Bluetongue and epizootic haemorrhagic disease in wildlife with emphasis on the South American scenario

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Summary
Bluetongue (BT) and epizootic haemorrhagic disease (EHD) are two OIE-listed vector borne diseases of domestic and wild ruminants caused by two orbiviruses, BT virus (BTV) and EHD virus, respectively. Also wildlife can be infected by these two viruses. They can manifest a variable range of clinical signs and lesions. Some species appear to be extremely susceptible showing, indeed, high mortality rates, others may act only as reservoirs, playing however, an important epidemiological role. The purpose of this review is to describe the clinical and pathological manifestations related to these diseases in wildlife species and to review available literature on BTV/EHDV-infected wildlife species with emphasis for the south American scenario.

Bluetongue virus and epizootic haemorrhagic disease virus history and distribution

Bluetongue (BT) and epizootic haemorrhagic disease (EHD) are vector-borne infectious diseases caused by bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV), respectively. Both viruses belong to the genus *Orbivirus* within the family *Reoviridae*. These diseases affect domestic and wild ruminants with variable susceptibility depending on the species involved (Duarte *et al.* 2001). The first report of BT was published in the early 20th century in Africa, but it was only in 1943, when an outbreak occurred in Cyprus, that the disease was officially identified on other continents (Gibbs and Greiner 1994). Currently, 1-24 classical and several novel atypical serotypes (which do not cause BT) are described (reviewed in Cappai *et al.* 2019).

Besides the recent events in Europe (reviewed in MacLachlan *et al.* 2019), BT has been found to circulate widely in Africa, the Middle East, Australia, the South Pacific, North and South America, and Asia, and it may be present in some regions without causing clinical manifestations (Werther and Kawanami 2014).

Epizootic haemorrhagic disease virus was first isolated in 1955 from a white-tailed deer in New Jersey; the animal was probably infected during an outbreak that led to the death of an estimated number of 500 to 700 deer (Shope *et al.* 1960). Epizootic haemorrhagic disease has been reported causing clinical cases and seropositive animals in North America, Australia, Asia, Africa and South America (Alfieri *et al.* 2007, Werther and Kawanami 2014, Favero *et al.* 2013). Currently at least eight serotypes are recognized (OIE 2009).

Vector abundance and distribution, among other factors, lead to the variation in geographic distribution of haemorrhagic diseases as BT and EHD. These factors, as well as the specific serotype present, pathogenicity, host immunity, and genetic variation contribute to the geographic distribution of EHDV and BTV (Stallknecht *et al.* 2002).

Among domestic species, sheep are notably susceptible to BTV (Erasmus 1975). Clinical signs are not common in cattle and goats however, these animals may develop reproductive disorders as a result of infection (Arida *et al.* 2007). In many endemic countries, cattle rarely present clinical signs. They however can show a prolonged period of viraemia (Katz *et al.* 1994, Singer *et al.* 2001) and act as a reservoir of the virus (Arida *et al.* 2007). Nevertheless, a higher occurrence of the disease

was reported in cattle than in sheep during the BTV-8 outbreaks in northern Europe. The clinical signs observed include loss of body condition, hyperthermia, nasal discharge and ulcers on the oral mucosa (Thiry *et al.* 2006).

Information on the impact of BTV and EHDV in wildlife in South America is rarely reported. While some studies indicate seroprevalence in wild populations and captive animals, information about circulating serotypes, distribution and species affected is extremely rare. Therefore, the aim of this study is to review the information currently available on BTV and EHDV in different species of wildlife in South America with the emphasis on clinical and pathological manifestations caused by these viruses.

Clinical and pathological manifestations in wildlife species

Vosdingh and colleagues (Vosdingh *et al.* 1968) 10 white-tailed deer (*Odocoileus virginianus*) were experimentally expose to the California BTV-8 strain. All infected animals developed clinical signs. Infection was fatal for all of the seven fawns and an adult female. The clinical signs observed in the fawns included: increase in temperature, anorexia, weakness, bloody diarrhoea and cyanotic tongues. Gross lesions such as subendocardial haemorrhage, enteritis and haemorrhage in the tongue were present in most of the animals. Histopathology revealed congestion haemorrhage, necrosis, thrombosis, and the most frequently affected organs were the tongue, heart, spleen, kidneys and lymph nodes. The histological lesions in deer resembled the lesions seen in infected sheep, and the major differences between these species appear to be the absence of extensive buccal erosions and foot lesions and a greater tendency for vascular thrombosis in deer (Karstadt and Trainert 1967). Contrarily to the findings in white-tailed deer, experimental infections with BTV and EHDV in black-tailed deer (*Odocoileus hemionus columbianus*) were unable to cause clinical disease other than elevated temperatures (Work *et al.* 1992). European red-deer (*Cervus elaphus*) do not develop clinical signs after experimental infection with BTV serotype 1 (BTV-1) and BTV serotype 8 (BTV-8). The authors of this study suggested that red-deer may act as a carrier host for BTV, maintaining the virus for long periods as RNA was detected in the blood until the end of the experiment at 112 days post infection (dpi) (López-Olivera *et al.* 2010).

American bisons (*Bison bison*) also do not show clinical signs after infection with BTV (Tessaro and Clavijo 2001).

Pronghorn (*Antilocapra americana*) is also susceptible to BTV. At least 3,500 animals died during the two outbreaks of BTV-17 in Wyoming, USA, in 1972 and 1984 (Thorne *et al.* 1988). Gross lesions included hemorrhages and oedema in the pericardial, subepicardial and subendocardial areas on the tunica adventitia of the dorsal aorta and pulmonary artery. Hemorrhages were also present in the gastrointestinal tract, lymph nodes, urinary bladder and synovial surfaces of joint capsules (Thorne *et al.* 1988).

Camelids appear to be resistant to the development of the clinical signs caused by both orbiviruses. Batten and colleagues (Batten *et al.* 2011) described an experimental infection with BTV-1 in three adult camels. The animals were observed for 75 days. Camels did not show clinical signs, however, they seroconverted in approximately 11 days. Viraemia was detected at 7 dpi, and replication magnitude of the virus was lower in the experimentally infected camels than that observed in sheep. The same study revealed for the first time that BTV could be isolated from the blood of infected camels with potential epidemiological consequences. Several studies described BTV seropositive camels in India, Morocco and Tunisia (Chandel *et al.* 2003, Touil *et al.* 2012, Lorusso *et al.* 2016, Hassine *et al.* 2017) and seropositive dromedaries were also found in the United Arab Emirates (Wernery *et al.* 2013). Also llamas (*Lama glama*) and alpacas (*Vicugna pacos*) experimentally infected with BTV did not show clinical signs. Furthermore, viral RNA levels in the blood of the infected animals were low and disappeared soon after seroconversion (Schulz *et al.* 2011). Despite the results of the previous study, BTV was detected in the spleen of a 15-year-old female alpaca that died after weakness, recumbency and respiratory distress. The necropsy revealed hydrothorax, hydropericardium, marked pulmonary oedema, and acute superficial myocardial haemorrhage affecting the left ventricle. To the author's knowledge this was the first report of lethal BT in a camelid in the Americas (Ortega *et al.* 2010). There is no report of EHDV causing clinical signs in South American camelids.

Wild sheep are susceptible to BT. In 2007, several European mouflons (*Ovis aries musimon*) from a game reserve in Spain developed clinical signs and died after a natural infection with BTV-1. The pathological lesions were inflammation of the mucous membranes, congestion, swelling and haemorrhage, which suggests that, like domestic sheep, this species is highly susceptible to the virus (Fernández-Pacheco *et al.* 2008). The susceptibility of bighorn sheep (*Ovis canadensis*) was also suggested during an outbreak of BTV-17 in 1991, when 13 sheep showed clinical signs of BT and died (Singer *et al.* 1998).

Despite the absence of clinical signs, several African carnivores were demonstrated to have antibodies to BTV, including African wild dogs (*Lycaon pictus*), jackals (*Canis* spp.), cheetahs (*Acinonyx jubatus*), lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*) and genets (*Genetta maculata*) (Alexander *et al.* 1994). Domestic cats and dogs also demonstrated to have detectable antibodies (Alexander *et al.* 1994, Oura and Harrak 2010). Another study, conducted by Jauniaux and colleagues (Jauniaux *et al.* 2008), reported a natural infection caused by BTV-8 in lynx (*Lynx lynx*) kept in a zoo in Belgium. The outbreak led to the death of two animals. The lynx had been fed with ruminant fetuses from an area in which BT cases had been lately confirmed. Necropsy findings were anemia, subcutaneous hematomas, petechial haemorrhage, pneumonia besides lung congestion and oedema. Microscopic examination showed oedematous vascular walls, enlarged endothelial cells, and vasculitis in muscle, myocardium, peritoneum, and lung. The infection of carnivores with BTV suggests that the number of natural BTV hosts may be much larger than previously supposed. The possibility that the two lynx were infected by vectors cannot be ruled out, but this case strongly suggests that BTV may also be transmitted by oral route to carnivores, while the development of clinical signs brings concern upon the extent of the disease in wildlife populations and questions upon the role of these species in the transmission and maintenance dynamics of BTV. A study evaluated the presence of antibodies to BTV in 187 domestic dogs from Morocco finding a prevalence of 21%. As these dogs were consumed canned food only, and had no access to other meat products, it was suggested that the most likely source of infection was through infected *Culicoides* midges (Oura and Harrak 2011).

BTV and EHDV are closely related to the African horse sickness virus which is known to infect carnivores, such as domestic dogs, hyenas, lions, jackals, cheetah and genets (Alexander *et al.* 1995). Similar to what was discussed above about BTV, a case of African horse sickness was reported in a domestic dog that died after a period of illness without apparent ingestion of horse meat (Van Sittert *et al.* 2013). Another study had reported that *Culicoides impunctatus* from Scotland had a blood meal on dogs, although less frequently that on other hosts, even when present in the same site as a rumimant host (Blackwell, *et al.* 1995). However, more studies are required to elucidate this question about possible BTV transmission from carnivore species.

In South America, the most abundant vector of BTV is *Culicoides insignis* although *Culicoides pusillus* has been described as the main biological vector of orbiviruses in Central America (Mo *et al.* 1994).

Infections caused by EHDV are also reported in several wildlife species. During an EHDV-2 outbreak in Colorado-USA, which caused the death of two white-tailed deers, other species maintained in the same facility, including bisons, elks (*Cervus canadensis*), domestic cattle, and domestic goats did not show clinical signs (Nol *et al.* 2010). However, disease and death were observed in yaks (*Bos grunniens*) after natural infection by EHDV-2. Clinical signs included anorexia, nasal discharge, conjunctivae and sores on the dental pad. Necropsy revealed exudate from the nares, the conjunctivae were oedematous, were ulcerated areas on the dental pad and under the tongue. Multiple petechiae were present on the serosal surface of the rumen, epicardial surface of the heart, papillary muscles, and the pulmonary artery, and serosanguineous fluid was found in the abdomen and thoracic cavities (Campen *et al.* 2013).

Elk experimentally infected with EHDV did not manifest clinical signs of infection (Hoff and Trainer 1973). When experimentally infected with EHDV, white-tailed deer demonstrated petechial and ecchymotic haemorrhages, and the peritoneal cavity contained clear straw-coloured or slightly blood-tinged fluid. Petechial and ecchymosis were also found on the serosal surfaces of the stomachs and intestines and haemorrhage and congestion were present in several organs such as liver, lungs and lymph nodes. Histopathology demonstrated extravasated red blood cells in the parenchyma of the spleen, stomachs, heart musculature, and lymph nodes (Shope *et al.* 1960). Given variable results on the susceptibility of white-tailed deer to EHDV, Gaydos (Gaydos 2002) measured the immune response and clinical signs in two subspecies, *Odocoileus virginianus borealis* and *Odocoileus virginianus texanus* experimentally infected with EHDV-2. The virus caused severe clinical disease with high mortality in *O. virginianus borealis* fawns, whereas in *O. virginianus texanus* fawns the disease was mild or non-detectable. Despite the difference in manifestation of the disease, the viral titers and humoral immune response were similar in both subspecies. The results suggest that differences may be explained by innate disease resistance of *O. virginianus texanus*, which occurs in southern areas, such as Texas, Oklahoma, New Mexico Colorado and Kansas, that are known to be endemic for the EHDV.

Bluetongue and epizootic haemorrhagic disease in South American wildlife

Free-living wildlife animals

The studies involving free-living animals in South

America are based on serological techniques. During the flooding of the Porto Primavera hydroelectric dam in Brazil's São Paulo state, marsh-deer (*Blastocerus dichotomus*) were captured and studied. A total of 81 individuals were tested for antibodies to BTV and EHDV. A seroprevalence of 88% and 74% to BTV and EHDV, respectively, was reported (Pandolfi *et al.* 1998a). In the same area, another study was carried out on peccaries (*Pecari tajacu*) reporting a seroprevalence of 39% to BTV (Gerber *et al.* 2012). The seroprevalence of peccaries in Madre de Dios, Peru, was reported to be 7.5% for BTV and 29.2% for another orbivirus of the same serogroup, possible EHDV (Rivera *et al.* 2013).

In Buenos Aires, Argentina, seven pampas deer (*Ozotoceros bezoarticus*) tested negative to BTV and EHDV antibodies (Uhart *et al.* 2003). Forty-nine animals of this species tested negative to BTV in Pantanal, Brazil (Tomich *et al.* 2009). The Gray brocket deer (*Mazama gouazoubira*), in Gran Chaco - Bolivia showed 0% and 7% of BTV and EHDV seropositivity, respectively (Deem *et al.* 2004).

A serosurveillance study conducted on Guanacos

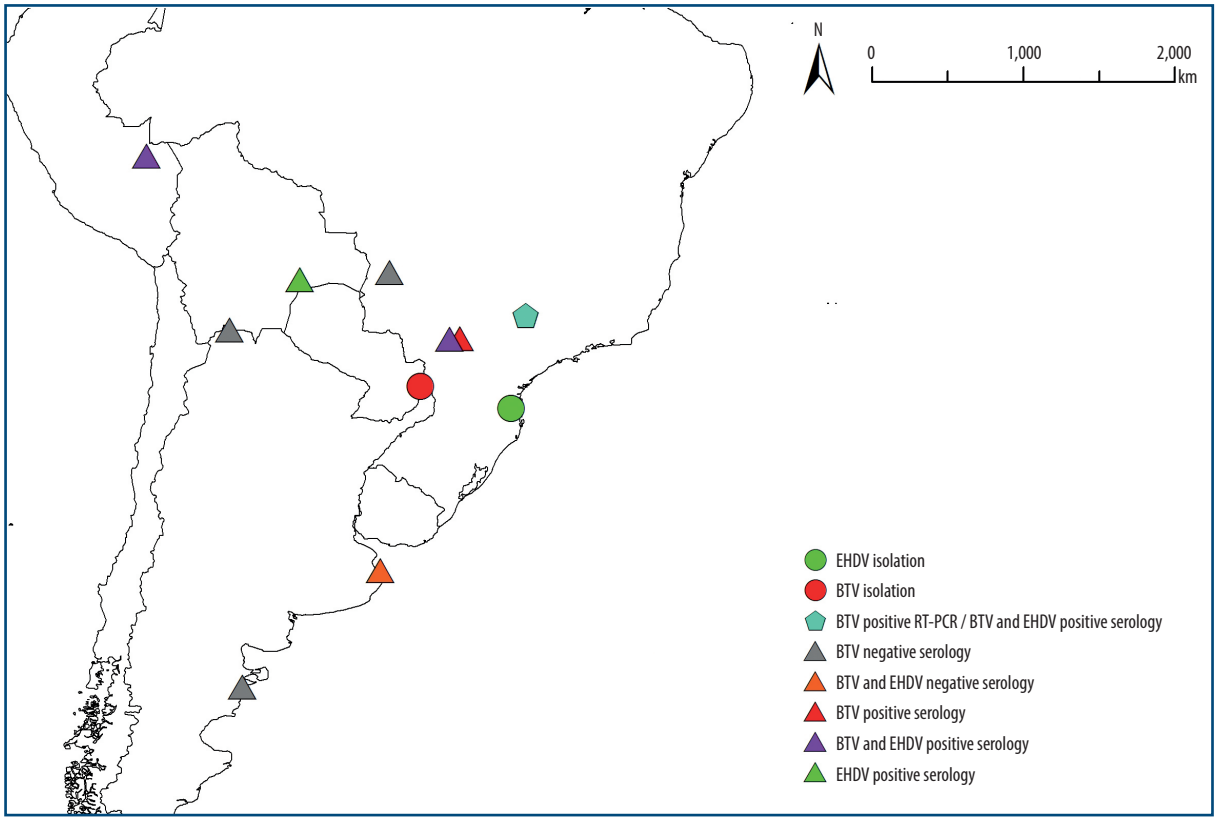

Figure 1. *Studies involving Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV) in wildlife in South America.*

Table I. *Bluetongue and epizootic haemorrhagic disease serological studies in free-living wildlife in South America.*

Country	Region	Specie	Number of animals	BTV	EHDV	Reference
Brazil	Porto Primavera - SP	*Blastocerus dichotomus*	81	88%	74%	Pandolfi *et al.* (1998b)
Brazil	Pantanal - MS	*Ozotoceros bezoarticus*	49	0%		Tomich *et al.* (2009)
Argentina	Buenos Aires	*Ozotoceros bezoarticus*	7	0%	0%	Uhart *et al.* (2003)
Bolivia	Gran Chaco	*Mazama gouazoubira*	15	0%	7%	Deem *et al.* (2004)
Argentina	Chubut	*Lama guanicoe*	20	0%		Karesh *et al.* (1998)
Argentina	Cieneguillas	*Vicugna vicugna*	128	0%		Marcoppido *et al.* (2010)
Brazil	Porto Primavera - SP	*Pecari tajacu*	49	*39		Gerber *et al.* (2012)
Peru	Madre de Dios	*Pecari tajacu*	106	7.5%	**29%	Rivera *et al.* (2013)
Brazil	Pontal do Paranapanema - SP	*Tapirus terrestris*	35	14.29%		May-Júnior (2011)

*Positive to orbivirus (BTV/ EHDV); **Orbivirus of the same serogroup, possibly EHDV

(*Lama guanicoe*) and vicugnas (*Vicugna vicugna*), from Argentina, demonstrated that these animals were negative for BTV antibodies (Karesh *et al.*1998, Marcoppido *et al.* 2010). In Pontal do Paranapanema, in São Paulo - Brazil, 35 tapires (*Tapirus terrestres*) were tested for BTV and 14.29% of the animals showed antibodies to the virus (May-Júnior 2011).

Studies involving free-living wildlife in South America are summarized in Figure 1 and in Table I.

Wildlife in captivity

The seroprevalence of EHDV and BTV in Brazilian deer belonging to a scientific breeding center in São Paulo state, Brazil, was investigated. Out of 22 animals, 23% presented antibodies to BTV and 9% to EHDV (Pandolfi 1998b).

A retrospective study performed in the same institution by Kawanami and colleagues (Kawanami *et al.* 2018) analyzed paraffin samples of organs from 42 animals that died showing clinical signs of haemorrhagic disease. These animals were marsh-deer (*Blastocerus dichotomus*) and brocket-deer (genus *Mazama*). Seven of the 42 animals tested positive to BTV RNA presented clinical signs that included loss of appetite, lethargy, lesions on the tongue or mouth, diarrhoea or soft faeces, emaciation, drooling, and oedema of the head. The most relevant macroscopic findings observed were haemorrhagic intestinal contents, petechiae in organs such as the heart, tongue and stomachs, reddish gastrointestinal mucosa and necrosis/ulceration in the mouth or tongue.

In 2008 EHDV was isolated in cell culture from a Brazilian dwarf brocket deer (*Mazama nana*) kept in a zoo in South Brazil. This animal died and necropsy revealed hemorrhages in several organs. This was the first isolation of EHDV ever recorded in Brazil (Favero *et al.* 2013).

Another study identified BTV as most likely cause of death of deer from a conservation center in South Brazil. This shelter has been suffering from outbreaks of haemorrhagic disease leading to the death of several deer for a period of 15 years (Baldini *et al.* 2018). Five BTV serotypes (BTV-3, BTV-14, BTV-18, BTV-19 and BTV-22) were isolated from samples collected from five Brazilian dwarf brocket deer that had died in recent years (OIE 2018). None of these serotypes had previously been recorded in Brazil. Interestingly, from only one out of 32 animals was possible to evidence BTV antibodies. This aspect suggests that this species is extremely susceptible to this disease (Baldini *et al.* 2018).

Gaps and considerations for the future

The role of wildlife species in the epidemiology of BT and EHD in South America appears to be variable. Some species are extremely susceptible while others are less and some others may act only as maintenance hosts. It is of great importance to understand how wildlife species respond to BTV and EHDV since this may have a considerable economic impact if wildlife species are serving as spillover source for domestic species. These viruses also impact the conservation of wildlife such as some species of deer and other ruminants like pronghorns (*Antilocapra americana*). As already written, BTV and EHDV are closely related to the African horse sickness virus, which is known to affect carnivores (Lubroth 1992). Reports of seropositive canids and felids in addition to the isolation of BTV from a lynx suggest that the range of hosts could be much wider than previously suggested.

Only few studies on EHD and BT epidemiology and clinical outcomes in free-living deer and other species are available from South America. However, evidence suggests that endangered species such as the Brazilian dwarf brocket deer may show high mortality related to the haemorrhagic manifestations of the disease. Unfortunately, the dense forest present in some South America biomes, and the shy behavior of most deer, hamper the visualization of clinical signs and the evaluation of diseases. Indeed, these animals are rarely captured to evaluate their health status.

South American camelids do not appear to play an important role in the epidemiology of BTV, possibly due to their innate resistance and the adverse climate conditions of their habitat for the survival of vector species.

South America has a colossal biodiversity, unique biomes and in most parts of its territory BTV and EHDV are endemic. More studies are necessary to identify vectors responsible for the transmission of BTV and EHDV in this continent and to better comprehend the additional potential host species and the epidemiological role they play in the maintenance and spread of these viruses.

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