Tularaemia: clinical aspects in Europe

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Tularaemia is a zoonotic disease caused by *Francisella tularensis*, a Gram-negative, facultative intracellular bacterium. Typically, human and animal infections are caused by *F tularensis* subspecies *tularensis* (type A) strains mainly in Canada and USA, and *F tularensis* subspecies *holarctica* (type B) strains throughout the northern hemisphere, including Europe. In the past, the epidemiological, clinical, therapeutic, and prognostic aspects of tularaemia reported in the English medical literature were mainly those that had been reported in the USA, where the disease was first described. Tularaemia has markedly changed in the past decade, and a large number of studies have provided novel data for the disease characteristics in Europe. In this Review we aim to emphasise the specific and variable aspects of tularaemia in different European countries. In particular, two natural lifecycles of *F tularensis* have been described in this continent, although not fully characterised, which are associated with different modes of transmission, clinical features, and public health burdens of tularaemia.

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Introduction

Francisella tularensis is a Gram-negative bacterium causing tularaemia.[1] The bacterium was first isolated in 1911 by McCoy and Chapin from ground squirrels in Tulare County (CA, USA). The first isolate from a human being was obtained in 1914 by Wherry and Lamb (OH, USA).[1] Edward Francis extensively described the clinical, epidemiological, and diagnostic aspects of tularaemia in the early 20th century.[1,2] The global incidence of tularaemia in human beings then declined, probably because of a more urban way of life and less frequent exposure to infected wild animals than before. After the anthrax attack in the USA in 2001,[3] renewed scientific and medical interest in *F tularensis* arose because of this bacterium's classification as a category A potential agent for bioterrorism by the US Centers for Disease Control and Prevention (CDC), based on the following arguments:[2,4] *F tularensis* is a highly infectious organism in human beings and many animals; the bacterium could be spread by aerosols to an entire population leading to a high number of severe pneumonia cases with fatality of up to 30%; survivors could be burdened with debilitating diseases for prolonged periods; the bacteria could remain in the water and soil environment for prolonged periods, leading to a high number of secondary infections in human beings and animals; no vaccines are available; antibiotic treatment options are very scarce and could be further reduced if engineered antibiotic resistant strains of *F tularensis* are used; and this pathogen was used for the manufacture of biological weapons during World War 2, although their use has never been reported.

Although tularaemia is encountered throughout the northern hemisphere,[1] for decades the medical literature has been largely dominated by reports from the USA.[5] Scientific literature for tularaemia in Europe has expanded recently, although principally describing highly endemic areas.[1] Advances have shown that *F tularensis* subspecies and genotypes vary according to geographical areas,[6,7] as do the clinical, epidemiological, therapeutic, and prognostic aspects of tularaemia.[1] This Review focuses on tularaemia in human beings and animals in Europe, emphasising the many and varied aspects of this disease in different European countries.

Francisella tularensis

The genus *Francisella* comprises seven species, including *F tularensis*, which is divided into four subspecies:[1] *tularensis* (type A strains), *holarctica* (type B strains), *mediasiatica*, and *novicida*. The subspecies *tularensis* and *holarctica* are the main cause of tularaemia worldwide.[1] Type A strains are mainly found in North America,[1] but a few have been isolated from environmental sources and arthropods in Slovakia and Austria.[8] Type B strains are reported throughout the northern hemisphere,[1] but isolates have also been identified in Tasmania.[9] Only type B strains cause tularaemia in Europe.

F tularensis subspecies *holarctica* strains are typically separated into three biovars.[10] Biovar 1 (erythromycin sensitive) are reported in western Europe and biovar 2 (erythromycin resistant) are found in eastern European countries, although these strains overlap in their geographical spread. Biovar japonica (ferment glycerol) is mainly found in Japan, but was also reported in China and Turkey.[11,12]

F tularensis subspecies *holarctica* has a small genome (roughly 1·89 Mb), with low genetic diversity between strains.[13] However, whole-genome sequencing coupled with canonical single nucleotide polymorphisms with or without insertion and deletion analyses have identified several phylogenetic groups within this subspecies, including four main genetic clades (B.4, B.6, B.12, and B.16) with many subgroups.[13–15] Clade B.12 predominates in central and eastern Europe (Austria, Czech Republic, Hungary, Ukraine, Romania, Russia, and Slovakia).[13,16] Subclade B.FTNF002–00 is the only native subclade (one subclade of B.6) found so far in Spain, France, Italy, and the Netherlands, although some B.12 isolates have been imported to France and Italy through live brown hares (*Lepus europaeus*).[13,16,17] Both clade B.12 and subclade B.FTNF002–00 are identified in Germany and Switzerland.[18,19] Clades B.4, B.6, and B.12 (and several of their subclades) are reported in Norway, Finland, and

Sweden.[13,20–22] Different subclades of clades B.6, B.12, and B.16 (biovar Japonica) have been described in the Asian part of Turkey.[12,23] Further genetic diversity can also be described with multilocus variable-number tandem repeat analysis, by use of whole-genome sequence comparison of annotated genes (multilocus sequence typing) or whole-genome sequencing and analysis of single-nucelotide polymorphisms.[6,24–26] These methods can be applied to differentiate outbreak isolates. For some tularaemia outbreaks, several *F tularensis* genotypes were simultaneously implicated, which suggests that epidemics are triggered by ecological factors rather than the increased infectivity of a specific *F tularensis* clone.[20,25–28] By contrast, the clonal identity of isolates originating from epizootic events in zoos has also been described.[26]

The high virulence of *F tularensis* in human beings and animals is mainly related to the bacterium's intracellular lifestyle.[29] The bacterial genome contains a duplicated francisella pathogenicity island, which encodes a type VI-like secretion system associate with bacterial virulence. After phagocytosis by macrophages, *F tularensis* escapes the phagosomal compartment and multiplies in the cytosol. The bacterial lipopolysaccharide does not trigger a high proinflammatory response in macrophages, which reduces the immune response of the infected host.

Reservoirs and modes of transmission

Natural reservoirs and vectors of *F tularensis*

F tularensis has many animal reservoirs, including both vertebrates and invertebrates. Among wild animals, small rodents and lagomorphs are thought to be the key reservoirs and amplification hosts of *F tularensis*, and important sources of infection in human beings.[30] Because these animals often develop fatal infections, other unidentified long-term animal reservoirs might exist. Domestic animals (especially sheep and cats in the USA) might be carriers of, or infected with, *F tularensis* and transmit the disease to human beings.[31]

One family of animal reservoirs is the European Leporidae, and more specifically, species belonging to the genus *Lepus*,[32] including *Lepus europaeus* (European brown hare) in most European countries; *Lepus capensis* (Cape hare or common hare) in Corsica and Cyprus; *Lepus timidus* (mountain hare) in northern Europe (especially Scandinavia) and the Alps; *Lepus granatensis* (Granada hare) in Spain and Portugal; and *Lepus corsicanus* (Corscian hare) in Corsica and southern Italy.

European small rodents possibly carrying *F tularensis* include several families:[32] Sciuridae (squirrels); Castoridae (beavers), especially the European beaver (*Castor fibre*); Hystricidae (porcupines); Myocastoridae (coypu or river rat); Gliridae (dormice); Spalacidae (mole rats); Cricetidae (hamsters, lemmings, voles, and muskrats); and Muridae (mice and rats), including the *Apodemus* species. Mice, rats, voles, beavers, and lemmings are most often associated with tularaemia cases in human beings.[33]

Arthropods can be contaminated by *F tularensis* from animal and environmental water sources,[1,34] and transstadial transmission of *F tularensis* has been experimentally shown for *Ixodes ricinus* ticks[35] and mosquitoes.[36] By contrast, experiments by Genchi and colleagues[37] revealed no transovarial transmission of *F tularensis* subspecies *holarctica* in ticks, suggesting that these arthropods might not be a competent reservoir for this pathogen. In Europe, the most prevalent Ixodidae ticks belong to the genera *Dermacentor*, *Haemaphysalis*, *Hyalinella*, *Ixodes*, *Boophilus*, *Hyalomma*, and *Rhipicephalus*.[32] *Dermacentor reticulatus* and *I ricinus* are tick species most commonly infected with *F tularensis*.[30] Mosquitoes are often associated with the transmission of *F tularensis* to human beings in Sweden[34] and Finland,[38] where *Aedes* species (especially *Aedes cinereus*) are primarily implicated.

Most *Francisella* species and subspecies (including *F tularensis* subspecies *novicida*) are deemed to be aquatic bacteria and might infect human beings through contaminated water exposure.[39] Present clinical and epidemiological data strongly suggest that *F tularensis* subspecies *holarctica* can maintain a long-term aquatic cycle: tularaemia is primarily a mosquito-borne disease in Sweden and Finland,[34,40] and mosquito larvae are infected with *F tularensis* during their aquatic lifecycle;[36] waterborne outbreaks of tularaemia from this subspecies have occurred in Norway,[41] Kosovo,[42] Bulgaria,[43] Spain,[44] and Turkey;[45] *F tularensis* has been recovered from water sources, especially in Norway and Turkey;[46] a type B tularaemia case was reported in a French patient after a near-drowning accident;[47] and *F tularensis* has been multiplied in vitro in amoebae.[48,49]

Modes of *F tularensis* transmission to human beings

Three major modes of *F tularensis* transmission to human beings exist:[1] direct transmission from the animal reservoir, arthropod bites, and transmission through contaminated water and soil environments. Direct transmission might occur through handling an infected animal (especially a hare), ingestion of undercooked meat prepared from an infected animal, or an animal bite (especially from small rodents, cats, and dogs).[9,31,50] Arthropod-borne tularaemia cases might occur after a tick bite, a mosquito bite, or, rarely, bites from other arthropods. These cases might account for 10% to more than 90% of human infections.[50] Because *F tularensis* can persist for weeks to months in the environment,[51] human infections might occur through contact with contaminated water or soil (eg, during landscaping),[52] or ingestion of contaminated water (eg, drinking water from tanks and wells).[42,43,45]

Lifecycle of *F tularensis* subspecies *holarctica* in Europe

In Europe, two lifecycles of *F tularensis* subspecies *holarctica* (terrestrial and aquatic) are suspected on the

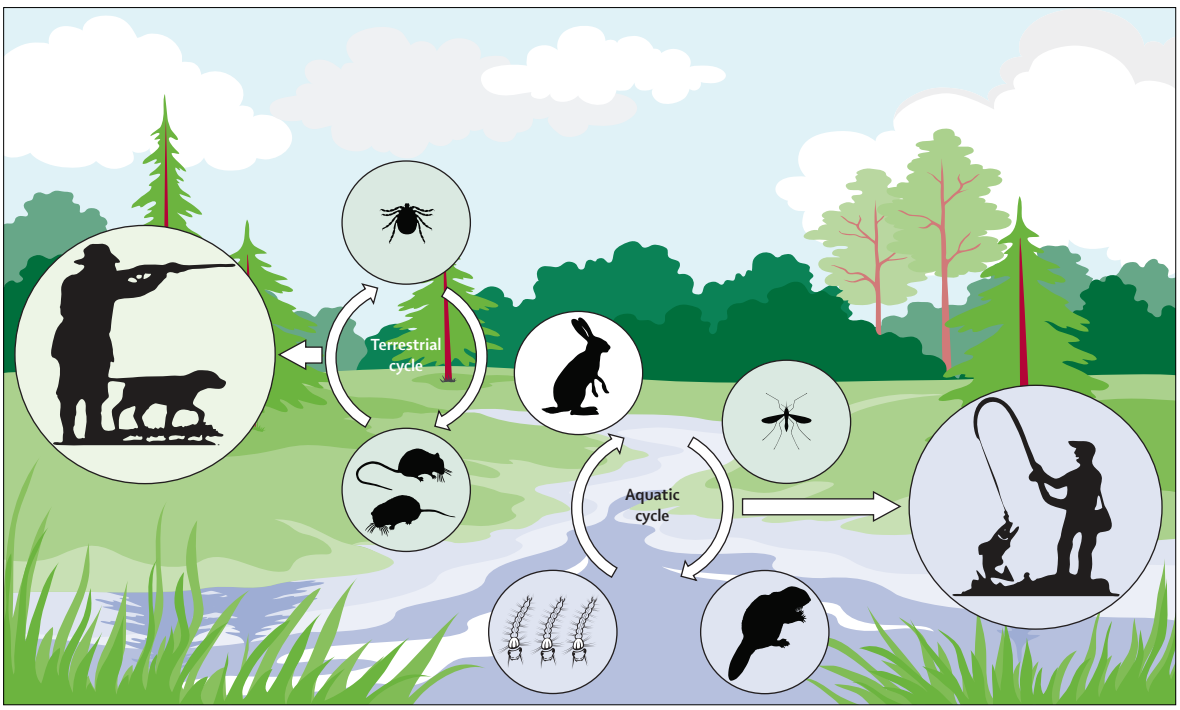

Figure 1: **The two main lifecycles—terrestrial and aquatic—of *Francisella tularensis* in Europe**
Ticks and rodents are reservoir hosts in the terrestrial cycle. Lagomorphs, mosquitoes, mosquito larvae, and rodents are reservoirs in the aquatic cycle.

basis of clinical and epidemiological data from scientific literature (figure 1).

The terrestrial lifecycle is predominant in most European countries, including Austria,[53] France,[50] Germany,[54] Hungary,[55] Switzerland,[56] Slovakia,[57] and the Czech Republic.[58] In this lifecycle, the lagomorphs, terrestrial rodents, and ticks are the main source of human infections. Typically, human tularaemia cases are rare and sporadic, although familial outbreaks might occur after contact with infected animals or consumption of contaminated food.[50]

The aquatic cycle has been reported in Bulgaria,[43] Kosovo,[42] Turkey,[45,46] Sweden,[34,40] and Finland.[59] In this life cycle, the primary sources of human infections are aquatic environments, including rivers, lakes, ponds, and water wells that are contaminated by excrement and carcasses of infected animals (especially semi-aquatic rodents).[41–43,45] Human tularaemia cases from aquatic sources are more common than in the terrestrial cycle, and, more importantly, often occur as large outbreaks. Human infections occur through consumption of contaminated water and the oropharyngeal forms of tularaemia predominate, such as in Bulgaria, Kosovo, and Turkey.[42,43,45,46] Alternatively, tularaemia cases might correspond to mosquito-borne infections, and the ucleroglandular and glandular forms predominate, such as in Sweden and Finland.[34,40,59] The two lifecycles might be encountered in the same country, in different areas, or at different times, as was the case in Spain.[44,60]

Clinical presentations of tularaemia

Human tularaemia

Tularaemia has a short incubation period (usually 3–5 days, maximum 2 weeks).[1,61] Early clinical presentation corresponds to influenza-like symptoms, including fever, chills, headaches, myalgia, and arthralgia. Six major clinical forms of tularaemia are recognised, depending mainly on the portal of entry of bacteria.[1,61] The ulceroglandular form combines a skin inoculation ulcer (often long-lasting with chronic suppuration) and a localised lymphadenopathy in the lymphatic system draining the corresponding skin area.[1,61] An isolated lymphadenopathy without detectable skin ulcer corresponds to the glandular form. The oropharyngeal form comprises chronic pharyngitis occurring after oral contamination, sometimes with mucosal ulcers and often with swollen and painful cervical lymph nodes.[62] The oculoglandular form is a painful conjunctivitis with localised lymphadenopathy (pretragal, submandibular, or cervical) occurring after conjunctival contamination.[63,64] Pneumonic tularaemia corresponds to *F tularensis* lung involvement after inhalation of a contaminated aerosol or through haematogenous spread of bacteria to these organs.[52,65] Typhoidal tularaemia (mimicking symptoms of typhoid) is typically a severe systemic disease, whatever the portal of entry of bacteria, with acute onset, high fever, asthenia, headaches, myalgia, and neurological symptoms (including confusion, stupor, and behavioural changes), but no localised lesions (such as skin ulcers and lymphadenopathy).

Tularaemia complications might include skin rashes including erythema nodosum,[66] soft tissue abscesses,[50,67] lymph node suppurations,[1] otitis media,[68] meningitis and brain abscesses,[69,70] and other secondary locations due to haematogenous spread of bacteria. Up to 30% of patients with lymphadenopathy develop chronic lymph node suppuration and occasionally skin fistulisation.[1,50] Thus, tularaemia is often a prolonged and debilitating disease, including in Europe. A worse prognosis is usually reported in patients with tularaemia and delayed treatment or an immunocompromised status.[4] In the preantibiotic era, death occurred in less than 1% of cases in Europe, compared with 3–5% of cases (up to 30% of pneumonic cases) in North America.[52] Death is now extremely rare in Europe in patients receiving appropriate antibiotic treatment.[1,4] By contrast, death rates vary according to the associated genotype—for example, in the subtypes of *Francisella tularensis* subspecises *tularensis* in the USA: 24% for A1b, 4% for A1a, 0% for A2, and 7% for type B strains.[7]

Although these characteristics are valid on a global basis, specificities exist for tularaemia cases occurring in different areas of Europe, including variations in the sources and modes of disease transmission to human beings, the median age and sex ratio of the infected patients, the seasonality of the disease, and the predominant clinical presentation. The ulceroglandular and glandular forms of tularaemia predominate in most countries. However, these clinical forms occur more often in men during the winter (hunting) season than in women, and skin ulcers and lymphadenopathies predominate in the upper part of the body when these clinical forms are caused primarily by direct contact with game animals.[50] By contrast, infections occur during the warm seasons in children and adult women as commonly as adult men, and skin ulcers and lymphadenopathies are reported in the upper and lower parts of the body when infections are caused primarily by arthropod bites (ticks and mosquitoes).[71,72] Oropharyngeal tularaemia predominates in countries and periods where precarious living conditions (including war time) favour the consumption of unsafe water and food,[42] but also in some developed countries, such as Norway.[41] Oculoglandular tularaemia, a rare cause of Parinaud oculoglandular syndrome (compared with *Bartonella henselae*, the bacterium of cat-scratch disease) in Europe, usually occurs in areas where handling of infected game animals is a key risk factor.[63,64] The pneumonic and typhoidal forms of tularaemia are usually deemed to be rare diseases in Europe, although probably underestimated because the clinical signs are unspecific. However, high incidences (up to 20%) have been notified in some countries,[50,60] possibly because of reporting bias but also because of differences in predominant modes of human infection. In our experience, typhoidal tularaemia cases often occurred in elderly or immunocompromised patients after consumption of a large amount of highly contaminated food (typically hare meat and rabbit terrine).[50,73] The disease is usually much less severe in Europe than in the USA.[50,73] Severe acute pneumonia seems very rare in Europe.[50,60,65] Most pneumonic tularaemia cases present as a subacute or chronic infection, with prolonged fever, coughing, weight loss, and enlarged mediastinal lymph nodes suggesting tuberculosis or non-infectious diseases (eg, lymphoma and sarcoidosis).[50,74]

	Total number of cases	Median per year (range)	Incidence per 100 000 inhabitants	Years of peak incidences
Austria	86	3 (0–19)	0·00–0·20	1998
Belgium	1	0 (0–1)	0·00–0·01	..
Bulgaria	308	7 (0–96)	0·00–1·20	1998, 2003
Croatia	97	4 (0–29)	0·00–0·60	1999
Czech Republic	1490	64 (36–255)	0·34–2·20	1999, 2008
Denmark	6	0 (0–4)	0·00–0·10	..
Estonia	9	0 (0–2)	0·00–0·15	..
Finland	4223	116 (15–926)	0·28–17·90	2000, 2003
France	325	16 (0–104)	0·00–0·16	2008
Germany	172	5 (0–31)	0·00–0·04	..
Hungary	1173	69 (15–148)	0·15–1·40	1997, 2006, 2010
Italy	125	4 (0–43)	0·00–0·07	2008
Latvia	6	0 (0–6)	0·00–0·29	..
Lithuania	15	0 (0–4)	0·00–0·14	..
Netherlands	1	0 (0–1)	0·00–0·00	..
Norway	529	18 (0–180)	0·00–3·66	2011
Poland	46	2 (0–8)	0·00–0·02	..
Romania	5	0 (0–4)	0·00–0·02	..
Slovakia	520	23 (5–133)	0·09–2·50	2002
Slovenia	21	1 (0–4)	0·00–0·20	..
Spain	1181	1 (0–585)	0·00–1·50	1997, 2007
Sweden	4622	241 (14–698)	0·20–7·80	2000, 2003, 2010
Switzerland	105	0 (0–40)	0·00–0·50	..
UK	2	0 (0–1)	0·00–0·00	..

Table 1: **Number of human tularaemia cases and incidence rates reported in European Union countries from 1997 to 2013**[17,83,84]

Animal tularaemia

Animals infected with *F tularensis* are usually easy to catch, moribund, or even dead.[75] The clinical features are unspecific and the pathological effects vary substantially between different animal species and geographical locations. Tularaemia can be very acute in highly susceptible species (eg, the house mouse, *Mus musculus*), with development of sepsis, spleen and liver enlargement, and pinpoint white foci in the affected organs.[32] The disease can be subacute in moderately susceptible species (eg, European brown hare in central Europe), with granulomatous lesions mainly in the lungs, pericardium, and kidneys.[76] Both acute and subacute diseases were recorded in mountain hares in Sweden.[32]

Tularaemia has been occasionally reported in non-human primates in European zoos.[77] All monkeys develop a disease similar to human ulceroglandular tularaemia, which might develop into bacteraemia and multifocal granulomatous pneumonia, hepatitis, and splenitis.

Tularaemia might be detected only by seropositivity in animals that resist *F tularensis* infection totally or partly, including rare cases in domestic animals in Europe and in some wild animal species (such as red fox, wild boar, and European brown hare), for which the slide agglutination test is a useful field screening method.[30,76,78]

In dead animals, heart-blood samples and tissue samples (from the spleen, liver, lung, kidney, and bone marrow) are useful to confirm tularaemia with serological, histological, immunohistochemistry, culture, and PCR methods.[30,76,79] *F tularensis* from carcasses must be isolated with media supplemented with antibiotics and antifungals to prevent overgrowth of other bacterial species.[80] Tissue suspensions can also be inoculated into laboratory mice for isolation and purification of *F tularensis* strains.[81]

Tularaemia in different European countries

Epidemiological data for tularaemia in Europe have shown that tularaemia is a notifiable disease in most European countries.[82] Although this disease is deemed to be underdiagnosed or under-reported, incidence data are available from the European Centre for Diseases Prevention and Control[83] and the European Commission Health and Consumers Directorate (table 1).[84] Figure 2 shows the main European areas for tularemeia endemics and outbreaks. Table 2 summarises the main epidemiological and clinical findings recorded for European countries where at least 50 tularaemia cases were notified during the past decade.

A few European countries are deemed to be free of tularaemia: Cyprus, Greece, Iceland, Ireland, Latvia, Luxembourg, Macedonia, Malta, and the UK (only imported cases).[83,84] No data for tularaemia exist in the scientific literature for Andorra, Liechtenstein, Moldova, Monaco, Montenegro, San Marino, and Vatican City. Very few cases of human tularaemia (reported annual incidences ranging from 0·0 to 0·1 cases per 100 000 inhabitants) have occurred in Albania,[84] Belgium,[92] Bosnia and Herzegovina,[84] Denmark,[93,94] Germany,[54,86] Italy,[87] Switzerland,[56] the Netherlands,[17] Poland,[95] Romania, and Slovenia.[83,84,95,96] Slightly higher annual incidences (up to 1 case per 100 000 inhabitants) during the same period of 1997 to 2013 have been reported in Austria,[53,85] Croatia,[91] Estonia,[83] France,[50,97,98] Lithuania,[83] Ukraine,[99] and Serbia.[91] Tularaemia cases in human beings have occurred almost every year, with maximum annual incidence rates higher than 1 case per 100 000 inhabitants in Scandinavia (ie, Sweden, Finland, and Norway),[40,41,59,88,100] Bulgaria,[43] the Czech Republic, Hungary, Kosovo, Russia, Slovakia, and Spain.[83,84]

Tularaemia is highly prevalent and primarily a mosquito-borne disease in Sweden,[40,100] where several type B genotypes coexist.[16,20] Most cases (including several outbreaks) have occurred in northern and central regions.[34,71,100] The ulceroglandular and glandular forms of the disease predominate,[71,72] but pneumonic, oropharyngeal, oculoglandular, and typhoidal forms have also been reported. Tularaemia is also primarily a mosquito-borne disease in Finland, although oropharyngeal and pneumonic forms have also been reported.[101] In Norway, water-borne oropharyngeal forms of the disease predominate, owing to the consumption of contaminated water from private wells and with lemmings as a key rodent reservoir.[41,88,102] Ulceroglandular forms of the bacterium (after rat or arthropod bites) have also been reported.[102] Most human infections (including outbreaks) have occurred in central and northern Norway.[41,88,101]

Human tularaemia was first recognised in Spain during a large outbreak occurring in the northwestern Castile and Leon region in 1997,[60] although cases had probably been detected before.[102] During this outbreak, the major risk factor was direct contact with hares (97% of cases), and most patients had ulceroglandular (61%) or glandular (9%) forms of tularaemia.[60] In July, 1998, a second outbreak of ulceroglandular tularaemia occurred

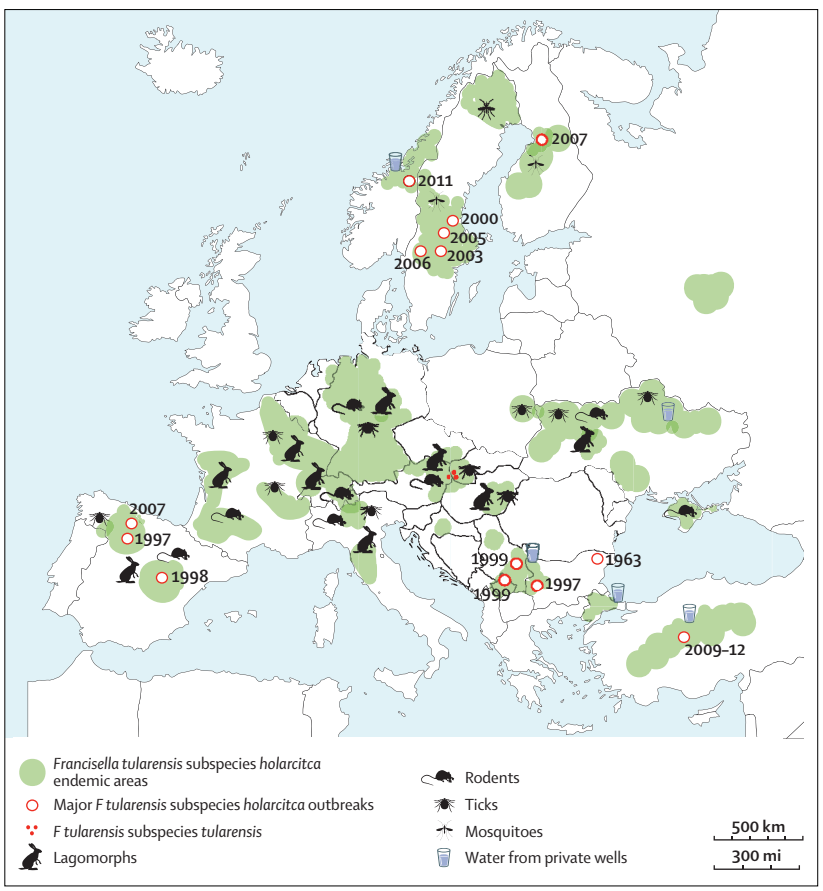

Figure 2: **Endemic areas and main outbreaks reported in the literature in Europe, with the main sources of human infections for selected countries**

	Most predominant environment for lifecycle	Most predominant modes of transmission	Most predominant modes of clinical forms	Known endemic areas
Austria[53,85]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Lower Austria, Burgenland, Vienna, Styria, and Upper Austria
Bulgaria[43]	Aquatic	Consumption of contaminated water	Oropharyngeal	Sofia and Pernik provinces
Czech Republic[58]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Moravia
Finland[59]	Aquatic	Mosquito bites	Ulceroglandular and glandular, oropharyngeal	Oulu
France[50]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Eastern, central, and southwestern parts of the country
Germany[54,86]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Bavaria, Hesse, Baden-Wuerttemberg, Thuringia, and Lower Saxony
Hungary[55]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Csongrád, Békés, Hajdú-Bihar, Győr-Moson-Sopron, Heves, and Jász-Nagykun-Szolnok
Italy[87]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Lombardy, and Tuscany
Kosovo[42]	Aquatic	Consumption of contaminated water	Oropharyngeal	Rural areas in the whole country
Norway[22,41,88]	Aquatic	Consumption of contaminated water	Oropharyngeal	Central and northern parts of the country
Serbia[89]	Aquatic	Consumption of contaminated water	Oropharyngeal	Nišava, Zajecar, Pirot, Pčinja, Toplica, Rasina, and Belgrade districts
Slovakia[44]	Terrestrial	Direct contact with animal reservoirs, through tick bites	Ulceroglandular and glandular	Western part of the country
Spain[44,60,90]	Terrestrial and aquatic	Direct contact with animal reservoirs, through tick bites, catching crayfish	Ulceroglandular and glandular, typhoidal	Valladolid, Palencia, Leon, and Cuenca provinces
Sweden[28,83]	Aquatic	Mosquito bites	Ulceroglandular and glandular	Northern and central parts of the country
Turkey[91]	Aquatic	Consumption of contaminated water	Oropharyngeal	Central Anatolia

Table 2: **Epidemiological and clinical aspects of the most recent tularaemia cases in European countries**

in patients after catching crayfish in central Spain (Castile-La Mancha region).[44] A third large outbreak transpired in 2007, in the Castila and Leon region,[103] mainly in men (roughly 80%) with typhoidal forms of the disease (about 60%). Despite variations in clinical and epidemiological features of the disease over time, the genetic diversity of strains isolated during these outbreaks is very low.[104]

Tularaemia is a widespread disease in the Czech Republic. The ulceroglandular forms related to contact with infected hares and tick bites predominate, but waterborne oropharyngeal cases also exist.[58] Rodents (especially the common vole, *Microtus arvalis*) and ticks (especially *D reticulatus*) are judged as key reservoirs of *F tularensis*.[58] Tularaemia is also endemic in Hungary, with incidences of 0–7 cases per 100 000 inhabitants per year between 1984 and 2010, correlating with brown hare and common vole abundance.[55] In Kosovo, a large outbreak of oropharyngeal forms occurred in 1999–2000, after the end of the Kosovo War,[42] owing to ingestion of water or food containing contaminants from the rodent population.[105] A second large outbreak occurred between 2001 and 2002[42] and the disease is still endemic in this country.[106] Tularaemia is also endemic in the western part of Slovakia (especially in the Nitra region), with several outbreaks reported.[57,107] The ulceroglandular, glandular, pneumonic, and oropharyngeal forms of the disease have been noted.[57] Exposure to contaminated animals and tick bites (especially *D reticulatus*) is thought of as a major risk factor. Worryingly, type AI strains of *F tularensis* were isolated from fleas and mites collected from small terrestrial mammals between 1978 and 1996, in the Danube region near Bratislava, Slovakia, probably representing environmental contamination from a laboratory stock of a Schu S4 North American strain.[8] Tularaemia cases are prevalent in western Russia and adjoining countries, including outbreaks in the Moscow region. The disease is also endemic in Ukraine, mainly in the western, northern, and Crimean regions.[99] Tularaemia is emerging as a waterborne disease in Turkey, where most patients have oropharyngeal infections, probably because of ingestion of water from wells contaminated with rodent carcasses.[91,108,109]

Diagnosis

Worldwide, tularaemia diagnosis is primarily based on compatible clinical and epidemiological data and a positive serological test.[33,61,110] The microagglutination test and the indirect immunofluorescence assay are most often used

in diagnosis,[33,61,110] although ELISA-type and western blot assays have been recently developed.[33,61,110] The live vaccine strain of *F tularensis* subspecies *holarctica* is often used to prepare whole-cell antigens because of this bacterium's attenuated virulence in human beings. A seroconversion or a four-fold or greater rise in antibody titre between acute and convalescent sera are deemed to be the diagnostic criteria. The cutoff titres might vary between laboratories because of an absence of standardisation of serological methods. Clinically significant antibody titres are usually detected only 1–2 weeks after symptom onset and therefore are usually absent in patients presenting with early clinical features.[33,50] Antibody titres peak at 3–4 weeks after progression of clinical symptoms,[33,50] and then progressively decrease, although residual titres might persist for months to years. Tularaemia serological tests are deemed to be highly specific despite a few serological crossreactions (eg, with *Brucella* species).

An *F tularensis* strain is isolated in less than 10% of tularaemia cases,[41,60,61,110] but greater proportions have been reported.[28,50] This bacterium might be isolated from the blood, lymph node punctures or biopsies, skin lesions, conjunctival and oropharyngeal specimens, and rarely from other sources (eg, cerebrospinal fluid). *F tularensis* is a highly infectious bacterium and cultures should be handled with appropriate biosafety conditions.[111] Laboratory infections still occur because *F tularensis* is often isolated from patients with non-specific symptoms, especially in automated blood culture systems, before the diagnosis of tularaemia is postulated.[50,111] *F tularensis* is a fastidious bacterium that grows aerobically in aminoacid-enriched media (eg, cysteine/cystine),[33] including the polyvitamin-enriched chocolate agar and the cysteine heart agar with 9% chocolate blood, after 24–48 h of media incubation at 37°C. Identification of *F tularensis* is difficult with conventional methods.[112] This bacterium can be obtained with a commercially available carbon-source utilisation test,[81] matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry,[113] or more reliable molecular methods.

PCR-based assays (classic, real-time, and multiplex)[50,63,114–119] are useful to detect *F tularensis* DNA directly from samples of human beings,[33,61,109] animals,[120] and the environment.[46] These assays can also identify the *F tularensis* subspecies or genotypes, either from isolated strains or directly from clinical samples.[114,115,121–125] Target genes have included the insertion sequence *ISFTu2*,[46,50,114] *tul4* coding a surface protein, a gene coding a 23-kDa protein,[114] *fopA* coding an outer membrane protein,[116] and *lpn*A coding a lipoprotein.[117] PCR testing of ticks must differentiate *F tularensis* from francisella-like endosymbionts.[126] Several methods have been developed for differentiation of *F tularensis* subspecies and genotypes, including PCR-sequencing, PCR-array, random amplified polymorphic DNA, PCR of enterobacterial repetitive intragenic consensus DNA sequences, PCR of repetitive extragenic pallendromic sequences, multilocus sequence typing, the study of canonical single nucleotide polymorphism and canonical insertions and deletions, and variable number tandem repeat markers.[6,13–15,24–26,50,115]

F tularensis DNA has been detected in several clinical specimens, including blood and serum samples,[50] skin lesions,[127] conjunctival[63,64] and oropharyngeal swabs,[45] lymph node suppurations and biopsies,[50,128] and lung, liver, and spleen tissue.[128] Multiplex PCR assays have been developed for simultaneous detection of several biological threat agents[129] or the various pathogens responsible for chronic lymphadenopathy.[120] PCR-based assays are more sensitive than cell culture for detection of *F tularensis* in skin ulcers and lymph node suppurations or tissues.[124,127] PCR testing of pharyngeal or conjunctival exudates is also useful for diagnosis of oropharyngeal and conjunctival tularaemia at symptom onset[63,64] when serological tests are negative. PCR testing of respiratory samples and lung tissue is useful for rapid confirmation of *F tularensis* pneumonia, especially in the context of bioterrorism.[128]

WHO has proposed criteria for tularaemia case definition:[33] a suspected case has an exposure history consistent with risks known to be associated with tularaemia together with clinical symptoms consistent with tularaemia; a presumptive case has suggestive clinical symptoms and a clinical sample that tests positive for tularaemia by antigen or DNA detection, and a single positive serum is also deemed to be presumptive; a confirmed case has recovery of an isolate and identification of the culture as *F tularensis* by antigen or DNA detection, or paired serum specimens with a four-fold (tube or microagglutination assay) or a substantial (ELISA) difference in titre, with at least one serum positive.

The diagnostic strategy of human tularaemia might vary according to the predominant clinical forms. We would like to emphasise that, at least in Europe where pneumonic tularaemia often presents as a chronic mediastinal lymphadenopathy,[6] *F tularensis* should be considered a second-line cause of tuberculosis-like pneumonia.

Treatment and prophylaxis

Antibiotic susceptibility testing of *F tularensis* strains should be done with appropriate biosafety conditions,[111] by use of the method recommended by the Clinical and Laboratory Standards Institute.[130] The Epsilometer test is an easier to use alternative to antibiotic susceptibility testing,[93,131] although eukaryotic cell models might allow the assessment of antibiotic activity against intracellular *F tularensis*.[132,133] Routine testing of *F tularensis* strains is not essential for patient care because acquired antibiotic resistance of this bacterium has never been reported in clinical settings. By contrast, emergence of resistance should be monitored in reference laboratories, especially because antibiotic-resistant mutants of *F tularensis* have been selected in vitro,[134–136] and overexpression of *F tularensis* efflux pumps could lead to multidrug resistance.[137,138]

Being a β-lactamase producer,[139] *F tularensis* is naturally resistant to β-lactam antibiotics; the poor penetration of

	Dosage in adults	Dosage in children	Duration	Comment
Ciprofloxacin	500·0 mg orally, twice daily	10·0–15·0 mg/kg orally, twice daily	2 weeks	Has been used successfully in children aged 1–10 years[145]
Levofloxacin	500·0 mg orally, once a day	Not recommended	2 weeks	..
Doxycycline	100·0 mg orally, twice daily	2·2 mg/kg orally twice daily	3 weeks	Preferably should be avoided in children younger than 8 years
Gentamicin	5·0 mg/kg intravenously in one or two doses	2·5 mg/kg intravenously three times daily	10 days	Use in children* with severe forms of tularaemia, with or without ciprofloxacin

*Gentamicin is preferred to tetracyclines and fluoroquinolones in children younger than 8 years because of potential side effects.

Table 3: **Frequently used antibiotic treatment regimens for tularaemia in Europe**[33,146]

these antibiotics into eukaryotic cells also further undermines their effectiveness.[132] Aminoglycosides (streptomycin and gentamicin), tetracyclines (especially doxycycline), and fluoroquinolones (ciprofloxacin and levofloxacin) are the main effective antibiotics in vitro, in cell-free media,[85,131,140–142] and in cell systems.[132,133] Rifampicin is also active against *F tularensis* in vitro.[131,143] Erythromycin and most other macrolides are poorly active, with the biovar 2 strains *F tularensis* subspecies *holarctica* being naturally much more resistant than other biovars because of an *A2059C* mutation on the gene coding for 23S ribosomal RNA.[85] Azithromycin and telithromycin are more effective in vitro than erythromycin.[132,144] The phenicol compounds and co-trimoxazole also have poor in-vitro activity.[132,143]

In view of the innate resistance of *F tularensis*, β-lactams are typically not thought of as effective treatments of tularaemia.[61,110] Aminoglycosides (streptomycin and gentamicin), fluoroquinolones (especially ciprofloxacin), and tetracyclines (mainly doxycycline) are the foundation of antibiotic treatment of tularaemia in human beings (table 3).[33,61,109,146] Streptomycin was deemed to be very effective.[61,110] Gentamicin is now often used instead in patients with systemic diseases and in children,[61,110] especially in countries where streptomycin is no longer available, although this antibiotic has been associated with higher relapse rates than streptomycin.[147] Fluoroquinolones and tetracyclines, given for 2–3 weeks, are first-line drugs for treatment of mild to moderate tularaemia cases that predominately occur in Europe. The fluoroquinolones are associated with lower relapse rates than the tetracyclines (roughly 5–10% *vs* 10–15%).[61,110] Treatment failures and relapses are particularly common in patients with delayed treatment or suppurated lymphadenopathies,[50,60,147] for which a 2–3 week treatment course is usually insufficient for cure. Fluoroquinolones might potentially induce fetal malformations and cause damage to young children's musculoskeletal system. However, ciprofloxacin has been reported as a safe and effective treatment of tularaemia in children aged 1–10 years.[145] Azithromycin might be useful in pregnant women infected with type B biovar 1 strains, which are naturally susceptible to macrolides and usually induce mild diseases,[148,149] although this alternative would be limited to the western part of Europe, where these strains are found. Gentamicin should be used in pregnant women with severe tularaemia to avoid potential deleterious obstetrical consequences.[150] New treatment alternatives for tularaemia are needed for better management of patients with severe or prolonged diseases, safer treatment of young children and pregnant women, and better preparedness in case of bioterrorism involving antibiotic-resistance strains.[151]

The attenuated live strain of *F tularensis* subspecies *holarctica* has been used for decades as a tularaemia vaccine, especially for laboratory workers.[152] However, it is no longer in use because of its overall small efficacy and concerns about reversion to virulence. Novel vaccines against tularaemia are in development but not yet licensed for use in human beings.[152] The major measures to prevent tularaemia cases are those that restrict human contact with the reservoirs of *F tularensis*, which vary depending on the specific endemic area considered. Antibiotic prophylaxis (eg, ciprofloxacin) is useful in patients with proven *F tularensis* exposure.[33,146] Safe laboratory practices are essential to prevent laboratory-acquired tularaemia.[111] Translocation of hares for sporting purposes and enhancement of existing populations can introduce tularaemia into new areas, and therefore should be controlled. No European Union regulation specific for tularaemia exists, the pre-export screening regulation of hares is only based on bilateral agreements between specific countries (eg, between Italy and Hungary).[32] Tularaemia is a notifiable disease to the World Animal Health Information Database.

For the **World Animal Health Information Database** see http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home

Conclusion

Tularaemia is endemic in many European countries, and has emerged recently in some (eg, Spain). The knowledge of clinical and epidemiological aspects of this zoonosis in Europe has improved since the early 2000s, when the classification of *F tularensis* as a bioterrorism agent led to closer monitoring of the disease. Although almost all tularaemia cases are caused by *F tularensis* subspecies *holarctica* on this continent, a wide variability exists among different countries in the modes of human contamination, the sporadic or epidemic nature and seasonality of the disease, the characteristics of the implicated population (sex ratio and mean age), the predominant clinical forms and their severity, and the health burden in human and animal populations. Different life cycles of *F tularensis* (terrestrial or aquatic) could partly explain these

Panel: Next steps

We have identified some lines of research to improve knowledge of the clinical, epidemiological, and therapeutic aspects of tularaemia in Europe.

- The true animal reservoirs of *Francisella tularensis* in different geographical areas must be better defined. Further characterisation of wild animal species that can carry *F tularensis* for extended periods in these animals' natural habitat is needed.
- Long-term survival of *F tularensis* in terrestrial and aquatic environments (including in free-living protozoa) must be further characterised.
- The reservoir competence of arthropods (especially ticks) for *F tularensis* subspecies *holarctica* must be further assessed. The role of mosquitoes in the transmission of tularaemia outside Scandinavia must be researched more actively.
- As for the clinical aspects of tularaemia in Europe, many human infections are probably undiagnosed (as for many other zoonoses), especially because of discrete self-limiting clinical symptoms. Seroepidemiological surveys could help to define the true prevalence of these asymptomatic infections.
- By contrast, better predictions are needed for the development of chronic suppuration and other complications in patients with tularaemia. Further diagnostic methods and prognostic markers are thus needed to optimise patients' management.
- New therapeutic alternatives are needed to reduce the rate of treatment failures and relapses, and find safer alternatives in children and pregnant women than at present.
- The ideal management of patients with tularaemia complications should be better defined. Guidelines should further describe the most appropriate therapeutic strategies in the corresponding patients.

geographical variations. Several *F tularensis* genotypes have also been characterised according to various geographical areas, but for now are not correlated with different characteristics of tularaemia in these areas. Although rarely fatal, tularaemia in human beings is often a prolonged and debilitating disease in Europe, especially in patients who have chronic suppurative lymphadenopathy. Furthermore, because of a subacute or chronic course of disease, the pneumonic forms of tularaemia often mimic more severe diseases, including tuberculosis and lymphoma. A better understanding of specific aspects of tularaemia in Europe is needed to better prevent infectious complications and improve the monitoring of this disease in the context of a biological threat (panel).

Contributors

The authors contributed equally to the preparation of the manuscript. MG was in charge more specifically of describing the clinical aspects of tularaemia in animals, and MM the clinical aspects of human tularaemia.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed for articles published in English from January, 1980, to August, 2015, by use of the terms "Tularemia", "Tularaemia", "*Francisella*", "*Francisella tularensis*", "Taxonomy", "Phylogeny", "Epidemiology", "Outbreaks", "Diagnosis", "Treatment", and "Prophylaxis". Relevant articles published during this time were identified and cited in the references section. Because we wanted to emphasise specific aspects of tularaemia in Europe, the term "Tularemia" was also crossed with each of the names of European countries.

Declaration of interest

We declare no competing interests.

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