Network for wildlife health surveillance in Europe

Diagnosis Card

COVID-19 (Infection with SARS-CoV-2)

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Etiology

Severe acute respiratory syndrome virus coronavirus type 2 (SARS-CoV-2), belongs to genus *Betacoronavirus*, family *Coronaviridae*. The virus is enveloped positive-sense single-stranded RNA viruses. The virion consists of structural proteins, including spike (S), envelope (E), membrane (M) and nucleocapsid (N).

Affected species (wildlife, domestic animals, humans)

SARS-CoV-2 likely originates from horseshoe bats (Rhinolophus spp.). SARS-CoV-2 currently circulates, mainly in humans from which virus can spill over to both wildlife and domestic animals. In field, SARS-CoV-2 can infect a broad range of mammals including tigers (Panthera tigris), lions (Panthera leo), snow leopards (Panthera uncia), gorillas (Gorilla gorilla), American mink (Neovison vison), domestic cats and domestic dogs.

Epidemiological characteristics and disease course

Most of SARS-CoV-2-infected animals were in close contact with SARS-CoV-2 confirmed or asymptomatic humans (e.g. owners, keepers and visitors). Group housing of large numbers of animals increases risk of animal-to-animal transmission as shown in outbreaks in mink farms.

Exact routes of human-to-animal and animal-to-animal transmission are still unclear, but it could occur directly through droplets or indirectly through inhalation of virus-contaminated aerosols. It cannot be excluded that the virus possibly can be shed by feces of some infected animal species.

SARS-CoV-2 causes acute infection which usually lasts from 2-3 days up to 4 weeks. The incubation period can take between 2 and 14 days. Outcome of disease is varied, from full recovery (after producing a specific immune response) to death.

Clinical signs

Captive wild animals: Mild respiratory signs including coughing, congestion, wheezing, runny nose and lethargy.

American mink: Respiratory disease, including labored breathing and watery to mucoid nasal exudates and inappetence, with variable severity ranging from mild to very severe which can lead to death. Asymptomatic infections can also occur in animals.

Gross lesions

Captive wild animals: Due to full recovery from infection, gross lesions have not been reported.

American mink: Adults: diffusely consolidated and dark red lungs. Juveniles: More subtle lesions than in adults, including diffuse red-brown discoloration and wet appearance of the lungs.

Histo-pathological features

Captive wild animals: Due to full recovery from infection, histological lesions have not been reported.

American mink: Multifocal to coalescing areas with thickening and degeneration of alveolar septa indicating diffuse alveolar damage. Alveolar lumina are infused with mononuclear inflammatory cells, desquamated cells and few neutrophils. Lesions in trachea include diffuse loss of cilia and swelling and flattening of epithelial cells. Lesions in nasal conchae include multifocal swelling and degeneration of epithelial cells with a mild infiltration of inflammatory cells.

Differential diagnosis

Other diseases with respiratory or gastrointestinal signs.

Criteria for diagnosis

Epidemiological factors and clinical signs related to SARS-CoV-2 infections should be evaluated together with laboratory testing. Laboratory diagnosis is necessary for a final diagnosis. Viral RNA detection by PCR-based methods can provide the information whether the virus is present in an individual at the time of sampling. Detection of specific anti-SARS-CoV-2 antibodies can provide the information about past infection.

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

(a) Viral RNA detection/direct isolation:

In live animals, samples of choice for viral RNA detection/direct isolation of SARS-CoV-2 are nasal swabs, oropharyngeal swabs, nasal washes, tracheal swabs and/or rectal swabs. Fecal samples are recommended in cases where direct sampling is not possible due to risk to the animal or staff. The samples should be kept at 4°C for up to 48 hours storage or -70°C for longer storage. In dead animals, fresh tissue samples of choice include lung, trachea, liver, spleen, kidney, intestinal tract, pancreas and brain. For immunohistochemistry, samples of the same tissues are fixed in 10% neutral-buffered formalin and embedded in paraffin.

- *Real-time reverse-transcription polymerase chain reaction (RT-PCR)*: The gold standard with high sensitivity and specificity. Various RT-PCR tests developed to detect unique viral RNA sequences in N, E, S, or RNA-dependent RNA polymerase (RdRp) genes.
- *Virus isolation*: Inoculation of clinical specimen or postmortem tissue sample in a susceptible cell line, Vero E6 or Vero CCL-81 cell line. The test needs to be performed in a BSL-3 laboratory.
- *Immunohistochemistry*: Detection assay of virus antigen in postmortem tissue samples.

(b) Detection of specific anti-SARS-CoV-2 antibodies:

Samples for detection of specific anti-SARS-CoV-2 antibodies are serum or plasma stored at -20°C and heat-inactivated at 56°C for 30 minutes prior to analysis.

- *Virus neutralization test (VNT)/ Plaque reduction neutralization test (PRNT)*: The gold standard to assess whether an individual has antibodies against SARS-CoV-2. The test needs to be performed in a BSL-3 laboratory.
- *Surrogate virus neutralization test (sVNT)*: Measures receptor binding domain-binding antibodies in various animal species. The test can be performed in a BSL-2 laboratory. Commercially available.
- *ELISA*: In-house assays (multi-species ELISAs and species-specific competition ELISAs) and commercially available (multi-species ELISAs). The test can be performed in a BSL-2 laboratory.

EWDA proposed harmonized protocol (for harmonization at large scale)

RT-PCR is recommended to test for viral nucleic acid in a sample taken directly from an animal by targeting at least two specific genome regions or a single genomic region followed by sequencing of a secondary target. Whole genome sequencing is recommended for virus typing and epidemiological analysis.

Laboratories that can be contacted for diagnostic support

Wageningen Bioveterinary Research (WBVR), the Netherlands (see: https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Diagnostiek/Diagnostiek-corona.htm)
OIE Wildlife Working Group (see: www.oie.int)
Department of Viroscience, Erasmus Medical Centre, Rotterdam, the Netherlands (see: https://www.erasmusmc.nl/nl-nl/patientenzorg/laboratoriumspecialismen/klinische-virologie)

Recommended literature

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