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Mediterranean spotted fever in Algeria — new trends

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Summary
Introduction: Mediterranean spotted fever (MSF) due to *Rickettsia conorii* is the most important tick-borne disease occurring in North Africa. However, there are only a few fragmentary reports on the epidemiology and clinical aspects of rickettsioses in North Africa, and cases are still rarely documented. We report herein a prospective study conducted in Oran, the second largest city in Algeria. This disease has not been properly described in Oran or in other Algerian cities.
Methods: A total of 167 cases of Mediterranean spotted fever were documented for the first time by the use of reference methods including immunofluorescence serology and Western blot and absorption studies, including isolation in culture by the shell-vial techniques, and molecular tools.
Results: Although some aspects of MSF were found to be in accordance with the general epidemiology of the disease, uncommon aspects were found, including increased incidence and the presence of multiple inoculation eschars in 12% of patients. The role of climatic changes in alterations of host-seeking and feeding behaviors of the vectors, including the brown dog tick *Rhipicephalus sanguineus*, is discussed. Also, 49% of patients were hospitalized with a severe form. The global death rate was 3.6%, but it was 54.5% in patients hospitalized with major neurological manifestations and multiorgan involvement.
Conclusions: The present report gives a unique panel of clinical aspects of MSF as well as new trends in this disease. Entomological, climatic, and molecular studies are needed to better understand both epidemiological and clinical aspects of MSF.

Introduction

Tick-borne rickettsioses are caused by the obligate intracellular bacteria spotted fever group (SFG) *Rickettsia spp* within the family *Rickettsiaceae* in the order *Rickettsiales*.[1] These zoonoses are among the oldest known vector-borne diseases, but they are also now recognized as emerging or reemerging

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human infections worldwide, with a dozen new tick-borne rickettsial species or subspecies recognized as human pathogens since 1984.[1,2] These diseases share characteristic clinical features, including fever, rash, and sometimes an inoculation eschar at the bite site, depending on the rickettsial agent that is involved.[1]

The first case of Mediterranean spotted fever (MSF) was reported in Tunisia, North Africa in 1910.[3] In the 1930s, the role of the brown dog tick, *Rhipicephalus sanguineus,* and the causative agent (*Rickettsia conorii*) were described.[4] Recently, the nomenclature of the several strains recognized as belonging to the so-called *R. conorii* complex has been modified and several species have been named including *R. conorii* subsp. *conorii* subsp. nov. (type strain = Malish, ATCC VR-613), the agent of MSF.[5]

There are only a few fragmentary reports on the ecology and epidemiology of rickettsioses in North Africa.[6–8] In Algeria, MSF due to *R. conorii* has been the sole human tick-borne rickettsiosis known by clinicians, although tick-borne rickettsial agents other than *R. conorii* have recently been detected in ticks, including *Rickettsia aeschlimannii* and *Rickettsia massiliae*. We report herein a prospective study conducted in Oran, the second largest city in Algeria.

Methods

Patients

All patients seen at the infectious diseases consultation department of the Oran Teaching Hospital with a suspicion of rickettsiosis (high fever, skin rash, headache, myalgia, arthralgia, and/or eschar) during the period between January 2004 and December 2005 were included in the study. Clinical and epidemiological data, laboratory results, treatments, and outcome were collected on a standardized form. A severe form of the disease was defined in hospitalized patients by extracutaneous organ dysfunction of at least one organ (neurological signs, renal insufficiency, shock, respiratory failure, etc.).[9,10] Informed consent was obtained from all patients.

Laboratory procedures

For each patient, an acute-phase serum sample was obtained within 2 weeks after the onset of symptoms and, when possible, a convalescent-phase serum sample (collected 1 to 2 weeks later) was also obtained. Sera were sent to the World Health Organization (WHO) collaborative center for rickettsial diseases in Marseille, France. IgG and IgM antibody titers were estimated by the immunofluorescence (IF) assay, using nine spotted fever group rickettsial antigens (*R. conorii conorii*, *R. conorii israelensis*, *Rickettsia africae*, *Rickettsia sibirica mongolotimonae*, *R. aeschlimannii*, *R. massiliae*, *Rickettsia helvetica*, *Rickettsia slovaca*, and *Rickettsia felis*) and a typhus group antigen, *Rickettsia typhi*.[11] The rationale for the antigen screening panel was supported by the presence of rickettsial species or subspecies in the Mediterranean area. The IF assay was considered positive if: (1) IgG titers were $\geq$128 and/or IgM titers $\geq$64 for *R. conorii* and (2) IgG titers were $\geq$64 and/or IgM titers $\geq$32 for other rickettsial antigens.[11,12] When cross-reactions were noted between several rickettsial antigens, the standard procedure of the Unité des Rickettsies was followed. This includes Western blotting and cross-adsorption studies to complement the IF assay, and is comprised of three steps:[13] (1) A rickettsial antigen was considered to represent the infectious agent if titers of IgG and/or IgM antibody against this antigen were at least two-fold higher than titers of IgG and/or IgM antibody against other rickettsial antigens. (2) When the difference in titers between several antigens was lower than two-fold, Western blot assays were performed. A rickettsial antigen was considered to represent the infectious agent if acute-phase or convalescent-phase sera reacted only with its specific antigens. (3) When Western blot assays were not diagnostic and IgG/IgM titers were $\geq$128/32, cross-absorption studies were performed. Specific diagnostic criteria after cross-absorption studies included: (a) positive IF serologic test results for a single antigen or (b) a Western blot assay showing exclusive reactivity with the specific proteins of a single agent.

Inoculation eschars were biopsied and frozen at −70 °C or kept in ethanol. Histologic analyses and immunohistochemical detection were performed as described previously.[14,15] Attempted cultivation of rickettsiae from skin biopsy specimens was performed using the shell-vial cell culture technique.[13,14] DNA was extracted from ground eschar biopsy specimens by use of the QIAamp tissue kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's recommendations. These extracts were used as templates in previously described PCR assays incorporating the primers 190–70 and 190–701, which amplify a 630-bp fragment of the *omp*A gene of SFG *Rickettsia spp*.[14,16] As negative controls, we used sterile water processed as described above, and DNA extracted from the heart valve of a patient with degenerative valvulopathy was incorporated into every six specimens. As a positive control, we used DNA from *Rickettsia montanensis*. The sequences of all positive PCR products were obtained, assembled, edited, and compared to those available in GenBank using a BLAST search, as previously described.[16] Clinical and epidemiological data were entered into and analyzed with EpiInfo version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Results

Mediterranean spotted fever confirmed cases

A total of 277 patients were included in the 2-year study, including 51% patients living in the city of Oran and 49% in surrounding rural areas. Most of the patients (80%) were included during the summer months from July to September. Half of the patients had received beta-lactam antibiotic regimens before being included in the study. Among the 277 patients included in this study, serum samples of 248 patients were studied. Among these patients, 191 (77%) had raised antibodies against SFG rickettsial antigens. After the Western blot and cross adsorption assays, a total of 161 cases were definitely confirmed to be *R. conorii* infections (Figure 1). The species involved could not be determined for 27 cases. Skin biopsy fragments of inoculation eschars were obtained from 44 cases. A total of 29 were positive by PCR, including those from six patients who tested negative by IF using their early course