Network for wildlife health surveillance in Europe

Diagnosis Card

Tularaemia in European wild mammals

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Etiology

Tularaemia is a disease caused by *Francisella tularensis*, a bacterium that is considered one of the most infectious known pathogens. It is an obligate aerobe, small (<1 µm), gram-negative, non-motile, pleomorphic coccobacillus with fastidious growth requirements, which include the need of cysteine-enriched media. Four subspecies (ssp.) of *F. tularensis* are described, the most important ones being the highly virulent *F. tularensis* ssp. *tularensis* (Type A) and the moderately virulent *F. tularensis* ssp. *holarctica* (Type B), which is the causative agent of tularaemia in Europe. *F. tularensis* ssp. *holarctica* is found throughout the Northern Hemisphere and occurs in many countries across Europe except the United Kingdom, Ireland and Iceland.

Affected species (wildlife, domestic animals, humans)

The number of animal species that have been found infected with *F. tularensis* is exceptionally high. 145 vertebrate and 111 invertebrate species are currently known to be susceptible to the infection. The principal vertebrate hosts of *F. tularensis* are wild mammals, in particular lagomorphs and rodents. The European brown hare (*Lepus europaeus*) and the mountain hare (*Lepus timidus*) are primarily associated with tularaemia in Europe, while the European rabbit (*Oryctolagus cuniculus*) can be infected but is relatively resistant to clinical disease. Voles (*Microtus* spp., *Arvicola amphibius*) and mice (*Mus musculus*, *Apodemus* spp.) are most frequently involved in tularaemia epizootics, and other rodent species are also found infected. Wild carnivores and scavenger species can get infected from eating moribund or dead animals. They do not develop clinical signs but a detectable antibody response. In addition, *F. tularensis* is transmitted by a wide spectrum of arthropod vectors, particularly ticks and mosquitoes. Domestic animals, in particular sheep, cats, dogs, pigs and horses can be affected as well. However, most cases in domestic animals have been reported in America after infection with *F. tularensis* ssp. *tularensis*, while limited information is available in Europe about the susceptibility of domestic animals to *F. tularensis* ssp. *holarctica*. Humans are susceptible to both subspecies of *F. tularensis* and show different clinical presentations and severity depending on the route of entry of the bacterium.

Epidemiological characteristics and disease course

The diversity of the ecosystems involved in the ecological cycle of *F. tularensis*, the high number of susceptible species, the different routes of infection and the presence of bacterial subspecies with different pathogenicity make the epidemiology of tularaemia very complex and not completely understood yet. There are two main ecological cycles of tularaemia: a terrestrial life cycle and a water-associated life cycle. In the former, hares and rodents are the most important mammalian hosts, while haematophagous arthropods play a role as vectors. Hares and rodents can contaminate the

environment through their body discharges, and stress-related aggression and cannibalism contribute to disease transmission among rodents. In the aquatic cycle, beavers, muskrats, and voles serve as main hosts, shed live bacteria into the environment, and can further contaminate the waters with their carcasses. That might be relevant e.g. in Scandinavia where mosquitoes the infection from contaminated waters and transmit the disease to humans. Exceptions to these general epidemiological patterns may occur.

Humans can easily become infected through different routes of entry, in particular through direct and even intact skin contact with infected animals or bites of arthropod vectors, ingestion of water or contaminated food, and inhalation of contaminated dusts or aerosols.

After entering the body, the bacteria multiply locally causing ulceration and necrosis, then spread to regional lymph nodes, where they cause formation of granulomas and necrosis. Further haematogenous and lymphatic spread may result in septicaemia and dissemination of the organisms to several organs (lung, kidney, pericardium, spleen, liver, bone marrow, etc.).

Clinical signs

Clinical manifestations of tularaemia in wildlife are not always evident or even recognized. Infected animals are usually found moribund or dead. Rarely, clinical signs in hares can range from exhaustion, tameness, stupor, depression in behaviour, easy capture, recurrent spasms and stagger. No clinical sign is usually reported in other animal species, such as ruminants, pigs, and dogs. In cases observed in primates in zoological parks and also after experimental infection, clinical signs included increased body temperature, peak cardiac and mean blood pressure following airborne infection, or stomatitis and an ulceroglandular syndrome after alimentary infection. Tularaemia in humans may occur in ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic forms.

Gross lesions

The pathology of tularaemia differs among different animal species. Gross lesions are not always observed. In highly susceptible species with acute disease (e.g. mice, mountain hares), an enlarged spleen and, less frequently, liver is the usual macroscopic finding. Pinpoint white foci can be seen in these organs. Subcutaneous petechiae can be seen in mountain hares. In moderately susceptible species (e.g. European brown hare), a more chronic form of disease can be observed, characterized by granulomatous lesions particularly in the lungs, pericardium, kidneys and occasionally liver, spleen, bone marrow, and lymph nodes. Nonetheless, splenomegaly without granulomatous lesions has often been the only lesion observed in native hares in Italy (unpublished authors' data).

Histological lesions

In highly susceptible species that develop acute disease (e.g. mice), multifocal coagulation necrosis is characteristic in the spleen, liver, lymph nodes, bone marrow and lungs. Karyolysis, pyknosis and infiltration with inflammatory cells such as macrophages andgranulocytes are observed in less acute cases.

In moderately susceptible species (e.g. European brown hare) where a more chronic form of disease is observed, the foci identified by gross pathological examination correspond to focal or coalescing granulomatous inflammation, which completely replace the normal tissue structure of the affected organs. Macrophages are the dominant constituent cell type, but other cells including lymphocytes, neutrophil/heterophil granulocytes, multinucleated giant cells and fibrocytes are also found occasionally. Focal or multifocal necrosis is often observed in the centre of these lesions.

Differential diagnosis

The differential diagnosis of tularaemia includes brucellosis (especially B. suis biovar 2 infection of hares), pseudotuberculosis, pasteurellosis, staphylococcosis, tuberculosis, European Brown Hare Syndrome (EBHS), toxoplasmosis and infection with Capillaria hepatica.

Criteria for diagnosis

The epidemiological situation, namely the presence of susceptible species and competent vectors coupled with the finding of dead or moribund rodents and lagomorphs, is suggestive of tularaemia. Necropsy findings are of limited use as they can be observed in other diseases as well (see "Differential diagnosis" above).

Diagnosis is based on the detection of *F. tularensis* by a direct method (see details in the next section).

A presumptive diagnosis can be made based on the detection of specific antibodies by an indirect method. Confirmation of diagnosis is achieved after exclusion of cross-reactivity of antibodies with other bacteria (see details in the next section).

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

Note: advantages and disadvantages are marked in the following text with "P" (pros) and "C" (cons).

Direct methods are performed primarily on dead animals.

culture: (P) highly specific, allows isolation and typing of the strain, (C) characterized by a lower sensitivity compared to PCR, associated with a high risk of laboratory infection and consequently allowed only in biosafety level 2/3 (BSL 2/3) laboratories;

PCR: (P) highly sensitive and specific, allows typing of the strain, reduces the risk of laboratory-acquired infections, (C) does not allow to establish the viability of the bacterium;

immunohistochemical assay (IHC): (P) sensitive, plenty of bacterial antigen can be detected in the foci of necrosis, reduces the risk of laboratory-acquired infections, (C) does not allow strain characterization;

direct fluorescent assay (DFA): (P) sensitive, plenty of bacterial antigen can be detected in the foci of necrosis, reduces the risk of laboratory-acquired infections, (C) does not allow strain characterization.

Target Samples: Warning: the carcasses of suspected animals and their organs should be handled carefully with gloves and placed in sealed containers to prevent the transmission of *F. tularensis* to humans.

Granulomas of different organs (e.g. lungs, pericardium, kidneys in European brown hare) or, in case of absence of granulomas (e.g. in rodents) a pool of spleen, liver, lung and kidney possibly supplemented with other organs (e.g. bone marrow) are recommended.

Storage: organs can be stored between +2 and +4 °C for maximum 2-3 days before their submission to the laboratory. Alternatively, organs can be frozen (-18 / -20°C) for several days following the above-mentioned precautions.

Indirect methods are performed on sera from live animals which either are relatively resistant to disease (e.g. carnivores, scavengers) or develop a chronic form of disease (e.g. European brown hare) and therefore seroconvert. They are:

the slide agglutination test: (P) useful field method for both diagnosis and epidemiological investigations, rapid, economic, applicable to all animal species, can be also performed with whole blood, (C) cross-reactions may occur with *Brucella* spp. and *Yersinia* spp. antigens and antisera, titer cannot be determined;

the micro-agglutination test (MAT) / tube agglutination test: (P) useful method for epidemiological investigations, rapid, economic, accurate, sensitive, applicable to all animal species, titer can be determined (1:40 is suspect, while 1:80 and above is considered positive), (C) cross-reactions may occur with *Brucella* spp. and *Yersinia* spp. antigens and antisera;

ELISA: (P) method with good sensitivity and specificity, allows for an early diagnosis, (C) expensive, requires availability of specific immunoglobulins for each animal species, is only performed in very few labs.

Storage: Sera can be stored between +2 and +4 °C before analysis up to 5 days, otherwise they should be stored frozen (-18 / -20°C). For long-term storage, lyophilization should be considered.

APHAEA protocol (for harmonization at large scale)

Direct diagnosis: PCR or Real Time PCR is recommended for direct diagnosis on tissues, vectors or other suspected samples. It is fast, sensitive and performed in many laboratory.

Indirect diagnosis: Microagglutination test is more sensitive respect slide agglutination test, gives an antibodies titer and is performed in the lab. Slide agglutination test is recommended during difficult work conditions as include the benefit to be made directly in many laboratory.

Laboratories that can be contacted for diagnostic support

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Recommended literature

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