Original article **UDC: 616.993(497.11)**
doi:10.5633/amm.2017.0105

TULAREMIA IN SOUTH-EASTERN SERBIA IN TWELVE-YEAR FOLLOW-UP

Marina Đorđević-Spasić[1], Miodrag Vrbić[1,2], Maja Jovanović[1,2], Lidija Popović-Dragonjić[1,2], Aleksandar Ranković[1]

Tularemia is a serious bacterial zoonosis caused by the highly infectious agent *Francisella tularensis*. Microbiological diagnosis of tularemia mainly relies on serology. The occurrence of a tularemia epidemic in the Southeast of Serbia in 1998/999 initiated an epidemiological as well as a clinical and microbiological research in this area.

Objective was establishing the correlation between the clinical-epidemiological and serological diagnosis of tularemia as well as the clinical and serological follow-up of patients in the period from 1 to 12 years since the disease onset.

From the beginning of 1999 until the end of 2011, 113 patients diagnosed with tularemia were examined. The control group was formed of 111 patients with lymphadenopathy of different origins. The following serological methods were used: microagglutination test (MAT), immunoensyme assays: ELISA (VMA, Belgrade), Serion ELISA IgG i IgM, Serazym ELISA and ELISA in house and immunochromatographic test (ICT).

Clinical-epidemiological diagnosis of tularemia was confirmed serologically in all 113 patients. The high sensitivity and specifity were found for all the examined tests. IgG Virion ELISA demonstrated the highest sensitivity (97.4%) and specificity (93.1%). IgG and IgM class of antibodies remained positive in the serum in a high percentage, even as long as 12 years from the infection. Oropharyngeal form (93.8%), with predominant unilateral cervical lymphadenopathy (91.5%), was the most common clinical form. Complications, such as suppurative lymphadenitis and recurrent lymphadenitis, were seen in 41.6% of patients.

A positive correlation between clinical-epidemiological and serological diagnosis of tularemia has been established. Serological findings must be interpreted only within the clinical picture of tularemia. A finding of IgM and IgG class antibodies or total antibodies of *F. tularensis* in the sera of patients without clinical disease manifestations, from one to 12 years from the disease onset, does not indicate an acute but a past infection. *Acta Medica Medianae 2017;56(1):31-38.*

Key words: *tularemia, Francisella tularensis, diagnosis, microagglutination test, ELISA, immunochromatografic test*

Infectious Diseases Clinic, Clinical Centre Niš, Niš, Serbia[1]
University of Niš, Facullty of Medicine, Niš, Niš, Serbia[2]

Contact: Marina Đorđević-Spasić
Vizantijski bulevar 94/9, 18000 Niš, Serbia
E-mail: marina_djordjevic@yahoo.com

Introduction

Tularemia is a bacterial zoonosis, typically characterized by lymphonodal involvement. All around the world, there are different names for this disease: rabbit fever, rodent plague, deer-fly fever, Francis' disease, O'Hara's disease, epidemic lymphadenitis (1, 2).

It is caused by the highly infectious agent *Francisella tularensis*: gram negative coccobacillus and facultative intracelular pathogen. It involves two major species: *F. tularensis* subsp. tularensis (type A) – existing in almost whole North America, very virulent and a pathogen for many hosts, and *F. tularensis* subsp. holarctica (type B) – prevalent along the northern hemisphere, mainly in Eurasia, causing milder disease in humans and animals.

Human infection develops after the contact with infected rodents or vectors (tics, deer-flies, and mosquitoes), by contaminated food, water and aerosol. There is no human to human transmission (1-4).

After mutiplication on the inoculation site (primary affect), *Francisella tularensis* spreads to regional lymph nodes (primary complex), then from the lymph nodes by the lymphohematogenous route to other organ systems. After acute inflammatory reaction on the site of inoculation,

similar as with other intracellular pathogens, a granuloma is formed, occasionally with caseous necrosis (1-4).

Depending on the site of infection, tularemia has six characteristic clinical forms: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal.

Ulceroglandular tularemia is the most common type, representing 75% of all forms. The port of infection are lesions on the hand skin, followed by cubital and or axillar lymphadenitis, or tick bites, followed mostly by inguinal lymphadenitis (5).

Oropharyngeal (tonsilloglandular) tularemia is the predominant form of tularemia in our country (6) and the region. The primary affect is on the tonsilla. It looks like a unilateral ulceromembranosus or ulceronecrotic pharingitis, with ipsilateral lymph node enlargement.

Glandular tularemia is pathogenetically identical to ulceroglandular and oropharyngeal forms, but with a weakly expressed primary affect that remains unrecognised.

Secondary skin manifestations are also possible in tularemia, and they are often misdiagnosed or overlooked. There are maculopapular, vesicopapular, urticarial rash, as well as numerous immune-related skin changes, including erythema nodosum, erythema multiforme and Sweet's syndrome (7).

The most frequent complications of tularemia are lymph node suppuration and reccurent lymphadenitis (8).

The diagnosis of tularemia is based on the recovery of an isolate, antigen or molecular detection and serology. Serological tests represent the gold standard in the diagnosis of tularemia. Agglutination in one titer ≥ 1:160 or microagglutination ≥ 1:128, or a four-fold or higher increase of the titer in consequent sera samples confirm the diagnosis (5, 9). Immunoenzyme assay (ELISA) is more sensitive and specific than agglutination tests and this test can detect individual immunoglobulin classes (10-12). Immunochromatographic test is fast, very sensitive and specific (13, 14). Indirect immunofluorescence test (IIF) is very specific and it can detect specific antibodies as well as antigens in the serum and in other clinical samples of patients (15). The tests used for antigen confirmation are direct immunofluorescence test (DIF) and immunohistochemistry (IHH) used in animal models with the use of monoclonal antibodies against *F. tularensis* antigen (16, 17). Molecular methods such as PCR are available in reference laboratories (18).

Objective

Establishment of a correlation between the clinical-epidemiological and serological diagnosis of tularemia, as well as clinical and serological follow-up of tularemia patients in the period from one to twelve years.

Patients and methods

From the beginning of 1999 untill the end of 2011, 113 patients diagnosed with tularemia were examined. The control group was consisted of 111 patients with lymphadenopathy of different origin. Tularemia patients were clinically observed and serologically monitored using adequate tests, in the period mentioned above. In newly diagnosed patients, paired serum samples were taken: the first sample was taken from 2nd to 4th week from the beginning, and the second, from 4th to 8th week.

Clinical-epidemilogical, microbiological-immunodiagnostic examination were conducted in all patients. Serological methods included: microagglutination test (MAT), immunoenzyme assays: ELISA (VMA, Belgrade), Serion ELISA IgG i IgM, Serazym ELISA and ELISA in-house (Friedriech-Loeffler Instiute, Jena) and immunochromatographic test (ICT)-VIRapid.

The multiple research was performed at the Clinic for Infectious Diseases, Clinical Centre Niš, primary care units in Sokobanja, Pirot, Aleksinac, Military Medical Acadamy in Belgrade, "Neolab" laboratory in Niš and Federal Veterinary Institute for bacterial zoonoses -Friedriech-Loeffler, Jena, Germany.

The results of the study were systematized and presented in tables and graphs (Excel 2007, Word 2007), processed using statistical descriptive and quantitative methodology (SPSS 16.0 for Windows 2007).

Results

The total of 144 patients with differential diagnosis of tularemia were clinically observed, diagnosed and monitored in the interval from 1 to 12 years, since the beginning of 1999 until the end of 2011. In order to definitely confirm the diagnosis of tularemia, the clinical, epidemiological suspicion had to be confirmed by serological immunodiagnostic tests. The clinical criteria included dominant unilateral lymphadenopathy and/or tonsillopharyngitis with fever and possible skin le-

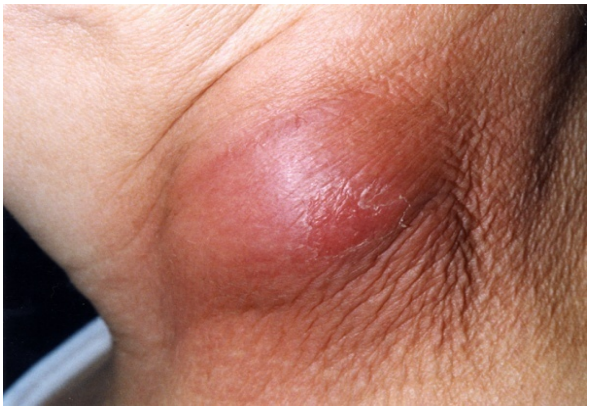

Figure 1. Unilateral suppurative lymphadenitis

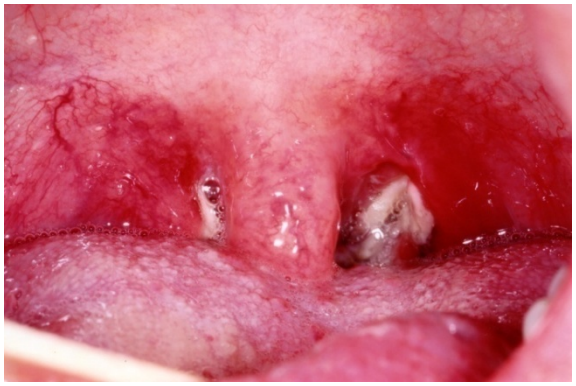

Figure 2. Exudative unilateral tonsillopharyngitis

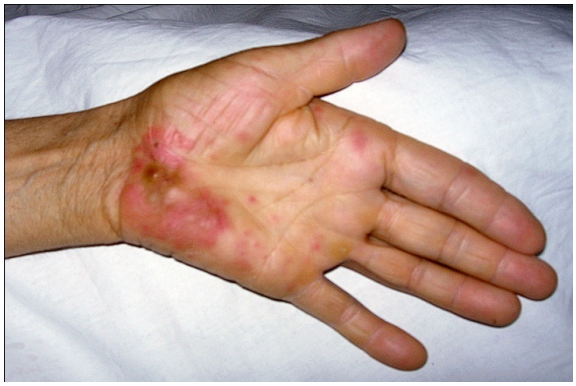

Figure 3. Erythema exudativum multiforme on the palms

sions, mostly on hands and shins. The most frequent clinical form was oropharyngeal (93.8%): with tonsiloglandular (47.8%) and glandular (46 %), manifested as a predominant unilateral form (92%).

The most common clinical manifestation of tularemia was lymphadenopathy in 95.6% of patients, predominantly cervical (88.5%) and unilateral (93%) (Figure 1). The unilateral cervical lymphadenopathy form is statistically significantly more frequent in tularemia patients, in comparison to the control group ($p < 0.05$).

Tonsillopharyngitis was diagnosed in 54 (47.8%) patients with a predominant unilateral localization in 44 (81.5%) patients. The most frequent form was unilateral exudative tonsillopharyngitis (63%) (Figure 2).

Fever was present in 100 of total 113 tularemia patients (88.5%). Two thirds of all patients (75%) presented with high fever > 38°C. Hyperpyrexia (≥ 39.1°C) was significantly more widespread in tularemia cases compared to the control group ($p = 0.005$). The fever of tularemia patients lasted for 7.5 days on the average.

The most frequent types of skin manifestations were erythema exudativum multiforme (25%) (Figure 3), erythema nodosum (25%) and ulceration as primary affect. The skin changes were located mostly on the hands (44.4%) and shins (37%).

Complications occurred in 47 out of 113 patients (41.6%). The most common complication was suppurative lymphadenitis (38%), followed by recurrent lymphadenitis (16.8%). Suppurative lymphadenitis was statistically more common in tularemia cases, than in the control group ($p < 0.05$) (Figure 1).

The patients with clinical-epidemiological diagnosis of tularemia (n = 113) were observed and serologically monitored in the period from one to twelve years. The results of the previous serum testing by MAT (VMA) and ELISA (VMA) were compared with the results of testing with commercial tests IgG Serion ELISA, IgM Serion ELISA, Serazym ELISA i VIRapid and noncommercial ELISA in house test. The results of testing with indirect-immunofluorescence test (IIFT) were used to detect the antigens of *F. tularensis* in pharyngeal swabs and lymph node punctates (6).

The schematic presentation of comparative immunodiagnosis is given in Diagram 1.

In patients suffering from tularemia (n = 113), the final results of repeated serum testings by MAT were as follows: 87 positive, 5 negative and 10 borderline findings. In the remaining 11 patients, MAT testing was not done. Of all 15 serums in which the specific antibodies on *F. Tularensis* were not found by MAT, in 11 of them ELISA (VMA) test was positive. Indirect immunofluorescence tests were positive in pharyngeal swabs and lymph-node punctates in the remaining 4 patients. All 15 serums negative by MAT were analyzed with the tests mentioned above (in 13 patients all 6 tests were positive; in one patient 5 tests were positive; in one patient, 4 tests were positive). The sera of all 11 patients not tested with MAT were positive after testing with IgG Serion ELISA, IgM Serion ELISA, Serazym ELISA, ELISA in house and VIRapid (Diagram 1).

In the control group, the results of previous testing by MAT were obtained: in 23 patients tularemia was exluded, in 2 patients tularemia was confirmed and 6 patients were not tested. In the folowing sera simultaneously tested with five immunodiagnostic tests, tularemia was excluded in 2 patients who were previosly positive on ELISA (VMA). Of 23 patients with negative sera on MAT, ELISA (VMA) was positive in the sera of 5 patients. After the simultaneous testing with immunodiagnostic tests, tularemia was exluded in all 5 patients. In all 6 patients who were not previosly tested by MAT, tularemia was excluded by immunodiagnostic tests (Diagram 1).

The characteristics of the diagnostic tests performed in all the examined patients were shown in Table 1. The results indicated the highest sensitivity in Serazym ELISA (97.4%) and VIRapid (97.4%), then IgG Serion ELISA (95.5%), IgM Serion ELISA (92.2%), and the lowest in In-house ELISA (77.9%).

Regarding specificity, the highest specificity was noted in In-house ELISA (94.4%) and IgG Se-

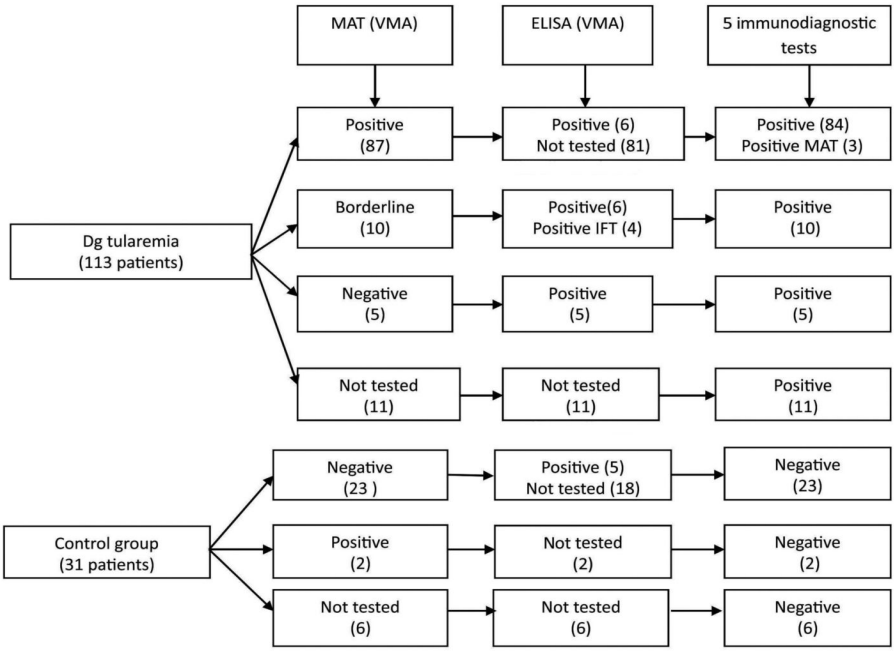

Diagram 1. Comparative immunodiagnosis of the patients affected by tularemia and control group

Table 1.: Characteristics of diagnostic tests in all examined serum samples

	IgG Serion ELISA	IgM Serion ELISA	Serazym ELISA	In-house ELISA	VIRapid
N° of serum samples	213	211	261	262	262
Sensitivity	0.9548	0.9221	0.9742	0.7792	0.9740
Specificity	0.9310	0.8421	0.6792	0.9444	0.8519
PPV*	0.9737	0.9404	0.8162	0.9524	0.9036
NPV**	0.8852	0.8000	0.9474	0.7500	0.9583
test efficiency	0.9484	0.9005	0.8544	0.8473	0.9237

* PPV- positive predicitive value
**NPV- negative predictive value

Abbreviations:

DIF**-**direct immunofluorescence
ELISA- enzyme-linked immune sorbent assay
F. tularensis - Francisella tularensis
ICH- immunochromatographic test
IHC- immunohistochemistry
IIF- indirect immunofluorescence
MAT- microagglutination test
VMA- engl. MMA - Military Medical Academy

rion ELISA (93.1%). The highest positive predictive value was found in IgG Serion ELISA (97.37 %), then In-house ELISA (95.2%) and IgM Serion ELISA (94%). The highest negative predictive value was seen in VIRapid (95.8%) and Serazym ELISA (94.7%) tests. The highest diagnostic efficacy was observed in IgG Serion ELISA (94.8%), VIRapid (92.4%) and IgM Serion ELISA (90.0%). The best test to confirm the diagnosis of tularemia, according to the factors of sensitivity and specificity, was IgG Serion ELISA test (Table 2).

Analyzing the presence of IgG and IgM antibodies in the serum of patients in correlation with the period of time (1-12 years), the persistence of positivity for longer period of time was seen in both classes of antibodies. IgG Serion test showed a 100% of positivity of IgG antibody, until 9 years of the disease onset. After that, the percentage of positivity slightly decreased; after 10 years it was 85.7%. IgM class of antibody presented high and irregular activity during the follow-up period. The finding of IgM positivity varied from 71.4% to 100.0% (Table 2).

Table 2.: Finding of F. tularensis IgG i IgM antibodies in a 11-year period

Time pariod (years)	N° patients n	Positive finding IgG n (%)	Positive finding IgM n (%)
1	5	5 (100.0)	5 (100.0)
2	6	6 (100.0)	5 (83.3)
3	5	5 (100.0)	5 (100.0)
4	-	-	-
5	7	7 (100.0)	5 (71.4)
6	14	14 (100.0)	14 (100.0)
7	10	10 (100.0)	9 (90.0)
8	16	16 (100.0)	15 (93.8)
9	1	1 (100.0)	1 (100.0)
10	7	6 (85.7)	7 (100.0)
11	17	16 (94,1)	14 (82.4)
Total	88	86 (97.7)	80 (90.9)

Discussion

During the first epidemic of tularemia in the Sokobanja region in 1999, the oropharyngeal form of tularemia was not recognized (6, 19). The disease was associated with an excessive regional neck lymphadenitis and/or tonsillopharyngitis; although, remarkably, the lymph node enlarge-ment was most often unilateral, as well as tonsi-llitis. Even though, it was the most common form (93.8 %) and typically unilateral (92%), oropha-ryngeal form could often be misinterpreted as some other infectious or non-infectious diseases, such as streptococcal angina, infectious mononucleosis, tuberculosis and lymphoma. An absence of response to B-lactamic antibiotics, raised suspicion that it was some other disease, which was in ac-cordance with the experience of other authors (20-22).

The data on the most frequent cervical unilateral localization of lymphadenopathy and tonsillopharyngitis, mostly exudative unilateral (6, 19, 23), corresponded to the results of those authors who described oropharyngal tularemia as the most common form (in Bosnia, Bulgaria and Turkey) (24-27). On the contrary, the involvement of axillar and inguinal lymph nodes is most frequent in ulceroglandular tularemia, as reported by other authors (28, 29).

In the first cases of tularemia, rash was predominant in the clinical picture, especially on the hands, which caused diagnostic dilemmas (19) (Figure 3). The most frequent skin manifestations were erythema exudativum multiforme (25%) and erythema nodosum (25%). The similar skin changes, erythema nodosum, macular, popular and morbiliform rash, were reported by Golubović. Erythema multiforme-like skin lesions on the upper and lower extremities were noticed in 14% of tularemia patients, as reported by Christensen et al. (1984) (30). According to Akdis et al. (1993), 14.1% of cases developed erythema nodosum. These patients had a high level of polyclonal circulating immune complexes in comparison to the patients without skin lesions (31).

The complications such as suppurative lymphadenitis seen in 38% of our patients, were also described in Turkey (40%), caused by a delayed diagnosis and treatment during the epidemic of oropharyngeal form of tularemia type B (8). Recurrent lymphadenitis, noted in 16.8% of our patients, was also reported by Golubović et al. (1996) in the Republic of Srpska in 1995. According to this author, 17% of 141 patients had a recurrence, in some cases recurring 2-3 times (24).

Serology is commonly used to confirm tularemia due to low sensitivity of bacterial culture and the fact that molecular methods are not widely available. The results of serological tests should always be interpreted in the context of clinical suspicion of tularemia. Serological analysis should only be performed in patients with a real possibility of having tularemia, and not as a screening test for feverish patients.

A negative result of MAT, in the case of a clinically-epidemiologically clear picture of tulare-mia does not exclude tularemia, but suggests that higher sensitivity tests, such as ELISA and immune blot, should be used (15, 32).

In an early phase of the infection, false-negative results of MAT are often seen, and it is thus necessary to test one more serum sample after two weeks. While some patients have never developed seroconversion, others have stayed seropositive for years. These seropositive patients could have had crossed antibodies because of the infections with other bacteria (Yersinia, Brucella) (33).

The latest results of serum sample examinations with commercial (IgG Serion ELISA, IgM Serion ELISA, Serazym ELISA) and non-commercial (ELISA in-house) immunoenzyme assays have shown a higher sensitivity and specificity of all tests (sensitivity 98-99%, specifity 90-97%) compared with the results from the previous testing with ELISA (VMA) (sensitivity 93%, specifity 94.4 %) (6, 15).

It has been confirmed that ELISA is more sensitive and specific than agglutination tests (10, 34). The advantage of ELISA is that it can determine separately the different antibody classes: IgM, IgG and IgA. In contrast to agglutination which can detect mainly IgM antibodies and at least two serum samples are required to confirm the disease, ELISA requires only one sample for diagnosis. Furthermore, ELISA can detect antibodies in infection earlier compared to agglutination; the percentage of detection during the first week is 43%, vs 11% for agglutination (35). The disadvantage of this method is that it can not differentiate between an acute and past infection, because all classes of antibodies, IgM, IgA and IgG, rise and decline at the same time (18). The monitoring of IgG and IgM titer levels and determination of the IgG/IgM ratio, can be important in detecting the onset of infection (34).

According to results of Koskela, the antibody responses against *F. tularensis* are generally detectable 10-20 days post-infection. Among the three used tests, ELISA, agglutination test and comple-

ment-fixation ELISA, the most efficiant is ELISA IgM, IgA and IgG for an early serodiagnosis of tularemia (36).

Analyzing the tests results, it was confirmed that most tests shared similar high sensitivity and specifity values. Based on the estimation of accordance between all the tests, the best accordance was seen for IgG Serion ELISA and Serazym ELISA (k = 0.802), then IgM and VIRapid (k = 0.780), IgG and IgM Serion ELISA (k = 0.766), IgM and VIRapid (k = 0.780), and IgG and VIRapid (k = 0.760). It was established, that the best test to confirm the diagnosis of tularemia, based on sensitivity and specificity, was IgG Serion ELISA test.

In our research, the persistence of both IgM and IgG antibodies was seen in the sera of patients, even as long as 12 years after the infection. This is in agreement with the results of several authors who monitored humoral immunity in a longer period of time after natural infection (Ericsson: 25-year follow-up; and Koskela: 8-year follow- up) (33, 36).

Patient is considered to suffer from tularemia when he has clinical symptoms compatible with tularemia, as well as a positive serology on *F. tularensis* antibodies. The persistence of antibody positivity for longer periods of time without clinical manifestations of the disease, indicates a prior, not a current infection. In our experience, there was not any cases of reinfection, and thus it was not necessary to control consecutive serum samples in two weeks, nor to determine the IgG/IgM ratio.

The reason for such an extremely long persistence of *F. tularensis* antibodies after infection, shown by different authors, is still under investigation (33, 37). According to the study by Ericsson, the humoral immune response decreases 25 years after natural infection with *F. tularensis*. Serum agglutinins titers were low: out of 53 patients, only two had titers >40 (33). The role of serum antibodies which develop after the infection is less known (37).

Tularemia is not present in a latent form and the patient can not be reinfected even in a state of immune deficiency. The patients are completely cured from this bacterial disease.

Conclusion

Tularemia in South-eastern Serbia is an endemic disease, according to the earlier clinical-epidemiological studies (6). A positive correlation between the clinical-epidemiological and serological diagnosis of tularemia has been established. Serological findings have to be interpreted only within the context of clinical diagnosis of tularemia, not independently and separately from the clinical picture. A detection of IgM and IgG classes of antibodies or total antibodies to *F. tularensis* in the sera of patients without clinical manifestations of the disease, up to 12 years from the infection onset, does not indicate a current, but a past infection.

References

1. Penn RL. Epidemiology, microbiology, and pathogenesis of tularemia. "cited 2013 Sep 10". Available from: http://www.uptodate.com/contents/epidemiology-microbiology-and pathogenesis-of-tularemia.
2. Jacobs RF. Tularemia. In: Harrison TR, Fauci AS, Braunwald E, editors. Harrison's Principles of Internal Medicine. 15th ed. New York: McGraw-Hill Companies; 2001. p. 990–3.
3. Sjöstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. Ann N Y Acad Sci 2007; 1105:1–29. [CrossRef] [PubMed]
4. Tärnvik A, Berglund L. Tularaemia. Eur Respir J 2003; 21(2):361–73. [CrossRef] [PubMed]
5. World Health Organization. World Health Organization Guidelines on Tularaemia. Geneva: World Health Organization; 2007.
6. Đorđević M. Kliničko epidemiološke karakteristike i značaj imunodijagnostičkih testova kod tularemije [magistarski rad]. Niš: Medicinski fakultet, Univerzitet u Nišu; 2008.
7. Syrjala H, Karvonen J, Salminen A. Skin manifestations of tularemia: a study of 88 cases in northern Finland during 16 years (1967–1983). Acta Derm Venereol 1984; 64(6):513–6. [PubMed]
8. Helvaci S, Gedikoğlu S, Akalin H, Oral HB. Tularemia in Bursa, Turkey: 205 cases in ten years. Eur J Epidemiol 2000; 16(3):271–6. [CrossRef] [PubMed]
9. Tarnvik A, Chu MC. New approaches to diagnosis and therapy of tularemia. Ann N Y Acad Sci 2007; 1105:378–404. [CrossRef] [PubMed]
10. Syrjälä H, Koskela P, Ripatti T, Salminen A, Herva E. Agglutination and ELISA methods in the diagnosis of tularemia in different clinical forms and severities of the disease. J Infect Dis 1986; 153(1):142–5. [CrossRef] [PubMed]
11. Porsch-Ozcürümez M, Kischel N, Priebe H, Splettstösser W, Finke EJ, Grunow R. Comparison

of enzyme-linked immunosorbent assay, Western blotting, microagglutination, indirect immunofluorescence assay, and flow cytometry for serological diagnosis of tularemia. Clin Diagn Lab Immunol 2004; 11(6):1008–15. [CrossRef] [PubMed]
12. Schmitt P, Splettstösser W, Porsch-Ozcürümez M, Finke EJ, Grunow R. A novel screening ELISA and a confirmatory Western blot useful for diagnosis and epidemiological studies of tularemia. Epidemiol Infect 2005; 133(4):759–66. [CrossRef] [PubMed]
13. Splettstoesser W, Guglielmo-Viret V, Seibold E, Thullier P. Evaluation of an immunochromatographic test for rapid and reliable serodiagnosis of human tularemia and detection of *Francisella tularensis*-specific antibodies in sera from different mammalian species. J Clin Microbiol 2010; 48(5):1629–34. [CrossRef] [PubMed]
14. Kiliç S, Celebi B, Yeşilyurt M. Evaluation of a commercial immunochromatographic assay for the serologic diagnosis of tularemia. Diagn Microbiol Infect Dis 2012; 74(1):1–5. [CrossRef] [PubMed]
15. Ristanović E. Tularemija vojnička bolest. Savremena mikrobiološka dijagnostika. Beograd: Novinsko izdavački centar; 2002.
16. Hotta A, Uda A, Fujita O, Tanabayashi K, Yamada A. Preparation of monoclonal antibodies for detection and identification of *Francisella tularensis*. Clin Vaccine Immunol 2007; 14(1):81–4. [CrossRef] [PubMed]
17. Gyuranecz M, Szeredi L, Makrai L, Fodor L, Mészáros AR, Szépe B, et al. Tularemia of European Brown Hare (*Lepus europaeus*): a pathological, histopathological, and immunohistochemical study. Vet Pathol 2010; 47(5):958–63. [CrossRef] [PubMed]
18. Splettstoesser WD, Tomaso H, Al Dahouk S, Neubauer H, Schuff-Werner P. Diagnostic procedures in tularaemia with special focus on molecular and immunological techniques. J Vet Med B Infect Dis Vet Public Health 2005; 52(6):249–61. [CrossRef] [PubMed]
19. Kostić V, Jovanović B, Krstić M, Mitrović G, Spasić M, Veličković Z. Tularemia. Naša prva iskustva. Acta medica Medianae 2000; 1:73–9.
20. Alonso Ovies A, Redondo Gonzalez LM, Lobo Valentin P, Bachiller Luque P, Martin Luquero M, Verrier Hernandez A. Tularemia in the differential diagnosis of cervical lymph node enlargement. An outbreak of tularemia in Castilla-Leon, Spain. Acta Otorrinolaringol Esp 2000; 51(1):62–7. [PubMed]
21. Evans ME, Gregory DW, Schaffner W, McGee ZA. Tularemia: a 30-year experience with 88 cases. Medicine (Baltimore) 1985; 64(4):251–69. [CrossRef] [PubMed]
22. Luotonen J, Syrjälä H, Jokinen K, Sutinen S, Salminen A. Tularemia in otolaryngologic practice. An analysis of 127 cases. Arch Otolaryngol Head Neck Surg 1986; 112(1):77–80. [CrossRef] [PubMed]
23. Djordjevic-Spasic M, Potkonjak A, Kostic V, Lako B, Spasic Z. Oropharyngeal tularemia in father and son after consumption of under-cooked rabbit meat. Scand J Infect Dis 2011; 43(11-12):977–81. [CrossRef] [PubMed]
24. Golubović S, Verhaz A, Rodić Ž, Tešić Z. Klinički fenomeni kod oboljelih od tularemije u ratom zahvaćenim područjima 1995. In: Zbornik radova Drugog kongresa ratne medicine sa međunarodnim učešćem; 1996 April 24-27; Banja Luka, Bosnia and Herzegovina; 1996. p. 11-6.
25. Kantardjiev T, Ivanov I, Velinov T, Padeshki P, Popov B, Nenova R, et al. Tularemia outbreak, Bulgaria, 1997-2005. Emerg Infect Dis 2006; 12(4):678–80. [CrossRef] [PubMed]
26. Meric M, Willke A, Finke EJ, Grunow R, Sayan M, Erdogan S, et al. Evaluation of clinical, laboratory, and therapeutic features of 145 tularemia cases: the role of quinolones in oropharyngeal tularemia. APMIS 2008; 116(1):66–73. [CrossRef] [PubMed]
27. Celebi G, Baruönü F, Ayoğlu F, Cinar F, Karadenizli A, Uğur MB, et al. Tularemia, a reemerging disease in northwest Turkey: epidemiological investigation and evaluation of treatment responses. Jpn J Infect Dis 2006; 59(4):229–34. [PubMed]
28. Eliasson H, Back E. Tularaemia in an emergent area in Sweden: an analysis of 234 cases in five years. Scand J Infect Dis 2007; 39(10):880–9. [CrossRef] [PubMed]
29. Ohara Y, Sato T, Homma M. Arthropod-borne tularemia in Japan: clinical analysis of 1,374 cases observed between 1924 and 1996. J Med Entomol 1998; 35(4):471–3. [CrossRef] [PubMed]
30. Christenson B. An outbreak of tularemia in the northern part of central Sweden. Scand J Infect Dis 1984; 16(3):285–90. [CrossRef] [PubMed]
31. Akdis AC, Kilicturgay K, Helvaci S, Mistik R, Oral B. Immunological evaluation of erythema nodosum in tularemia. Br J Dermatol 1993; 129(3):275. [CrossRef] [PubMed]
32. Chaignat V, Djordjevic-Spasic M, Ruettger A, Otto P, Klimpel D, Wolfgang Müller W, et al. Performance of seven serological assays for diagnosis of tularemia. BMC Infect Dis 2014; 14:234. [CrossRef] [PubMed]
33. Ericsson M, Sandstrom G, Sjostedt A, Tamvik A. Persistence of cell-mediated immunity and decline of humoral immunity to the intracellular bacterium *Francisella tularensis* 25 years after natural infection. J Infect Dis 1994; 170(1):110–4. [CrossRef] [PubMed]
34. Carlsson HE, Lindberg AA, Lindberg G, Hederstedt B, Karlsson KA, Agell BO. Enzyme-linked immunosorbent assay for immunological diagnosis of human tularemia. J Clin Microbiol 1979; 10(5): 615–21. [PubMed]
35. Viljanen MK, Nurmi T, Salminen A. Enzyme-linked immunosorbent assay (ELISA) with bacterial sonicate antigen for IgM, IgA, and IgG antibodies to *Francisella tularensis*: comparison with bacterial agglutination test and ELISA with lipopolysaccharide antigen. J Infect Dis 1983; 148(4):715–20. [CrossRef] [PubMed]
36. Koskela P, Salminen A. Humoral immunity against *Francisella tularensis* after natural infection. J Clin Microbiol 1985; 22(6):973–9. [PubMed]
37. Tarnvik A. Nature of protective immunity to *Francisella tularensis*. Rev Infect Dis 1989; 11(3):440–51. [CrossRef] [PubMed]

Originalni rad **UDC: 616.993(497.11)**
doi:10.5633/amm.2017.0105

TULAREMIJA U JUGOISTOČNOJ SRBIJI U DVANAESTOGODIŠNJEM PERIODU PRAĆENJA

Marina Đorđević-Spasić[1], Miodrag Vrbić[1,2], Maja Jovanović[1,2], Lidija Popović-Dragonjić[1,2], Aleksandar Ranković[1]

Klinika za infektivne bolesti, klinički centar Niš, Srbija[1]
Univerzitet u Nišu, Medicinski fakultet Niš, Sbija[2]

Kontakt: Marina Đorđević-Spasić
Vizantijski bulevar 94/9, 18000 Niš, Srbija
E-mail: marina_djordjevic@yahoo.com

Tularemija je ozbiljna bakterijska zoonoza uzrokovana visoko infektivnim agensom *Francisella tularensis*. Mikrobiološka dijagnoza tularemije se uglavnom zasniva na serološkim testovima. Pojava epidemije tularemije na jugoistoku Srbije 1998/1999. godine pokrenula je kako epidemiološka tako i klinička i mikrobiološka istraživanja u ovoj oblasti.

Cilj rada bio je utvrđivanje korelacije između kliničko-epidemiološke i serološke dijagnoze tularemije kao i kliničko i serološko praćenje bolesnika u periodu od jedne do 12 godina od početka oboljevanja.

Ispitivanjem je obuhvaćena grupa od 113 bolesnika obolelih od tularemije u periodu od početka 1999. do kraja 2011. Kontrolna grupa je obuhvatila 111 ispitanika sa limfadenopatijama različite geneze. Korišćeni su serološki testovi: mikroaglutinacioni test (MAT), imunoenzimski testovi: ELISA (VMA) i ELISA "in house", Serion ELISA classic *Francisella tularensis* IgG i Serion ELISA classic *Francisella tularensis* IgM, Serazym Anti-*Francisella tularensis* ELISA i imunohromatografski test (ICT)-VIRapid.

Kod svih 113 bolesnika sa kliničko-epidemiološkom dijagnozom tularemije bolest je potvrđena serološki. Potvrđena je visoka senzitivnost i specifičnost ispitivanih testova. ELISA IgG Serion test je pokazao najveću osetljivost (97,4%) i specifičnost (93,1%). IgM i IgG antitela se održavaju u serumu obolelih u visokom procentu i posle 12 godina od infekcije. Orofaringealna tularemija je najzastupljenija klinička forma bolesti (93,8%) sa dominantnom jednostranom cervikalnom limfadenopatijom (91,5%). Komplikacije je imalo 41,6% bolesnika, sa najvećom zastupljenošću apscedirajućeg i recidivnog limfadenitisa.

Utvrđena je pozitivna korelacija između kliničko-epidemiološke i serološke dijagnoze tularemije. Rezultati dobijeni serološkim testovima se moraju tumačiti u sklopu kliničke slike tularemije. Nalaz antitela IgM i IgG klase ili ukupnih antitela na *F. tularensis* u serumima bolesnika bez kliničke slike posle 12 godina od početka bolesti nije pokazatelj akutne, već preležane bolesti. *Acta Medica Medianae 2017;56(1):31-38.*

Ključne reči: *tularemija, Francisella tularensis, dijagnoza, mikroaglutinacioni test, ELISA, imunohromatografski test*