be restricted in Africa and parts of the Middle East, bluetongue has now become a concern in sheep-rearing countries around the world. In the past 10 years, severe outbreaks have occurred in Europe with important economic consequences; of these, the 2006–2008 outbreak in Europe was caused by a serotype 8 strain and the 2014 outbreak in Greece and the other countries of south-east Europe was caused by a serotype 4 strain, suggested to be a reassortant strain with genome segments from lineages of serotype 1, 2 and 4. Immunisation campaigns can be implemented for successful control and limiting of the disease. Nevertheless, in both of the above outbreaks, late application of vaccinations led to a wide spread of the disease, which subsequently resulted in significant losses in livestock in the affected regions. In view of that, standardisation of control measures in the future will be beneficial for efficiently limiting outbreaks of the disease.

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1. Introduction

Bluetongue is a vector-borne viral disease of domesticated or wild ruminants, which affects clinically mainly sheep and is a significant cause of financial losses in affected farms. The disease is caused by *Bluetongue virus*, a member of the genus *Orbivirus* (family Reoviridae). *Bluetongue virus* includes 26 serotypes (1–26). The virus is principally transmitted by biting midges of the genus *Culicoides*. The disease does not have a zoonotic potential.

Bluetongue was first reported in South Africa in the early 1900s (Spreull, 1902,1905) and initially, until the first half of the 20th century, was considered endemic only in parts of Africa and Cyprus. However, in subsequent years the disease was diagnosed in Israel (1951), the USA (1952), the Iberian peninsula (1956), the Indian subcontinent (1961) and Australia (1975) (Hardy and Price, 1952; Manso-Ribeiro et al., 1957; Sapre, 1964; St George et al., 1978). Later (1979), outbreaks of the disease also occurred in the Greek islands near the Asian mainland (Vassalos, 1980). By the end

of the 20th century, the distribution of bluetongue coincided with the geographic distribution of the most prevalent vectors of the causative virus: *Culicoides imicola*, *C. soronensis* and *C. brevitarsis*, i.e., in the area between 40° N and 35° S (Mellor et al., 2000; Mellor and Wittmann, 2002). This dogma was challenged especially during the first decade of the 21st century, when the disease emerged in areas of Europe, where *C. imicola* (the virus' principal vector in south Europe) was not present, thus implicating other *Culicoides* species transmission of the virus.

The most significant outbreak of the disease was caused by a serotype 8 virus, between 2006 and 2008. It occurred in north and west Europe, up to Scandinavia, as well as in the British isles, and caused financial losses exceeding 200 million Euros in the Netherlands alone (Institute for Animal Health, 2006; Department for Environment, Food and Rural Affairs, 2007; European Food Safety Authority, 2007; Losson et al., 2007; Velthuis et al., 2010). This led the European authorities to encourage vaccine manufacturers to develop and produce efficient inactivated vaccines against the virus and national authorities to initiate major immunisation campaigns as part of programs to control the disease (Wilson and Mellor, 2009; Baetz, 2014). Other outbreaks

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of the disease in Europe caused by various serotypes (e.g., 1, 2, 4, 6, 9, 11, 16, 25) have been dealt with by implementation of temporary restriction zones or widespread vaccination for minor or major incidents, respectively (Rodriguez-Sanchez et al., 2008; Wilson and Mellor, 2009; Eschbaumer et al., 2010a; Maclachlan and Guthrie, 2010; Maclachlan and Mayo, 2013).

Objective of this review is to present a general account of the disease, at the same time also summarising data currently (July 2015) available regarding the ongoing outbreak of the disease in Greece and the neighbouring Balkan countries in south-east Europe, which started in May 2014.

2. Bluetongue virus and disease

Bluetongue virus was initially classified as an arbovirus, due to its transmission by biting midges. However, later studies, which pointed out a stability in lipid solvents (indicating lack of a lipid membrane), and further genetic analyses led to its re-classification as the type-species of the genus *Orbivirus* of the Reoviridae family (Mertens et al., 2005; Roy, 2013). Virions under the electron microscope appear round in shape and have a diameter between 68 and 70 nm. They possess double-stranded RNA genome that consists of 10 segments and encodes 7 structural (VP1 to VP7) and 4 non-structural proteins (NS1 to NS4) (Belhouche et al., 2011). VP2 is the major component of the outer capsid and the principal antigen (together with VP5) that determines the serotype of each strain. Serotype-specific neutralising antibodies produced by infected or vaccinated animals are mainly directed against VP2 (Mertens et al., 1987; Pierce et al., 1995). Solid immunity is offered only against strains of the same serotype, while only partial protection has been observed against isolates that belong to other serotypes (Neitz, 1948; Noad and Roy, 2009). However, the fact that the original serotype 4 isolate ('Theiler's strain') has been in use as a vaccine strain for over 80 years indicates that genetic drift within a serotype is minimal (Verwoerd and Erasmus, 2004). Thusfar, 26 antigenically distinct serotypes have been identified, with the most recent one (26), isolated from small ruminants in Kuwait in 2010 (Maan et al., 2011).

Bluetongue virus infects domesticated or wild ruminant species, particularly sheep. Clinical manifestations of bluetongue vary widely between different animal species, as well as virus strains. Often, the infection remains subclinical (Kahn and Line, 2010). However, other animals may exhibit disease with high fever and depression, followed by hyperaemia of the buccal and nasal mucosa, increased salivation, lacrimation and nasal discharge. These signs may be accompanied by oedema of the tongue, lips, face, eyelids and ears and lameness, due to hyperaemia of the coronary bands and haemorrhages around the hoofs. Additionally, pregnant animals may abort. Severe swelling of the tongue, which can become cyanotic ('blue tongue'), has offered the name to the disease, but is not common. In acute cases of disease, animals may die within ten days of infection, mainly due to pulmonary oedema (Verwoerd and Erasmus, 2004; Darpel et al., 2007).

In general, *Bluetongue virus*, like any vector-borne pathogen, can emerge into a virus-free area by two main events: the introduction of infected livestock and the movement of infected vector insects by the wind. Nevertheless, a third, unknown route of introduction of this virus may not be excluded (Wilson and Mellor, 2009).

Principally, *Bluetongue virus* is transmitted between animals by haematophagous insects of the genus *Culicoides*. These biting midges are found in all continents (bar Antarctica), but only around 50 out of the 1500 known species can transmit the virus to ruminants (Wilson and Mellor, 2009). Due to the seasonality of the vector's presence, transmission of the virus was believed to be limited during the warmer months of the year. However, recent evidence from California indicated the possibility that the virus can

survive through the winter in long-living *C. soronensis* female midges, which had been infected during the previous flying activity period (Mayo et al., 2014).

Limited evidence furthermore indicates that, possibly, at least certain strains of the virus, may also be transmitted transplacentally, orally or through direct contact (Menziez et al., 2008; Darpel et al., 2009; Mayo et al., 2010; Veronesi et al., 2010; Batten et al., 2014). A possible iatrogenic transmission of the virus (e.g., during vaccinations) cannot be explicitly ruled out, as the virus has been isolated from the skin of infected sheep, even after two months post-clinical recovery (Takamatsu et al., 2003; Wilson et al., 2008). The significance of these atypical routes of transmission in the epidemiology of the disease has not been fully assessed. The role of wild ruminants as a reservoir for virus and vector maintenance is also considered to be potentially important; a hypothesis, suggesting that two disease cycles, one prevalent in wild and one in domestic ruminants, linked through shared *Culicoides* vectors coexisting in certain regions of the Mediterranean Basin has been recently proposed (Ruiz-Fons et al., 2014).

3. Bluetongue virus vector

The genus *Culicoides* (Family Ceratopogonidae) consists of very small flies, which are commonly referred to as 'biting midges' and are abundantly occurring haematophagous insects. Female *Culicoides* feed also on mammals (including humans) and birds, additionally to consuming sugar-containing plant juice, whilst males feed only on that. These insects ingest blood at intervals of three to four days, if available, since they need it for egg deposition. They have a painful bite and can cause irritation and annoyance to animals. The main parts of animals that they attack are the head and the neck. Their biting has been associated with a hypersensitivity reaction and moreover they can transmit various pathogens. They are, among others, vectors for *Bluetongue virus* and *Schmallenberg disease virus* (Taylor et al., 2007; Koenraadt et al., 2014).

Specifically for *Bluetongue virus*, *Culicoides* species act as the vector within which virus-replication occurs. Virus ingested by midges replicates in the insects' midgut cells, spreads to its salivary glands and then can be transmitted to another ruminant. The spread of *Bluetongue virus* thus coincides with the distribution of the vector species.

There are over 1500 *Culicoides* species, most significant of which are *C. imicola*, *C. obsoletus*, *C. variipennis*, *C. pulicaris*, *C. sonorensis*, *C. nubeculosus*, *C. dewulfi* and *C. chiopterus*. However, only a small number of these have been shown to act as biological vectors, while in experimental work some more species have also been found to be suitable hosts (Mellor et al., 2000; Carpenter et al., 2006; Mehlhorn et al., 2007). *C. imicola* is the principal vector in Africa, the Middle East, most of south-east Asia and parts of south Europe, *C. soronensis* is the principal vector in North America and *C. brevitarsis* in Australia (Wilson and Mellor, 2009). Bluetongue outbreaks in north and west Europe have shown that in the area north of 40° N, *obsoletus*- and *pulicaris*-group *Culicoides* species can facilitate transmission of the virus as well (Hoffmann et al., 2009; Mehlhorn et al., 2009).

Adult *Culicoides* spp. are 1.0–5.0 mm-long, with the thorax humped over a small head, which is equipped with a short piercing proboscis. Their wings are generally mottled in pattern, covered with microscopic hairs and at rest they held like a closed pair of scissors over the abdomen. Egg laying takes place in damp marshy ground or in decaying vegetable matter, in a variety of breeding sites. Egg hatching occurs after two to nine days, depending on the species and temperature, but temperate species may overwinter as eggs (Mehlhorn et al., 2007). Larvae have a small dark head, segmented body and terminal anal gills. In warm climates, larval

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