

Original Article

First Seroprevalence Survey of Children with Tularemia Infection in Chaharmahal va Bakhtiari Province, Iran

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ABSTRACT

Background and Objectives: An increasing number of tularemia was reported in all over the world. This infection is characterized by different clinical syndromes that can be considered in differential diagnosis of infectious disease. Despite effective antibiotics against *Francisella tularensis*, this infection is still as one of the agent of mortality and disability among infectious disease. The aim of this study was investigation of seroepidemiological of *F. tuleransis* among children between 2-18 years old in a risky zone in Iran.

Methods: This cross-sectional, laboratory-based study in two distinct villages Saragha seyed and Khoeye in Chaharmahal va bakhtiari Province involved 183 children, adolescents who had no sign and symptom of disease and were screened for tularemia immunoglobulins G (IgG), using the ELISA-based quantitative assay.

Results: In general, from 183 children 11 persons (6%) were seropositive, compared with 172 persons (94%) were seronegative.

Conclusion: According to the high prevalence of antibodies against *F. tuleransis* in this study, this infection must be considered as differential diagnosis of infectious disease in suspect patients.

Key words: Tularemia, Seroprevalence, Children, Iran

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Introduction

Tularemia, also known as rabbit fever or deer-fly fever, is a potentially fatal, multi-systemic disease of humans and some animals caused by the bacterial pathogen *Francisella tularensis*. The disease can be transmitted by ticks, biting flies, water exposure, food, and aerosols. *Francisella tularensis* occurs around the northern hemisphere including North America, Europe, and Asia (1). Infection often is water-associated and disease is produced by the various species and subspecies in humans and an array of other animals including domestic animals, wild small mammals, and fish (2). Human infections often occur after direct contact with infected animals, especially lagomorphs and small rodents (3, 4).

There is growing evidence that the occurrence of tularemia is substantially underestimated in many parts of the world (5). In Asia the distribution of *F. tularensis* is little known, Turkey one of neighbor country of Iran has reported increases in tularemia incidence in recent year (6). The first investigation about tularemia in Iran return back to near forty years ago (7). Several factors can be responsible for existence and transition of tularemia infection.

There is not any study about the prevalence of tularemia in Iran. The aim of this study was to determine the seroprevalance of tularemia in children between 2- 16 years of rural area that were more prone to be infected with this agent. Because isolation of *F. tularensis* is difficult and there is a high risk of accidental infection as a result of which serology is most commonly used for diagnosis (8, 9).

Materials and Methods

The cross-sectional, laboratory-based study was conducted in two distinct villages Saragha seyed and Khoeye in Chaharmahal va bakhtiari Province

that were 2 kilometer far from each other and were completely isolated because of their geographical condition. Total population was 2200 person almost all people have livestock, sheep, cattle and dog and the health level was low. We chose these rural areas in this study because previous study in 1973 suggests that this region is more susceptible to have tularemia infection because of special rodents that carrying *F. tularensis* (7). This factor led to selecte convenient in the risky village of Iran.

Totally, 183 children 2-18 years were chosen from Health House in June 2011. This study had the medical university Ethical Committee's approval and obtained informed consent from their parents. They had examined and their data recorded in a standard questionnaire by pediatric specialist and then they were screened for tularemia immunoglobulin G (IgG) using the ELISA-based quantitative assay, at the laboratory of the district center. Children, adolescents who had no sign and symptom of disease such as fever, skin sore, and lymphadenopathy were recruited for the study.

Ten milliliters of blood was collected by an aseptic technique from veins of the volunteers into laboratory bottles. Antibodies of IgG were assayed by the Plate ELISA Method. Quantitative IgG results were expressed in international units (IU), with calibration performed against reference standards of Serion Immundiagnostica GmbH kit for IgG, according to the manufacturer's instruction. *F. tularensis* has common antigens with *Brucella* and there is existence of serological cross reaction. In order to confirmation tests for IgG antibodies the IgG seropositive samples were further analyzed for IgG Brucellosis using Wright test according to the manufacturer's instructions (Brusel IgG kit Gen WayBioTech, Inc. catalog number 40-101-325075). According to kit there is not any cross-reaction with any other microorganisms. Principles of the IgG assay

were done according to manufactures instruction of Serion Immunodiagnostic GmbH kit Germany (ESR142G, Date of expire of kits were 2013). Samples with an OD in 450 nm value < 0.23 were classified as negative, between 0.23 and 0.31 were classified as borderline and samples with OD value upper than 0.31 were classified as positive. The sensitivity and specificity of the IgG assay of this kit were 99% and 96.9% respectively. To compensate for normal test variations and also for test run control a standard sample was used in each individual test run. For this control serum a reference value with a validity range was determined by the quality control of the producer.

Data Analysis

Data were expressed as percentages and differences between groups were assessed by the Chi-square (χ^2) test using SPSS software,

version 16.5. A P -value of <0.05 was considered statistically significant.

Results

The mean age of the study population was 10.18 ± 4.6 year. From this population 94 (51.3%) were female and 89 (48.7%) male.

The majority, 172 (94%) were seronegative for IgG antibodies, compared with 11 persons (6%) were seropositive.

Although 88 (51.2%) persons with negative IgG seromarkers were women, the difference was not significant between sex and IgG serostatus ($P=0.583$). There was no statistically significant difference between the age groups, history of contact with domestic animals, contact with dogs, Exact sig (1-sided) were $P=0.554$, $P=0.480$ and $P=0.510$ respectively. Table 1 shows IgG serostatus of the study population.

Table 1- IgG serostatus of the study population

	Seropositive n (%)	Seronegative n (%)	Total n (%)
Female	6(6.3)	88(51.2)	94(100)
Male	5(5.6)	84(48.8)	89(100)
Less than 5 years old	2(5)	38(95)	40(100)
5 year or more	9(6.3)	134(93.7)	143(100)
Contact with domestic animals	9(6)	141(94)	150(100)
Contact with dog	5(6.7)	69(93.3)	74(100)

Discussion

This study is the first report on the prevalence of tularemia in Iran after thirty years in human. Tularemia ecology is only partially understood with many knowledge gaps about the disease reservoir and vectors (3). This disease is a rare and primarily rural disease which may be transmitted by ingestion, inhalation or by direct skin contact with rabbits, other rodents and by blood sucking arthropods (10).

Study epidemiological information of this disease is very useful to control outbreak of the disease. In

this study 11 persons (6%) were seropositive for tularemia IgG antibodies and were seronegative for brucellosis IgG antibodies. Volunteers had not fever, skin sore, lymphadenopathy or other sign and symptom of disease. It was revealed that 6% (11 out of 183) of children in these rural areas were seropositive for tularemia IgG by ELISA. Worldwide, cases of tularemia are less common than before, mostly because of reduced exposure to infected animals. In 1939, there were 2291 reported cases in the US, while only 125 occurred between 1993 and 2005 (11). Cases

in Scandinavia are common, with an average in Finland and Sweden of 3.4 cases/100,000 people (3). In southern Europe, infection is uncommon but has been reported from Italy and France (12). Cases have been reported in the former Soviet Union, Japan, Canada, and Mexico as well. Outbreaks may be associated with exposure to arthropods, infected animals, food, or water, fomites, or occasionally aerosol-borne bacteria. In Spain, a large outbreak of 585 people occurred associated with exposure to infected hares (13). An outbreak on Indian reservations in South Dakota was associated with very high dog populations and corresponding increases in American dog tick, *Dermacentor variabilis*, infestation of dogs and people (14). Large outbreaks in Turkey and Bulgaria were associated, respectively, with drinking water exposure (15, 16). The first study of *F. tularensis* in Iran came back to about forty years ago that evaluate the tularemia in domestic and wild mammals (7). First human's tularemia case in Iran was reported by Karimi in 1981 (17). According to our search we did not find any other study about human tularemia in Iran. In view of the natural occurrence of known wild reservoir and vector species in Iran, the presence of large numbers of sheep, goats, and cattle, and the reports of tularemia infection from adjacent and ecologically similar areas, it seemed reasonable to expect to find the infection in rural areas during the present study.

Arata *et al.* in 1973 demonstrated that tularemia occurs in cattle and sheep in Iran (7). In view of the prevailing conditions for its natural occurrence it seems likely that tularemia also occurs in man. This study indicates presence of tularemia in Iran. Although in this study the sample size was small 9% positive result from rural area in Iran is interesting.

The focus of this study is 46.5 km far from Shahrekord, central of Chaharmahal Va Bakhtiari Province. This area has special characters

because of its geographical location and being isolated from nearest city for almost six months. Because of snowy winter and corrupt the roads. The population study chosen came from this rural background and has some risk factors. For example their parents task mostly farmers and shepherds that have close contact with animals such as dog, cattle and other domestic animals that heavily infested with ticks therefore more susceptible to infection from *F. tularensis*.

Obtained findings from the study were nearly consistent with other studies in different regions of the world. Knowledge of the prevalence of tularemia in these rural areas is important because tularemia is contagious and should be kept in mind as a differential diagnosis in children that suffer from FUO or lymphadenopathy in our country. One reason for the lack of human tularemia cases, despite positive serological test and presence of animal cases might be not considering tularemia as a possibility when making a diagnosis and also symptoms may be compatible with other pathogens that are more common in the region. Because when epidemiological information is unknown it is difficult to link symptoms to tularemia. In this study we used ELISA. Sensitivity and specificity of the IgG assay were 99% and 96.9% respectively and this technique can be an appropriate tool for evaluation seroprevalence. Because isolation of *F. tularensis* is difficult and there is a high risk of accidental infection as a result of which serology is most commonly used for diagnosis (8, 9). Results indicate further need for more specific attention and extensive field evaluation about tularemia in Iran.

Conclusion

The findings described in this study emphasize the importance to develop a national program and protocol for epidemiology of tularemia in Iran.

This is crucial; especially considering the fact that there is a wide variety of clinical manifestation for the disease therefore physicians must pay more attention to tularemia in differential diagnosis.

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