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Massilia timonae Infection Presenting as Generalized Lymphadenopathy in a Man Returning to Belgium from Nigeria[▽]

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We report a case of apparent malaria infection presented with a syndrome of painless, generalized lymphadenopathy without granulomas shortly after exposure to fresh water in rural West Africa. Residual infection with *Massilia timonae* was diagnosed and successfully treated with co-trimoxazole.

CASE REPORT

A 52-year-old man, born in Nigeria and living in Belgium since 1996, was admitted to our hospital with a 7-day history of fever, myalgia, and lymphadenitis. The fever was intermittent, with spikes of fever following chills on alternating days. Two weeks earlier, he had returned from a 2-month visit to his family in Nigeria. He did not take any malaria prophylaxis and reported swimming in a lake outside Lagos. Once returned in Belgium, he continued his usual life as an unemployed person, spending most of his time indoors. His medical history revealed diabetes mellitus and previous malaria.

At the time of admission, the patient was conscious and alert; he had a temperature of 38.5°C, a pulse rate of 120 beats/min, and blood pressure of 120/80 mm Hg. Physical examination revealed multiple firm and nontender cervical, axillary, and inguinal lymphadenopathies, with a size up to 2 cm. Abdominal examination showed tenderness in the right upper quadrant. The rest of the physical examination was unremarkable.

Hematological investigations showed a hemoglobin level of 11.5 g/dl (reference values, 12.9 to 16.4 g/dl), a platelet count of 69×10^9/liter (reference values, 142×10^9 to 340×10^9/liter), and a total leukocyte count of 13.1×10^9/liter (reference values, 3.45×10^9 to 9.76×10^9/liter) with a normal differentiation. Biochemical investigations were normal, except for mildly elevated lactic dehydrogenase (LDH) (790 U/liter [reference values, 313 to 618 U/liter]) and elevated C-reactive protein (CRP) (30 mg/dl [reference value, <0.5 mg/dl]). A rapid diagnostic test (RDT) for malaria (BinaxNOW malaria test; Inverness Medical Binax, Inc., Scarborough, ME) was positive for *Plasmodium falciparum* protein antigen. According to the manufacturer, the overall sensitivity for *P. falciparum* of the BinaxNOW malaria test in an endemic population is 95.3% (95% confidence interval [CI], 93 to 97%) and the overall specificity for the same antigen is 99.8% (95% CI, 99 to 100%). Similar performance characteristics of the test were found in rounds 1 and 2 of the WHO RDT testing scheme (sensitivity, 100% in high-parasite density samples; specificity, 95%). Blood and urine cultures were sterile. Computed tomography scans were performed and showed diffuse lymphadenopathies with a diameter of 2 to 3 cm in the cervical, mediastinal, axillary, and pelvic regions. The liver and spleen were normal in size.

The patient was treated for malaria with oral quinine (1,500 mg daily) and doxycycline (100 mg twice daily) for 7 days. The clinical state of the patient improved, and he became afebrile within 3 days.

The enlargement of the lymph nodes persisted, and therefore a biopsy of a cervical lymph node was performed 2 weeks later. Histocytological analysis of the biopsy specimen revealed a diffuse nonspecific inflammation with infiltration by small and large lymphocytes. Immunohistochemical staining for the B-cell marker CD20 showed a preserved architectural structure of the lymph node with no arguments for B-cell lymphoma. There was some positivity for CD30, but the absence of cells with typical Reed-Sternberg morphology and negative staining for CD15 of these CD30-positive cells made a diagnosis of Hodgkin lymphoma very unlikely. In conclusion, no arguments for hematologic disease could be found.

Direct examination of the lymph node by Gram staining and Ziehl-Neelsen staining was negative. Lymph node culture on blood agar at 37°C showed growth of nonfermentative Gram-negative bacilli after 2 days. Tests for oxidase and catalase were positive.

Using a standardized disk diffusion technique with Neo-Sensitabs (Rosco Diagnostica A/S, Taastrup, Denmark) for *Pseudomonas aeruginosa*, the Gram-negative bacilli appeared to be susceptible to meropenem, piperacillin-tazobactam, ceftazidime, amikacin, ciprofloxacin, and co-trimoxazole and resistant to ampicillin, amoxicillin-clavulanate, and cefuroxime. The patient was treated with co-trimoxazole, and the lymphadenopathy resolved within 3 weeks.

To identify the bacterial isolate, a 16S rRNA sequence analysis was performed on the culture originating from the lymph node. Following DNA extraction, PCR amplification was executed using the MicroSeq 500 16S rDNA PCR kit (Applied Biosystems). After purification of the PCR product, analysis was performed with the ABI PRISM 310 genetic analyzer. The obtained sequences of the strain were aligned with the EMBL

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TABLE 1. Summary of six reported cases in literature of *M. timonae* infection

Patient age (yr), sex	Underlying medical condition	Diagnosis	Type of isolate	Reference
25, male	Common variable immunodeficiency	Meningoencephalitis	Blood	La Scola et al. (8)
36, male	None	Wound infection following elective orthopedic surgery	Pus collected intraoperatively	Sintchenko et al. (10)
29, male	None	Osteomyelitis	Femur	Lindquist et al. (9)
49, female	None	Cerebral pseudotumor	Cerebrospinal fluid	Lindquist et al. (9)
41, male	End-stage renal disease secondary to diabetic nephropathy and hypertension, hemodialysis	Sepsis	Blood	Lindquist et al. (9)
39, female	None	Sepsis	Blood	Lindquist et al. (9)

Nucleotide Sequence Database (accession number U54470) and displayed 99.7% homology for *Massilia timonae*. Based on the morphological characteristics, conventional biochemical tests results, and 16S RNA sequence analysis, the diagnosis of *M. timonae* lymphadenitis was made.

The genus *Massilia* belongs to the family *Oxalobacteraceae* (*Betaproteobacteria*) and, up to now, comprises five species: *M. timonae*, *M. dura*, *M. albidiflava*, *M. plicata*, and *M. lutea* (17). *M. timonae* was first described by La Scola et al. in 1998 based on a single isolate from the blood of an immunocompromised patient with meningoencephalitis (8). It was classified as a novel bacterium based on its unique phenotypic and genotypic characteristics. The use of 16S rRNA sequence analysis has led to the identification of five additional cases of *M. timonae* infection in humans (Table 1) and resulted in an emended description of the species in 2003 (9, 10). The organisms are Gram-negative medium straight rods. They are motile, predominantly by means of a single polar flagellum, but lateral flagella may also be present. Tests for oxidase and catalase are positive. Growth occurs at 25 and 35°C, on MacConkey agar, and in nutrient broth with 0% NaCl. Growth does not occur at 42°C, on SS agar, or in nutrient broth with 6% NaCl. The species is sensitive to polymyxin B.

The six reported cases in the literature demonstrate the wide range of clinical presentation of *M. timonae* infections. The source of infection appears to be unclear in all cases. In two patients with a tentative diagnosis of sepsis, the strain was isolated from the blood. One strain was isolated from cerebrospinal fluid of a patient with cerebral pseudotumor, one was isolated from a wound infection following elective orthopedic surgery, and one was isolated from bone with signs of osteomyelitis. The six reported cases had no common predisposing condition. One patient had a variable immunodeficiency, and one patient had an end-stage renal disease secondary to diabetic nephropathy and hypertension. No underlying medical conditions were known in the other four patients. In our patient, *M. timonae* was isolated from the lymph node and the infection manifested mainly as a generalized lymphadenopathy.

The sources of the infection are unknown in all reported cases. In one patient, abscessed teeth were suspected to be the source, suggesting that *M. timonae* may occur as part of the transient normal oral flora. In our patient, it is likely that the contamination with this organism occurred in Nigeria, possibly while swimming in a lake. Indeed, there is a growing body of evidence that *M. timonae* is an environmental organism. Comparison of 16S rRNA gene sequences with those of closely related species demonstrated that *M. timonae* is located within a cluster of soil-living bacteria (8). Furthermore, the isolation from soil samples of phenanthrene-degrading (3), protease-producing (5), and N-acyl homoserine lactone-producing strains (14) related to the genus *Massilia*, as well as four species of the genus *Massilia* isolated from different soil samples from southeast China (17, 18), confirms the environmental nature of the species. Recently, other *Massilia* strains have been isolated from air (11–13) and drinking water (6).

Malaria is widespread in tropical and subtropical regions, including parts of Africa, Asia, and Latin America. It is a mosquito-borne infectious disease caused by a microorganism of the genus *Plasmodium*. For travelers to areas in which the disease is endemic, malaria is a serious health hazard, and the disease is often diagnosed on return to the country of residence. Coinfection with malaria and a second pathogen is rarely reported in travelers. This could be explained by the fact that infection with a species of *Plasmodium* is responsible for most of the fevers in travelers to countries in which the infection is endemic. According to surveillance data from GeoSentinel, the global surveillance network of the International Society of Travel Medicine, and the Centers for Disease Control and Prevention, malaria is responsible for 62% of systemic febrile illness in travelers returning from sub-Saharan Africa (15). Additionally, malaria is easier to diagnose than many other infectious diseases, and consequently, other infections are not suspected unless patients remain symptomatic after treatment for malaria. Simultaneous infection and malaria have been reported with dengue (1), leptospirosis (16), brucellosis (2), nontyphoidal *Salmonella* (7), and Q fever (4). To our knowledge, this is the first report of *M. timonae* and malaria coinfection in the literature. Because the association of two diseases may result in an atypical clinical presentation, it is not easy to diagnose a coinfection based only upon clinical and epidemiologic characteristics. Therefore, extensive biological testing is crucial to establish the presence of coinfections when other signs and symptoms of residual disease persist after malaria treatment.

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