



SANDFLY – PAPPATACI FEVER IN BOSNIA AND HERZEGOVINA: THE NEW-OLD DISEASE

MIRSADA HUKIĆ*, IRMA SALIMOVIĆ-BEŠIĆ

Institute of Clinical Microbiology, University of Sarajevo Clinics Centre,
Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

Sandfly fever viruses (SFV) are endemic in the Mediterranean, Middle East, northern African and western Asian countries. Toscana virus (TOSV), serotype of Sandfly fever Naples virus, is among of the three most prevalent viruses associated with meningitis during the warm seasons in northern Mediterranean countries. The historical data of the sandfly fever (Pappataci fever) indicates its origin in Bosnia and Herzegovina at the end of 19th century. There is a long period of time for which there are no data on research related to the SFV in Bosnia and Herzegovina. The purpose of the study was to investigate the presence of sandfly fever in Bosnia and Herzegovina in recent years. The 68 of serum samples were obtained from February 2006 until September 2008 from a group of patients with febrile illness of unknown etiology. The sera were tested on the presence of IgG and IgM antibodies against TOSV by specific serology test- *recom*Line Bunyavirus IgG/IgM immuno-line assay. The recent TOSV-infection was confirmed in the patients in each year during the study: 10,71% (3/28) in 2008; 9,38% (3/32) in 2007 and 12,50% (1/8) in 2006. The presence of specific antibodies to TOSV in the sera of the patients in recent years indicates re-emerging character of the disease in this region. It would be necessary to make biological, epidemiological and clinical research on the TOSV and related phleboviruses to elucidate the problem of SFV in Bosnia and Herzegovina.

KEY WORDS: *Phlebotomus*, Sandfly fever viruses, TOSV, phlebotomus fever, Pappataci fever

INTRODUCTION

Sandfly fever viruses are classified among family Bunyaviridae, genus *Phlebotomus*. They are endemic in the Mediterranean, Middle East, northern African and western Asian countries, where they represent a significant health problem, especially for non-native and non-immune visitors (1). These viruses are responsible for a human disease known as sandfly fever, phlebotomus fever or Pappataci fever. Although the genus *Phlebotomus* consists currently of 68 distinct virus serotypes, eight serotypes, namely Alenquer, Candiru, Chagres, sandfly fever Naples, Punta Toro, Rift Valley fever, SFSV and sandfly fever Toscana virus (TOSV), have been linked to disease in humans (2). TOSV is endemic in central Italy and described as the most frequent cause of aseptic meningitis in children in that region (3, 4). As a consequence of the life cycle of *Phlebotomus*, TOSV is more frequent during summer, with a peak in August. The most common presentation is an acute febrile illness or meningitis, and more rarely a meningoencephalitis (4-6). Most of the studies on TOSV have been done in central Italy, but the occurrence of TOSV in other countries such as France, Spain, Slovenia, Greece, Cyprus, Turkey and Egypt, has also been reported recently (7).

History of sandfly fever

The first who described the disease was Austro-Hungarian military doctor of medicine from Trebinje (Herzegovina), Czech Alois Pick. In 1886, he published an article entitled "Pathology and the therapy of the domestic endemic disease" in "Wiener medizinischen Wochenschrift" (8). He rejected the use of the name of the disease "gastroenteritis endemica" which was in use by many physicians, because of its medical nomenclature, and introduced a new term of the disease "the dog disease". The new term was commonly used by people and soldiers. In 1887, Pick was intensively involved in study of "the dog disease" (9), when the disease occurred around Trebinje and Stolac (Herzegovina). He knew that similar disease occurred in Mostar and Ljubuški (Herzegovina), and in Korčula and Dubrovnik (Croatia), as well. Foreigners were more exposed to the disease than native population. Also, other scientists were study about "the dog disease". Without knowledge of Pick, Gabel investigated the disease in Mostar, Trebinje and Stolac (Herzegovina), and thought on "acclimatization disease", that had never been noted in Spring, Autumn and Winter time (10). In 1904, Taussig performed broad clinical and epidemiological research and found out the existence

of "Pappataci" mosquitos in some military barracks in Herzegovina (11). These "Pappataci" mosquitos hit only during the night. "The dog disease" was appeared just in places where "Pappataci" mosquitos occurred. In 1907, Giovanni Battista Grassi (1854-1925) studied about "*phlebotomus papatasii*" in Rome and described this type of mosquito (12). In summer 1908, leadership of Committee constituted of physicians Franz, Taussig and Doerr took the mission to explore the infective agent of the disease (13). Doerr found out that a virus is a causative agent and the infected pappataci mosquito is vector of the disease. That was assumed by Taussig. Committee made the issue to term the disease as "Pappataci fever", respectively, in honor of Grassi "Phlebotomus fever" (13). However, at the beginning of the history of development of the disease is the name of Alois Pick, who gave the origin of the disease in Bosnia and Herzegovina. During the World War II, the disease affected large numbers of soldiers serving in the Mediterranean operations, especially USA Army personnel. The disease was first recognized among American soldiers when the morbidity rate of febrile cases increased during the period they spent in North Africa (end of April 1943). Study of the infected military personnel led to the clinical description, the discovery of the vector of the disease (*Phlebotomus papatasii*) and the isolation of the responsible viruses, namely sandfly fever Sicilian virus (SFSV) and sandfly fever Naples virus (SFNV) (14). After World War II, the disease occurred in Serbia and the first epidemic of pappataci fever in this Republic was described by Karakašević in 1947 (15). The epidemic affected East Serbia, then Belgrade, where there were several thousand sick persons. In the same year, the first cases occurred in Banat, and in 1950 a new epidemic struck the southern part of that region, where over 80% of the inhabitants were taken ill (16). The epidemics remained without etiological confirmation, and the presumptive diagnosis was based on the clinical picture, epidemiological data and entomological findings.

There is a long period of time for which there are no data on research related to the sandfly fever (phlebotomus fever or Pappataci fever) in Bosnia and Herzegovina. According to the historical data of the phlebotomus fever in Bosnia and Herzegovina and emergence of Toscana Virus in Europe, finding disease in this region is to expect. Aim of this study was to investigate the presence of sandfly fever in Bosnia and Herzegovina (B&H) in a group of patients with febrile illness of unknown etiology, from 2006 till 2008.

MATERIAL AND METHODS

The study was retrospective. The 68 serum samples were collected from February 2006 until September 2008 from a group of patients with febrile illness of unknown etiology. All the patients were hospitalized in University of Sarajevo Clinics Centre, Bosnia and Herzegovina. Samples sera were tested on the presence of IgG and IgM antibody directed against TOSV by the *recomLine* Bunyavirus IgG/IgM immuno-line assay (Mikrogen GmbH, Germany) according to the manufacturer's instructions. The test is based on specific recombinant antigens for the determination of IgG- and IgM-antibodies against Hantavirus (serotypes Hantaan, Puumala, Seoul and Dobrava) as well as Sandfly Fever Virus (serotype Toscana) in human serum or plasma. According to the manufacturer's instructions a positive IgM-finding without a positive IgG-finding is evaluated as borderline. A serum with a borderline or even negative IgM result and positive IgG result could indicate a recent infection. In acute cases of the disease, IgG and IgM antibodies should be detected, confirming the diagnosis.

RESULTS

Anti-TOSV IgG and IgM were analyzed in 68 human serum samples. Recent infection was found in seven patients (10,29%). The findings are presented in Table 1, Figure 1 and 2.

Year	Results	<i>recomLine</i> Bunyavirus IgG/IgM immuno-line assay	
		<i>anti-TOSV IgG</i>	<i>anti-TOSV IgM</i>
2008.	Positive	3 (10,71%)	2 (7,14%)
	Negative	25 (89,29%)	26 (92,86%)
	Recent infection	3 (10,71%)	
	Total samples	28 (100%)	
2007.	Positive	3 (9,38%)	
	Negative	29 (90,62%)	32 (100%)
	Recent infection	3 (9,38%)	
	Total samples	32 (100%)	
2006.	Positive	1 (12,50%)	1 (12,50%)
	Negative	7 (87,50%)	7 (87,50%)
	Recent infection	1 (12,50%)	
	Total samples	8 (100%)	
TOTAL CASES WITH RECENT INFECTION		7 (10,29%)	
TOTAL SAMPLES		68 (100%)	

TABLE 1. Prevalence of anti-TOSV antibodies detected by *recomLine* Bunyavirus IgG/IgM immuno-line assay (Mikrogen GmbH, Germany) in group of patients with febrile illness of unknown etiology in BiH during the years 2006-2008.

Positive IgM-finding without a positive IgG-finding was found in three samples of sera. They are evaluated as borderline (Figure 1).

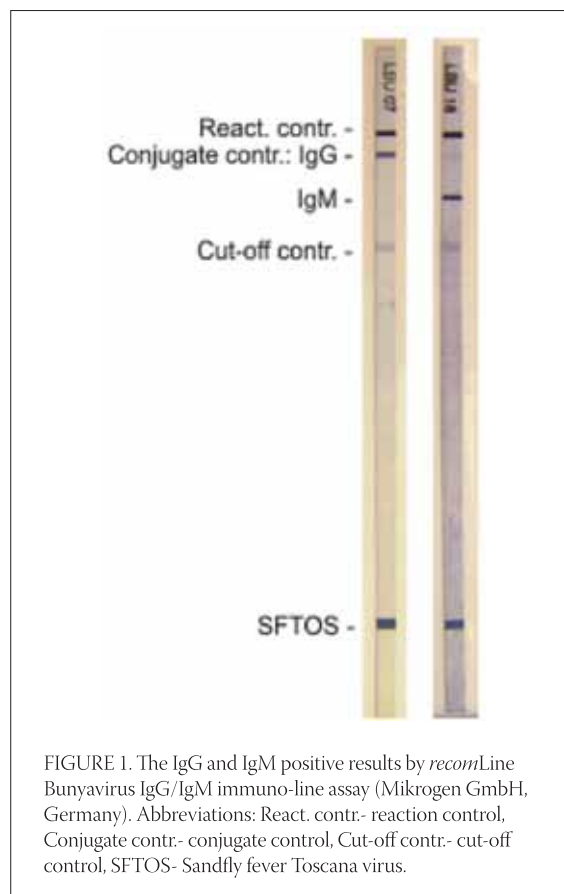
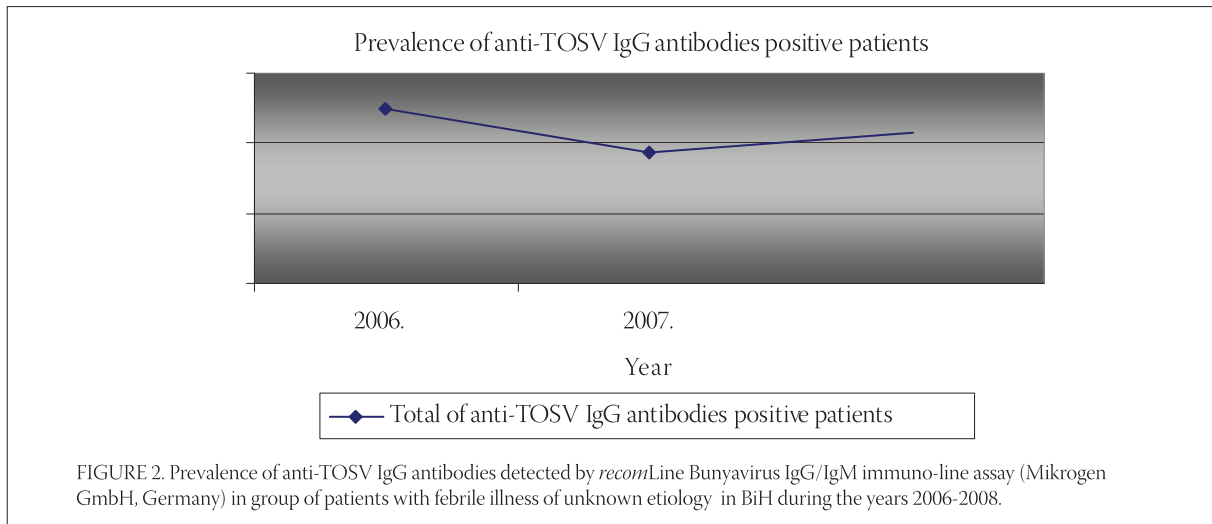


FIGURE 1. The IgG and IgM positive results by *recomLine* Bunyavirus IgG/IgM immuno-line assay (Mikrogen GmbH, Germany). Abbreviations: React. contr.- reaction control, Conjugate contr.- conjugate control, Cut-off contr.- cut-off control, SFTOS- Sandfly fever Toscana virus.

There was not a case with IgM and IgG antibodies against TOSV present at the same time. The recent infection was confirmed in the patients in each year during the study: 10,71% (3/28) in 2008; 9,38% (3/32) in 2007 and 12,50% (1/8) in 2006 (Figure 2).

DISCUSSION

Before World War II, the pappatasi fever in the former Yugoslavia was known mainly in Herzegovina, Dalmatia, Montenegro and especially in Macedonia, where its prevalence coincided with that of *Phlebotomus pappatasi*. The first cases of pappatasi fever in Bosnia and Herzegovina were detected at the end of 19th century (8, 9). Most of the clinical and epidemiologic studies have been conducted in Bosnia and Herzegovina between 1886 and 1962 (18). After that period, interest for the pappatasi fever has disappeared. There is no published data about the TOSV infection after 1971 in the B&H, the year when the virus was originally isolated in central Italy (19). Our study presents the evidence of occurrence of the TOSV infection among the selected group of patients with febrile illness of unknown etiology. We have found



IgM positive results in three cases (two in 2008, and one in 2006). However, no positive findings in IgG were detected for these patients. These results were evaluated as borderline and it was recommended to retest the new serum samples. We did not have the results of the retested samples so we could not confirm or reject the diagnosis of TOSV-infection. In all other cases, IgG positive findings represented the recent infection. In 2007, our laboratory experiences showed a large number of bacteriological sterile liquor samples (687/720; 95,4%) of patients with CNS disease (unpublished data). This could imply that virus was ethological agent of the infection in those patients. The recent infection was found in 9,38% to 12,50% of patients during the period of three years (2006 – 2008). The percentage of the positive sera in that period is less than forty years ago. In 1975-1976 Tesh et al. (20) made the first extensive serological investigations in this field, reported their findings, indicating the high prevalence of the antibodies to Naples and Sicilian sandfly fever viruses in population of Dalmatia, North Croatia and Kosovo. On the island of Brač, 57,6% of the population had antibodies for the Naples virus and 15,6% for the Sicilian virus; in Kosovo- 27,9% had Naples antibodies and 9,6% had Sicilian. In this research on the prevalence of the arbovirus infection, Terzin et al. (18) discussed the data on the detection of residual antibodies for

the Naples virus in the inhabitants of Bosnia and Herzegovina in 1962. In subsequent work (21), it has been seen that the prevalence of the Naples virus infection reached 62,1%. The significant importance was the finding of antibodies in 3,0% of children aged 7-16 years. The similar results were obtained by Salja and associates in Kosovo, in 1980 (22). These results indicated the continual circulation of Naples virus in nature. The results of faunistic and ecological investigations of the sandfly vectors associated with this virus have been published (16,23-32). The oldest data on the study of sandflies in former Yugoslavia, exactly in Bosnia and Herzegovina, are in the works of Strobl (33,34), where the detection of the *Phlebotomus papatasi* was first mentioned. The virus of Naples pappataci fever was the first causative agent of the disease isolated in former Yugoslavia (35). Finding the TOSV positive results as member of the genus *Phlebovirus* in clinical samples after more than forty years (19), means that the virus has still circulating in this region, but has not been the point of interest of the local explorers. It is very important to direct the attention of clinicians to sandfly fever (pappataci fever) because the disease was unrecognized in B&H in the last time. It would be necessary to make the research on the TOSV and related phleboviruses in its natural reservoir and in men, as well as to compare the results with data available in literature.

CONCLUSION

This study confirms that TOSV as a serotype of Sandfly fever Naples virus circulates in Bosnia and Herzegovina for centuries. The presence of such phlebovirus in recent years indicates re-emerging character of the TOSV infection in this region.

Despite increasing evidence of its major role in medicine as an emerging cause of CNS infections, TOSV remains an unstudied pathogen. The recent data on TOSV circulation in Mediterranean countries and so in Bosnia and Herzegovina raises concern about potential implications for blood donations, because of the asymptomatic course of illness in majority of cases.

REFERENCES

- (1) Tesh R.B. The genus Phlebovirus and its vectors. *Annu. Rev. Entomol.* 1988; 33: 169–181.
- (2) Liu D.Y., Tesh R.B., Travassos Da Rosa A.P. et al. Phylogenetic relationships among members of the genus Phlebovirus (Bunyaviridae) based on partial M segment sequence analyses. *J. Gen. Virol.* 2003; 84: 465–473.
- (3) Valassina M., Cuppone A.M., Bianchi S., Santini L., Cusi M.G. Evidence of Toscana virus variants circulating in Tuscany, Italy, during the summers of 1995 to 1997. *J Clin Microbiol.* 1998; 36: 2103-2104.
- (4) Valassina M., Meacci F., Valensin P., Cusi M. Detection of neurotropic virus circulating in Tuscany: the incisive role of Toscana Virus. *J. Med. Virol.* 2000; 60: 86-90.
- (5) Dionisio D., Valassina M., Ciufolini M.G. et al. Encephalitis without meningitis due to sandfly fever virus serotype Toscana. *Clin. Infect. Dis.* 2001; 32: 1241-1243.
- (6) Baldelli F., Ciufolini M.G., Francisci D. et al. Unusual presentation of life-threatening TOSV meningoencephalitis. *Clin. Infect. Dis.* 2004; 38: 515-520.
- (7) Charrel R.N., Gallian P., Navarro-Mari J.M. et al. Emergence of Toscana virus in Europe. *Emerg. Infect. Dis.* 2005; 11: 1657-63.
- (8) Pick A. Zur Pathologie und Therapie einer eigenthümlichen endemischen Krankheitsform. *Wien. Med. Wschr.* 1886; 33: 1141-1145.
- (9) Pick A. Beiträge zur Pathologie und Therapie einer eigenthümlichen Krankheitsform (Gastro-enteritis climatica). *Prager Med. Wschr.* 1887; 12: 364.
- (10) Gabel W. Eine acute Infections- und Acclimatisationskrankheit. *Wien. med. Wschr.* 1900; 50: 164.
- (11) Taussig S. Die Hundskrankheit, endemischer Magenkatarrh in der Herzegowina. *Wien. klin. Wschr.* 1905; 18: 126-136, 163-169.
- (12) Grassi G.B. Ricerche sui flebotomi. *Roma* 1907, zit. Nach R. Doerr und V.K. Ruß: Weitere Untersuchungen über das Pappataciefieber. *Archiv f. Schiffs- und Tropenhygiene* 1909; 13: 706.
- (13) Doerr R., Franz K. und Taussig S. Das Papatasi Fieber. Leipzig-Wien: Franz Deuticke, 1909.
- (14) Sabin A.B., Philip C.B., Paul J.R. Phlebotomus (pappataci or sandfly) fever: a disease of military importance; summary of existing knowledge and preliminary report of original investigations. *JAMA* 1944; 125: 603–606.
- (15) Karakašević B. O prvoj epidemiji papatačijeve groznice na teritoriji NR Srbije. *Vojno sanitetski pregled* 1947; IV, 9/10, 224-228.
- (16) Simić Ć. O posleratnoj pojavi papatačijeve groznice u Srbiji i Banatu. *Glas Srpske akademije nauka CCIV. Odeljenje medicinskih nauka* 1951; 4: 143-152.
- (17) Doerr R., Franz K. und Taussig S. Das Papatasi Fieber. Leipzig-Wien: Franz Deuticke, 1909.
- (18) Terzin A.L., Matuka S., Fornazarić M.R., Hlača D.M. Antibodies against some arboviruses and against the Bedsonia antigen in sera of men, sheep and cattle in Bosnia and Herzegovina. *Acta Medica Yugoslavica* 1962; XVI: fasc.3-4, 301-317.
- (19) Verani P., Ciufolini M.G., Nicoletti L. et al. Ecological and epidemiological studies of Toscana virus, an arbovirus isolated from Phlebotomus. *Ann Inst Super Sanita.* 1982; 18: 397-399.
- (20) Tesh R.B., Saidi S., Gaidamovich S.J., Rodhain F., Vesenjaj-Hirjan J. Serological studies on the epidemiology of sandfly fever in the Old World. *Bull. World Health Organ.* 1976; 54: 663-674.
- (21) Vesenjaj-Hirjan J., Galinović-Weiglass M., Urlič V. et al. Occurrence of Arboviruses in the Middle and the South Adriatic (Yugoslavia). *Arboviruses in the Mediterranean Countries, Zbl. Bakt. Suppl.* Gustav Fischer Verlag, Stuttgart- New York, 1980; 303-310.
- (22) Salja S., Imami O., Galinović-Weiglass M., Calisher C.H., Lazuić J.S., Vesenjaj-Hirjan J. Arbovirus infections in the region of Kosovska Kamenica (Yugoslavia). *Arboviruses in the Mediterranean Countries. Zbl. Bakt. Suppl.* 9, Gustav Fischer Verlag, Stuttgart-New York, 1980; 285-289.
- (23) Simić Ć. Prilog poznavanju flebotomus-a u Jugoslaviji. *Vojnosanitetski pregled*, 1945; II, 10/11, 40-54.
- (24) Simić Ć., Živković V. Prilog poznavanju faune naših flebotomina, I deo. Makedonija, južni deo Srbije i Kosmet. *Glas Srpske akademije nauka CXCIV, Odeljenje medicinskih nauka* 1949; 1: 151-181.
- (25) Simić Ć., Kostić D., Nežić E., Živković V. Prilog poznavanju flebotomina Jugoslavije. VI deo. Flebotomine Vojvodine, Bosne, Hercegovine, Dalmacije i Istre. *Glas Srpske akademije nauka CCII, Odeljenje medicinskih nauka* 1951; 3: 81-86.
- (26) Živković V. Prilog poznavanju faune naših flebotomina. VI deo. Istočna, zapadna i severna Srbija. *Glas Srpske akademije nauka CXCVII, Odeljenje medicinskih nauka* 1950; 2: 153-168.
- (27) Živković V. Flebotomine (Diptera, Psychodidae) jugoistočne i istočne Srbije. *Glas Srpske akademije nauka i umetnosti CCLXXI, Odeljenje medicinskih nauka* 1967; 20: 179-188.
- (28) Živković V., Mišćević Z. Fluctuations des populations de flebotomes (Diptera, Psychodidae) dans les habitations humaines et les abris des aminateux domestiques dans deux villages de la region de Niš. *Ekologija* 1972; 7: 197-206.
- (29) Živković V., Mišćević Z. Sandflies (Diptera, Psychodidae) in human dwellings and animal quarters of the village of Azbresnica (region of Niš). *Acta veterinaria, Beograd* 1973; 23: 225-233.
- (30) Živković V., Mišćević Z. Le cycle d'activite nocturne des phlébotomes (Diptera, Psychodidae) a la lumière artificielle dans les agglomérations. *Acta parasitologica Yugoslavica* 1979; 10: 3-13.
- (31) Mišćević Z. Faunistička i ekološka ispitivanja flebotomina (Diptera, Psychodidae) u jugoistočnoj Srbiji na području Dobriča, sa posebnim osvrtom na ishranu i mogućnost prenošenja uzročnika oboljenja. *Doktorska disertacija* 1979; pp. 240.
- (32) Živković V. Faunistic and ecological investigations of Sandflies (Diptera, Psychodidae) in Serbia. Part I. Phlebotomus papatasi (Scopoli 1786) and Phlebotomus sergenti Parrot 1917. *Beograd, Acta veterinaria* 1980; 30: 67-88.
- (33) Strobl P.G. Dipterenfauna von Bosnien, Herzegovina und Dalmatien. *Wiss. Mitt. Bosn. Herz.* 1900; VII: 552-670.
- (34) Strobl P.G. Neue Beitrage zur Dipterenfauna der Balkanhalbinsel. *Wiss. Mitt. Bosn. Herz.* 1904; IX: 519-581.
- (35) Gligić A., Mišćević Z., Tech R.B., Travassos da Rosa A., Živković V. First isolation of Naples sandfly fever virus in Yugoslavia. *Mikrobiologija* 1982; 19: 167-175.