Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Review

Tularaemia: A challenging zoonosis

C.L. Carvalho, I. Lopes de Carvalho, L. Zé-Zé, M.S. Núncio, E.L. Duarte

A R T I C L E   I N F O

Article history:
Received 16 October 2013
Received in revised form 28 December 2013
Accepted 4 January 2014

Keywords:
Tularaemia
Francisella tularensis
Zoonosis
Category A biowarfare agents
Wild animals

A B S T R A C T

In recent years, several emerging zoonotic vector-borne infections with potential impact on human health have been identified in Europe, including tularaemia, caused by Francisella tularensis. This remarkable pathogen, one of the most virulent microorganisms currently known, has been detected in increasingly new settings and in a wide range of wild species, including lagomorphs, rodents, carnivores, fish and invertebrate arthropods. Also, a renewed concern has arisen with regard to F. tularensis: its potential use by bioterrorists. Based on the information published concerning the latest outbreaks, the aim of this paper is to review the main features of the agent, its biology, immunology and epidemiology. Moreover, special focus will be given to zoonotic aspects of the disease, as tularaemia outbreaks in human populations have been frequently associated with disease in animals.

© 2014 Elsevier Ltd. All rights reserved.
1. Introduction

Seventy-five per cent of emerging infectious diseases are zoonotic [1]. Some wildlife species have been recognised as being major reservoirs for infectious diseases and the proximity of wildlife habitats and the existence of arthropod vectors with a wide geographical spread have rendered epidemiological cycles more complex [1].

Tularaemia is a zoonosis caused by the Francisella tularensis bacterium, which was first isolated in 1912 in Tulare County, California, by George McCoy and Charles Chapin [2–4]. Initially termed Bacterium tularensense, it was allocated to a new genus and named F. tularensis in honour of the pioneer of research on the organism, Edward Francis [2,4]. Arthropod-borne transmission of tularaemia was first demonstrated by Francis in 1919 when he isolated the etiologic agent in a patient with “deer fly fever” [2,5,6].

Tularaemia was recognised as an important disease in the last century and since then there has been a growth in enthusiasm for research on this pathogen [7,8]. Interest has arisen with regard to F. tularensis as it has emerged in new locations, populations and settings, and increasingly figured in scientific research gauging its potential use in bioterrorism [7,9]. The European Centre for Disease Control and Prevention (ECDC) 2012 surveillance report refers a total of 891 confirmed cases of tularaemia in a number of European countries in 2010, with Sweden reporting the highest confirmed case rate, followed by Finland and Hungary [10]. Tularaemia is considered an unusual disease and the confirmed case rate in Europe has remained stable from 2006 to 2010. Recent outbreaks of tularaemia have occurred in several European countries, presented in Table 1, including the Czech Republic, Kosovo, Bulgaria, Germany, Sweden, Finland, Spain, Turkey, France and Norway [11–20]. Besides these outbreaks, sporadic case notifications have occurred in Austria, Estonia, Italy, Lithuania, Poland, Romania, Slovakia and the United Kingdom [10]. Although there are no reports of tularaemia for Denmark during this period, a confirmed case of the disease in a human was recorded there in 2003 [21]. In Portugal, the bacterium has been detected in the blood of an asymptomatic man and in a Dermacentor reticulatus tick by molecular methods [9].

2. Microbiology and phylogeography of F. tularensis

F. tularensis is one of the most virulent microorganisms currently known, while as few as ten microorganisms can cause potentially fatal disease in man and animals [7,22]. This high rate of infectivity has led the Centre for Disease Control and Prevention (CDC) to classify F. tularensis as a Category A biowarfare agent [23].

F. tularensis is a gram-negative, catalase-positive, pleomorphic and non-motile cocobacillus, characterised as a facultative intracellular pathogen that can grow within different types of cells including macrophages, hepatocytes and epithelial cells [2,22,24,25]. The cell wall of F. tularensis has an unusually high level of fatty acids with a unique profile for the genus, and wild strains have a lipid-rich capsule, with neither toxic nor immunogenic properties [2,5,6]. Capsule loss has been related to a decrease in virulence, although the viability or survival of the bacterium within neutrophils may remain unaltered.

F. tularensis is a gamma (γ)-Proteobacteria of the Francisellaceae family [2,4,22]. F. tularensis is the most common and pathogenic species and is formally divided into three subspecies with different pathogenicities and geographic distributions: tularensis, holarctica and mediiasiatica. The species Francisella novicida is currently widely accepted as a fourth subspecies of F. tularensis [3,4,26–31], as it shares with F. tularensis an average of 99.2% nucleotide identity over a 1.1 Mbp of genome sequence [4,26,27,30]. However, some objections to the transfer of F. novicida to the subspecies rank of F. tularensis have been recorded, based on recent multiple genome sequencing results, which show divergent evolutions for F. tularensis and F. novicida populations. Therefore, separate species may be retained [32].

The F. tularensis subspecies tularensis, regarded as the most virulent subspecies and classified as Type A, occurs predominantly in North America [3,4,6,22,33]. Two distinct genetic sub-populations have been identified, Al and All, which have different geographic distributions, hosts and vectors [3,4,6,26,30,34]. Sub-population Al has been additionally sub-divided into groups Ala and Alb [3,6,30,35]. The subspecies holarctica, related to milder forms of the disease and classified as Type B, occurs throughout the Northern Hemisphere [3,22,30,33]. Human infection with Alb strains usually have a fulminating clinical progression and are associated with high mortality rates, in contrast with infections by Al and All strains or Type B tularaemia [25,30,35]. Recently, this subspecies has also been detected in Tasmania, Australia [36]. Subspecies mediasiatica presents a similar virulence to subspecies holarctica, but its geographic distribution is restricted so far to Central Asia [26,33]. F. novicida is less virulent and has been isolated in North America, Australia and Thailand [3,6,29–33,37]. Based on a high degree of similarity between 16S rRNA gene sequences, other microorganisms have been classified as probable members of the Francisellaceae family; these include the Francisella-like endosymbionts or FLEs [6,8,38]. FLEs belong to a distinct phylogenetic clade from F. tularensis species [39]. The effect of FLEs, if any, on vector competency and in the transmission of F. tularensis by ticks is still unknown [6]. FLEs have a worldwide distribution and are vertically transmitted by hard and soft ticks of the genera Amblyomma, Dermacentor, kordes and Ornithodoros [39–42]. FLEs have been detected in ticks in North America
| European country   | First report  | Latest report   | Suspected animal host                                      | Tularaemia transmission to humans                                      | Number of cases (year(s)) | References |
|--------------------|---------------|-----------------|------------------------------------------------------------|------------------------------------------------------------------------|-----------------------------|------------|
| Czech Republic     | 1936 (humans and hares) | 2000 | Small mammals, particularly wild hares, rodents          | Contact with tissues of infected animals, aerosols, contaminated food and water, tick bite | 48 (2000)                  | [11,87–91] |
| Kosovo             | 1999–2000     | 2001/2002–2010  |                             | Ingestion of contaminated food or water                               | Ranging from 25 to 327 (2001–2010)                                   | [12]        |
| Bulgaria           | 1962 (muskrat) | 1997, 2003–2005 | Wild hares                                               | Contaminated food and water, tick bite                                | 285 (1997–2005) 24 (2003–2004)                                    | [13,92]     |
| Germany            | 1949          | 2004–2005       | Wild hares, rodents                                     | Contact with tissues of infected animals                              | 270 (2000) 698 (2003) 39 (2004–2005)                                | [14,93,94]  |
| Sweden             | 1931          | 2000–2005       |                             | Contaminated water, aerosols (farming), Mosquitoes, aerosols (farming), tick bite | 270 (2000) 698 (2003) 39 (2004–2005)                                | [15,95–97]  |
| Finland            | a             | 2000, 2003, 2007 |                             | Mosquitoes, aerosols (farming), tick bite                            | 270 (2000) 698 (2003) 39 (2004–2005)                                | [16,21,76,97–99] |
| Spain              | 1997 (Human, wild hares) | 2007 | Small mammals (especially hares and rodents)         | Aerosols, wild lagomorphs, canids, rodents, sheep, haematophagus vectors, crayfish | 507 (2007)                  | [17,51,100,101] |
| Turkey             | 1936          | 2000, 2004–2010 |                             | Contaminated water                                                   | 12 (2000) 61 (2004–2005) 12 (2005–2006) 40 (2010)                   | [18,102–107] |
| France             | a             | 1997–2011       | Wild hares                                               | Contact with tissues of infected animals, aerosols, tick bite        | 144 (2007–2008) 51 (2011)                                          | [19,108–110] |
| Norway             | a             | 2003, 2005, 2007, 2011 | Rodents (lemmings) and hares                             | Contaminated water or food                                           | 5 (2005) 9 (2007) 39 (2011)                                        | [20,111,112] |

* Information unavailable.
(Texas, California, Minnesota), Canada (Alberta) and European countries such as Spain, Portugal, Hungary, Serbia and Bulgaria [38–45]. Their pathogenicity to humans is undetermined. They have recently been detected in free-living small mammals in Europe, suggesting the possible transmission of some FLE types from ticks to small mammals, although, to date, attempts to demonstrate it have failed [39,41,42,45,46]. The phylogeographic distribution of *F. tularensis* is given in Fig. 1; the geographic locations where FLEs have been detected in ticks are also indicated.

### 3. Epidemiology of *F. tularensis*

In nature, *F. tularensis* has been detected in a high number of wild species including lagomorphs, rodents, insectivores, carnivores, ungulates, marsupials, birds, amphibians, fish, and invertebrates [6,22,27,39,46–48].

Lagomorphs and rodents are considered as the main reservoirs of *F. tularensis* [6,22,46]. Wild lagomorphs, such as the European brown hare (*Lepus europaeus*), are thought to be suitable sentinels for *F. tularensis* and disease surveillance [46,47]. Recently, there have been serological evidences that foxes and raccoon dogs could also act as biological indicators for tularemia [48].

Natural infections with *F. tularensis* have also been documented in different arthropods, although only a subset of these have been identified as important in *F. tularensis* transmission to humans. Still, few pathogen shows the adaptability of *F. tularensis* to such a wide range of arthropod vectors capable of infection dissemination [6]. Arthropod found infected in nature include ticks of the genera *Amblyomma*, *Dermacentor*, *Ixodes* and *Ornithodoros*, mosquitoes of the genera *Aedes*, *Culex*, *Anopheles* and *Ochlerotatus excrucians*, and flies from the Tabanidae family (*Tabanus* spp., *Chrisozone* spp. and *Chrisops* spp.) [6,22,27,49]. Nevertheless, vector competence has only been demonstrated in ticks of the genera *Dermacentor* [35].

Tick-borne transmission of *F. tularensis* usually results in sporadic cases, although occasional outbreaks have also been reported [6]. Although regarded as merely mechanical vectors, mosquitoes have been associated with widespread epidemics of tularemia and are capable of transient disease transmission [6,50]. Both ticks and mosquitoes may be infected in the larval phase. Transtadial transmission has been demonstrated in ticks although in mosquitoes evidences for transtadiatal transmission are only based in molecular methods [35,50]. Although transovarial transmission of *F. tularensis* in ticks was reported [2,6,51], a recent study in *Dermacentor variabilis* has proved otherwise [52]. Despite dissemination to ovaries and then to the oocytes, the pathogen was not recovered from the subsequently hatched larvae. Tabanid flies are regarded as mechanical vectors for *F. tularensis* and the long-term survival of this bacterium does not occur in these arthropods [6].

The epidemiologic characteristics of vector-borne tularemia vary throughout the northern hemisphere and also within a given geographic location. This is thought to be related to the abundance of different vectors and host species. This could explain why, in the USA, Sweden, Finland and Russia, the arthropod bite is a common mode of transmission to humans, whilst in Western and Central Europe, contact with infected animals and the ingestion of contaminated food or water have been reported as more common transmission modes. Differences in transmission patterns have also been recorded within the USA: in western states, both ticks and deer flies are considered to be important vectors of tularemia, while in the east only ticks are considered relevant. In Sweden and Finland, mosquitoes have been identified as the primary vectors [6].

In Portugal, the role of ticks and small mammals in the transmission of tularaemia is still the subject of research. A collection of 4949 mosquitoes belonging to the genus *Culex* (63.97%), *Ochlerotatus* (35.34%), *Anopheles* (0.42%), *Culiseta* (0.14%) and a small number of *Aedes aegypti* females from the island of Madeira (0.12%) have been analysed, although all the results were found to be negative [53]. So far, this is in accordance with previous findings regarding the epidemiology characteristics of vector-borne tularaemia, suggesting that, in Portugal, mosquitoes have no role in the transmission of this disease. Ticks are thought to be the most important vectors of tularaemia in the majority of countries where tularaemia is endemic [53]. Nevertheless, major on-going research on tularaemia, aiming at gauging the overall impact of the disease in Portugal, is expected to throw further light on the main *F. tularensis* sources.

In endemic areas, tularaemia is a seasonal disease, with higher incidence in late spring, summer and autumn, occurring annually over a 5-year period or unreported for more than a decade. Often, the number of cases varies widely from 1 year to another, which is thought to be due to temperature or precipitation variability. However, the association between climactic conditions and tularaemia outbreaks has yet to be demonstrated [49]. *F. tularensis* has been found to be extremely resistant to environmental stress, surviving for weeks in soil, water and animal carcasses, at low temperatures [22].

Human tularaemia outbreaks are often preceded by animal outbreaks, particularly in wild lagomorphs and rodents. This is usually related to an increase in the numbers of these species, increasing the probability of exposure to infected animals [4,22,27,49].

The transmission of tularaemia to humans can occur either by direct contact with infected animals or indirectly due to arthropod vector bites, the ingestion of contaminated water, food or aerosols inhalation. Aerosols can be dispersed by ventilators, farming, and the deposition of contaminated hay, either intentionally or unintentionally [22]. Domestic dogs and cats can also transmit tularaemia to humans after contact with an infected animal, environment or infected ticks [54–56]. Person-to-person transmission has not been described so far [22,49,54].

Tularaemia has been reported to occur in any age group. Men tend to present a higher prevalence than women [2,49]. Professions that are prone to contact with reservoirs or arthropod vectors have been associated with a higher infection risk: these include laboratory technicians, hunters, farmers, veterinary surgeons, and anyone handling the flesh of infected animals [22,27].
4. *F. tularensis* life cycle

Few pathogens show the adaptability of *F. tularensis* to varying vector, host and environmental conditions. Variations occur in local transmission cycles in association with differing ecologies. Both *F. tularensis* type A and type B are associated with different life cycles in which different animal hosts and arthropod vectors intervene [6]. Type A tularemia is more commonly associated with the terrestrial cycle of the disease, with wild lagomorphs such as rabbits and hares acting as vertebrate hosts in which amplification of the agent occurs and where arthropods are disease-disseminating vectors [6,22,54,57]. Type B tularemia is more frequently associated with the aquatic cycle, although outbreaks of tick-borne tularemia involving subspecies *holarctica* have been reported [2,6,57]. In this life cycle, *F. tularensis* circulates in rodents such as beavers, muskrats and voles, and can be introduced in water courses from animal carcasses [6,22,27,54]. There is also evidence that *F. tularensis* can persist in water courses in association with amoebas [27,49,58]. Contaminated water can be the source of infection to humans, flies and mosquitoes [49]. An unusual waterborne outbreak of human tularemia has been described in Spain associated with crayfish (*Procambarus clarkii*) caught in a contaminated freshwater stream. The crayfish acted as mechanical vectors, through mud- or water-contaminated carapaces, although the presence of *F. tularensis* in crayfish stomach and hepatopancreas could indicate their eventual role as hosts [51]. A diagrammatic representation of the terrestrial and aquatic cycles of tularemia is shown in Fig. 2.

5. Immunopathogenesis

*F. tularensis* is a remarkable bacterial pathogen that can invade and multiply in a wide range of cell types [4,22,24,25,59]. Antigen-presenting cells (APC) such as macrophages or dendritic cells, appear to be the primary cell types targeted by the bacterium at the onset of infection [59]. The virulence of the bacterium is directly related to its capacity to replicate within the cytosol of infected cells [60]. *F. tularensis* clearly possesses several mechanisms by which it manipulates immunity. The bacterium evades detection at the point of entry in the host in three ways: (a) it has modified cell-surface structures that enable it to avoid interaction with host receptors that are associated with the induction of inflammation; (b) it targets cells that lack co-receptors which facilitate binding to receptors that might alert the host cell to invasion; (c) it utilises receptors that fail to initiate the production of pro-inflammatory cytokines [60].

6. Innate immune response

The entry of *F. tularensis* in macrophages occurs by means of a specific mechanism inherent to *Francisella* spp. [24]. The bacterium induces the macrophage to produce asymmetric spacious pseudopod loops in a “looping phagocytosis” process [4,61]. Uptake of *F. tularensis* is markedly
enhanced by serum opsonisation, which depends on serum intact complement factor C3 and host cell receptors (CR3), involving bacterial surface polysaccharides [4,62].

Utilisation of CR3 (and of mannose receptors of dendritic cells (MR) under non-opsonising conditions) is considered to be a fairly innocuous route for entry of *F. tularensis*, since it is not associated with the induction of signalling cascades that result in pro-inflammatory cytokines production. When opsonised by serum, *F. tularensis* binds iC3b and gains entry to host cells via the CR3 receptor [59].

The lipopolysaccharide (LPS) of subspecies *tularensis* is only moderately inflammatory and acts as an extremely weak toll-like receptor (TLR) 4 agonist stimulating a reduced production of pro-inflammatory cytokines [59,63]. These is attributed to the presence of only four acyl groups on the LPS that do not bind to the "LPS-binding proteins", subverting TLR4 recognition [4,25,59]. In addition to LPS, *F. tularensis* possesses two other TLR agonists [59]: Tu4 and FTT1103 lipoproteins. These interact with TLR2 and may alert the host cell for the presence of the bacterium prior to phagocytosis [4,25,59]. TLR2/myeloid differentiation primary response gene 88 (MyD88) signalling is essential for the production of pro-inflammatory cytokines and is critical for host defence against *Francisella* infection [24,61,63,64].

*F. novicida* has been used as a model organism to study immunity to *F. tularensis*. Nevertheless, *F. novicida* expresses a structurally distinct chemotype of LPS that is more pro-inflammatory in mice than the dominant LPS chemotype, and is expected to result in different inflammasome activations [25]. *F. novicida* escapes the phagosome and replicate in the cell cytosol where it is recognised by the inflammasome signalling system [24,25,60,64]. Inflammasome *stimuli* activate the pro tease cysteine aspartate-specific Caspase-1, promoting the release of potent pro-inflammatory cytokines responsible for cell apoptosis [24,60]. This results in *F. novicida* release from infected cells and enables the infection of new ones [24,60].

*F. tularensis* survival and replication within macrophages is enabled by a large set of virulence genes that include the “macrophage growth locus” (mgl) A and B and the "Francisella Pathogenicity Island", FPI [24]. FPI encodes for a putative type VI secretion system [4,8] and contains 19 genes that have been demonstrated as essential for intra-cellular growth and virulence [24]. Less virulent *F. novicida* presents only one copy of FPI in contrast with *F. tularensis* subspecies *tularensis* and *holarctica* that present two copies [4,24]. Genes within the FPI are regulated by mglA [4]. Although current knowledge of the gene’s functions is far from complete, this is one of the most active areas of Francisella research [8].

Following phagocytosis of opsonised *F. tularensis* by polymorphonuclear cells (PMN), the bacterium actively inhibits superoxide anion generation (ROS) via NADPH oxidase. This allows *F. tularensis* to evade the phagosome and persist in the cell cytosol. The contribution of polymorphonuclear cells seems to be related to the secretion of cytokines and chemokines that recruit effector cells to the infection site [25]. However, an excessive recruitment of neutrophils, modulated by an increase in metalloprotease-9 from the matrix, plays an important role in modulating leukocyte recruitment and seems to be directly related to *F. tularensis* pathogenesis [24,25].

Natural killer (NK) cells from the liver, spleen and lung also play an important role in the innate immune response, in particular by producing INF-α following primary infection by *F. tularensis* [25].

7. Acquired immune response

As *F. tularensis* is an intracellular pathogen, cellular immune response is believed to be the main defence mechanism. Memory effector T cells CD4+ and CD8+ are clearly important for the primary control of infection. These cells produce Type I cytokines like INF-γ, TNF-α and IL-2 that are critical for the initial response to *F. tularensis* infection [25].

Although the role of humoral immunity in *F. tularensis* infection is believed to be less important, some studies have demonstrated the enhanced recovery of infected humans that have received hyper-immune serum [59]. Also, infection-specific IgM, IgA and IgG antibodies produced are good exposure indicators and may interfere with the ability of bacteria to infect host cells [25,49,59]. The contribution of B cells in defence is thought to be dependent on strain virulence [8,25]. Research on anti- *Francisella* antibodies targets is expected to allow for the identification of new diagnostic or reactive antigens and the development of vaccines [8].

Furthermore, *F. tularensis* is capable of influencing multiple pathways, and continued research into the specific mechanisms by which *F. tularensis* evades, modulates and suppresses the host immune response will improve our understanding of tularemia pathogenesis and the regulation of host immunity [59].

8. Clinical manifestations of tularemia

8.1. Humans

Relevant clinical disease has been reported with *F. tularensis* subsp. *tularensis* and *holarctica*. Clinical manifestations of tularemia depend on strain virulence, infective dose and infection route, the extent of systemic involvement and host immune status [2,4,49]. The incubation period averages 3–5 days but ranges from 1 to 20 days. The disease has an acute onset, with the occurrence of fever (38–40°C), chills, fatigue, generalised myalgia and headaches, resembling a flu-like syndrome [22,49]. The subspecies *tularensis* (Type A) causes severe disease, potentially fatal if untreated. The subspecies *holarctica* (Type B) causes less severe disease and fatalities are rare [49]. Depending on the route of infection, the following forms of the disease are described: ulceroglandular, glandular, oculeoglandular, oropharyngeal, pneumatic, typhoidial and septic [22,49].

Ulceroglandular and glandular forms of the disease are the most common and frequently result from an arthropod bite or animal contact [2,4,49]. In ulceroglandular tularémia, a soft, painless ulcer develops at the inoculation
site and evolves to a scar [6,22]. This presentation is associated with fever, lymphadenopathy and, in Type A tularemia, pneumonia and pleural effusion can occur [49]. In glandular tularemia, the primary ulcer is unrecognisable [2,6,22,49].

Direct contamination of the eye through contaminated fingers, splashes or aerosols, may be followed by oculoglandular tularemia. Unilateral conjunctivitis, with ulcers or papules in some patients, photophobia and epiphora are the main signs of this form of the disease [2,49].

Oropharyngeal tularemia is acquired by means of contaminated food or water intake and aerosol inhalation [22]. It develops with ulcerative and exudative stomatitis and pharyngitis [49].

Pneumonic tularemia occurs by means of contaminated aerosol inhalation but can also arise as a complication of any of the other disease forms by haematogenous generalisation [2,22,49]. Initial disease development is characterised by fever, cough, pleuritic chest pain and dyspnoea, along with other unspecific symptoms. Type A tularemia is associated with significantly severer and more fulminant forms of pneumonia [2,49].

Typhoidal tularemia refers to a systemic and febrile form of the disease in which no route of infection acquisition can be established [2,49].

Septic tularemia is a severe and often fatal form of the disease that can occur as a complication of the ulceroglandular form in Type A tularemia [2,22,49]. Patients can present unspecific and neurologic symptoms, and septic shock, SIRS (systemic inflammatory response syndrome), DIC (disseminated intravascular coagulation), haemorrhages, SARS (severe acute respiratory syndrome) and multiple organ failure [2,22,49]. In Type B tularemia, complications of meningitis and septicaemia have only occasionally been described [49].

8.2. Animals

Clinical manifestations largely depend on the susceptibility of animal species to F. tularensis [49]. In wild animals, clinical signs of tularemia are not well documented, and post-mortem findings are highly unspecific and include splenomegaly and punctual necrotic lesions in the liver and spleen [49,54].

In one experimental study in European brown hares (Lepus europaeus), clinical signs developed 1-day post-inoculation with a F. tularensis subspecies holarctica strain. These included fever, lethargy and anorexia. Two of the five hares in the study succumbed to the infection on days 5 and 9 following inoculation. Pathological findings included splenomegaly, diffuse spleen necrosis and focal liver necrosis with hepatocytes vacuolisation. The remaining three hares were euthanised and revealed no pathological lesions. Both bacterial culture and mouse inoculation test failed to produce F. tularensis isolation [46]. In a natural outbreak of tularemia in brown hares in France, all eight hares involved presented splenomegaly, congestion and haemorrhagic lesions of several organs, tracheitis and bronchitis [65]. A similar study carried out in Hungary on European brown hares naturally infected with F. tularensis subspecies holarctica also showed very similar results [47].

In another study, 20 female New Zealand white rabbits (Oryctolagus cuniculus) were exposed to Type A tularemia aerosols, with three different doses. Seven of them died while the others developed fever, anorexia and weight loss, with all infecting doses. Haematological findings in six rabbits included lymphopenia, monocytopenia and thrombocytopenia. A labial pneumonia and gastrointestinal tract gas distension were the only radiological findings. Necropsy findings demonstrated hepatosplenomegaly with extensive spleen necrosis and small white nodules. Some of the rabbits presented nodular lesions in the lungs while others showed haemorrhagic lesions [66].

A situation of particular public health significance, given the risk of pet-to-human transmission, is associated with infected prairie dogs (Cynomys ludovicianus) sold as pets in the USA and exported internationally [67,68]. A ban was put in place in the European Union and other countries regarding the import of prairie dogs and other rodent species after the USA monkeypox outbreak in 2003 [68,69]. Wild-caught prairie dogs are particularly susceptible to environmental stress, such as capture, transit and crowding, which can enhance disease manifestations. Clinical signs include lethargy, dehydration and grossly enlarged cervical lymph nodes. Prairie dogs can produce specific antibodies against F. tularensis and survive tularemia infection, suggesting their potential role as F. tularensis reservoirs in nature. Moreover, one study found that all seropositive animals harboured live infectious bacteria, suggesting persistent infection [67].

Tularemia has also been described in domestic dogs and cats [49,55], which may be infected by means of arthropod bites, direct contact with infected animals, their ingestion, or contaminated aerosols [70,71].

Cats usually develop severe illness with unspecific clinical signs like fever, lethargy, prostration, vomiting and anorexia, dehydration, regional or generalised lymphadenopathy, splenomegaly, tongue and oropharyngeal ulceration and jaundice [49,72,73]. Pathological findings include multiple necrotic foci on the lymph nodes, spleen, liver and lungs. Frequently, panleukopenia with toxic degeneration of the neutrophils and hyperbilirubinaemia with bilirubinuria are present [73].

Dogs are less susceptible and rarely manifest signs of the disease [55,56]. Nevertheless, they can act as carrier hosts [70] and transmit the bacterium by means their fur after contact with contaminated dead animals or soil [74]. In most cases, infection is self-limiting and recovery is spontaneous. However, only few cases of natural infection in dogs have been reported [55,56].

9. Laboratory diagnosis

9.1. Samples

In humans, samples should preferably be collected before the onset of antibiotherapy and depend on the clinical form of the disease. Samples may include non-heparinised whole blood, serum, respiratory tract
secretions and washes, swabs from visible lesions, lymph node aspirates or biopsies, urine, and autopsy materials [49].

In animals, serum is the preferential sample for all disease forms, but plasma and dry blood on paper filters can also be used. Blood samples should be collected at least 14 days after the onset of the symptoms. Lymph nodes or bone marrow aspirates, organs (lung, liver, spleen) and cerebrospinal fluid can also be used [49].

In the context of an outbreak or epidemiologic studies, samples should include arthropod vectors as well as environmental samples like water, soil and rodent faeces [49,54].

9.2. Culture

Culture is the gold standard for *F. tularensis* and must be carried out in biosafety level 3 facilities (BSL-3) [2,22,49]. *F. tularensis* is a fastidious microorganism. Optimal growth conditions occur at 37 °C and pH 6.9 [5,24]. Cysteine-enriched media, such as enriched chocolate agar (CA) or 9% cysteine heart agar with blood medium (CHAB) must be used for this purpose [22,49,54]. Growth in a CHAB medium enables the presumptive identification of *F. tularensis* by characteristic growth at 24–48 h of round and smooth green opalescent shiny colonies, 2–4 mm in diameter [4,22,27,49,54]. Antibiotic supplementation of CHAB is possible in order to optimise growth and inhibit contaminants [22,49,54]. For cultures made from blood, the use of the BACTEC™ (BD) system or equivalent, BacT/Alert™ (Biomerieux) is recommended [49,54]. Liquid media is not suitable for *F. tularensis* growth, even when supplemented with cysteine [4,27,54].

10. Microbiologic identification of *F. tularensis*

Basic biochemical tests provide a presumptive identification of isolates and may be further complemented by immunological and molecular methods. Some additional biochemical tests, such as the ability to ferment glucose or glycerol, or the presence of the citrulline ureidase pathway are useful for subtyping purposes [54].

The commercial Microlog Microstation™ System (Bilog Inc., Hayward, CA) based on the ability to ferment glucose has been successfully used for differentiating between subspecies *tularensis* and *holarctica* [54,67]. Also, the commercially available Microbial Identification System (MIS) and Library Generation System (LGS) (MIDI, Inc, Newark, NJ) enables cell-wall fatty-acid analysis and can be used for the identification of *Francisella* at the genus level. It has also enabled the identification of atypical *F. tularensis* strains lacking cysteine requirements [54,75].

Immune based techniques have also been employed for identification: immunoblot analysis and immunofluorescence microscopy, either from grown cultures or clinical samples [54].

11. Serology

Antibodies against *F. tularensis* reach detectable levels 10–20 days post-infection [49]. A fourfold increase in the titre between acute and convalescent sera or a titre of 1:160 or greater of agglutinating antibodies is considered for diagnostic purposes [27,49,76]. Titres peak at a level of 320–1280 and decline slowly [76]. Serologic methods include the whole-cell agglutination test (Widal’s reaction), the tube agglutination test, microagglutination assays, haemagglutination, ELISA (Enzyme-linked immunosorbent assay) and immunoblot [22,22]. ELISA has repeatedly been more sensitive than agglutination assays, with the additional advantage of determining separately different antibody classes (IgM, IgG and IgA) [54].

A combination of a first ELISA screening test complemented by an immunoblot confirmatory test, with higher specificity, is the current recommended two-step approach for the serological diagnosis of tularemia [54].

The same approach can be used for animals. Serology has a limited use in highly susceptible species since death usually precedes the development of specific antibodies [47]. However, in endemic areas, antibodies for *F. tularensis* are frequently detected in wild animals that have developed immunity, including foxes and coyotes. This serconversion is suspected as being related to subspecies *holarctica* infection since infection by the subspecies *tularensis* is expected to be fatal [27,49].

12. Molecular methods

Molecular methods are valuable diagnostic tools whenever culture is either not possible or is negative [2,22,49]. Moreover, they reduce the high risk of laboratory-acquired infections over conventional biochemical typing [2,21,77].

During recent years, polymerase chain reaction (PCR)-based methods have been successfully used for the rapid identification and classification of *Francisella* isolates, with increased sensitivity and specificity [54,78]. However, false positive results related to non-pathogenic closely related *Francisella* subspecies, occurring naturally in the environment, may hamper species and subspecies identification [78].

Conventional PCR targets are *tul4* and *fopA* genes, which encode for *F. tularensis* superficial membrane lipoproteins. Both protocols show a good level of sensitivity and reasonable specificity in *F. tularensis* detection and may be used in blood, tissue or aerosol samples [4,49,54]. PCR product specificity is confirmed by sequencing, reverse-line blotting (RLB) or restriction fragment-length polymorphism (RFLP) [54].

Real time PCR for *F. tularensis* detection has been developed, in particular, TaqMan™ (Applied Biosystems) real time PCR multiple assay shows high specificity and sensitivity using four target genes: ISFtu2, 23 kDa, *tul4* and *fopA* [49,54]. Real-time PCR for the differentiation between the subspecies *tularensis* and *holarctica* is also now available [79].

Further discrimination has been achieved using high-resolution genotyping methods including pulse-field gel electrophoresis (PFGE), amplified fragment-length polymorphism (AFLP), ribotyping, 16S rDNA gene sequencing, canonic insertion deletions and paired-end sequence mapping [26,27,34,80]. Still, as *F. tularensis* exhibits highly
conserved genomic sequences among strains of diverse origin, genetic polymorphisms allowing for individual strain typing have been difficult to find [77]. As for other bacteria, more recent PCR-based techniques such as variable-number tandem repeats (VNTR), multiple-locus VNTR analysis (MLVA) and short-tandem repeats (STR) typing have been successfully used for identification at the subspecies level and for molecular epidemiology purposes [54,77,80]. One of the most discriminatory methods for the molecular subtyping of F. tularensis is MLVA, which consists of a series of VNTR loci that are PCR amplified via flanking primer sites and examined for size variation [79]. One MLVA system designed for F. tularensis is based on polymorphisms of 25 VNTR loci, Ft-M01 to Ft-M25. This MLVA typing system has a greater discriminatory power when applied to a worldwide set of F. tularensis isolates and provides accurate classification at the subspecies level [77]. This MLVA system has recently been improved by redesigning the subset of the 25 previously identified VNTRs to produce a new optimised, multiplexed MLVA system with a similar level of discrimination but with fewer time and cost requirements [79]. Ten of the previously described VNTR loci were selected based on their discrimination ability within the subspecies: Ft-M02, Ft-M03, Ft-M04, Ft-M05, Ft-M06, Ft-M010, Ft-M20, Ft-M22, Ft-M23, and Ft-M24. Locus Ft-M20 was split into two loci, Ft-M20A (which contains the originally described 12 bp repeat and is polymorphic across subspecies) and Ft-M20B (which contains the insertion with its 15 bp repeat and varies only among type A II and F. novicida isolates) [79].

While providing discrimination among strains, VNTRs are unsuited for determining deeper phylogenetic relationships due to mutational saturation. In this case, more accurate and alternative markers should be used, such as whole-genome sequence single nucleotide polymorphism (SNPs) [79]. Additional studies have shown a remarkable degree of discrimination of the F. tularensis phylogenetic structure, using a combined analysis with canonical whole-genome SNPs for major clade typing, and MLVA for high-resolution typing [26,79]. In a different study, the combined analysis of insertion-deletion markers, for subspecies and major clade typing, along with MLVA, was used [80].

Microarrays have also allowed for the differentiation of the four F. tularensis subspecies and have been proven useful for pathogenicity and virulence marker identification [54].

13. Treatment

Tularaemia usually responds to antibiotic therapy. Historically, aminoglycosides have been the drugs of choice for humans. Although clinically effective, they are rarely used now due their ototoxicity and nephrotoxicity. Nevertheless, gentamicin has been used for treatment of pneumonic tularemia and aminoglycosides are now generally used in the most serious cases. Chloramphenicol is effective but seldom the first choice due to its possible irreversible effects on haematopoiesis. Tetracyclines have been associated with high relapse rates on withdrawal. Fluoroquinolones, such as ciprofloxacin, have been shown to be highly effective in per os and are the best choice for uncomplicated tularemia. Also, ciprofloxacin has proved suitable and effective in the treatment of tularemia in children and pregnant women [4,49]. In domestic animals, gentamicin, enrofloxacin, doxycycline and chloramphenicol are referred to as therapeutic options for dogs [55,70]. In cats, there are reports of the use of doxycycline or enrofloxacin and amoxicillin-clavulanic acid as being beneficial in the early stages of the disease [81].

14. Vaccination

Currently, there is no available licensed vaccine against F. tularensis although an attenuated Type B strain, known as the Live Vaccine Strain (LVS) was developed in the United States during the 1950s and used to vaccinate military personnel and laboratory workers [4,49,82–84]. LVS failed to uniformly protect against pneumonic tularemia and when delivered in high titres caused mild tularemia as an undesirable side-effect [85].

One focus of current research work in the USA and in Europe is to develop a vaccine for protection against F. tularensis intentional release [49]. The restricted efficacy of the LVS has fostered extensive research with a view to providing alternative vaccine formulations, including the exploration of different live and killed attenuated strains and immunogenic components to produce subunit vaccines [4,82]. In view of its immunogenic antigens, an effort has been made to develop attenuated strains of SchuS4, a representative strain of Type A tularaemia, for vaccine production. In fact, between LVS and SchuS4 strains there are about 35 genes that encode for different protein sequences, whose functions are not well defined, and may represent important immunogenic antigens. Still, given the increased virulence of the SchuS4 strain, only a small number of bacteria should be required to generate effective protection against wild type F. tularensis [85]. A recently published study demonstrated that inoculation with low doses of specific attenuated mutants of the F. tularensis strain SchuS4 provided protection against parenteral and intranasal challenge with a fully virulent wild type SchuS4 strain [86]. This favours the role of T-cell memory response as a critical determinant of F. tularensis immunity, additionally to the humoral response. This feature is the basis of the challenges foreseen for vaccine development, aiming at identifying antigen determinants that elicit an effective cellular-mediated immune response [4,82,84,85]. Cell-mediated immunity was found to persist three decades after tularemia vaccination. A recent study sought to identify the T-cell responses present in immune individuals in order to characterise F. tularensis–specific immune response [84,86]. The findings showed that the production of INF-γ, macrophage inflammatory protein (MIP)-1β and CD107a (lysosome-associated membrane protein 1 or LAMP-1) by peripheral blood mononuclear cells appeared to be a characteristic of protective immune responses and that a correlation exists between these parameters and immunity [84].
15. Conclusions

Several factors such as human demographics and behaviour, international travel and commerce, including the animal trade, climatic changes and microorganism adaptation, have a potential impact on disease ecology and the emergence of zoonosis. The same factors are thought to be related to the emergence of tularemia. Special concerns regarding this bacterium exist in relation to its high infectivity, and easy dispersion through aerosols and contaminated water, which make it a potential bioterrorism weapon. Also, tularemia presents a wide geographic distribution and has recently emerged in new settings, particularly in Europe. In Portugal, an on-going research project on tularemia aims to increase our knowledge about the disease, particularly its impact in this country, which is still poorly understood, in view of the fact that there is little information available to risk population and health professionals, with the result that there is a possible underestimation of prevalence in man and animals. To this regard, efforts have been made by the National Institute of Health to increase awareness of the disease among risk populations, particularly hunters and health professionals. In accordance with the preliminary results, on-going research will further identify and characterise F. tularensis circulating strains and develop molecular and typing methods with increased sensitivity, specificity and discriminatory power. The role of autochthon wild lago-morphs in the F. tularensis life cycle, their involvement in animal-to-human transmission and their suitability as tularemia sentinels will be accessed. Moreover, considering the economic and social relevance of hunting-related activities in this country, with very few studies having acknowledged its relation to zoonotic disease transmission risks, research into infection in game species is of major importance.

F. tularensis is also associated with a considerably wider range of hosts and vectors than most zoonotic pathogens, although there is little information on bacteria mechanisms for adaptation to such a wide diversity of arthropod vectors. Despite our increasing knowledge of tularemia and its etiological agent, many aspects of F. tularensis biology and epidemiology need to be further examined, particularly its pathogenicity and virulence, vaccine development, and the specific mechanisms by which F. tularensis evades, modulates and suppresses the host immune response. As with any zoonotic emergent disease, the role of wild and domestic animals in F. tularensis epidemiology needs to be further evaluated, in particular, those which may act as reservoirs. Other epidemiologic data such as the population dynamics of susceptible animals, particularly lagomorphs and rodents in Europe, should be part of surveillance programmes, as they are thought to be directly associated with disease transmission patterns. From a public health perspective, disease surveillance in animals is crucial in order to prevent and monitor human outbreaks, particularly in endemic areas, where contact between humans and wildlife reservoirs or vectors is likely. Although tularemia is not regarded as a common disease, and there is little awareness of the disease among health authorities and practitioners, its eventual future impact as an emergent zoonosis should not be neglected.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

We acknowledge the work carried out by Ana Sofia Santos, Fátima Amaro, Hugo Osório, Maria João Alves and Rita de Sousa from the Doctor Ricardo Jorge National Health Institute, I.P., Centre for Vectors and Infectious Diseases Research. Funding was provided by the Portuguese Science and Technology Foundation (FCT) (PTDC/SAU-ESA 104947/2008). C. Carvalho has been awarded a FCT PhD scholarship (SFRH/BD/79225/2011).

References

[1] Vorou RM, Papavassiliou VG, Tsiodras S. Emerging zoonosis and vector-borne infections affecting humans in Europe. Epidemiol Infect 2007;135(8):123–47.
[2] Mandell GL, Bennett JE, Dolin R. Mandell, Douglas, and Bennett’s principles and practice of infectious diseases, vol. 2. Elsevier, Churchill Livingstone; 2005. p. 2674–83.
[3] Hansen CM, Vogler AJ, Keim P, Wagner DM, Huerfert K. Tularemia in Alaska, 1938–2010. Acta Vet Scand 2011;53(1):61.
[4] Oyston PC. Francisella tularensis: unraveling the secrets of an intra-cellular pathogen. J Med Microbiol 2008;57(Pt. 8):921–30.
[5] Collier I, Barlows A, Sussman M, Topley and Wilson’s Microbiology and Microbial Infections, vol. 2; 1998. p. 1347–9.
[6] Petersen JM, Madsen PS, Schriefer ME. Francisella tularensis: an arthropod-borne pathogen. Vet Res 2000;40(2):7.
[7] Petersen JM, Schriefer ME. Tularemia: emergence/re-emergence. Vet Res 2005;36(3):455–67.
[8] Sjôstedt A. Special Topic on Francisella tularensis and tularemia. Front Microbiol 2011;2:86.
[9] de Carvalho I, Escudero R, García-Amil C, Falcão H, Anda P, Núncio MS. Francisella tularensis, Portugal. Emerg Infect Dis 2007;13(4):666–7.
[10] European Center for Disease Control and Prevention. Annual Epidemiologic Report. Reporting on 2010 surveillance data and 2011 epidemiic intelligence data. Stockholm: ECDC; 2013. p. 119–21.
[11] Pazdriová P, Morávková I, Nocarová D, Velkoborská M, Valecková K. A water-borne epidemic of tularemia in Chlumcany. Epidemiol Mikrobiol Imunol 2002;51(1):23–5.
[12] Grunow R, Kalaveshi A, Kühn A, Mulligi-Osmani G, Ramadani N. Surveillance of tularemia in Kosovo, 2001 to 2010. Euro Surveill 2012;17(28), pii:20217.
[13] Kantardjiev T, Ivanov I, Velinov T, Padeshki P, Popov B, Nenova R, et al. Tularemia outbreak, Bulgaria, 1997–2005. Emerg Infect Dis 2006;12(4):678–80.
[14] Hauri AM, Hofstetter I, Seibold E, Kaysser P, Eckert J, Neubauer H, et al. Investigating an airborne tularemia outbreak, Germany. Emerg Infect Dis 2010;16(2):238–43.
[15] Wik O. Large tularemia outbreak in Värmland, central Sweden, 2006. Euro Surveill 2006;11(9):E060921.1.
[16] Junii U, Renko M, Uhari M. An outbreak of holarctic-type tularemia in pediatric patients. Pediatr Infect Dis J 2010;29(2):160–2.
[17] Allue M, Sopeña CR, Gallardo MT, Mateos I, Vian E, García MJ, et al. Tularemia outbreak in Castilla y León, Spain, 2007: an update. Euro Surveill 2008;13(32), pii:18948.
[18] Dikici N, Ural O, Sümer S, Oztürk K, Albayrak Yigit Ö, Katlanır E, et al. Tularemia in Konya region, Turkey. Mikrobiyol Bul 2012;46(2):225–35.
[19] Decors A, Lesage C, Jourdain E, Giraud P, Houbron P, Vanhem P, et al. outbreak of tularemia in brown hares (Lepus Europaeus) in France, January to March 2011. Euro Surveill 2011;16(28), pii:19913.
et al. 2011;53:2.

Vogler AJ, Birdsell D, Price LB, Bowers JR, Beckstrom-Sternberg SM, Auerbach RK, et al. Phylogeography of Francisella tularensis: global expansion of a highly fit clone. J Bacteriol 2009;191(8):2474–84.

Ellis J, Oyston PCF, Green M, Titball RW. Tularemia. Clin Microbiol Rev 2002;15:629–46.

Huber B, Escudero R, Busse HJ, Seibold E, Scholz HC, Anda P, et al. Description of Francisella hispaniensis sp. nov., isolated from human blood, reclassification of Francisella novicida (Larson et al., 1955) Olsufiev et al., 1959 as Francisella tularensis nov subspecies comb. nov. and emended description of the genus Francisella. Int J Syst Evol Microbiol 2010;60(6):Pt. 8:1887–96.

Bret M, Dompierre DJ, Espinosa-Kingry LB, Myers D, Husband B, Pollard K, et al. Francisella novicida bacteriaemia after a near-drowning accident. J Clin Microbiol 2012;50(8):2826–9.

Birdsell DN, Stewart T, Vogler AJ, Lawaczek E, Diggs A, Sywester TL, et al. Francisella tularensis subsp. novicida isolated from a human in Arizona. BMC Res Notes 2009;2:224.

Siddaramappa S, Challacombe JF, Petersen JM, Pillai S, Hogg G, Kuske CR. Common ancestry and novel genetic traits of Francisella novicida-like isolates from North America and Australia as revealed by comparative genomics analysis. Appl Environ Microbiol 2011;77(15):5110–22.

Johansson A, Celli J, Conland W, Elkins KL, Forsman M, Keim PS, et al. Objections to the transfer of Francisella novicida to the subspecies rank of Francisella tularensis. Int J Syst Evol Microbiol 2010;60(6):Pt. 8:1717–8.

Whipp MJ, Davis JM, Lumm G, de Boer J, Zhou Y, Bearden SW, et al. Characterization of a novicida-like subspecies of Francisella tularensis isolated in Australia. J Med Microbiol 2003;52(9):893–942.

Fey PD, Dempsey MM, Olson ME, Christowski MS, Engle JL, Jay JJ, et al. Molecular analysis of Francisella tularensis subspecies tularensis and holarctica. Am J Trop Med Hyg 2010;83(3):645–52.

Jackson J, McGregor A, Cooley L, Ng J, Brown M, Ong CW, et al. Francisella tularensis subspecies holarctica, Tasmania, Australia, 2011. Emerg Infect Dis 2012;18(6):1484–5.

Leelaporn A, Yongyod S, Limsviravichakorn S, Yongyuen T, Kiratisin P, Francisella novicida bacteriaemia, Thailand. Emerg Infect Dis 2008;14(12):1935–7.

Kugeler KJ, Curfield N, Creek JG, Mahoney KS, Versage JL, Petersen JM. Discrimination between Francisella tularensis and Francisella-like endobionts when screening ticks by PCR. Appl Environ Microbiol 2005;71(11):7594–7.

Escudero R, Toledo A, Gil H, Kovácsiová K, Rodrigues-Vargas M, Jado I, et al. Molecular method for discrimination between Francisella tularensis and Francisella-like endobionts. J Clin Microbiol 2008;46(9):3139–43.

De Carvalho IL, Santos N, Soares T, Zê-Zê L, Núncio MS, Francisellalike endobionts in Dermacentor reticulatus collected in Portugal. Vector Borne Zoonotic Dis 2011;11(2):185–8.

Ivanov IN, Mitkova N, Reye AL, Hübchen JM, Watcheva-Dobrevska RS, Dobreva EG, et al. Detection of new Francisella-like endobionts in Hyaunlessa spp and Rhizoplosophus spp. (Acari: Ixodidae) from Bulgaria. J Med Entomol 2003(40):5355–8.

Dergousoff SJ, Chilton NB. Association of different genetic types of Francisella-like organisms with the rocky mountain wood tick (Dermacentor andersoni) and the American dog tick (Dermacentor variabilis) in localities near their northern distributional limits. Appl Environ Microbiol 2012;78(4):965–71.

Scoles GA. Phylogenetic analysis of the Francisella-like endobionts of Dermacentor ticks. J Med Entomol 2004;41(3):277–86.

Séret-Lanz Z, Szell Z, Séret T, Máríaligeti K. Detection of a novel Francisella in Dermacentor reticulatus: a need for careful evaluation of PCR-based identification of Francisella tularensis in Eurasian ticks. Zoonoses Public Health 2009;56(1):123–6.

Baldridge GD, Scoles GA, Burkhardt NY, Schroeder B, Kurtti TJ, Munderloh UG. Transovarial transmission of Francisella-like endobionts and Anaplasma phagocytophilum variants in Dermacentor variabilis (Acari: Ixodidae). J Med Entomol 2009;46(3):625–32.

Gyurancz M, Szeredi L, Makrai L, Fodor L, Mészáros AR, Szébé L, et al. Tularemia of Brown European Brown Hare (Lepus europaeus): a pathologic, histological and immunohistochemical study. Vet Pathol 2010;47(5):794–9.

Bandouchova H, Pohanka M, Vlkova K, Damkova V, Peckova L, Sedlackova J, et al. Biochemical responses and oxidative stress in Francisella tularensis infection: a European brown hare model. Acta Vet Scand 2011;53:2.

Kuehn A, Shulze C, Kutzer P, Probst C, Hlinák A, Ochs A, et al. Tularemia serorepertoire of captured and wild animals in Germany: the fox (Vulpes vulpes) as a biological indicator. Epidemiol Infect 2013;141(4):833–40.

World Health Organization working group on Tularemia. WHO Guidelines on Tularemia; 2007, 115p.

Lundström JO, Anderson AC, Bäckman S, Schäfer ML, Forsman M, Thelaus J. Transtadiational transmission of Francisella tularensis holarctica in mosquitoes, Xenos, Emerg Infect Dis 2011;17(5):794–9.

Anda P, Segura del Pozo J, Diaz Garcia JM, Escudero R, Garcia Peña FJ, Lopez Velasco MC, et al. Waterborne outbreak of tularemia associated with crayfish fishing. Emerg Infect Dis 2011;17(3 Suppl.):575–82.

Mami R, Reichard MV, Morton RJ, Kocan KM, Clickendeard KD. Biology of Francisella tularensis subspecies holarctica live vaccine strain in the tick vector Dermacentor variabilis. PLoS ONE 2012;7(4):e35441.

Carvalho CL, Zê-Zê L, Duarte EL, Núncio MS, de Carvalho IL. Screening of mosquitoes as vectors of F. tularensis in Portugal. In: 7th International Conference on tularemia. 2012.

Splettstoesser WD, Tomas H, Al Dahshouk S, Neubauer H, Schuff-Weerner P. Diagnostic procedures in tularemia with special focus on molecular and immunological techniques. J Vet Med B Infect Dis Vet Public Health 2005;52(6):249–61.

Meinkoth KR, Morton RJ, Meinkoth JH. Naturally occurring tularemia in a dog. J Am Vet Med Assoc 2004;225(4):545–7.

Gustafson BW, DeBoves LJ. Tularemia in a dog. J Am Anim Hosp Assoc 1996;32(4):339–41.

Zarrrella TM, Singh A, Bitsaksit C, Rahman T, Sahay B, Feustel PJ, et al. Host-adaptation of Francisella tularensis alters the bacterium’s surface–carbohydrates to hinder effectors of innate and adaptive immunity. PLoS ONE 2011;6(7):e22335.

Abd H, Johansson T, Golovlev I, Sandström G, Forsman M. Survival and growth of Francisella tularensis in Acanthamoeba castellanii. Appl Environ Microbiol 2003;69(1):600–6.

Bossio CM. The subterranent immune system by Francisella tularensis. Front Microbiol 2011;2:9.

Henry T, Brotcke A, Weiss DS, Thompson LJ, Monack DM. Type I interferon signaling is required for activation of the inflammasome during Francisella infection. J Exp Med 2007;204(5):987–94.

Cremer TJ, Butcher JP, Trindadpani S, Francisella subverts innate immune signaling: focus on PI3K/Akt. Front Microbiol 2011;5:13.

Chong A, Celli J. The Francisella intracellular life cycle: toward molecular mechanisms of intracellular survival and proliferation. Front Microbiol 2011;2:138.

Jones JW, Broz P, Monack DM. Innate immune recognition of Francisella tularensis: activation of type-I interferons and the inflammasome. Front Microbiol 2011;2:16.

Jones CL, Weiss DS. TLR2 signaling contributes to rapid inflammasome activation during F. novicida infection. PLoS ONE 2011;6(6):e20609.

Decors, Lesage C, Jourdain E, Giraud P, Houborn P, Vanhem P, et al. Outbreak of tularemia in brown hares (Lepus europaeus) in France, Emerg Infect Dis 2010;16(3):465–8.

Reed DS, Smith L, Dunsmore T, Trichel A, Ortiz LA, Cole KS, et al. Pneumonic tularemia in rabbits resembles the human disease as illustrated by radiographic and hematological changes after infection. PLoS ONE 2011;6(9):e24004.

Petersen JM, Schirfer ME, Carter LG, Zhou Y, Sealy T, Bawiec D, et al. Laboratory analysis of tularemia in wild-trapped, commercially traded prairie dogs, Texas, 2002. Emerg Infect Dis 2004;10(3):419–25.
Avashia SB, Petersen JM, Lindley CM, Schriever ME, Gage KL, Cetron M, et al. First reported prairie dog-to-human transmission, Texas, 2002. Emerg Infect Dis 2004;10(3):483–6.

European Commission bans import of prairie dogs from the US and rodents and squirrels from sub-Saharan Africa. Euro Surveill 2003;7(25):pii:2243.

Feldman KA. Tularemia. J Am Vet Med Assoc 2003;31(6):725–30.

Kayszer P, Seibold E, Matz-Rensing K, Pfeiffer M, Essbauer S, Splettstoesser WD. Re-emergence of tularemia in Germany: presence of Francisella tularensis in different rodent species in endemic areas. BMC Infect Dis 2008;8:157.

Müller W, Bocklisch H, Schüler G, Hotzel H, Neubauer H, Otto P. Detection of Francisella tularensis subsp. holarctica in a European brown hare (Lepus europaeus) in Thuringia, Germany. Vet Microbiol 2007;123(1-2):225–9.

Vogler W, Woods JP, Crystal MA, Morton RJ, Panciera RJ. Tularemia in two cats. J Am Vet Med Assoc 1998;212(7):81–3.

Baldwin CJ, Panciera RJ, Morton RJ, Cowell AK, Wauryniazi RJ. Acute tularemia in three domestic cats. J Am Vet Med Assoc 1991;199(11):1602–5.

Siret V, Barataud D, Prat M, Vaillant V, Ansart S, Coustumier AL, et al. An outbreak of airborne tularemia in France, August 2004. Euro Surveill 2006;11:58–60.

Bernard K, Tessier S, Winstenley J, Chang D, Borczyk A. Early recognition of atypical Francisella tularensis strains lacking a cytotoxic requirement. J Clin Microbiol 1994;32(2):551–3.

Cirnivka I, Berglund L. Tularemia. Eur Respir J 2003;21(2):361–73.

Johansson A, Farlow J, Larsson P, Dukerich M, Chambers E, Byström M, et al. Worldwide genetic relationships among Francisella tularensis isolates determined by multiple-locus variable-number tandem repeat analysis. J Bacteriol 2004;186(17):5808–18.

Ahlinder J, Ohrman C, Svensson K, Lindgren P, Johansson A, Forsman M, et al. Increased knowledge of Francisella genus diversity highlights the benefits of optimized DNA-based assays. BMC Microbiol 2012;12:120.

Vogler AJ, Birdsell D, Wagner DM, Keim P. An optimized, multiplexed multi-locus variable-number tandem repeat analysis system for genotyping Francisella tularensis. Lett Appl Microbiol 2009;49(1):140–4.

Larsson P, Svensson K, Karlsson L, Guala D, Granberg M, Forsman M, et al. Canonical insertion–deletion markers for rapid DNA typing of Francisella tularensis. Emerg Infect Dis 2007;13(11):1725–32.

Valentine BA, Deely BM, Sonn RJ, Stauffer LR, Pietsch LG. Localized cutaneous infection with Francisella tularensis resembling ulceroglandular tularemia in a cat. J Vet Diagn Invest 2004;16(1):83–5.

Zvi A, Rotem S, Cohen O, Shafferman A. Clusters versus affinity-based approaches in F. tularensis whole genome search of CTL epitopes. PLoS ONE 2012;7(5):e36440.

Klimyuk SL, Twine SM. The Francisella tularensis proteome and its recognition by antibodies. Front Microbiol 2011;1:143.

Eneslatt K, Normark M, Björk R, Rietz C, Zingmark C, Wolfraim LA, et al. Signatures of T cells as correlates of immunity to Francisella tularensis. PLoS ONE 2012;7(3):e32367.

Rockx-Brouwer D, Chong A, Wehrly TD, Child R, Crane DD, Celli J, et al. Low dose vaccination with attenuated Francisella tularensis strain SchuS4 mutants protects against tularemia independent of the route of vaccination. PLoS ONE 2012;7(5):e37752.

Eneslatt K, Rietz C, Ryden P, Stevens S, House RV, Wolfraim LA, et al. Persistence of cell mediated immunity three decades after vaccination with the live vaccine strain of Francisella tularensis. Eur J Immunol 2011;41(4):974–80.

Tomase E. Tularemia in Czechoslovakia and Austria during 1936 and 1937. Am J Public Health Nations Health 1937;27(5):443.

Pikula J, Tremf L, Beklová M, Holeovská Z, Pikulová J. Ecological conditions of natural foci of tularemia in the Czech Republic. J Epi- demiol 2003;13(1):1091–5.

Cerný Z. Changes of the epidemiology and the clinical picture of tularemia in Southern Moravia (The Czech Republic) during the period 1936–1999. Eur J Epidemiol 2001;17(7):637–42.

Cerný Z. Tularemia – history, epidemiology, clinical aspects, diagnosis and therapy. Cas Lek Cesk 2002;141(9):270–5.

Tremf L, Hubálek Z, Halouzka J, Juricová Z, Hunady M, Janík V. Analysis of the incidence of tularemia in the Breclav District 1994–1999. Epidemiol Mikrobiol Imunol 2001;50(1):4–9.

Komitova R, Novena R, Padeski P, Ivanov I, Popov V, Petrov P. Tularemia in Bulgaria 2003–2004. J Infect Dev Ctries 2010;4(11):689–94.

Payne L, Arneborn M, Tegnell A, Giesecke J. Endemic tularemia, Sweden 2003. Emerg Infect Dis 2005;11(9):1440–2.

Eliasson H, Lindbäck J, Nuortti JP, Arneborn M, Giesecke J, Tegnell A. The 2000 tularemia outbreak: a case–control study of risk factors in disease-endemic and emergent areas, Sweden. Emerg Infect Dis 2002;8(9):956–60.

Seppäinen M. Insect-borne diseases and insect bites in Finland. Duodecem 2011;127(11):1393–400.

Rydén P, Björk R, Schafer ML, Lundström JO, Petersén B, Lindblom A, et al. Outbreaks of tularemia in a boreal forest region depends on mosquito prevalence. J Infect Dis 2012;205(2):297–304.

Martín C, Gallardo MT, Mateos I, Vían E, García MJ, Ramos J, et al. Outbreaks of tularemia in Castilla y León, Spain. Euro Surveill 2007;12(11):E071108.1.

M.Pérez-Castrellón JL, Bachiller-Luque P, Martín-Luque M, Mena-Martin FJ, Herrera V. Tularemia epidemic in northwestern Spain: clinical description and therapeutic response. Clin Infect Dis 2001;33(4):573–6.

Gurcan S, Karabag O, Karadenizli A, Karagol C, Kantardjiev T, Ivanov IN. Characteristics of the Turkish isolates of Francisella tularensis. Jpn J Infect Dis 2008;61(3):223–5.

Bayhan-Tas GI, Tanir G, Celebi B. Two cases of glandular tularemia from Turkey. Turk J Pediatr 2012;54(2):203–6.

Yesilyurt M, Kilic S, Celebi B, Gül S. Tularemia: are hunters really a risk group? Mikrobiol Bul 2012;46(1):153–5.

Celebi G, Baroumi F, Ayoglu F, Cinar F, Karadenizli A, Ugur MB, et al. Tularemia re-emerging disease in northwest Turkey: epidemiological investigation and evaluation of treatment responses. Jpn J Infect Dis 2006;59(4):229–34.

Ozdernir D, Sencan I, Annakayya AN, Karadenizli A, Guclu E, Sert E, et al. Comparison of the 2000 and 2005 outbreaks of tularemia in the Duzce region of Turkey. Jpn J Infect Dis 2007;60(1):51–2.

Sahin M, Atabay HI, Bicakci Z, Unver A, Ortuu S. Outbreaks of tularemia in Turkey. Kobe J Med Sci 2007;53(1-2):37–42.

Maurin M, Pelloux I, Brion JP, Del Banó JN, Picard A. Human tularemia in France, 2006–2012. Clin Infect Dis 2013;53(10):e133–41.

Fournier PE, Bernabeu B, Schubert B, Muttillo M, Roux V, Raoult D. Isolation of Francisella tularensis by centrifugation of shell vial cell culture from an inoculation eschar. J Clin Microbiol 1998;36(9):2782–3.

Li W, Raoult D, Rolain JM, La Scola B. Evidence of circulation of an epidemic strain of Francisella tularensis in France by multispace typing. Eur J Clin Microbiol Infect Dis 2011;30(9):1135–8.

Brantsaeter AB, Krogh T, Radtke A, Nygaard K. Tularemia outbreak in northern Norway. Euro Surveill 2007;12(3):E070329.2.

Hagen IJ, Aandalh E, Hasseltvedt V. Five case histories of tularemia infection in Oppland and Hedmark Counties, Norway. Euro Surveill 2005;10(8):E050317.4.

Akıncu A, Kaya YA. Francisella-arthropod vector interaction and its role in patho-adaptation to insect mammals. Front Microbiol 2011;2:34.