

Susceptibility of the Common Hamster (*Cricetus cricetus*) to *Francisella tularensis* and Its Effect on the Epizootiology of Tularemia in an Area Where Both Are Endemic

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ABSTRACT: *Francisella tularensis* is a highly infectious zoonotic agent causing the disease tularemia. The common hamster (*Cricetus cricetus*) is considered a pest in eastern Europe, and believed to be a source of human tularemia infections. We examined the role of the common hamster in the natural cycle of tularemia using serologic methods on 900 hamsters and real-time polymerase chain reaction (PCR) on 100 hamsters in an endemic agricultural area. We collected 374 *Ixodes acuminatus* ticks from the hamsters and tested them by real-time PCR. All tests were negative. To examine clinical signs, pathology, and histopathology of acute tularemia infection similar to the natural infection, two hamsters were infected with a large dose of a wild strain of *F. tularensis* ssp. *holarctica*. After a short period of apathy, the animals died on the eighth and ninth days postinfection. The pathologic, histopathologic, and immunohistochemical examination contributed to the diagnosis of septicemia in both cases. Our results confirmed previous findings that common hamsters are highly sensitive to *F. tularensis*. We conclude that although septicemic hamsters may pose substantial risk to humans during tularemia outbreaks, hamsters in interepizootic periods do not act as a main reservoir of *F. tularensis*.

Key words: *Francisella tularensis* ssp. *holarctica*, immunohistochemistry, real-time polymerase chain reaction (PCR), tularemia, wildlife, zoonosis.

Francisella tularensis is the etiologic agent of tularemia, a life-threatening zoonotic disease and a potential biological warfare agent that has been placed on the list of Class A biothreat agents (Ellis et al., 2002). Humans are highly susceptible to *F. tularensis*, and infection often occurs during hunting, trapping, and skinning of infected wildlife (Dennis et al., 2001). Three subspe-

cies of *F. tularensis* are recognized: the highly virulent *F. tularensis* ssp. *tularensis* (Type A), the moderately virulent *F. tularensis* ssp. *holarctica* (Type B), and *F. tularensis* ssp. *mediasiatica* (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [DSMZ], 2010). *Francisella tularensis* ssp. *holarctica* is the causative agent of tularemia in Europe.

Natural infections with *F. tularensis* have been reported in a range of vertebrates including mammals, birds, amphibians, fish, and certain invertebrates (Mörner, 1992). Despite the broad host range, tularemia is primarily a disease of the orders Lagomorpha and Rodentia, and hemathophagous arthropods have a substantial role both in the maintenance of *F. tularensis* in nature and in disease transmission (Friend, 2006). Ticks are believed to be the most important arthropods in the ecology of tularemia (Friend, 2006).

Rodents are of great importance for maintaining enzootic foci of tularemia in Eurasia (Friend, 2006). The common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), and the water vole (*Arvicola amphibius*) are the species most frequently involved in tularemia epizootics. During outbreaks 4.5% (4/88) and 5.2% (4/79) prevalences of infection were determined by bacterial isolation from common voles in Austria (Gurycová et al., 2001) and 8.0% (2/25) among common voles, 10.0% (1/10) among field voles, and 15.0% (6/40) among water voles with real-time polymerase chain reaction (PCR) in Germany

(Kaysser et al., 2008). Many other species (*Ondatra zibethicus*, *Castor* spp., *Lemmus* spp., *Rattus rattus*, *Mus musculus*, *Apodemus* spp., *Myodes glareolus*, *Microtus agrestis*, *Clethrionomys* spp., *Tamias sibiricus*, *Sciurus vulgaris*, etc.) were also found infected (Friend, 2006; Gurycová et al., 2001; Kaysser et al., 2008; Mörner and Addison, 2001).

The common hamster (*Cricetus cricetus*) is a species of hamster native in western, central and eastern Europe, central Russia, and Kazakhstan. The common hamster was considered to be a dangerous pest throughout Europe, but its population has declined in its western range during the last decades (Nechay, 2000). Nevertheless, it is still a common species in eastern Hungary, which supports the strongest European hamster population (Bihari and Arany, 2001). Trapping of hamsters for pest control and fur collection for sale is a widespread practice in eastern Hungary. Trappers, who skin more than half a million hamsters a year (Bihari, 2003) regularly become infected with tularemia in this area (Münnich and Lakatos, 1979). In spite of this, trappers are not aware of the risk of infection and there is no public health effort to reduce risk. *Cricetus* spp. are highly sensitive to tularemia and were classified as a Class 1 species, which means that acute disease occurs after inoculation of only 1–10 bacteria (Olsufiev and Dunayeva, 1970; Sjöstedt, 2007).

The aim of our present study was to investigate the role of hamsters in the natural cycle of tularemia by collecting samples from a subpopulation of common hamsters in an 80-km² agricultural area in eastern Hungary. To examine clinical signs, pathology and histopathology of acute tularemia resembling natural infection in two trapped hamsters infected intramuscularly and orally with a large dose of *F. tularensis* ssp. *holarctica*.

Estimation of hamster population size in the study area was based on the number of active burrows/hectare (Bihari, 2003).

Overall 900 hamsters were trapped with kill traps in the study area: 250 in May 2008, 500 in May 2009, and 150 in October 2009. Hamsters were screened with the slide agglutination test with the use of stained *F. tularensis* (Bioveta Inc., Ivanovice na Hané, Czech Republic) and tube agglutination test (World Organization for Animal Health [OIE], 2008) using whole blood taken from the heart and thoracic cavity. Lung, liver, spleen, and kidney tissue pools (a total of about 100 mg) were collected from 50 individuals trapped in May and from 50 animals trapped in October 2009 and stored at –20 C until PCR examination. Ticks were also collected from these 900 animals and kept in 70% ethanol. After identification to species, development stage, and sex, ticks were pooled (10 or fewer) and stored at –20 C until PCR examination.

Pools of ticks and organs were homogenized in 1,000 µl Tris-EDTA (pH 8.0) buffer using the TissueLyser high-throughput disruption instrument (Qiagen Inc., Hilden, Germany) according to the manufacturer's recommendations. Homogenized pools were centrifuged at 12,000 × G for 5 min at 4 C. A 100-µl supernatant from each sample was used for DNA extraction conducted on an X-tractor Gene automated nucleic acid extraction robot (Corbett Robotics Pty. Ltd., Queensland, Australia) with the use of the Total RNA Isolation Kit, Nucleospin 96 RNA (Macherey-Nagel GmbH & Co. KG, Düren, Germany) in accordance with the manufacturer's instructions, except for the DNase incubation step. DNA was eluted in 50-µl elution buffer. A part of the *tul4* gene was amplified with the use of a real-time TaqMan PCR system as described earlier (Versage et al., 2003). A liver sample (about 50 mg) from an experimentally infected mouse served as positive control.

Two adult, male hamsters were trapped alive with box traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) and housed in individual cages (730×530×250 mm), in a biosafety level 3 (BSL-3)

compartment. In order to induce acute infections resembling natural cases, both animals were infected with a wild *F. tularensis* ssp. *holarctica* strain isolated from a European brown hare (*Lepus europaeus*) in 2007 (Permit number for animal challenge: 22.1/2703/003/2009). One animal was infected intramuscularly on its hind leg (case 1) with 10^3 colony-forming units (CFU), as ticks harbor high infectious doses of *F. tularensis* (Gurycová et al., 1995), and one orally with 10^5 CFU (case 2), a dose used in earlier studies in similar Class 1 species, in voles (*Microtus pennsylvanicus* and *Microtus rossiaemerdionalis*) (Olsufiev and Dunayeva, 1970; Bell and Stewart, 1983; Olsufiev et al., 1984; Sjöstedt, 2007). Hamsters were checked three times a day to record clinical symptoms. The animals were necropsied immediately after natural death, and tissue samples (brain, heart, lung, liver, spleen, kidney, stomach, small and large intestine, submandibular, mediastinal and mesenteric lymph nodes, testicle, bone marrow, and brain) were collected for routine histologic examination. Histologic and immunohistochemical examination was performed as described (Gyuranecz et al., 2010). Livers were used for reisolation of *F. tularensis* on a modified Francis agar plate (chocolate agar plate containing 1% glucose and 0.1% cysteine). Win Episcope 2.0 program was used for data analysis.

The estimated population size in the study area was 400,000 hamsters (50 burrows/hectare) in 2008 and 80,000 (10 burrows/hectare) in 2009. Thus, at an assumed 0.5% antibody prevalence the probability of diagnosing at least one positive animal was 100% from the 250 screened hamsters in 2008 and 96.2% from the 650 animals in 2009. The serologic testing of all 900 hamsters yielded negative results both with slide and plate agglutination. Only one tick species, *Ixodes acuminatus*, was found on the hamsters. Overall, 368 females and 6 nymphs were collected. *Francisella tular-*

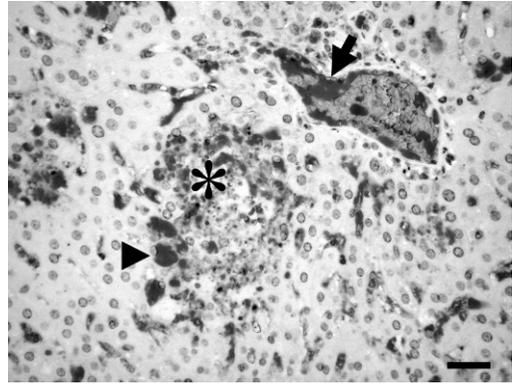


FIGURE 1. Focal acute necrosis (asterisk) presenting significant amounts of *Francisella tularensis* antigen in the liver of a common hamster (Case 1). There is strong immunoreactivity within a blood vessel (arrow), in sinusoids, endothelial cells, hepatocytes (arrowhead), and in a few macrophages. Immunohistochemistry, mouse monoclonal antibody to *F. tularensis*, and hematoxylin counterstain. Bar=50 μ m.

ensis DNA was not detected in any of the tick pools or organ pools of the 100 hamsters tested.

The two infected hamsters died on day 8 (case 1) and on day 9 (case 2) postinfection. Clinical signs were observed on the day before death, when animals were found apathetic and quickly entered a moribund state. Both animals were antibody negative by the slide-agglutination test. Gross pathologic lesions were found only in the spleens, which were enlarged and congested. In case 1, the spleen presented several randomly distributed pinpoint white necrotic foci on the serosal and cut surfaces. Histologically, severe acute necrosis was found in almost the entire section of the spleens. Focal or multifocal acute necrosis was also evident in the livers (Fig. 1), lymph nodes, and bone marrow. Additional findings of diffuse severe acute glomerulo- and tubulonephrosis, moderate lymphohistiocytic interstitial bronchopneumonia, and acute multifocal hemorrhage in the lungs were observed in both cases. Bacterial emboli were found in the glomeruli and interstitial blood vessels of the kidney in both

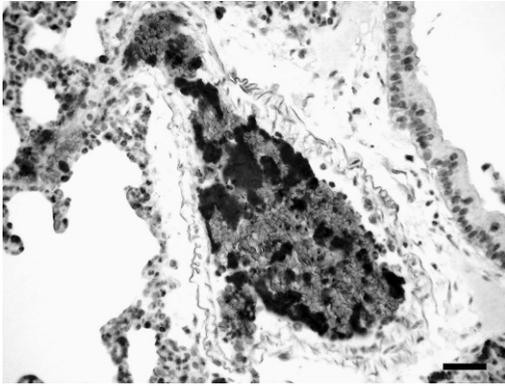


FIGURE 2. Moderate lymphohistiocytic interstitial pneumonia and strong *Francisella tularensis* immunoreactivity within a blood vessel, in endothelial cells, pneumocytes, and macrophages in the lung of a common hamster (Case 1). Immunohistochemistry, mouse monoclonal antibody to *F. tularensis*, and hematoxylin counterstain. Bar=50 μ m.

cases and in the blood vessels of the lung and in the sinusoids and blood vessels of the liver and spleen of case 1. *Francisella tularensis* antigen was found in large aggregates or small dots within the blood vessels or sinusoids of all organs examined (Fig. 2). Immunolabeling was more intensive and showed wider distribution in the organ samples of case 1. Intracytoplasmic bacterial antigen, visualized as small dots or diffuse granular staining, was frequently observed in macrophages, reticulocytes, endothelial cells, pneumocytes, enterocytes, hepatocytes, neurons, glial cells, epithelial cells of testis and stomach mucosa, and heart muscle cells. *Francisella tularensis* antigen accumulated in areas of necrosis. Bacteria were rarely found in the lumen of small intestine and seminiferous tubuli of the testes. *Francisella tularensis* was reisolated from both hamsters. The pathologic, immunohistochemical, and bacteriologic results contributed to the diagnosis of septicemia.

Tularemia infection is chronic in the European brown hare, a reservoir species that serves as a good indicator for the occurrence of the disease in central Europe (Mörner, 1994; Gyuranecz et al., 2010). The study area was considered a

tularemia-endemic region during the past decade and during the study period, based on the 1–1.2% infection rate in the local European brown hare population (estimated from 3,930 hares by slide-agglutination test and isolation). Additionally, 14 human clinical cases including hamster trappers occurred in the surrounding villages during the same time period. We suspect that our study was conducted in an interepizootic period, as there were no human cases during the study period; the negative results of the tick and organ pools further support this hypothesis.

Because of the high sensitivity of common hamsters, preliminary diagnosis of tularemia cannot be based on gross pathologic lesions, as it can in the European brown hare (Gyuranecz et al., 2010). Foci are not always found in the spleen, and apart from septicemia these same lesions may also be induced by shock. Bacterial isolation and the immunohistochemical assay were effective in diagnosing *F. tularensis* infection in hamsters. The intramuscular bacterial challenge in case 1 showed that a shorter incubation period was associated with more severe gross pathologic lesions, and larger amounts of bacterial antigen. Because the number of the animals in the trial was limited, this result may require confirmation and infection studies with different inoculation doses and routes may also be needed.

Francisella tularensis may cause large epizootics among rodents, and infection is considered to be a factor in population regulation, preventing overpopulation of these species in nature (Friend, 2006). Our results confirmed previous data (Olsufiev and Dunayeva, 1970; Sjöstedt, 2007) that common hamsters are highly sensitive to *F. tularensis* infection and die after a short incubation period. The negative results of the serologic survey confirm the high sensitivity of hamsters to tularemia. Because they do not survive the infection, there are no antibody-positive individuals in the population after the outbreak. We conclude that although

septicemic hamsters may pose substantial risk to humans during tularemia outbreaks, hamsters in interepizootic periods are not an important constituent of the natural cycle of the disease in that they do not act as a main reservoir of *F. tularensis*.

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