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## 1 Review

3 BTV infection in wild ruminants, with emphasis on red deer:  
4 A review5 Caterina Falconi<sup>a,c,\*</sup>, Jorge Ramón López-Olvera<sup>b</sup>, Christian Gortázar<sup>a</sup>6 <sup>a</sup> Instituto de Investigación en Recursos Cinegéticos (IREC; CSIC-UCLM-JCCM), Ciudad Real, Spain7 <sup>b</sup> Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain8 <sup>c</sup> ASL 8, Dipartimento di Prevenzione, Servizio Veterinario Sanità Animale – Area A, Cagliari, Italy

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## ABSTRACT

The distribution of bluetongue virus has changed, possibly related to climate change. Vaccination of domestic ruminants is taking place throughout Europe to control BT expansion. The high density of wild red deer (*Cervus elaphus*) in some European regions has raised concerns about the potential role that unvaccinated European wild ungulates might play in maintaining or spreading the virus. Most species of wild ruminants are susceptible to BTV infection, although frequently asymptotically. The red deer population density in Europe is similar to that of domestic livestock in some areas, and red deer could account for a significant percentage of the BTV-infection susceptible ruminant population in certain regions. High serum antibody prevalence has been found in red deer, and BTV RNA (BTV-1, BTV-4 and BTV-8) has been repeatedly detected in naturally infected European red deer by means of RT-PCR. Moreover, red deer may carry the virus asymptotically for long periods. Epidemiological studies suggest that there are more BT cases in domestic ungulates in those areas where red deer are present. Vector and host density and environmental factors are implicated in the spatial distribution of BT. As in domestic ruminants, BTV transmission among wild ruminants depends almost exclusively on *Culicoides* vectors, mainly *C. imicola* but also members of the *C. obsoletus* and *C. pulicaris* complex. However, BTV transmission from red deer to the vector remains to be demonstrated. Transplacental, oral, and mechanical transmissions are also suspected. Thus, wild red deer contribute to the still unclear epidemiology of BTV in Europe, and could complicate BTV control in domestic ruminants. However, further research at the wildlife host–vector–pathogen interface and regarding the epidemiology of BT and BT vectors in wildlife habitats is needed to confirm this hypothesis. Moreover, red deer could be used as BT sentinels. Serum and spleen tissue of calves sampled from late autumn onwards should be the target samples when establishing a BTV surveillance program.

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25 **1. Introduction**

26 Formerly considered an exotic viral disease of wild and  
 27 domestic ruminants, several outbreaks of bluetongue (BT)  
 28 virus serotypes 1, 2, 4, 6, 8, 9, 11, and 16 have challenged  
 29 Europe since 1998. Nowadays, BT is a vector borne  
 30 infectious disease with significant changes in its geograph-  
 31 ical range (Purse et al., 2005; Enserink, 2008; Rodrí-  
 32 guez-Sánchez et al., 2008; Eschbaumer et al., 2009;  
 33 European Commission, 2009). Recently, BTV serotype 8  
 34 (BTV-8) infection has become widespread in livestock in  
 35 Central Europe since an initial outbreak in the Nether-

lands in 2006. In addition, BTV-1 has also expanded north- 36  
 and eastwards since its initial entry through southern 37  
 Spain in 2007 (Rodríguez-Sánchez et al., 2008) (Fig. 1). 38  
 Vaccination of domestic ruminants is taking place 39  
 throughout Europe to control BT expansion. The target 40  
 of the vaccination campaign is to achieve at least 80% 41  
 coverage of susceptible ruminants using killed vaccines 42  
 (Enserink, 2008). The high density of wild red deer (*Cervus* 43  
*elaphus*) in some European regions has raised concerns 44  
 about the potential role European wild ungulate popula- 45  
 tions, and red deer in particular, might play in maintaining 46  
 or spreading BT. 47

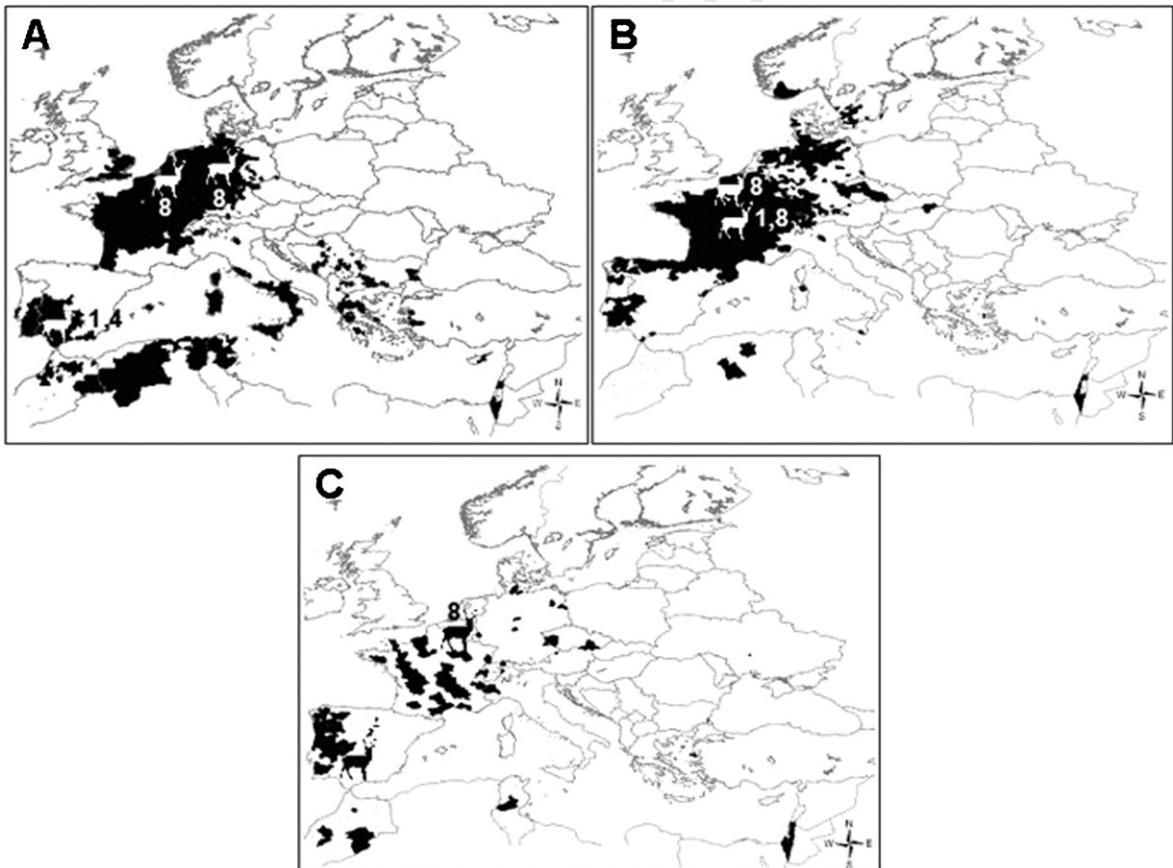


Fig. 1. BTV circulation in Europe from 2000 to 2010 ((A) since 1st of August 2000 to 30th of April 2008; (B) since 1st of May 2008; (C) since 1st of May 2009), including detection of BTV or antibodies against BTV in European red deer. Adapted and modified from the Epidemiological report of the European surveillance network for bluetongue (<http://eubtnet.izs.it/btnet.reports/EpidemiologicalSituation.html>), Linden et al. (2008), Ruiz-Fons et al. (2008), Conraths et al. (2009), García et al. (2009), Rossi et al. (2009), Linden et al. (2010), Rodríguez-Sánchez et al. (2008), and unpublished data (Falconi et al., in preparation).

48 Herein, we review the current knowledge on BT  
49 epidemiology in wild ruminants with an emphasis on  
50 red deer, discussing on the role of red deer in BTV  
51 epidemiology. Finally, areas open to future research are  
52 identified, and management implications stated.

## 53 2. General characteristics of bluetongue

54 Bluetongue virus (BTV) belongs to the Orbivirus genus  
55 in the Reoviridae family, closely related to the epizootic  
56 haemorrhagic disease virus (EHDV), the causative agent of  
57 epizootic haemorrhagic disease (EHD) (Mertens et al.,  
58 2005). Twenty-four serotypes of BTV have been described.  
59 Bluetongue (BT) affects both domestic and wild rumi-  
60 nants, and its origin is probably African. It was first  
61 identified in South Africa in Merino sheep in the late 18th  
62 century, and it is thought that cattle has now replaced  
63 antelope as a maintenance host of the virus (Gerdes,  
64 2004). Both BT and EHD are vector-borne pathogens,  
65 transmitted by several species of biting midges belonging  
66 to the *Culicoides* genus (Diptera: Ceratopogonidae) (Mel-  
67 lor and Wittmann, 2002). The potential for economic  
68 losses makes BT a disease reportable to the World  
69 Organisation for Animal Health.

70 BTV pathogenesis is very similar in sheep and cattle  
71 (MacLachlan, 1994), and probably also in wild ruminants.  
72 After inoculation of the virus through the skin by the bite of  
73 a competent and infected vector, BTV travels to the  
74 draining regional lymph node, where the immunological  
75 response of the host is initiated. Peripheral mononuclear  
76 blood cells carry the virus to secondary sites of replication,  
77 including lymphoid organs and the lungs (Stallknecht  
78 et al., 1997; Dal Pozzo et al., 2009).

79 Because of their number and susceptibility to BTV  
80 infection, domestic ruminants are key in BT epidemiology.  
81 Sheep are the most common host for BTV, often showing  
82 clinical consequences of the disease. The severity of the  
83 clinical expression in sheep can be influenced by the breed  
84 and the immunological conditions, as well as by the  
85 virulence of the BTV strain involved and other external  
86 factors. Goats are also infected but rarely show clinical  
87 signs (MacLachlan, 2004). However, a 25% case fatality rate  
88 in BTV-8 infected goats was recently reported from  
89 Germany (Conraths et al., 2009). Infection in cattle is  
90 commonly asymptomatic and characterized by a long  
91 viraemia, but after the emergence of BTV-8 in Northern  
92 and Central Europe, clinical illness and reproductive  
93 disorders have also been reported in cattle (Dal Pozzo  
94 et al., 2009).

95 Most if not all of the species of wild ruminants are  
96 susceptible to BTV infection. However, infected animals  
97 usually do not show clinical disease, particularly  
98 indigenous animals in regions where BTV is endemic  
99 (Quist et al., 1997; Johnson et al., 2006). While European  
100 Union compulsory control measures have been put in  
101 place to control the spread of the disease in livestock, the  
102 role of wildlife as susceptible hosts should be consid-  
103 ered, too, in any strategy to control BT (Fernández-  
104 Pacheco et al., 2008). In Europe, only numerically  
105 relevant wild ruminant species, and therefore poten-  
106 tially able to play a significant role in BT epidemiology

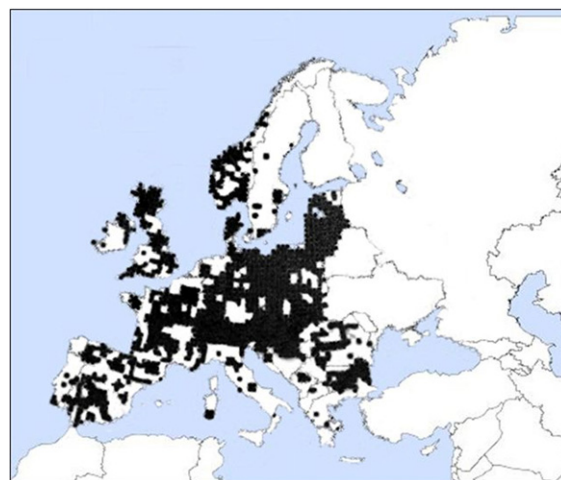


Fig. 2. Red deer (*Cervus elaphus*) distribution in Europe. Adapted and modified from <http://www.european-mammals.org>.

and its control in livestock, may be a cause of real  
concern. One of these species is red deer.

## 3. Red deer abundance and distribution

The red deer is a native wild ruminant of the northern  
hemisphere. It is present in most European countries,  
generally with a clustered distribution pattern (Fig. 2).  
Red deer population density in Europe ranges from 2 to 70  
individuals per square kilometre (Acevedo et al., 2008;  
Lovari et al., 2009). Hence, some local densities are not  
much lower than some livestock stocking rates, and red  
deer could account for a significant percentage of the total  
BTV-infection susceptible ruminant population in certain  
regions. The red deer is also a domestic animal, as deer  
farms are run to produce either venison and velvet, or  
trophy stags for the hunting industry. Farmed deer can  
eventually be vaccinated and managed as other livestock.

## 4. Bluetongue virus infection in wild ruminants

In the research aimed at understanding the role of wild  
ungulates in the epidemiology of BT, several species of wild  
ruminants have been investigated to elucidate their  
potential influence on BTV control, as well as the  
occurrence of clinical disease. 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 (Robinson  
et al., 1967; Fernández-Pacheco et al., 2008). Experimental  
infection of pronghorn antelope (*Antilocapra americana*),  
American bison (*Bison bison*), and African buffalo (*Syncerus  
caffer*) also produced clinical disease (Howerth et al., 2001;  
Tessaro and Clavijo, 2001), whereas blesbock (*Damaliscus  
pygargus*) (Bender et al., 2003) and mountain gazelle  
(*Gazella gazelle*) (Barzilai and Tadmor, 1972) did not show  
clinical signs after natural or experimental infection.

Table 1 presents data on viraemia and antibody kinetics  
in experimentally infected wild cervids. BTV infection and  
antibody response kinetics as well as clinical disease after

**Table 1**  
**Q3** Viraemia and serum BTV-specific antibody detection in BTV experimentally infected North American and European wild ruminants.

Species	Viraemia duration	Viraemia onset	Viraemia analytical methods	1st antibodies	Antibody duration	Reference
Black-tailed deer ( <i>Odocoileu shemioneus columbianus</i> )	NA	NA	NA	From 9 to 12 dpi	Over 692 dpi	Patton et al. (1994)
Black-tailed deer ( <i>Odocoileu shemioneus columbianus</i> )	From 1 to 10 days	From 2 to 9 dpi; peak at 7 dpi	Virus isolation	From 6 to 13 dpi	Over 692 dpi	Work et al. (1992)
White-tailed deer ( <i>Odocoileu svirginianus</i> )	3 days (2–5 dpi)	2 dpi	Virus isolation	NA	NA	Hoff and Trainer (1974)
White-tailed deer ( <i>Odocoileu virginianus</i> )	From 1 to 8 days	From 2 to 7 dpi	Virus isolation	NA	NA	Vosdingh et al. (1968)
American bison ( <i>Bison bison</i> )	From 1 to 4 days	From 4 to 7 dpi; 1 up to 28 dpi	Virus isolation	From 11 to 28 dpi	Over 127 dpi	Tessaro and Clavijo (2001)
European red deer ( <i>Cervus elaphus</i> )	Over 112 days	From 1 to 112 dpi	Virus isolation and RNA detection (only 12 DPI)	From 1 to 7 dpi	Over 112 dpi	López-Olvera et al. (2010)
North American elk ( <i>Cervus elaphus canadensis</i> )	From 3 to 8 days (2 days) <sup>a</sup>	From 2 to 10 dpi (106 dpi) <sup>a</sup>	Virus isolation	From 2 to 5 dpi	>7 weekdpi	Murray and Trainer (1970)

dpi, days post-infection

<sup>a</sup> After injection with 5.0 mg Flumethasone.

143 experimental infection have been reported in several  
 144 North American deer species belonging to the subfamily  
 145 *Odocoileinae*, namely white-tailed deer (*Odocoileus virgi-*  
 146 *nianus*) (Thomas and Trainer, 1970; Hoff and Trainer, 1974)  
 147 and mule deer (*Odocoileus hemionus*) (Work et al., 1992).  
 148 Maternal antibodies against BTV conferred partial immu-  
 149 nity in white-tailed deer fawns, although they disappeared  
 150 by 17–18 weeks of age (Gaydos et al., 2002). It has been  
 151 suggested that white-tailed deer may be an important BTV  
 152 host in nature (Thomas and Trainer, 1970). Overall,  
 153 *Odocoileinae* deer BT pathogenesis is probably similar to  
 154 that in cattle (Howerth et al., 2001).

155 Regarding cervids of the subfamily *Cervinae*, data on  
 156 experimental BTV infection are available for North  
 157 American elk and European red deer (both *C. elaphus*).  
 158 The results obtained indicate that both elk and red deer  
 159 have a potential role in BTV epidemiology, meriting further  
 160 investigation (Murray and Trainer, 1970; López-Olvera  
 161 et al., 2010).

162 Camelids have also been reported to be susceptible to  
 163 BTV infection. Experimental BTV infection induced anti-  
 164 bodies against BTV but coursed asymptotically in two  
 165 llamas (*Llama glama*) (Afshar et al., 1995), whereas a severe  
 166 clinical form with lethal disease was reported in naturally  
 167 infected llamas (Meyer et al., 2008).

168 Although ruminants are the natural hosts of BTV,  
 169 antibody and virus have been reported in carnivores  
 170 (Alexander et al., 1994). Infection, disease and death of two  
 171 Eurasian lynx (*Lynx lynx*) fed with lambs from BTV-8  
 172 affected farms have been reported in a zoo in Belgium  
 173 (Jauniaux et al., 2008).

## 174 5. Bluetongue clinical signs and lesions in wild 175 ruminants

176 BTV infection of the more susceptible wild ungulates can  
 177 result in a wide variety of lesions and clinical signs, ranging

178 from sudden death to asymptomatic infection (Vosdingh  
 179 et al., 1968; Murray and Trainer, 1970; Hoff and Trainer,  
 180 1974; Prestwood et al., 1974; Howerth and Tyler, 1988;  
 181 Howerth et al., 2001). Only serotypes 1, 8 and 17 have been  
 182 associated with clinical signs in either experimental or  
 183 natural infections in wild white-tailed deer (Vosdingh et al.,  
 184 1968; Howerth and Tyler, 1988; Johnson et al., 2006).  
 185 Clinical signs and lesions have been reported both in  
 186 experimental and natural BTV-infections of white-tailed  
 187 deer, and three forms of BTV-infection have been reported in  
 188 this species peracute, acute and persistent (Table 3).

189 Clinical signs reported in white-tailed deer include  
 190 severe depression, weakness, fever, loss of fear, anorexia,  
 191 hyperemic oral mucosa, muco-purulent nasal discharge,  
 192 crusts in the nares, severe respiratory distress, sub-  
 193 mandibular oedema, reddening of the muzzle and ears,  
 194 oedema around the eyes, swollen and cyanotic tongue,  
 195 excessive salivation, oral ulceration, multifocal haemor-  
 196 rhage in the skin and mucosa, severe bloody diarrhoea and  
 197 laminitis. The only appreciable haematological change is  
 198 leucopenia and neutropenia (Vosdingh et al., 1968; Hoff and  
 199 Trainer, 1974; Prestwood et al., 1974). Oedema of the head  
 200 and neck was reported in white-tailed deer with peracute  
 201 BT, whereas haemorrhages throughout the body were the  
 202 main clinical sign in the classic acute form of BT (Prestwood  
 203 et al., 1974). In European mouflon, taxonomically consid-  
 204 ered as a subspecies of domestic sheep, the main domestic  
 205 BTV hosts and clinical signs reported include inflammation  
 206 of mucous membranes, congestion, swelling and haemor-  
 207 rhages (Fernández-Pacheco et al., 2008).

208 Conversely, BTV-infection of less susceptible wild  
 209 ruminants is asymptomatic or causes only mild clinical  
 210 signs. In experimentally infected black-tailed deer (*Odo-*  
 211 *coileus hemionus columbianus*), the only clinical sign was  
 212 hyperthermia, body temperature ranging from 40 °C to  
 213 41.2 °C (Work et al., 1992). In European red deer no clinical  
 214 signs have been reported, both after natural and experi-

215 mental BTV-infection (Ruiz-Fons et al., 2008; García et al.,  
216 2009; Rossi et al., 2009; López-Olvera et al., 2010;  
217 Rodríguez-Sánchez et al., 2010).

218 Most of the clinical signs reported for BTV-infected wild  
219 ruminants originate from experimental infections, but in  
220 domestic sheep the clinical response to BTV-infection is  
221 more severe in sheep infected via vector bites than in those  
222 inoculated with infective blood (Luedke et al., 1976), which  
223 could mean that naturally infected wild ruminants could  
224 show some more clinical signs than experimentally infected  
225 ones. Nevertheless, BTV clinical signs are very difficult to  
226 appreciate in field conditions, particularly in those species  
227 where the disease is silent or asymptomatic, including the  
228 red deer.

229 Lesions due to BTV-infection have also been thoroughly  
230 reported in white-tailed deer (Table 3). Organs most  
231 frequently affected include tongue, heart, spleen, lymph  
232 nodes and kidneys (Hoff and Trainer, 1974; Howerth and  
233 Tyler, 1988; Howerth et al., 2001). In the acute classical  
234 form, haemorrhagic lesions are due to endothelial damage  
235 and disseminated intravascular coagulation (Howerth and  
236 Tyler, 1988). Cardiovascular lesions occur within 12 days of  
237 infection only and consist in pericardial, subpericardial and  
238 subendocardial haemorrhages, as well as small haemor-  
239 rhages in the papillary muscles of the left ventricle, at the  
240 base of the aorta and at the base of the pulmonary artery  
241 (Howerth et al., 2001).

242 Typical findings in the persistent form of BT in white-  
243 tailed deer include erosion and ulceration of dental pad,  
244 hard palate, tongue, gingiva, forestomachs and abomasum;  
245 gastritis and enteritis; and foot lesions, ranging from  
246 coronitis and laminitis to complete sloughing of the hooves  
247 (Prestwood et al., 1974; Howerth et al., 2001).

248 Among North American Odocoileinae, both clinical  
249 signs and lesion patterns are different in endemic or  
250 epidemic disease: cases from endemic areas are char-  
251 acterized by hoof and rumen lesions, whereas in epidemic  
252 areas BT is characterized by high levels of mortality, with a  
253 predominance of acute and peracute forms (Stallknecht  
254 and Howerth, 2004). However, differences in clinical  
255 response could also be related to the combined effects  
256 of maternal antibody transfer (Gaydos et al., 2002),  
257 acquired immunity through previous challenge (Quist  
258 et al., 1997), and innate resistance within specific host  
259 populations (Stallknecht et al., 1991).

## 260 6. Bluetongue in European wild ruminants 260

261 BT is considered endemic in wild ruminants in large  
262 parts of Africa and North America (Stallknecht et al., 1996;  
263 Gerdes, 2004). However, the role of European wild  
264 ruminants is still under debate (Durand et al., 2010;  
265 Rodríguez-Sánchez et al., 2010). BTV specific antibodies  
266 have been detected both in free-ranging and farmed wild  
267 ruminants in several European countries (De Curtis et al.,  
268 2007; Fernández-Pacheco et al., 2008; Gür, 2008; Linden  
269 et al., 2008, 2010; Ruiz-Fons et al., 2008; Conraths et al.,  
270 2009; García et al., 2009; Rossi et al., 2009; Rodríguez-  
271 Sánchez et al., 2010; ~~Lorca et al., 2010~~) (Table 2). The Q1  
272 highest serum antibody prevalence has been reported for  
273 red and fallow deer (*Dama dama*), both belonging to the  
274 Cervinae subfamily (Table 2; García et al., 2009; Linden  
275 et al., 2008, 2010; Rossi et al., 2009; Ruiz-Fons et al., 2008).  
276 Moreover, BTV RNA has been repeatedly detected in  
277 naturally infected European red deer by means of RT-PCR,  
278 including BTV-8 in free-ranging red deer from Belgium

Table 2

Published results on prevalence of BTV specific serum antibodies in free-ranging European wild ruminants.

Host species	Serotype	Country	Seroprevalence	Sampling year	Reference
Red deer ( <i>Cervus elaphus</i> )	1	Spain	66.3%	2006–2007	García et al. (2009)
Fallow deer ( <i>Dama dama</i> )	1	Spain	50.0%	2006–2007	García et al. (2009)
Red deer ( <i>Cervus elaphus</i> )	8	Belgium	52.3%	2007	Linden et al. (2010)
Red deer ( <i>Cervus elaphus</i> )	8	Belgium	40.4%	2007	Linden et al. (2008)
Goitered gazelle ( <i>Gazella subgutturosa</i> )	–	Turkey	40.2%	2005	Gür (2008)
Red deer ( <i>Cervus elaphus</i> )	1, 8	France	37.7%	2008	Rossi et al. (2009)
Fallow deer ( <i>Dama dama</i> )	1	Spain	35.4%	2005–2007	Ruiz-Fons et al. (2008)
Red deer ( <i>Cervus elaphus</i> )	8	Belgium	34.0%	2008	Linden et al. (2010)
Mouflon ( <i>Ovis aries musimon</i> )	1	Spain	33.3%	2006–2007	García et al. (2009)
Barbary sheep ( <i>Ammotragus lervia</i> )	1	Spain	25.0%	2005–2007	Ruiz-Fons et al. (2008)
Red deer ( <i>Cervus elaphus</i> )	1	Spain	21.9%	2005–2007	Ruiz-Fons et al. (2008)
Mouflon ( <i>Ovis aries musimon</i> )	1	Spain	13.2%	2005–2007	Ruiz-Fons et al. (2008)
Spanish ibex ( <i>Capra pyrenaica</i> )	1	Spain	10.8%	2006–2007	García et al. (2009)
Spanish ibex ( <i>Capra pyrenaica</i> )	–	Spain	5.8%	2009	Santiago-Moreno et al. (2010)
Roe deer ( <i>Capreolus capreolus</i> )	1	Spain	5.1%	2005–2007	Ruiz-Fons et al. (2008)
Spanish ibex ( <i>Capra pyrenaica</i> )	1, 4	Spain	4.0%	2006–2009	<del>Lorca et al. (2010)</del>
Roe deer ( <i>Capreolus capreolus</i> )	8	Belgium	2.8%	2007	Linden et al. (2010)
Roe deer ( <i>Capreolus capreolus</i> )	8	Belgium	2.6%	2006	Linden et al. (2010)
Roe deer ( <i>Capreolus capreolus</i> )	8	Belgium	1.7%	2008	Linden et al. (2010)
Ibex ( <i>Capra ibex</i> )	1, 8	France	1.6%	2008	Rossi et al. (2009)
Red deer ( <i>Cervus elaphus</i> )	8	Belgium	1.5%	2006	Linden et al. (2010)
Roe deer ( <i>Capreolus capreolus</i> )	1, 8	France	1.2%	2008	Rossi et al. (2009)
Southern chamois ( <i>Rupicapra pyrenaica</i> )	1, 8	France	1.1%	2008	Rossi et al. (2009)
Fallow deer ( <i>Dama dama</i> )	–	Italy	0.5%	2004–2005	De Curtis et al. (2007)

**Table 3**

Clinical signs and lesions reported for both experimentally and naturally BTV-infected North American white tailed-deer.

Serotype and reference	Infection	Clinical signs	Lesions
BTV-8 (Vosdingh et al., 1968)	Experimental	<ul style="list-style-type: none"> <li>• Hyperthermia</li> <li>• Anorexia and weight loss</li> <li>• Weakness</li> <li>• Loss of fear of man</li> <li>• Leucopenia</li> <li>• Clear mucopurulent nasal discharge</li> <li>• Crusts in the nares</li> <li>• Severe bloody diarrhoea</li> <li>• Excessive salivation</li> <li>• Recumbence</li> </ul>	<ul style="list-style-type: none"> <li>• Swollen and cyanotic tongue</li> <li>• Subendocardial and tongue haemorrhages</li> <li>• Enteritis</li> <li>• Generalised congestion</li> </ul>
BTV-8 (Thomas and Trainer, 1970)	Experimental	<ul style="list-style-type: none"> <li>• Acute rapidly fatal</li> <li>• Subclinical with persistent viraemia</li> </ul>	
BTV-17 (Howerth and Tyler, 1988)	Experimental	<ul style="list-style-type: none"> <li>• Hyperthermia</li> <li>• Erythema</li> <li>• Facial oedema</li> <li>• Coronitis</li> <li>• Stomatitis</li> <li>• Excessive bleeding and haematoma formation</li> <li>• Dehydration</li> <li>• Diarrhoea</li> </ul>	<ul style="list-style-type: none"> <li>• Oral lesions</li> <li>• Widespread haemorrhage (from petechiae to massive haematoma)</li> <li>• Endothelial damage and disseminated intravascular coagulation</li> </ul>
BTV-10 after EHDV-2 challenge (Quist et al., 1997)	Experimental	<ul style="list-style-type: none"> <li>• Hyperthermia</li> <li>• Skin redness</li> <li>• Depression and lethargy</li> <li>• Dehydration</li> <li>• Subcutaneous swelling about head</li> <li>• Bleeding tendencies</li> </ul>	<ul style="list-style-type: none"> <li>• Haemorrhagic disease</li> <li>• Cranioventral bronchopneumonia</li> <li>• Mild pericardial effusion</li> </ul>
BTV-8 (Hoff and Trainer, 1974)	Natural	<ul style="list-style-type: none"> <li>• Depression</li> <li>• Severe respiratory distress</li> <li>• Cyanotic tongue</li> <li>• Foetal death</li> </ul>	<ul style="list-style-type: none"> <li>• Ecchymosis</li> <li>• Haemorrhages: <ul style="list-style-type: none"> <li>- Digestive</li> <li>- Trachea (petechial)</li> </ul> </li> <li>• Oedema: <ul style="list-style-type: none"> <li>- Liver</li> <li>- Skeletal musculature</li> <li>- Kidney</li> <li>- Tongue</li> </ul> </li> <li>• Oedema and haemorrhages: <ul style="list-style-type: none"> <li>- Heart and vessels</li> <li>- Larynx</li> </ul> </li> </ul>
BTV, serotype not specified (Prestwood et al., 1974)	Natural	<ul style="list-style-type: none"> <li>• Peracute: <ul style="list-style-type: none"> <li>- Respiratory, head and neck oedema</li> </ul> </li> <li>• Acute: <ul style="list-style-type: none"> <li>- Dental pad, hard palate and tongue erosions</li> <li>- Coronitis</li> </ul> </li> <li>• Persistent: <ul style="list-style-type: none"> <li>- Coronitis</li> <li>- Ulcers on dental pad, hard palate and tongue</li> <li>- Severe necrotizing glossitis</li> <li>- Coronitis, laminitis, sloughing of the hoof</li> <li>- Emaciation, malnutrition</li> <li>- Abscess formation due to secondary infection</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Acute: <ul style="list-style-type: none"> <li>- Haemorrhages of the digestive tract</li> </ul> </li> <li>• Persistent: <ul style="list-style-type: none"> <li>- Ulceration and sloughing of forestomach lining</li> <li>- Gastritis and enteritis</li> </ul> </li> </ul>
	Experimental	<ul style="list-style-type: none"> <li>• Depression and weakness</li> <li>• Reddening of muzzle and ears</li> <li>• Oedema around the eyes and muzzle</li> <li>• Swollen tongue</li> <li>• Laminitis</li> <li>• Dyspnea</li> <li>• Blood in faeces</li> <li>• White frothy nasal discharge</li> <li>• Recumbence and death</li> </ul>	<ul style="list-style-type: none"> <li>• Stomatitis</li> <li>• Laminitis</li> <li>• Oedema</li> <li>• Haemorrhages (particularly digestive)</li> </ul>

279 (Linden et al., 2010), BTV-1 and BTV-8 in free-ranging red  
 280 deer in France (Rossi et al., 2009), BTV-1 in free-ranging red  
 281 deer in Spain (Arenas-Montes et al., 2010), and BTV-4 and  
 282 BTV-1 in farmed red deer in Spain (Rodríguez-Sánchez  
 283 et al., 2010). Red deer infection with BTV does not cause  
 284 clinical disease or high mortality, and therefore it does not  
 285 have a significant effect on the dynamics of the affected

population (Linden et al., 2008; Rossi et al., 2009; López-  
 Olvera et al., 2010). 286

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293 potential use of deer (and probably other wild ruminants)  
294 as BT sentinels in Europe (Simpson, 2009). Recent findings  
295 pointing in this direction include: clinical fatal disease  
296 caused by BTV-1 in mouflons (Fernández-Pacheco et al.,  
297 2008); widespread and elevated antibody prevalence in  
298 wild ungulates (Ruiz-Fons et al., 2008); the occasional  
299 detection of antibodies against BTV in wild ungulates in  
300 regions with no reports from livestock (García et al., 2009);  
301 detection of BTV RNA in naturally infected red deer (Rossi  
302 et al., 2009); a high susceptibility of red deer to BTV  
303 infection, albeit asymptotically (López-Olvera et al.,  
304 2010; Rodríguez-Sánchez et al., 2010); and a higher  
305 incidence of BT in domestic ungulates in areas with  
306 presence of red deer (Allepuz et al., 2010). In addition to  
307 blood and serum, target organs for BT surveillance in wild  
308 red deer should include the spleen, since this is the organ  
309 with more consistent BTV isolation in white-tailed deer  
310 (Vosdingh et al., 1968) and cattle (MacLachlan et al., 1990).

## 311 7. BTV transmission in wild ruminants

312 As for domestic ruminants, BTV transmission among  
313 wild ruminants depends almost exclusively on *Culicoides*  
314 vectors. BTV prevalence and outbreaks follow a seasonal  
315 pattern in Europe, infection generally occurring in late  
316 summer and early fall. This seasonal variation is probably  
317 related to changes in vector abundance, which depends on  
318 climate factors, namely precipitation and temperature  
319 (Sleeman et al., 2009). In Europe, different species of the  
320 *Culicoides* genus have been identified as competent vectors  
321 of BTV (Rodríguez-Sánchez et al., 2008). *Culicoides imicola*,  
322 the most important BTV vector in Africa and in the  
323 Mediterranean basin (Mellor and Wittmann, 2002), has  
324 extended its geographical range of activity northwards  
325 under the influence of a warming global climate (Purse  
326 et al., 2005), and climate change has been proposed as an  
327 explanation for the emergence of BT in Europe (Enserink,  
328 2008). Nevertheless, the occurrence of BT in Northern and  
329 Central Europe has been associated with the involvement  
330 of other species of the *Culicoides* genus, such as the *C.*  
331 *obsoletus* and *C. pulicaris* complex, which have proven to be  
332 competent vectors of BTV once the virus has entered their  
333 distribution areas (Mehlhorn et al., 2007). Modelling  
334 predicts higher abundances but limited range expansion  
335 for *C. imicola* in the coming decades, presumably affecting  
336 the intensity of spread of BTV (Acevedo et al., 2010).

337 Apart from the annual seasonality, there are inter-  
338 annual cycles in the occurrence of BT outbreaks. In  
339 endemic areas of North America, deer populations are  
340 infected on one to three year cycles, whereas in epidemic  
341 areas disease occurs in an eight to ten-year cycle  
342 (Stallknecht and Howerth, 2004). These cycles can be  
343 related to combined effects of herd immunity and  
344 fluctuations in vector populations, and could be further  
345 complicated by the possibility that these short and long-  
346 term cycles may occur concurrently (Stallknecht and  
347 Howerth, 2004). Moreover it has been reported that  
348 vector, host and environmental factors are implicated in  
349 the spatial distribution of BTV (Acevedo et al., 2010).  
350 Hence, wild ruminants may influence the epidemiology of  
351 BT through different mechanisms, interacting both with

the virus and with the vectors (Acevedo et al., 2010; 352  
Durand et al., 2010). However, studies on vector popula- 353  
tions in wildlife habitats with few or no domestic 354  
ruminants, and studies on host/vector interaction in wild 355  
ruminants are scarce (Smith et al., 1996). Moreover, BTV 356  
can exceptionally be transmitted by alternative vectors, 357  
either mechanically, as reported for *Melophagus ovinus* 358  
(Luedke et al., 1965), or through vector infection, as 359  
reported for ticks and BTV-8 (Bouwknegt et al., 2010). 360

361 Exceptionally, BTV can be transmitted directly from  
362 ruminant to ruminant in absence of the vector. Transpla-  
363 cental, oral (including colostrum), semen and mechanical  
364 (wound) contacts have been suggested as possible  
365 mechanisms for BTV transmission, either in domestic or  
366 wild ungulates (Vosdingh et al., 1968; Thomas and Trainer,  
367 1970; Stott et al., 1982; Menzies et al., 2008; Backx et al.,  
368 2009; López-Olvera et al., 2010; Mayo et al., 2010;  
369 Santiago-Moreno et al., 2011). Further research is needed  
370 regarding these newly reported transmission routes to  
371 determine their importance for BTV maintenance.

## 372 8. BTV infection in red deer

373 Both the European red deer and its close relative the  
374 North American elk (*Cervus elaphus canadensis*) have  
375 been experimentally infected with BTV (Murray and  
376 Trainer, 1970; Ellis et al., 1993; López-Olvera et al.,  
377 2010). In all cases, the deer became asymptotically  
378 infected or showed only mild signs, such as a transient  
379 increase of body temperature, conjunctivitis and diar-  
380 rhoea containing small amounts of blood and mucus.  
381 Both subspecies had BTV in blood with a significant  
382 magnitude and duration, comparable to that of experi-  
383 mentally infected domestic cattle (Puentes et al., 2008).  
384 The epidemiological importance of a species in BTV  
385 transmission is directly correlated to viraemia duration  
386 (Koumbati et al., 1999). BTV RNA has been found in  
387 blood up to 112 days post-infection in red deer (López-  
388 Olvera et al., 2010), and 180 days post-infection in cattle,  
389 the known key maintenance species in BTV epidemiol-  
390 ogy (Katz et al., 1994).

391 Moreover, red deer develop antibodies by the second or  
392 third week after exposure until up to seven months  
393 (Murray and Trainer, 1970; López-Olvera et al., 2010).  
394 Overall, BTV RNA and antibody kinetics were very similar  
395 to those of domestic cattle. Interestingly, BTV could be  
396 isolated again from elk blood at 106 dpi after an  
397 experimental cortisone treatment. These elk had rendered  
398 negative results to BTV isolation from 10 to 105 dpi, when  
399 cortisone was injected. The cortisone treatment appar-  
400 ently re-stimulated BTV viraemia (Murray and Trainer,  
401 1970), suggesting that latently infected red deer could  
402 eventually return to viraemic under certain circum-  
403 stances, such as stress. However, the Murray and Trainer  
404 (1970) study had no control animal, so the findings remain  
405 questionable. A long viraemia may contribute to BTV  
406 maintenance, and it is likely that in deer, like in cattle, the  
407 virus adheres in the red blood cell surface until the red  
408 blood cell is removed from circulation, resulting in rather  
409 long term circulation of virus in the blood (Stallknecht  
410 et al., 1997).

Altogether, literature indicates that both elk and red deer have the potential to contribute to BTV maintenance, meriting further investigation (Murray and Trainer, 1970). However, to ensure that red deer can be a maintenance host for BTV, it remains to be determined whether they are capable of infecting *Culicoides* sp. vectors, thus allowing virus transmission from deer.

### 9. Red deer as a bluetongue virus sentinel

Although the role of European red deer in BT epidemiology is still unclear, several studies have shown its potential as a sentinel for bluetongue virus. Antibodies against BTV have been reported in European red deer shortly after being detected in domestic livestock (Linden et al., 2008; Ruiz-Fons et al., 2008; Rossi et al., 2009; Rodríguez-Sánchez et al., 2010). Occasionally, contact of wild ruminants with BTV has been reported in geographic regions where BTV had not been found in domestic livestock (García et al., 2009). In other cases, BTV specific antibodies or RNA was detected in wild ruminants long after its successful control in domestic ruminants (Rodríguez-Sánchez et al., 2010). Seroprevalence among yearlings should more accurately reflect the level of exposure to infected insect vectors in a Q2 season, since seropositive subadults and adults 2008 could have been infected in previous vector seasons (Linden et al., 2008). Nevertheless, care must be taken when interpreting the presence of antibody in serum samples from calves, since they can be due to transmission of maternal immunity via colostrum. Since maternal antibodies have been reported to last up to 17-18 weeks of age in white-tailed deer (Gaydos et al., 2002), European red deer calves should not be sampled before late autumn for data to be reliable.

BTV RNA has also been directly detected in the spleen of naturally infected European red deer by means of RT-PCR (Rossi et al., 2009; Arenas-Montes et al., 2010; Linden et al., 2010; Rodríguez-Sánchez 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 European red deer for BTV-1 and BTV-8 (López-Olvera et al., 2010).

Altogether, the susceptibility to BTV, the detection of antibodies and of BTV RNA in spleen, and the wide European distribution of red deer, make this species a good sentinel for surveillance of BTV (Ruiz-Fons et al., 2008; Rodríguez-Sánchez et al., 2010). Serum, for antibody detection, as well as spleen tissue for PCR in fawns sampled from late autumn onwards, should be the target samples when establishing a BTV surveillance program in this species.

### 10. Red deer as a maintenance host for BTV

Wild ruminants are maintenance hosts for BTV in South Africa, with up to 30% antibody prevalence, although cattle appear to have largely replaced antelope in this role (Gerdes, 2004). In parts of North America, both BTV and EHDV are endemic among wild white-tailed deer (Stallknecht et al., 1996). In Europe, if BTV circulates in red deer

and in other wild ruminants independently of its situation in sheep, goat and cattle livestock, a wildlife maintenance host will exist. Information contained in this review suggests that red deer do have the potential to interfere with current BT control schemes in Europe, but evidence are still not sufficient to establish the role of European red deer in BT epidemiology. Current knowledge is as follows:

1. BTV RNA was consistently detected in experimentally BTV-1 and BTV-8 infected red deer for up to 112 days after inoculation (dpi), although isolation was only successful during the viraemia peak at 12 dpi (López-Olvera et al., 2010). In North American elk, cortisone treatment possibly re-stimulated viraemia at 105 dpi (Murray and Trainer, 1970). Viraemia duration is directly related to the significance of a species in BT epidemiology (Koumbati et al., 1999). Moreover, in naturally infected red deer, BTV RNA has been detected in spleen up to eighteen months after the last detection in sympatric domestic ruminants (Rossi et al., 2009; Arenas-Montes et al., 2010; Linden et al., 2010; Rodríguez-Sánchez et al., 2010). Overall, data on red deer suggest that this species has the potential to contribute to BTV infection maintenance in the wild, especially where deer occur at high densities.
2. Detection of antibodies against several serotypes of BTV in red deer concurrently with domestic livestock outbreaks has been repeatedly reported (Ruiz-Fons et al., 2008; Linden et al., 2008; García et al., 2009; Rossi et al., 2009; Rodríguez-Sánchez et al., 2010). However, seroprevalence against BTV declines both in red and white-tailed deer after an epidemic event or domestic livestock vaccination, time required for this decline ranging from one to five years (Stallknecht et al., 1991; Linden et al., 2010). This would suggest that even high densities of unvaccinated red deer are not capable of maintaining circulating BTV for longer periods.
3. Finally, BTV can be transmitted from infected vectors to deer, and potential mechanisms of transmission independent of the presence of the vector have been reported in deer: transplacental transmission (Stott et al., 1982) and direct transmission (Vosdingh et al., 1968; Thomas and Trainer, 1970; Menzies et al., 2008; Backx et al., 2009; López-Olvera et al., 2010). But BTV transmission from red deer to a competent vector remains to be demonstrated, as well as the time after infection when *Culicoides* vectors can get infected from red deer. This point needs to be elucidated in order to more accurately establish the potential epidemiological risk of European red deer in BTV dynamics.

These facts, along with the locally high densities of wild red deer in some European regions, support the need to consider red deer and possibly also other wild ruminants when dealing with BT epidemiology and control. Based on current knowledge, we hypothesize that wild red deer have the potential to maintain BTV circulation in certain regions in Europe, at least for a few years after the initial outbreak. A natural experiment to test this hypothesis is currently running: livestock has largely been vaccinated (European Commission, 2009), but annual re-vaccination of strains that are no longer detected in livestock is unlikely to be

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maintained for long due to logistic and budget constraints. Vaccine induced immunity against a given BTV strain can last for over one year (e.g. Hamers et al., 2009). However, immunity acquired by vaccination or after natural infection is lost after a certain time if no annual boost occurs. Hence, if repeated BTV outbreaks occur in regions with high wild ruminant densities and no clear link with livestock movements or vaccination failures, the deer reservoir hypothesis will be confirmed. If, in contrast, no BTV circulation takes place and livestock vaccination alone is successful in eradicating bluetongue, the hypothesis will be rejected (López-Olvera et al., 2010).

## 11. Management implications

1. Results reviewed in this document contribute to the opinion that wild red deer could potentially play a role in the still unclear epidemiology of BTV in Europe, and should be taken into account in BT control. Movement restrictions and vaccination schemes applied to domestic animals should be adapted to include farmed or translocated red deer and other wild ruminants.
2. Deer are good BTV sentinels. Monitoring of red deer contact with BTV should combine serology (ELISA, eventually followed by serum neutralization) and PCR testing of blood and/or lymphoid organs such as the spleen. Samples can be obtained either from hunter-harvested deer or from farmed deer. Calves sampled in late autumn and winter, after losing any maternal antibodies, should be the preferred target, since they would be good indicators of the current status of BTV, rather than adult deer, whose antibodies and viral RNA could have an older origin. Regions with high deer densities should set up appropriate surveillance schemes.
3. Based on current knowledge, no impacts of BTV on farmed or wild red deer populations are expected. However, sporadic cases and possible sporadic reproductive disorders cannot be excluded, depending on BTV-serotype, as well as effects on other wild ruminant species, in particular wild sheep species such as the mouflon.
4. Vector, host density and environmental factors are possibly implicated in the spatial distribution of BTV. Wild ruminants may influence the epidemiology of BT by different mechanisms, because while different types of habitat influence the spread of BTV (Durand et al., 2010), the populations of hosts, including wildlife, influence the abundance of vectors. Moreover, changes in the abundance of vectors will continue in the coming decades, affecting the intensity of spread of BTV (Acevedo et al., 2010). Further research is needed at the wildlife host–vector–pathogen interface and regarding the epidemiology of BT and BT vectors in wildlife habitats.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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