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Bluetongue Virus in wild ruminants in Europe: Concerns and facts, with a brief reference to bluetongue in cervids in Greece during the 2014 outbreak☆

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ABSTRACT

Bluetongue is a constant threat to ruminants, affecting primarily the sheep industry. *Bluetongue Virus*, the causative agent, has a worldwide distribution and a large number of antigenically different serotypes (at least 26), with vector-borne transmission. All these make effective control measures difficult to implement. Expansion of the virus to Northern Europe changed the epidemiology of the disease and highlighted the existence of hitherto unknown vectors and susceptible hosts. A possible epidemiological role of wildlife as hosts of the virus must be considered, complicating the control strategies. In this review, facts regarding the disease in Europe and its impact in wildlife are presented; concerns are also discussed regarding implication of wild ruminants in surveillance of the disease and formulation of novel control strategies. Further, preliminary results of a study into the presence of *Bluetongue Virus* in cervids in Greece, performed during the outbreak currently (2014–15) prevailing in the country, have indicated (by using molecular techniques) presence of the virus in 3 of 19 samples from roe deer in areas adjoining domestic ruminant farms. It is concluded that wild ruminants should be included in surveillance programs and in strategies for controlling *Bluetongue Virus* infections in a region.

1. Introduction

Bluetongue is a vector-borne viral disease of ruminants with a worldwide distribution and great socioeconomic impact (Erasmus, 1990; Walton, 2004). The causative agent of the disease is a double stranded RNA (ds-RNA) virus (*Bluetongue Virus*), member of the *Orbivirus* genus in the Reoviridae family (Borden et al., 1971; Fenner et al., 1974; Gould et al., 1992; Gould and Hyatt, 1994). Bluetongue has been diagnosed in almost all ruminant species (wild or domestic), as well as in carnivores, rodents and reptiles. Except Antarctica, bluetongue has been reported in almost every corner on earth, either as enzootic (Africa, North America) or epidemic ruminant disease (Gibbs and Greiner, 1994; Ward, 1994; Kirkland, 2004; Lager, 2004; Tabachnick, 2004; Mellor et al., 2008; MacLachlan, 2011).

The *Bluetongue Virus* may cause subclinical or clinical infections, mainly depending on the host species. Severity

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and rate of infection further depend on the virulence of the infective strain and the immunological status of the affected animals (Maclachlan et al., 2009). The clinical form of bluetongue occurs mainly in sheep, causing a febrile disease characterised by haemorrhagic clinical signs and increased mortality rates (Moulton, 1961). In non-endemic regions, a bluetongue outbreak usually causes significant losses and requires remarkable resources for its control and eradication. In regions where it is endemic, bluetongue is a continuous threat and a permanent financial concern for the farming industry (MacLachlan and Osburn, 2006; Saegerman et al., 2008). Due to the importance of the disease, the increasing virus spread around the world and its significant direct or indirect consequences, the disease has been included in List A of notifiable diseases compiled by the World Organisation for Animal Health (World Organisation for Animal Health, 2009).

Bluetongue in wild ruminants often is subclinical (Garcia et al., 2009; Casaubon et al., 2013). Lack of clinical signs allows these species to play a crucial role in the epidemiology of the disease, enabling multiplex interactions with competent vectors and developing interesting relationships with other susceptible hosts (Billinis, 2013; Miller et al., 2013; Lorca-Oró et al., 2014; Ruiz-Fons et al., 2014).

Objectives of this review are (i) to describe a poorly understood impact of *Bluetongue Virus* in wildlife, (ii) to highlight the facets of bluetongue in wild ruminants, (iii) to resume the available data regarding bluetongue in European wild cervid species and (iv) to discuss the potential of wild cervid species implicated in the current bluetongue outbreak occurring in Greece.

2. General features of *Bluetongue Virus* and the disease

The *Bluetongue Virus* is closely related to the epizootic *Haemorrhagic Disease Virus* (Gould and Hyatt, 1994). *Bluetongue Virus* genome is composed of ten dsRNA linear segments that encode seven structural (VP1–VP7) and five nonstructural (NSP1–NSP3, NSP3/A, NSP4) proteins (Verwoerd et al., 1972). The various strains are further classified based on their antigenic relationships and genomic characteristics. Currently, 26 different serotypes have been detected (Chaignat et al., 2009; Maan et al., 2011, 2012). Each serotype may infect various hosts and has distinct geographical spread, although such limits have often been superseded. Furthermore, the increasing amount of viral genome sequence analysis data has allowed recently the molecular classification of the virus in two lineages (western, eastern) and the demonstration of a few local topotypes (Carpi et al., 2010).

Bluetongue Virus is transmitted primarily through bites of haematophagous insects. The haematophagous midges of *Culicoides* genus (Diptera: Ceratopogonidae) are the major biological vector of the virus (Du Toit, 1944). Beyond these, several other midge species have been certified as maintenance vectors and natural source of infection. Interestingly, from a large number of different *Culicoides* species, only a few (approximately 30) are usually involved in the epidemiology of the disease. The most prevalent vector with a worldwide distribution is *Culicoides imicola*. Other species with significant role in the expansion of the infection are *C. bolitinos* in Africa (Paweska et al., 2002), *C. furvus* in Australia (Standfast et al., 1985) and *C. sonorensis*, *C. insignis* and *C. pusillus* in America. The recent dramatic increase of bluetongue cases in northern Europe have probably taken place due to an involvement of palearctic *Culicoides* species, such as *C. obsoletus* and *C. pulicaris* complexes (De Liberato et al., 2005; Savini et al., 2005; Meiswinkel et al., 2007, 2008; Wilson and Mellor, 2008; Carpenter et al., 2009; Brugger and Rubel, 2013).

A direct transmission of the virus can also occur, primarily during the mating and parturition period of the various susceptible host species. During the mating season, female animals may be infected by means of contaminated semen from males with prolonged *Bluetongue Virus* viraemia. Additionally, during the parturition period, a direct contamination or digestion of contaminated placental or foetal tissue may lead to infection and disease of individual animals (De Clercq et al., 2008; Menzies et al., 2008). Finally, transmission of the virus to pregnant females may lead to transplacental infections, resulting in foetal deaths, stillbirths or births of neonates that would die in their early life (Osburn, 1994; Mayo et al., 2010; Rasmussen et al., 2013; van der Sluijs et al., 2013).

After invasion into the host, *Bluetongue Virus* passes through the subcutaneous tissues and enters into the bloodstream. Target cells of the virus are the endothelial cells of blood vessels (Maclachlan et al., 2009). However, virus particles have also been found in phagocytic, dendritic and/or mononuclear cells (Hemati et al., 2009). The mechanisms involved after virus attachment remain unclear. According to the prevailing opinion, *Bluetongue Virus* infection induces a significant increase of endothelium paracellular permeability, leading to mild to severe vascular leakage (DeMaula et al., 2001, 2002).

Clinical features of bluetongue vary a lot, ranging from mild, difficult to characterise signs to severe disease that may result to death (Erasmus, 1975). Clinical severity depends mainly upon the infected host and the virulence of each infective strain (Schwartz-Cornil et al., 2008). Historically, introduction of a new emergent strain in a naïve population usually leads to severe disease with devastating consequences for the infected population. Sheep (*Ovis aries*) is by far the most susceptible host of the virus, usually presenting a high morbidity and mortality course of disease. Nowadays, certain breeds of sheep (e.g., those not indigenous to the African continent) are considered more likely to develop severe disease (Gerdes, 2004; Caporale et al., 2014). In cattle and goats, bluetongue often remains subclinical or with mild or transient clinical signs (Barratt-Boyes and MacLachlan, 1994, 1995). In any case, infected goats may also develop clinical signs, although traditional views have been expressed that they were only infected subclinically. In both species, persistent viraemia may cause notable indirect losses, which are associated with loss of body weight, reduction in milk production and reproductive disorders (Dal Pozzo et al., 2009).

Clinical signs of bluetongue reflect the multiple vascular injuries, causing moderate fever, oedema and congestion of lips, mouth, nose and eyelids and, in some cases,