EXPERT REVIEWS

Tularemia: current diagnosis and treatment options

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Tularemia is an infection caused by *Francisella tularensis* with a worldwide distribution and diverse clinical manifestations. Limitations in both culture and serologic testing have led to substantial research into new diagnostic techniques and their clinical application, with PCR testing as the best example. This review focuses on the utility of culture, PCR and serologic testing for tularemia. In addition, we also review the evidence to support different therapeutic options for tularemia, highlighting both the most effective supporting evidence for therapeutic recommendations as well as gaps in current knowledge. We conclude the article with suggestions regarding potential areas for future research.

KEYWORDS: antibiotics • diagnosis • *Francisella tularensis* • PCR • treatment • tularemia

Tularemia is an infection caused by the Gram-negative coccobacilli *Francisella tularensis*, which was first documented as a pathogen in ground squirrels in Tulare County (CA, USA) in 1911 [1]. Since that time, tularemia has been described as a clinical infection in temperate climates throughout the world. Tularemia has received attention recently owing to its potential for use as a biologic weapon [2]. In particular, *F. tularensis* is able to cause infection via the inhalation of only a very low number of organisms. The organism has been categorized amongst the pathogens that are most dangerous (Category A) as biologic weapons on the US CDC list. Owing to its relevance both as a cause of human infection and as a biologic weapon, we will review the current diagnostic and therapeutic approaches for this pathogen. We will focus the diagnostic discussion on clinical diagnostic testing, without reviewing identification and strain-typing techniques, or issues associated with the environmental detection.

F. tularensis has multiple subspecies, with the subspecies *tularensis* and *holarctica* being the most clinically relevant. Subspecies *tularensis* is often designated Type A, and is the predominant cause of tularemia infection in the USA, [3], although a case report recently documented a subspecies *tularensis* infection in Europe [4]. Subspecies *holarctica* (Type B) is responsible for almost all tularemia infections in Europe. Type B tends to cause less severe clinical manifestations and is associated with a lower mortality rate [5]. This difference is important when evaluating clinical therapeutic studies, since the two organisms have different clinical courses. Another notable strain of *F. tularensis* is the live vaccine strain (LVS) [6]. This strain was obtained by the USA from the Soviet Union in the 1950s and developed into a vaccine to protect laboratory workers at the US Army Medical Research Institute of Infectious Diseases. Protection for laboratory workers was documented in an observational study in which the incidence of ulceroglandular and pneumonic tularemia declined after the vaccine was introduced [7].

Tularemia causes a wide variety of clinical manifestations, usually related to the route of infection. The different forms of tularemia are listed in Table 1. The ulceroglandular form is the most commonly reported form of tularemia in most case series, particularly those from the USA [8]. In Japan, the glandular form of tularemia predominates [9]. The oropharyngeal and pneumonic manifestations tend to be associated with outbreaks. The largest published outbreak of pneumonic tularemia occurred in Sweden, in which infected voles contaminated barns and subsequent aerosolization of the organism caused pneumonic infection [10]. In general, the mortality associated with untreated tularemia is 10% for Type A and 1% for Type B [5]. Even with antibiotic treatment, tularemia may be associated

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14. ABSTRACT

Tularemia is an infection caused by Francisella tularensis with a worldwide distribution and diverse clinical manifestations. Limitations in both culture and serologic testing have led to substantial research into new diagnostic techniques and their clinical application, with PCR testing as the best example. This review focuses on the utility of culture, PCR and serologic testing for tularemia. In addition, we also review the evidence to support different therapeutic options for tularemia, highlighting both the most effective supporting evidence for therapeutic recommendations as well as gaps in current knowledge. We conclude the article with suggestions regarding potential areas for future research.

15. SUBJECT TERMS

Francisella tularensis, tularemia, diagnosis, therapy, treatment options, PCR, serology

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with 2% mortality [11]. Reports of tularemia infection prior to antibiotic availability suggest that the infection causes prolonged disabling symptoms, with some patients developing a chronic, debilitating condition [12].

F. tularensis is characterized as an intracellular pathogen and substantial research has described its mechanisms for survival within the macrophage. Recently characterized defense mechanisms include the following:

- Production of an acid phosphatase (AcpA), which mitigates the macrophage's respiratory burst-killing mechanism [13]
- Inhibition of Toll-like receptor signal transduction pathways [14–16]
- Prevention of acidification in the phagosome [17]
- Escape from the phagosome [18], mediated by the 23-kDa protein made by the *igiC* gene [19,20]

Survival in the macrophage is one of the essential attributes that allows *F. tularensis* to infect humans, particularly with a low number of organisms. Only a few organisms are required to cause infection. A subcutaneous inoculation of ten organisms caused the ulceroglandular form of tularemia in human volunteers [21]; and only ten to 50 organisms were required to cause infection by the inhalational route [22]. The ability to evade innate immune responses of macrophages and dendritic cells (particularly pulmonary dendritic cells [23,24]) allows the organism to survive and leads to infection. However, this innate immune response is also crucial for protection from lethal infection, particularly in the mouse LVS infection model [25]. Knockout mice, depleted of T lymphocytes needed for an appropriate cell-mediated immune response, survive for multiple weeks in the murine intradermal LVS infection model [26,27]. In addition, a recent report noted that the virulent forms of *F. tularensis* may have a substantial extracellular phase in mice [28]. More research is needed to accurately assess the impact of this extracellular phase on the overall pathogenesis of the organism.

Protection against tularemia is related to the ability of the host immune response to control and, eventually, eradicate infection. Numerous studies have emphasized the importance of the cell-mediated immune response (reviewed by Tarnvik *et al.* [29]), and natural infection can induce a T-cell memory response that can last for 25 years, or longer [30]. The γδ T lymphocytes, which are thought to be important in the control of intracellular pathogens, increase as a response to tularemia infection [31]. In addition, neutrophils [32] and natural killer cells [25] also assist in the protective immune response.

The role of antibodies is less certain, with evidence in humans that a serologic response to a killed tularemia vaccine was not protective for laboratory workers, although these laboratory workers may have had milder symptoms as a result of prior vaccination [7]. One murine model suggested that passive transfer of antibodies was protective in a lethal LVS mouse model but not protective (only time to death was increased) in a virulent *F. tularensis* model [33]. A review of prior animal and human data on humoral immunity in tularemia concluded that the humoral response had minimal effect on the overall control of *F. tularensis* infection [29]. Other reviews have suggested that passive immune transfer may be useful in certain circumstances [34]. A recent study in mice demonstrated the passive immunization of immune serum-protected mice in a LVS infection model [35]. Further study on the role of antibodies in protection against tularemia infection is important for both vaccine development and possible therapeutic products.

Table 1. Categories of clinical forms of tularemia and their characteristics.

Type of tularemia	Clinical symptoms	Exposure risk	Descriptive examples	Ref.
Ulceroglandular	Painful ulcer, regional lymphadenopathy and fever	Direct contact with infected animals and arthropod bite	Infected animals include rabbits and voles Vectors include deer flies and ticks Laboratory-acquired infection	[7,8]
Glandular	Lymphadenopathy without ulcer	Direct contact with infected animals, arthropod bite and possibly inhalation	Most common form in Japan, challenging to recognize owing to extensive differential diagnosis	[9,81]
Oculoglandular	Conjunctivitis with yellow nodules, ocular swelling and periauricular lymphadenopathy	Direct contact with infected animals	Initial case of human infection described in the USA	[82]
Oropharyngeal	Exudative pharyngitis and cervical adenopathy	Contaminated food or water	Outbreaks in Bulgaria and Turkey	[83,84]
Pneumonic	Fever and pulmonary infiltrates	Inhalation	Mowing lawns in Cape Cod, MA, USA Laboratory-acquired infection	[85,86] [7]
Typhoidal	Chronic fever without adenopathy, weight loss and fatigue	Inhalation	Probably associated with a higher mortality rate	[11]

Diagnosis

Culture

The primary diagnostic challenge with tularemia infections is the difficulty encountered when trying to culture the organism from a clinical sample. *F. tularensis* requires media that are enhanced with cysteine, or another compound containing sulfhydryl groups, and incubation in a CO_2-rich environment. Various agars are acceptable for growing *F. tularensis*, which include cysteine blood agar, Thayer–Martin agar and cysteine heart agar with 9% heated sheep red blood cells (CHAB) agar. On the CHAB agar plates, *F. tularensis* colonies appear small (2–4 mm), round and greenish-white in colour [36]. *F. tularensis* grows slowly, even on appropriate media; therefore, 2–4 days are often required for growth to be observed on agar plates. In liquid media, enhancement of the broth with cysteine or compounds containing sulfhydryl groups is required. Options for broth include modified Mueller–Hinton and thioglycollate broth, with some experts recommending enhancement of the broth with 0.025% ferric pyrophosphate [36].

However, even when conditions are optimized, cultures from patients with documented tularemia infections are often negative. It is possible that the yield of cultures is improved by bedside or rapid inoculation into broth or culture plates since there is often a delay between the acquisition of a sample and its arrival at the microbiology laboratory. An improved yield was observed by rapid plate inoculation of necropsy samples from infected prairie dogs [37]. In addition, this study reported that utilizing media with antibiotics also improved the yield of cultures. In a study that involved obtaining swabs of the inoculation after LVS tularemia vaccination, we observed that neither immediate nor delayed inoculation yielded a high percentage of positive tests by culture [38]. The yield of culture may also be influenced by the mode of tularemia infection. In a human challenge model, a patient infected with tularemia by inhalation had positive culture results from nasal and gastric washes up to 1 week after treatment with tetracycline [39]. These results may reflect the inability of tetracycline to rapidly eliminate *F. tularensis* owing to its bacteriostatic properties, but also indicates the success of culture as a diagnostic test in this model.

Tularemia is rarely isolated from blood cultures, with existing literature consisting primarily of case reports. Haristoy *et al.* reviewed and summarized 20 prior case reports, particularly noting that most of these cases (16 out of 20) were associated with pneumonic tularemia, while only two of the cases were associated with typhoidal tularemia [40]. Most of the cases described did not occur in immunocompromised patients, however, a recent case report noted tularemia bacteremia as a cause of fever in a kidney transplant recipient [41]. The case report also noted that blood cultures performed on this patient led to a laboratory exposure to *F. tularensis*, requiring the laboratory worker to receive postexposure prophylaxis. The yield of blood cultures may be improved by blind subculture (inoculating agar plates with material from a blood culture bottle, even when Gram stain of the blood culture material is negative) after a period of incubation. One report describes three examples of cases in which blood cultures were negative and Gram stain of the broth was also negative; however, blind subculture allowed for *F. tularensis* to be identified [42].

PCR

A wide variety of PCR assays have been developed for the detection of *F. tularensis* (Table 2). These PCR assays have typically been tested against a wide variety of other pathogens, with minimal false-positive results. The limit of detection of these assays varies by the type of sample. In general, blood samples contain inhibitors to PCR reactions, such the heme component of red blood cells [36]. These inhibitors cause the limit of detection of the organism to be higher.

Refinements to PCR techniques have focused on improving the limits of detection and processing time and developing the testing for field use. One group investigated the use of a handheld thermocycler, compared with laboratory-based PCR platform and culture of tissue homogenates from infected mice [43]. Culture of these tissues proved to be more sensitive than both PCR platforms. In order to improve detection of *F. tularensis* from complex specimens, such as environmental samples, Versage *et al.* developed a multitarget PCR, with assays for the *isftu2* element and the *23kDa* and *tul4* genes [44], and this platform proved to be more sensitive than culture when comparing samples from infected animal carcasses.

PCR is becoming increasingly utilized in the clinical diagnosis of infection. Initially, case reports and observational studies described the effective use of PCR for the diagnosis of tularemia, particularly ulceroglandular tularemia. Two studies from Sweden provided useful evidence for the employment of PCR for testing ulcer samples from ulceroglandular tularemia patients [45,46]. The first study noted PCR was positive in wound samples from 29 out of 40 patients with serologically confirmed ulceroglandular tularemia infections [46].

In a follow-on study, PCR was compared with culture among patients with ulceroglandular tularemia. *F. tularensis* DNA was detected in 30 out of 40 (75%) of patients by PCR, whereas culture was positive in only 25 out of 40 patients [45]. In this study, there were three patients with negative cultures and serologic tests (even after 12-week follow-up testing) who had clinical symptoms suspicious for tularemia and a positive PCR result. In these patients, T-cell stimulation assays were positive. These results may be attributable to the early use of antibiotics after diagnosis, which prevented the development of a serologic response. The results have important implications for the overall incidence of tularemia since serologic testing is often considered the 'gold standard' diagnostic test. Further study of tularemia infections, with a combined assessment using culture, PCR, serology and clinical symptoms, is needed to determine the optimal testing combination for cases of tularemia. More recent retrospective testing from the research group in Sweden confirmed the utility of PCR for the diagnosis of ulceroglandular

Table 2. PCR platforms for *Francisella tularensis*.

Authors	Target gene(s)	LOD	Comments	Ref.
Christensen *et al.*	*fopA* *tul4*	50 fg/27 genomic equivalents for both assays	Comparison of three different, real-time PCR platforms	[87]
Fulop *et al.*	*fopA*	1 CFU direct detection, 10^2 CFU/ml in blood	None	[88]
Fujita *et al.*	*fopA*	Ten DNA copies	None	[89]
Grunow *et al.*	*tul4*	10^2 CFU/ml in saline, 10^3–10^4 in spiked serum	Detected *F. tularensis* from tissue samples from infected European brown hares	[60]
Higgins *et al.*	TaqMan 5′ nuclease assay: *fopA* PCR-enzyme immunoassay: *tul4*	<100 CFU for both assays	Successful detection of *F. tularensis* in mouse tissue and ticks	[90]
Junhui *et al.*	Three primers		PCR (38 out of 46) better than cultures (22 out of 46) for blood samples from infected mice	[91]
Skottman *et al.*	*23kDa*	1 fg sensitivity	Part of multiplex PCR for biodefense detection. Tested in infected hares	[52]
Tomioka *et al.*	*tul4*	50 CFU/ml in spiked human blood samples	Part of multiplex PCR array for biodefense detection. Used LVS *F. tularensis* for testing	[51]
Versage *et al.*	*isftu2* element and the *23kDa* and *tul4*	*tul4*: 1 CFU *isftu2* element and *23kDa*: <1 CFU	More sensitive than culture during assessment of environmentally contaminated samples	[44]

CFU: Colony-forming unit; LOD: Limit of detection; LVS: Live vaccine strain.

tularemia [47]. This study noted the importance of lifting the crust of the ulceroglandular lesion and culturing the ulcer underneath the crust, as 35 out of 36 samples were positive when this procedure was performed on an encrusted lesion [47].

PCR of the affected lymph nodes in cases of glandular or oropharyngeal tularemia may be an effective technique for diagnosis of this infection. In an analysis of two outbreaks of oropharyngeal tularemia in Turkey, PCR of the lymph node aspirates was positive in all seven cases [48]. A similar observation in another outbreak investigation in Turkey was observed in the lymph node aspirate from one patient [49]. An additional advantage of PCR is the possibility of obtaining positive results even after the patient has been receiving antibiotic therapy. In the tularemia outbreaks in Turkey, PCR was positive in the seven lymph node aspirates, even though the patients had been receiving antibiotics for more than 2 weeks [48].

A description of the use of PCR for the diagnosis of oculoglandular tularemia was published recently, noting positive results from conjunctival swab in one patient and a positive PCR result from a conjunctival nodule in another [50]. In these two patients, PCR testing of the blood was also positive. From another two patients, oculoglandular tularemia was diagnosed with positive cultures and PCR results from cervical lymph nodes.

Future diagnostic testing will probably employ multiplex PCR platforms to simultaneously test for multiple possible pathogens using a single sample. This multiplex technology offers the advantage for testing an unknown clinical or environmental sample that may indicate the use of a biologic weapon. Recently published reports have demonstrated the feasibility of this technology [51,52], with further modifications leading to multiplex PCR-coupled liquid-bead array, with a process that is completely automated, utilized primarily for environmental samples [53]. Further testing of clinical samples is still needed.

PCR has disadvantages, including false-positive and false-negative results, the expense of the test and the inability to perform antimicrobial susceptibility testing on the identified organism. False-positive results can occur owing to laboratory contamination. This emphasizes the importance of effective training and quality control procedures in PCR diagnostic laboratories. The specificity of PCR for clinical samples tends to be high (with few false-positive results), with one platform, utilized in Sweden, noting only rare positive results in patients not suspected of having clinical tularemia infection [46,47]. False-negative results can occur in the presence of inhibitors in the clinical sample, such as heme in whole blood samples as mentioned previously. Antimicrobial susceptibility testing can only be performed when live organisms are recovered from culture. Although naturally occurring *F. tularensis* tends to be susceptible to appropriate antibiotics, this testing is useful for surveillance purposes, as well as if the intentional use of this organism as a biologic weapon occurred. Another disadvantage is that the cost of the reagents may prohibit their routine usage in clinical microbiology laboratories, particularly in resource-limited environments. A final issue with PCR is that techniques are not standardized between laboratories [54].

Serologic testing

Serologic testing has been the most consistently utilized method for the diagnosis of tularemia over the past 50 years. The typical diagnostic criteria involve an upper-limit cut-off for a single test (≥1:160 for example) or a fourfold rise in titers between acute and convalescent samples. Convalescent samples are usually collected at least 2 weeks after the acute samples. One large case series describes the use of agglutination reactions (tube agglutination and slide) for the diagnosis of tularemia from 1949–1979 [11]. Microagglutination is a similar technique that has been utilized for the diagnosis of tularemia and is also utilized in monitoring response to LVS tularemia vaccination [55]. ELISA is another test for measuring antibodies to *F. tularensis*. Various antigens can be utilized for the ELISA, including lipopolysaccharide [56] or ether extract antigens [57,58]. Titers can remain detectable for many years after infection; one series reported a detectable titer in 23 out of 52 patients when measured 25 years after infection [30].

The advantages of serology include the relative simplicity of conducting the test and the rarity of false-negative results in patients with symptoms for an extended duration. The disadvantages of serology include the possibility of a negative test in early infection, the frequent need for convalescent samples to confirm the diagnosis and the cross-reactivity with other infections producing false-positive results. However, tularemia serologic testing tends to only rarely have cross-reactive results [59]. In addition, the persistence of antibodies can cause a false-positive result in patients with prior tularemia infection. As mentioned previously, it is also possible that serology never becomes positive if patients self-medicate and/or are prescribed antibiotics.

Other techniques

Other groups have attempted to develop alternative techniques for the rapid detection of *F. tularensis*. One product is a handheld immunochromatographic assay that has a limit of detection of 10^6–10^7 bacteria/ml in human serum [60]. Further research will hopefully yield diagnostic products with both speed and accuracy. More recently, a description of gene expression after tularemia infection was published [61]. If *F. tularensis* causes a unique signature of gene expression after infection, this technique may be useful for the future diagnosis of tularemia cases. However, much further investigation is required before this technique could have practical clinical utility.

Treatment

Antibiotic treatment

The traditional, preferred therapy for all forms of tularemia is streptomycin or tetracycline (or doxycycline), based on the amount of clinical evidence supporting their use. Streptomycin was the first antibiotic utilized for the treatment of tularemia and case series have documented its utility for treating tularemia patients [11]. At concentrations achieved in humans, streptomycin is bactericidal. One review highlighted the effectiveness of streptomycin, noting a 97% cure rate without relapse [62]. Other investigations have noted higher relapse rates. The disadvantages of streptomycin include a side-effect profile of vestibular toxicity and nephrotoxicity, both of which require close monitoring. In addition, it can only be administered through the intravenous or intramuscular routes, which causes inconvenience and risks additional complications. Finally, streptomycin is often difficult to obtain owing to a lack of availability of the medication. As a possible solution to the inability to procure streptomycin, gentamicin has been suggested as a suitable alternative. Observational studies have suggested that gentamicin is an effective option, as it has shown to be as successful as streptomycin [62–64]. Aminoglycosides, administered early in the course of infection, may be associated with better outcomes as described in one case review [65].

Case reports and small case series have evaluated the use of other antibiotics for tularemia. One case series described eight individuals receiving ceftriaxone, with none having a favorable therapeutic response [66]. Another review mentioned that imipenem/cilastatin was successful in treating one case of tularemia [62]. One case report described the successful use of erythromycin to treat tularemia [67]. However, erythromycin is generally considered ineffective for the treatment of tularemia with many strains, especially in Europe, being resistant [59,68]. In some series of *in vitro* susceptibility testing, *F. tularensis* strains are sensitive to rifampin and rifamicin [68]. However, it is unclear if rifampin is useful for treating patients. In one case series, six patients were treated with chloramphenicol and relapse was observed in three patients [11].

Tetracycline was demonstrated to be effective for the treatment of tularemia infections occurring by inhalation in a human challenge model [39]. In this model, antibiotics were initiated at the onset of fever. Tetracycline administered twice daily for 15 days was associated with no relapsed infection in 20 volunteers. Murine studies support the importance of an adequate duration of therapy. When doxycycline or ciprofloxacin were administered as single agents of therapy, 10-day duration was required to prevent relapsed infection [69]. In a case series of 42 personnel with laboratory-acquired tularemia infection, 32 patients were successfully treated with tetracycline or related compounds and relapsed infection was noted in only five patients [70]. Relapsed infection occurred only among the 15 patients who received treatment within the first week of symptoms. Out of 16 patients treated after the first week of infection, none had a relapse of their clinical infection.

The fluoroquinolone class of antibiotics has the potential for being effective first-line therapy for tularemia. Fluoroquinolones have the following advantages: bactericidal effects, the ability to be orally administered and excellent *in vitro* activity against *F. tularensis* [71,72]. Fluoroquinolones are classified as acceptable alternatives in reviews discussing options for the treatment of tularemia used as a biologic weapon [2]. However, none of the fluoroquinolones have a US FDA-labeled indication for the treatment of tularemia.

Studies in mice have verified the successful use of fluoroquinolones in treating experimental tularemia, [69] including newer fluoroquinolones such as gatifloxacin and moxifloxacin [73,74]. One retrospective review described the successful treatment of 41 out of 43 patients with ciprofloxacin during a tularemia outbreak in Sweden [75]. Another case series documented relapse in seven out of 14 patients treated with ciprofloxacin, but the onset of treatment in comparison with the onset of symptoms was not mentioned [76]. One particular study from Spain noted a high percentage of therapeutic failure (22.5%) in patients treated for tularemia, with failure rates much lower for ciprofloxacin (one out of 22, or 4.5%) than for streptomycin (22 out of 94, or 23.4%) or for doxycycline (six out of 14, or 42.8%) or for other antibiotics (three out of 6, or 50%) [77]. However, this study was not randomized and, therefore, selection bias may have caused more severe cases to be treated with streptomycin. Ciprofloxacin has also been utilized to treat tularemia in children. In a case series of 12 children, ciprofloxacin was effective, except when it was discontinued before completing a 10-day course due to the development of a rash [78]. Case reports also describe the successful use of levofloxacin for the treatment of tularemia [79,80]. It is important to note that most of the clinical reports supporting the use of fluoroquinolones for the treatment of tularemia infection described patients infected with the Type B (*F. tularensis* subspecies *holarctica*). As mentioned previously, this subspecies tends to be less virulent; therefore, proof of fluoroquinolone effectiveness needs to be established for Type A infection [59].

The duration between the initial development of symptoms and commencement of appropriate antibiotic therapy has a substantial impact on outcome. In two outbreaks of oropharyngeal tularemia in Turkey, investigators observed a large number of patients who developed symptoms many weeks before they received correct treatment [48]. In this study, treatment failure was defined as a lack of resolution of lymphadenopathy, including suppuration or surgical excision of the affected nodes. Of eight patients who received appropriate antibiotics within 3 weeks of the onset of symptoms, six recovered completely (75%). Of the remaining 41 patients, only 11 had complete recovery (26.8%) with the rest being defined as treatment failures. In this study, patients received either doxycycline, a fluoroquinolone or streptomycin, or a combination of these antibiotics, but the antibiotic treatment itself was not associated with treatment success or failure. Another chart review noted worse outcome in tularemia patients if their time to treatment after development of symptoms was delayed [65]. The study highlighted the importance of effective diagnosis and expeditious initiation of therapy.

The available publications on the treatment of tularemia are based on anecdotal reports and observational studies. There are no available randomized, controlled trials comparing the two therapeutic options. In addition, some of the observational studies commented on the treatment of different forms of tularemia, with a wide range of symptoms, such as lymphadenopathy, and varying durations of symptoms prior to presentation. As a consequence, it is difficult to accurately assess the optimal treatment options; rather, we can conclude that aminoglycosides, tetracycline compounds and fluoroquinolones are observed to be effective for tularemia when administered for an adequate duration of time; however, randomized, controlled trials are needed to determine the optimal therapeutic option and regimen.

Postexposure prophylaxis

The issue of postexposure prophylaxis applies to both the use of tularemia as a biologic weapon, as well as a food- or waterborne outbreak of the disease. A high-risk laboratory exposure to *F. tularensis* should also be considered for postexposure prophylaxis [2]. In a scenario in which postexposure prophylaxis is needed after a *F. tularensis* biologic weapon attack, doxycycline or ciprofloxacin is recommended for 14 days duration [2].

Tetracycline has been evaluated in postexposure prophylaxis in a macaque model. In these 11 animals, tetracycline was administered either 24 or 60 h after respiratory exposure [39]. Two of the animals became ill during the course of postexposure prophylaxis, while all 11 animals relapsed after treatment was concluded. It is possible that the tetracycline dose was inadequate (200 mg intragastric, daily, for 13 days). In a human challenge model with the same investigators, tetracycline was administered starting 24 h after an inhalation of 25,000 organisms of virulent *F. tularensis* [39]. In eight patients taking every-other-day dosing, all eight either developed infection on therapy or relapsed after the completion of therapy. None of the patients receiving tetracycline 1 g twice daily for 14 days or 1 g daily for 28 days had evidence of symptoms while taking antibiotics, or relapsed infection after completion of the course of therapy. Streptomycin was utilized effectively in cases of relapsed infection after tetracycline therapy.

Antibodies/passive immune serum

As mentioned previously, transfer of serum from mice immunized against *F. tularensis* protected mice challenged in a LVS respiratory infection model [35]. Normal sera or immunoglobulin-deplete sera were not protective. An additional observation from the study was that immune sera can be administered as late as 2 days after exposure and is still benefitial. These data may suggest a role for immune sera for both therapy and postexposure prophylaxis but these results need validation in other tularemia infection models.

Expert commentary

The optimal diagnostic approach to tularemia combines the three methodologies: culture, PCR and serologic testing. Each test has a particular unique utility: culture allows for antimicrobial susceptibility testing, PCR is useful because *F. tularensis* is difficult to culture, particularly with patients receiving antibiotic therapy, and serologic testing is useful in situations of prolonged

infection when both culture and PCR are negative. PCR appears to be a very promising technique for the diagnosis of tularemia, particularly the ulceroglandular form. However, larger clinical studies are needed in order to define the performance characteristics of this diagnostic test, particularly in a variety of clinical samples such as blood, lymph node aspirates or sputum.

Antibiotics, such as streptomycin and tetracycline-related compounds, have been successfully utilized for decades for the treatment of tularemia. However, the lack of an oral formulation with streptomycin and the associated toxicity are disadvantages to using this antibiotic. Tetracycline and doxycycline are associated with some relapsed infection after therapy is completed. Fluoroquinolones offer an alternative bactericidal therapy with oral administration. However, there are no randomized trials supporting the use of this antibiotic compared with either an aminoglycoside or tetracycline-related antibiotic. There is minimal evidence available to support the use of other antibiotics for tularemia therapy.

Five-year view

We anticipate that PCR will be become increasingly available and affordable so that numerous microbiology diagnostic laboratories worldwide will apply this technology to tularemia. As a consequence of rapid diagnosis, more cases of tularemia will be recognized and a better understanding of the epidemiology of this infection will become apparent. In addition, publications clarifying the optimal diagnostic techniques with tularemia will be distributed so that the performance characteristics of PCR will be available to healthcare practitioners.

Fluoroquinolones will be increasingly applied to the treatment of tularemia, while aminoglycosides, such as gentamicin, will be utilized instead of streptomycin for more severe infections. A substantial improvement in the understanding of the pathogenesis of tularemia will occur and some of these discoveries may lead to potential therapeutic products. However, we do not suspect that any novel therapy, such as immunomodulatory treatment, will be developed and licensed for tularemia in the next 5 years.

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Key issues

- Three modalities are currently available for the diagnosis of tularemia: serologic testing, culture and PCR. Each method has advantages and disadvantages; optimal diagnostic capability utilizes all three modalities.
- Advances in PCR platforms have allowed for the application of this technology to the diagnosis of clinical infection. In particular, PCR may be useful in cases in which antibiotic therapy has already been initiated. Further clinical assessment studies are needed to accurately define performance characteristics of PCR for the various manifestations of tularemia.
- Streptomycin has been the traditional antibiotic for the treatment of tularemia, with demonstrated effectiveness. As an oral antibiotic alternative choice, tetracycline and doxycycline have been widely utilized. An appropriate duration of therapy and post-treatment monitoring is needed for tetracycline or doxycycline, as the occurrence of relapsed infections is well described.
- Fluoroquinolones are promising alternatives for the treatment of tularemia owing to their *in vitro* activity against the pathogen and oral administration. However, further studies are needed to demonstrate the clinical utility of this class of antimicrobials for tularemia.

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