

**Brief Original Article****Tularemia in children during the last outbreak in Kosovo**Izet Sadiku<sup>1</sup>, Sadie Namani<sup>1</sup>, Vera Ndrejaj Berisha<sup>1</sup>, Albina Ponosheci Biçaku<sup>1</sup>, Saranda Hyseni<sup>1</sup><sup>1</sup> Clinic of Infectious Diseases, University Clinical Center of Kosovo, Prishtinë, Kosovo**Abstract**

**Introduction:** Tularemia is a zoonotic disease that primarily affects adults and children in rural areas. Late diagnosis in children is often associated with treatment failure and accessory surgical procedures.

**Objective:** To analyze the diagnostic and treatment options of pediatric tularemia during the last outbreak in Kosovo during years 2014 and 2015.

**Methodology:** This retrospective study includes 36 children treated for Tularemia at pediatric department. The diagnosis was based on clinical, serological, and PCR testing.

**Results:** Of the 230 patients treated for tularemia, 36 (16%) were children with a median age of 9.4 years old (range 2–15 years). Major clinical manifestations included fever (97%) and swelling of lymph glands (94%), and the duration of symptoms prior to hospitalization was two weeks (range 3–60 days). Leukocytosis (41%), along with an elevated erythrocyte sedimentation rate (97%) characterized the laboratory findings. Both serology and PCR were used to confirm tularemia in children in 100% of cases. Due to abscess formation, suppuration, and high prevalence of tuberculosis, surgical procedures were used as accessory therapy and for diagnosis in half of the patients (50%). Gentamycin was the first drug of choice (97%), while 3 patients experienced relapses. Since the majority of the patients (72%) used unsafe water from wells in rural regions, the outbreak was thought to be water-related.

**Conclusions:** Every febrile child with swollen glands should be suspected of having tularemia. Gentamycin continues to be the preferred treatment for unilateral cervical glandular type. Successful therapy depends on early diagnosis and supplemental surgical procedures.

**Key words:** *Francisella tularensis*; tularemia; children; gentamycin.

*J Infect Dev Ctries* 2022; 16(8):1364–1369. doi:10.3855/jidc.14886

(Received 10 February 2021 – Accepted 21 June 2022)

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**Introduction**

Tularemia is a zoonotic infectious disease caused by intracellular, Gram-negative bacillus (resistant to various types of drugs and are increasingly resistant to most available antibiotics) *Francisella tularensis* (*F. tularensis*). *F. tularensis* is divided into 4 subspecies: *holarctica*, *tularensis*, *mediasiatica*, and *novicida*. Tularemia in humans is caused by the subspecies *tularensis* and *holarctica*. The subspecies *novicida* can cause pathology in immunocompromised subjects.

Tularemia is reported in North America and in many European and Asian countries as sporadic cases or outbreaks. The most typical *F. tularensis* reservoirs are mice, rats, rabbit ticks, mosquitoes, and flies [1]. Direct contact with ill animals, eating infected meat, drinking contaminated water, coming into direct touch with polluted water, and inhaling aerosols containing bacteria are all ways that humans might contract the disease [2]. There is no human-to-human transmission [1]. Based on modes of transmission of *F. tularensis*, clinical forms of infection may be presented as external

(glandular, ulceroglandular, oropharyngeal, and oculoglandular), and internal forms (respiratory and typhoidal).

Following the war in Kosovo, there were 247 cases of the first Tularemia outbreak (1999–2000). In Kosovo, there were 5.2 cases of tularemia per 100,000 population each year between 2001 and 2010 [3]. The study's goal is to highlight the diagnostic and treatment alternatives for pediatric tularemia in the most recent outbreak in Kosovo between 2014 and 2015. Since there are very few reports of tularemia in children worldwide, this is a rare occurrence.

**Methodology**

During 2014 through 2015, information was gathered on every child under the age of 15 who visited the pediatric department at the Clinic of Infectious Diseases in Pristina with tularemia suspicion. Every febrile child with swollen lymph nodes was thought to have tularemia, and the diagnosis was verified by serology testing at the National Institute of Public

Health of Kosovo, in Prishtina. From serology tests, microagglutination and enzyme-linked immunosorbent assay (ELISA) testing were used. The department of microbiology used an agglutination test of BD *F. tularensis* Antigen (Becton, Dickinson and Company, USA) in accordance with manufacturer recommendations to identify antibodies against *F. tularensis*.

According to manufacturer instructions, ELISA testing was carried out using SERION ELISA classic *F. tularensis* IgG and IgM (Institut Virion-Serion GmbH, Würzburg, Germany), and Real-Time PCR testing was carried out using the *F. tularensis* genesig Standard Kit (Primerdesign LTD, UK).

Agglutination titers more than 1:80 and a rise in the titer of fourfold were regarded as positive results. We submitted lymph node aspirates to Slovenia for molecular testing in accordance with interstate cooperation. Fine-needle aspiration (FNA) of the swollen lymph nodes was carried out by a maxillofacial surgeon, and the sample was then transferred to the Microbiology Institute in Ljubljana, Slovenia, for molecular analysis. Additionally, from the samples received from Kosovo, the *F. tularensis* subspecies *holartica* was discovered in Ljubljana for the first time.

## Results

The conclusive results from 36 cases (16%) were from children under the age of 15 out of the total 230 patients treated for tularemia at the Clinic of Infectious Diseases in Prishtina. The average age of the patients was 9.4 years (range 2-15 years). Furthermore, 21 (58%) of the cases were male, with no statistically significant difference. Since all of the patients were from rural areas, most of them, 26 patients (72%), drank

contaminated water from wells (Figure 1). The length of symptoms in children prior to tularemia diagnosis was roughly 13 days (range 1-45 days). The majority of tularemia cases in children occurred in the winter (69%) with the largest prevalence occurring in February (39%) (Figure 2). The most recent Tularemia outbreak in Kosovo affected eleven out of the 36 municipalities. The disease was mainly concentrated in 3 municipalities that contained 58% of all cases (Vushtrri, Drenas, and Lipjan) (Figure 3).

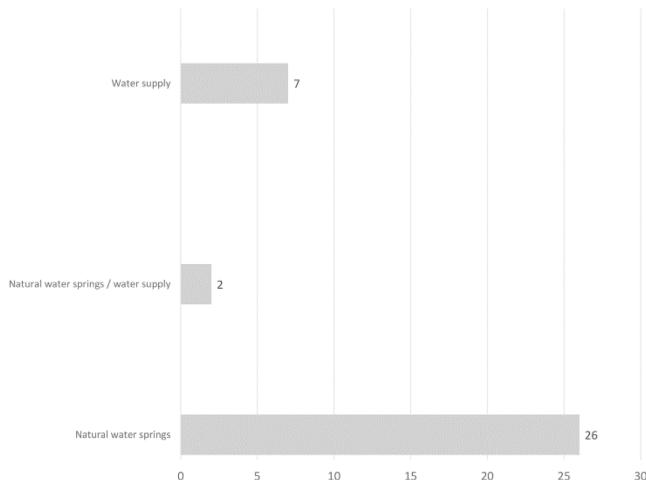
The majority of the children (97%), suffered neck pain (55%), tiredness (92%), and unilateral lymph node swelling (94%). Ninety-two percent of swollen lymph nodes were seen in the cervical area, one in the axillary region and two children (5.5) had oropharyngeal forms (Figure 3 and 4). In 18 kids (about 50%), neck sonography was done. The typical length of stay in the hospital was 14 days (range 4-28 days).

According to laboratory findings, 35 patients (97%) had high erythrocyte sedimentation rates, 15 (41%) had leukocytosis, and 20 cases (55.5%) had lymphocytosis. 28 patients (78%) had high C-Reactive Protein (CRP), with an average level of 43 mg/L.

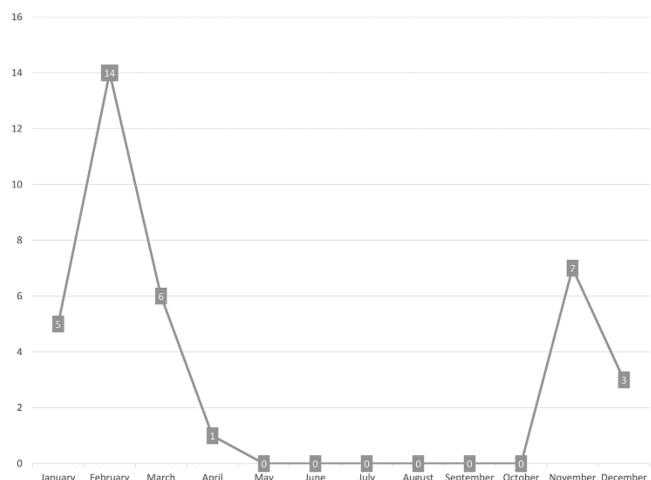
Based on the clinical signs, serology results, and polymerase chain reaction (PCR) of lymph node aspirates, tularemia was determined to be the cause. While ELISA testing was positive in 8 children (22%), the micro-agglutination test was positive in 32 children (89%), with antibody titers 1:320 in 58% of cases. A maxillofacial surgeon performed fine needle aspiration biopsy of lymph nodes on 9 patients (25%) and validated the diagnosis of tularemia using PCR at the Microbiology Institute in Ljubljana, Slovenia.

For the first 10 to 14 days of treatment, almost all children received intravenous micro infusions of

**Figure 1.** Water supply sources.



**Figure 2.** Distribution of tularemia cases per month.



gentamycin at a dose of 5-7 mg/kg twice daily. Erythromycin and streptomycin were used to treat only one child. Surgical treatments were used as adjunctive therapy and for diagnosis in 50% of the patients due to abscess development and suppuration of cervical lymph nodes. Streptomycin was used to treat three individuals (8%) who experienced relapses.

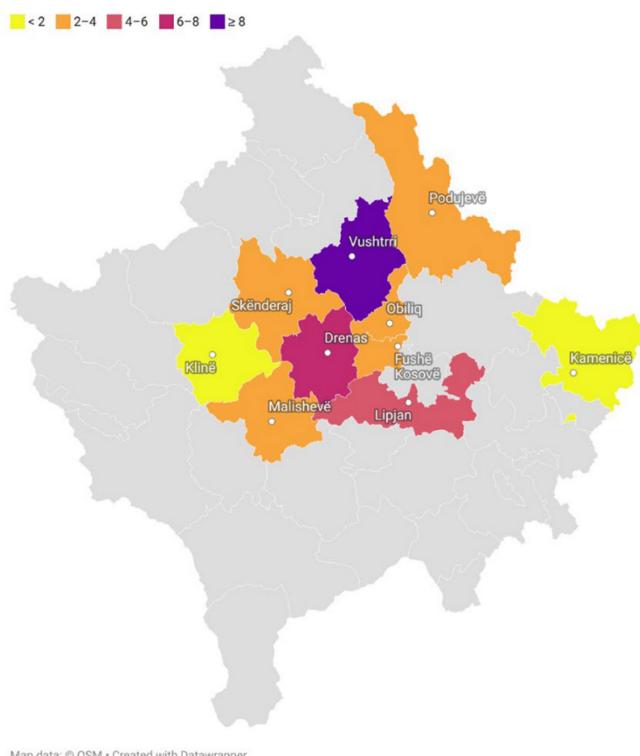
## Discussion

The zoonotic disease tularemia, which is extremely contagious, continues to be a major public health issue in many countries. Over the past few decades, isolated cases and outbreaks of water-borne tularemia have been reported all over the world [4]. Tularemia is present throughout the Northern Hemisphere, and cases are on the rise in some regions of Europe, particularly in the Balkans, Turkey, and Scandinavian nations [5].

While *F. tularensis* subspecies *tularensis* is more widespread in North America, subspecies *holoarctica* is more frequently associated with human and animal infection and can be found in Europe, Asia, and North America [6]. In the majority of European countries, tularemia is endemic [7]. At least once every ten years, epidemics involving hundreds of cases are reported in various countries, including Finland and Sweden [7]. History demonstrates that tularemia epidemics are linked to unsanitary conditions, particularly in post-war and wartime settings, along with a significant rise in rodent populations and the following mass deaths of these animals [7,8].

Following the 1999 war, there was a first-ever outbreak of tularemia in Kosovo. The Kosovo tularemia outbreak was impacted by the unfavorable socioeconomic and hygienic conditions, as well as the numerous human and animal deaths. Although suspected, *F. tularensis* as a possible bioterrorism agent could not be shown to be responsible for a tularemia outbreak in Kosovo in 1999 and 2000 [8]. Tularemia was clinically suspected, and the diagnosis was serologically confirmed on 14<sup>th</sup> April 2002, at the World Health Organization (WHO) regional reference

**Figure 3.** Distribution of tularemia cases in municipalities of Kosovo.



Map data: © OSM • Created with Datawrapper

laboratory in Rome [8]. Epizootic rodent tularemia was caused by environmental conditions in the war-torn region of Kosovo, which spread to newly relocated rural communities who were living in substandard housing, hygiene, and sanitation conditions [9].

After the war in Kosovo, there were many recorded tularemia outbreaks, with the most recent one occurring in 2014–2015 and including about 500 cases with an annual rate of 11.35 cases per 100,000 population. 1764 cases of tularemia were reported in Kosovo between 1999 and 2016. The most prevalent clinical symptoms were glandular (79%) and ulceroglandular (21%) types, with contaminated food and drinking water being the most frequent sources of infection. The most impacted occupational categories in prior reports of tularemia

**Figure 4.** a) Oropharyngeal form, b) cervical glandular and ulceroglandular form and c) axilar glandular form of tularemia.



epidemics in Kosovo were housewives and farmers [3,9]. Additionally, nearly 20% of children and adolescents (under the age of 20) had the infection [3].

During last outbreak of tularemia in Kosovo, from a total of 230 patients treated at the Clinic of Infectious Diseases in Prishtina, children were infected in 16% of cases. Other publications presented a higher incidence of tularemia in children aged  $\leq 15$  years such as in Bursa (Turkey) at 22% [10]. According to a report on tularemia in the United States from 2001 to 2010, the annual incidence was highest in men between the ages of 65 and 69 and in children between the ages of 5 and 9 [11].

The median age of the youngsters in this study was 9.4 years (range 2–15 years).

As in other reports, children developed tularemia during the winter or the autumn, with the largest prevalence occurring in February as a result of meteorological factors that directly affected the quality of well drinking water [10]. The most likely causes of infection in Kosovo between 2001 and 2010 were contaminated food and drinking water, according to earlier reports on the disease's surveillance [3]. People in the afflicted areas reported a significant rise in rodent populations, particularly field, forest, and domestic mice, before the outbreak among humans, which was a defining feature of the two previously documented outbreaks [3].

The most strikingly positive results in the antigen identification of *F. tularensis* were detected in the feces of tiny rats, which the investigation teams frequently discovered in foodstuffs stored in the affected homes' food stores during the reported outbreaks [3]. Epidemiologic and environmental data also pointed to the contribution of unprotected and unboiled water to the outbreak [9].

There were no cases of tularemia in children from May through October. This was probably due to use of fresh food during these months due to hot weather and not storing it in food warehouses as practiced in the autumn and winter months. All children came from rural places and most of them consumed unsafe food and water supply. Eleven of the thirty-six municipalities in Kosovo were affected during last outbreak of tularemia but the disease was mostly concentrated in 3 municipalities that accounted for more than a half of total cases (Vushtrri, Drenas and Lipjan).

From previous reports, the most common clinical signs are sore throat, fever, swelling on the neck, skin, mucosa, or conjunctival inoculation lesions [1,12-15]. In this report, the most common symptoms of children with tularemia were fever (97%) and unilateral swelling

of lymph nodes (94%). As almost all the tularemia patients during 2001 to 2010, as in the first reported outbreak [9], had the oropharyngeal form with fever and a unilateral cervical lymph node enlargement as the main symptoms, obviously the main route of infection was alimentary ingestion of *F. tularensis* [3].

Ulceroglandular and glandular tularemia are by far the most common types of the illness in terms of pandemic frequency. They may comprise more than 95% of epidemics in European nations [1]. Unilateral glandular and ulceroglandular forms of tularemia in children were also the most frequently reported clinical forms in this outbreak. Transmission of infection by contaminated food and water supply favored cervical glandular form in 92% of cases but not the oropharyngeal form which only appeared in two children. Previous reports of tularemia in Kosovo [3,9] stated that the oropharyngeal variant of the disease was the most clinically evident.

Elevated ESR (97%) and elevated CRP (78%) were very significant, according to test results.

The golden standard for the diagnosis is the isolation of *F. tularensis* in sterile body samples [16]. The diagnosis of tularemia is based on clinical signs and serological findings because this method is extremely challenging and dangerous for laboratory employees [17,18].

Based on the clinical manifestation, serology results, and PCR of lymph node aspirates, tularemia was determined to be the cause. 89% of cases tested positive for microagglutination, 22% tested positive for ELISA, and 25% had tissue samples tested for PCR to confirm the diagnosis of tularemia (lymph node aspirates).

A variety of antibiotics, including aminoglycosides (gentamycin and streptomycin), tetracyclines, azithromycin, quinolones, and chloramphenicol, are used to treat tularemia [10,16,19-21]. Streptomycin had a cure rate of 97% with no relapses, while gentamicin had an 86% cure rate with a 6% failure rate, according to published research [22]. In our study, gentamicin was the first-line treatment for tularemia, and 97% of the children had a good outcome. For 10 to 14 days, gentamicin was administered intravenously as micro infusions.

Due to the high incidence of tuberculosis in our country, streptomycin was not used as a first line drug for the treatment of tularemia in children. Only 3 relapse cases (8%) were treated successfully with streptomycin given by intramuscular route. No cases of ototoxicity and nephrotoxicity have been observed from the use of aminoglycosides in our cases.

Children's tularemia symptoms typically lasted two weeks (range 1- 45 days). Suppuration and the development of abscesses were linked to early treatment delays. In 50% of cases, surgery was performed as an adjunctive therapy to promote quicker healing and pus drainage. Since Kosovo, the state in Southeastern Europe, has a very high number of TB cases in this area, aspiration biopsy of cervical lymphadenitis was recommended for the differential diagnosis between tularemia cases and tuberculous lymphadenitis [22]. In suspected tularemia cases, Albayrak *et al.* advised keeping tuberculous lymphadenitis in mind, and those patients should also be examined concurrently for the existence of tuberculous lymphadenitis [23].

Children in Kosovo manifested external forms of tularemia due to infection with *F. tularensis* subspecies *holartica*. There were no deaths among children infected with tularemia.

This research has several limitations. Firstly, the study's sample size is small, and it excluded children who had ambulatory tularemia treatment at our clinic. The absence of information on tularemia cases in children treated in other Kosovo municipalities is the study's second drawback.

In conclusion, tularemia has emerged as a new health threat for children in Kosovo since the initial epidemic 20 years ago. Every febrile youngster with unilateral cervical lymphadenitis should have tularemia suspected by practicing professionals. Further research is needed to know the exact inclusion of children with tularemia. New strategies should be implemented for earlier diagnosis and treatment of tularemia in children.

## Acknowledgements

Authors would like to thank the personnel of the Clinic of Infectious Diseases of Prishtina, for their support during this study.

## Authors' Contributions

Designed the study: Izet Sadiku and Sadie Namani. Collected data: Vera Ndrezaj Berisha, Albina Ponosheci Biçaku and Saranda Hyseni. Analyzed the data: Sadie Namani and Izet Sadiku. Wrote the paper: Sadie Namani.

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**Conflict of interests:** No conflict of interests is declared.