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Tularaemia

A. Tärnvik*, L. Berglund#

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ABSTRACT: Tularaemia is a zoonotic bacterial disease of the Northern hemisphere. The causative agent, *Francisella tularensis*, is spread to humans by direct contact with infected rodents or lagomorphs, aerogenic exposure, ingestion of contaminated food or water, or by arthropod bites. The prevalence of tularaemia shows a wide geographic variation. In some endemic regions, outbreaks occur frequently, whereas nearby rural parts of a country may be completely free.

***F. tularensis* is a facultative intracellular pathogen and its primary mammalian target cell is the mononuclear phagocyte. When tularaemia is acquired *via* the skin, a primary ulcer is often detected and in general, regional lymph nodes become prominently enlarged. When contracted by inhalation, the disease may present with pneumonia. Nearly as frequent, however, is the development of fever and general illness with no respiratory symptoms and no pulmonary radiological changes. When present, the changes vary widely and may sometimes include hilar enlargement indistinguishable from that of lymphoma.**

Within an outbreak, the first case of tularaemia is not always readily diagnosed. A decade may have lapsed since the disease was encountered and its existence may be more or less forgotten. The difficulty refers especially to the respiratory form, in which symptoms are less specific. In cases of atypical pneumonia or acute febrile disease with no local symptoms, a history of exposure to hares or rodents or merely living in an endemic region should be sufficient to include tularaemia among differential diagnoses.

The microbiological diagnosis of tularaemia relies mainly on serology, and the treatment on broad-spectrum antibiotics. For decades, a live vaccine has been successfully used in risk groups but is presently not available due to difficulties in standardisation.
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*Dept of Clinical Microbiology (Infectious Diseases), Umeå University, Umeå and the #Primary Health Care Centre, Ljusdal, Sweden.

Correspondence: A. Tärnvik
Umeå University
Infectious Diseases
SE-901 85 Umeå
Sweden
Fax: 46 90133006
E-mail: arne.tarnvik@infdis.umu.se

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When describing the clinical aspects of tularaemia, a distinction has to be made between disease acquired on the North American continent, on the one hand, and the Eurasian continent on the other. Due to a difference in virulence between two major subspecies of *Francisella tularensis*, tularaemia is a more threatening disease in North America, especially when associated with pulmonary involvement. Actually, the virulence of the "North American" subspecies is an obstacle for laboratory work. However, work on the "European" subspecies has resulted in the development of new diagnostic and therapeutic measures, which are all applicable, irrespective of causative subspecies and geographic occurrence of the disease.

History

The discovery of tularaemia is mainly an American achievement, including isolation of the causative agent, linkage of the agent to human disease, and elucidation of the histopathology and epidemiology. After the major San Francisco earthquake in 1906, G.W. McCoy, Director of the US Public Health Service plague laboratory, undertook bacteriological investigations of bubonic plague in ground squirrels and rats in afflicted areas. In samples from some ground squirrels, with changes typical of plague, attempts to isolate the specific pathogen failed [1]. When more nutritive culture media were tried, McCoy and Chapin [2] successfully isolated a novel organism, which was named *Bacterium tularense* after Tulare county in central California, the site of the original discovery. In human disease, the agent was first isolated in a case of conjuctivitis with regional lymphadenopathy [3].

In 1919, E. Francis was sent as a public health officer from Washington D.C. to Utah to investigate deer fly fever. Francis isolated *B. tularensis* from the blood of severely ill patients and undertook extensive studies on the pathological changes of the disease in guinea pigs and rabbits as well as in humans. Due to the recovery of the agent from human blood, the disease was named tularaemia. Francis developed culture-based and serological diagnostic methods for tularaemia and described laboratory-acquired human cases, thereby identifying the organism as a laboratory

For editorial comment see page 201.

hazard [4–6]. The agent was renamed *Francisella tularensis* [7] to honour the achievements of E. Francis.

In 1925, during an intense period of research on tularaemia by Francis, Hachiro Ohara described a disease in Japan, similar in clinical expression to tularaemia [8]. Mrs Riki Ohara volunteered to be the subject of experiments, by allowing Ohara to rub the dorsal surface of the left hand with tissues from an infected rabbit [8]. Mrs Ohara developed fever and lymphadenopathy and from a lymph node biopsy, bacteria were recovered and later identified by Francis as *F. tularensis*.

During the 1930s, vaccine research on tularaemia was initiated. Preparations based on killed *F. tularensis* were found to induce only a marginal protection towards infection with virulent strains [9, 10]. In the Soviet Union, however, Gaiskii and El′bert successfully attenuated a natural isolate of *F. tularensis* into a safe and effective vaccine, which was introduced for mass vaccination in the Soviet Union in 1946 [11, 12].

In 1956, an ampoule containing viable vaccine bacteria was transferred from the Gamaleia Institute in Moscow to the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. After investigations in animal models, one of two bacterial colony variants was selected and tested for safety and efficacy in humans [13, 14], named *F. tularensis* Live Vaccine Strain (LVS), and used for vaccination of at-risk personnel. After the introduction of LVS at Fort Detrick in 1960, the incidence of respiratory tularaemia fell from 5.7 to 0.27 cases per 1,000 at-risk employees [15]. The incidence of ulceroglandular tularaemia remained unchanged, although signs and symptoms became less pronounced. Regrettably, the vaccine is no longer available and its future is undetermined [16].

The causative agent

F. tularensis is a short rod-shaped or coccoid, faintly staining, strictly aerobic Gram-negative bacterium. It requires cysteine or cystine for growth. On suitable solid media, such as cystine heart blood agar supplemented with 1% haemoglobin, distinct convex opalescent colonies are formed within 2–4 days of incubation. *F. tularensis* produces acid but no gas from a limited number of carbohydrates and is not easily differentiated from other Gram-negative bacteria by use of conventional diagnostic kits. It is characterised by a unique composition of long-chain saturated and unsaturated fatty acids, and can be identified by gas-liquid chromatography [17]. A test amenable for rapid and simple use on suspect colonies is the agglutination of suspended bacteria by specific antiserum. Due to the recent development of molecular methods, polymerase chain reaction (PCR)-based assays can now be performed for a rapid final confirmation of bacterial isolates [18–20].

F. tularensis comprises two predominant subspecies, *F. tularensis* spp. *tularensis* (Jellison type A) and *F. tularensis* spp. *holarctica* (Jellison type B). The original bases for the differentiation were biochemical properties, epizootology, and virulence assay in rabbits [21]. More recently, subtle differences in nucleic acid sequences have been established [18–20].

F. tularensis type A is isolated in North America and is so far the main subspecies of the continent. It is transmitted most frequently by ticks from rabbits to man but also by direct contact with infected animals. Type A strains are highly virulent and before the advent of effective antibiotics, a mortality of 5–10% was reported in humans. The infective dose in humans is extremely low, 10 bacteria when injected subcutaneously and 25 when given as an aerosol [9, 10, 14]. Nonetheless, transmission among humans does not occur.

F. tularensis type B is spread more widely over the Northern hemisphere and is the sole subspecies isolated in European countries. It is associated with rodents and hares and is transmitted to man by direct contact with animals, aerogenic exposure, intake of contaminated food or water, or by arthropod bites. Type B is less virulent than type A and is nonlethal in humans. Nonetheless, it may cause severe disease and in case of delay of insertion of appropriate antibiotic treatment, the course may be long-lasting and complicated.

Epidemiology

Tularaemia occurs endemically in most countries of the Northern hemisphere, within a range of 30 to 71° latitude [22]. On the American continent, the disease is reported from Canada, the USA, and Mexico. In the USA, human cases have been reported from all states except Hawaii. Since the 1950s, the overall incidence of tularaemia has decreased from >5 to 0.5 cases per 1 million USA inhabitants [23], although with a stationary endemic centre in the southern states and Arkansas and Missouri in particular [24, 25]. An isolated small endemic area is Martha′s Vineyard in Massachusetts, where tularaemia has been reported repeatedly during the last decades [26, 27]. The seasonal distribution in the USA is bimodal with a peak in November to February, associated with hunting, and another in the summer, associated with exposure to tick bites.

In Japan, tularaemia occurs in the north-eastern part of the main island [28]. After a peak in the 1950s, associated with consumption of hares, the incidence has decreased to less than 10 cases per year.

Tularaemia is widely distributed over the Eurasian continent. A high prevalence is found in the former Soviet Union and the Nordic countries, whereas the British Islands seem to be free from the disease. In 1940–1942, during World War II, East European countries were afflicted with epidemics comprising 10,000–100,000 cases each year, including large outbreaks of water-borne tularaemia [11]. Also more recently, tularaemia has been associated with disruption of environmental conditions. In the postwar period of Kosovo in 1999–2000, 327 cases of tularaemia were reported, the outbreak being associated with rodent-caused contamination of water and food [29].

In the Nordic countries and in Sweden in particular, outbreaks of ulceroglandular tularaemia are associated with arthropod bites and occur at least once or twice per decade. In Sweden, the disease occurs endemically only in the northern part of the country

[30]. In both Sweden and Finland, aerogenic exposure in farming has been reported to cause a few outbreaks comprising several hundreds of cases [31, 32].

In comparison with the Nordic countries, the incidence is generally lower in central and southern Europe. In a territory including parts of Moravia, Slovakia, and Austria, tularaemia occurs endemically, the annual incidence varying from <1 to >5 cases per 100,000 inhabitants [33]. In Germany, only one or a few cases are reported each year [34]. A few cases have been reported from Italy [35, 36] and France [37]. In Turkey, 205 cases occurred at the end of the 1990s, associated with use of drinking water from a natural aqueduct [38]. In Spain, tularaemia in humans was first reported in 1996, when 585 cases occurred associated with hare hunting [39].

The reservoir of *F. tularensis* in nature is unknown [22]. Although outbreaks of type B tularaemia are associated with an increased occurrence among rodents and lagomorphs, these animals do not seem capable of harbouring the bacteria between the outbreaks. It is true that chronic shedding of *F. tularensis* by urine has been observed in partly immune voles [40]. In general, however, rodents and lagomorphs do not survive the infection and if they do, experimental evidence strongly indicates that they are capable of eradicating the bacteria.

Hence a natural reservoir of *F. tularensis* has to be sought elsewhere in the environment. *F. tularensis* survives in water and mud for months [22, 41, 42] and the distribution of tularaemia in Eastern European areas and in Sweden is related to natural water. In a survey of serum antibodies to *F. tularensis* in various mammals, the beaver showed a prevalence as high as 57% [43]. This may suggest that the beaver is heavily exposed in its natural habitat and besides, and that it survives tularaemia at a higher extent than do other wild mammals.

Obviously, streams might be contaminated by infected mammals and so become a source of infection. This does, however, not explain the sudden burst of the disease at intervals of several years, intervened by periods of a complete absence. Possibly, some waterborne nonmammalian host cell may harbour the organism and allow it to replicate intracellularly, similar to the protozoan reservoir of *Legionella pneumophila*, another facultative intracellular human pathogen. In that case, outbreaks would depend on the living conditions and distribution of the host cell.

Work aiming to find the reservoir of tularaemia has been hampered by the extremely conserved nature of *F. tularensis* and a subsequent difficulty in finding individual phenotypic or genotypic traits among various isolates of a subspecies. Recently, however, interstrain variation in short tandem nucleotide repeats has been identified [19, 20]. By use of PCR-based techniques, isolates derived from patients at different periods of time or from various regions within the Nordic countries can now be differentiated [20].

Clinical forms of tularaemia

The clinical expression of tularaemia depends on the route of entrance (table 1). Ulceroglandular tularaemia is most frequently caused by vector-borne transmission, in the USA and Central European regions by ticks [33] and in Northern Europe by mosquitoes [45, 46]. Hunters may contract the disease by dressing a hare or by merely touching the animal, without noticing any skin lesion or accident during the handling. Ulceroglandular tularaemia is the most prevalent form of the disease and in Sweden, it comprises more than 90% of outbreaks. Glandular tularaemia is conceptually identical to the ulceroglandular form, although the local infection is too slight to be noticed.

Table 1. – Symptoms of tularaemia in two Swedish outbreaks

Symptoms	Probable source of infection	
	Hay[#,+]	Hare/mosquito bite[¶,§]
Fever	88	94–96
Chills	69	70–83
Headache	59	30–76
Malaise	46	22–53
Sore throat	28	0–22
Conjunctivitis	26	9–17
Lymphadenitis	10	83–87

[#]: based on 140 patients in an outbreak in 1966 [32]; [¶]: based on two outbreaks, comprising 23 cases in 1966 [32] and 344 in 1981 [44]; [+]: percentage of cases presenting with symptoms; [§]: range of percentages.

Also oculoglandular tularaemia is closely related to the ulceroglandular form. The bacteria may be transmitted by the fingers of the patient, or possibly by aerogenic exposure. The form, which is characterised by conjunctivitis and an enlarged preauricular gland, comprises one or a few per cent of all cases of tularaemia. Oropharyngeal tularaemia is contracted by ingestion of contaminated food or water. The primary ulcer is localised in the mouth, and lymph nodes of the neck region are enlarged. In Western countries, oropharyngeal tularaemia comprises a few per cent of cases of tularaemia.

Respiratory tularaemia is contracted by inhalation of aerosolised *F. tularensis*. In the original classification of clinical types of tularaemia [47], this form was not included. Instead, tularaemia with no local signs of aquisition were classified as typhoidal or cryptogenic. Strong circumstantial evidence later justified the inclusion of respiratory tularaemia among forms defined according to route of transmission [48, 49]. Compared with the ulceroglandular form, outbreaks of respiratory tularaemia occur infrequently but tend to comprise large numbers of cases.

Tularemic pneumonia is a manifestation rather than a discrete form of tularaemia. Pulmonary changes may be present or absent in all forms of tularaemia, including the respiratory form. Primary pneumonia indicates pulmonary involvement as a result of inhalation, whereas secondary pneumonia is believed to be caused by hematogeneic spread during the course of disease.

Enteral tularaemia is a form caused by ingestion of contaminated food or water. It is well known from the former Soviet Union but is virtually absent in Western

literature. The term typhoidal tularaemia, finally, should be reserved for cases with no indicated route of entrance [48, 49].

Clinical manifestations

The incubation period of tularaemia is usually 3–5 days, but may range from 1–21 days. The onset of disease is typically sudden, including high fever, chills, fatigue, general body aches, headache, and nausea. A dry cough occurs frequently, also in the absence of pneumonia, and a sore throat is not a sign restricted to oropharyngeal tularaemia. In both type A and type B tularaemia, there is a wide individual variation in severity of disease. The duration of fever may vary from a few days to several weeks. In type A infection, the general condition tends to be more fulminate and may develop into rhabdomyolysis and septic shock [50–52]. After the advent of effective antibiotics, the overall estimated mortality rate of type A tularaemia has declined from 5–10% to 1–2%. Type B tularaemia is virtually nonlethal in humans, even when appropriate treatment is not inserted.

In ulceroglandular tularaemia, a local cutaneous papule develops at the time of onset of general symptoms [53]. Within a few days, the papule may become pustular and ulcerate. It soon heals, leaving a more or less visible scar, similar in appearance to that of the bacille Calmette-Guérin (BCG) vaccination. Within a few days of onset of disease, regional lymph node enlargement is noticed by the patient. The gland tends to grow to a considerable size. It is tender and may be surrounded by redness and oedema in skin and subcutaneous tissue.

The lymph node enlargement is a usual cause for medical attention, whereas the primary ulceration is seldom a matter of concern and may be noticed only by physical examination. Provided appropriate therapy is inserted within a week of onset, the gland will soon decrease in size. If appropriate treatment is not afforded or inserted >2 weeks after onset of disease, the risk of abscess development will be >20% [54, 55] (A. Berglund†, Boden Hospital, Boden, Sweden, personal communication). In most of the latter cases, incision or spontaneous rupture will ensue, necessitating wound care for several weeks. According to old experience, incision within a couple of weeks should be avoided because of risk of local spread of the infection [54].

Oropharyngeal tularaemia presents as stomatitis and pharyngitis. Physical examination shows redness and pustular changes in the mouth and pharyngeal mucous membranes, together with enlargement of regional neck lymph nodes [56–58]. If tularaemia is not suspected for epidemiological reasons, the diagnosis will most likely be missed and appropriate therapy not prescribed. In a Turkish outbreak of oropharyngeal type B tularaemia, where treatment was generally delayed, suppurating neck lymph nodes occurred in ~40% [38]. In Sweden, one single isolated outbreak of oropharyngeal tularaemia has been reported so far [30]. In the beginning of October, when mosquitoes were no longer present, nine members of a family were infected by water from a contaminated well. After receiving two subsequent courses of therapy with ineffective antibiotics, three of the subjects developed lymph node abscesses. Finally, tularaemia was serologically confirmed in all subjects.

Oculoglandular tularaemia is a unilateral lesion, presenting as an intense conjunctivitis requiring medical attention [54, 56, 59, 60]. It is associated with preauricular lymph node enlargement, which often becomes extensive enough to change the contour of the face.

Respiratory tularaemia may present with symptoms of pneumonia, including dry cough, dyspnoea, and chest pain. However, nearly as frequent is the development of fever and general illness with no respiratory symptoms. In respiratory tularaemia, there is a clear distinction between the severity of type A and type B disease and these types will be described separately.

Primary pneumonia of type A tularaemia includes many of the most fulminate cases ever encountered in the disease [48, 53, 55, 61–64]. The onset is often abrupt with a chill, fever, dyspnoea, cough, pain in the chest, and profuse sweating. The cough may or may not be productive and the patient appears extremely ill. Prior to the advent of effective antibiotics, the mortality rate of the form was 30–60%. The disease may remind of typhoid fever, due to the severity of general symptoms and a deteriorated consciousness. In half the number of cases of type A tularaemia, a pulse dissociation has been demonstrated.

When secondary to ulceroglandular or glandular type A tularaemia, symptoms of pneumonia may appear from 1–2 days to many months after onset of disease. There is a wide individual variation in severity and in more severe cases, pulmonary symptoms usually predominate [48, 55].

The radiographic features of pneumonic type A tularaemia are highly variable and may be confused with common bacterial pneumonias, tuberculosis, lymphoma, or carcinoma of the lung. In a review of tularaemia in Arkansas, where type A predominates, 66 cases were classified as ulceroglandular disease and 22 as typhoidal [53]. Pneumonia was diagnosed in 31% of patients with the ulceroglandular form and 83% of those with typhoidal tularaemia. In 36 of 37 patients, parenchymal infiltrates were demonstrated, in 12 cases together with pleural effusion. All areas of the lungs were affected. Only one case of enlarged hilar lymphadenopathy was recorded. In the review [53], no distinction in radiographic features was made between ulceroglandular and typhoidal disease.

Large outbreaks of respiratory type B tularaemia have been described from the Nordic countries. Among 140 patients of a Swedish outbreak in 1967, probably infected through contact with contaminated hay, only 7% had symptoms suggestive of pneumonia [32]. In an outbreak in Finland in 1982, comprising 53 farmers presumed to have contracted tularaemia by aerogenic transmission, 11% were classified as mild (fever enduring <1 week), 55% as moderate (1–2 weeks) and 34% as severe (⩾3 weeks) [31]. All patients had fever and most of them had general symptoms such as headache, myalgia, and arthralgia. Dry cough was reported

in one-half of the cases, and a similar proportion had retrosternal discomfort, pleural pain or dyspnoea. Radiologically, hilar adenopathy was the most frequent change, occurring in 13 of 38 patients. Pneumonic infiltration occurred in five cases and pleural effusion in one. In 12 cases, chest radiography was normal [31]. Together these studies suggest that in respiratory type B tularaemia, pneumonia occurs in <50% of patients and presents with signs and symptoms that are generally much milder than those of type A disesase.

In type B tularaemia, pneumonic involvement occurs only more seldom as a secondary manifestation. In a Swedish outbreak, mainly of ulceroglandular disease, pneumonia was diagnosed in only one of 400 cases [44]. Even in southern European countries, where the disease is less easily recognised and the start of effective therapy subsequently delayed, only 1–4% of cases of ulceroglandular or oropharyngeal tularaemia are associated with pneumonia [38, 65].

In addition to pneumonia, tularaemia may be complicated by a variety of manifestations. In regions endemic for type A disease, life-threatening or fatal conditions are described, such as septicaemia [52], meningitis [66], endocarditis [67], and more or less severe hepatic [53, 68, 69] and renal [70, 71] failure. In type B tularaemia, septicaemia [72, 73] and meningitis [74] have been described, although with a more favourable outcome than in type A disease. Finally, various immune-mediated skin manifestations are observed in tularaemia, including erythema nodosum and erythema multiforme [38, 53, 64, 75].

Blood chemistry

Routine assays of blood chemistry show no abnormalities specific to tularaemia. In the Arkansas study of 88 patients, presumably type A tularaemia, white blood cell counts ranged 5,000–22,000 cells·mm^{-3} (median 10,400) and the differential count showed some preponderance of lymphocytes. Liver enzyme values were somewhat increased [53].

In a study of type B tularaemia, comprising 52 cases of the pulmonary and 42 cases of the ulceroglandular and glandular forms, the mean leukocyte count was $8.3\times10^{9}\cdot L^{-1}$ and the differential count was usually normal [76]. C-reactive protein (CRP) values were generally lower than expected for an invasive bacterial disease. The mean CRP level peaked at 53 mg·L^{-1} during the first week of disease and normalisation was reached within three or four weeks. Erythrocyte sedimentation rate more closely conformed with values found in invasive bacterial diseases. Mean values increased from 30 to 50 mm·h^{-1} during the first week and remained high for the first whole month [76].

Case reports

From an outbreak of tularaemia in 1998 in Ljusdal, a hyperendemic area of central Sweden, four patients with pulmonary involvement will be described here, of whom at least three conformed with the respiratory form.

Case one

A 24-yr-old male sought medical attention on July 31, due to a 3-day history of hoarseness without cough and a temperature of 37.5–39.5°C. The patient was a farmer and had been heavily exposed to hay during the summer. The patient's CRP level was 75 mg·L^{-1} and phenoxymethyl penicillin V was prescribed for a 10-day course. After 8 days of treatment, fever still prevailed. Pulmonary auscultation was normal. A chest radiograph showed a somewhat prominent hilar enlargement with no parenchymal changes (fig. 1). Lymphoma and tularaemia were the main differential diagnoses. Doxycycline, 200 mg daily, was given for 14 days and tularaemia was serologically confirmed. Ten weeks after onset, a follow-up radiograph showed normalisation.

Case two

A 63-yr-old male, smoker since 40 yrs, sought medical attention on August 18, 3 days after onset of fever and frontal headache. On the days preceding onset of disease, the patient had been working with hay. Physical examination disclosed rales at the basal, dorsal left side of the thorax. There were no palpable lymph nodes. The CRP level was 89 mg·L^{-1} and a chest radiograph showed basal right-sided infiltrates. Phenoxymethyl penicillin was prescribed and 13 days later, the patient's condition was improved although not yet satisfactory, with a temperature of 38.3°C. A new radiograph showed left-sided pleuritis with a 2-cm fluid layer but no pneumonia-like infiltrate. Doxycycline 200 mg daily was instituted for a 14-day course. Tularaemia was serologically confirmed. One month after onset of disease, the patient reported full recovery.

Case three

A 34-yr-old male sought attention on August 24, with a 1-week history of fever at 38°C, headache, and minor cough. The patient had handled cattle and been harvesting hay. There were no palpable lymph nodes. A chest radiograph showed a prominent lower hilus enlargement, tularaemia was suspected, and a 10-day course of oral ciprofloxacin 0.75 g *b.i.d.* was instituted. Tularaemia was serologically confirmed and the patient recovered. At a 1-month follow-up, a radiograph showed unchanged conditions.

Case four

A 16-yr-old male sought attention on October 12, complaining of upper respiratory symptoms, a peak temperature of 40°C, and productive cough for 5 days. Since the patient already felt better and was afebrile, no antimicrobial therapy was instituted. Four days later, however, fever recurred and phenoxymethyl penicillin was prescribed for a 10-day course. At a visit within 2 days of treatment, continuing fever and a

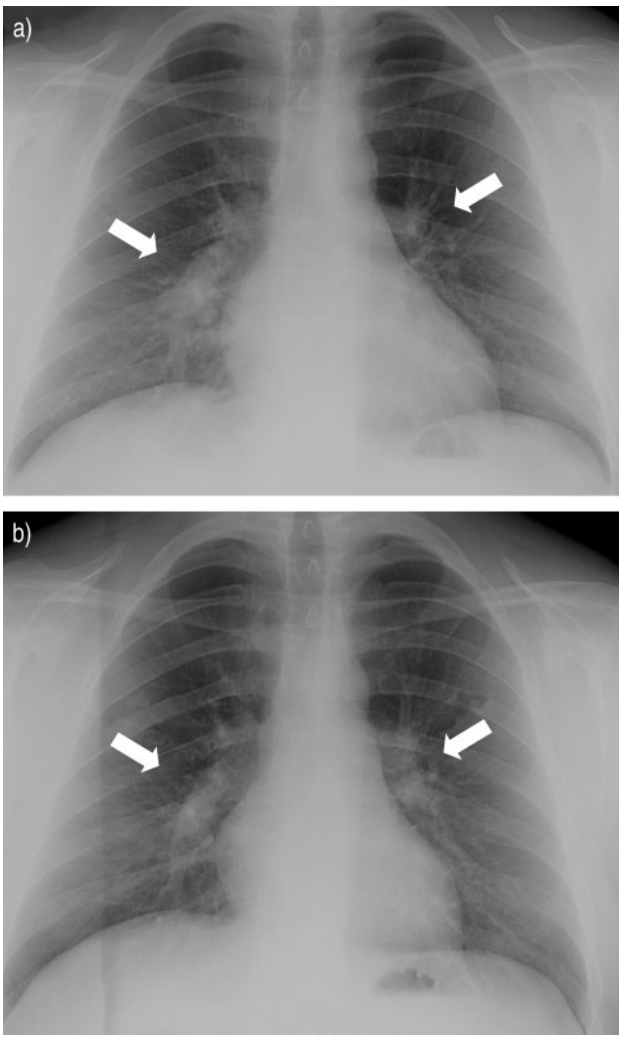

Fig. 1. – Hilar enlargement (arrows) in a 24-yr old farmer (case number 1) with fever but with no lower respiratory tract symptoms. Radiography was performed a) 13 days and b) 10 weeks after onset of disease.

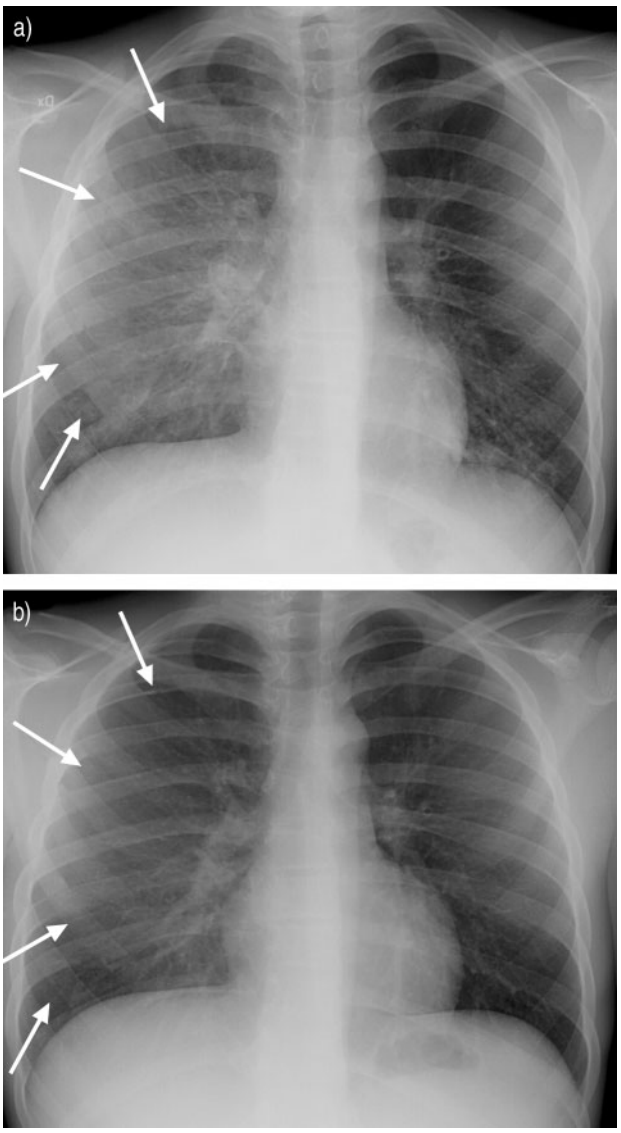

Fig. 2. – Extended right-sided consolidation (arrows) in a 16-yr-old male (case number 4) with fever and productive cough. Radiography was performed a) 11 days and b) 2 months after onset of disease.

cervical lymphadenitis were recorded. Pulmonary auscultation was normal, with a CRP level of 45 $mg \cdot L^{-1}$, and a chest radiograph showed right-sided, extended consolidation and also basal left-sided infiltrates (fig. 2). Tularaemia was suspected and ciprofloxacin was instituted. One day later, therapy was changed to doxycycline 200 mg daily for a 13-day period. Tularaemia was serologically confirmed. The patient recovered and 2 months after onset of disease, a radiograph showed normalisation.

Diagnosis

The duration from a first medical consultation until tularaemia is clinically suspected depends on the form of disease and the prevalence of tularaemia in the geographical area. In a tularaemia-endemic region of the southern USA [53], the mean duration from first medical contact to start of therapy was 11 days (range 0–51) among patients with ulceroglandular tularaemia and 17 days (range 0–66) in typhoidal disease. In the four present cases of respiratory tularaemia, occurring in a hyperendemic area, the interval from the first visit to start of effective therapy was 11, 13, 0, and 6 days, respectively. In Spain, where tularaemia had not been previously reported, an outbreak comprising 142 cases, mainly of the ulceroglandular form [77], was associated with a mean delay from onset of symptoms to diagnosis of 47.5 days (range 3–145).

Respiratory tularaemia does not present with specific signs or symptoms. Depending on epidemiological circumstances, a variety of microbial agents may need to be considered (table 2). Since tularaemia cannot be serologically confirmed before 10–14 days after onset of disease, institution of therapy may be warranted, based only on known occurrence of tularaemia in the past in the area where the patient lives. In

Table 2. – Differential microbial aetiology of tularaemic pneumonia caused by zoonotic and environmental agents

Bacillus anthracis
Brucella species
Chlamydia psittaci
Coxiella burnetii
Francisella tularensis
Hantavirus
Influenza A virus
Legionella species
Leptospira species
Mycobacteria
Pasteurella multocida
Ricketssia rickettsii
Toxoplasma gondii
Yersinia pestis

endemic areas of Finland and Sweden, farming is a risk factor for aquisition of respiratory tularaemia.

The microbiological diagnosis of tularaemia relies mainly on serology. The tube agglutination test shows high sensitivity and specificity. Cross reactions may only be seen with serum from patients with brucellosis and yersiniosis [5, 78, 79]. A four-fold increase of the titre or a titre $\geqslant$160 of agglutinating antibodies confirms a clinical suspicion of tularaemia. Titres peak at a level of 320–1,280 and decline slowly. In a study of 53 patients contracting tularaemia in 1967, a median titre of 640 (range 160–2,560) was found in the acute phase. When re-investigated 25 yrs later, 23 of 53 subjects still had detectable agglutinins [80]. Thus, the presence of agglutinins in a case less typical of tularaemia must be cautiously interpreted, and the patient should be asked about episodes complying with tularaemia several years back.

During the last decades, agglutination techniques for bacteriological serology, including tularaemia, have been replaced by enzyme-linked immunosorbent assay (ELISA) [81]. The ELISA enables determination of immunoglobulin (Ig) class-specific antibodies, an advantage which is, however, of limited value in tularaemia and may even be misleading. IgM, IgG and IgA antibodies appear in parallel during the course of tularaemia, and they all decline at a similar slow rate [82, 83]. Hence, the presence of IgM antibodies to *F. tularensis* is not a reliable indicator of recent infection.

Due to risks associated with laboratory work, culture is not routinely performed in tularaemia, and especially not in North America where type A is endemic. According to recent recommendations by a USA working group on civilian biodefence, routine culture of specimens possibly containing *F. tularensis* can be performed in biological safety level 2 (BSL-2), whereas work on colonies and manipulations that might involve aerosol formation requires BSL-3 conditions [16].

In regions endemic for type B tularaemia, culture is more often performed. Before sending a specimen from a suspect case, however, the laboratory should be called. For wound specimens, a commercial transport medium based on Amies agar with charcoal is recommended [84]. In a recent study of ulceroglandular tularaemia, *F. tularensis* was isolated from ulcers in 62% of the patients [84]. A higher sensitivity may need invasive sampling because in some patients with ulceroglandular disease, the ulcer is dry and innocent and may be difficult to distinguish from a noninfected mosquitoe bite.

In tularaemic pneumonia, sputum may yield growth of *F. tularensis*. The experience is, however, based only on old sporadic cases [85, 86] and sputum culture is not routinely performed. Although blood culture is not routinely performed in tularaemia, *F. tularensis* is occasionally isolated from patients hospitalised with fever for unknown reason [52, 73, 87]. When growth of *F. tularensis* is suspected, a reference laboratory should be consulted for safe handling and further identification.

For rapid diagnosis of tularaemia, demonstration of bacterial antigen by immune assay and ribonucleic acid hybridisation have been tried [88–91], without being widely applied. PCR-based methods have yielded highly promising results and will probably become established more generally in endemic regions. Detection of deoxyribonucleic acid (DNA) encoding a conserved 17-kD lipoprotein of *F. tularensis* [92] allows for specific diagnosis [93–95]. In two studies, each comprising 40 patients with ulceroglandular tularaemia, PCR on wound specimens detected *F. tularensis* DNA in 73% and 75% of the cases [84, 95]. In the latter study, four of eight patients with clinically suspected tularaemia but with negative culture and serology showed detectable DNA. As for culture, the availability of wound secretion was a probable limitation of PCR-based methods. Degradation of DNA during transportation did not seem to occur [84].

Treatment

For decades, therapy against tularaemia has consisted of aminoglycosides, tetracycline, and in the early days, chloramphenicol [96]. Since 1947, when streptomycin was introduced, aminoglycosides have been the preferred drugs for treatment of severe cases [97–99]. Aminoglycosides are bactericidal and the relapse rate is low. The main drawbacks are their toxicity and the necessity for parenteral administration. Tetracycline is bacteriostatic and associated with a relatively high risk for relapse [96, 100]. Nonetheless, doxycycline is the preferred drug in most cases of type B tularaemia. To minimise the risk for relapse, treatment with a bacteriostatic drug should preferably be extended to cover the first 2 or even 3 weeks of onset of disease. Before that, a cell-mediated immune response is not yet fully developed [101, 102] and the infection is not effectively contained by the host defence.

Betalactams, macrolides, lincosamides, and trimoxazole are not reliable for treatment of tularaemia. It should be remarked that, in spite of reported susceptibility *in vitro*, parenteral cephalosporin has been found ineffective for clinical use [103].

In less severe paediatric cases of tularaemia, antibiotics for oral administration are not easily found. Due to side effects of tetracycline at ages $\leqslant$8 yrs, children may often need hospitalisation only to receive parenteral administration of an aminoglycoside. Among 67 Scandinavian paediatric cases, drugs ineffective against *F. tularensis* were administered

in 20 [104]. The mean duration of symptoms in the 67 children was 26 days (range 8–92 days). Obviously, the lack of oral alternatives for children may have contributed to prolong the course of disease.

Quinolones offer new options for the treatment of tularaemia. According to *in vitro* tests, they are highly active against *F. tularensis*. Minimal inhibitory concentration (MIC) values are within the range of 0.01–0.1 $mg \cdot L^{-1}$, which is two orders of magnitude lower than concentrations achieved in tissue at ordinary doses. Actually, the relationship between MIC values and achievable tissue concentrations is more favourable for quinolones than for alternative agents [105–108]. Reports on first-line treatment with ciprofloxacin of ~80 patients with various forms of type B tularaemia have shown a high efficacy [77, 106, 109, 110]. Moreover, levofloxacin was successfully used in two compromised patients with severe type B tularaemia [111]. A few cases of failure with ciprofloxacin have been reported [112]. A common factor in the latter cases was a long interval between onset of disease and initiation of treatment, a factor known to be associated with a poor outcome irrespective of the drug used (including streptomycin).

There is currently an increasing use of quinolones in various paediatric conditions. The risk of arthropathy, as described for juvenile animals, is currently regarded to be low or even negligable [113, 114]. Of 12 children with ulceroglandular tularaemia treated as outpatients with oral ciprofloxacin, all responded satisfactorily [106].

There is, as yet, no experience on quinolones in treatment of type A tularaemia. However, type A isolates of *F. tularensis* show similar low MIC values as type B [115]. Altogether, quinolones seem to be promising options for treatment of tularaemia, irrespective of age of patient and subspecies of *F. tularensis*.

Pathophysiology

F. tularensis belongs to the group of facultative intracellular pathogens [116], also including agents such as Mycobacteria, Listeria, Legionella, and Brucella. In experimental animals as well as humans, tularaemia may induce changes indistinguishable from those seen in tuberculosis [117–119]. Due to a more rapid bacterial replication, however, tularaemia develops much more rapidly.

After cutaneous inoculation, the bacteria multiply locally and spread to regional lymph nodes. Dissemination to various organs ensues, including the liver, spleen, and lungs. This phase probably includes bacteraemia. In all tissues invaded, an intense inflammatory response is induced. Polymorphonuclear cell invasion and a varying degree of necrosis are followed by an accumulation of mononuclear cells, including macrophages and T-lymphocytes. Eventually, granuloma formation may be seen, with epitheloid cells, giant cells, and caseous necrosis. In fulminate cases of type A disease, tissue necrosis may be excessive and death may precede the development of granuloma. Many of these cases show lobular or confluent lobular pneumonia, involving one single, several, or all lobes [48, 53, 55, 62, 63].

Survival seems to depend on a capability of the host to induce an effective immune response before the bacterial burden becomes too large. *F. tularensis* type A multiplies more rapidly within the host than type B organisms and may reach lethal numbers before an effective host response is mobilised. The molecular basis for this difference in virulence is unknown. *F. tularensis* has not been shown to cause any potent toxin [120].

In type B tularaemia, changes are generally milder. In a study of seven patients putatively infected by airborne transmission, bronchoscopy disclosed haemorrhagic oedema progressing to mononuclear inflammatory reaction [49]. In most cases, hilar enlargement was disclosed by radiography and the authors suggested the changes to correspond to the primary ulcer and regional lymph node reaction of the ulceroglandular form. In pleural fluid, changes similar to those of tuberculosis have been observed [121, 122].

Immunity

Like the defence to other intracellular bacteria, the host defence to *F. tularensis* depends on cell-mediated immunity [116]. The bacteria replicate intracellularly in macrophages and the outcome is determined by the state of the macrophages. When exposed to pro-inflammatory cytokines and interferon (IFN)-γ in particular, the macrophages change from target cells susceptible to unrestricted bacterial growth into a more resistant state. Work on murine models shows that as early as the initial stage of infection, cells of the innate immune system can provide IFN-γ and cause retardation of bacterial replication. Thereafter, an effective control of the infection requires the involvement of T-cells [123–127]. Both CD4 and CD8 T-cells proliferate in response to *F. tularensis* and differentiate into IFN-γ-producing effector cells. CD4 cells are probably the most important, whereas CD8 T-cells depend on CD4 cell help for their capability of IFN-γ production [128].

In human tularaemia, an immunospecific T-cell response can be demonstrated *in vitro* after the first 2 weeks of onset of disease [101, 102, 129]. The T-cells recognise a vast number of bacterial proteins [80, 128, 130–135] and the strength of the response seems to depend on the sum of T-cell specificities involved rather than on cells recognising one or a few immunodominant antigens [136].

Tularaemia is followed by development of a long-lasting protective immunity. Only 12 instances (among nine individuals) of re-infection are known in the literature [15]. In accordance, a long-lasting T-cell memory can be demonstrated [80, 133]. In assays performed 25 yrs after an outbreak of tularaemia, a vigorous T-cell response to *F. tularensis* was still demonstrable, including a capacity of IFN-γ production upon *in vitro* stimulation. During the 25-yr period, tularaemia had been reported only rarely in the region where the subjects lived, suggesting that an antigen-specific T-cell memory may persist for a

lifetime after tularaemia, independently of re-exposure to *F. tularensis*.

Thus, T-cells specifically sensitised towards *F. tularensis* are the main denominator of protective immunity. Besides these CD4+ and CD8+ cells, which express the αβ T-cell receptor, there is also a subset of γδ T-cell receptor-expressing cells. In tularaemia, similar to other intracellular bacterial diseases, γδ T-cells utilising one specific combination of gene sequences, the Vγ9Vδ2 T-cells (or Vγ2Vδ2 according to an alternative nomenclature), increase in blood from 1–5% to 15–30%, thereby becoming the predominant specific T-cells in circulation [137, 138]. The Vγ9Vδ2 T-cells recognise nonpeptidic low molecular weight phosphorylated compounds, so-called phosphoantigens. Upon stimulation *in vitro*, Vγ9Vδ2 T-cells produce large amounts of pro-inflammatory cytokines, and tumour necrosis factor-α and IFN-γ in particular, and exert cytotoxicity. Their consistent increase in two intracellular bacterial diseases, as different in their clinical expression as tularaemia and Pontiac fever [139], suggests that they are pathophysiologically important in the type of infection. In work aiming to understand the role of Vγ9Vδ2 T-cells, animal models are of limited value, because cells analogous to the subset cannot be detected in mice or other nonprimate mammals.

Vaccine

Immunospecific protection against tularaemia is afforded by vaccination with the live attenuated strain *F. tularensis* LVS. LVS affords excellent protection against respiratory tularaemia and mitigates the course of ulceroglandular disease [15]. LVS-vaccinated individuals show a memory T-cell response to *F. tularensis* [140–142], lasting for at least 10 yrs [143]. Due to difficulties with standardisation of the live vaccine, LVS is no longer licensed and in Western countries, no effective vaccine is currently available [16].

Perspectives for development of a subcellular, well-defined tularaemia vaccine are uncertain. Similar to experience from work on mycobacteria, the need for live vaccine seems difficult to overcome. Due to the multitude of T-cell reactive bacterial proteins involved and the relation of T-cell epitopes to the major histocompatability complex haplotype of the individual, a large cocktail of bacterial polypeptides may be required. This is not enough, however, as illustrated by the failure of inducing protection by use of crude killed preparations of *F. tularensis*, even in the presence of strong adjuvant [144]. Prerequisites necessary for induction of an effective T-cell response *in vivo* remain to be understood, including measures to obtain an appropriate cytokine induction [145, 146].

Concluding remarks

F. tularensis is one of the most potent pathogens known in human medicine and evokes great concern as a bioterrorism agent. Despite improvements in therapy over the last decades, type A tularaemia is still associated with fatal outcome. The molecular reason for the difference in virulence between type A and B organisms is unknown. Bacteria of the two types share antigens and a live vaccine based on attenuated type B organisms protects against disease caused by type A bacteria.

Due to its lower virulence, *F. tularensis* type B is amenable to experimental work. This applies not only on vaccine research, where type B strains have been the basis for the progress so far, but also on work aiming to improve diagnostic methods and therapy. Recent achievements are the development of PCR-based methods for rapid and accurate laboratory diagnosis under safe conditions and the introduction of quinolones for treatment.

F. tularensis offers some special advantages as a model for studies of immunity against intracellular bacteria in general. Experiments can be done in animals which are naturally infected and the vaccine strain *F. tularensis* LVS can be used for work under safe conditions. When infected with LVS, rodents show changes similar to those of tularaemia in humans [123, 147–149]. In work on tularaemia, the interpretation of immunological results is facilitated by a low background reactivity of T-cells from non-primed individuals to various protein antigens of *F. tularensis* [136], possibly related to the fact that *F. tularensis* lacks close relation to bacteria in the commensal or pathogen human flora [18].

A challenge for the near future will be the unravelling of natural reservoirs of *Francisella tularensis*. In this work, the current development of molecular techniques for differentiation of isolates of *Francisella tularensis* within a given subspecies [19, 20] will be instrumental. When the reservoir becomes disclosed, measures to predict and restrict epidemic outbreaks may appear.

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