ium difficile in a hospital in Japan and risk factors for infection. *Eur J Clin Microbiol Infect Dis* 2003; 22: 525–529.
14. Barbut F, Mastrantonio P, Delmée M *et al.* Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterization of the isolates. *Clin Microbiol Infect* 2007; 13: 1048–1057.
15. Lyras D, O'Connor JR, Howarth PM *et al.* Toxin B is essential for virulence of *Clostridium difficile*. *Nature* 2009; 458: 1176–1179.
16. Van den Berg RJ, Claas EC, Oyib DH *et al.* Characterization of toxin A-negative, toxin B-positive *Clostridium difficile* isolates from outbreaks in different countries by amplified fragment length polymorphism and PCR ribotyping. *J Clin Microbiol* 2004; 42: 1035–1041.
17. Baines SD, O'Connor R, Freeman J *et al.* Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*. *J Antimicrob Chemother* 2008; 62: 1046–1052.
18. Peláez T, Cercenado E, Alcalá L *et al.* Metronidazole resistance in *Clostridium difficile* is heterogeneous. *J Clin Microbiol* 2008; 46: 3028–3032.
19. Cai W, Lu Q, Zhang J *et al.* Analysis of antibiotic use in Shanghai second and third class hospitals in 2006. *China Pharmacy* 2008; 19: 810–813 (in Chinese).

Epidemiology and diagnostics of visceral leishmaniasis in Serbia

Z. D. Dakic[1], M. R. Pelemis[2], G. D. Stevanovic[2], J. L. Poluga[2], L. S. Lavadinovic[3], I. S. Milosevic[2], N. K. Indjic[4], I. V. Ofori-Belic[1] and M. D. Pavlovic[2]

1) *Parasitological Laboratory,* 2) *Department for Tropical Diseases* 3) *Intensive Care Unit, Institute for Infectious and Tropical Diseases, Bulevar oslobodjenja, Belgrade, and* 4) *Microbiological Laboratory, Military Medical Hospital, Bulevar Zorana Djindjica bb, Nis, Serbia*

Abstract

A retrospective epidemiological and diagnostic study of visceral leishmaniasis (VL) was carried out during the period 2001–2007 and included patients suspected of VL who had been diagnosed at the Parasitological Laboratory at the Institute for Infectious and Tropical Diseases, Belgrade. Diagnosis of VL was confirmed by microscopic examination of Giemsa-stained bone marrow (BM) smears. BM smears from 134 patients were examined; 22 cases of VL were diagnosed, the majority of which involved individuals who had been on holiday at the Montenegrian sea coast. The sensitivity of the initial BM smears was inadequate; this required the application of a serological test, adapted for routine use, for the diagnosis of VL.

Keywords: Bane marrow smear, diagnosis, epidemiology, indirect hemagglutination assay, rapid dipstick rK39 test, visceral leishmaniasis

Original Submission: 25 August 2008; **Revised Submission:** 19 November 2008; **Accepted:** 24 November 2008
Editor: G Pappas
Article published online: 21 April 2009

Clin Microbiol Infect 2009; **15:** 1173–1176
10.1111/j.1469-0691.2009.02768.x

Corresponding author and reprint requests: Z. Dakic, Institute for Infectious and Tropical Diseases, Parasitological Laboratory, Bulevar oslobodjenja 16, 11000 Belgrade, Serbia
E-mail: zorda_dakic@yahoo.com

The epidemiology of leishmaniasis is extremely diverse, with 12 million people infected worldwide [1]. In the Mediterranean basin, visceral leishmaniasis (VL) is caused by *Leishmania infantum*, known as 'Mediterranean kala-azar' (MVL). The diagnosis of MVL is complex and requires detection of parasites in bone marrow (BM) smears [2]. Sporadic autochthonous cases of VL have occurred in southern Serbia (unpublished data). An epidemiological and diagnostic study of VL was carried out at the Institute of Infectious and Tropical Diseases in Belgrade, where most VL cases are treated.

The study was carried out from January 2001 to December 2007 and included two groups (one of patients with suspected VL and one control group). Informed consent was obtained from all patients. Suspected VL was defined on the basis of a history of fever of ≥14 days with either clinical splenomegaly or wasting syndrome. Clinical suspicion was strengthened if the patient was from an endemic area or had travelled to one in the recent past. Definitive diagnosis of VL was based on direct microscopic demonstration of amastigotes in the BM smear. If the initial BM smear was negative but the clinical index of suspicion was high, parasitological investigation was repeated, or the diagnosis was based on the clinical picture and positive serology.

All patients with suspected VL (n = 134) were examined by performing a Giemsa-stained BM smear. BM aspirates were obtained by sternal puncture or, if the quality of the sternal aspirate was poor, by iliac crest biopsy. Starting in December 2004, 26 patients were further tested using the strip-test [3] and an indirect haemagglutination assay (IHA). The Rapid Dipstick rK39 test (strip-test; DiaSys Europe Ltd, Wokingham, UK) is a qualitative membrane-based immunoassay using the recombinant antigen K39, which is part of the *L. chagasi* kinesin-related protein and is specific for all members of the *L. donovani* complex [4]. In the IHA (Behring

Diagnostics GmbH, Marburg, Germany), human erythrocytes sensitized with *L. donovani* antigen agglutinate in the presence of *L. donovani* antibodies. The IHA was considered positive at a titre of >1:64.

The control group included 57 patients with other diagnoses, who were tested using IHA and the strip-test, but no BM aspiration.

In evaluation of each of the three methods, sensitivity and specifcity were calculated, sensitivity was calculated as (TP)100/(TP+FN), specificity was callculated as (TN)100 (TN+FP), where TP is the number of patients with true-positive results, FN is the number of patients with false-negative results, TN is the number of patients with false-positive results [5].

A total of 159 BM smears from 134 patients was examined: 134 of sternal punctures and 25 of iliac crest biopsies. Twenty-two patients had VL. The infections were contracted in Montenegro (n = 16, 72%), Herzegovina (n = 3, 13%), southern Serbia, Kosovo, or another southern European country [n = 1 (5%) each]. Six patients (27.3%) had different pre-existent diseases. The medium duration of illness before presenting to the investigating hospital was > 4 months. The initial examination of BM smears was successful in 86.36% (19/22) of the patients.

Among 26 patients suspected of having VL who were tested with the three diagnostic methods, 10 had VL. At the first examination, two had negative BM smears. In only one patient was the parasitological investigation repeated and VL confirmed. In another patient, the diagnosis was based on the clinical picture, serology and therapeutic effect (Table 1). The strip-test was the most sensitive test (100%), followed by IHA (90%) and the initial examination of BM smears (80%). Nine patients with VL had significant antibody titres as determined with IHA. All patients with imported malaria and other infectious and non-infectious diseases scored negative according to both the strip-test and IHA (Table 2).

In former Yugoslavia, VL was endemic in Macedonia, southern Serbia, the Montenegro coast, the south of Herzegovina and Dalmatia [6]. During the period 1945–1955, three epidemic waves of VL were recorded in Serbia. In the subsequent 3 years, only 17 cases were reported, the result of eradication of malaria vectors [7]. Rare autochthonous cases were noticed in 1968 and 1969 in Nis, where *Phlebotomus major, P. simići* and *P. perfiliewi* were identified [6].

According to epidemiological data, 39 VL cases were reported in Serbia and Montenegro from 1991 to 2000, with only one case being imported. In central Serbia, the incidence was 0.01/100 000 in 2007. The transmission was probably zoonotic, but animal reservoirs were unknown.

The predominant VL risk in the study patients was a stay at the Montenegrian sea coast, where as many as ten cases have been diagnosed each year in Bar [8]. Although a single case originated in southern Serbia, this could imply a dormant focus and a necessity of surveillance.

The long average duration of disease before a diagnosis was established in the study patients is the result of (i) pre-

TABLE 1. Characteristics of ten VL patients tested in parallel using three diagnostic methods from December 2004 to December 2007

Patient	Age/ gender	Origin of patients	Clinical presentation	Pre-existent diseases	IHA titre	Strip-test result	Initial BM smear
1	24/M	South Serbia	Fever, sweats, weight loss, pancytopenia, hepatosplenomegaly	No	1:1024	+	+ Numerous amastigotes
2	68/F	Montenegro sea-coast	Fever, pancytopenia, hepatosplenomegaly	Sarcoidosis	1:256	+	+ Moderate amastigotes
3	27/M	Herzegovina	Fever, heavy sweating, bicytopenia, hepatosplenomegaly	No	1:1024	+	+ Numerous amastigotes
4	28/F	Montenegro sea-coast	Fever, weight loss, splenomegaly	Ulcerative colitis	1:128	+	–[b]
5	68/F	Herzegovina	Fever, pancytopenia, hepatosplenomegaly	Diabetes mellitus, anemia	1:2048	+	–[c]
6	63/F	Montenegro sea-coast	Fever, fatigue, cough, weight loss, muscular pain, hepatosplenomegaly	No	1:16384	+	+ Numerous amastigotes
7	44/M	Montenegro sea-coast	Thrombocytopenia, skin rash, hepatosplenomegaly	No	1:16384	+	+ Numerous amastigotes
8	33/F	Montenegro sea-coast	Fever, pancytopenia, moderate hepatosplenomegaly	Chronic meningo -encephalitis[a]	1:2048	+	+ Numerous amastigotes
9	22/M	Herzegovina	Fever, night sweats, cough, fatigue, weight loss, pancytopenia, hepatosplenomegaly	No	1:32±	+	+ Rare amastigotes
10	69/M	Montenegro sea-coast	Fever, headache, bicytopenia, cough, night sweats, fatigue, muscular pain, arthralgia, hepatosplenomegaly	Chronic renal failure, diabetes mellitus	1:6536	+	+ Rare amastigotes

[a]Surgery for lumbar meningocoelae; congenital hydrocephalus.
[b]Parasitological investigation was repeated and Vh was confirmed (rare amastigotes were found).
[c]BM aspiration was not repeated. Diagnosis was based on clinical picture, positive serology and therapeutic effect.
M, male; F, female; +, positive; –, negative.

TABLE 2. Serological reactivities of patients with documented VL, patients with suspected VL, and patients with other diseases using IHA and the strip-test for the detection of anti-leishmania IgG antibodies

Study group	No. of positive patients			
	Total	IHA	Strip-test	BM smears
(i) Patients with suspected VL[a]	26	9 (34.6%)	10 (38.5%)	8 (30.8%)
Patients with documented VL[b]	10	9 (90%)	10 (100%)	8 (80%)
(ii) Control group[c] (x + y + z)	57			
(x) Protozoan infections	10			
Imported malaria	9	0	0	/
Toxoplasmosis	1	0	0	/
(y) Bacterial and viral infections	24			
Abscess	3	0	0	/
Sepsis	5	0	0	/
Brucellosis	2	0	0	/
Hepatic listeriosis	1	0	0	/
Infectious mononucleosis	2	0	0	/
Pneumonia	3	0	0	/
Varicella	1	0	0	/
Urinary tract infection	4	0	0	/
Hepatitis C	1	0	0	/
Coxsackie virus infection	1	0	0	/
Salmonellosis	1	0	0	/
(z) Other	23			
Lymphoma	10	0	0	/
Other malignancies	5	0	0	/
Diabetes mellitus	2	0	0	/
Thyroiditis	1	0	0	/
Juvenile rheumatoid arthritis	2	0	0	/
Systemic connective tissue disorders	2	0	0	/
Systemic lupus erythematosus	1	0	0	/

BM, bone marrow; IHA, indirect haemagglutination assay; VL, visceral leishmaniasis.
[a]Patients with a history of fever of ≥14 days with either clinical splenomegaly or wasting syndrome.
[b]Diagnosis of VL was confirmed on direct microscopic demonstration of *Leishmania* amastigotes in the BM smear or based on clinical picture and positive serology.
[c]Patients with the imported malaria and other infectious and non-infectious diseases.

existing diseases that can mimic VL [9,10], (ii) the diversity of the clinical pictures, and (iii) the rare incidence of VL (explaining why clinicians are not familiar with this disease) [1].

The sensitivity of BM smear examination is estimated at 70% or lower [11]. The number of fields and the duration of observation may augment the sensitivity [12]. In our hands, high sensitivity (86.36%) was expected since our microscopic examination included up to 1000 oil immersion fields, depending on the density of amastigotes. Smears were reported negative after only 1 h of microscopic examination.

The strip-test has the advantage of easy handling and quick results and it allows the detection of active VL [4,13]. However, the sensitivity varied considerably in different endemic areas. Sensitivity was highest in India and Nepal (100%) [11,13], but was significantly lower in southern Europe (71.4%) [3] and Sudan (67%) [14]. These variations may be the result of different subspecies of *L. donovani* complexes, of differences in the genetic background of the patients, and/or of differences in the duration and severity of the disease [15].

The IHA and strip-test were negative with sera of patients in the control group, contrary to previous reports [16]. The density of *Leishmania* amastigotes and the antibody titres according to IHA were not always correlated with each other (patient 10) or with the clinical condition (patients 4 and 9). Introduction of molecular methods for the detection of *Leishmania* species could contribute to explain these discrepancies.

The inadequate sensitivity of BM smear examination required the application of serological tests, adapted for routine use, to diagnose VL reliably. Although the sensitivity and specificity of both tests appeared satisfactory, their use in only a small number of cases precludes definitive conclusions. Their further evaluation in the study population is recommended.

Acknowledgements

The authors wish to thank A. Mijovic for assistance with the English language. Epidemiological data concerning VL in Serbia and Montenegro were obtained from the published reports of the former Federal Institute for Health Protection and the Institute of Public Health of Serbia 'Dr Milan Jovanovic Batut'.

Transparency declaration

The authors have no dual or conflicting interests to declare.

References

1. Iqbal J, Hira PR, Saroj G *et al.* Imported visceral leishmaniasis: diagnostic dilemmas and comparative analysis of three assays. *J Clin Microbiol* 2002; 40: 475–479.
2. Lachaud L, Dereure J, Chabbert E *et al.* Optimized PCR using patient blood samples for diagnosis and follow-up of visceral leishmaniasis, with special reference to AIDS patients. *J Clin Microbiol* 2000; 38: 236–240.
3. Jelinek T, Eichenlaub S, Löscher T. Sensitivity and specificity of a rapid immunochromatographic test for diagnosis of visceral leishmaniasis. *Eur J Clin Microbiol Infect Dis* 1999; 18: 669–670.
4. Burns JM, Shreffler WG, Benson DR, Ghalib HW, Badaró R, Reed SG. Molecular characterization of a kinesin-related antigen of

Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. *Proc Natl Acad Sci USA* 1993; 90: 775–779.
5. Ancelle T. *Statistique épidémiologie*, 2nd edn. Maloin: Paris, France, 2002.
6. Petrovic Z. *Epidemiology of kala-azar in Serbia*. Belgrade: Institute for Medical Research, 1980 (Serbian).
7. Milovanovic M, Popovic D. Addition to research of kala-azar epidemic in NR Serbia. *Voice Hyg Inst* 1960; 9: 23–27 (Serbian).
8. Ivovic V, Ivovic M, Miscevic Z. Sandflies (*Diptera*: *Psychodidae*) in the Bar area of Montenegro (Yugoslavia). *Ann Trop Med Parasitol* 2003; 97: 193–197.
9. Colovic MD, Jankovic GM, Colovic NR, Cemerikic-Martinovic V. Kala-azar and myelodysplastic syndrome in the same patient. *Haema* 2002; 5: 246–248.
10. Suvajdzic N, Pavlovic M, Misic S, Cemerikic V, Atkinson HDA, Colovic M. Secondary myelofibrosis in visceral leishmaniasis—case report. *Haematologia (Budap)* 2001; 31: 167–171.
11. Bern C, Jha SN, Joshi AB, Thakur GD, Bista MB. Use of the recombinant K39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. *Am J Trop Med Hyg* 2000; 63: 153–157.
12. Da Silva MRB, Stewart J, Costa CHN. Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. *Am J Trop Med Hyg* 2002; 72: 811–814.
13. Sundar S, Reed SG, Singh VP, Kumar PCK, Murray HW. Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* 1998; 351: 563–565.
14. Zijlstra E, Nur Y, Desjeux P, Khalil E, El-Hassan A, Groen J. Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from the Sudan. *Trop Med Int Health* 2001; 6: 108–113.
15. Carvalho SFG, Lemos EM, Corey R, Dietze R. Performance of recombinant K39 antigen in the diagnosis of Brazilian visceral leishmaniasis. *Am J Trop Med Hyg* 2003; 68: 321–324.
16. Islam MZ, Itoh M, Shamsuzzaman SM *et al.* Diagnosis of visceral leishmaniasis by enzyme-linked immunosorbent assay using urine samples. *Clin Diagn Lab Immunol* 2002; 9: 789–794.

Methicillin-resistant *Staphylococcus saprophyticus* in Sweden carries various types of staphylococcal cassette chromosome *mec* (SCC*mec*)

B. Söderquist[1,2] and C. Berglund[1]

Departments of 1) *Clinical Microbiology and* 2) *Infectious Diseases, Örebro University Hospital, Örebro, Sweden*

Abstract

Staphylococcus saprophyticus is a common cause of uncomplicated urinary tract infections and is usually susceptible to the antimicrobial agents used for their treatment. However, *S. saprophyticus* resistant to β-lactam antibiotics and carrying *mecA* has been reported. Eight Swedish isolates of *mecA*-positive *S. saprophyticus* with diverse origin carrying at least three different types of staphylococcal cassette chromosome *mec* (SCC*mec*) are described here.

Keywords: *mecA* gene, staphylococcal cassette chromosome *mec*, *staphylococcus saprophyticus*, urinary tract infection

Original Submission: 30 June 2008; **Revised Submission:** 3 September 2008; **Accepted:** 12 September 2008
Editor: J-L. Mainardi
Article published online: 18 May 2009

Clin Microbiol Infect 2009; **15:** 1176–1178
10.1111/j.1469-0691.2009.02771.x

Corresponding author and reprint requests: B. Söderquist, Department of Clinical Microbiology and Infectious Diseases, Örebro University Hospital, SE-70185 Örebro, Sweden
E-mail: bo.soderquist@orebroll.se

Staphylococcus saprophyticus, a coagulase-negative staphylococcus, is a common cause of uncomplicated urinary tract infections (UTI) in young and sexually active women. Routine laboratory identification of *S. saprophyticus* is mainly made on the basis of resistance to novobiocin, absence of haemolysis, and negative tests for coagulase and/or DNase. *Staphylococcus saprophyticus* is usually susceptible to the antimicrobial agents used for treatment of UTI.

However, during the late summer in 2007, three isolates of *S. saprophyticus* were noted at the Department of Clinical Microbiology, Örebro University Hospital, Sweden, that were resistant to ampicillin and cephadroxil as determined by disc diffusion test (AB Biodisk, Solna, Sweden) according to the Swedish Reference Group for Antibiotics (SRGA, http://www.srga.org). Detection of *mecA* was performed by using a multiplex PCR adapted for the LightCycler System with SYBR Green I as described previously [1], and the three isolates were found to carry the *mecA* gene. A retrospective survey of *S. saprophyticus* isolated from the period 2000–2008 revealed in 15 urine samples containing *S. saprophyticus* regarded as resistant to cephadroxil. Five of these isolates had been stored at −70°C and were subsequently further analysed. In addition, these five isolates were *mecA*-positive. Altogether, eight isolates were characterized by pulsed-field gel electrophoresis (PFGE) of chromosomal *SmaI* digests as described [1] and determined to the species level by ID32Staph (bioMérieux, Marcy l'Etoile, France).

Determination of the staphylococcal cassette chromosome *mec* (SCC*mec*) type I, II, III, IV and V was performed as described [2] except for the class B *mec* complex, which was identified according to Okuma *et al.* [3].