LETTERS

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Natural Infection of a European Red Squirrel (*Sciurus vulgaris*) with *Francisella tularensis* subsp. *holarctica*

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ABSTRACT: Postmortem examination and immunohistochemical and bacteriologic analyses on a free-ranging European red squirrel (*Sciurus vulgaris*) revealed a systemic infection with *Francisella tularensis*. Genome sequencing and single-nucleotide polymorphism analysis were consistent with *F. tularensis* subs. *holarctica* clade B.45. Tularemia has not previously been reported in this species.

Francisella tularensis is a gram-negative, intracellular bacterium and the causative agent of the zoonotic disease tularemia. The ecology of tularemia is complex and includes partially overlapping aquatic and terrestrial, sylvatic cycles, arthropod reservoirs, and environmental persistence (Luque-Larena et al. 2017). Currently, the subspecies *holarctica* is the only subspecies isolated in Europe (Origgi et al. 2014; Hestvik et al. 2015; Pilo 2018; Wittwer et al. 2018; Kittl et al. 2020). Despite its broad host range, including free-ranging and captive wildlife, domestic animals, humans, and arthropods in Central Europe, *F. tularensis* has been primarily detected in free-ranging rodents and lagomorphs, such as European brown hares (*Lepus europaeus*) and European rabbits (*Oryctolagus cuniculus*; Pilo 2018). In humans, tularemia occurs in several clinicopathologic forms, depending on the transmission route (Hestvik et al. 2015). In Europe, most cases have been linked to contact with infected lagomorphs, and outbreaks may follow population increases in murid rodents, especially voles (subfamily Arvicolinae; Rossow et al. 2014; Otto et al. 2015; Luque-Larena et al. 2017). Tularemia in other rodent suborders has rarely been described in Europe (Hestvik et al. 2015). The European red squirrel (*Sciurus vulgaris*) is a native tree squirrel (family Sciuridae), widespread in European forests and periurban habitats. Population declines have been observed where eastern gray squirrels (*Sciurus carolinensis*) have been introduced (Chantrey et al. 2014).

Although European red squirrels have been shown to be susceptible to *F. tularensis* infection and died in experimental trials (Olin 1942), natural infections in free-ranging tree squirrels have exclusively been reported in fox squirrels (*Sciurus niger*; Vincent et al. 2020), and eastern (White et al. 1975) and western gray squirrels (*Sciurus griseus*; Nelson et al. 2014) in North America. Here, we report findings supporting a natural infection in a free-ranging European red squirrel with *F. tularensis* subsp. *holarctica*.

An adult female European red squirrel showing tremors and lethargy was found in September 2017 in a field in northwestern Switzerland (Riggisberg, 46°48′N, 7°28′E). Euthanasia was performed by intracardial injection of pentobarbital (Esconarcon, 300 mg/mL, Streuli, Uznach, Switzerland).

Gross necropsy revealed moderate emaciation and infestation with ticks (*Ixodes* sp.). The right popliteal lymph node was pale tan, firm, and severely enlarged (0.8×0.6×0.4 cm) with a scalloped surface and a 0.1–0.3-cm-thick capsule (Fig. 1A). The surrounding connective tissue and musculature were slightly firm and edematous. The spleen was mildly enlarged (1.5×3.0×0.5 cm) with minimally rounded borders and multifocal to

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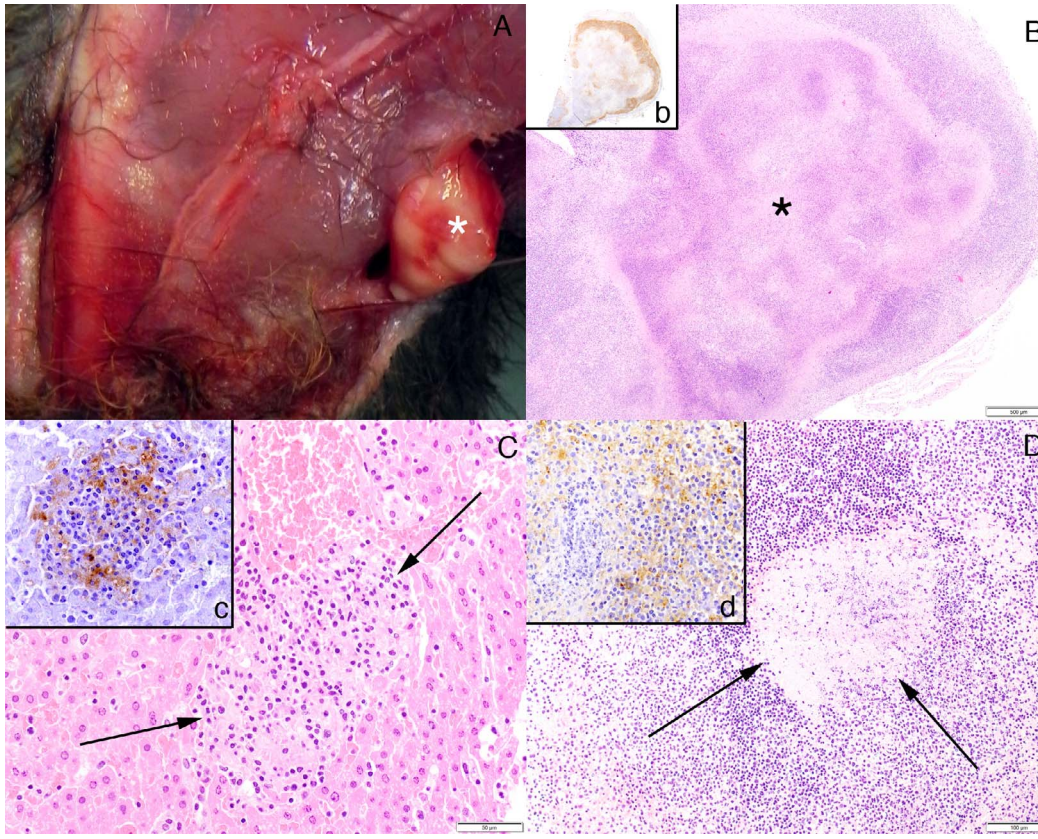

FIGURE 1. Macroscopic and microscopic findings (H&E stain; insets, immunohistochemistry) of a European red squirrel (*Sciurus vulgaris*) naturally infected with *Francisella tularensis* subsp. *holarctica*. (A) Severe lymphadenomegaly. Popliteal lymph node (white asterisk), medial view. (B) Necrotizing lymphadenitis. A large area of necrosis (black asterisk) expands the medullary portion of the lymph node. Bar=1 mm. By immunohistochemistry, a large amount of antigen is collecting along the cortical layer (inset b). (C) Granulomatous hepatitis. A large granuloma (arrows) expands the liver parenchyma compressing the surrounding hepatocytes. Bar=50 µm. Co-localization of *F. tularensis* is shown by immunohistochemistry (inset c). (D) Necrotizing splenitis. A large area of necrosis (arrows) with associated lymphoid loss is shown. Bar=50 µm. Co-localization of bacterial antigen (immunohistochemistry) is shown (inset d).

coalescent, variably extensive, light-tan foci (0.1–0.2×0.1–0.2×0.1 cm) on cross-section.

Sections of lymph node, spleen, liver, brain, lung, heart, kidney, urinary bladder, adrenal gland, and gastrointestinal tract were fixed in 4% neutral-buffered formaldehyde (Formafix, Hittnau, Switzerland), stained with H&E, and processed for immunohistochemistry, as previously described (Origgi and Pilo 2016). Histopathology revealed severe, focal-extensive, necrotizing lymphadenitis (Fig. 1B); moderate, multifocal, granulomatous hepatitis (Fig. 1C) and splenitis (Fig. 1D); mild to moderate, multifocal, lymphocytic meningoencephalitis and granulomatous cystitis; and histiocytic choroiditis. Lipopolysaccharide antigens of *F. tularensis* were detected by immunohistochemistry in the lymph node (Fig. 1B), lung, liver (Fig. 1C), and spleen sections (Fig. 1D), mostly associated with granulomatous and necrotic lesions.

Bacterial colonies from lymph node and spleen samples were identified as *F. tularensis* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (microflex LT/SH, Bruker, Bremen, Germany) using an in-house database (Institute of Veterinary Bacteriology, Bern, Switzerland), as previously de-

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scribed (Origgi and Pilo 2016; Kittl et al. 2020). The isolated strain, 17OD1646, was characterized genetically through Illumina whole-genome sequencing (GenBank accession no. PRJNA664901) and canonical single-nucleotide polymorphism (CanSNP) analysis (CanSNper 1.0.8), as previously described (Origgi et al. 2014; Wittwer et al. 2018; Kittl et al. 2020) and was phylogenetically classified as *F. tularensis* subsp. *holarctica* CanSNP clade B.45.

Clade B.45 is widespread in Central Europe and has been detected in European brown hares, a stone marten (*Martes foina*), ticks (*Ixodes ricinus*), and humans in Switzerland (Wittwer et al. 2018; Kittl et al. 2020). The extent and severity of the pathologic lesions were preterminal. The liver and spleen changes were consistent with those reported in brown hares and a yellow-necked mouse (*Apodemus flavicollis*) in Europe (Origgi and Pilo 2016; Hestvik et al. 2018). The meningoencephalitis was not associated with *F. tularensis* immunohistochemical labeling; however, that might be related to low bacterial load in that organ. In the absence of other obvious pathogens, the brain lesions were probably associated with the systemic spread of *F. tularensis*. Interestingly, neuromeningeal involvement is observed only rarely in humans and European brown hares (Origgi and Pilo 2016). Importantly, brain examination may not be performed routinely during outbreaks in wildlife; therefore, central nervous system–associated lesions may go undetected.

This report represents the first documented natural occurrence of tularemia in a free-ranging European red squirrel, suggesting a presumptive susceptibility to *F. tularensis*, which needs to be confirmed by additional cases and studies. It is unclear whether the previous reports of tularemia in tree squirrels of North America are only secondary to a lack of disease or pathogen detection in European tree squirrels, to differences in susceptibility of squirrel species, or to different subtypes of *F. tularensis* (subsp. *tularensis* vs. *holarctica*) occurring in North America and in Europe (Pilo 2018).

Tularemia has been increasingly detected in both humans and hares in past decades in Europe (Hestvik et al. 2015), including Switzerland, where the incidence in humans has risen from 0.0018% to 0.0172% between 2010 and 2019 (Kittl et al. 2020). This may be due to improved disease awareness and surveillance efforts or to an emergence of the pathogen, which might also lead to spillover to relatively less-conventional hosts, such as the European red squirrel.

Finally, detection of *F. tularensis* in a European red squirrel should encourage consideration of tularemia as a differential diagnosis when compatible pathology is observed in tree squirrels, contributing to better clarification of their potential role in the ecology of *F. tularensis*. Additionally, these findings suggest renewed attention for personnel operating in wildlife rehabilitation centers and veterinary services, who may be exposed to both conventional and less-conventional wildlife hosts potentially harboring zoonotic pathogens.

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