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Re-emergence of *Francisella tularensis* in Germany: fatal tularaemia in a colony of semi-free-living marmosets (*Callithrix jacchus*)

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SUMMARY

Francisella tularensis was identified as the cause of a die-off which occurred among a colony of semi-free-living common marmosets (*Callithrix jacchus*). During the outbreak 5 out of 62 animals died of tularaemia in a research facility located in the district of Goettingen, Germany. All animals had been born at the facility suggesting an endemic infection. A total of five culture isolates were recovered and characterized as *F. tularensis holarctica*, biovar I. These cultures represent the first isolates obtained in the Federal Republic of Germany for more than 45 years. The outbreak area shows several geographical and ecological characteristics known to favour long-term presence of *F. tularensis*. Persistence of the pathogen in the remote region along the former German–German border, continuous re-introduction from eastern European countries after destruction of the 'Iron curtain' or introduction through migrating birds are testable hypotheses which could explain the emergence of tularaemia in this particular region.

INTRODUCTION

Francisella tularensis is the causative agent of tularaemia, a severe bacterial disease with numerous enzootic and endemic foci [1, 2]. Human disease is primarily associated with two subspecies: *F. tularensis tularensis* (type A), which is highly virulent and found only in North America, and the less virulent *F. tularensis holarctica* (type B), which is thought to be endemic throughout the Northern Hemisphere [1]. In humans, tularaemia may present with various clinical forms depending on the route of inoculation and the virulence of the *F. tularensis* strain involved. The ulceroglandular form, an eschar-like cutaneous lesion and painful lymphadenitis, and the glandular form, in which the primary lesion is lacking, are the most common clinical pictures and are caused by handling of contaminated materials or bites of infectious arthropods. The oropharyngeal form develops following ingestion of contaminated food or water [3, 4]. Typhoidal and ocular diseases are less frequent, while inhalation of infectious aerosols containing minute amounts of *F. tularensis* can result in severe pulmonary symptoms including fatal pneumonia [5]. For this reason, *F. tularensis tularensis* is considered to be a biological warfare agent that poses a substantial risk to public health [6].

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In most countries, human as well as animal disease show seasonal patterns especially when examining arthropod-borne tularaemia. Contact with infected hares or rodents accounts for most cases of human tularaemia in Western and Central Europe as well as the European part of the former Soviet Union, where water-related cases are also common. In Fennoscandinavia, mosquito-, tick- and airborne infections have also been described. Large outbreaks are generally associated with direct contact with infected game or contaminated water. In North America, human infections are mainly caused by tick bites or contact with rabbits [1].

After a steady decline in case reports starting in the early 1960s, there is growing evidence of the emergence or re-emergence of tularaemia in several European countries (Denmark, France, Austria, Slovakia, Bulgaria, Croatia, Former Republic of Yugoslavia, Spain, Turkey) [3, 7–12] over the last decade. The cause of recent outbreaks in hitherto unaffected regions, some of which have involved hundreds of cases, is unknown.

In Germany, tularaemia in hares has not been reported to the Food and Agriculture Organization of the United Nations (FAO) since 1992 and notified human cases are in the range of only 0–5 cases per year (1960–2004). Historically, outbreaks of tularaemia in Germany have occurred in four federal states: Brandenburg, Mecklenburg-Western Pomerania, Bavaria and Schleswig-Holstein [13, 14]. Outside of these foci, human disease was reported only sporadically. In the district of Goettingen, Lower Saxony, which is located in the geographical centre of Germany, tularaemia has never been previously reported (Fig.).

In November 2004, an epizootic of tularaemia was identified as the cause of a die-off among a colony of semi-free-living common marmosets (*Callithrix jacchus*) in an ethological research facility in the district of Goettingen. Marmosets, New World primates originating from Brazil, have been rarely associated with tularaemia but have been shown to be extremely susceptible to infection with *F. tularensis* [15, 16]. We describe the epidemiological and laboratory findings of this unusual outbreak.

METHODS

Epidemiological and environmental investigation

From 14 September to 21 October 2004, five marmosets, housed in an outdoor area, died with signs of an acute and generalized infection within a few hours after the onset of symptoms. Initial microbiological investigations employing standard media and protocols revealed no specific results. Salmonellosis and yersinosis were excluded. An initially suspected *Arenavirus* infection was not confirmed. Histopathological findings were compatible with tularaemia and therefore specimens were sent to the German reference laboratory for *F. tularensis*. In the same period, two other animals died from a clinically different disease showing a more prolonged course without laboratory proof of *F. tularensis* infection.

Starting in early December, four visits were made to the epidemic site. Interviews and examinations were conducted at the facility to obtain relevant information regarding similar fatalities in the past, the environmental situation and basic epidemiological data.

At the outdoor area, 199 ticks were collected by flagging and 38 rodents were trapped next to the enclosures where the primates were housed in the winter months (November to April). Ticks were pooled resulting in 37 samples (5–10 larvae or nymphs/pool, 1–2 adults/pool). In addition to the ticks collected at the facility, fleas ($n=19$, two pools) and ticks ($n=105$, ten pools) found during the necropsy of the captured rodents were also examined. All samples were stored at $-20\ ^{\circ}C$.

Data on elevation, regional mean annual air temperature, precipitation and sunshine hours (1961–2004) were obtained from the Federal Meteorological Service (Deutscher Wetterdienst, Offenbach am Main, Germany, personal communication).

Laboratory investigations

Direct detection of F. tularensis

Dead marmosets were necropsied and all organs were immediately frozen and stored at $-20\ ^{\circ}C$. Specimens of the spleens and livers (all seven fatal cases) as well as of the captured rodents were homogenized using the FastPrep™ System (QBiogene, Heidelberg, Germany). Briefly, tissues were placed into 2-ml screw-cap tubes containing different amounts of ceramic medium; 0·9 ml sterile PBS was added, and the tubes were shaken vigorously for 20 s in the FastPrep™ instrument. All specimens were tested for the presence of *F. tularensis*-specific LPS using a capture (c)ELISA [17].

PCR amplification and product detection were performed in a LightCycler instrument (Roche, Mannheim, Germany). Real-time PCR protocols

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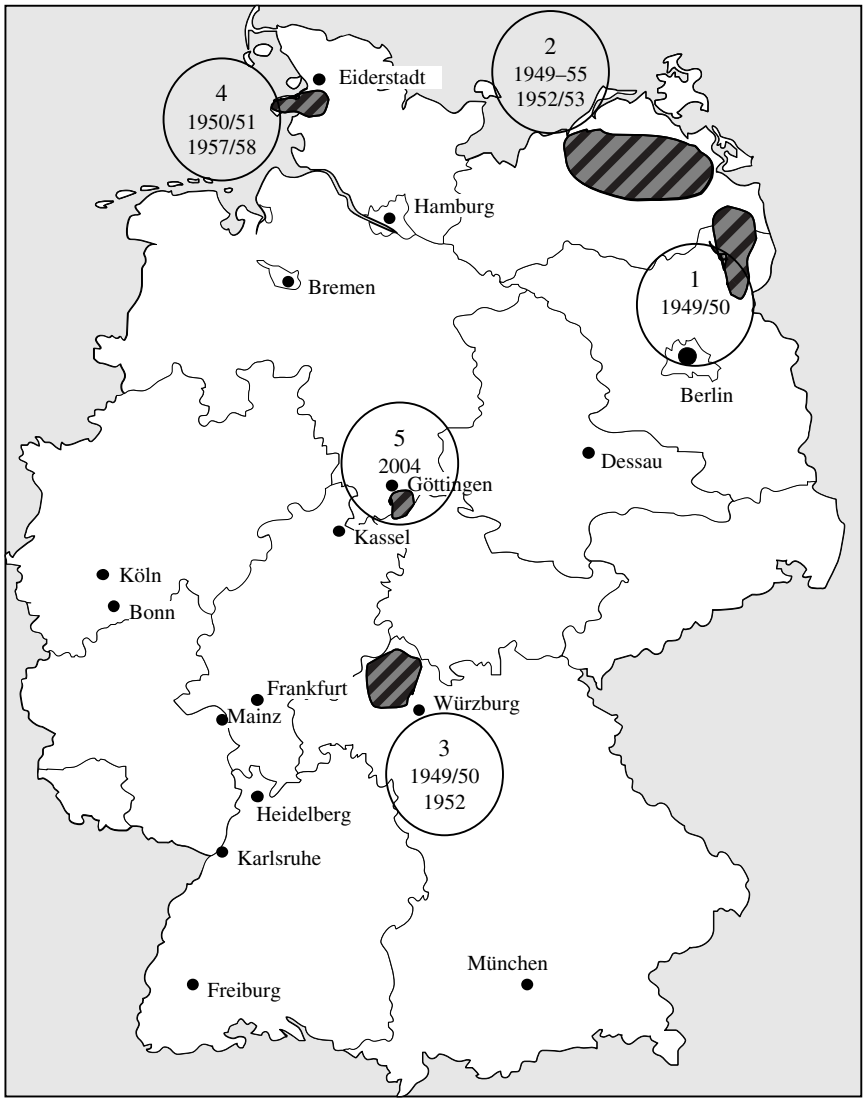

Fig. Location of tularaemia endemic regions within Germany. The time periods of the last notified outbreaks in humans and wildlife are given. 1, Brandenburg; 2, Mecklenburg-Western Pomerania; 3, Bavaria; 4, Schleswig-Holstein; 5, Lower Saxony.

targeting the *tul4* gene [18], and *iglC* and *ISFtu2* genes were performed as described previously [19]. Positive and negative controls were included in each run.

Homogenized arthropod samples were investigated using a commercially available real-time PCR kit (TibMolBiol, Berlin, Germany) for the specific detection of a nucleotide sequence within the 16S rRNA gene of *F. tularensis* [20].

Culture recovery of F. tularensis

For cultivation of *F. tularensis*, spleen and liver homogenates were used. An aliquot of 50 μl was spread onto cysteine heart agar supplemented with 9% sheep blood (HCA). Culture plates were then incubated at 37 °C for 10 days and investigated daily for bacterial growth. Additional aliquots were plated onto Columbia blood agar, McConkey agar and modified Thayer–Martin medium containing antibiotics (Merck, Darmstadt, Germany).

Colonies showing characteristic growth on HCA as well as typical bacteriological characteristics (motility, catalase, cefinase and oxidase activity, all reagents: Becton Dickinson, Heidelberg, Germany) were further investigated by a hand-held test kit (HHTK) based on an immunochromatographical method [17], slide agglutination with a *F. tularensis*-specific monoclonal antibody (mAb 11/1/6) [21] and *tul4*, *iglC* and *ISFtu2* PCR.

To confirm the presence of *F. tularensis* subspecies *holarctica*, partial sequencing of the 16S rRNA gene

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was performed [3, 20]. Additionally, multiple-locus variable-number tandem repeat analysis (MLVA) was completed for all isolates to verify the subtyping and to determine the genetic relationship of the isolates [8, 22].

In vitro antimicrobial susceptibility of the isolates was determined for 24 antimicrobial agents using Etest™ strips (Viva Diagnostics, Munich, Germany) on cysteine heart agar plates supplemented with 10% sheep blood [18].

Serological testing

Sera of all employees ($n=4$) working at the facility were examined by ELISA and immunoblot as previously described [23]. To screen for *F. tularensis*-specific antibodies in marmosets, a published competitive ELISA [24] was adapted using a LPS-specific peroxidase-conjugated monoclonal mouse antibody (clone 11-1-6) [21]. A decrease of more than 50% of the specific signal obtained with the monoclonal antibody was considered positive for the presence of anti-*Francisella*-specific antibodies.

Criteria for the confirmation of a F. tularensis *infection*

Marmosets or rodents were considered positive when *F. tularensis* was recovered from tissue samples. Animals were considered presumptive positive if tissues tested positive by cELISA or PCR, but no isolate was recovered. Animals were considered negative if all diagnostic tests (direct antigen detection, culture, serological testing) failed to detect any evidence of *F. tularensis* infection.

RESULTS

Descriptive epidemiology

The animal husbandry where the outbreak occurred is divided into an outdoor and an indoor area in the vicinity of Goettingen, Lower Saxony, Germany (Fig.). The facility is located within a region intensively used for agriculture. It was founded in 1994 and covers 60 000 m². In total, 62 marmosets were kept for ethological studies. All animals were born at the facility and had no contact with other primates imported from other parts of Germany or other countries. About 50% of the animals lived in the indoor area which consisted of various large cages within a closed building, preventing any contact with rodents or birds. From this group, only one subpopulation (12 animals, group D) had access to a series of small outdoor cages by leaving the building through a cross-barred tube.

The remaining marmosets represented the main ethological study group and lived freely in the outdoor area in three separated groups (A–C), each consisting of one family. Each primate carried a transponder which allowed monitoring of the animals' movement within the area by continuous data registration. From late spring to early autumn, escape from the facility was only prevented by the dense vegetation of grass and lawn of a height of 150–200 cm. Four persons were responsible for the care of all the primate groups which were regularly fed at the enclosures which were also the winter homes for the outdoor groups. The animals of all groups (outdoor and indoor) were fed with the same food and tap water and also received the same straw bedding material. The marmosets living outdoor were exposed to arthropods (e.g. ticks, mosquitoes) and rodents. Some primates were observed catching and eating mice or birds. Thus no rodent control using rodenticides was possible and consequently the rodent population within the facility was high. The whole area was also highly infested with ticks (*Ixodes* spp.). Starting in late August, the employees noticed dead rodents next to the enclosures. Additionally, carcasses were found on the walks connecting the main building and the outdoor area.

On 14 September, marmosets of the outdoor area began to die. Five animals showed a very similar clinical picture of an acute and fatal systemic disease and comparable histopathological changes of parenchymateous organs were observed. In addition to the main findings listed in Table 1, all cases showed a severe lymphadenitis particularly involving the submandibular lymph nodes. In the last two primates, which died at the end of October, the clinical course differed and deaths could not be attributed to tularaemia (Table 1).

Being study objects in the ethological research project, all animals had been intensively observed and telemetric data were available from the days and hours before the death of each animal. Interestingly, only three (groups B, C, D) of the four outdoor groups were affected. Most fatalities occurred in the south-eastern part of the facility which was located next to a small creek and bordered by trees.

Due to the fulminant course of disease, no treatment could be started. No signs of infection were noticed in any surviving primate. This outbreak was unique and

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Table 1. *Clinical course and histopathological findings in all seven marmosets* (Callithrix jacchus) *which died during the outbreak*

Onset of disease (time of death)	ID, sex	Clinical symptoms	Histopathological findings
14 September (same day)	1255, female	Apathy, hypothermia	Mild multifocal necrotizing hepatitis, severe mulitfocal haemorrhagic enteritis
16 September (same day)	1275, female	No obvious symptoms	Multifocal necrotizing and granulomatous hepatitis and spleenitis
25 September (same day)	1155, male	No obvious symptoms	Multifocal necrotising and granulomatous hepatitis and spleenitis, severe mulitfocal hemorrhagic enteritis
15 October (same day)	1267, male	Apathy, hyperthermia	Multifocal necrotizing and granulomatous hepatitis, mild spleenitis, lymphadenitis (Lnn. axillaries and submandibularis)
21 October (same day)	1238, male	No obvious symptoms	Multifocal necrotizing and granulomatous hepatitis
22 October (23 October)	1060, female	Fever, shaking, antibiotic treatment started, progressing dizziness	Chronic degenerative hepatitis, acute spleenitis, lung emphysema
20 October (27 October)	1198, male	Orchitis, cellulitis, apathy, antibiotic treatment started	Acute to peracute necrotising hepatitis, haemorrhagic orchitis, mulitfocal haemorrhagic enteritis, *E. coli* septicaemia (culture proven)

ID, Identification number of the animal.

similar cases had never been registered at the facility before September 2004.

After an infectious aetiology of the die-off was suspected and tularaemia was presumptively diagnosed, restriction of movement was introduced and all animals were contained in the enclosures normally serving as winter lodgings. Sanitation and food and water hygiene were improved with special emphasis on the prevention of water contamination with rodent urine and faeces. Next to the enclosures, the rodent population was reduced by clap-trapping. Lawn-mowing allowed an additional and even more pronounced reduction of the rodent population by the ubiquitous birds of prey living next to the facility.

Laboratory findings

Infection with *F. tularensis holarctica* was confirmed in five of the seven dead marmosets using at least three independent laboratory methods (Table 2). *F. tularensis*-specific LPS was detected by cELISA in spleen or liver homogenates from affected primates. Targeting three different genomic gene sequences, *F. tularensis*-specific DNA was detected in the same specimens which were positive in the cELISA. Although three specimens were heavily contaminated with a mixed bacterial flora, *F. tularensis* could initially be grown on HCA from two out of five PCR-positive samples. When additional plates containing antibiotics as well as antibiotic disks were used to suppress growth of contaminating bacterial populations, *F. tularensis* could be isolated from the tissues of each suspected case (Table 2).

Classical bacteriological methods and molecular methods were used to confirm the identification of the five isolates as *F. tularensis* and to further subtype the isolates into subspecies. In addition to characteristic growth on HCA, all strains were non-motile, weakly catalase- and β-lactamase positive and showed a negative oxidase reaction. Slide agglutination and a lateral-flow assay-based rapid test (HHTK) were positive. All applied PCR assays identified the isolates as *F. tularensis* strains (Table 3).

All five isolates were tested for their antimicrobial susceptibility to 24 antibacterial agents and demonstrated inhibitory concentrations consistent with those recently published for *F. tularensis holarctica* from Austria [18] with one exception: all strains were sensitive to erythromycin, characterizing the strains as *F. tularensis holarctica*, biovar I.

A fragment of the 16S rRNA gene (nucleotide position 355–1205 corresponding to *Escherichia coli* nomenclature) was sequenced from one strain. The sequence of isolate 1255 shared 100% homology with the sequence from *F. tularensis holarctica* (GenBank

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Table 2. *Laboratory findings in all seven marmosets* (Callithrix jacchus) *which died during the outbreak*

ID	Specimen	Ag cELISA	PCR (tul4, iglC, ISFtu2)	Bacterial culture
1255	Spleen	+	+/+/+	*F. tularensis holarctica*, mixed indigenous flora
1275	Spleen	+	+/+/+	*F. tularensis holarctica*, pure culture
1155	Spleen	+	+/+/+	*F. tularensis holarctica*, mixed indigenous flora
1267	Liver	+	+/+/+	*F. tularensis holarctica*, mixed indigenous flora
1238	Liver	+	+/+/+	*F. tularensis holarctica*, pure culture
1060	Liver	−	−/−/−	Mixed indigenous flora
1198	Liver	−	−/−/−	*Escherichia coli*, pure culture

ID, Identification number of the animal; Ag cELISA, antigen capture ELISA; +, positive result; −, negative result.

acc. no. L26086) and showed the Type B-specific signature nucleotide (nt 1153: A) [3, 20].

MLVA [22, 25] demonstrated identical repeat numbers at all 25 loci analysed and showed Ft-M19 fragments considered to be specific for *F. tularensis holarctica* (Type B).

Serological findings

F. tularensis-specific antibodies could not be detected in any of the surviving marmosets ($n = 55$), suggesting that exposure to *F. tularensis* resulted consistently in severe, fatal infection. Serological examination was also offered to all persons taking care of the primates during the time interval when the outbreak had occurred. Only one person, who remembered ulceration at the left ankle after a tick bite in early spring 2004, was seropositive in the ELISA and immunoblot.

Ecological study

The available epidemiological data revealed that tularaemia has never been reported from the county of Goettingen where the research facility is located (Fig.). The outbreak area is characterized by the environmental data summarized in Table 4. The weather data reported from 1960 to 2004 demonstrated a striking abnormality for the year 2003. This year was characterized by a very hot and extremely dry period with very low precipitation and long sunshine duration, especially during the summer months. Mean temperature in June and August 2003 (18·1 °C and 20·3 °C, respectively) was about 3 °C higher than the mean value in the 30-year period before. The lowest annual precipitation between 1994 and 2004 was recorded (464 mm), which was only 71% of the long-term mean precipitation (645 mm, 1961–1990). The sunshine duration in 2003 (1948 h) was about 30% higher than the average in a 30-year period (1421 h).

Table 3. *Characterization of the* Francisella tularensis *isolates obtained from the fatal tularaemia cases* (Callithrix jacchus) *during the Goettingen outbreak* (*all five cultural isolates showed the same features*)

Key features	Results
Slide agglutination with anti-*F. tularensis* mAb 11-1-6	+
Positive hand-held test kit (HHTK)	+
Cysteine required for growth	+
Growth on McConkey agar	−
Motility	−
Oxidase (Kovac's) activity	−
Catalase activity	+
β-Lactamase activity	+
Erythromycin sensitive	+
PCR positive for	
tul4	+
iglC	+
ISFtu2	+

+, Positive result; −, negative result.

Liver and spleen samples of 38 rodents or insectivores [seven *Sorex araneus*, five *Microtus* (*M.*) *arvalis*, *one M. agrestis*, three *Microtus* spp., six *Apodemus* (*A.*) *flavicollis*, nine *A. agrarius*, one *A. sylvaticus*, three *Clethrionomys glareolus*, and three rodents of the family of Arvicolidae from which the species was not determined] were examined for the presence of *F. tularensis*. In three rodents (*S. araneus*, *A. flavicollis*, *M. agrestis*), positive signals were obtained using PCR. To confirm the morphologically based host species determination of the animals, partial sequencing of mitochondrial cytochrome b was performed for *A. flavicollis* (GenBank acc no. DQ379300), and *M. agrestis* (GenBank acc no. DQ480084). Only one spleen sample (*S. araneus*) gave weakly positive signals using PCR, whereas *F. tularensis*-specific DNA was found in the liver of all three carcasses. The

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Table 4. *Geographic and ecological characteristics of the Goettingen outbreak area favouring long-term persistence of* Francisella tularensis

Characteristics of natural habitats correlated with a high number of tularaemia foci in the Czech Republic*		Geographic and ecological features of the outbreak region in Germany (1994–2004)
Elevation above sea level:	Up to 200 m	175 m
Mean annual air temperature:	8·1–10·00 °C	9·2 °C
Mean annual precipitation:	450–700 mm	642 mm
Habitat:	Alluvial forests	Single trees along a creek, alluvial forest-like
	Field biotopes	Field biotope, surrounded by areas of intensive agriculture
Mean annual sunshine duration:	2001–2200 h	1506 h

* In 2003, Pikula *et al*. [28] published a report describing the ecological conditions of natural foci of tularaemia in the Czech Republic. Comparing two 15-year periods, the authors proved that tularaemia persists in specific areas of natural foci. The general environmental features of theses foci can be defined and make it possible to predict the occurrence of tularaemia in other areas of suitable conditions. Using a geographic information system and a database on the natural foci of tularaemia, Pikula *et al*. could calculate the relation of these foci to various ecological conditions. The characteristics of habitats showing the highest correlation with the number of tularaemia foci are given in column 2. As shown in column 3, the outbreak region in Germany shared several features favouring the occurrence or persistence of *F. tularensis* in the environment.

PCR-positive specimens were cultured on HCA, blood, McConkey and Thayer–Martin agar, but no growth of *F. tularensis* was observed. The cELISA was negative in all samples.

Neither the 37 primary tick pools nor the ten pools of ticks and two pools of fleas collected from the rodent carcasses were PCR-positive.

DISCUSSION

In our study, we described the epidemiological findings and laboratory results from an unusual outbreak of fatal tularaemia among semi-free-living marmosets (*Callithrix jacchus*) in Central Germany, county of Goettingen. For the first time, tularaemia has been documented among non-human primates in Germany. This has been the first outbreak of tularaemia for about 40 years. To the best of our knowledge, *F. tularensis* has not been isolated within the Federal Republic of Germany for more than 45 years.

A murine tularaemia epizootic was the probable source of infection for the affected primates. The most likely route of transmission was ingestion of *F. tularensis* as all infected marmosets displayed striking involvement of submandibular lymph nodes, a typical sign of oropharyngeal tularaemia. All primates physically separated from the outdoor groups (approximately half of the population at the facility) were negative for *F. tularensis* infection, although risk factors commonly known in animal husbandry like straw bedding of cages, tap water and food were virtually identical in both major groups. The finding that captured rodents but not arthropods harboured *F. tularensis* supports our hypothesis that ingestion of *F. tularensis* infected rodents or contact with contaminated water or food was the route of transmission. Consequently, containment in the winter refuges and food protection were suitable measures to stop the outbreak.

In 1989, a very similar incident occurred in the Cheyenne Mountain Zoo, Colorado Springs, United States. Twelve non-human primates of four species developed typhoidal tularaemia during a 6-week epizootic in a zoological collection. Clinical signs and histological findings were almost identical to our observations. At that time, a murine tularaemia epizootic preceding the outbreak was identified as the source of infection with *F. tularensis holarctica* [26]. In Europe, two cases were reported affecting New World primates. In 1996, septicaemic tularaemia was observed in a Swiss marmoset found moribund in an outdoor cage. Acute necrosis in the jejunum, oropharyngeal, cercical and mesenterical lymph nodes suggested an oral route of infection. None of the six cage mates showed any evidence of *F. tularensis* infection. The source of infection remained obscure [16]. Another case of fatal typhoidal tularaemia occurred in a captive golden-headed lion tamarin (*Leontopithecus chrysomelas*) in the Zoo of Zurich, Switzerland. Once more, no definite source of infection could be found [15]. As in our case, serological studies had confirmed that no subclinical cases had occurred and that tularaemia seems to be a severe, unvaryingly fatal disease in New World primates.

Finally, our study confirmed the re-emergence of this highly infectious pathogen in Central Germany which is of major public health concern. In Germany, a central European country, with a very high population density and intensive agriculture, tularaemia has long been assumed to be virtually absent. Hence, most physicians in Germany are not aware of tularaemia being a differential diagnosis for patients of typical populations at risk (e.g. hunters or farmers). Therefore, diagnosis is often delayed and the frequency of reported cases is low when compared to neighbouring countries like the Czech Republic, Austria, and France. This problem was exemplified in the current study, where we could disclose a hitherto unknown human case of tularaemia. Current data on the epidemiology or the distribution of natural foci in Germany are not available. This fact is critical regarding the extraordinary importance of *F. tularensis* as a category A select agent [6]. The unexpected emergence of an unusual infection must lead to an intensive investigation to rule out an intentional release, e.g. by a bioterrorist attack. For this reason, data on the natural 'background level' of dangerous pathogens are urgently needed. Increasing awareness and the application of new and more efficient diagnostic tools will overcome the currently assumed underreporting of tularaemia in Germany [27].

The confirmation of autochthon infections in Germany will also stimulate the historical discussion about the origin of tularaemia in Central Europe. Our analysis demonstrated that the outbreak area showed favourable ecological conditions for the manifestation of a natural focus [28] (Table 4).

According to Jusatz, tularaemia was introduced into Central and Western Europe very recently [14]. Starting in the early 1920s in Western Siberia, it spread in three waves and reached France and Turkey at the end of World War II. Alternatively, tularaemia might have been spread over the whole continent (except for Great Britain) for several centuries, being capable of re-emerging in endemic foci favouring enzootic cycles [29]. Both hypotheses could explain why tularaemia occurred at this particular place near Goettingen. The area used to be a very rural and rarely visited region due to the former inner German border which had been located at a distance of only 2 km and prevented any traffic to neighbouring eastern counties. A swathe of land about 500–700 m in width and 1378 km in length, located at both sides of the demarcation line could not be used for agriculture for more than 40 years. This remote area might have served as a natural biotope for *F. tularensis*. Tularaemia could have cycled here in suitable hosts unobserved for decades. The abrupt onset of the disease in 2004 might be explained by the sharp rise in rodent density (gradation) observed in Germany, Belgium and France in this year. Interestingly, a rise in other rodent-associated diseases (e.g. Hantavirus infection) was noticed simultaneously in all three countries [30].

Otherwise, re-introduction of tularaemia from Eastern Europe, after the borders were removed in 1990, could not be excluded. Migrating game, boars (*Sus scrofa*), foxes (*Vulpes vulpes*), diverse species of rodents or new species like the raccoon-dog (*Nyctereutes procyonoides*) might carry the pathogen over long distances.

However, the comparison of the reported outbreak in Germany with the described cases in Switzerland, the recent description of the first tularaemia case in Denmark, the repeated outbreaks in Spain, Bulgaria, Kosovo and in the Turkish Black Sea area [3, 7–11] give support to a third hypothesis: the transmission of *F. tularensis* by migrating birds. Migratory birds could be involved in the dispersal of *F. tularensis* as infected vectors or as mechanical carriers of infected haematophagous ectoparasites (e.g. *Ixodes* spp.). Many pathogens of homeothermic vertebrates, including humans, are associated with free-living migratory birds [31]. It has to be assumed that rodent and tick populations of a non-endemic area are very susceptible to a newly introduced highly infectious and virulent pathogen. This may explain the spot-like or patchy emergence of tularaemia in hitherto unaffected areas.

Thus, in contrast to the current opinion, we propose that true endemic areas of *F. tularensis* are very rare. The pathogen may totally disappear from an adverse habitat where it was able to establish itself for a short period of time. Consequently, *F. tularensis* was not continuously endemic in Central Europe but was repeatedly re-introduced by the irregular invasions of migratory birds normally living in the endemic areas of Fennoscandinavia. The finding that *F. tularensis* is nowadays totally absent from the German Eiderstedt region at the North Sea coast (Fig.) supports this idea [32]. This hypothesis has to be proved by comparing the genetic and spatial diversity patterns among the European *F. tularensis holarctica* strains as recently done for *F. tularensis* in the United States [25].

CONCLUSION

We describe the re-emergence of *F. tularensis* in Germany affecting highly susceptible non-human primates. After almost 50 years, *F. tularensis* was isolated in Germany again. Isolates are now available for further genetic studies to locate the origin of the German *F. tularensis holarctica* biovar I strains within Europe.

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DECLARATION OF INTEREST

None.

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