Network for wildlife health surveillance in Europe

Diagnosis Card

Bluetongue and Epizootic Haemorrhagic Disease

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Etiology
Bluetongue (BT) and Epizootic Haemorrhagic Disease (EHD) are caused by Orbiviruses within the family *Sedoreoviridae*. Both viruses are multi-typic. Currently, the Bluetongue virus (BTV) consists of over 30 serotypes, with serotypes 1 to 24 being reportable to the World Organisation for Animal Health (WOAH). In comparison, the EHD virus (EHDV) has 7 officially recognized serotypes. Both viruses are also arboviruses and are usually transmitted biologically between their hosts by the bites of *Culicoides* (Diptera, Ceratopogonidae) biting midges.

Affected species (wildlife, domestic animals, humans)
BTV can infect all ruminant species but in domestic ruminants, severe disease is usually restricted to improved breeds of sheep. Although not as commonly as sheep, cattle can also experience severe disease. Due to their prolonged viraemia, they can act as covert virus reservoirs. Goats and camelids rarely exhibit obvious clinical signs.
In African wild ruminants the infection is inapparent. In North American wild ruminants, acute disease and death occurs in white-tailed deer (*Odocoileus virginianus*), bighorn sheep (*Ovis canadensis*) and pronghorn antelope (*Antilocapra americanus*). Disease has also been reported in various wild deer species and captive wild ruminants such as European bison (*B. bonasus*) and musk ox (*Ovibos moschatus*), although disease is not always symptomatic. The occurrence of disease in North American and Asiatic wild ruminants suggests the historical link between them and BTV is more recent than with native African ruminants.
Among carnivores, domestic dogs are susceptible when inoculated with BTV and may die or abort. BTV antibodies have also been detected in the larger African carnivores and captive Eurasian lynx (*Lynx lynx*) have died from BT. However, carnivore species are not considered epidemiologically important.
EHDV is probably able to infect all ruminant species but infection is usually subclinical or mild. However, a variant of EHDV-2 (Ibaraki) has caused morbidity and mortality in Japanese cattle and EHDV-6 and EHDV-7 have also been reported in cattle in North America, northern Africa, Israel and Turkey between 2006 and 2007. EHDV-8 was first reported in Tunisia in 2021 and subsequently made its debut in Europe in 2022 causing clinical disease in cattle and red deer (*Cervus elaphus*). In other wildlife, EHDV has not been reported as causing disease in most species though it may occasionally do so in elk (*C. canadensis*), bighorn sheep, mule deer (*O. hemionus*) and pronghorn antelope. An exception to this rule is a severe and fulminating haemorrhagic disease that often occurs in white-tailed deer in North America.

Epidemiological characteristics and disease course
BTV and EHDV infections usually occur via the bites of vector insects - *Culicoides* biting midges. Consequently, the distribution of these viruses is limited to areas where vectors occur, to seasons when they are active and to regions where ambient temperature is sufficient to allow virus replication in them. In tropical areas transmission occurs year-round. In temperate areas peak transmission is usually limited to summer and autumn. In such areas BTV and EHDV are usually unable to overwinter and annual re-introduction is necessary. However, oral and transplacental transmission of some BTV-8 and BTV-3 strains has been demonstrated which potentially facilitates virus persistence between years in regions as far north as North Europe. After inoculation by vector bite both viruses are disseminated throughout the host body. Replication occurs in monocytes, macrophages, dendritic cells and vascular endothelial cells. Virus may be isolated from the circulation from 3-6 days p.i. and viraemia peaks at 7-8 days p.i. However, as most circulating virus is cell-associated and protected from antibody there may be an extended viraemia (up to 60 days in cattle).

Clinical signs
Animals with acute BT may have combinations of fever, anorexia, dyspnoea, excessive salivation, nasal

and ocular discharges, petechial and ecchymotic haemorrhages in oro-nasal mucosa, oral erosions, lameness, coronitis, oedema of head and neck, and cyanosis of the tongue. Pregnant animals may abort. Most animals succumbing to acute BT die within 14 days. Wool of sheep and hooves may be shed, and chronically affected animals may die from secondary infections. EHD is clinically indistinguishable from BT.

Gross lesions

Characteristic lesions of BT and EHD are congestion, haemorrhage and oedema. The mucosa of the digestive tract may be oedematous with petechial haemorrhages and bloody diarrhoea. There may also be subcutaneous haemorrhages. Haemorrhage at the base of the pulmonary artery has been considered pathognomonic but is not always seen. Interlobular and interstitial oedema in the lungs leads to froth in the bronchial tract and dyspnoea. A gelatinous, reddish fluid may infiltrate subcutaneous and inter-muscular connective tissue. The skeletal musculature may be greyish and marbled due to degeneration.

Histological lesions

Depending on the stage of infection there can be widespread damage to the endothelium of small blood vessels resulting in vascular permeability, thrombosis and tissue infarction. In epithelial tissues this can lead to sloughing. Haemorrhages, mononuclear cell infiltration and necrosis of the myocardium may occur.

Differential diagnosis

Pox, foot and mouth disease, Akabane disease, pest des petits ruminants, contagious ecthyma, vesicular stomatitis, Rift Valley fever, pneumonia, photosensitization, and copper deficiency.

Criteria for diagnosis

Because clinical disease in many animal species is rare and the clinical signs are largely unspecific, diagnosis usually requires isolation and/or identification of BTV or EHDV, or their nucleic acids.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Virus and/or nucleic acid detection: using a group-specific BTV real-time RT-PCR assay (WOAH recommended assay targets genome segment 10) and then type-specific real-time RT-PCRs (targeting genome segment 2) to confirm serotype. Real-time assays are preferred due to increased sensitivity and specificity compared to conventional PCR assays.

Isolation of BTV and EHDV via cell culture (KC cells derived from C. sonorensis have been shown to be highly sensitive for orbivirus isolation): followed by viral identification, first using group-specific real-time RT-PCRs, then type-specific real-time RT-PCRs. Further characterization can be performed through Sanger sequencing of segment 2 or full genome sequencing. Despite high sensitivity, specificity and speed, a major disadvantage of PCRs is that they detect nucleic acid and cannot distinguish live virus. Isolation must be used in addition to PCRs to confirm the presence of live/infectious virus.

Samples: Blood (10 ml) collected into EDTA tubes during the febrile phase or any solid tissue from the haematopoietic system (10 g). Spleen and whole blood are preferred. Tissue samples should be stored at +4˚C. Erythrocytes should be washed to remove any antibody before virus isolation procedures commence. Long term storage of virus samples should be at -80˚C.

Serology: Identification of BTV or EHDV VP7 antibodies using a group-specific ELISA. Serotyping can be performed by type-specific SNTs, however this requires access to reference viruses and cell culture facilities. Serum samples may be stored at -20˚C.

EWDA proposed harmonized protocol (for harmonization at large scale)

Refer to WOAH Terrestrial Manual chapter 3.13.

Laboratories that can be contacted for diagnostic support

BT and EHD are notifiable. The WOAH reference laboratories for BTV are: The Pirbright Institute UK, Onderstepoort Veterinary Institute RSA, IZS dell 'Abruzzo e del Molise Italy and The Commonwealth Scientific and Industrial Research Organization Australia (see: woah.org). The WOAH reference for EHD is ANSES (French Agency for Food, Environmental and Occupational Health & Safety) Animal Health Laboratory (see: woah.org).

Recommended literature

1. MacLachlan, N.J., C. P. Drew, K.R. Darpel, and G. Worwa. 2009. The pathology and pathogenesis of bluetongue. Journal of Comparative Pathology 141: 1 – 16.
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4. Ruiz-Fons, F. *et al*. (2024) Emergence of epizootic haemorrhagic disease in red deer (*Cervus elaphus*), Spain 2024 Veterinary Microbiology 292, 110069 https://doi.org/10.1016/j.vetmic.2024.110069.
5. WOAH (2021). WOAH Terrestrial Manual, Chapter 3.1.3 Infection with bluetongue, 20pp.