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Case report: tularaemia in a white-handed gibbon (*Hylobates lar*), Germany

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ABSTRACT

In 2021, a white-handed gibbon (*Hylobates lar*) succumbed to illness shortly after transfer from one zoo to another in Germany, due to *Francisella tularensis* subsp. *holarctica* infection. To determine the source of infection, whole genome sequencing of the gibbon-derived isolate was performed and wild pest rodents (and captive squirrels) from both zoos were screened for *F. tularensis*. The *F. tularensis* whole genome sequence obtained from the gibbon was closely related to previous subclade B.281 sequences obtained from hares from Baden-Wuerttemberg, the same region where the gibbon was first housed. However, *F. tularensis* DNA was detected in one Norway rat from the receiving zoo. Therefore, neither zoo can be excluded as the source of infection.

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1. Introduction

Tularaemia is a zoonotic disease caused by the Gram-negative, intracellular bacterium *Francisella tularensis* [1]. In Germany, most human or animal infections result from contact with European brown hares (*Lepus europaeus*) infected with *F. tularensis* subsp. *holarctica* [1]. However, infections may also occur through bites from infected arthropods; ingestion of contaminated food or water; contact with a contaminated hydrotelluric environment; or the inhalation of infectious aerosols [2]. Depending on the route of transmission, the infection may present differently [3]. Several vole species and yellow-necked field mice (*Apodemus flavicollis*) have also been shown to be potential carriers of the pathogen in Germany [4,5].

Tularaemia re-emerged in Germany in 2004 with infections of hare hunters in Hesse [6] and the death of five common marmosets (*Callithrix jacchus*) at a research facility reported in Lower Saxony [7]. Reports of human cases have been steadily increasing, with 435 cases reported between 2002 and 2019 – though the number of undiagnosed cases is believed to be substantially higher [8].

2. The study

In September 2021, a female white-handed gibbon (*Hylobates lar*, born April 2014) was transferred from a zoo in the federal state Baden-Wuerttemberg to a zoo in Schleswig-Holstein and succumbed to illness ten days later. No other animals or caretakers from either zoo showed symptoms of infection. At the receiving zoo, the gibbon was housed with outdoor access, adjacent to an outdoor rabbit exhibit, during the day. Ultrasound showed fluid accumulation in the abdomen, predominantly around the liver and bladder (Figure 1). The gibbon presented with diarrhoea and general discomfort (rectal 38.7°C). Auscultation of the heart and lungs, and palpation of the abdomen found no abnormalities. Amoxicillin (105 mg, Duphamox® LA) and Metamizole (350 mg, Metapyrin®) were administered subcutaneously. The following day, the gibbon was found unresponsive, presenting with yellowish mucosa, and a weak and irregular heartbeat (rectal temperature 36.5°C). An abdominal ultrasound revealed hyperechogenic liver and free abdominal fluid caudal of the liver and around the bladder. X-rays showed this in slightly reduced detail and mild intestinal dilatation. Enrofloxacin (1.4 ml, Baytril® 2.5%), Metamizole (350 mg, Metapyrin®) and Dexamethason (1.8 mg) were administered through intravenous fluids. After no improvement in condition and poor prognosis, the animal was humanely euthanized for animal welfare reasons.

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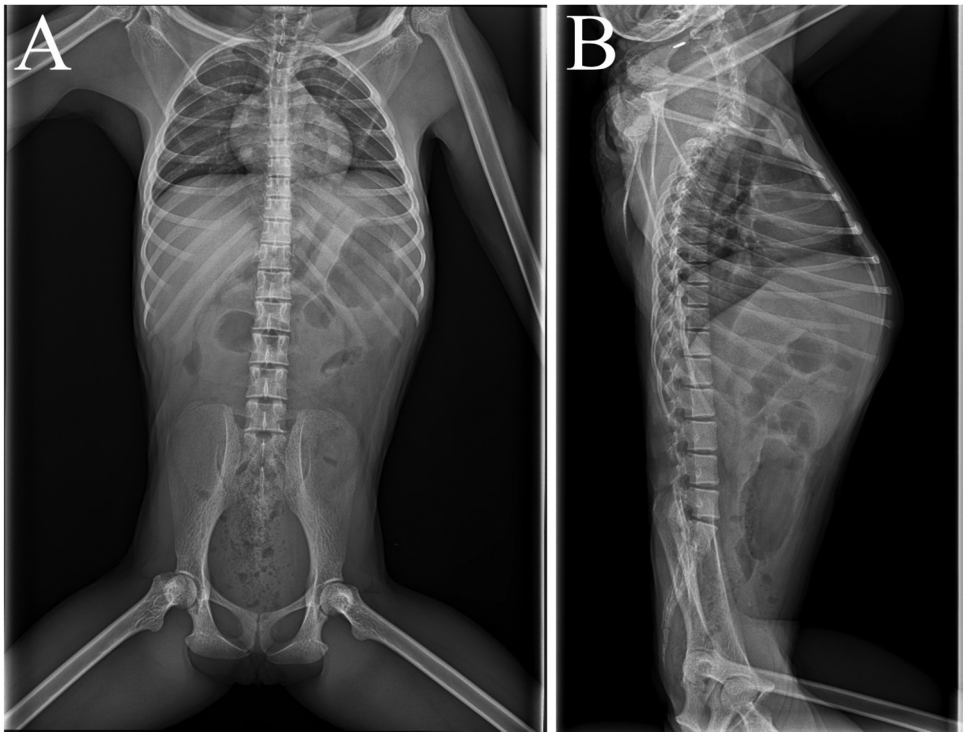

Figure 1. (A) ventrodorsal and (B) laterolateral x-rays of white-handed gibbon with *Francisella tularensis* subsp. *holarctica* infection show mild intestinal dilatation.

The gibbon cadaver was sent to the German Primate Center for necropsy. Necropsy revealed severe acute haemorrhagic enteritis in both the small and large intestines. Within the liver and spleen, a high-grade multifocal necrotizing hepatitis and splenitis could be demonstrated. Furthermore, erosive ulcerative lesions were present within the tongue followed by necrotizing inflammation within associated salivary gland and lymph node. The elevated necropsy and histologic findings were typical for tularaemia. Immunohistochemistry using a specific monoclonal anti-*F. tularensis* antibody confirmed the widespread distribution of *Francisella* antigen within the typical lesions in liver and spleen.

Important differential diagnostic diseases like toxoplasmosis or herpes simplex virus infection were excluded by immunohistochemistry, listeriosis and salmonellosis by bacteriologic investigation and echinococcosis by histology. Furthermore, special stains like Gram's, Giemsa, PAS-reaction, Grocott, and Ziehl-Neelsen failed to demonstrate other microorganisms in any of the affected organs. A mild perivascular lymphocytic encephalitis and focal necrotizing pneumonia were important secondary findings indicating severe systemic infection.

Due to the typical pathologic findings an oropharyngeal and enteral form of tularaemia was diagnosed. Taking the incubation period of up to 14 days into consideration, the infection with the agent could have happened in both facilities.

To identify the source of the infection, pest rodents were collected from both zoos between late 2020 and early 2022 as part of routine pest management (using snap traps). These, as well as captive squirrels, were frozen at −20°C until dissection (FLI Riems) (Table 1) followed by the PCR screening of spleen tissue (FLI Jena). A single rat (*Rattus norvegicus*) from the receiving zoo was positive for *F. tularensis* by PCR, but sequencing of the PCR product and isolation were not possible.

Table 1. Rodents screened for *Francisella tularensis* DNA.

Species	Number of positive animals/total number of animals
Sending zoo - Baden-Wuerttemberg	
Field mice (*Apodemus* sp.)	0/2
Bank vole (*Clethrionomys glareolus*)	0/3
House mouse (*Mus musculus*)	0/101
Norway rat (*Rattus norvegicus*)	0/53
Receiving zoo - Schleswig-Holstein	
Field mice (*Apodemus* sp.)	0/5
Common vole (*Microtus arvalis*)	0/1
Norway rat (*Rattus norvegicus*)	1/85
Swinhoe's striped squirrel (*Tamiops swinhoei*)	0/10

F. tularensis subsp. *holarctica* was isolated from the gibbon and identified as previously described [9]. Bacteria were isolated after being incubated at 37°C with 5% CO_2 for 72 hours. Following the manufacturer's instructions, DNA was extracted from the gibbon spleen tissue using the QIAGEN Genomic-tip 20/G and QIAGEN Genomic DNA buffer. Sequencing was then performed as previously described [9]. The genome sequence of 21T0109 (isolated from the gibbon) was compared to 306 previously sequenced German strains [9]. Raw sequencing data were all analyzed using the WGSBAC pipeline version 2.2.0 [9]. The raw read quality was controlled using FastQC version 0.11.7 [10]. Reads were assembled using Shovill v. 1.0.4 [11] and assessed using QUAST v. 5.0.2 [12]. Contamination was checked with Kraken2 v2.1.1 [13]. CanSNPer [14] and CanSNPer2 [15] were used for genotyping based on pre-defined canonical single nucleotide polymorphisms (SNPs). Additionally, Snippy 4.3.6 [16], which also created a core-genome (cg) alignment of all strains, was used to perform mapping-based SNP typing using OSU18 as a reference. Based on the alignment of the core genome, Snps-dists version 0.63 [17] estimated the pairwise SNP distances between the strains. Using Randomized Axelerated Maximum Likelihood (RAxML) version 8 [18], a phylogenetic tree based on the core-genome alignment was created using the generalized time reversible model (GTR) with a gamma distribution and visualized using the interactive Tree of Life (iTOL) version 4 web application [19]. Five hundred repetitions of bootstrapping were performed and values larger 20 were visualized.

The *F. tularensis* whole genome sequence (21T0109, labelled in red) obtained from the gibbon-derived isolate clustered in major clade B.6, CanSnper1 subclade B.61, CanSnper2 further subdivided B.61 into subclade B.281 (Supplementary Figure S1). The most closely related sequence (16T0051, labelled in green) originated from the cadaver of a European brown hare (*Lepus europaeus*) in 2016, Stuttgart, Baden-Wuerttemberg (Figure 2). Of note, all other sequences within subclade B.281 originate from Baden-Wuerttemberg.

3. Discussion and conclusions

Although the *F. tularensis* infection in the white-handed gibbon could not be traced back to the source, several possible infection routes were identified. Firstly, the strain in the gibbon was most similar to an isolate from a hare in Stuttgart and fell within a subclade (B.281) solely represented by genome sequences obtained from hares from Baden-Wuerttemberg – the same federal state as the sending zoo. These sequences belonged to major clade B. 6 which can only rarely be

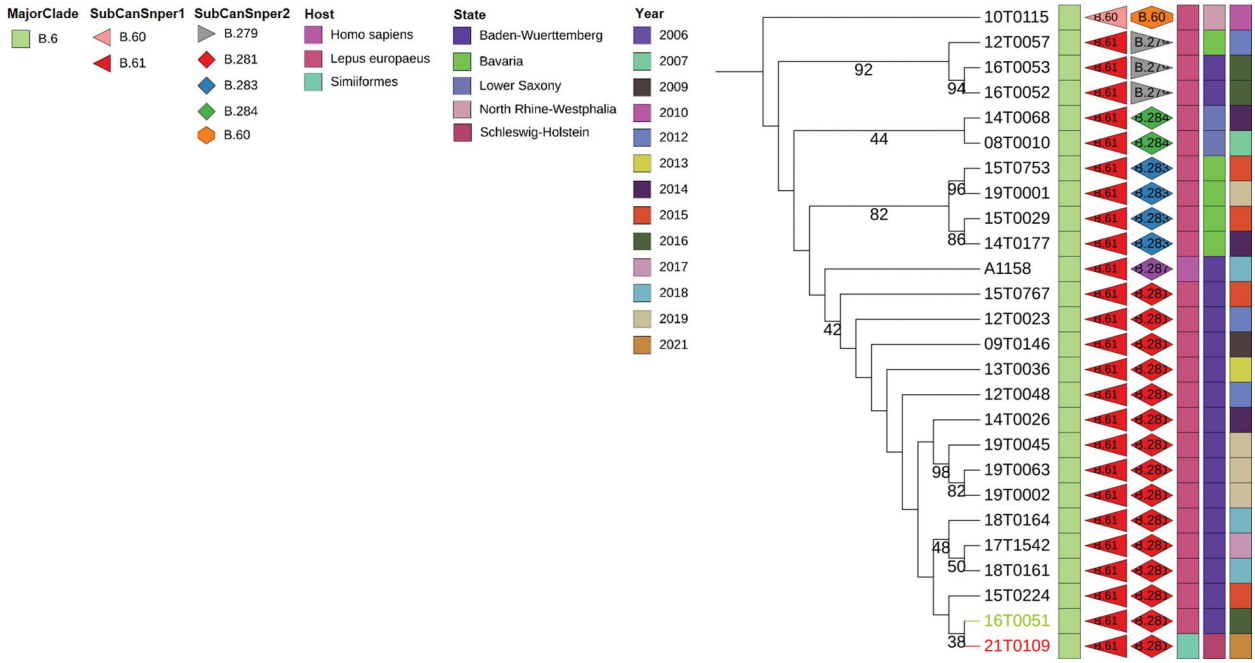

Figure 2. Subtree of the relevant clade of the phylogenetic tree of *Francisella tularensis* in Germany as shown in Supplementary Figure S1. This subtree is an enlarged section of the clade to which the gibbon sample clustered, indicated in the original tree by a black box. Symbols (colours and shapes) besides branches are explained in the corresponding columns of the legend (top left). Tree was calculated using published full genome sequences and visualized in iTOL. Reference strain OSU18 was used for rooting. Bootstrap values > 20 are given at the branches. Sequence from the white-handed gibbon (*Hylobates lar*) – derived isolate is shown in red (21T0109). Its closest relative in the data set, an isolate from a hare in Baden-Wuerttemberg (BW; 16T0051), is marked in green. As given in both panels in the left-hand upper corner: colours and shapes illustrate information for each sample (column 1 = major clade, column 2 = CanSnper1 subclade, column 3 = CanSnper2 subclade, column 4 = host species, column 5 = federal state, column 6 = year).

found in northern Germany [9]. However, because no comprehensive study on the distribution and sequence diversity exists for *F. tularensis* in Germany, it is possible that similar sequences can be found in other regions, namely the northern part of Germany where the receiving zoo is located. Secondly, at the receiving zoo in Schleswig-Holstein, a single rat was found PCR-positive for the bacterial DNA, however isolation of the bacterium was not possible. Additionally, symptoms typically present within 3–5 days of exposure [20], suggesting exposure may have occurred at the receiving zoo (the gibbon was asymptomatic at the time of transfer). However, depending on the dose, symptoms can also take up to 21 days to manifest [20]. Based on the necropsy results (bloody intestinal contents, accumulation of fluid in the abdomen, necrotizing splenitis and hepatitis) the exposure route was presumed to be oral [21,22] – potentially from contaminated water or food.

Similar to this study, the German Primate Center had an outbreak of *F. tularensis* in 2007 which showed similar pathology in infected cynomolgus monkeys (*Macaca fascicularis*) that were housed outdoors [22]. The authors described a similar clinical picture, including necrotizing hepatitis and splenitis, and suggest local wild rodents as the source of infections. Indeed, multiple small mammal species in Europe have been identified as potential sources of *F. tularensis* infection [4]. Furthermore, multiple studies have shown the risk of *F. tularensis* infection in non-human primates housed outdoors, almost all presenting with similar symptoms and many of which implicated rodents in the transmission of the pathogen [6,20,21]. In this study, there is evidence for the possible source of infection from both the sending and receiving zoos and more intensive screening and whole genome sequencing of *F. tularensis* isolates are necessary to confirm the source. Although the site at which the pathogen transmission occurred cannot be determined here, there is mounting evidence for the risk of *F. tularensis* infection from wild rodents, highlighting the need for pest rodent management in zoos.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

All data are given within the manuscript and its Supplementary material.

Ethical approval statement

The samples were collected during necropsy of an animal humanely euthanized according to animal welfare reasons. The animal was euthanized because of poor prognosis to avoid further pain and distress.

Ethical review and approval were not required for the animal study because all data used for this study were collected during clinical treatment and pathological examination and were obtained to diagnose the clinical case. All animal work followed relevant national and international guidelines. Good veterinary practice was followed in all procedures whenever animals were handled. Evaluation of data took place retrospectively. Written informed consent was obtained from the zoo owners for the participation of their animals in this study.

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