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IMPORTED PARASITIC INFECTIONS IN SERBIA

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Background: Travel to the tropics is associated with a risk of parasitic infection, which is increasing in parallel with the rise in travel to these areas. We thus examined the prevalence and trend in the occurrence of parasitic infections in Serbian travelers. *Methods:* A retrospective analysis of the medical records of all travelers returning from tropical and subtropical areas, who presented at the Institute for Infectious and Tropical Diseases in Belgrade between January 2001 and January 2008, was performed. *Results:* Of a total of 2440 travelers, 169 (6.9%) were diagnosed with a parasitic infection, including malaria in 79, intestinal parasites in 84 (pathogenic species in 30 and non-pathogenic in 54), filariasis in four, and visceral leishmaniasis and fascioliasis in one patient each. Importantly, of the whole series only 583 (23.9%) were symptomatic, of which 19.4% were found to be infected with a parasite. The single pathogenic parasite occurring in asymptomatic patients was *Giardia intestinalis. Conclusions:* Parasitic infection causing symptomatic disease among travelers returning from tropical areas to Serbia is not infrequent. In view of the expected increase in travel to the tropics, diagnostic protocols for tropical parasitic diseases should take these data into account.

Keywords: imported parasitic infections, malaria, intestinal parasites, Serbia, travelers

Introduction

Every year, millions of people travel abroad, exposing themselves to various diseases. The proportion of those who report some illness associated with travel is variable and ranges from 20–70% [1, 2]. Of these, tropical parasitic infections make a significant part, with malaria [3], schistosomiasis [4], leishmaniasis [5], strongyloidiasis [6], and filariasis, along with intestinal parasites [7], being the most significant ones.

The issue of imported tropical diseases was first brought up in Serbia in 1961 when a group of 96 children from Congo was accepted by the Yugoslav Red Cross. Tropical parasitic infections were found in even 74 (77%) children. It was the first time that filariasis due to *Wuchereria bancrofti* was ever diagnosed and treated in Serbia [8]. Over the next 15 years, a total of 244 cases of *Ancylostoma duodenale*, 132 *Strongyloides stercoralis*, 82 *Schistosoma haematobium*, 55 *S. mansoni*, 14 *S. intercalatum*, three *Leishmania donovani*, two *L. tropica*, one *Fasciolopsis buski*, 12 *W. bancrofti,* 10 *Dipetalonema perstans*, one *Loa loa*, one *Oncocerca volvulus,* and 140 cases of malaria, were diagnosed [8]. Between 1973 and 1983, Kecmanović et al. found even 21 cases of loiasis in travelers [9].

Imported parasitic diseases are increasing in non-endemic countries worldwide due to a highly increased number of travels. However, in Serbia, due to the conflicts and resulting economic devastation in the Western Balkans during the nineties, the number of travels was greatly reduced, a by-result of which was a low number of imported diseases. Since 2000, travel of Serbian citizens to tropical areas has been slowly but steadily increasing. Thus, this study was undertaken to examine the trend of imported tropical parasitic infections in the last decade.

Materials and methods

A retrospective analysis of the clinical history data of all travelers returning from tropical and subtropical areas, who presented at the Institute for Infectious and Tropical Diseases in Belgrade between January 2001 and January 2008, was performed. The Institute is the single reference center for tropical diseases in Serbia. The study series involved a total of 2440 travelers, and included those with (n=583; 23.9%) and without (n=1857; 76.1%) clinical symptomatology. The epidemiological data included age, sex, travel category (business, visiting friends and relatives, tourism), and travel destination. Post-travel examination is not mandatory in Serbia, and thus, in contrast to traveler populations in most developed countries, our series involved a majority of business-related travelers (mostly construction and forest workers or air crew members), as companies they work for advise them to report to our Institute after completion of travel, and a small number of tourists.

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The study was approved by the Clinical Center of Serbia Ethics Committee.

Irrespective of the occurrence of clinical symptomatology, all 2440 travelers were examined for malaria and intestinal parasites. In addition, diagnosis of specific infections caused by other species was attempted if clinically suspected, i.e. filariasis was searched for in 69, visceral leishmaniasis (VL) in nine, cutaneous leishmaniasis (CL) in three, schistosomiasis in 20, fascioliasis in three and extraintestinal amoebiasis in 25 patients.

Parasitological diagnosis was carried out using conventional techniques. Diagnosis of malaria was based on microscopic examinations of Giemsa-stained thick and thin blood smears. From December 2004 onwards, the rapid HRP2-based diagnostic test for *Plasmodium falciparum* (Visitect Malaria Pf, Omega Diagnostics Ltd., England) was also routinely used in patients with suspected malaria. Intestinal parasites were examined in stool samples by direct wet smear (with and without Lugol) and by the formol-ether concentration (FEC) according to Ritchie and the Lörincz flotation concentration techniques. In addition, specific methods were performed, including the *Baermann* technique or "agar-plate" methods for strongyloidiasis; rapid antigenic tests for *Giardia intestinalis* and *Cryptosporidium* spp. (Ridaquick Giardia; Ridaquick Cryptosporidium) or ELISA for the identification of parasitic copro-antigens (Ridascreen ELISA for *G. intestinalis, Cryptosporidium, Entamoeba histolytica*), and Kinyoun's acid fast and modified Ziehl-Neelsen staining methods for *Cryptosporidium*. Methods for parasites other than *Plasmodium* and intestinal parasites, applied according to clinical need, included the following: for filariasis, Knott's concentration method and Giemsa stained thick blood film were performed on blood samples drawn at different times during 24 hours. Diagnosis of VL was based on direct microscopic demonstration of amastigotes in the bone marrow (BM) smear, and/or serology which included the rapid dipstick rK39 test (strip-test; DiaSys Europe Ltd, Berkshire, England) and indirect hemagglutination assay (IHA) (Behring Diagnostics GmbH, Marburg, Germany). CL was examined in smears prepared by the skin scraping method and stained by Giemsa. Schistosomiasis was examined by the detection of schistosomal eggs and by serology. *Schistosoma* ova were looked for in stool specimens by direct smear and FEC methods, and in sedimented urine (collected between 10 am and 3 pm). Detection of *Schistosoma*-specific antibodies was performed using IHA (Behring Diagnostics GmbH, Marburg, Germany). Fascioliasis was examined by the detection of fluke eggs in multiple faecal samples. Extraintestinal amoebiasis (liver absces) was examined by serology, using IHA (Behring Diagnostics GmbH, Marburg, Germany) and IgG ELISA (Novagnost™, Dade Behring, Germany).

All commercial tests were performed and the results were interpreted according to the respective manufacturers' instructions.

Differences among groups were assessed by the chi-square test. The level of statistical significance was less than 5% ($p \leq .05$).

Results

Basic characteristics of the traveler series are presented in *Table 1.* Travelers were predominantly male (79.6%), and the majority were between 40 and 59 years of age (75.7%). The vast majority (98.5%) were Serbian natives. Most (76.1%) were asymptomatic, while among those with clinical complaints (23.9%), symptoms were unspecific and diverse, but fever was dominant (81.5%). Africa was by far the most visited region (96.1%), within which Nigeria, Cameroon, Benin, Equatorial Guinea and Ghana were the most visited countries.

All 2440 travelers were examined for malaria and intestinal parasitoses; 79 were diagnosed with malaria and 84 with intestinal parasites *(Table 2).* In addition, in travelers examined for other infections, four cases of filariasis, and one each of VL and fascioliasis were diagnosed. Among the four patients with filariasis, microfilaraemia was confirmed in one, while in three (two who returned from Equatorial Guinea and one from Côte d'Ivoire), the clinical diagnosis of loiasis was confirmed indirectly, by a successful therapeutic effect. Acute fascioliasis was diagnosed in one patient by EIA and Western blot at the Institute for Tropical Diseases in Hamburg in 2005, while repeated examinations of stool and duodenal aspirate were negative for *Fasciola hepatica* ova.

Overall, a total of 169 (6.9%) travelers were diagnosed with a parasitic infection. Of these, malaria accounted for 46.7% and intestinal parasites for 49.7%. Other infections including filariasis, VL and fascioliasis, were sporadic (3.6%).

All 79 malaria patients presented with fever as the chief symptom; of all febrile travelers, malaria was diagnosed in 16.6%. It was predominantly imported from Africa (96.1%). The most common causative species was *P. falciparum,* identified in 59 (75%) of all malaria cases, alone or in mixed infections. *Plasmodium* was not seen microscopically in nine cases (11%), and in those, the diagnosis was based on clinical presentation, travel history and therapeutic effect.

Of the intestinal parasites detected, 54 (64.3%) were mostly non-pathogenic intestinal protozoa (*Entamoeba coli*, *Entamoeba hartmanni*, *Dientamoeba fragilis*, *Chilomastix mesnili*, *Endolimax nana* and *Blastocystis hominis*), while only 30 (35.7%) were pathogenic species. Accordingly, the detected parasite caused symptomatic infection in 113 patients (malaria in 79, intestinal parasitic infections in 28, filariasis in four, VL and fascioliasis in one each), indicating parasitic etiology of disease in 19.4% of the 583 symptomatic patients. Conversely, parasitic infection remained asymptomatic in 56 travelers (3.0%, 56/1857); these included the 54 cases of infection with mostly non-pathogenic intestinal parasites and two cases of *G. intestinalis. G.*

Table 1. Characteristics of the series of 2440 travelers who presented at the Institute for Infectious and Tropical Diseases in Belgrade, Serbia, between 2001 and 2008

Characteristic	Number	%
Gender		
Male	1943	79.6
Female	497	20.4
Age		
< 10	20	0.8
10–19	15	0.6
20–29	110	4.5
30–39	366	15
40–49	1117	45.8
50–59	729	29.9
≥ 60	83	3.4
Travel destination		
Africa	2344	96.1
Asia	47	1.9
South Europe	34	1.4
South America	13	0.5
North America	2	0.1
Purpose of travel		
Work/business	2295	94.1
Tourism	80	3.3
Foreigners	37	1.5
Visiting friends/relatives	28	1.1
Clinical presentation		
Asymptomatic	1857	76.1
Symptomatic	583	23.9
Chief complaint or finding		
Fever	475	81.5
Respiratory symptoms	32	5.5
Gastrointestinal (diarrhea)	20	3.4
Eosinophilia	15	2.6
Hepatosplenomegaly	9	1.5
Skin or soft tissue infection	7	1.2
Other	25	4.3

intestinalis was the single pathogenic parasite found in both symptomatic and asymptomatic travelers, equally distributed ($p = 0.058$) among these groups *(Table 2).*

Discussion

This retrospective analysis of imported tropical parasites in 2440 Serbian travelers over a 7-year period revealed a low overall infection rate of 6.9%. Although conducted in a single center in Serbia, the study is quite representative of the entire traveler population seeking medical attention upon returning from tropical areas as all such patients are referred to the Institute for Infectious and Tropical Diseases in Belgrade. Another specificity of the setting is that the traveler series involved practically only the native population (98.5%), as opposed to most studies in which the traveler population is composed of native travelers as well as immigrants and refugees, mostly from areas endemic for tropical diseases. Thus, a recent Canadian study showed a prevalence of 29% of imported parasitic infections in a series of 3528 symptomatic patients, which included 24.5% immigrants and travelers visiting friends and relatives, the particular subgroups found to most likely be burdened with parasites [10].

Occurrence of parasitic infection, expectedly, differed greatly between symptomatic (19.4%) and asymptomatic (3%) travelers. Since the leading health problem in our se-

Table 2. Imported tropical parasitic infections in the series of 2440 travelers according to occurrence of clinical disease and travel destination

Parasitic infection	No. (%) of infected travelers	No. of infected symptomatic travelers	No. of infected asymptomatic travelers	Travel destination
Malaria	79 (3.2)	79	0	
P. falciparum	52 (2.1)	52	0	Cameroon (7), Equatorial Guinea (22), Ethiopia (1), Gabon (3), Ghana (3), Mali (1), Nigeria (11), Tanzania (1), Uganda (1), multiple African countries (1), multiple African countries and India (1)
P. vivax	10 (0.4)	10	0	Equatorial Guinea (3), Ethiopia (4), India (1), Pakistan (1), Uganda (1)
P. malariae	1 (0.04)	1	0	Democratic Republic of Congo (1)
mixed infections *	7 (0.3)	7	0	Equatorial Guinea (2), Ethiopia (2), Ghana (1), Nigeria (1), multiple African countries (1)
Plasmodium not detected	9 (0.4)	9	0	Brazil (1), Equatorial Guinea (4), Madagascar (1), Nigeria (1), South Africa (1), Zanzibar (1)
Intestinal parasites	84 (3.4)	28	56	
E. histolytica/dispar	18 (0.7)	18	0	Benin (2), Cameroon (2), Equatorial Guinea (4), Ethiopia (4), Gabon (1), Ghana (1), Nigeria (3), Uganda (1)
G. intestinalis	5 (0.2)	3	2	Benin (2), Cameroon (2), Nigeria (1)
Cryptosporidium spp.	4 (0.2)	4	0	Equatorial Guinea (1), Nigeria (1), South European countries (1), Uganda (1)
S. stercoralis	1 (0.04)	1	0	Multiple African countries (1)
T. trichiura	2 (0.08)	2	0	Equatorial Guinea (1), Nigeria (1)
Mostly non-pathogenic protozoa **	54 (2.2)	0	54	Cameroon (9), Democratic Republic of Congo (1), Equatorial Guinea (9), Ethiopia (6), Ghana (4), Nigeria (24), Uganda (1)
Other	6 (0.2)	6	0	
Filariasis	4 (0.2)	4	0	Equatorial Guinea (2), Côte d'Ivoire (1), South Europe (unclear travel history)
VL	1 (0.04)	1	0	multiple Asian countries (1)
Fascioliasis	1 (0.04)	1	0	South Europe (1)

* 4 mixed infections *P. falciparum* + *P. vivax* and 3 mixed infections *P. falciparum* + *P. malariae*
** *Entamoeba coli, Entamoeba hartmanni, Dientamoeba fragilis, Chilomastix mesnili, Endolimax nana, Blastocystis hominis*

ries was fever, it is not surprising that malaria was the most frequent imported parasitic infection in Serbia, occurring in 16.6% of all febrile patients. This is quite similar to other reports showing malaria in 17.9%, 21%, 25.7% and 16.7% of all febrile patients coming to Canada, France, Spain and the US, respectively [10–13]. The most commonly isolated species, as in other studies in European travelers [14, 15], including Croatia, another Western Balkan country [16], was *P. falciparum*. This is important since *falciparum* malaria may be fatal in the previously unexposed.

Most travelers with malaria visited Africa as forest or building workers in Equatorial Guinea and Ghana, or as air crew in Nigeria, Benin and Cameroon. Destination has been identified as the most important risk factor for acquiring malaria [17], and other tropical parasitic infections, during travel [1]. Another factor significantly contributing to these infections is the reason for travel. While in Switzerland three quarters of all reported cases of malaria occur in travelers who have been visiting friends and relatives [18], in our study the reason for travel was almost exclusively work/business. This also explains why our series consisted of an unproportionally higher number of male subjects.

The prevalence of imported intestinal parasites in studies mostly involving refugees and immigrants has been reported to range from 11% to 80% [7, 14, 19]. We found a remarkably lower prevalence (3.4%) of these infections but we were looking almost exclusively at our native travelers. However, among all those diagnosed with a parasitic infection, not unlike the 58% showed in Canadian travelers [10], intestinal parasites accounted for almost one half. Of

these, one third was infected with a pathogenic species, while two thirds harbored non-pathogenic species. Moreover, autochthonous origin of some intestinal parasites, especially non-pathogenic protozoa and *G. intestinalis,* may not be excluded, since a prevalence of 1.4% of non-pathogenic protozoa (including *E. coli* 1.3%, *E. hartmanni* 0.02% and *I. bütschlii* 0.02%) and of 6.8% of *G. intestinalis* has been reported in a series of 5981 healthy schoolchildren from throughout Serbia [20].

Finally, we observed few cases of filariasis, VL and fascioliasis, and interestingly no cases of extraintestinal amoebiasis, CL, or of schistosomiasis, whose prevalence as an imported infection in non-endemic countries is reported to be rising [21]. However, because only a small number of travelers were investigated for these diseases, the observed frequencies cannot be interpreted as their prevalence in the studied population.

In conclusion, the presented results show a relatively low presence of parasitic infections in the traveler population as a whole; however, among those who presented clinical signs and symptoms, parasitic infections accounted for as many as one fifth of all cases. These findings question the current practices in Serbia of screening the whole traveler population for malaria and intestinal parasites. According to the presented results, it seems more appropriate to examine for these infections only travelers who present with symptoms. Moreover, for malaria, molecular diagnosis, not available at our Institute at the time of the study (but currently introduced), is needed for the detection and species identification of *Plasmodium* in submicroscopical cases [22, 23].

Imported tropical parasites raise the issue of their establishment and spread in areas where they are not autochthonous. Although there is no such imminent danger at this time, global climatic changes and the resulting changing environmental conditions may favor such a development. In view of the expected increase in the volume of travel from the Western Balkan Countries to tropical areas in the future, development of adequate diagnostic protocols for imported tropical parasitic infections is needed.

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References

1. Steffen R, Rickenbach M, Wilhelm U, Helminger A, Schär M: Health problems after travel to developing countries. J Infect Dis 156, 84–91 (1987)
2. Ryan ET, Wilson ME, Kain KC: Illness after international travel. New Engl J Med 347, 505–516 (2002)
3. Sabatinelli G, Ejov M, Joergensen P: Malaria in the WHO European Region (1971–1999). Euro Surveill 6, pii=213 (2001)
4. Jelinek T: Imported schistosomiasis in Europe: preliminary data for 2007 from TropNetEurop. Euro Surveill 13, pii=8038 (2008)
5. Malik AN, John L, Bruceson AD, Lockwood DN: Changing pattern of visceral leishmaniasis, United Kingdom, 1985–2004. Emerg Infect Dis 12, 1257–1259 (2006)
6. Nuesch R, Zimmerli L, Stockli R, Gyr N, Christoph Hatz FR: Imported strongyloidosis: a longitudinal analysis of 31 cases. J Travel Med 12, 80–84 (2005)
7. Miller JM, Boyd HA, Ostrowski SR, Cookson ST, Parise ME, Gonzaga PS, Addiss DG, Wilson M, Nguyen-Dinh P, Wahlquist SP, Weld LH, Wainwright RB, Gushulak BD, Cetron MS: Malaria, intestinal parasites, and schistosomiasis among Barawan Somali refugees resettling to the United States: a strategy to reduce morbidity and decrease the risk of imported infections. Am J Trop Med Hyg 62, 115–121 (2000)
8. Kecmanović M, Šuvaković V (1976): Tropical and parasitic diseases. In: Fifty Years of Institute for Infectious and Tropical Diseases "Dr Kosta Todorovic" in Belgrade, 1926–1976, Galenika, Belgrade, pp. 111–115
9. Kecmanović M, Pavlović M, Šuvaković V, Bakalović S, Mišić S, Gavrilović G, Brmbolić B (1983): Filariasis Loa. 4th Yugoslavian Congress of Infectologists, Belgrade, October 18–21. Proceedings, pp. 113–116
10. Boggild AK, Yohanna S, Keystone JS, Kain KC: Prospective analysis of parasitic infections in Canadian travelers and immigrants. J Travel Med 13, 138–144 (2006)
11. Ansart S, Perez L, Vergely O, Danis M, Bricaire F, Caumes E: Illnesses in travelers returning from the tropics: a prospective study of 622 patients. J Travel Med 12, 312–318 (2005)
12. Zamarrón Fuertes P, Pérez-Ayala A, Pérez Molina JA, Norman FF, Monge-Maíllo B, Navarro M, López-Vélez R: Clinical and epidemiological characteristics of imported infectious diseases in Spanish travelers. J Travel Med 17, 303–309 (2010)
13. Hill DR: Health problems in a large cohort of Americans traveling to developing countries. J Travel Med 7, 259–266 (2000)
14. Monge-Maillo B, Jiménez BC, Pérez-Molina JA, Norman F, Navarro M, Pérez-Ayala A, Herrero JM, Zamarrón P, López-Vélez R: Imported infectious diseases in mobile populations, Spain. Emerg Infect Dis 15, 1745–1752 (2009)
15. Jelinek T, Peyerl-Hoffmann G, Mühlberger N, Wichmann O, Wilhelm M, Schmider N, Grobusch MP, von Sonnenburg F, Gascon J, Laferl H, Hatz C, Alifrangis M, Burchard G, McWhinney P, Schulze M, Kollaritsch H, da Cunha S, Beran J, Kern P, Gjørup I, Cuadros J: Molecular surveillance of drug resistance through imported isolates of Plasmodium falciparum in Europe. Malaria J 1, 11 (2002)
16. Perić D, Skrobonja I, Skrobonja A: Malaria in Croatia in the period between 1987 to 2006. Lijec Vjesn 131, 192–195 (2009)
17. Antinori S, Galimberti L, Gianelli E, Calattini S, Piazza M, Morelli P, Moroni M, Galli M, Corbellino M: Prospective observational study of fever in hospitalized returning travelers and migrants from tropical areas, 1997–2001. J Travel Med 11, 135–142 (2004)
18. Fenner L, Weber R, Steffen R, Schlagenhauf P: Imported infectious disease and purpose of travel, Switzerland. Emerg Infect Dis 13, 217–222 (2007)

19. Garg PK, Perry S, Dorn M, Hardcastle L, Parsonnet J: Risk of intestinal helminth and protozoan infection in a refugee population. Am J Trop Med Hyg 73, 386–391 (2005)
20. Nikolić A, Djurković-Djaković O, Bobić B: Intestinal parasitic infections in Serbia. Srp Arh Celok Lek 126, 1–5 (1998)
21. Bierman WF, Wetsteyn JC, van Gool T: Presentation and diagnosis of imported schistosomiasis: relevance of eosinophilia, microscopy for ova, and serology. J Travel Med 12, 9–13 (2005)
22. Rougemont M, Van Saanen M, Sahli R, Hinrikson HP, Bille J, Jaton K: Detection of four Plasmodium species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. J Clin Microbiol 42, 5636–5643 (2004)
23. Rantala MA, Taylor SM, Trottman PA, Luntamo M, Mbewe B, Maleta K, Kulmala T, Ashorn P, Meshnick SR: Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malaria J 9, 269 (2010)