Network for wildlife health surveillance in Europe

Diagnosis Card

Rabies

Author(s) (*corresponding author)

Thomas Mueller*, Friedrich-Loeffler-Institute - Riems , Germany, Thomas.Mueller@fli.bund.de

Conrad Freuling, Friedrich-Loeffler-Institute - Riems, Germany, Conrad.Freuling@fli.bund.de

Ad Vos, IDT Biologika GmbH, Germany, Ad.Vos@idt-biologika.de

Reviewers

Charles E. Rupprecht, Centers for Disease Control and Prevention, Georgia, USA

Reto Zanoni, Swiss Rabies Centre, Institute of Veterinary Virology, University of Bern, Switzerland, reto.zanoni@vetsuisse.unibe.ch

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Etiology

Negative-strand RNA viruses of the genus *Lyssavirus* (Family: *Rhabdoviridae*).

Affected species (wildlife, domestic animals, humans)

Rabies virus: All mammals, but only a few species are important as reservoirs for the disease, e.g. in Europe the red fox (*Vulpes vulpes*) and the raccoon dog (*Nyctereutes procyonoides*).

Bat lyssaviruses: Presently, four different lyssaviruses have been isolated from bats in Europe: European Bat Lyssavirus Type 1 and Type 2 (EBLV-1 and EBLV-2); West Caucasian bat virus; and Bokeloh bat lyssavirus. Most commonly infected bat species are: *Eptesicus serotinus* (EBLV-1) and *Myotis daubentonii/dasycneme* (EBLV-2). Worldwide, bats are reservoirs for the majority of lyssavirus species.

Epidemiological characteristics and disease course

Viral transmission is most commonly through a bite from an infected animal. Incubation period varies enormously from two weeks to several months but even shorter and longer periods have been reported. Lyssaviruses are highly neurotropic and propagate through the neuronal network from the entry site to the central nervous system. In late stages, non-neuronal tissues such as the salivary glands are infected by centrifugal dissemination, allowing virus transmission through biting.

Rabies virus: A peak in the number of fox rabies cases occurs during late winter and early spring as a result of the mating season. During summer months case numbers are fewest. In autumn, there is an increase that has been linked to juvenile dispersal. The temporal epidemiology of raccoon dog rabies is similar although at higher latitudes it can be influenced by reduced activity of this reservoir species during winter months.

Bat lyssaviruses: Knowledge is still too limited for specific epidemiological characteristics beyond designation as an acute, progressive encephalitis with viral excretion in the saliva.

Clinical signs

Rabies is a fatal viral encephalitis where clinical observations can only be used for identifying suspicious rabid animals.

Terrestrial animals: In the prodromal phase first signs of rabies may include lethargy, fever, vomiting, and anorexia followed within days by a progression of signs to cerebral dysfunction and cranial nerve dysfunction. The clinical signs are often divided in two groups: dumb and furious rabies. Dumb rabies is characterized by ascending paralysis causing abnormal facial expressions, drooping heads, sagging jaws and paralysed hind limbs. Other signs are ataxia, weakness, seizures, laboured breathing, difficulty swallowing, and excessive salivation. Animals with furious rabies show extreme excitement and aggression towards any object. However, this distinction between dumb and furious may cause confusion because bouts of furious rabies alternate with periods of depression resembling dumb rabies. Bitonal barking in infected dogs is also often observed. For ruminants, continuous bellowing has been described.

Bats: Some clinical signs observed in rabid bats are disorientation, muscular spasms, paralysis and lethargy. These signs often result in losing their ability to fly, or flying in an uncoordinated fashion. Animals also show agitation and sometimes overt aggression. However, bats do not necessarily show any of these clinical signs.

Gross lesions

No typical gross lesions, at least not in terms of being characteristic for the disease. Meningeal vessel congestion, mild cerebral oedema and focal congestion of the white matter can be present. Self-inflicted trauma may be evident. Evidence of pica may be obtained from an examination of gastro-intestinal contents at necropsy.

Histological lesions

Inflammatory lesions appear to be restricted primarily to the brain stem and spinal cord. Evidence of non-specific encephalomyelitis can be observed when brain tissues from rabid animals are examined microscopically: mononuclear cell infiltration; perivascular cuffing with lymphocytes or polymorphonuclear cells; Babès' nodules (aggregations of glial cells). Additionally, presence of Negri bodies (intracytoplasmic eosinophilic inclusions), considered specific for rabies.

Differential diagnosis

Carnivores: Canine distemper (morbillivirus), Aujeszky's disease (pseudorabies, suid herpesvirus 1 infection) and other central nervous system disorders; intoxication.

Bats: Trauma; physiological lethargy from torpor or hibernation; intoxication; other infectious diseases.

Criteria for diagnosis

Detection of viral antigens directly in the infected brain, or isolation of virus, preferably using tissue culture techniques. Detection of viral RNA (RT-PCR, etc.) is considered for confirmation rather than for primary diagnosis, and allows further virus characterization (sequencing).

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

Care must be taken not to damage the head during euthanasia, decapitation, and brain removal. Post mortem diagnosis should be performed on fresh brain tissues, preferably from multiple locations, e.g. brainstem (medulla oblongata), cerebellum, and hippocampus (Ammon's horn). Gold standard for rabies diagnosis is the direct fluorescent antibody test (FAT). In case of inconclusive FAT results, or in negative FAT cases with known human exposures, additional confirmatory tests may be recommended. For virus isolation, two different assays are available; the mouse inoculation test and the rabies tissue culture infection test. Wherever possible, the latter should be chosen.

Haematoxylin-and-eosin-staining of tissue sections is no longer recommended for primary diagnosis. Immunohistochemical techniques for specific detection of viral antigen in tissue sections are available and are much more sensitive.

Shipment and sample storage: Specimens for rabies diagnosis should be shipped refrigerated, frozen, or in 50% glycerine-saline solution (temperature: +4 °C or –20 °C) according to the national and international regulations for shipment of infectious substances to avoid exposure. For long-distance shipment of isolates or tissues, proper packing and freezing on dry ice or in liquid nitrogen is recommended. Upon arrival in the laboratory, specimens preferably should be stored refrigerated or frozen (-20°C) for a short period before testing. Infected brain samples also may be transported at ambient temperature as dried smears on filter paper.

APHAEA protocol (for harmonization at large scale)

Direct fluorescent antibody test (FAT).

Laboratories that can be contacted for diagnostic support

Wildlife Zoonoses and Vector-Borne Diseases Research Group, Animal Health and Veterinary Laboratories Agency – Weybridge, Woodham Lane, Surrey KT15 3NB, United Kingdom (http://vla.defra.gov.uk/science/sci_rabies.htm)

Institute of Molecular Biology, Friedrich-Loeffler Institute, Südufer 10, 17493 Greifswald - Insel Riems, Germany (http://www.fli.bund.de/en/startseite/institutes/institute-of-epidemiology/reference-laboratories/oie-and-nrl-for-rabies.html)

ANSES – Laboratoire de la rage et de la faune sauvage de Nancy, Technopole agricole et vétérinaire, BP40009, 54220 Malzeville, France (www.afssa.fr/PNQ4I0.htm)

Recommended literature

CDC Rabies diagnostic page, http://www.cdc.gov/rabies/diagnosis/index.html

Meslin FX, Kaplan MM, Koprowski H. 1996. *Laboratory techniques in rabies*. World Health Organization, Geneva, Switzerland, 493 pp.

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