

FRANCISELLA TULARENSIS: A ZOONOTIC PATHOGEN AMONG WILD RODENTS AND ARTHROPODS - A POSSIBLE THREAT IN FUTURE

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SUMMARY: *Francisella tularensis* is a Gram-negative coccobacillus and an aerobic bacterium. It causes a zoonotic disease called tularemia in humans. Four subspecies have been found in *F. tularensis* as *F. tularensis* subsp. *Tularensis* (Type A strains), *F. tularensis* subsp. *Holarctica* (Type B strains), *F. tularensis* subsp. *mediasiatica*, and *F. tularensis* subsp. *Novicida*. Rearing rabbits and different kinds of rodents as pets are becoming popular in Sri Lanka, veterinarians need to be knowledgeable on emerging pathogens such as *F. tularensis*, to diagnose the disease within a short time. Therefore, the objective of this paper is to update veterinarians on possible emerging infections to improve the health of pets and to minimize possible zoonotic infections. The clinical outcome caused by *Francisella* is a debilitating febrile disease in humans. *Francisella* has been isolated from hundreds of animal species in the world. Being a diverse host range, associated ecological factors relating transmission of *Francisella* in the environment is largely unknown. *F. tularensis* type A was reported to be common in North America while occasionally found in Europe. Type B was found commonly in the Northern hemisphere and in Australia. Tularemia is a sporadic disease, and a small infectious dose is required for an infection in humans. The clinical signs and symptoms of tularemia depend on the route of infection. Six types of clinical forms were identified as ulceroglandular, glandular, oropharyngeal, oculoglandular, pneumonic and typhoidal in humans. Diagnosis of tularemia in humans is based on epidemiology, clinical findings and laboratory confirmation. Microagglutination test, indirect immunofluorescence assay (IFA) and Enzyme-linked immunosorbent assay ELISA are widely used as diagnostic tests. Several conventional and qPCR have been optimized to detect the organism in clinical samples. Antimicrobials such as aminoglycosides, tetracycline, quinolones, and chloramphenicol were used to minimize clinical complications. Utilization of treated water, usage of gloves on handling wild rabbits and rodents, thorough cooking of bush meat, usage of insect repellents, protection of stored food from rodents, wearing masks, ticks-free clothes, keeping away from weeds, cleaning pets from external parasites have been identified as the main preventive strategies against tularemia in human. No commercial vaccine is found in the market yet against *F. tularensis*. This can be an emerging and threatening disease in the future with ongoing changes in arthropod parasites in the ecosystem followed by climatic changes in the world.

KEYWORDS: Tularemia, *Francisella tularensis*, human, rodents

INTRODUCTION

Francisella tularensis is a Gram-negative coccobacillus, an aerobic bacterium and grown well at 35-37°C (Ramakrishnan, 2017; Freudenberger Catanzaro and Inzana, 2020). It is a non-spore forming, non-motile organism, encapsulated and a facultative intracellular microorganism (Freudenberger Catanzaro and Inzana, 2020). The bacterium is a fastidious organism and cysteine is required for the optimum growth under 5% CO₂ in laboratory conditions (Caspar and Maurin, 2017; Telford and Goethert, 2020). *Francisella* requires supplementation of sulfhydryl compounds and cysteine enriched media for the optimum growth in the laboratory (Kinkead and Allen, 2016). A gray

colony in 4 mm diameter green coloured medium is the unique feature of glucose cysteine blood agar (Kinkead and Allen, 2016). However, different strains may have different colony morphology in the same medium (Kinkead and Allen, 2016). The recommended incubation temperature is 35°C and colonies appear after 2-4 days of incubation (Kinkead and Allen, 2016).

In classification, four subspecies have been found in *F. tularensis* as *F. tularensis* subsp. *Tularensis* (Type A strains), *F. tularensis* subsp. *Holarctica* (Type B strains), *F. tularensis* subsp. *mediasiatica*, and *F. tularensis* subsp. *Novicida*. Both type A and type B have been reported in human with zoonotic

infection (Zellner and Huntley, 2019). The type A can be further divided into three sub types as A1a, A1b and A2 and A1b is responsible for more serious infection in human (Zellner and Huntley, 2019). *F. tularensis* causes a zoonotic disease called tularemia, also called as “rabbit fever”, “Pahvant Valley plague”, “deer fly fever”, and “Ohara’s fever” (Santic and Abu Kwaik, 2013; Faber *et al.*, 2018; Hennebique *et al.*, 2019; Yeni *et al.*, 2021). In addition, “epidemic lymphadenitis,” “Plague-like lymphadenitis,” and “Influenza-like disease of water hole hunters” were synonyms for the disease (Faber *et al.*, 2018).

It is a highly infectious agent that spreads through aerosol, requires a low infectious dose and results in a high degree of virulence in human (Hennebique *et al.*, 2019). Therefore, It is categorized as “A” potential biological agent by Centre for Disease Control and Prevention (CDC), USA (Hennebique *et al.*, 2019). However, *F. tularensis* subsp. *Tularensis* causes severe pulmonary infection, specially in people who go for hunting frequently in North America (Hennebique *et al.*, 2019). In addition, *F. philomiragia* is a closely related species of bacteria which causes disease in immune compromised patients occasionally (Celli and Zahrt, 2013). As rearing of rabbits and rodents as pets has become popular and emerging trend in Sri Lanka, veterinarians need to be aware of emerging potential pathogen such as *F. tularensis*, especially in imported rodents. Therefore, the objective of this review was to update the knowledge of local veterinarians on possible emerging infections in rodent pets in order to diagnose them accurately and minimise potential zoonotic transmission to the owners.

EPIDEMIOLOGY

Tularemia is a debilitating febrile disease in human (Celli and Zahrt, 2013). *F. tularensis* is first reported in Tulare county of California in 1911. This disease is common among hunters of rabbits and hares (Yeni *et al.*, 2021). *F. tularensis* type A has been reported to be common in North America while occasionally found in Europe (Zellner and Huntley, 2019). Type B was found to be common in Northern hemisphere and in Australia. Type A was reported to cause severe clinical diseases in humans than type B (Gunnell *et al.*, 2016). *F. tularensis* sub species *mediastica* has caused high degree of virulence in experimental mice models while it was shown to be causing less virulence in humans (Gunnell *et al.*, 2016). Only the high virulent strains were capable

of fermenting glycerol and citrulline in Europe and North America (Pilo, 2018). However, the low virulent isolates in Asia also have shown similar characteristics of fermenting glycerol and citrulline (Pilo, 2018). Therefore, fermentation technique is not a good test to evaluate the degree of virulence of the organism in the laboratory. In addition, *F. tularensis* sub species *novicida* has shown high virulence in mice but it rarely causes disease in human (Gunnell *et al.*, 2016).

As mentioned previously, Type A infection of *F. tularensis* was found in North America. Type B of *F. tularensis* has been reported in Eurasia, North America, Scandinavia, Russia and Japan (Gunnell *et al.*, 2016; Yeni *et al.*, 2021). The reported prevalence of the disease is widely varied with different factors such as environment, social habits, reservoirs, vectors, pathogen and host- associated factors. Tularemia is an endemic disease in France with compulsory notification to the health authorities, over 99 human cases were reported in the period of 2006-2010 (Gaci *et al.*, 2017). The mosquito-based infection had been reported in Sweden and Finland in Scandinavians region and both *Aedes* and *Culex* mosquitoes were identified as vectors (Abdellahoum *et al.*, 2020). Over 50% of total clinical cases in Sweden and Finland were reported in the month of August which is the peak season of mosquitoes in the region. Most patients (74%) were shown ulceroglandular form of tularemia in the region (Abdellahoum *et al.*, 2020). Furthermore, the lesions were limited to lower limb with inguinal lymphadenopathy, while lesions in arms, face, or neck were found with axillary or cervical lymphadenopathy (Abdellahoum *et al.*, 2020). These findings implied that the lesions had been mainly found in exposed part of the body in humans where mosquitoes have ready access. However, the role of mosquitoes on transmission of *F. tularensis* was reported minimum in other parts of the Europe (Abdellahoum *et al.*, 2020). Importantly, the disease has not been reported in some part of the Europe such as Greece, Iceland, Ireland, Luxembourg, Malta, and the United Kingdom other than in few people visited endemic countries (Seiwald *et al.*, 2020). The organism has been isolated among ticks in Japan and the ticks may thought to have a significant role in dissemination of the disease in Japan (Suzuki *et al.*, 2016). In addition, serological evidence has been observed in ranches in Iran although no sign of infection was reported (Ahangari Cohan *et al.*, 2021). Livestock farming communities who rear small ruminants had high sero prevalence of *F. tularensis* in Jordan

hence, livestock farmers have been described as risk category for the diseases (Obaidat *et al.*, 2020). The same study highlighted that the age, region of residence and practising horticulture as risk factors for acquiring serological evidence for tularensis in humans (Obaidat *et al.*, 2020). In addition, tularensis has been reported in tropical countries including India although not a single case has been reported in Sri Lanka (Nirkhiwale *et al.*, 2015). Furthermore, *F. tularensis* has been isolated in bed bugs in Madagascar although the role of bed bugs on transmission of diseases is not well known (Peta *et al.*, 2022).

Francisella has been isolated from hundreds of animal species in the world (Pilo, 2018). Therefore, veterinarians must always be on alert of tularemia as an emerging infection in livestock, pets, especially the exotic pets and wild animals.

The disease has also been reported in, birds, fish, amphibians, arthropods, and protozoa other than in mammals (Seiwald *et al.*, 2020; Yeni *et al.*, 2021). Although both mice and guinea pigs were shown to be susceptible to *F. tularensis* in experimental conditions, the natural infection has not been investigated (Santic and Abu Kwaik, 2013; Kingry and Petersen, 2014). Furthermore, sudden death was noticed in rodents with severe degree of infection (Appelt *et al.*, 2020; Seiwald *et al.*, 2020). Interestingly the white rats appeared to be less susceptible to the disease in experimental conditions (Kingry and Petersen, 2014). The presence of the organism is not an indicator of a disease in wild animals and domestic animals, while some strain or lineage were limited to unique mammalian host (Telford and Goethert, 2020). Therefore, diagnosis and interpretation of the clinical disease is a challenging task in veterinary medicine.

Two cycles of *Francisella* have been identified in North America as a terrestrial or sylvatic cycle and an aquatic cycle. In addition, lagomorphs and ticks play vital role in sylvatic cycle (*F. tularensis*). In contrast, Semi aquatic rodents involve in aquatic cycle (American beaver, *Castor canadensis* and the muskrat *Ondatra zibethicus*) involving *F. holarctica* (Pilo, 2018). Conversely, several rodents, ticks, and mosquitoes play a vital role in Eurasia in the life cycle of *F. hallartica* (Telford and Goethert, 2020). Rabbits (*Sylvilagus* spp.) and hares (*Lepus* spp.) were considered as carriers/reservoirs of *F. tularensis* subsp. *Tularensis* (Yeni *et al.*, 2021). *F. tularensis* has been identified with multiple reservoirs including lagomorph and small rodents,

whereas Ixodidae ticks are the main vector for the bacterium (Hennebique *et al.*, 2019). In addition, mosquitoes and deer flies are considered as vectors for *F. tularensis* in this region (Zellner and Huntley, 2019). Furthermore, waterborne infection of *F. tularensis* has also been reported (Hennebique *et al.*, 2019). The pathogenic bacterium could survive in water and environment for several months (Caspar and Maurin, 2017). Furthermore, the organism had multiplied in a protozoan, amoeba in water (Caspar and Maurin, 2017). Humans get the infection through multiple routes directly or indirectly through infected animals, carcasses, ticks, mosquitoes, contaminated water, soil and food (Appelt *et al.*, 2020). Majority of humans infections were associated with direct or indirect contact with infected animals (Seiwald *et al.*, 2020). Lung infection was mostly associated with farming activities (Seiwald *et al.*, 2020). The periprosthetic joint infections have been reported in many occasions (Steiner *et al.*, 2014; Chrdle *et al.*, 2019).

Basically, three main types of animals have been categorised by World Health Organization (WHO) based on the high diversity of host range of *F. tularensis*; and identification of incidental or reservoir host are also challenging (Pilo, 2018). The classification is based on susceptibility and sensitivity or severity of the infection such as acute disease after 1-10 organism inoculation with rapid mortification within blood and tissue (Pilo, 2018). The second class or category is reported cause fatalities after inoculation of 10^8 - 10^9 organism and animal may survive with low dose of infection with development of immunity (Pilo, 2018; WHO, 2007). The class three host are anyway resistant to the infection (Pilo, 2018). The disadvantage of this classification is that the hosts are classified only based on challenge experiments though blood or lymphatic routes (Pilo, 2018). However, other natural routes of infection and accidental host or reservoirs host have not been evaluated. Furthermore, tularaemia has been reported in companion animals such as dogs and cats in North America (Pilo, 2018). According to the recent finding, 50% of human tularemia infection were cat associated while only 3% were associated with canine infection in humans in USA (Seiwald *et al.*, 2020).

Tularaemia is also a disease in wild and captured animals and identification of the organism in animals and humans have been reported simultaneously (Faber *et al.*, 2018). Neurological and respiratory signs are found common in infected animals. Meningoencephalitis, pneumonia and

myocarditis were observed with unusual mortalities in fox squirrels in USA (Vincent *et al.*, 2020). The seropositive cases had been encountered in wild animals as hares, foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes procyonoides*), wild boar (*Sus scrofa*), bank voles (*Myodes glareolus*), water voles (*Arvicola terrestris*), field voles (*Microtus agrestis*), common voles (*Microtus arvalis*), yellow-necked field mice (*Apodemus flavicollis*) and zoo animals in Europe previously (Faber *et al.*, 2018). The feline tularemia has only been reported in North America and canine tularemia has been observed in Europe (Pilo, 2018). In addition, tularemia has also been noticed in sheep as livestock spices (Pilo, 2018).

The first genome of *F. tularensis* had been sequenced and published in 2005, genetic similarity between A and B types were observed as 97.63% (Gunnell *et al.*, 2016). The size of genome is round 1.7 to 2.0 Mb and 16S rDNA gene does not have sufficient discriminative power to differentiate each sub-species (Gunnell *et al.*, 2016). Some of the tools cannot be applied in molecular epidemiology with *F. tularensis*, especially in outbreak investigation. Repetitive extragenic palindromic element PCR (REP-PCR), enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), random amplified polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE), and restriction fragment length polymorphism (RFLP) assays were with limited success (Pilo, 2018). However, the multiple loci variable number tandem repeats (VNTR) markers have been devolved and proven success in molecular epidemiology. Geographically specific clades have been identified with VNTR makers in Europe and North America (Pilo, 2018). In contrast, the lack of standard databases is the limiting factor in VNTR analysis. However, VNTR markers may not work in phylogenetic analysis, while canonical single nucleotide polymorphism (canSNP) and canonical insertions/deletions (INDELs) have shown success in such analysis (Pilo, 2018).

Pathogenesis, immune responses and virulence mechanism

The average incubation period of the infection caused by Francisella was as low as 3-5 days in humans, and maximum time reported was two weeks (Abdellahoum *et al.*, 2020). Humans show nonspecific signs such as flu-like symptoms including fever, lymphadenopathy, headache, chills, myalgia, and arthralgia (Seiwald *et al.*, 2020). Tularemia is a sporadic disease with a low infectious dose required on pathogenesis (Celli and Zahrt,

2013). The severity of infection depends on portal of entry, infectious dose, and subspecies (biovar) of the infecting strain (Celli and Zahrt, 2013). Six clinical forms were identified as ulceroglandular, glandular, oropharyngeal, oculoglandular, pneumonic and typhoidal form (Celli and Zahrt, 2013). The first two forms (ulceroglandular, glandular) were mostly associated with skin inoculation of bacteria either by arthropod bites or contact with the skin (hair) which are the main routes of infection through infected animals (Caspar and Maurin, 2017; Yeni *et al.*, 2021). The contaminated hand may touch conjunctiva of the eye and organism enter the body through conjunctival route (Yeni *et al.*, 2021). The contaminated food and water are also suggestive sources of infection in animal and human through which a number of outbreaks have been reported (Yeni *et al.*, 2021). Inhalation of infected aerosol is considered another important method of infection especially contaminated dust which had caused clinical disease in human and animals (Yeni *et al.*, 2021). Furthermore, untreated cases can result up to 60% mortality in tularemia while infectious dose was as low as 10^3 cfu in *F. tularensis* subsp. Holarctica (Type B strains) (Celli and Zahrt, 2013). However, it can be as far low as 10 cfu in *F. tularensis* subsp. Tularensis (Type A strains) (Celli and Zahrt, 2013). Conversely, *F. tularensis* is named as potential weaponized bacterium or type A agent by CDC due to the characteristic of severe form of clinical disease, low infectious dose and having a life-threatening outcome (Celli and Zahrt, 2013). However, human to human transmission has not been reported (Caspar and Maurin, 2017).

Innate immune responses against *F. tularensis* have not been fully understood (Kubelkova and Macela, 2019). The innate immunity is the first line defense mechanism against an infection, *F. tularensis* represses the activation of inflammasome at the cellular level to bypass innate immune responses (Krocova *et al.*, 2017). In addition, Francisella had enhanced protein secretion in the infected cells (Krocova *et al.*, 2017). The pathogen had invaded different mammalian cell types such as macrophages, dendritic cells, polymorphonuclear neutrophils, hepatocytes, endothelial, and type II alveolar lung epithelial cells (Celli and Zahrt, 2013; Krocova *et al.*, 2017). The serum opsonisation was important in the process of uptaking of the organism into the phagocytic cells (Celli and Zahrt, 2013). Furthermore, the scavenger receptor A, Fcγ receptors, neucliolin, lung surfactant protein A also play a vital role on uptaking serum opsonised Francisella into the macrophages (Celli and Zahrt,

2013). *Francisella* may interfere with the host metabolism including glycosylation pathway of human macrophages (Barel and Charbit, 2017; Ziveri *et al.*, 2017). In addition, the pathogen utilises host cells substrates as nutritional requirements of the organism (Ziveri *et al.*, 2017). The bacteria survive and reside in early phagosome and interact with early and late endocytic compartment except lysosome (Celli and Zahrt, 2013). Bacteria disrupt early phagosome cell membrane and rapid replication occurs in cytosol followed by cell death, bacterial release, and subsequent infection. In addition, *Francisella* inhibits the NADPH oxidase activity, limiting activation of polymorphonuclear cells and the pathogen halts oxidative burst by reactive oxygen in macrophages (Celli and Zahrt, 2013).

O antigen found in LPS has been identified as main virulence factors in *Francisella* species (Nicol *et al.*, 2021). Simultaneously, O antigen has shown negative activity of IgM and complement mediated mechanism within the host (Rowe and Huntley, 2015). The capsule like complex/CLC protein and high molecular weight carbohydrate have shown to induce pathogenesis (Nicol *et al.*, 2021). In addition, ability to LPS alteration and presence of pili in the cells are considered as protection mechanism against host immune mechanism (Rowe and Huntley, 2015). Pathogenicity Island consists of 17 open reading frames which is believed to be essential for the pathogenesis (Steiner *et al.*, 2014). The reactive oxygen species (ROS) and nitrogen species is also an essential component of *Francisella* virulence mechanism, enzyme KatG deactivates these two-protective mechanisms of the host cell (Steiner *et al.*, 2014). *SodB* and *SodC* which encoded for superoxide dismutase are also required for the resistance against superoxide radicals (Steiner *et al.*, 2014). *Francisella* enter into the macrophages with a specific mechanism called “loosing phagocytosis” which consists of a large volume of space around the bacterium, surface receptors playing a vital role in phagocytosis such as mannose receptors, Fc receptors and complement receptors (Steiner *et al.*, 2014). In addition, Iron is also an essential element for survival of *Francisella* in a host cell (Steiner *et al.*, 2014). Importantly, bacterial sepsis and inflammation cause death than pneumonic condition in infected human with *F. tularensis* (Steiner *et al.*, 2014). In addition, high levels of inflammatory cytokines and chemokines including IL-6, macrophages inflammatory protein, chemokine ligand 2 released in lung and spleen lead to the death (Steiner *et al.*, 2014). Excessive

neutrophil recruitment is also an important factor in pathogenesis (Steiner *et al.*, 2014).

The clinical infection of *F. tularensis* had been reported in dogs and lethargy, pyrexia, anorexia, lymphadenopathies were found as the common clinical signs (Kwit *et al.*, 2020). The prognosis was shown good in hospitalised canine hosts (Kwit *et al.*, 2020). Some of the dogs had fever and lethargy 2 to 4 days after a hunting session and had recovered automatically (Kwit *et al.*, 2020). In addition, the disease had been reported in cats, *F. tularensis* had been isolated in a number of feline post-mortem carcasses in Europe (Stidham *et al.*, 2018). Domestic cats also play a vital role in spreading *Francisella* infection in humans in Europe (Frischknecht *et al.*, 2019).

Diagnosis and laboratory tests

Diagnosis of tularemia in humans is based on clinical findings, epidemiology and serological testing (Kinkead and Allen, 2016). The fever with lymphadenopathy and proven contact with animals can be suspected as tularemia in humans (Kinkead and Allen, 2016). Q fever, Plague, Psittacosis has been noted in the top of the differential diagnosis (Kinkead and Allen, 2016). Micro agglutination test (MAT), indirect immunofluorescence assay (IFA) and ELISA are being used widely in the field as serological tests to detect tularaemia in humans (Kinkead and Allen, 2016; Kubelkova and Macela, 2019). However, cross reactivity has been observed common with a number of bacterial organisms such as *Salmonella*, *Brucella*, *Legionella* and *Yersinia* species (Kinkead and Allen, 2016). As a rule of thumb, four-fold rising of antibody titers against *Francisella* within a period of 2-4 weeks period was identified as suspected tularaemia infection in human (Kinkead and Allen, 2016). MAT and IFA were proven the ability to test specific antibodies at 2-3 weeks of post infection against *F. tularensis*. In addition, tularaemia can be diagnosed at a stage as early as 2 weeks of post infection by ELISA (Maurin, 2020). However, high percentages of false positive were also observed in ELISA (Maurin, 2020). The cross reactivity with other bacterial pathogen and long-term persistence of natural antibodies from previous infections resulted in a negative impact on serological tests of acute infections in humans (Maurin, 2020). Furthermore, Immunochromatography and immunoblot test have also being used in research laboratories with variable success (Maurin, 2020).

The conventional isolation and identification of *Francisella* is not practiced since it can be done only in special laboratory facility such as Bio Safety Level III (BSL-III) (Kinkead and Allen, 2016). Therefore, Conventional methods cannot be performed in local context in Sri Lanka since such BSL-III facility is not found in many laboratories except one or two in the country. A number of conventional and qPCR protocols have been optimized to detect the organism in clinical submission including multiplex PCR assays (Gunnell *et al.*, 2016). In addition, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MOLDI TOF MS) is used for rapid identification of the bacterial organism with high level of accuracy (Kinkead and Allen, 2016).

Treatment

The efficacy of antimicrobial treatment in human infections depends on time of initiation of therapy; it had greatly been reduced in the clinical cases in which antimicrobial therapy had been started 24-48 hours of post infection (Caspar and Maurin, 2017). Antimicrobials are used for a comparatively long time as 10-21 days to minimize the clinical complications. According to literature, Aminoglycosides, tetracycline, quinolones, and chloramphenicol had been used in the past (Kinkead and Allen, 2016). Antimicrobial susceptibility testing (AST) is challenging since BSL-III facilities is required and thus, automated AST facilities are recommended. In addition, beta lactams, rifampicin and linezolid have also been used (Caspar and Maurin, 2017). Conversely, antimicrobial treatment is based on a number of other factors such as physiological condition of the patient, degree of dehydration, presence of metabolic or immune-compromised diseases well.

According to Caspar and Maurin, recovery rate of human tularaemia was 60-100% and it depends on the type of antimicrobial, time of commencement of the treatment, duration of treatment, and presence of other clinical complications (Caspar and Maurin, 2017). Information regarding antimicrobial resistance was scarcely found in *Francisella* due to the limited studies. However, the resistance had been reported for penicillin, cephalosporins, carbapenems, macrolides, and clindamycin (Kinkead and Allen, 2016). In contrast, moxifloxacin had been proven successful against the cases of delayed diagnosis in humans (Caspar and Maurin, 2017). A combination of aminoglycoside with tetracycline, quinolone and chloramphenicol

are used for meningitis and endocarditis while pediatric cases were often treated with gentamicin (Kinkead and Allen, 2016). Ciprofloxacin and doxycycline are widely used in mild to moderate cases of tularaemia in adults while azithromycin is used widely in pregnant women.

Prevention and control

A number of guidelines have been developed by different authorities including WHO to prevent the infection in humans. Utilization of treated water for daily activities, usage of gloves when handling wild rabbits and rodents, thorough cooking of bush meat, using of insect repellent when traveling outside, protection of stored food from rodents and wearing masks have been recommended to prevent tularemia in humans. In addition, protection from ticks, minimum contact with weeds (when traveling in natural trails), controlling external parasites in pets are considered as strategies to prevent *Francisella* infection in the endemic regions (Kinkead and Allen, 2016).

Vaccination is practised as an alternative method to control the infection in endemic regions. However, there is no commercial vaccine found in the market. Killed, live attenuated and subunit vaccine have been developed in different countries (Kinkead and Allen, 2016) and live attenuated vaccine had been tried in Russia with variable success (Celli and Zahrt, 2013; Kinkead and Allen, 2016). A number of studies are underway to determine the efficacy and potency of *Francisella* vaccine around the world (Elkins *et al.*, 2016; Mulligan *et al.*, 2017). Importantly, live attenuated vaccine has been developed with significant reduction of clinical incidence in the experimental models (Mulligan *et al.*, 2017). Being an intracellular parasite, studies are targeting on T cell responses in experimental models (Elkins *et al.*, 2016). A number of animal models have been suggested and the efficacy of live vaccine has been evaluated including rats, rabbits, mice and nonhuman primates (Roberts *et al.*, 2018). Although extensive studies are required for a solid conclusion, variable degree of immune responses have been resulted from different routes of infection in human (Nicol *et al.*, 2021).

CONCLUSION

F. tularensis is considered as a potential risk to humans and animals in the future due to the trend of rearing rodents as a habit in the country. In addition, the varied host range, low infective dose and

severity of the infection may aggravate the risk together with emerging antimicrobial resistance. On the other hand, existence of a wide gap between the information on pathogenesis of *Francisella* species and virulence mechanism in humans and animal is a limiting factor which warrants further research.

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