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Published in:
Transboundary and Emerging Diseases

Link to article, DOI:
[10.1111/tbed.13078](https://doi.org/10.1111/tbed.13078)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Jeske, K., Tomaso, H., Imholt, C., Schulz, J., Beerli, O., Suchomel, J., Heroldova, M., Jacob, J., Staubach, C., & Ulrich, R. G. (2019). Detection of *Francisella tularensis* in three vole species in Central Europe. *Transboundary and Emerging Diseases*, 66(2), 1029-1032. <https://doi.org/10.1111/tbed.13078>

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Article type : Short Communication

Detection of *Francisella tularensis* in three vole species in Central Europe

Short running title: *Francisella tularensis* in voles

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tbed.13078

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Funding information: European Union, EMIDA-ERA-NET project “harmonized Approaches in monitoring wildlife Population Health, and Ecology and Abundance” (APHAEA) through Bundesamt für Landwirtschaft und Ernährung (BLE) (2811ERA117); Federal Environment Agency (UBA) within the Environment Research Plan of the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) (grant number 3713 48 401).

Abstract

Francisella tularensis is a zoonotic, gram-negative bacterium that causes tularemia in humans. Depending on its subspecies and the route of transmission, mild to lethal courses have been reported. *F. tularensis* subsp. *holarctica* is the only subspecies found in Europe and affects a plentitude of vertebrates including lagomorphs and rodents. Population outbreaks of certain rodent species are likely to be involved in the transmission of this pathogen. This molecular survey aims to evaluate the presence of *F. tularensis* in small mammals from three Central European countries. Using a real-time polymerase chain reaction, *F. tularensis* DNA was detected in common voles (*Microtus arvalis*) from Switzerland and in field voles (*Microtus agrestis*) and a bank vole (*Myodes glareolus*) from Germany, but not in any other small mammal species. All common voles from the Czech Republic were negative for *F. tularensis* DNA. The prevalence in the three vole species varied between 1.3% and 3.0%. In conclusion, *Francisella tularensis* DNA was detected in three vole species in two of three countries investigated. The observed low prevalence raises questions on the role of voles for the transmission of *Francisella tularensis* in Central Europe.

KEYWORDS: Bank vole, Common vole, Czech Republic, Field vole, Germany, Switzerland, Tularemia

1 INTRODUCTION

Tularemia is a zoonotic disease in humans with a flu-like onset. Later, symptoms differ depending on the route of pathogen entry. The disease is caused by the gram-negative bacterium *Francisella tularensis*, which can infect a broad range of invertebrates and vertebrates. In Europe, rodents and

lagomorphs are thought to act as main amplification hosts (Rossow et al., 2014). Transmission to humans can occur through contact with infected mammals, arthropods or contaminated water (Luque-Larena et al., 2017). The objective of this molecular survey was to evaluate the occurrence of *F. tularensis* in small mammals, and voles in particular, in Central Europe.

2 MATERIAL AND METHODS

Small mammals were collected according to a standard protocol for snap trapping (Drewes et al., 2016): (1) during late fall 2014 at three grassland grids close to Brno, Czech Republic, (2) during spring, summer and fall 2014 at grassland and forest grids in three federal states of Germany, and (3) at two sites in Zurich, Switzerland, namely Almend and Fildern (Figure S1). In the Czech Republic trapping was performed under the supervision of a person with a certificate of professional competence to design experiments and projects according to § 15d paragraph 3 of law 246/1992 (certificate CZ 01270). In Germany, all procedures involving animals were conducted according to relevant legislation and by permission of the federal authorities (Regierungspräsidium Stuttgart: 35-9185.82/0261, Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern: 7221.3-030/09, Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz: 22-2684-04-15-107/09). Trapping in Switzerland was performed under the direct supervision of a veterinary specialist, and according to the Swiss law, the guidelines on Animal Welfare and the specific regulations of the Canton of Zurich (permit number 17/2013 by the Veterinary Office and the Ethics Committee of the Canton of Zurich; Kantonales Veterinäramt Zürich, Zürich, Switzerland). Small mammal dissection and species confirmation followed established standard protocols (Fischer et al., 2018). DNA was isolated from spleen tissue samples using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and analyzed by a real-time polymerase chain reaction (PCR) assay targeting the *F. tularensis*-specific *tul4* gene (Versage et al., 2003). A conventional duplex PCR was used for the identification of subspecies *holarctica* (Fabbi et al., 2016). An isolation attempt using spleen tissue was performed on cysteine heart agar at 37°C and 5% CO₂ (Tomaso et al., 2018). The 95% confidence interval (CI) was estimated using the statistical software package R (<http://www.r-project.org>).

3 RESULTS

A total of 767 small mammals were collected for this molecular survey, with 151 common voles originating from Brno, Czech Republic, 347 small mammals from Germany and 269 voles of four species from Switzerland (Table 1). Nine out of 737 (1.2%, CI: 0.6-2.3%) small mammals were tested to be positive for *F. tularensis* DNA (Table 1). Subspecies identification revealed exclusively *F. tularensis* subspecies *holarctica*. The isolation attempt failed for all samples (data not shown). In Switzerland five of 167 (3.0%, CI: 1.0-6.8%) common voles were found to be *Francisella* DNA positive (Table 1), whereas in Germany none of the common voles, but three of 104 (2.9%, CI: 0.6-8.2%) field voles and one of 78 (1.3%, CI: 0.0-6.9%) bank voles were found to be *Francisella* DNA positive (Table 1; Fig. S2A). All common voles from the Czech Republic were tested negative. Positive animals were all trapped in fall 2014 (Figs. S2A and S2B). In Germany only animals from one of three grids at Gotha, Thuringia, in grassland habitat with a nearby forest, were found to be positive. All animals from the other grids in Gotha and the other sites in Mecklenburg-Western Pomerania and Baden-Wuerttemberg were negative (data not shown).

4 DISCUSSION

The detection of *Francisella* DNA in common vole, field vole and bank vole is in agreement with previous studies in Germany and Finland (Kaysser et al., 2008; Rossow et al., 2014), and confirms the susceptibility of these vole species for *F. tularensis* subsp. *holarctica*. Its detection in voles from Germany and Switzerland is also in line with its previous detection in potential vectors and reservoirs in these countries and an increasing incidence of human tularemia cases (Kaysser et al., 2008). However, in Switzerland *F. tularensis* was detected before in house mice (*Mus musculus domesticus*), but not in voles (Origgi et al., 2015). In contrast to an earlier detection of this pathogen in a common vole in the Czech Republic (Trembl et al., 2001), the current investigation failed to detect any *Francisella* DNA in common voles from this country. This finding might be explained by a focal occurrence of the pathogen (Pavlovsky & Levine, 1966). Similarly, *Francisella* DNA was detected only in voles from one of three grids in Thuringia, but not in Baden-Wuerttemberg, although human tularemia cases were reported in 2014 from both German federal states (Robert-Koch-Institut, 2015).

A similar spatial *Francisella* occurrence was observed in a study in Sweden where subpopulations of *F. tularensis* could only be detected within areas of 2 km² (Svensson et al., 2009).

The average prevalence of *F. tularensis* in this study was 1.2%, which is comparable to results of studies in wild small mammal populations in Finland and Iran during non-outbreak situations with prevalences ranging from 0.9 to 1.9% (Rossow et al., 2014; Mostafavi et al., 2018). In case of known foci or recent outbreaks of *F. tularensis* in China, Germany and Spain prevalence may reach 4.8% to 20% depending on area and rodent population dynamics (Kaysser et al., 2008; Rodriguez-Pastor et al., 2018; Zhang et al., 2006). In our study the prevalences in bank voles (1.3%), field voles (2.9%) and common voles (3.0%) were lower than the prevalences observed during an outbreak study in Lower Saxony, Germany, with prevalences of 4.5%, 10% and 8%, respectively (Kaysser et al., 2008). The highest prevalence in common voles was measured during a recent tularemia outbreak in Spain with 34% (Rodriguez-Pastor et al., 2018).

The failure of the isolation attempt might have been caused by our workflow including snap-trapping and freeze-thaw-cycles of carcasses and spleen samples. Previous isolation attempts using fresh spleen samples from hares demonstrated the successful performance of the isolation protocol in general (Tomaso et al., 2018).

In conclusion, this study detected *Francisella* DNA in voles of three species in Germany and Switzerland. Future detailed studies are warranted to confirm and characterize the potential hotspot distribution of *Francisella* in Central Europe and to evaluate if this could be used in control measures to prevent human infections. For this purpose a widespread network of wildlife monitoring and surveillance in Europe should be established for *F. tularensis* and other relevant pathogens.

ACKNOWLEDGEMENTS

We kindly acknowledge Marie-Pierre Ryser-Degiorgis, Thijs Kuiken, Ezio Ferroglio and Christian Gortazar for setting up and supporting the project APHAEA. We are grateful to Thijs Kuiken and Daniel Hegglin for critical reading of the manuscript, Kerstin Cerncic, Anja Hackbart and Byrgit Hofmann for excellent technical assistance, Franz J. Conraths and Peter Deplazes for their support, Ann-Kathrin Güttner for statistical analyses, Patrick Wysocki for generation of Figure S1, Christin

Accepted Article

Trapp and Daniela Reil for small mammal trapping in Germany and Stefan Fischer, Dörte Kaufmann, Anke Mandelkow, Marie Luisa Schmidt, Hanan Sheikh Ali, Stephan Drewes and Elisa Heuser for support of rodent trapping, dissections and cytochrome *b* analysis.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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TABLE 1. Detection of *Francisella tularensis* in small mammals from the Czech Republic, Germany and Switzerland. Positive results are shown in bold.

Country	Species		Number of captured animals	Number of PCR positive/ total number of sampled animals and percentage [CI]
Czech Republic	Common vole	<i>Microtus arvalis</i>	151	0/147*
Germany	Striped field mouse	<i>Apodemus agrarius</i>	4	0/4
	Yellow-necked mouse	<i>Apodemus flavicollis</i>	56	0/48*
	Wood mouse	<i>Apodemus sylvaticus</i>	6	0/6
	Field vole	<i>Microtus agrestis</i>	106	3/104* 2.9% [0.6-8.2%]
	Common vole	<i>Microtus arvalis</i>	50	0/44*
	Bank vole	<i>Myodes glareolus</i>	85	1/78* 1.3% [0.0-6.9%]
	Common shrew	<i>Sorex araneus</i>	31	0 /30*
	Crowned shrew	<i>Sorex coronatus</i>	7	0/7
	Eurasian pygmy shrew	<i>Sorex minutus</i>	2	0/1*
Sub-total			347	4/322* 1.2% [0.3-3.1%]
Switzerland	Montane water vole	<i>Arvicola scherman</i>	65	0/65
	Field vole	<i>Microtus agrestis</i>	9	0/9
	Common vole	<i>Microtus arvalis</i>	168	5/167* 3.0% [1.0-6.8%]
	Bank vole	<i>Myodes glareolus</i>	27	0/27
Sub-total			269	5/268* 1.9% [0.6-4.3%]
Total			767	9/737* 1.2% [0.6-2.3%]

CI, confidence interval

* Differences between total number of captured and analysed animals is caused by missing spleen samples for a few animals.

SUPPORTING INFORMATION

Supporting information can be found online.