The authors are responsible for the final contents of the card. Please refer to this card when you publish a study for which the APHAEA protocol has been applied. Reference suggestion: «This method is recommended by the EWDA Wildlife Disease Network (www.ewda.org)»; citation: Authors, Year, APHAEA/EWDA Diagnosis card: [name of disease], www.ewda.org
disseminated throughout the 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).

### Clinical signs

Animals with acute BT may have any combination of: fever, anorexia, dyspnoea, excessive salivation, nasal and ocular discharges, petechial and ecchymotic haemorrhages in oro-nasal mucosa, oral erosions, lameness, coronitis, oedema of the head and neck, and cyanosis of the tongue. Pregnant animals may abort. White-tailed deer and improved sheep breeds are very susceptible and may develop a bleeding tendency which is usually associated with consumptive coagulopathy in white-tailed deer. Most animals succumbing to acute BT die within 14 days. Survivors may have a long convalescence. In them muscle injury and necrosis may prevent normal gait and can lead to torticollis. Wool of sheep and, hooves of sheep and white-tailed deer may be shed and many chronically affected animals may succumb to secondary infections. EHD is clinically indistinguishable from BT.

### Gross lesions

Characteristic lesions of BT and EHD: congestion, haemorrhage and oedema. The mucosa of the digestive tract may be oedematous with petechial haemorrhages and sometimes 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 infiltrates subcutaneous and inter-muscular connective tissues. The skeletal musculature may be grayish 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. Haemorrhage, mononuclear cell infiltration and necrosis of the myocardium may occur.

### Differential diagnosis

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

### Criteria for diagnosis

Because clinical disease 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: first using group-specific then type-specific RT-PCRs. Real time assays are preferred (increased sensitivity and specificity) but conventional PCRs based upon seg. 7 may be used.
  - Isolation of BTV via embryonating hens eggs (preferred) or cell culture, and of EHDV via cell culture: followed by virus identification, first using group-specific conventional or real time RT-PCRs, then type-specific RT-PCRs or VNTs. PCRs are preferred as they do not require virus adaptation to cell culture.
  - Despite high sensitivity, specificity and speed, a major disadvantage of PCRs is that they detect RNA, not infective virus. Isolation must be used in addition to PCRs to confirm infective virus.
  - Samples: Blood (10 ml) collected into EDTA tubes during the febrile phase or any solid tissue from the haematopoietic system (10 g), e.g. spleen, lung, lymph nodes. Spleen and whole blood are preferred. Tissue samples should be stored at +4OC, as freeze-thawing will lyse the erythrocytes and release virus which may then be inactivated by antibody. The erythrocytes should be washed to remove any antibody before isolation procedures commence. Long term storage of virus samples should be at -80OC
  - Serology: Identification of BTV or EHDV antibodies using a group-specific antibody detection VP7 competition ELISA followed by the type-specific SNT. Serum samples may be stored at -20OC.

### APHAEA protocol (for harmonization at large scale)

A monoclonal antibody-based competitive ELISA that detects serogroup specific BTV antibodies is recommended and is an OIE prescribed test (OIE 2014). Should BTV nucleic acid detection/identification also be required, prescribed methods are similarly described in OIE (2014).
Laboratories that can be contacted for diagnostic support

BT and EHD are notifiable. The EU reference laboratory is the Pirbright Institute UK and OIE reference laboratories are: Onderstepoort Veterinary Institute RSA, National Veterinary Services Laboratories USA, Pirbright Institute UK, IZS dell'Abruzzo e del Molise, Italy and Australian Animal Health Laboratory Australia (see: www.oie.int).

Recommended literature

OIE (2014). OIE Terrestrial Manual, Chapter 2.1.3 Bluetongue, 18pp