Journal of Veterinary Diagnostic Investigation
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J VET Diagn Invest 2013 25: 519 originally published online 14 May 2013
DOI: 10.1177/1040638713489124

The online version of this article can be found at:
http://vdi.sagepub.com/content/25/4/519

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SAGE
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On behalf of:
AAVLD
Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.

Journal of Veterinary Diagnostic Investigation
25(4) 519–521

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jvdi.sagepub.com

Francisella tularensis infection in a stone marten (*Martes foina*) without classic pathological lesions consistent with tularemia

Francesco C. Origgi, Natacha Wu, Paola Pilo[1]

Abstract. The current report describes the isolation and typing of a strain of *Francisella tularensis*, the causative agent of tularemia, from the spleen of a stone marten (*Martes foina*) showing no classic lesions consistent with the disease. The identification of this bacterium, belonging to the World Health Organization risk 3 category and considered to have a low infectious dose, could be performed only because of an ongoing project screening *F. tularensis* in the environment sensu lato. The findings described herein should alert diagnostic laboratories of the possible presence of *F. tularensis* in clinical samples in countries where tularemia is endemic even in cases with no consistent anamnesis and from unsuspected animal species.

Key words: Diagnostics; *Francisella tularensis*; inconsistent pathological lesions; martens.

Tularemia is a very complex emerging zoonotic disease caused by the Gram-negative bacterium *Francisella tularensis.* Depending on the route of infection, involved strains, and hosts, tularemia presents numerous clinical forms.[6,14,15] It is assumed that people become infected via the environment sensu lato, mainly after contact with infected animals, contaminated water, and aerosols or by bites from blood-sucking arthropods.[6] However, besides outbreak situations, the source of infection remains essentially unknown and difficult to demonstrate.[17] Although certain wild animal species are recognized as playing a role in the biological cycle of this bacterium, especially lagomorphs and rodents, the real spectrum of naturally infected species remains hypothetical.[11] This is principally due to the fact that *F. tularensis* grows only on special culture media[5,18] and is not commonly considered in routine diagnostic investigations. Tularemia may be overlooked in some animal species and more particularly when anamnesis and/or tissue changes are inconsistent. Additionally, similarly to other fastidious organisms, the presence of *F. tularensis* might be masked by the overgrowth of other microorganisms or go undetected because of poor growth on inadequate isolation media. The current report describes the isolation and genetic characterization of *F. tularensis* subsp. *holarctica* belonging to the cluster circulating in Western Europe[4] from the spleen of a stone marten (or beach marten, *Martes foina*), with no pathological observations suggestive of tularemia.

A stone marten was found unresponsive and did not attempt to escape when approached by a game warden in Graenichen, Canton Aargau, Switzerland, in July 2012. The animal was culled and sent to the Centre for Fish and Wildlife Health, University of Bern, Switzerland, for a postmortem examination. A full necropsy was performed on the submitted marten, and representative tissue sections of multiple organs were routinely processed and stained with hematoxylin and eosin according to the standard protocol.[16] Additionally, special stains were used as appropriate.

Grossly, a 4-mm perforation of the skin just caudal to the left elbow region associated with the presence of subcutaneous light tan creamy material consistent with pus was observed. The suppurative exudate was dissecting the subcutis and extended over the entire left thoracic region and the cranial two-thirds of the left abdominal region. Additionally, a 0.4 cm × 1 cm perforating wound was found on the right front paw. No additional gross findings were observed.

Histologically, the lesions observed were consistent with complication of traumatic wounds, parasite infestation, and kidney disease. None of the lesions were suggestive of tularemia. The most severe tissue change was a chronic-active suppurative cellulitis with intralesional cocci observed in correspondence to the gross skin wounds. A mild myocarditis on a background of mild multifocal fibrosis and focal moderate thickening of the epicardium were seen in the heart. A parasitic pneumonia, with large numbers of nematode parasites, and multifocal parenchymal consolidation with rare small foci of parasite-associated necrosis were seen in the lungs. Grocott and Gram stains did not reveal the

From the Institute for Veterinary Bacteriology (Origgi, Pilo) and the Centre for Fish and Wildlife Health (Origgi, Wu), University of Bern, Bern, Switzerland.

[1]Corresponding Author: Paola Pilo, Institute for Veterinary Bacteriology, Vetsuisse Faculty, Länggassstrasse 122, 3012 Bern, Switzerland. paola.pilo@vetsuisse.unibe.ch

presence of detectable organisms. A mild multifocal lymphoplasmacytic interstitial nephritis with segmental tubular necrosis and mineralization along with occasional collection of tubular casts was seen in the kidneys. Multifocal mild mineralization in the lamina propria of the stomach and moderate numbers of cestodes in the lumen of the small intestine were seen in the gastrointestinal tract. Finally, a minute focus of coagulation necrosis was seen in the liver, while a diffuse, minimal lymphoid depletion was observed in the spleen.

Samples from spleen, lung, liver, kidney, and subcutaneous suppurative exudate were submitted for bacteriology analyses. Stone martens are unconventional hosts for tularemia. However, 2 factors were considered: an ongoing project investigating *F. tularensis* in the environment was being carried out in the laboratory, and an outbreak of tularemia in farmed *Mustela* sp., another genus belonging to the family *Mustelidae*, had been previously reported.[12] Hence, the decision was made to test the stone marten for *F. tularensis*, despite the absence of typical signs or lesions. Lysates[20] from the organs and a swab of the subcutaneous suppurative exudate were tested by real-time polymerase chain reaction (PCR) for *F. tularensis* as previously described.[22] The lung, spleen, and subcutaneous pus were positive by this *F. tularensis*–specific PCR.

All samples were cultivated onto trypticase soy agar (TSA) with 5% sheep blood,[a] chocolate agar with IsoVitaleX[b] (ChocIso), and *F. tularensis* selective agar (Ftsel; chocolate agar with brain–heart infusion broth[b] as base, 1% soluble hemoglobin powder,[a] 0.1% L-cysteine hydrochloride monohydrate, 0.1% D-glucose and Skirrow *Campylobacter* selective supplement[a]) for 3 days at 37°C with 5% CO_2. Mixed flora grew from all samples on TSA, ChocIso, and Ftsel. After colony purification, a strain with colony morphology consistent with *F. tularensis* could be isolated from Ftsel agar plates inoculated with the spleen.

The strain isolated from the spleen was then genetically characterized. A lysate[20] from the culture was prepared as DNA template. The strain was identified as subspecies *holarctica* by PCR.[1] An additional PCR targeting the region of difference 23 was performed as previously described,[4] and the amplicon size corresponded to the emerging clone from Western Europe of *F. tularensis* subsp. *holarctica* that is circulating in France, Germany, Italy, Spain, and Switzerland.[4,7,10,19] Sequence analysis of the single nucleotide polymorphism markers B.18 (derived state) and B.19 (ancestral state) confirmed the subclade B4.FTNF002-00,[21] which is endemic in Switzerland. Multiple-loci variable number of tandem repeats (VNTR) analysis[2,13,19] was performed, and the isolated strain showed a VNTR profile that is circulating in Switzerland.[19]

Awareness of tularemia is emerging in Europe, and increased resources are being invested in order to better understand this disease.[3,8,9,19] The focus on the improvement and refinement of diagnostic methods for *F. tularensis* identification of the past decade may contribute to an increase in the number of cases reported in human beings and in animals. However, only long-term systematic surveillance programs may answer if the present scenario is the outcome of an actual increment of *F. tularensis* on the territory or simply an increased attention for this bacterium.

In veterinary diagnostic laboratories, *F. tularensis* is normally investigated only in cases of suspicion of tularemia (mainly in primates, rodents, and lagomorphs) and typically only when consistent anamnesis is present. In the reported case, the marten was tested in the frame of a study investigating the biological cycle of *F. tularensis* in the environment sensu lato. It should be emphasized that the bacterium could be isolated only on an appropriate selective medium used specifically for this project and was present as a mixed culture. In other circumstances, it is very likely that this case would have been missed, which raises serious concerns not only for the missed diagnosis of a serious pathogen but also for biosafety, especially for that of the personnel who handled the carcass and samples. The isolation of *F. tularensis* from an animal species generally not screened for this bacterium and with no consistent pathological lesions may be challenging and dangerous for the personnel of diagnostic laboratories. Awareness of the potential presence of *F. tularensis* in unconventional hosts should be raised in countries where tularemia is endemic.

Finally, the biological role of martens, if any, in the environmental cycle of *F. tularensis* is not clear as is the case for many other wild animal species. More investigations are needed because the environmental "reservoir(s)" for tularemia is still unknown.

Acknowledgements

The assistance of the game wardens who submitted wild animals for the project is acknowledged.

Sources and manufacturers

a. Oxoid, Basel, Switzerland.
b. Becton Dickinson, Allschwil, Switzerland.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Swiss Federal Office for the Environment (12.0003.KP/L121-0964) and by the Swiss Expert Committee for Biosafety (grant no. 04.1240.PZ/L064-7521).

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