Journal of Veterinary Science & Medical Diagnosis

Review Article A SCITECHNOL JOURNAL

Francisella Tularensis, A Zoonotic Risk from Wild Rodents and Arthropods, Possible Threat in Future with Continuing Climatic Changes

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Received date: October 21, 2021; **Accepted date:** November 05, 2021; **Published date:** November 12, 2021

Abstract

Francisella tularensis is a Gram-negative coccobacillus and an aerobic bacterium. It causes a zoonotic disease called tularemia in human. Four subspecies have been found in Francisella tularensis as Francisella tularensis subsp. Tularensis (Type A strains), Francisella tularensis subsp. Holarctica (Type B strains), Francisella tularensis subsp. mediasiatica, and Francisella tularensis subsp. Novicida. The disease is called as tularemia which is a debilitating febrile disease in human. Francisella has been isolated from hundreds of animal species in the world. As a results of diverse host range observed, ecological factors relating transmission of Francisella in the environment is largely unclear. Francisella tularensis type A has been reported common in North America while occasionally found in some countries of Europe. Type B has been found common in Northern hemisphere and found in Australia as well. Type A has been reported severe clinical diseases than type B in human.

Tularemia is a sporadic disease with small infectious dose required. The symptom of tularaemia depends on route of infection, altogether six main clinical forms were identified as ulceroglandular, glandular, oropharyngeal, oculoglandular, pneumonic and typhoidal form in human. Diagnosis of tularemia in human is based on clinical finding, epidemiology, and serological testing. Micro agglutination test, Indirect Immunofluorescence Assay (IFA), and ELISA is used widely used as diagnostic test on tularemia. Several conventional, multiplex PCR assays and qPCR has been optimized to diagnose the organism in clinical submission. Antimicrobials are used widely to minimize the complication of the infection and aminoglycosides, tetracyclines, quinolones, and chloramphenicol with high relapse rates of 10-21 days.

Utilization of treated water for daily activities, usage of gloves when handling wild rabbits and rodents, thoroughly cooking of bush meat, using of repellent on insect specially traveling outside, protection of food at storage from rodents, wearing mask, checking the cloths for ticks, avoid touching of weed when traveling in natural trails, cleaned pets without ticks and other external parasites, vaccination of veterinarian and other staff who contacts animals and livestock are alternative preventing strategies against tularaemia in human. Vaccination of live attenuated, killed or subunit vaccines are an alternative method to control the infection in endemic regions with variable success. No commercial vaccine is found in the market. Francisella tularensis can be emerging and threatening disease in future with ongoing changes in arthropod parasites in the ecosystem followed by climatic changes in the world.

Keywords: Overview; Francisella tularemia; Human; Rodents

Introduction

Francisella tularensis is a Gram-negative coccobacillus and an aerobic bacterium and grown best at 35-37°C. It is a non-spore forming, nonmotile organism, encapsulated and a facultative intracellular parasite. The organism is a fastidious and cysteine is required for the growth under 5% CO_2 in laboratory conditions. Francisella tularensis causes a zoonotic disease called tularemia in human, also called as “rabbit fever”, “Pahvant Valley plague”, “deer fly fever”, and “Ohara’s fever”. In addition, “epidemic lymphadenitis,” “Plague-like lymphadenitis,” and “Influenza-like disease of water hole hunters” also were used synonym for the disease in the literature. It is a highly infectious agent as organism is spread through aerosol, low infectious dose and high degree of virulence in human. it is considered as category A potential biological agent by CDC, USA. In classification, four subspecies have been found in Francisella 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. 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. However, F. tularensis subsp. Tularensis causes severe pulmonary infection in North America. In addition, Francisella philomiragia causes disease rarely in immunocompromised patients.

Literature Review

Tularemia is a debilitating febrile disease in human. This disease is common among hunters of rabbits and hares. Francisella tularensis is first reported in Tulare country of California in 1911. Francisella tularensis type A has been reported common in North America while occasionally found in some countries of Europe. Type B has been found common in Northern hemisphere and found in Australia as well. Type A has been reported severe clinical diseases than type B in human [1]. Francisella tularensis sub species mediastica has shown high virulent in mice although still less virulence in human. The high virulent strain in North America and Europe were shown of fermenting glycerol and citrulline and other less virulent strain are not capable to ferment these two chemical compounds. Importantly, the bacterium isolated from Asia were also shown capacity of fermenting glycerol and citrulline although those isolates were described as less virulent. Francisella tularensis sub species novicida also shown high virulence in mice while rarely cause disease in human. Type B has been reported in Eurasia, North America, Scandinavia Russia and Japan. Toluremia is an endemic disease in France with compulsory notification to the health authorities in the country, over 99 human cases were reported between 2006-2010. However, disease has not been reported in Greece, Iceland, Ireland, Luxembourg, Malta, and the United Kingdom (Only imported cases) and considered as free of diseases. Serological evidence has been reported among ranches in

Iran recently while no sign of infection in livestock in the same study [2].

Francisella has been isolated from hundreds of animal species in the endemic region of the world. As a results of this diverse host range, ecological factors related to transmission of Francisella in the environment is largely unclear were found to be the carriers/reservoirs of F. tularensis subsp. Tularensis. Francisella tularensis has multiple reservoirs including lagomorph and small rodents, Ixodidae ticks is the main vector for the bacterium. In addition, mosquitoes and deer flies also be considered as vector for Francisella tularensis for short term. All these arthropodes may transmit the bacterium to human and animal. Water born infection of Francisella tularensis is also emerging around the world. The disease also has been reported in mammals, birds, fishes, amphibians, arthropods, and protozoa. The presence of the organism is not an indicator of a disease in wild animal and other animals. In addition, some strain or lineage of Francisella were limited to specific mammal host.

Two main cycles on Francisella have been identified in North America as a terrestrial or sylvatic cycle and an aquatic cycle, lagomorphs and ticks play the role in sylvatic cycle (Francisella tularensis). Semi aquatic rodents involve in aquatic cycle (American beaver Castor canadensis and the muskrat Ondatra zibethicus) (Francisella holarctica). In addition, several rodents, ticks, mosquitoes involve in Eurasia to complete the life cycle of Francisella hallartica.

The disease has been reported as sporadic cases or small family outbreaks and the organism is survived in water and environment for several months. Furthermore, the organism multiply within protozoan found in the water such as amoeba.

Tularemia is an also disease in wild animals and captured animal, identification of the organism from those animals have been reported concurrent to the human infections [3]. In Germany, seropositive cases had been detected in 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) and yellownecked field mice (Apodemus flavicollis) and zoo animals. Basically, three main types of animals have been categories by WHO due to the high diversity of host range on Francisella tularensis, identification of incidental or reservoir host also challenging. The classification is based on susceptibility and sensitivity or severity of the infection such as acute disease after 1-10 organism inoculation with rapid multifaction within blood and tissue. The second class or category is reported death after inoculation of 108-109 organism and animal may survive with low dose of infection with development of immunity. The class three host are anyway resistant to the infection. The disadvantage of the classification is that host are classified only based on challenge experiment though blood or lymphatic route of infection. However, other natural route of infection and accidental host or reservoirs host has not been evaluated. Furthermore, tularemia has bene reported in companion animal such as dogs and cats in North America. The feline tularemia has only reported in North America and canine tularemia has observed in Europe. In addition, tularemia has also been noticed in sheep as livestock spices.

The first complete whole genome sequence of Francisella tularensis was done in 2005 while extensive genetic similarity as 97.63% were observed in both type A and type B. The size of genome is round 1.7 to 2.0 Mb 16S rDNA gene does not have discriminative power to differentiate each species. 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 not success in Francisella due to lack of discriminative power and interlaboratory reproducibility in molecular epidemiology. The multiple loci VNTR markers has been devolved, geographically specific clades also been identified in Europe and North America. However, these markers were not used solid phylogenetic analysis, Canonical Single Nucleotide Polymorphism (canSNP) and on Canonical Insertions/Deletions (INDELs) are used in extensive phylogenetic analysis currently.

Pathogenesis

Tularemia is a sporadic disease with low infectious dose required on pathogenesis. The severity of the infection is dependent upon the portal of entry, infectious dose, and subspecies (biovar) of the infecting strain [4]. The symptom of tularaemia depends on route of infection and altogether six main clinical forms were identified as ulceroglandular, glandular, oropharyngeal, oculoglandular, pneumonic and typhoidal. The first two forms were mainly due to the skin inoculation of bacteria either by arthropod bites or contact with the skin by infected animals. However, human to human transmission has not been reported. Multiple routes of transmission to animal have been reported in Francisella tularensis in the environment such as through contaminated skin, via conjunctiva, and ingestion. The main route of infection is via skin either contact with infected animal (hairs) or arthropod bites. The contaminated hand may spread into conjunctiva of the eye and organism enter the body through conjunctiva. The contaminated food and water also a source of infection in animal and human a number of outbreaks have been reported. Inhalation of infected aerosol is considered another important method of infection specially contaminated dust causes clinical disease in human and animals. Furthermore, untreated cases can be grown up to 60% mortality in tularemia while infectious dose was around 103 cfu in F. tularensis subsp. Holarctica (Type B strains). However, it was less 10 cfu in F. tularensis subsp. Tularensis (Type A strains). Conversely, Francisella tularensis is named as potential weaponized or type A agent by United States Centers for Infectious Disease Control and Prevention due to the characteristic of sever form of disease, low infectious dose and being a life-threatening infection.

The innate immune response is the first line defense mechanism against Francisella infection in human, the organism represses the activation of inflammasome at the cellular level to survive against innate immune responses. Innate immune responses on Francisella tularensis has not been fully investigated and although it has been studied by many authors. In contrast, some other concluded that Francisella activates protein secretion process in innate immune responses. The whole pathogenesis mechanism has been reviewed with minor details; the organism is found in extracellularly in the infected mice in the phase of septicaemia. The organism invades different mammalian cell types such as macrophages, dendritic cells, polymorphonuclear neutrophils, hepatocytes, endothelial, and type II alveolar lung epithelial cells. Importantly, uptake of Francisella by macrophages has been studied extensively while entering the nonphagocytic cells has not been investigated thoroughly.

It has been studied that mannose receptors have a significant role in monopsonic uptake of Francisella novicida and Francisella tularensis by human monocyte derived macrophages and murine bone marrow derive macrophages. In addition, serum opsonization is also important

in the process of up taking of the organism into the phagocytic cells. Furthermore, the scavenger receptor A, Fcr receptors, neucliolin, lung surfactant protein A also run a vital role on up taking serum opsonized Francisella into the macrophages. Francisella interfere the host metabolism including glycosylation pathway of human macrophages. In addition, the organism utilizes host cells substrates as nutritional requirements. Once enter to the cells, the bacteria survive and reside in early phagosome and interact with early and late endocytic compartment except lysosome [5]. 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 and limiting activation of poly morphonuclear cells. Oxidative burst by reactive oxygen species in macrophages is being is haltered dur the action of the organism. Several phagosome escape mechanisms have been examined in Francisella organism. In summary, resistance to reactive oxygen species, escape form phagosome, replication within cytosol, avoidance of innate immune responses is considered as survival mechanism for Francisella. Human infection has been reported common in literature with short incubation period as 3-5 days, and maximum incubation period is two weeks. In recent past, endocarditis was reported in human, Europe. Human get nonspecific lesion such as flu-like symptoms, including fever, lymphadenopathy, headache, chills, myalgia, and arthralgia. Majority of infection was associated with directly or indirectly contact with infected animals ended up with 95% of human infection in human in the world. Lung infection is mostly associated with farming activities. However, common infection was observed in skin, lymph nodes, bloodstream, and lungs. The periprosthetic joint infections has been reported many occasions around world. Human get the infection through multiple route such as directly or indirectly through infected animals, carcasses, ticks, mosquitoes, and contaminated water, soil and food. The mosquito-based infection is quite common in Sweden and Finland in Scandinavians region both Aedes and Culex mosquitoes are vector for the infection. Most of the human cases such as 50% out of total human cases were observed in August, the peak season for mosquitoes in Sweden and Finland. 74% of suffered patients were having ulcer glandular form of tularemia in Finland. Most of human cases in these two countries is limited to lower limb and associated with inguinal lymphadenopathy, or lesions in arms, face, or neck with axillary or cervical lymphadenopathy. However, other part of the Europe, role of mosquitoes as main vector for transmission if Francisella tularensis was observed minimum. O antigen found in LPS has been identified as main virulence factors in the process of pathogeneses. The capsule like complex/CLC protein and high molecular weight carbohydrate may have role in pathogenesis although exact mechanism is not known.

The infection caused by Francisella tularensis was reported in high host range such as range of vertebrates, amphibians, fish and range of invertebrates. Both mice and guinea pigs are highly susceptible for Francisella tularensis in laboratory animal experimental models. High susceptibility was observed on rodent species and rodents often early death with severe degree of infection were noticed. However, virulence is changed in rabbits with Francisella species and infective dose is varied widely from 1 cfu to 109 cfu. In addition, white rats are less susceptible under laboratory experimental condition. In addition, virulence contribution by different part of the cells has been investigated O antigen found in polysaccharide layer in the cell has been identified as the alternative mechanism of preventing activity of IgM and compliment mediated mechanism within the host. In addition, ability to LPS alteration and presence of pili in the cells is considered as potential mechanism against natural immune mechanism of the host. According to the recent finding, 50% of human tularemia infection were cat associated in human in USA while 3% were associated with canine infection.

Virulence factors play a major role in pathogenesis, most of these virulence genes are found in Francisella pathogenicity island. The pathogenicity island consists of 17 open reading frame which is believed as essential for the pathogenesis. pdpA, pdpD, vgrG, IglI, dotU, IglC were some of the genes found in Francisella pathogenicity island. The Reactive Oxygen Species (ROS) and nitro¬gen species is also an essential component of Francisella virulence mechanism. The enzyme KatG deactivate these two-protective mechanisms of the host cell and Francisella will be survived within the cell. SodB and SodC which encoded for superoxide dismutase are required for the resistance against superoxide radicals.

Francisella enter to the macrophages with a specific mechnsiasm called “looing pahogocytosis” which consist of large volume of space around the bacterium. Surafce receptors play a vital role in phagocytosis such as mannose receptors, Fc receptors and complement receptors. In addition, Iron is also an essential element for survivale of Franceilla in a host cell.The bacterium actively modulate expression of transferrin reptors in host cell at the initil intra cellayr growth. The important factor of the pathogensis is the ability to multifly intracellularly by the organism. The sepsis and inflammation cyasing death than pneumonic condition in human. In addition, high level inflammatory cytokines and chemokines are released in lung and speeelin leading to the death including IL-6, macrophages inflamamrtyr protein, chemokine ligand. Excessive lebel of neutrophil recrutement is also important facor in pathogensisi.

Diagnosis

Diagnosis of tularemia is based on clinical finding, epidemiology and serological testing in human. The clinical disease can be suspected as fever with lymphadenopathy who has proven contact with animals. Q fever, Plague, Psittacosis are the other clinical conditions which important in differential diagnosis. Both Micro agglutination test, Indirect Immunofluorescence Assay (IFA), and ELISA is used widely used in the field as diagnostic test on tularemia. However, cross reactivity has been observed with a number of bacterial organisms such as Salmonella, Brucella, Legionella and Yersinia species. Furthermore, four-fold rising of anti Francisella antibody titters within a period of 2-4 weeks period is identified as tularemia infection in human.

MAT and IFA have capacity to detect specific antibodies against Francisella tularensis 2-3 weeks of post infection while ELISA is early as 2 weeks of post infection. High percentage of false positive were observed and considered as negative point of using ELISA. The long-term persistence of past antibodies from previous infection interferes badly in diagnostic serological test together with cross reactivity to another bacterial pathogen. Immunochromatographic tests, immunoblot test also being used in research laboratories with variable degree of success

The conventional isolation and identification of Francisella is not common since it is required special laboratory such as BSL-III facility. Francisella is a strict aerobe which requires supplementation of sulfhydryl compounds and cysteine enriched media for the optimum growth in the laboratory. 4 mm diameter gray colonies with colour

changed in the medium into green is typical feature in the medium of glucose cysteine blood agar. However, different strains may have different colony morphology under the laboratory condition. The required incubation temperature is 35°C and colonies are shown 2-4 days of incubation.

A number of conventional and qPCR has been optimized to diagnose the organism in clinical submission including multiplex PCR assays. In addition, MOLDI TOF MS is used for rapid identification of the bacterial organism with high level of accuracy.

Treatment

Several antimicrobial classes are used widely to minimize the complication of the bacterial infection such as Aminoglycosides, tetracyclines, quinolones, and chloramphenicol with high relapse rates such as 10-21 days depend on the type of antimicrobials. In addition, beta lactams, rifampicin and linezolid are also used. According to Caspar and Maurin, cure rate is 60-100% depend upon type of antimicrobial used, time of appropriate antimicrobial set up, duration of treatment, and presence of further clinical complication. In the same study, no resistance was reported against used antimicrobials and minimum inhibitory concentration for ciprofloxacin, levofloxacin, gentamicin and doxycycline were ≤ 0.002–0.125 mg/L, ≤ 0.002–0.125 mg/L, ≤ 0.016 to 2 mg/L0.064 to 4 mg/L respectively. However, resistance has been reported by other study against penicillin, cephalosporins, carbapenems, macrolides, and clindamycin. Importantly, efficacy of antimicrobial treatment greatly reduced when treatment is started 24-48 hours of post infection. In contrast, moxifloxacin was proven success against the cases of delayed diagnose in human. The selection of antimicrobials depends upon clinical experience, severity of the systemic infection. Aminoglycosides (gentamicin, streptomycin) are preferred on treating of prolonged or extensive systemic symptoms, sepsis with or without renal failure, typhoidal tularemia, and symptomatic pneumonic tularemia. A combination of Aminoglycoside with tetracycline, quinolone and chloramphenicol are used for meningitis and endocarditis wile pediatric cases often treated with gentamicin. Ciprofloxacin and doxycycline are widely used in mild to moderate cases of tularemia in adults while azithromycin is used widely in pregnant women.

A number of guidelines have been developed by different organization including WHO to prevent the infection in human. This guideline is limited to the endemic areas of the tularemia based on prevalence and risk factors of the disease. Utilization of treated water for daily activities, usage of gloves when handling wild rabbits and rodents, thoroughly cooking of bush meat, using of repellent on insect specially traveling outside, protection of food at storage from rodents, wearing mask, checking the cloths for ticks, avoiding of weed when traveling in natural trails, pets need to be cleaned for ticks and other external parasites, vaccinated veterinarian and other who handle animals and livestock are considered as methods to away from Francisella specially in the endemic region of the world .

Vaccination is an alternative method to control the infection in endemic regions of North America and Europe. However, no commercial vaccine is found in the market now. Live attenuated vaccine had been tried in Russia couple years back with variable success. Killed, live attenuated and subunit vaccine have been developed in different countries with variable success. However, many of studies are going on investigating the efficacy and potency of Francisella vaccine around the world. Live vaccinated vaccine has been developed with significant reduction of clinical incidence in the experimental models. Being intracellular parasite, studies are targeting on T cell responses on their experimental models and immunity to Francisella appears to be dominated by T cell mediated mechanism. Importantly, several animals' models have been suggested and evaluated to study the efficacy of live vaccine against tularemia at present including rats, rabbits, mice and nonhuman primates. According to the latest finding, different degree of immune responses was observed on route of infection in human, however, further investigate are required.

Conclusion

Francisella tularensis is a potential risk to the human and animal in future due to the high host range, low infective dose, severity of the infection together with emerging antimicrobial resistance. Importantly, vector range, presence of incidental host and natural reservoirs have given us alarming signal together with changes in ecosystem together with climactic changes in the world. On the other hand, there are Hugh gap in the information on pathogenesis and virulence mechanism in human and animal. Therefore, Francisella tularensis can be a potential risk in near future as an emerging disease.

Acknowledgement

Author would like to acknowledge the Fleming Fund Fellowship programme, UK, University of Hong Kong, Mentor Dr. Wu Peng for tremendous support, encouragement, and facilities for the fellowship programme.

References

1. McCormick MA, Slamovich EB (2003) Microstructure development and dielectric properties of hydrothermal BaTiO3 thin films. J Eur Ceram Soc 23: 2143–2152.
2. Wang Q, Varghese O, Grimes CA, Dickey EC (2007) Grain boundary blocking and segregation effects in yttrium-doped polycrystalline titanium dioxide. Solid State Ion 178: 187-194.
3. Hu W, Liu Y, Withers RL, Frankcombe TJ, Noren L, et al. (2013) Electron-pinned defect-dipoles for high-performance colossal permittivity materials. Nat Mater 12: 821-826.
4. Bashal AH, Saad MH, Khalafalla MA (2020) The effect of Nickel percentage on the dielectric properties of Bentonite. J Taibah Univ Sci 14: 496-499.
5. Wang Y, Li L, Huang X, Li Q, Li G (2015) New insights into fluorinated TiO 2 (brookite, anatase and rutile) nanoparticles as efficient photocatalytic redox catalysts. RSC Adv 5: 34302-34313.