Review

Bluetongue in Europe and the role of wildlife in the epidemiology of disease

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Abstract

The article reviews a current bluetongue (BT) epidemiological situation in Europe, BT restricted zones and the role of wild ungulates as a reservoir for bluetongue virus (BTV) and its transmission. BT has been eradicated from central and northern Europe, however it is still circulating in some regions of southern and south-eastern Europe. According to the recent information of the Directoriate General for Health and Consumer Affairs (DG SANCO) disease caused by BTV1 was spreading at the beginning of 2014 in Corsica (France). Moreover, four BTV1 cases were noticed in the west Spain (Cáceres province), 59 BTV4 outbreaks in south Spain (Andalusia), 10 in the region of Algarve in Portugal and about 200 outbreaks of BTV4 in Greece (Peloponesse and Evros regions). On 4th July the first outbreak of BTV4 was also confirmed at the south Bulgarian border and by 5th September 2014 disease was noticed in 21 of 28 administrative districts of Bulgaria. In August 2014 the BTV4 disease was reported in south-east of Romania and as for 8th September 184 outbreaks of BT were confirmed in 17 counties of this country. As of 3 September 2014 in Europe there has been fourteen BT-affected zones, in different regions of Italy, Spain, Portugal, Cyprus, Malta, France (Corsica), Greece, Bulgaria and Romania. Most species of wild ruminants and camelids are susceptible to BTV infection, although frequently asymptomatically. Wild sheep, bighorn and mouflon, are susceptible to BTV infection and can develop fatal clinical disease, as do domestic sheep. Experimental or natural infection of antelope, wapiti, musk, ox, bison, yak, white-tailed deer and African buffalo also produced clinical disease, whereas blesbock, mountain gazelle, roe deer, red deer and Eurasian elk did not show clinical sign after natural or experimental infection and infection was recognized by the presence of BTV viral RNA or specific antibodies. The wildlife due to the long-term carrier state may act as a reservoir for BTV and play an important role in its transmission.

Key words: bluetongue, Europe, wildlife, epidemiology

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Introduction

Bluetongue (BT) is an infectious disease of ruminants and camelids (Vervoerd and Erasmus 2004), caused by bluetongue virus (BTV), a RNA virus belonging to the Orbivirus genus in the Reoviridae family (Mertens et al. 2005) and transmitted by bitting midges of the genus *Culicoides* (Mellor et al. 2000). BT has a significant economic impact, mainly due to the disease effect on animals (morbidity, mortality, reproductive failure, reduction in milk yields and weight gain) and, most of all, to the disruption of international trade of animals and animal products (Saegerman et al. 2008). Up-to-date 26 immunologically distinct serotypes of BTV have been identified worldwide (Schwartz-Cornil et al. 2008, Chaignat et al. 2009, Mann et al. 2011). BTV is endemic in many tropical, sub-tropical and temperate regions of the world (America, Australia, Africa and some regions of Asia), between latitudes 40°S and 53°N, during times of the year that are optimal for vector activity (Mellor et al. 2000). Historically, Europe has experienced only sporadic incursions of BT, involving a single virus serotype on each occasion (Mellor and Boorman 1995). However, since 1998, probably due to climatic changes, BTV spread northwards into the Mediterranean Basin, where five BTV serotypes (1, 2, 4, 9 and 16) have been identified (Purse et al. 2005). In the summer of 2006, for the first time, the BTV has crossed latitude 50°N and BT outbreaks caused by BTV serotype 8 occurred in North-Western Europe: the Netherlands, Belgium, Germany, France, and Luxembourg (Wilson and Mellor 2008). In 2007-2008, the BT situation changed for the worse, BTV8 spread to the other regions of Europe and the number of outbreaks increased rapidly (about 50000 cases of BT were reported). Additionally, two new BTV serotypes, BTV1 and BTV6, were detected (Wilson and Mellor 2009). However, the implementation of BT compulsory vaccination programmes in Europe in spring 2008 resulted in reduction of BTV8 cases from about 50000 in 2007-2008 to 350 in 2009 and 19 in 2010. The last case of BT caused by BTV8 was reported in May 2011 in north Sardinia (Italy) (http://eubtnet.izs.it/btnet.reports/BTV8.htlm).

BT epidemiological situation and restriction zones in Europe

BTV has been eradicated from central and northern Europe, however it is still circulating in some regions of southern and south-eastern Europe. According to the information of the Directoriate General for Health and Consumer Affairs – European Commission (DG SANCO), in 2013 disease caused by BTV1 was spreading extensively over the territory of Italy (Sardinia, Sicily and mainland Italy) and more than 5000 BT outbreaks has been confirmed. At the beginning of 2014 the same BTV serotype has been isolated in Corsica (France) where 137 outbreaks of BTV4 were reported. Moreover, four BTV1 cases were noticed in the west Spain (Cáceres province), 59 outbreaks caused by BTV4 in south Spain (Andalusia) and 10 in the region of Algarve in Portugal. In the period from May to July 2014 about 200 outbreaks of BTV4 have been confirmed in Greece (Peloponesse and Evros regions). On 4th July the first outbreak of BTV4 was also reported at the south Bulgarian border (Burgas Region, near the border with Turkey) and by 5th September 2014 disease was confirmed in 21 of 28 administrative districts of Bulgaria. Additionally, on 21 August 2014 the first outbreak of disease was notified in south-east of Romania and as for 8th September 184 outbreaks of BTV4 were confirmed in 17 counties of this country (http://ec.europa.eu). The BT-affected zones in Europe, as of 3 September 2014, were as follows (Fig. 1):

– Zone B – BTV2 and 16, Italy, regions Abruzzo, Campania and Molise

– Zone D – BTV16, Italy, regions Basilicata and Umbria (excl. Zone Z)

– Zone G – BTV1, 2, 4 and 16, Italy, region Sardegna (excl. zone T)

– Zone H – BTV serotypes not specified, Malta, whole territory

– Zone I – BTV1 and 4, Spain, provinces Huelva, Cadiz, Malaga, Sevilla and Portugal, region Algarve

– Zone J – BTV1, Spain, whole territory (excl. zone I), Portugal, whole mainland territory (excl. zone I) and Italy, regions Lazio, Liguria, Marche and Toscana.

– Zone T – BTV1, 2, 4, 8 and 16, France, Corsica and Italy, region Sardegna (only the province of Olbia Tempio)

– Zone W – BTV1, 8 and 16, Greece, the island Lesbos

– Zone X – BTV4 and 16, Cyprus, whole territory and Greece, the islands of Dodekanisa and Samos

– Zone Y – BTV2, 9 and 16, Italy, region Puglia

– Zone Z – BTV1,16, Italy, region Lazio (provinces of Roma and Viterbo), Basilicata (province of Potenza) and Umbria (province of Terni)

– Zone A1 – BTV1, 2, 4, 9 and 16, Italy, regions Calabria and Sicilia

– Zone A2 – BTV1, 2 and 16, Italy, region Campania (only the province of Salerno) and Abruzzo (only the province of Aquila)

– Zone A3 – BTV4, Greece, the whole continental Greece plus the island Kerkyra and Lefkada,
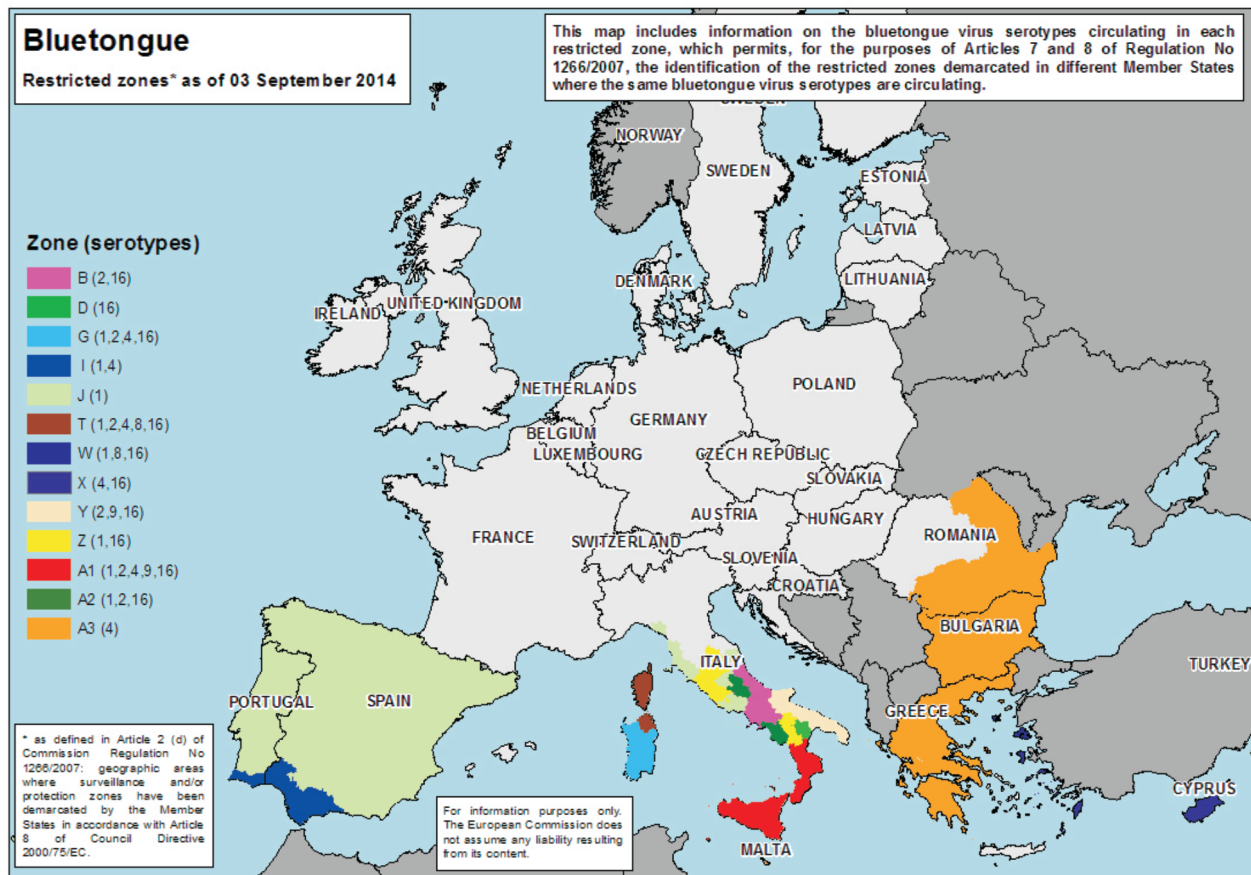
Fig. 1. Bluetongue – restricted zones in Europe as of 3 September 2014. (http://ec.europa.eu/food/animal/diseases/control-measures/bt_restrictedzones-map.jpg)

Bulgaria, whole territory and Romania, whole territory of counties: Iasi, Neamt, Bacau, Vaslui, Vrancea, Galati, Buzau, Braila, Ialomita, Calarasi, Prahova, Dambovita, Arges, Valcea, Gorj, Mehedinti, Dolj, Teleorman, Giurgiu and Ilfor, part of territory of counties: Botosani, Suceava, Tulcea, Constanta.

Bluetongue in wildlife

BT is considered endemic in wildlife in the large parts of Africa and North America (Gerdes 2004). Wild ruminants are included in the European Council Directive 2000/75/EC of 20 November 2000, laying down specific provisions for the control and eradication of bluetongue, but vaccination and movement restrictions can only be applied in farmed or managed ruminants, being almost impossible in wild free-ranging hosts of the virus. Most, if not all, of the species of wild ruminants are susceptible to BTV infection. However, infected animals usually do not show clinical signs of disease, particularly indigenous animals in region where BT is endemic (Johnson et al. 2006). While European Union compulsory control measures have been put in place to control the spread of BT in livestock, the role of wild ruminants as susceptible hosts should be considered in any strategy to control of BT (Fernandez-Pacheco et al. 2008). In order to know the role of wildlife in the epidemiology of BT, several species of wild ruminants and camelids have been investigated to elucidate their potential role on BTV control, as well as the occurrence of clinical disease. It was shown that wild sheep such as bighorn (*Ovis canadensis*) and mouflon (*Ovis aries musimon*) are susceptible to BTV infection and can develop fatal clinical disease, as do closely related domestic sheep (Fernandez-Pacheco et al. 2008). The clinical signs of BT were also produced after experimental infection of pronghorn antelope (*Antilocapra Americana*), American bison (*Bison bison*), and African buffalo (*Syncerus caffer*) (Tessaro and Clavijo, 2001). Under natural conditions the disease may also be present in wapiti (*Cervus elaphus canadensis*), axis deer (*Axis axis*), fellow deer (*Dama dama*), sika deer (*Cervus nippon*), musk deer (*Moschus moschiferus*), roe deer (*Capreolus capreolus*), Spanish ibex (*Capra pyrenaica*), and captive yak (*Bos grunniens grunniens* (Howerth et al. 2001, Fernandez-Pacheco et al. 2008, Ruiz-Fons et al. 2008, Rodriquez-Sanchez et al. 2010). BTV infection, antibody response and clinical disease

Table 1. Viraemia and seroconversion in BTV-infected wildlife.

Species	Viraemia duration	Viraemia onset	Viraemia detection methods	1st antibodies	Antibody duration
European red deer (*Cervus elaphus*)	over 112 days	1 dpi	virus isolation and RT-PCR	1-7 dpi	over 112 dpi
Camel (*Camelus dromedaries*)	28 days	5-7 dpi	virus isolation and RT-PCR	11 dpi	over 75 dpi
Black-tailed deer (*Odocoileus hemioneus columbianus*)	1-10 days	2-9 dpi	virus isolation	6-13 dpi	over 692 dpi
White-tailed deer (*Odocoileus virginianus*)	2-5 days	2 dpi	virus isolation	n.d.	n.d.
American bison (*Bison bison*)	1-4 days	4-7 dpi	virus isolation	11-28 dpi	over 127 dpi

dpi, days post-infection

n.d., not determined

after experimental infection have been reported in several North American deer species belonging to the subfamily *Odocoileinae*, such as: white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemioneus columbianus*) and mule deer (*Odocoileus hemionus)* (Work et al. 1992) (Table 1). Conversely, BTV-infection of less susceptible wild animals is asymptomatic or causes only mild clinical signs, e.g. after natural or experimental infection of blesbock (*Damaliscus pygargus*) and mountain gazelle (*Gazella gazella*) no clinical signs of BT have been shown (Shimshony et al. 1988, Bender et al. 2003). The BTV natural infection of moose (*Alces alces*) and roe deer (*Capreolus capreolus)* has been recognized on the basis of the presence of specific antibodies and/or viral RNA in samples of blood taken from infected animals (Linden et al. 2008, Rodriquez-Sanchez et al. 2010).

Camelids have also been reported to be susceptible to BTV infection; a severe clinical signs with lethal disease was reported in naturally infected llamas (Meyer et al. 2008) whereas experimental infection of two llamas induced specific antibodies against BTV but coursed asymptomatically (Afshar et al. 1995). Also alpacas (*Vicugna pacos*) after experimental infection with BTV8 displayed very mild clinical signs. Seroconversion was first measured 6-8 days after infection (dpi) and RNA levels in blood were very low, and quickly cleared after seroconversion. However, spleens collected post-mortem were still positive for BTV RNA, over 71 days after the last detection in blood samples (Schulz et al. 2012). In three camels (*Camelus dromedaries*) experimentally infected with BTV1 no clinical signs of BT were observed, however all animals seroconverted and developed BTV1 specific neutralizing antibodies after challenge (Table 1). All camels developed a viraemia from 7 dpi and BTV was isolated from the blood of all three animals suggesting that camels may act as a res-

ervoir for BTV and play an important role in its transmission (Batten et al. 2011). Although BT is a disease found in ruminants and camelids, under certain circumstances it can also be transmitted to carnivores. BT has been recorded in Eurasian lynxes (*Lynx lynx*) kept in a Belgian zoo after the cats were fed on aborted or still-born foetuses of ruminants raised on neighbouring farms (Jauniaux et al. 2008). Specific antibodies to BTV were also identified in African carnivores including lions, cheetahs, wild dogs, jackals, hyenas and genets (Alexander et al. 1994). These cases can also be explained by ingestion of flesh and organs of BTV-infected animals. Moreover, the presence of BTV antibodies have been found in Florida black bears (*Ursus americanus floridanus*) and Florida panthers (*Puma concolor coryi*) (Dunbar et al. 1998). The antibodies against BTV have also been found in the elephantidae (Bhat et al. 1998).

BTV infection of the susceptible wildlife can results in a wide variety of lesions and clinical signs, ranging from sudden death to asymptomatic infection (Howerth et al. 2001). BTV serotypes 1, 8 and 17 have been associated with clinical signs and lesions in either experimental or natural infections in wild-white deer (Howerth and Tyler 1988, Johnson et al. 2006). Clinical signs reported in this animal include severe depression, weakness, fever, anorexia, hyperemic oral mucosa, crusts in the nares, severe respiratory distress, reddening of the muzzle and ears. Moreover, swollen and cyanotic tongue, excessive salivation, oral ulceration, multifocal haemorrhage in the skin and mucosa, severe bloody diarrhea and laminitis were observed. Organs most frequently affected include tongue, hearth, spleen, lymph nodes and kidneys (Howerth et al. 2001). In the acute form, haemorrhagic lesions are due to endothelial damage and disseminated intravascular coagulation (Howerth and Tyler 1988). Cardiovasicular lesions consist in pericardial, subpericardial and subendocardial haemor-

rhages. Typical clinical signs in the persistent form of BT in white-tailed deer include erosion and ulceration of dental pad, hard palate, tongue, gingival, forestomachs and abomasums as well as food lesions, ranging from coronitis and laminitis to complete sloughing of the hooves (Howerth et al. 2001). In European mouflon, taxonomically considered as a subspecies of domestic sheep, the clinical signs reported include inflammation of mucous membranes, congestion, swelling and haemorrhages (Fernandez-Pacheco et al. 2008). BTV infection of less susceptible wild animals is asymptomatic or causes only mild clinical signs. In experimentally infected black-tailed deer (*Odocoileus hemionus columbianus*), the only clinical sign was hyperthermia, body temperature ranging from 40ºC to 41.2ºC (Work et al. 1992). In European red deer (*Cervus elaphus*) no clinical signs have been reported, both after natural and experimental BTV infection (Lopez-Olivera et al. 2010, Rodriquez-Sanchez et al. 2010). In African wild ruminants, BTV infection is asymptomatic and BTV was isolated from several species of screwhorn antelope: addax (*Addax nasomaculatus*), Nubian ibex (*Capra nubiana*), sable antelope (*Hippotraqus niger*) and African buffalo (*Syncerus caffer*) (Verwoerd and Erasmus 1994).

BT virological and serological surveillance of wildlife is carried out systematically for several years in different regions of Europe (Linden et al. 2008, Garcia et al. 2009, Rodriguez-Sanchez et al. 2010) (Table 1). The highest titres of BTV antibodies have been detected in samples of sera taken from free-ranging European red deer and fallow deer (*Dama dama*) from *Cervinae* subfamily (Ruiz-Fons et al. 2008, Garcia et al. 2009, Linden et al. 2009). The RNA of BTV serotype 8 was detected by Real-Time RT-PCR (rRT-PCR) in naturally-infected wild deer in Belgium (Linden et al. 2008) and BTV1 and BTV4 genetic material in blood of European red deer and mouflons in Spain (Garcia et al. 2009) while BTV1 and BTV8 RNA in red deer in France (Rossi et al. 2010). As for domestic ruminants, BTV transmission among wildlife depends almost exclusively on the presence of the *Culicoides* vector in the environment. BTV prevalence and outbreaks follow a seasonal pattern in Europe, infection generally occurring in late summer and early fall. This seasonal variation is related to vector abundance, which depends on climate factors, such as humidity and temperature (Sleeman et al. 2009). Apart from the annual seasonality, there are inter-annual cycles in the occurrence of BTV. In endemic areas of North America, deer populations are infected on-one to three-year cycles, whereas in epidemic areas BT occurs in an eight-to ten-year cycle. These cycles can be related to combined effects

of herd immunity and fluctuations in vector populations (Howerth at al. 2001). Moreover, BTV can exceptionally be transmitted by alternative vectors e.g. ticks (Bouwknegt at al. 2010). Transplacental, oral (including colostrums), semen and mechanical (wound) contacts have been suggested as possible mechanism for BTV transmission (Menzies at al. 2008, Lopez-Olivera at al. 2010, Santiago-Moreno at al. 2010).

The wildlife, particularly cervids, because of their wider distribution in Europe, could be used as sentinels for surveillance of BTV. Antibodies against BTV have been reported in European reed deer shortly after being detected in domestic livestock (Linden et al. 2008, Ruiz-Fons et al. 2008, Rodriquez-Sanchez et al. 2010). Occasionally, contact of wild ungulates with BTV has been reported in geographic regions where BTV has not been found in domestic livestock (Garcia et al. 2009). BTV-specific antibodies or RNA were detected in wild ruminants even one year after its successful control in domestic ruminants (Rodriquez-Sanchez et al. 2010). However, when interpreting the presence of antibody in serum samples from calves the presence of maternal antibodies should be considered. Since maternal antibodies have been reported to last up to 17-18 weeks of age in white-tailed deer, European red deer should not be sampled before late autumn for data to be reliable (Gaydos et. al. 2002). BTV RNA has also been directly detected in spleen of naturally-infected European red deer by means of RT-PCR (Rodriquez-Sanchez et al. 2010, Rossi et al. 2010). Both antibodies against BTV and positive RT-PCR results have been reported to last up to 112 days after experimental infection of this animal for BTV1 and BTV8 (Lopez-Olivera at al. 2010) (Table 1). On the basis of results confirming the high susceptibility of European red deer to BTV infection, the detection of specific BTV antibodies in blood and RNA in spleen, and wide European distribution of red deer, make this species a good sentinel for surveillance of BTV (Ruiz-Fons et al. 2008, Rodriquez-Sanchez et al. 2010). Besides, the epidemiological data confirm the more cases of BTV infection among domestic ruminants in the regions where there are wild animals, mainly from cervus family. In summary, it can be concluded that wild ungulates due to the long-term carrier state may act as a reservoir for BTV and play an important role in its transmission.

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