Network for wildlife health surveillance in Europe

Diagnosis Card

Tuberculosis in European Wild Mammals

Author(s) (*corresponding author)

Mariana Boadella*, IREC (CSIC-UCLM-JCCM), Spain, mariana.boadella@gmail.com
Christian Gortázar, IREC (CSIC-UCLM-JCCM), Spain, Christian.Gortazar@uclm.es

Reviewers

Dolores Gavier-Widén, National Veterinary Institute, Sweden, dolores.gavier-widen@sva.se

Mark Chambers, Animal Health and Veterinary Laboratories Agency, UK, mark.chambers@ahvla.gsi.gov.uk

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Etiology

Mycobacterium bovis and closely related members of the *Mycobacterium tuberculosis* complex.

Affected species (wildlife, domestic animals, humans)

Most mammals. Eurasian badgers (*Meles meles*), Eurasian wild boar (*Sus scrofa*) and deer of the subfamily Cervinae are most susceptible. Cases recorded in endangered Iberian lynx (*Lynx pardinus*) and European bison (*Bison bonasus*). Regarding domestic animals, mainly cattle, goats, sheep, domestic pigs and cats. Zoo animals. Humans (rarely).

Epidemiological characteristics and disease course

In most hosts, tuberculosis (TB) is a chronic infection. The clinical outcome depends on host species, age and sex, among other factors. Individuals of many species develop limited or even no visible lesions, while a few develop generalized TB, with the potential for extensive excretion of mycobacteria (e.g. red deer, *Cervus elaphus*).

Clinical signs

TB is a debilitating disease and the only sign may be weight loss in some of the infected animals with advanced disease.

Gross lesions

The presentation of TB can vary dramatically between species and depends on the stage of disease. The classic presentation is not always apparent; that is formation of yellowish, purulent, caseous, caseo-calcareous or calcified encapsulated granulomas. Tubercular lesions in some species (e.g. cats) may be atypical and Ziehl-Neelsen staining of histological specimens is always advisable. Lesions are often located in the lymph nodes (LN) of the head and thorax, and in the lung. Neutrophilic abscess-like lesions may also be found in the mesenteric LNs, particularly in deer. Mandibular LN lesions are characteristic of wild boar TB. Kidney lesions may be observed in badgers.

Histological lesions

The classical tuberculous granuloma is typically formed by a necrotic core, surrounded by a mixed population of inflammatory cells, giant cells and separated from the normal parenchyma by a peripheral fibrous capsule. The histological morphology of TB lesions varies according to the species of the host.

Differential diagnosis

Avian TB caused by Mycobacterium avium avium; Paratuberculosis (Johne's disease) caused by M. avium paratuberculosis. Actinomycosis, pseudotuberculosis and other infections causing granulomatous lesions, in particular parasitic granulomas.

Criteria for diagnosis

Clinical signs are not diagnostic. TB should be considered as possible diagnosis if TB-compatible lesions are found in host species and regions where TB is endemic.

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

Pros and cons of each technique are indicated after (P) and (C), respectively. The ideal post-mortem diagnosis combines detailed examination and culture of tissues individually.
TB diagnosis may be based on the detection of:

(a) *M. bovis* organisms (culture, DNA detection) or the host's pathological response to *M. bovis*:

- **Culture** of *M. bovis* is the definitive diagnosis of TB. It can be performed on fresh/-20ºC frozen tissues. In live animals, bronchial aspirate, urine or swabs (e.g. fecal, abscess, wounds) can be taken and cultured. At necropsy, 2 grams of sample should be collected from lesions, affected organs and from lymphoid tissues (including the tonsils and the main LN). (P) Culture is the only technique that allows further molecular typing (spoligotyping and VNTR) and has high specificity. (C) Low sensitivity (especially for samples from live animals), expensive and time-consuming.
- **PCR** is an obligatory final step for organism identification after culture, but can also detect *M. bovis* directly in clinical samples. (P) Can be performed on fresh/frozen tissues or paraffin-embedded tissue sections, but (C) with lower sensitivity than culture.
- **Pathology**. Can be highly sensitive. (P) It is cheap and can be applied in large-scale surveys. The combination of different pathology techniques increases the sensitivity. (C) However, it is only applicable to dead animals and needs culture or PCR confirmation.

  Gross pathology. Detection of granuloma-like lesions by systematic examination of head and thoracic lymph nodes, lungs and mesenteric lymph nodes (+ hepatic lymph node for badgers).

  Histopathology. Detection of microscopic granulomas. Detection of acid-fast bacilli (AFB) in contact smears from fresh tissues and in histopathology slides from formalin-fixed tissues, stained with Ziehl-Neelsen (ZN). (C) The presence of AFB is not specific of *M. bovis*.

(b) Diagnosis using cellular immune response to infection:

- **Tuberculin skin test**. Can be performed in many mammals (mostly ungulates) using bovine tuberculin alone, or as a comparative test that distinguishes reactions to other mycobacteria. (P) It can be performed in the field. (C) But it requires handling the animals twice, special facilities and individual identification. Sensitivity and specificity vary depending on different factors. It is unreliable in Eurasian badger. The TST is not evaluated or validated for most wildlife species and impractical in most cases.
- **Interferon assay** (IFN-γ). Performed on fresh blood. (P) It is useful in animals difficult to capture or handle, since only one capture is needed. It is also more sensitive than most serological methods. (C) However, it requires the test to be developed and validated for each target species as reagents rarely cross-react. The cattle commercial test has insufficient or unknown performance in some cervids since the monoclonal antibody is specific to bovine IFN-γ, and there is no commercial test for deer. The test has been developed for Eurasian badgers but is not available commercially. It requires a specialized laboratory and quick delivery of sample of fresh blood to the lab. It is relatively expensive.

(c) Antibody response to infection. Performance varies with target species and may be less sensitive than combining culture and pathology. Sensitivity tends to increase with increasing disease severity and bacterial load. Serological tests are quick and relatively easy to perform, and only small volumes of serum are needed.

- Plate **ELISAs**. (P) Inexpensive technique that gives high specificity and sensitivity in the wild boar. (C) Limited value in deer and badger due to lower sensitivity and cross-reactions with other mycobacteria.
- **Lateral-flow rapid tests**. Good performance in wild boar and fair in deer. Often work across species. (P) Rapid animal side testing. Can be performed on whole blood. (C) Low sensitivity in some species, such as Brushtail Possum (Trichosurus vulpecula), cervids or badger.

APHAEA protocol (for harmonization at large scale)

Badger and wild ruminants: Microbiological culture of pooled lymph nodes. Wild boar: ELISA.
In known-endemic sites, TB-compatible lesions are a good indicator in wild ungulates.

Laboratories that can be contacted for diagnostic support

VISAVET (EU reference laboratory for bovine TB), Spain (www.vigilanciasanitaria.es)
Animal Health and Veterinary Laboratories Agency (AHVLA), U.K. (www.defra.gov.uk/ahvla)
Anses Maisons-Alfort, France (https://www.anses.fr/en/content/maisons-alfort-laboratory-animal-health)

Recommended literature

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