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Original article

Clinical characteristics of and diagnostic approaches to human *Francisella tularensis* infection: a retrospective, monocentric case study from Germany

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ABSTRACT

Francisella tularensis, the causative agent of tularemia, poses a challenge for diagnosis and treatment due to its diverse clinical presentations and low incidence. Hence, the awareness among clinicians is comparatively low. This study reports the clinical characteristics, diagnostic approaches, and treatment outcomes of tularemia cases at one tertiary center in Germany over a 12-yearperiod.

This retrospective monocentric case series considered all tularemia cases diagnosed at Saarland University Medical Center in Homburg, Germany between January 2013 and December 2024. Cases were identified from electronic medical records, and the certainty of tularemia was graded as definite, probable and possible infection, based on results of serology, polymerase chain reaction (PCR) assays, or blood cultures. Clinical data were extracted from patient records and supplemented by follow-up information from the clinicians.

We identified 14 tularemia cases, including 6 definite as well as 3 probable and 5 possible cases. The clinical presentation was highly variable, with the (ulcero-)glandular form being the most common entity (10/14). Invasive diagnostics or surgery were required in eleven out of 14 patients. Initial misdiagnosis was common, leading to delayed diagnosis and multiple courses of ineffective antibiotics. Definite treatment included fluoroquinolones or doxycycline, and led to resolution of symptoms in most patients.

The varied clinical manifestations of tularemia, from classic (ulcero-)glandular forms to severe and atypical presentations illustrate its diagnostic and clinical complexity. Enhanced awareness and early consideration are crucial, especially in endemic areas or patients with anamnestic environmental exposures.

1. Introduction

Infections due to *Francisella tularensis*, the causative agent of tularemia, represent a complex and challenging differential diagnosis among clinical infectious diseases. This bacterial disease mainly occurs in the northern hemisphere (especially in the United States and some European and Asian countries) and is almost absent from tropical and southern regions (Yeni et al., 2021). Three distinct subspecies of *F. tularensis* exist, i.e. *tularensis* (type A, the most virulent form, which mainly occurs in Northern America (Gurycová, 1998)), *holarctica* (type B, the most widespread form) and *mediasiatica* (mainly found in Central Asia) (Sjöstedt, 2007). *Francisella novicida,* previously classified as a subspecies of *F. tularensis*, is now generally regarded as a distinct species, although this classification remains a topic of debate (Larsson et al., 2009). Notably, *F. novicida* is not considered an etiological agent of tularemia.

In Europe, tularemia accounts for approximately reported 800 cases annually, with the highest notification rates in Sweden, Norway, Finland, France and Germany. ("Surveillance Atlas of Infectious Diseases", https://atlas.ecdc.europa.eu/public/index.aspx) Transmission of *F. tularensis* occurs through multiple routes, including ingestion of contaminated food or water, handling of infected animals, arthropod bites (e.g. ticks, mosquitoes), aerosol exposure from contaminated dust, and accidental inoculation or exposure in laboratory settings (Maurin

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and Gyuranecz, 2016; Sjöstedt, 2007). The aquatic environment also represents a potential and frequently encountered source of human infection. The bacterium is able to survive for weeks in cold, moist environments such as water, soil, and decaying animal carcasses. Due to its potential for aerosolization and its low infective dose, *F. tularensis* was classified as a potential biowarfare agent, and it is indeed suspected that it may have been used as a biological weapon by Japan during World War II (Dennis et al., 2001; Hirschmann, 2018; Maurin, 2015; Oyston et al., 2004).

The clinical features of tularemia are many fold, with an incubation period typically ranging between 3 and 5 days, but extending up to 21 days depending on the mode of infection and infective doses. Early signs often resemble flu-like symptoms, including fever, fatigue, chills, and headache (Ohara et al., 1991). Depending on the transmission route, different clinical forms of the disease occur, including oropharyngeal, glandular/ulceroglandular, oculoglandular, pneumonic and typhoidal presentations, which are characterised by distinctive patterns (Ellis et al., 2002; Evans et al., 1985; Maurin and Gyuranecz, 2016):

1. The glandular and ulcero-glandular forms present with local, sometimes painful lymphadenopathy, with the latter variant involving a skin inoculation ulcer. These forms typically occur through skin inoculation of *F. tularensis*, which can happen via arthropod bites, animal bites, direct contact with infected animals, or exposure to contaminated vegetation, soil, or other environmental sources.
2. The oropharyngeal form is characterized by chronic pharyngitis and typically follows ingestion of contaminated water or food. Hand-borne oral contamination may also occur.
3. The oculo-glandular form manifests as conjunctivitis accompanied by local lymphadenopathy and results from contamination of the conjunctiva.
4. The pneumonic form involves lung infection and arises following inhalation of the bacteria or through haematogenous spread as a secondary pulmonary involvement of e.g. ulcero-glandular disease (Vacca et al., 2024).
5. The typhoidal form exhibits severe systemic symptoms (including organ failure and a septic shock) and can develop as a rare but life-threatening complication in patients with tularemia.

The distribution of these patterns varies significantly among different regions. In France, more than 50 % of cases are (ulcero-) glandular forms, while 10–15 % are pneumonic, 5–15 % are oropharyngeal, less than 5 % are oculo-glandular, and less than 10 % are typhoidal (Darmon-Curti et al., 2020; Mailles and Vaillant, 2014; Maurin et al., 2011). In Germany, the (ulcero-) glandular form is also by far the most common presentation of tularemia (Faber et al., 2018a; Hestvik et al., 2015). The mortality of infections due to *F. tularensis* ssp. *holarctica* is low (less than 1 %), while for *F. tularensis* ssp. *tularensis*, the case fatality rate can be up to 30–60 % for pneumonic or typhoidal forms without effective antibiotic therapy (Kugeler et al., 2009; Maurin and Gyuranecz, 2016).

Prevention measures focus primarily on avoiding contact with the bacterium. These measures include avoiding consumption of untreated surface water, using insect repellents to prevent arthropod bites, employing appropriate precautions when handling potentially contaminated biological samples and handling contaminated animals or their carcasses. A vaccine against *F. tularensis* is not available (Maurin and Gyuranecz, 2016).

The laboratory diagnosis of tularemia can be challenging due to its rarity and the frequently nonspecific symptoms. Laboratory confirmation typically involves molecular or serological methods, as cultivation of the bacterium is slow and requires specialised facilities. *Francisella tularensis* specific IgM and IgG antibodies become positive almost in parallel after 6 to 21 days after onset of the disease and both classes can remain positive for up to three years (Eliasson et al., 2008). To distinguish between acute and past infections, it is recommended to test two serum samples collected at least two weeks apart. A seroconversion or a fourfold or greater rise in antibody titers between the two samples is considered a diagnostic confirmation. Treatment of tularemia relies on antibiotic therapy, with fluoroquinolones, aminoglycosides, or tetracyclines (Maurin and Gyuranecz, 2016; Tomaso et al., 2017; World Health Organization, 2007). Prompt treatment significantly reduces the associated morbidity, underscoring the importance of early recognition and intervention in mitigating the impact of this pathogen.

In Germany, seroprevalences range from 0.2 % to 2 %, with higher rates observed among risk groups, such as hunters (Maurin, 2020). Research on seroprevalence suggests that tularemia is likely significantly underdiagnosed (Splettstoesser et al., 2009). This underdiagnosis could be due to a combination of factors: a low level of awareness among healthcare providers and a high proportion of mild or atypical courses that do not result in further diagnostic testing and resolve without antibiotic treatment.

To reflect the real-life clinical practice challenges caused by the diverse spectrum of disease presentations, we present the clinical and diagnostic features of all tularemia cases diagnosed at one German University medical center over a 12-year period.

2. Materials and methods

This retrospective single-centre case series was conducted at Saarland University Medical Center in Homburg, southwest Germany, covering the period from 1 January 2013 to 31 December 2024. No ethical approval was required for this retrospective analysis of patient data obtained during routine diagnostics.

Case identification was performed by searching the electronic records of the laboratory software used in the Institute for Medical Microbiology and Hygiene at Saarland University Medical Center (M/LAB, Dorner; Müllheim, Germany). The following patients were eligible for inclusion: All cases with either a positive serology, PCR, or positive blood culture for *F. tularensis*.

Serological testing was conducted using an immunochromatographic rapid antibody test (ICT) employing the Virapid Tularemia assay (Vircell; Granada, Spain). In accordance with the manufacturer's instructions, the results were determined visually using semi-quantitative measurements, with values >0.5 being considered positive. Positive results were always confirmed by further serological tests, which were in most cases conducted at the German National Reference Laboratory for tularemia in Munich. Such confirmatory testing included an initial polyvalent ELISA screening test for *F. tularensis* IgG/IgA/IgM antibodies, followed by a confirmatory immunoblot for *F. tularensis* lipopolysaccharide (LPS)-specific IgG antibodies. To further differentiate antibody subclasses, an additional ELISA was performed using the Virion/Serion *F. tularensis* LPS IgG/IgM ELISA (Virion/Serion, Germany). In selected cases, PCR testing was initially performed using the FilmArray® BioThreat Panel (BioFire Defense; Salt Lake City, United States of America), a multiplex PCR test that analyses 16 biohazardous pathogens and toxins, including *F. tularensis*. Although this method is not officially validated for this indication, it has previously been used successfully in our institute for the diagnosis of tularemia (Roth et al., 2021). Due to the lack of validation, all positive results were subsequently confirmed by validated species-specific PCR assays conducted at external reference laboratories. For species-level confirmation, a real-time PCR targeting the *F. tularensis* 16S rRNA gene was employed (LightMix®, TibMolbiol/Roche). Subspecies differentiation was performed using an in-house real-time PCR assay targeting the 23S rRNA gene of *F. tularensis* (unpublished).

Clinical data of the patients were retrieved from electronic medical records (R/3 IS-H/is-h*med, SAP; Walldorf, Germany), including demographics, principal symptoms, laboratory results, imaging findings, treatment modalities, and outcomes. Additional information was obtained through telephone calls with the attending physicians.

Patients were grouped into three categories based on the level of

diagnostic certainty:

- *Definite tularemia:* Cases with positive *F. tularensis* culture or PCR from blood culture or biopsy
- *Probable tularemia:* Cases with positive serology and typical clinical presentation (e.g., ulcers, fever, lymph node enlargement)
- *Possible tularemia:* Cases with positive serology (confirmed in an external reference laboratory), but atypical, not highly suggestive clinical presentation

3. Results

3.1. Epidemiology of F. tularensis cases

A total of 14 cases occurred in the study period; 6 patients (43 %) had definite tularemia, 3 patients (21 %) had probable and 5 patients (36 %) had possible tularemia.

There were more male than female patients (8 versus 6). The age distribution displayed in Fig. 1 was comparable to that of officially notified cases across Europe.

An increase of cases over time was observed, which was in accordance with overall data from Germany (Fig. 2).

3.2. Clinical and diagnostic characteristics

The major symptoms and clinical signs of patients included in this study as well as diagnostic and treatment modalities and individual outcome are presented in Table 1.

The route of transmission remained unclear in 8 out of 14 patients (57 %). These patients did not report any specific risk factors associated with tularemia, like occupational exposure or outdoor activities. Among the remaining cases, 2 patients reported tick bites (14 %), 2 had been bitten by unidentified insects (14 %), one patient had contact with dead hares during a camping trip (7 %), and another patient, who was a falconer, had regular contact with various wild animals in a local forest (7 %).

Among the ten patients presenting with glandular forms of tularemia, the localisation of the lymph nodes was as follows:

- Cervical lymphadenopathy: six patients
- Inguinal/proximal thigh lymphadenopathy: four patients
- Central lymphadenopathy: one patient

Fever was documented in six patients, highlighting its presence as a common, but not universal symptom. Night sweat or weight loss were reported in three patients, indicating that systemic symptoms suggestive of malignancy or tuberculosis can occur.

Fig. 3 illustrates the 'diagnostic delay', i.e. the time between the first medical consultation and the correct diagnosis. In 5 patients (36 %), the diagnosis was made within one month (in 3 of them within one week). In contrast, 3 patients (21 %) were diagnosed within 1 to 3 months, while 6 patients (43 %) took more than 3 months to be diagnosed.

Invasive diagnostics or surgical interventions were performed in eleven patients (79 %). 11 out of 14 patients (79 %) required hospitalisation due to the severity of symptoms (e.g. abscesses) or for diagnostic procedures (e.g. biopsy of lymph nodes).

3.3. Detailed appraisal of uncertain cases of F. tularensis infection

In one case (F, 60), a patient with pre-existing IgA nephritis presented with acute-on-chronic kidney injury, headache, malaise, enteritis, pleural and pericardial effusions. Despite extensive microbiological and virological diagnostics, including repeated blood cultures, mycobacterial testing, and comprehensive serological analyses, no causative pathogen could be identified except for a positive *F. tularensis* serology. Empiric antimicrobial treatment with piperacillin/tazobactam was initiated and subsequently escalated to meropenem, vancomycin, and caspofungin. Following the positive serology, therapy was switched to levofloxacin. Kidney biopsy revealed interstitial nephritis with non-necrotizing epithelioid cell granulomas, consistent with an infectious or post-infectious process. Although interstitial nephritis is not a typical presentation of tularemia, it has been previously described in the

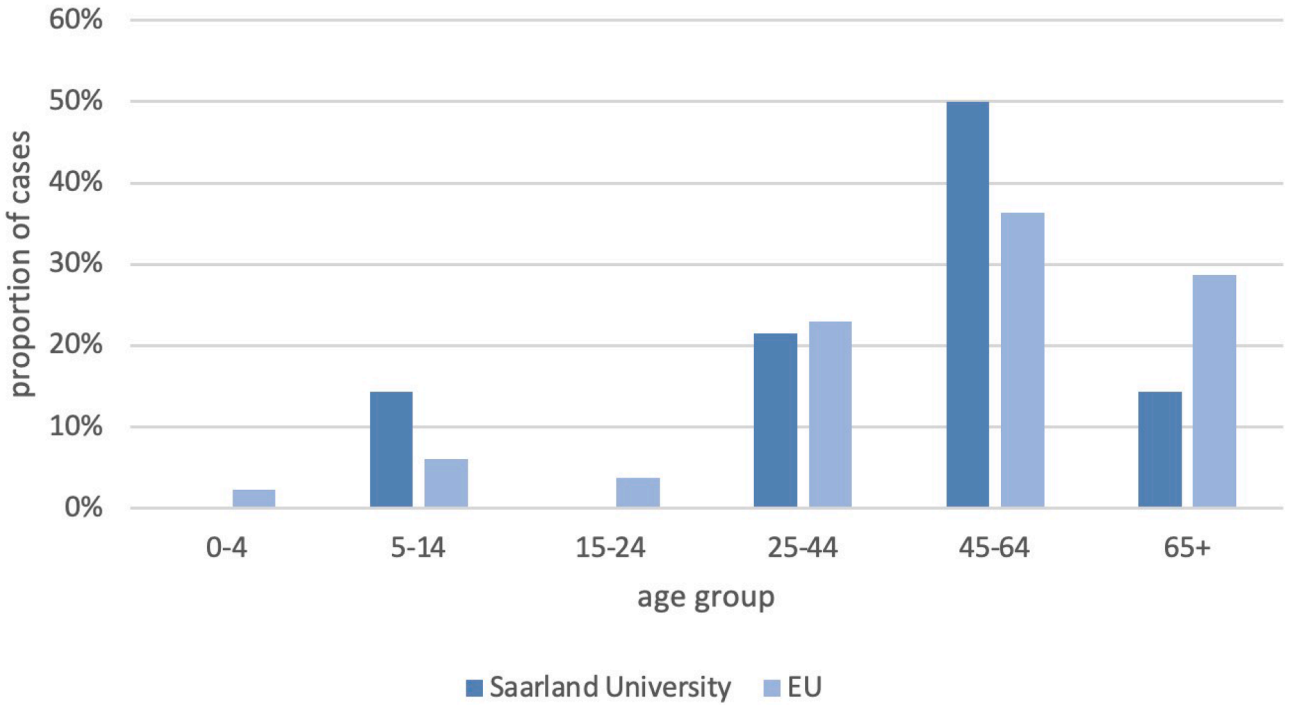

Fig. 1. Age range distribution (in years) of patients with tularemia at one hospital in southwest Germany as compared to European data from 2022 put forth by the European Centre for Disease Prevention and Control (ECDC) (Surveillance Atlas of Infectious Diseases, https://atlas.ecdc.europa.eu/public/index.aspx).

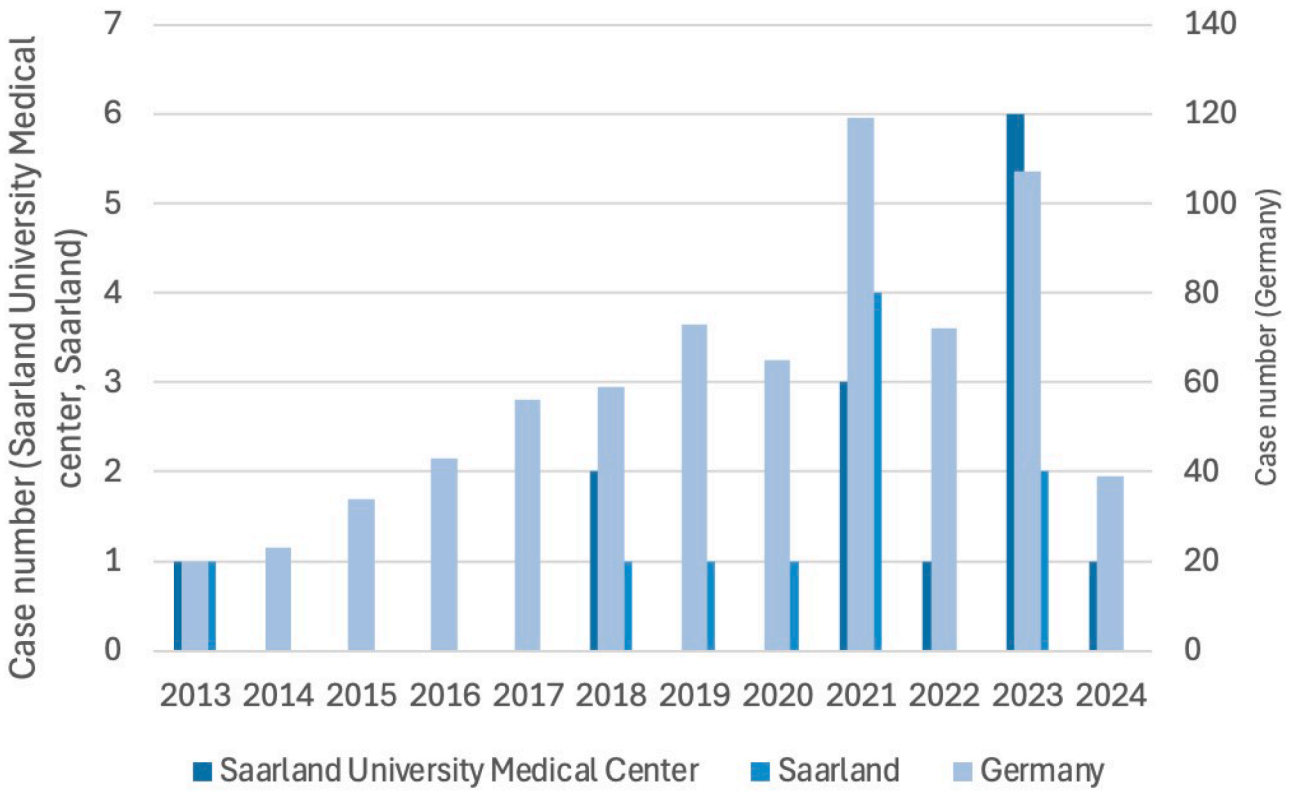

Fig. 2. Number of notified tularemia cases over time in Germany, in the German federal state of Saarland and one hospital in southwest Germany (Robert Koch-Institut: SurvStat@RKI 2.0, https://survstat.rki.de).

literature (Tilley et al., 1983).

Another notable case (F, 48) concerned a patient who presented with a septic picture, characterized by fever, a white blood cell count >50,000/µL, and a CRP value >300 mg/L. Clinically, the patient exhibited extensive ulcerations on both legs and neurological impairment. Initial antimicrobial therapy with meropenem and linezolid did not lead to clinical or laboratory improvement. The patient reported that the skin lesions had developed following a hornet sting. Imaging revealed enlarged inguinal lymph nodes. Given the history of an insect bite, serological testing for *F. tularensis* was performed and yielded a strongly positive result. Microbiological cultures from wound swabs and tissue samples showed colonization with various pathogens, but no definitive infectious agent could be identified. PCR testing for *F. tularensis* from a skin biopsy was negative, likely due to the superficial nature of the sample. Given the critical clinical condition, a treatment with ciprofloxacin was initiated, leading to a remarkable decline in inflammatory markers and clinical improvement. During the hospital stay, the patient was additionally diagnosed with varicella-zoster virus encephalitis, but this finding did not fully explain the severity of the clinical course. Although it cannot definitively be confirmed that tularemia was the underlying cause, it remains a plausible contributor to the clinical presentation.

In two cases (M, 74; M, 63), *F. tularensis* serology was positive, but no acute clinical symptoms indicative of active tularemia were present at the time of diagnosis. Both patients had no history of specific treatment for tularemia, suggesting that these were likely earlier infections with mild clinical courses that resolved spontaneously without targeted antimicrobial therapy.

3.4. Antiinfective treatment information

Treatment regimens varied among the patients: nine patients received fluoroquinolones, with ciprofloxacin being the primary agent, two patients were treated with doxycycline and two patients did not receive *F. tularensis*-specific therapy, likely due to initial diagnostic uncertainty or spontaneous symptom resolution. In one patient, a second course of antibiotics was necessary due to persisting lymphadenitis.

4. Discussion

Francisella tularensis remains a challenging pathogen to diagnose and manage due to its diverse clinical presentations and relatively low prevalence. This case series sheds light on the clinical characteristics, diagnostic methods, and treatment approaches of tularemia cases encountered at Saarland University Medical Center, Germany, between January 2013 and December 2024.

Our case presentations underline the diverse clinical manifestations of tularemia, which represent a key challenge in the diagnosis and treatment of this infection. Classic mild presentations, such as glandular and ulcero-glandular forms, were noted, characterized by local lymphadenopathy and skin inoculation ulcers. However, the series also included severe cases, such as a patient with a bloodstream infection presenting with severe epigastric pain and high fever, and cases with atypical presentations.,

Initial misdiagnosis was common, with some patients suspected of having flu-like illnesses, malignancies (due to symptoms like fever, weight loss, or night sweats), or tuberculosis. These diagnostic challenges often resulted in delayed diagnoses, with many patients undergoing several courses of ineffective antibiotics or surgical procedures before appropriate serological testing confirmed or suspected tularemia.

The frequently extended time to diagnosis in tularemia cases is likely due to a combination of various factors. Initially, the symptoms of tularemia, such as fever, chills, and fatigue, are nonspecific and can easily be mistaken for a common viral or bacterial infection. This misinterpretation often led to the prescription of broad-spectrum antibiotics like amoxicillin-clavulanic acid or clindamycin, which are

Table 1
Patient characteristics and major symptoms of *Francisella tularensis* infections in one hospital in southwest Germany. Data on specific diagnostic investigations, treatment and outcome are displayed. M, male; F, female.

Patient	(Presumed) disease form	Symptoms	Diagnostics					Therapy & outcome
			Laboratory values	Blood culture	Biopsy/tissue specimen	Serology	Histopathology	
Definite tularemia								
M, 68	Typhoidal	Epigastric pain, fever	CRP 112 mg/l, White Blood Cell Count (WBC) normal	Positive	Not done	Negative	Not done	Ciprofloxacin (duration and outcome unknown)
F, 14	(Ulcero-) glandular	Refugee from Ukraine, lived in the forest for a long time during the flight, reported many tick bites. Lymphadenopathy and abscess of the thigh	CRP 11 mg/l, WBC 12,4 × 10^9 /l	Not done	Positive PCR in lymph node tissue (FilmArray® BioThreat Panel, external laboratory)	Positive (IgM, IgG)	Epithelioid cell granulomatous inflammation with partial necrosis	Doxycycline for 14 days → resolution of symptoms (lymphadenopathy, abscess)
F, 34	(Ulcero-) glandular	Presented with swelling of the thigh, formation of abscess. Multiple operations with VAC therapy and several courses of ineffective antibiotics	WBC normal, CRP 23 mg/l	Not done	Positive PCR in lymph node tissue (FilmArray® BioThreat Panel, external laboratory)	Positive (IgG, IgM negative)	Acute and chronic granulating inflammation	Ciprofloxacin for 14 days → resolution of lymphadenitis
F, 10	Oro-pharyngeal	Ulcers of the nose, cervical lymphadenopathy, headache, sore throat, fever	WBC and CRP normal, slightly elevated liver enzymes	Not done	Positive PCR in lymph node tissue (external laboratory)	Positive	Ulcerative inflammation of the skin, reactive changes in the lymph nodes	Ciprofloxacin for 14 days → outcome unknown
M, 48	(Ulcero-) glandular	Cervical lymphadenopathy, several diagnostic lymph node extirpations (due to suspicion of tuberculosis or malignancies)	WBC normal, CRP 30 mg/l	Not done	Positive PCR in lymph node tissue (external laboratory)	Positive	necrotising granulomatous inflammation	Doxycycline → no residual lymphadenopathy, no fistula
F, 30	Oculo-glandular	Persistent cervical lymphadenopathy with submandibular abscess, sialadenitis of the parotid gland and conjunctivitis. Onset after tick bite.	WBC 14 × 10^9/l, CRP 15 mg/l	Not done	16S rRNA sequencing (wound swab lymph node) indicating *F. tularensis*	Positive (IgM, IgG)	necrotising granulomatous inflammation	Ciprofloxacin → lymphadenitis did not regress → second antibiotic course with addition of gentamicin
Probable tularemia								
M, 54	(Ulcero-) glandular	Persistent cervical lymphadenopathy after respiratory infection, no ulceration, no fever	WBC, CRP normal	Not done	Not done	Positive (IgM, IgG)	necrotising granulomatous inflammation	Ciprofloxacin for 14 days → outcome unknown
M, 54	(Ulcero-) glandular	High fever, inguinal lymphadenopathy, night sweat, onset after arthropod bite	WBC normal, CRP 8 mg/l	Negative	Not done	Positive (IgM, IgG)	Not done	Ciprofloxacin for 14 days → clinical improvement, regression of lymphadenopathy
M, 47	(Ulcero-) glandular	Initially respiratory infection, then cervical lymphadenopathy for four weeks, accompanied by weight loss. Lymph node extirpation was performed due to suspected malignancy	WBC, CRP normal	Not done	Not done	Positive (IgM, IgG)	Not available	No information available
Possible tularemia								
F, 48	(Ulcero-) glandular	Massive ulcerations on both legs after bite of an unknown insect, also unclear neurological symptoms. Septic patient with highly elevated inflammation parameters. No improvement under antibiotic therapy with meropenem, linezolid, clindamycin.	WBC >50 × 10^9/l, CRP 80 mg/l (max 250 mg/l)	Negative	Negative PCR in skin biopsy (external laboratory), not performed on lymph node tissue	Positive (IgM, IgG)	Abscessing inflammation in skin biopsy	Prolonged treatment in the intensive care unit with several complications (including candidemia), but significant clinical and laboratory improvement after 14 days of ciprofloxacin administration
F, 60	Not clearly attributable	Granulomatous interstitial nephritis most likely of infectious origin, enteritis, pericardial and pleural effusion.	Initial CRP 300 mg/l, WBC 10 × 10^9/l	Negative	Not done	Positive (IgM, IgG)	interstitial nephritis with epithelioid cell granulomas	Slight improvement after levofloxacin therapy, but persistent impairment of renal function

(*continued on next page*)

Table 1 (*continued*)

Patient	(Presumed) disease form	Symptoms	Diagnostics					Therapy & outcome
			Laboratory values	Blood culture	Biopsy/tissue specimen	Serology	Histopathology	
M, 74	(Ulcero-) glandular	Enlarged lymph node in the groin noticed during inguinal hernia diagnostics. Most likely previous infection, no further clinical information available.	CRP, WBC normal	Not done	Not done	Positive (borderline IgM, positive IgG)	Not done	No information available
M, 42	(Ulcero-) glandular	Presentation with central lymphadenopathy, suspected metastasised bronchial carcinoma or sarcoidosis, B symptoms, in particular night sweats. Had contact with dead hares during a camping holiday, followed by fever, night sweats, fatigue. At the time of presentation mainly fatigue. CT scan suspected myocarditis/pericarditis and central lymphadenopathy. Diagnosis of malignancies (lung and lymph nodes) without findings.	WBC max 15 × 10^9/l, CRP max 80 mg/l	Negative	Not done	Positive (IgM, IgG)	Florid erosive inflammation with granuloma formation	Treatment with ciprofloxacin was recommended, in control scan regression of lymphadenopathy
M, 63	(Ulcero-) glandular	Patient with unclear motor neurone disease who presented with a massively enlarged cervical lymph node. *F. tularensis* serology was positive, but the lymph node tissue also showed a positive PCR for *Mycobacterium tuberculosis* complex, so the leading cause of the lymphadenopathy remained unclear.	WBC normal, CRP 6 mg/	Not done	PCR in lymph node tissue negative (external laboratory)	Positive (IgM, IgG)	Chronic necrotising inflammation	Tuberculostatic treatment, no therapy for tularemia

ineffective against *F. tularensis*. As a result, the early flu-like symptoms might subside spontaneously during this ineffective therapy, possibly leading to the false impression of therapeutic efficacy. In reality, this initial improvement is likely due to the natural course of the disease. At such early stages, further diagnostics were seldom performed. By the time when patients present with persistent fever or lymphadenopathy, they are less likely to recall recent tick or mosquito bites or other possible environmental exposures. Of note, weight loss and night sweats, symptoms typically associated with chronic conditions, were more common in our cohort than in other tularemia case series, which may have further complicated the diagnostic process. As a result, clinicians, focusing on the presenting symptoms, often suspected chronic or more severe conditions such as malignancies or tuberculosis rather than tularemia. A case series on pediatric tularemia cases demonstrated similar diagnostic challenges, although the average time to diagnosis was shorter (a median of 10 days with a range from 1 to 40 days). Of note, 80 % of the reported patients initially received empiric treatment with beta-lactam antibiotics or clindamycin (Schöbi et al., 2022). In contrast, in Sweden, where the incidence of tularemia is significantly higher, physicians are more familiar with the disease, leading to earlier diagnoses and more appropriate treatment. This familiarity has contributed to fewer misdiagnoses and complications, as patients are treated more rapidly. Although alternative presumptive diagnoses such as unspecific viral infections, bacterial skin or soft tissue infections, and viral or bacterial respiratory infections are still common at initial presentation in Sweden, timely recognition and intervention are more prevalent (Plymoth et al., 2024). These differences may reflect varying levels of awareness and experience with tularemia among healthcare providers, which in turn affects the timeliness and accuracy of diagnosis.

Notably, when microbiologists or infectious disease specialists were involved early in the diagnostic process at our center, the correct diagnosis was made much sooner compared to other cases, as many clinicians did not consider tularemia as a differential diagnosis. In these cases, serological testing for tularemia was conducted more quickly. To further facilitate the diagnosis of tularemia, a serologic diagnostic "lymphadenitis" panel was introduced at Saarland University Medical Center in 2018, which clinicians could request and that included a comprehensive antibody testing for *F. tularensis* and other common and rare causes of infectious lymphadenitis. Additionally, emerging diagnostic methods were employed to accelerate the confirmation of the diagnosis: In two cases, lymph node tissue was analyzed using the FilmArray® BioThreat Panel, a commercially available multiplex PCR test for biohazardous pathogens. Such positive PCR results were able to support the clinical suspicion within a few hours, so that the targeted therapy was initiated earlier. While these cases emphasise the importance of interdisciplinary collaboration between clinicians, microbiologists and laboratory staff, they also highlight a crucial area for improvement in clinical practice. Enhanced awareness and consideration of tularemia in differential diagnoses, particularly in patients with atypical presentations or poor response to frequently used antibiotics with a potential environmental exposure or contact with vectors could reduce diagnostic delays.

One of the challenges in the diagnosis of tularemia is the limited sensitivity and specificity of many serological assays. According to the manufacturer, the Virapid Tularemia assay has a sensitivity of 99.13 % and a specificity of 98.58 %. However, it is widely known that real-life performance of such assays typically yields lower diagnostic accuracy levels. In this case series, all positive serum samples were sent to an

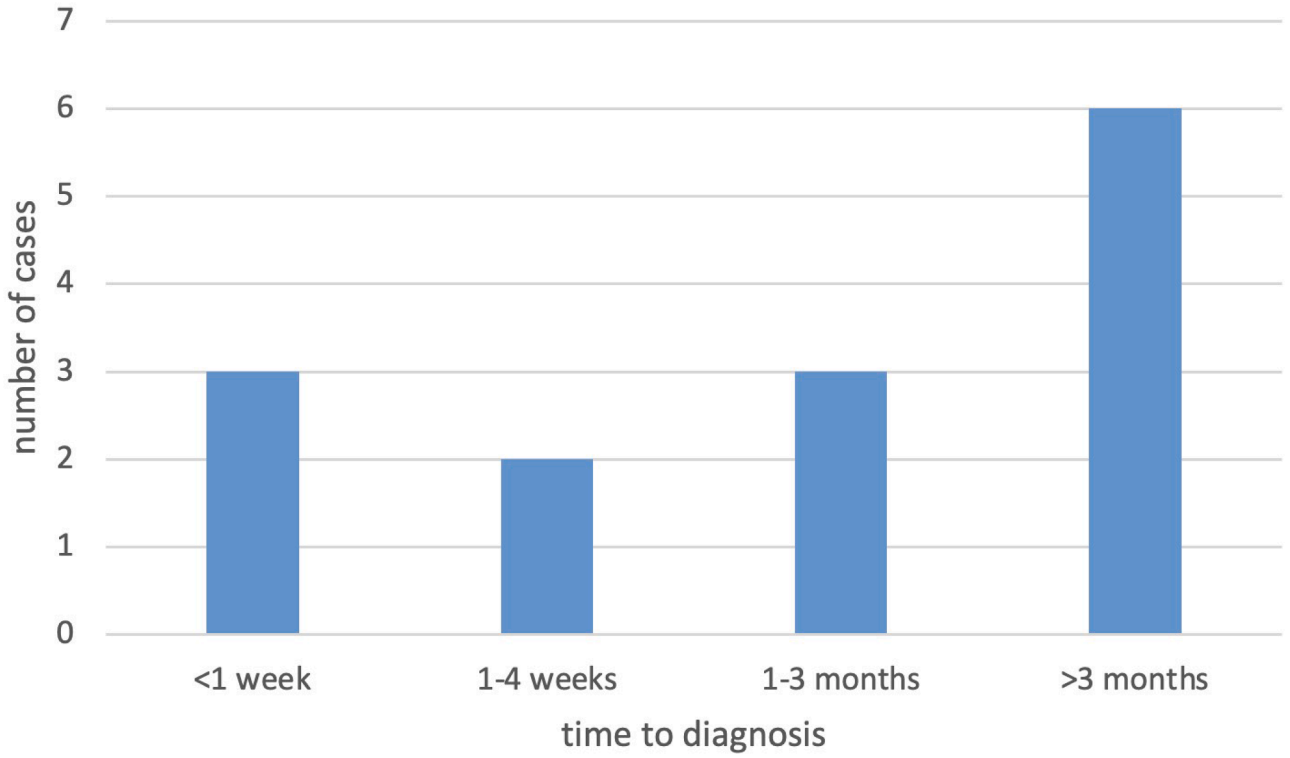

Fig. 3. Time evolved between first presentation and the establishment of definite/probable/possible tularemia diagnosis in patients seen at one German hospital.

external reference laboratory for confirmatory testing, thereby reducing the probability of false-positive results. Nonetheless, the possibility of false-negative results cannot be excluded, especially in the early stages of infection or in cases with low antibody titers.

Treatment modalities varied among the cases, reflecting the clinical judgment of treating physicians based on disease severity, patient age, and individual patient factors. Most patients received either ciprofloxacin or doxycycline, in accordance with current treatment guidelines recommending fluoroquinolones and tetracyclines as first-line therapies for tularemia. While established recommendations suggest the use of gentamicin in more severe or refractory cases (Dennis et al., 2001; Maurin and Gyuranecz, 2016; World Health Organization, 2007), in our cohort, it was administered in only one case, namely a patient with persisting symptoms after initial ciprofloxacin therapy. Notably, other patients with potentially severe disease, including one with typhoidal tularemia, were managed without aminoglycosides. This may be due to a cautious approach to aminoglycoside use in our hospital, likely influenced by concerns regarding their known toxicity profile. Nonetheless, the good clinical outcome observed in almost all patients for whom follow-up data were available demonstrate the efficacy of these treatment regimens. However, the renal function of the patient with tularemia-related acute renal failure did not fully recover, highlighting the potential for significant morbidity despite appropriate antimicrobial therapy.

The observed increase in tularemia cases at our center mirrors global trends. Indeed, tularemia is increasingly recognized as a re-emerging disease in Europe. Over the past three decades, the incidence of tularemia has quadrupled in Switzerland and increased ten-fold in Germany and Sweden (Bahuaud et al., 2021; Faber et al., 2018a; Imbimbo et al., 2020). There has also been a slight increase in reported cases outside Europe, e.g. in the United States of America and the Middle East (Sholeh et al., 2024; Zargar et al., 2015). *Francisella tularensis* has been absent from the southern hemisphere for a long time, but its geographic range is expanding, with at least four locally acquired cases reported from Australia (Eden et al., 2017; Jackson et al., 2012; Whipp et al., 2003). The presence of tularemia in Africa remains controversial, with reports from Kenya and Sudan requiring further confirmation through standard diagnostic methods (Mohamed et al., 2012; Njeru et al., 2017). Currently, South America is considered free of tularemia. Several factors might contribute to this rise, including increased awareness among healthcare providers, changes in leisure activities that increase human exposure to the pathogen, and expansions in the animal reservoir of *F. tularensis* (Hestvik et al., 2015).

The environmental reservoir of *F. tularensis* is diverse and includes both terrestrial and aquatic habitats. In Germany, various wild animals, including red foxes, hares, raccoon dogs, wild boars and beavers, have tested seropositive, suggesting their possible role in the maintenance and spread of the pathogen (Faber et al., 2018b; Kuehn et al., 2013; Müller et al., 2013). In addition, the aquatic environment may serve as an important reservoir, as *F. tularensis* has been shown to survive in water for extended periods of time (Berrada and Telford Iii, 2011). The potential interaction with free-living amoebae may further increase the stability of the pathogen in the environment, providing a significant and persistent reservoir (Buse et al., 2016; Hennebique et al., 2019). Climate change also favours the spread of vectors such as mosquitoes and ticks, possibly indicating that a further increase in the number of cases is to be expected (Rydén et al., 2009). Continued surveillance and research are thus urgently needed.

Our case series is limited by its retrospective and monocentric design with comparably low case numbers, which may limit the generalizability of our findings. Indeed, the completeness of datasets in retrospective analyses frequently shows some weaknesses, which is e.g. reflected in the absence of two consecutive serum samples being obtained for examination and the lack of specific quantitative serological titer reporting in our cohort. Prospective, multicentric studies or standardized patient registries with larger cohorts are needed to better understand the epidemiology, optimal management, and outcomes of tularemia.

5. Conclusion

This case series emphasizes the importance of considering tularemia

as a differential diagnosis among patients with enlarged lymph nodes who do not respond to beta-lactam antibiotics or clindamycin. The atypical presentations observed in some cases underscore the diverse clinical spectrum of tularemia. Healthcare providers, including laboratory staff, should remain vigilant for *F. tularensis*, particularly in regions where the disease is endemic or in individuals who may have come into contact with contaminated environments or vectors. Increased awareness of the various clinical manifestations and the usefulness of serological testing can help to detect tularemia early and treat it promptly, thereby reducing the diagnostic delay and improving patient outcomes.

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CRediT authorship contribution statement

Sophie E. Müller: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Sophie Schneitler:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Sabine Zange:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Maximilian Linxweiler:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Arne Simon:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Lorenz Thurner:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Sören L. Becker:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available due to data protection reasons but are available from the corresponding author on reasonable request.

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