Tularaemia – a diagnostic challenge

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Abstract

Introduction. Tularaemia is an infrequently occurring disease in Poland. It has therefore rarely been taken into account in the differential diagnosis of skin lesions, lymphadenitis, or soft tissue abscesses. This fact, accompanied by non-specific initial presentation, may lead to a delay in diagnosis and a more severe course of the disease.
Objective. The aim of the study is to present the current state of knowledge on tularaemia and convince medical professionals to take it into consideration in the diagnosis of skin lesions, lymphadenitis, and tissue abscesses.
Review methods. A literature review using PubMed and other online resources, using terms including 'tularaemia', 'lymphadenitis', etc., was undertaken. Papers were reviewed for relevance and scientific merit.
Abbreviated description of the state of knowledge. Tularaemia, also known as 'rabbit fever', is a zoonotic infection caused by Francisella tularensis, an aerobic, facultative intracellular, gram-negative bacteria. In Europe, it is mainly spread via tick bites and contact with wild animals such as lagomorphs and rodents. Clinical presentation may differ depending on the transmission route; the ulceroglandular and glandular forms of disease predominate. An early diagnosis and implementation of appropriate antibiotic therapy are the cornerstones of successful treatment and make it possible to avoid a surgical incision and drainage of suppurative complications.
Summary. Raised awareness and knowledge on tularaemia among health care professionals are required for timely diagnosis and treatment. Arrival from endemic areas, contact with wild animals, tick bites, and exclusion of more common etiologies of presenting signs should prompt consideration of tularaemia. More research is needed for a better understanding of the burden of the disease and its impact on public health in Poland.

Key words

abscess, Francisella tularensis, tick, skin lesion, lymphadenitis

INTRODUCTION

Tularaemia is an acute zoonotic disease caused by the small, gram-negative, aerobic bacteria *Francisella tularensis* [1]. Tularaemia has a broad geographical distribution and there is evidence suggesting its local emergence or re-emergence in Europe [2]. Poland is assumed to have a low tularaemia incidence with a prevalence rate from a few to two dozen cases per year [3]. However, this epidemiological data may be underestimated and underreported due to rare tularaemia consideration in the differential diagnosis of several conditions [4]. Some cases may be overlooked or misdiagnosed due to low accessibility to serological testing, which is limited predominantly to highly specialized units, and since in some patients, successful treatment with antibiotics efficient for *F. tularensis* is implemented before establishing the correct diagnosis.

Tularaemia may present with diverse clinical manifestations, including skin lesions, the development of granulomatous and suppurative lesions in the affected regional lymph nodes and various organs [1]. Due to its rarity and non-specific initial presentation, diagnosis and therapy of tularaemia are challenging. If mistaken for more common conditions, the applied treatment may be insufficient and lead to a more

severe course of the disease, with the development of several complications [5]. In this case, an aggressive therapeutic approach, including surgical incision and drainage of the suppurative lesions, may be required. Therefore, the early diagnosis of tularaemia and timely implementation of proper antibiotic therapy is essential [1]. Moreover, due to the natural resistance of *F. tularensis* to beta-lactams and macrolides, tularaemia may cause a serious therapeutic problem, particularly in children and pregnant women [6]. In these patients, bacteriological or serological confirmation is crucial before implementing therapy.

OBJECTIVE

The aim of the study is to present the current state of knowledge on tularaemia and to convince medical professionals to take it into consideration in the differential diagnosis of skin lesions, lymphadenitis, and tissue abscesses.

DISCOVERY OF *F. tularensis* AND POTENTIAL FOR BIOTERRORISM

F. tularensis was first isolated in 1912 by McCoy and Chapin from ground squirrels in Tulare County, California, USA [7, 8]. A few years later, the first human cases were reported. In 1928, Edward Francis described the clinical, epidemiological,

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and diagnostic aspects of tularaemia, summarizing 679 cases of the disease [9]. In that study, ulceroglandular was the most frequent form of tularaemia, caused by direct contact with rabbits or tick bites. Subsequently, new cases were reported in Russia, Norway, Sweden, and Austria [10, 11, 12]. A large outbreak of tularaemia, presumably caused by a semi-aquatic rodent, the European water vole (*Arvicola amphibious*), was noted during the Second World War [11]. To prevent disease, millions of people were vaccinated with live tularaemia vaccine.

F. tularensis, due to the high infectivity, ease of spread, and high pathogenicity, has the potential for use as a biological weapon. Studies on the use of the bacteria as a bioterrorism agent were carried out in the USA, USSR, and Japan before the Second World War. After the anthrax attack in the USA in 2011, interest in tularaemia was renewed worldwide [13]. At present, the bacterium is classified as a Category A (high-priority) bioterrorism agent by the United States Centers for Disease Control and Prevention (CDC) [14].

Microbiology. *Francisella tularensis* is formally divided into four subspecies with different pathogenicity and geographic distribution: *tularensis* (type A), *holarctica* (type B), *mediasiatica*, and *novicida* [1]. However, the current appropriate nomenclature for *Francisella tularensis* subspecies *novicida* remains controversial, thus a part of the scientists regard it as a separate species [15, 16].

The majority of human infections worldwide are caused by *F. tularensis* subspecies *tularensis* and subspecies *holarctica* [1]. The highly virulent *F. tularensis tularensis* (type A) is found in the USA and is predominantly transmitted by rabbits, ticks, and sheep [15]. It has an infectious dose of <10 colony-forming units (CFU) in humans and may cause severe disease. A less virulent *F. tularensis holarctica* (type B) has been reported throughout the northern hemisphere and seems to be the only cause of the disease in Europe [2, 17]. In this case, the human morbidity is low with a milder form of tularaemia predominant, which is only rarely lethal. *F. tularensis* subspecies *holarctica* strains are typically subdivided into three biovars [17]. Biovar 1, reported in western Europe, is sensitive to erythromycin; biovar 2, found in eastern Europe, is resistant to erythromycin; and biovar japonica ferments glycerol and is mainly found in Japan, but has also been reported in China and Turkey. Other species and subspecies of *Francisella* (e.g., *F. mediasiatica*, *F. novicida*) are considered to have low or unknown pathogenicity in humans [1].

Epidemiology. Epidemiology and ecology of tularaemia are complex and differ depending on the endemic area, inhabiting animals, and the *F. tularensis* strains involved [17]. To date, in most European countries it is a notifiable disease with the highest notification rate in the Scandinavian region (approximately 3,4 – 8 cases per 100,000/population) [18]. In that area, outbreaks comprising more than 100 cases are reported every 10 years [2, 17]. Nowadays, tularaemia is recognized as a re-emerging infectious disease and recent data reveals the increase in natural tularaemia outbreaks in Europe. In 2018, a major outbreak occurred in western France, which was the highest incidence reported in France since 2002 [18]. In 2019, a large outbreak of tularaemia (979 cases) was noted in Sweden [19]. Other countries have also reported greater incidence rates than in previous years, with the highest rate in the Czech Republic (1 case per 100,000/population) [18].

The first case of tularaemia in Poland dates back to 1931 [4]. Until recently, about 600 cases of the disease have been reported and the northern regions of Poland are considered to be endemic. The prevalence rate of tularaemia in Poland is estimated at 21 cases per year with a tendency to increase in recent years [3, 18]. However, these data are presumably understated [4]. Due to its uncommon occurrence and non-specific presentation, tularaemia has been rarely taken into account in the differential diagnosis of skin lesions, lymphadenitis, or soft tissue abscesses. Moreover, according to the study presented by Chróst et al. [4], not all tularaemia cases seem to be reported to the National Institute of Public Health – National Institute of Hygiene, the Polish national infection disease registry. Some cases of tularaemia might be misdiagnosed due to low accessibility to serological testing which is limited predominantly to highly specialized units, and since in some patients, successful treatment with antibiotics efficient for *F. tularensis* is implemented before establishing the correct diagnosis. This may lead to the underestimation of tularaemia epidemiologic data in Poland.

Until recently, all the *F. tularensis* strains isolated in Poland belong to the subspecies *holarctica*. The subspecies *tularensis* has not yet been identified in Poland [20].

SOURCES OF INFECTION AND ROUTES OF TRANSMISSION

Tularaemia can be transmitted to humans through multiple routes [1]:
- arthropod bites (predominantly tick or mosquito bites), which might cause 10 – 90% of tularaemia cases in humans in Europe [17, 19, 21, 22];
- direct transmission from an animal reservoir, which might occur through handling an infected animal (especially a hare), ingestion of undercooked meat prepared from an infected animal, or an animal bite (especially by small rodents, cats, and dogs);
- spread through direct contact or ingestion of contaminated water and soil;
- inhalation of infective aerosols, e.g. during farming activities.

Human-to-human transmission does not occur [1].

There are some geographical variations in the routes of transmission, predominant clinical forms of the disease, and its severity, which might be partly explained by the presence of various epidemiological lifecycles of *F. tularensis* subspecies *holarctica* in Europe [17].

Terrestrial lifecycle is predominant in most European countries [2, 17]. Lagomorphs, terrestrial rodents, and ticks are the primary source of human infections. The bacteria are transmitted to humans by direct contact with infected animals or ingestion of undercooked meat. In this lifecycle, human morbidity is low with the ulceroglandular form dominating.

Aquatic lifecycle of *F. tularensis* occurs in Sweden, Finland, Bulgaria, and Turkey [17, 23, 24, 25]. The aquatic environment contaminated by excrement and the carcasses of infected animals is the primary source of human infections. In this lifecycle, human tularaemia cases are more common and often

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occur as large outbreaks. Predominantly, the disease presents with an oropharyngeal form caused by the consumption of contaminated water. Alternatively, tularaemia cases might correspond to mosquito-borne infections as ulceroglandular and glandular forms [19].

In the past 50 years, most of the reported cases in Poland had a septic or typhoidal form and were acquired by handling hares [26]. Nowadays, the most common form of tularaemia in Poland is ulceroglandular or glandular, often developing after an arthropod bite [26, 27, 28]. The increasing role of ticks and mosquitoes in tularaemia transmission in Poland has been emphasized in recent years. As evidenced by some studies, the pathogen is found in ticks. Wójcik-Fatla et al. identified *F. tularensis*-positive samples collected from 1,391 ticks in eastern Poland during 2011–2012 [29], and Bielawska-Drózd et al. found 0.49% tularensis-positive samples among 1,551 ticks collected in the region of north-western Poland in 2017 [30]. However, the overall presence of *F. tularensis* in arthropods is uncommon. Contrary to previously mentioned studies, *F. tularensis* DNA was not detected in any of the 3,072 tick and 2,180 mosquito samples collected in southern, central-eastern, and central Poland in the study by Formińska et al.[20]. Moreover, in the study by Pancewicz et al., no significance of *Ixodes ricinus* ticks in the transmission of *F. tularensis* was reported [31].

Since the investigated arthropods were collected in the period preceding and corresponding to the time arthropod bite-related tularaemia cases were recorded, these findings suggest that a relatively small number of arthropods are infected with *F. tularensis* in Poland [20]. Nevertheless, the role of arthropods as vectors of animal and human diseases increases due to their expanding range and climatic changes. Increased exposure to tick bites during summer and autumn may conduce to the disease transmission. Therefore, the significance of ticks in spreading tularaemia should be monitored continuously.

PATHOGENESIS

The high virulence of *F. tularensis* in human beings and animals is mainly related to the bacterium intracellular lifestyle [32, 33, 34]. The pathogen replicates primarily in macrophages and uses several mechanisms to manipulate host immunity.

Bacterial virulence genes are often located within mobile genetic elements, such as plasmids, transposons, bacteriophages, and islands of pathogenicity that can be exchanged between bacteria through horizontal gene transfer. Many virulence factors of *F. tularensis* have been described, including capsule, lipopolysaccharide, type IV pili, MGlA regulator, Francisella Pathogenicity Island (FPI), outer membrane proteins (OMP), secretory proteins, and the secretion system [35]. The virulence mechanisms of *F. tularensis* have been summarized by Jones et al. [36].

The host immune response to *F. tularensis* is yet not clear. Cell-mediated immunity is believed to be the primary defence mechanism [34]. Memory effector T cells CD4+ and CD8+ are essential for the primary control of infection. These cells produce cytokines, such as INF-γ, TNF-α, and IL-2 [37]. Although the role of humoral immunity in *F. tularensis* infection is believed to be less important, several reports proved its impact on the immune response to the pathogen

[33]. The infection-specific IgM, IgA, and IgG antibodies constitute indicators of exposure and may interfere with the ability of bacteria to infect the host cells. The contribution of B cells to host immunity response is thought to be dependent on the virulence of the *F. tularensis* strain [34]. Both humoral and cellular mechanisms are critical to host immunity response; however, their significance to protective immunity is still unknown.

CLINICAL MANIFESTATIONS

The incubation period of tularaemia is usually 3 – 5 days, but it can extend up to 21 days [1]. The clinical presentation depends on the site of entry and dose of the bacteria, virulence of the *F. tularensis* strain, and the immune status of the host [1, 34]. Clinical presentation of tularaemia may range from asymptomatic to severe with progress to sepsis and fatal outcome if left untreated, particularly in immunocompromised patients.

Depending on the site of transmission, six different clinical forms occur: ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, and respiratory tularaemia [1]. However, the initial clinical presentation is very similar in all of them [1]. From the site of infection, bacteria spread through the lymphatic system to draining lymph nodes, where they replicate. The bacteria may then disseminate to various organs, including spleen, liver, lungs, kidneys, central nervous system, and skeletal muscles. During this time, infected humans present flu-like symptoms, including the rapid development of fever with chills, fatigue, headache, and general body aches. Within a few days of the onset of fever, the patient will perceive a regional lymph node enlargement. Besides fever and unspecific symptoms, lymphadenopathy is a major reason for drawing clinicians' attention [1].

Ulceroglandular and **glandular forms** predominate in Europe (95% of cases) and are caused by vector-borne transmission or direct contact with infected animals [19, 38, 39, 40]. Within the onset of fever, a small papule appears and after a few days develops into a pustule surrounded by an inflammation zone [1]. The ulcer heals soon, leaving a thin red area of 1 cm which eventually develops into a scar, resembling the Bacilli Calmette-Guérin (BCG) vaccination scar. In the ulceroglandular form, the local skin lesion is considered the site of infection. The glandular form is similar to the ulceroglandular form, but no primary skin lesion is detected. The disease progresses to the swelling of the regional lymph nodes, which may ulcerate and suppurate [5, 41]. The lymphadenopathy location also depends on the site of bacteria entry. In children, the neck and nape are commonly involved, while in adults, groin adenopathy occurs [42]. The swelling may progress within 7–10 days unless antibiotic therapy is initiated. The most serious complication of ulceroglandular and glandular forms of tularaemia caused by *F. tularensis* subspecies *holarctica* is lymph node suppuration, which may occur in 3–40% of cases [42].

Oropharyngeal form accounts for about 5% of cases [43, 44], transmitted via contaminated water or food. Typically, a patient presents with an acute sore throat and high fever. Ulcerating and exudative stomatitis may be accompanied by tonsillitis and the presence of lesions in the throat and

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larynx. Large cervical lymphadenopathy is usually unilateral. During examination, this form of tularaemia is often misdiagnosed as streptococcal pharyngitis [1]. Temporary clinical improvement (mostly abating fever) may also delay the correct diagnosis, suggesting a response to antibiotic treatment. The suspicion of pharyngeal tularaemia should be taken into consideration in endemic regions and in patients with an acute sore throat if penicillin treatment was unsuccessful and routine diagnostic tests showed no satisfactory results. An ongoing lymph node and soft tissue inflammation with the development of suppurative lesions should prompt consideration for tularaemia. In the case of delayed treatment, the risk of suppurative complications occurs in ~40% [45].

Respiratory form results from inhalation of airborne bacteria or the haematogenous spread of bacteria as a complication of other forms of tularaemia [46, 47]. The respiratory form of tularaemia is the severe form of the disease with a high rate of mortality if not treated [48]. There are some disparities between the clinical presentation of tularaemia caused by *F. tularensis* subspecies *tularensis* (type A) and *F. tularensis* subspecies *holarctica* (type B) [1]. Infection with *F. tularensis* subspecies *tularensis* (type A) is severe and potentially life-threatening, with a mortality rate of about 30–60%. It usually presents with an abrupt decline in general condition, fatigue, high fever, shortness of breath, chest pain, and cough. Joint pain, nausea or diarrhoea may also present. Tularaemia caused by *F. tularensis* subspecies *holarctica* (type B) usually presents as a milder form of the disease. Severe acute pneumonia seems to be rarely present in Europe [17]. Most cases present as subacute or chronic infections with relatively non-specific symptoms, including fever, cough, chest pain, and dyspnea. Radiological findings include hilar adenopathy, suggesting tuberculosis or non-infectious diseases (e.g., lymphoma, lung cancer, and sarcoidosis), lobar or multi-lobar infiltrates, and pleural effusion [41, 49]. Most commonly, enlarged mediastinal or hilar lymph nodes and pulmonary lesions are found on the chest CT. These lesions are typically nodular and multiple, frequently located peripherally, with a blurred delimitation. Signs of necrosis, cavities, or bronchogram may be present.

According to Kravdal et al., the most common routes of bacteria transmission are wood chopping, farming, carpentry, hunting, and other outdoor activities [49]. Most of the patients in this study became infected in the autumn. In regions with high tularaemia prevalence, the occupational outbreak of respiratory tularaemia, caused by inhalation of agricultural dust contaminated with particles of rodent origin, may occur [45]. Such outbreaks caused by the inhalation of bacteria during machine washing of sugar beets contaminated by rodents were described in the former Soviet Union, Hungary, Austria, and Czechoslovakia [11]. Since respiratory tularaemia is potentially life-threatening, it may represent an important clinical problem in developing countries.

Oculoglandular form refers to a clinical presentation involving painful conjunctivitis and regional adenopathy. This form occurs mostly following direct contamination of the eyes, for example, when rubbed after handling an infected animal carcass or when blood from a compressed tick sprays into an eye [50]. Oculoglandular form, as a localized form of glandular tularaemia was initially described by a Polish ophthalmologist Gałęzkowski working in France and a French Pairnaud (Gałęzowski-Parinaud syndrome) [42].

Typhoidal form is typically a severe systemic disease characterized by a high mortality rate ranging up to 50% of cases [51, 52, 53]. It spreads via contaminated water or food, and direct animal contact. However, the typhoidal form may develop due to infection via any of the possible routes of transmission. Abrupt onset with high fever, asthenia, myalgia, neurological symptoms, and manifestations of kidney, liver, or joint inflammation are characteristic. Nausea, vomiting, diarrhea may also occur. In this form of tularaemia, neither a primary lesion nor regional lymphadenopathy is observed. According to Maurin and Gyuranecz, typhoidal tularaemia often occurs in elderly or immunocompromised patients after the consumption of a large amount of highly contaminated food [17].

Respiratory and typhoidal are the most severe forms of tularaemia and may represent an important clinical problem, particularly in developing countries. The oculoglandular and typhoidal forms of tularaemia are infrequent in Europe [17].

COMPLICATIONS

Complications of tularaemia mainly include soft tissue abscesses and lymph node suppurations [5, 54]. After two weeks of delay in diagnosis, the risk of lymph node suppuration increases over 20% [1, 45]. In such cases, the surgical incision and drainage of necrotic tissue may be necessary. Complications are more likely to be found in patients with immunocompromised status. Other complications include skin rashes, such as Sweet's syndrome, erythema nodosum [55], otitis media, meningitis [56], abscesses in the brain [57] and other locations due to the haematogenous spread of the bacteria [58, 59]. Death is currently scarcely reported in Europe [17].

DIAGNOSIS

The diagnosis of tularaemia depends on medical history, clinical presentation, and laboratory findings [1]. The laboratory diagnosis of tularaemia is based on bacteriological, molecular, and serological investigations. Methods used in the diagnosis of tularaemia are presented below.

Serological methods include the whole-cell agglutination test (Widal's reaction), the tube agglutination test, microagglutination assays, haemagglutination, ELISA (enzyme-linked immunosorbent assay), and the immunoblot test [34]. The ELISA and microagglutination assay are the methods most often used for establishing the diagnosis [60, 61]. The indirect ELISA is particularly applicable for routine serodiagnosis, as well as seroepidemiological studies because it is highly sensitive and specific. On the other hand, the tube agglutination test is cheap and easy to perform. Recently, the latex agglutination test was developed as a specific, sensitive, fast, easy-to-perform, and cost-efficient tool for the detection of antibodies against *F. tularensis*. It may be used as a screening test in the routine diagnosis of tularaemia, particularly in small, mobile laboratories [62].

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From the clinical point of view, the early identification of the disease is very important for initiating appropriate treatment to avoid severe complications. Antibodies against *F. tularensis* reach detectable levels 10 – 20 days after the onset of symptoms, and therefore are usually absent in patients presenting with early clinical features. A seroconversion or fourfold increase in the titers between acute and convalescent sera (obtained at a two-week interval) is considered for the diagnostic purpose [63]. Antibodies titres peak at 3 – 4 weeks after the progression of the clinical symptoms, and then gradually decrease, although the residual titres might persist for months or even years. In comparison to agglutination assays, ELISA is more sensitive and additionally allows the determination of different antibody classes (IgM, IgG, and IgA) [34]. However, cross-reactivity between *F. tularensis* and *Salmonella, Brucella, Legionella*, and *Yersinia spp.*, has been reported. A combination of initial ELISA screening tests complemented by an immunoblot confirmatory test is the currently recommended two-step approach for establishing the serological diagnosis [34].

F. tularensis **culture** must be carried out in biosecurity level three facilities due to the high bacterial virulence [1]. Samples should be collected preferably before the onset of antibiotic therapy and may include blood, serum, urine, respiratory tract secretion, swabs from visible lesions, lymph node aspirates, or biopsies [1, 17, 34]. *F. tularensis* grows on several types of cysteine/cystine-supplemented agar, including enriched chocolate agar (CA), cystine heart agar with 9% blood (CHAB), buffered charcoal yeast extract (BCYE), thioglycollate-glucoseblood agar (TGBA), and GC Agar II with 1% haemoglobin and 1% IsoVitaleX [1, 17, 34]. Additionally, growth of the bacteria on the CHAB provides presumptive identification of *F. tularensis* due to its characteristic appearance on this medium (green, opalescent, raised, shiny colonies at 24–48 h) [1].

Antigen detection can be useful for either direct identification of *F. tularensis* in clinical specimens or confirmatory identification of isolates recovered in culture. Antigen detection directly in clinical specimens can be performed by antigen-capture ELISA, or by direct fluorescent antibody staining using a FITC (fluorescein isothiocyanate)-labelled rabbit antibody against whole killed *F. tularensis* cells [1]. The classical slide agglutination tests and the latex agglutination tests (LAT) are used for the rapid identification of *F. tularensis* isolates [64].

Molecular methods are the valuable diagnostic tools allowing the identification of *F. tularensis* directly from the samples of human beings, animals, and the environment by amplification of target sequences of nucleic acids with the use of specific primers (the polymerase chain reaction, PCR-based methods) [1, 65, 66]. However, false-positive results related to non-pathogenic closely related *Francisella* subspecies, occurring naturally in the environment, may limit the species and subspecies identification [34]. A new technique, MALDI-ToF mass spectrometry (matrix-assisted laser desorption/ionization – time-of-flight mass spectrometer), has been evaluated recently as a useful tool for rapidly identifying and typing the isolated *F. tularensis* strains [67]. The accuracy of this analysis is highly dependent on the available mass spectrum databases. However, MALDI-ToF MS-analysis provides results in accordance with the PCR assay. The new cartridge-based assay can rapidly detect *F. tularensis* directly in the whole blood at the early stages of infection with a sensitivity superior to other methods [68].

Histopathology has limited sensitivity in tularaemia. Due to its non-specific presentation, any suspicion of this disease should be confirmed by other diagnostic methods [69, 70]. Granulomas, necrosis, and suppurative inflammation extending to extracapsular areas are usually found [69]. The fine needle aspiration cytomorphology of the lymph nodes shows the presence of suppuration and abscesses. Rare epithelioid histiocytes and granulomas or phagocytosed bacilli-like microorganisms may be observed [69]. Fine needle aspirations of the lymph nodes can be useful for providing material for PCR and culture in the early phase when the serology is negative and the treatment is more effective.

DIAGNOSTIC CRITERIA

The diagnosis of tularaemia is established when the clinical manifestations and patient history are supported by laboratory tests, including microbiological, immunological, morphological studies, and PCR [1]. The World Health Organization (WHO) has proposed the following criteria for tularaemia case definition:
- a suspected case – clinical symptoms suggesting tularaemia associated with a history of exposure to risk factors (e.g., tick bite);
- a presumptive case – suggestive clinical symptoms of tularaemia in association with positive testing for tularaemia (single elevated serum antibody titre; antigen or DNA detection);
- a confirmed case – isolation and identification of *F. tularensis* in the clinical specimen, or the fourfold or greater change in serum antibody titre to *F. tularensis* antigen between acute and convalescent specimens.

Although the isolation of *F. tularensis* in sterile body samples is the gold standard in establishing the diagnosis, in clinical practice it often depends on clinical signs and serological findings [17]. Hence, the culture of *F. tularensis* is very difficult and risky for laboratory staff.

DIFFERENTIAL DIAGNOSIS

The rarity and non-specific manifestations of tularaemia make it a diagnostic challenge. This disease may be mistaken for various conditions presenting with fever and enlargement of the lymph nodes [1, 42]. Predominant ulceroglandular form of tularaemia may be misdiagnosed with staphylococcal or streptococcal infection, cat-scratch disease, toxoplasmosis, mycobacteriosis, sporotrichosis, anthrax, or plague. Oropharyngeal tularaemia, as a rare condition, may be considered when more common etiologies of pharyngitis – adenoviral, *cytomegalovirus* (CMV), *Epstein-Barr virus* (EBV), and streptococcal infections are excluded. Differential diagnosis of respiratory tularaemia includes pneumonia caused by community-acquired pathogens: *Mycoplasma pneumoniae, Chlamydophila pneumoniae,*

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Legionella pneumophila, Chlamydia psittaci, Coxiella burnetii, Mycobacterium tuberculosis or nontuberculous mycobacterial infection. Oculoglandular tularaemia should be differentiated with cat-scratch disease, adenoviral, *Herpes simplex*, or bacterial infection. The differential diagnosis of typhoidal form includes Q fever, malaria, rickettsioses, *Salmonella typhi, Brucella spp., Legionella spp.* and *Plasmodium spp.* infections. The most common diseases that should be differentiated from tularaemia are presented below [1].

Table 1. Most common conditions that should be taken into account in differential diagnosis of tularaemia [1]

Disease	Differences from tularaemia
Staphylococcus aureus infection	General symptoms are less frequent [71]. Skin lesions include furunculosis, abscesses, folliculitis, or impetigo. The infection course is dynamic with inflammation in deeper skin layers, fasciae, and lymph nodes, which is more intensified than in tularaemia.
Streptococcus spp. infection	Increased local inflammation of the skin and subcutaneous tissue, present as irregular erythema, with vesicular lesions and epidermis exfoliation in severe cases [71]. On the contrary, tularaemic skin lesion is regular, sharply demarcated from the surrounding tissue with elevated edges.
Toxoplasmosis (*Toxoplasma gondii*)	Toxoplasmosis is oligosymptomatic or asymptomatic in immunocompetent patients [72]. Manifestations may include fever and lymph node enlargement. Ulcerative lesions are non-typical. Serology is confirmative.
Cat-scratch disease (*Bartonella henselae*)	The disease is acquired by contact with cats, most often involving scratches. Erythematous papule develops at the infection site leaving an eschar [73]. Typically, it is associated with regional lymphadenopathy. In 20 – 30% of patients, lymph nodes produce suppuration with purulent fistulas to the skin [73]. The general symptoms are less prominent than in tularaemia. Serology is confirmative.
Mycobacterium tuberculosis, nontuberculous mycobacterial infection	Clinical course and histopathology of lymphadenitis may be very similar to respiratory, ulceroglandular or glandular form of tularaemia [70, 74]. Infection with *Mycobacterium tuberculosis* or atypical mycobacteria is suggested when enlarged lymph nodes develop over weeks to months, and become fluctuant or matted without significant inflammation or tenderness. It is occasionally associated with fever. The diagnosis is confirmed by demonstrating acid-fast bacilli, a culture of the bacteria, and PCR. The Interferon-γ release assays and tuberculin skin tests are essential in establishing the diagnosis of *Mycobacterium tuberculosis* infection. The pulmonary nontuberculous mycobacterial infection most often occurs in immunocompromised patients or those with chronic lung diseases.
Cytomegalovirus (CMV) infection	Infection presents with unspecific manifestations, such as fever and lymphadenopathy [75]. Skin lesion, lymphadenitis, and suppurative complications are non-typical. Serology or antigen assays are confirmative.
Mononucleosis (*Epstein-Barr Virus* infection)	Typically, mononucleosis is characterized by the triad of moderate to high fever, pharyngitis, and lymphadenopathy [76]. There are no skin lesions or necrotic lymphadenitis. Antibody or antigen assays are confirmative.
Adenoviral infection	Infection most often presents with high fever, pharyngitis, conjunctivitis, lymph nodes enlargement, and gastroenteritis [77]. Ulcerated lesions and necrotic lymphadenitis are not typical.
HIV infection	Clinical presentation includes unspecific manifestations, such as fever and lymphadenopathy [78]. Ulcerative lesions or lymph node necrosis are non-typical. Serology is confirmative.
Lymphoma	Lymphoma should be concerned in the differential diagnosis of glandular and respiratory tularaemia. Manifestations include no-specific symptoms, such as fever, night sweating, bodyweight loss, fatigue, and general lymph node enlargement [79]. No clinical improvement is noted despite antibiotic therapy. Imaging findings are critical for forming an appropriate diagnosis.
Anthrax (*Bacillus anthracis*)	Cutaneous anthrax may present with a blister that undergoes necrosis and forms a black eschar [80]. Although similar to tularaemic ulcers, the anthrax lesion is typically painless and associated with extensive tissue damage. Respiratory anthrax may develop more rapidly than tularaemia into a toxic, fatal state, which occurs irrespectively of antibiotic therapy.
Pasteurellosis (*Pasteurella multocida*)	Pasteurellosis occurs after dog, cat, and pig bites or scratches, and presents with intense local inflammation, including erythema, oedema, and regional adenopathy, which is less prominent than in tularaemia [81, 82]. General symptoms, such as fever, may be present. *Pasteurella multocida* is easily isolated from wound specimens.
Rickettsioses (*Rickettsia spp.*)	Rickettsioses are spread by ticks, likewise tularaemia [83]. The spotted fever group of rickettsioses develops with fever, exanthema, and eschar. In Poland, tick-borne rickettsioses are reported sporadically (3 cases reported in 2000–2009) [83]. Serology is confirmative.
Plague (*Yersinia pestis*)	*Yersinia pestis,* found in small mammals and their fleas [84], causes 2 primary clinical forms of plague: bubonic and pneumonic. Painful swollen lymph nodes characterise common bubonic plague. With fulminant general symptoms, the course of the disease is usually more rapid than ulceroglandular tularaemia, and has a higher fatality rate (30 – 60%). Diagnosis can be confirmed by usual bacteriological techniques, serological examination, and PCR. According to the report of the European Centre for Disease Prevention and Control (ECDC), since 2017, no plague cases were reported in Europe [85].
Brucellosis (*Brucella spp.*)	Brucellosis presents with general manifestations, such as fever, myalgia, and fatigue [86]. Diagnosis is confirmed by blood culture and serology.
Leptospirosis (*Leptospira spp.*)	Leptospirosis, predominantly spread by rodents, also has an abrupt onset and presents with fever, headaches, and myalgia [87]. Other manifestations, such as non-productive cough, nausea, or vomiting can occur. Conjunctival suffusion is a hallmark clinical sign of leptospirosis [88]. In Poland, its incidence in humans is very low, although the spirochetes are found in animals [89]. Most cases are mild or self-limited. Diagnosis is confirmed by serology.
Sporotrichosis (*Sporothrix spp.*)	Sporotrichosis may manifest with ulcerated nodules with seropurulent material at the site of inoculation and lymphatic involvement. General symptoms are unusual, although fever and chills may be present [90, 91]. Diagnosis is established by biopsy specimen and immunofluorescence, or by culture.
Atypical pneumonia (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila*)	Atypical pneumonia caused by *Mycoplasma pn., Chlamydophila pn.,* or *Legionella pn.,* may resemble respiratory tularaemia [92]. Spread occurs by close human contact (*Mycoplasma pn., Chlamydophila pn.*) and air-conditioning systems (*Legionella pn.*). Serology is confirmative.
Herpes simplex infection	Differentiation between the oculoglandular form of tularaemia and viral infection can be difficult by visual inspection alone. HSV infection often presents with a vesicular rash, fever, and local lymphadenitis, which are less prominent than in tularaemia [93]. Diagnosis is confirmed by PCR or serology.

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Co-infections with *F. tularensis* and other tick-borne pathogens have been described so far in ticks and reservoir animals. They may result from a single bite of the tick infected with several pathogens, or from multiple bites by ticks, each infected with one pathogen. Results of serological testing indicate that *Rickettsia spp./F. tularensis* and *Borrelia spp./F. tularensis* co-infections may occur in humans in Poland [94, 95].

TREATMENT

An early diagnosis and initiation of effective antibiotic treatment are still the cornerstones of successful therapies [1]. Anti-microbial therapy should be applied to prevent complications in *F. tularensis* infections, shorten the recovery period, and decrease mortality. *F. tularensis* is resistant to several antibiotics – beta-lactams (due to the production of beta-lactamases), macrolides (regarding biovar 2 strains of *F. tularensis* subspecies *holarctica*), and presumably clindamycin [96]. Aminoglycosides (streptomycin and gentamicin), tetracyclines (especially doxycycline), and fluoroquinolones (ciprofloxacin) are the main effective antibiotics. According to the WHO guidelines [1], ciprofloxacin and doxycycline are recommended in mild and moderate disease or a mass casualty setting. In severe tularaemia cases requiring hospitalization, parenteral administration of an aminoglycoside is the first choice of treatment. Gentamicin is preferred and streptomycin is given alternatively by intramuscular injection. Monitoring of the gentamicin serum concentration is recommended.

Pregnant women and children. When considering the therapeutic approach in these cases, potential side-effects should be weighed against the benefits of the treatment. Ciprofloxacin is recommended for mild and moderate tularaemia [1]. In severe cases requiring hospitalization, parenteral administration of gentamycin is preferred. The monitoring of gentamicin serum concentration is recommended; alternatively streptomycin might be used. Azithromycin might be a first-line treatment to overcome the side-effects of gentamicin, and ciprofloxacin in some cases [97, 98]. This might be useful in patients infected with the type B biovar 1 strain, which is naturally susceptible to macrolides and usually induces mild diseases [17].

The treatment period depends on the clinical response and may comprise more than 10 days [1]. To avoid relapses, aminoglycosides are usually administered for 10 days, fluoroquinolones for 14 days, and doxycycline for 15 days. However, the treatment duration may be extended to 21 days in cases involving meningitis and endocarditis [1, 99].

ANTIBIOTIC RESISTANCE

Although treatment failures have been documented in human cases of tularaemia, they are not associated with spontaneous antibiotic resistance, but rather a delay in therapy initiation [1, 17]. Antibiotic resistance to frontline therapeutics recommended for tularaemia treatment has never been identified in naturally occurring strains of *F. tularensis*. Due to the potential use of *F. tularensis* as a biological weapon, antibiotic resistance remains of concern. For experimental purposes, streptomycin- and tetracycline-resistant strains of *F. tularensis* have been developed, and presumably, the resistance to quinolones may also be quickly introduced [1].

NOVEL THERAPEUTIC APPROACHES

Several reasons make it necessary to search for new therapeutic alternatives: the potential toxicity of the first-line drugs, high rate of treatment relapses and failures, particularly in severe and suppurated forms of tularaemia, and possible use of antibiotic-resistant strains as a biological weapon [100].

Potential novel therapeutic strategies for tularaemia include the application of new antibiotics or developing new techniques for using existing ones, reduction of *F. tularensis* virulence, and enhancement of the innate and adaptive immune response of the host [100, 101, 102, 103]. A new insight into the therapeutic approach in tularaemia is provided by the development of a new dye uptake assay to test the activity of antibiotics against intracellular *F. tularensis* [104]. It revealed novel therapeutic agents such as linezolid [104] and resazurin [105].

PREVENTION

Individuals can protect themselves from infection by minimizing the risk of exposure to *F. tularensis*, especially in the endemic regions [1]. The following precautions should be taken:
- avoidance of drinking unboiled water;
- disinfection of water used for washing or brushing teeth;
- avoidance of bathing and swimming in water which may be contaminated with animal faeces;
- protection of water sources from contact with rodents;
- avoidance of eating uncooked meat;
- avoidance of contact with wild animals;
- using insect repellents and avoidance of exposure to haematophagous arthropods by wearing long-sleeved clothing;
- using impermeable gloves and clothes when skinning, handling, or dressing wild animals, especially rabbits;

Table 2. Tularaemia antibiotic treatment according to WHO guidelines [1]

Antibiotic	Daily dosage	Duration of treatment
Gentamicin	Adults: 5 mg/kg daily divided into 2 doses (parenterally). Children: 5–6 mg/kg daily divided into 2–3 doses (parenterally).	10 days
Streptomycin	Adults: 2 g daily divided into 2 doses (by intramuscular injection). Children: 30 mg/kg daily divided into 2 doses, up to 2 g daily (by intramuscular injection).	10 days
Ciprofloxacin	Adults: 800–1,000 mg daily divided into 2 doses (orally). Children: 30 mg/kg daily divided into 2 doses, up to 1g daily (orally).	10–14 days
Doxycycline	200 mg daily divided into 2 doses (orally).	15 days

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- using protective masks against infected dust and aerosols by members of professional groups, such as farmers or gardeners;
- regular inspections of domestic animals for signs of the disease; in case of an outbreak, avoidance of close contact with domestic animals such as dogs and cats.

Although immunotherapy of tularaemia has received increased attention in recent years, and numerous immunotherapeutics have demonstrated protection in animal models of tularaemia, there is no available or approved vaccine against tularaemia for humans [1, 106, 107]. Many of these studies were conducted using strains that do not cause the disease in humans and, therefore, do not correspond with preventing infections caused by virulent *F. tularensis* strains. It has been suggested that each subtype of *F. tularensis* should require a different approach when creating a vaccine, with a careful balance between attenuation of the pathogen and the ability of a vaccine to develop protection [107]. Furthermore, due to the intracellular adaptation of *F. tularensis*, the new vaccine development initiatives should aim to provide remarkable cell-mediated immunity for long-term protection.

CONCLUSIONS

Tularaemia is a rare but notifiable disease in many European countries, and there is evidence of its local emergence or re-emergence. In Poland, it is assumed to be an infrequent disease; however, current data may be underestimated. The rarity, non-specific initial presentation, resemblance to more common conditions, and natural resistance to beta-lactams and macrolides make tularaemia a serious clinical problem. Its diagnosis should be taken under consideration primarily in patients with lymphadenitis, skin lesions, and soft tissue inflammation, particularly if necrosis and abscesses are present or empirical therapy is ineffective. However, tularaemia may present with diverse and variable manifestations. Therefore, its diagnosis can be challenging. Timely implementation of the appropriate antibiotic therapy holds out the prospect of successful treatment. This is why thorough knowledge on how to diagnose tularaemia is crucial among medical professionals. Arrival from endemic areas, contact with wild animals or history of a tick bite, and exclusion of more common etiologies of presenting signs should prompt the consideration of tularaemia.

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