Clinical and laboratory study

RICKETTSIAL INFECTIONS IN SLOVENIA

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ABSTRACT

Using indirect immunofluorescence, complement fixation, and enzyme immuno assay *Rickettsia conorii* and *Coxiella burnetii* infections were followed in suspected cases of rickettsial disease.

The study of patients sera for *Rickettsia conorii* antibodies elucidated two positive cases with tache noire, and nine cases with pathological skin changes and lymphadenopathy. The immune response to rickettsia antigens was found also in 10 out of 150 patients with an episode of tick bite.

Patients with a respiratory disease suffered from Q fever in 25 to 58%. In 30% of patients sera with symptoms of a viral disease *Coxiella burnetii* was involved as well as in 19% of those with hepatitis and in 24% of endocarditis cases.

KEY WORDS

Rickettsia conorii, Coxiella burnetii, Mediterranean spotted fever, Q fever

INTRODUCTION

The epidemic typhus (typhus exanthematicus) appears from time to time in an epidemic form, mostly during war-time. It is caused by an intracellular bacteria *Rickettsia prowazekie* and transmitted by the body louse. During the 2nd World War epidemics were observed in certain regions of former Yugoslavia.

The medical doctors are less aware of the tick-mediated Mediterranean spotted fever (MSF) (fievre boutonneuse) appearing mostly in the Mediterranean countries, in India and Africa (1,2). This rickettsiosis is caused by *Rickettsia conorii* and transmitted through bites by a variety of ticks (dog ticks and Ixodes species). The ethiologic agent of MSF belongs to the "spotted fever group" (SFG) agents, which include also the Rocky Mountain Spotted Fever (RMSF), caused by *Rickettsia rickettsii,* and numerous other species spread all over the world (3).

After the inoculation of rickettsia into the skin a small ulcer with black necrotic centre and a red areola (tache noire) develops on the site of the tick bite within 5 to 7 days. Later on the systemic symptoms may develop: headache, malaise, joint and stomach pains and, rarely even mental confusions. The elevated body temperature (39-40°C) lasts for 7-14 days. During this time, maculopapular or purpuric eruptions of the skin appear. In the past, MSF was considered a benign disease but several recent studies have shown a mortality rate of approximately 2% to 6% (4,5,6,7,8).

The other rickettsial disease, Q fever, appears in Slovenia accidentally when infection breaks out in domestic animals.

It is caused by *Coxiella burnetii*. This zoonosis is widespread in several domestic animals. The organism is being found in animals' placenta, mammary gland, and milk, through ticks can be involved in the transmission of agent as well (9). Cases of Q fever can often be traced to farms, tanneries, and slaughterhouses. The disease is well-known in Southern Europe, Northern Africa and Middle East (10).

Q fever has acute and chronic forms. The diagnosis of acute Q fever is seldom established except in setting of an epidemiologically investigated common source outbreak. Acute disease is characterized by the sudden onset of fever, headache and chills. Usually there is no rash. The incubation period is 2 to 4 weeks. The disease lasts for 5 do 30 days if patient is not properly treated. Many cases are subclinical, and appear as a mild or moderate self-limited respiratory disease but more often presented as atypical pneumonia and hepatitis. Hepatitis is evaluated and diagnosed usually in patients with granulomas seen in hepatitic biopsy specimens or in patients with culture-negative endocarditis. Rarely the affected person develops chronical infection, e.g. subacute endocarditis or other complications (11). The mortality rate in such cases may be as high as 60%.

In this study the results of the serological evaluations of patients suspected to be affected by MSF or Q fever are presented.

PATIENTS AND METHODS

Patients:
Sera of 679 patients suspected to be infected with *Rickettsia conorii* and *Coxiella burnetii* or sent to exclude rickettsial infections were collected. Out of these, 417 specimen were taken of the patients who might have had Mediterranean spotted fever and 262 for the evaluation of the *Coxiella burnetii* IgG antibodies. Additionally, we analyzed also 40 patients sera from the region where the coxiella zoonosis outbreak occurred.

Table 1. Patients sera tested for Rickettsia conorii infection

Clinical diagnosis	No. 417	Rickettsia conorii CF (titer >1:16)	Rickettsia conorii IIF-IgG (titer >1:160)
Febris mediterranea susp.	2	2	2
Rickettsioses susp.	62	0	0
Erythema nodosum	15	2	3
Exanthema maculopapulare	58	0	4
Lymphadenopathia	38	1	2
Borreliosis Lyme (tick bite)	150	5	10
St. febrilis	17	0	1
Others	75	1	2

Table 2: Serological analyses for Coxiella specific antibodies

Clinical diagnosis	No 262	Coxiella burnetii CF (%) (titer >1:16)	Coxiella burnetii IIF-IgG (%) (titer >1:160)
Bronchitis	2	0	0
Bronchopneumonia	24	14 (58)	14 (58)
Pneumonia	2	0	0
Pneumonia atypica	113	26 (23)	28 (25)
St. febrilis	6	0	0
Virosis i.o.	27	7 (26)	8 (30)
Endocarditis, myocarditis	17	3 (18)	4 (24)
Hepatitis	16	3 (19)	3 (19)
Acute renal failure	1	NT	0
Others	54	NT	0

Methods:
All sera were tested according to procedures accepted for laboratory diagnosis of rickettsial infections. We tried to detect specific IgG antibodies with the indirect immunofluorescence test (IIF-IgG) and with complement fixation test (CF). Some sera were tested for specific IgM and IgG antibodies to *Coxiella burnetii* phase I and phase II antigen by enzyme-immuno assay (ELISA).

RESULTS

The results of the analyses of patients sera assayed for antibodies to *Rickettsia conorii* or in whom this infection was to be excluded are shown in Table 1.

Routinely we looked for *Coxiella burnetii* specific IgG antibodies in the case of a suspected acute or chronic infection (Table 2).

The analyses of 40 patients' sera for phase II and phase I antibodies in ELISA test showed that 5 specimen taken from 10 patients with endocarditis and hepatitis reacted in IgG immune response to phase I. Specific IgM directed to phase II was detected in 3 cases of atypical pneumonia and in 1 case with clinical suspicion of virosis but IgG was proved in 13 out of 30 patients.

DISCUSSION

The ecosystem of Rickettsia in the Subalpine region of Slovenia is not yet completely understood. In the past we collected data on rickettsial infections among rodents and domestic animals in three Slovene regions: Vipava, Novo mesto and Ilirska Bistrica. The results showed that the prevalence of antibodies to *Rickettsia conorii* was high in the house mouse *Mus musculus* and field mouse *Apodemus agrarius* species (70 -90%). Antibodies to *Coxiella burnetii*

*Table 3. Mediterranean Spotted Fever Diagnostic Score**

	Points	Patient
Epidemiologic criteria		
Life or recent travel in endemic area	2	_____
Onset between May and September	2	_____
Contact with ticks	2	_____
Clinical criteria		
Temperature higher than 39 °C	5	_____
Eschar tache noire	5	_____
Maculopapular or purpuric eruption	5	_____
Two or three clinical criteria	3	_____
Three clinical criteria together	5	_____
Unspecific biologic criteria		
Platelet count <150 x 10^9 / L	1	_____
Liver enzymes >50 IU / L	1	_____
Bacteriologic criteria		
Isolation of Rickettsia conorii from blood	25	_____
Detection of Rickettsia conorii in skin biopsy using IFA	25	_____
Serologic criteria (immunofluorescence)		
Sole serum with total Ig >1:128	5	_____
Sole serum with IgG >1:128 and IgM >1:64	10	_____
Two sera with fourfold titer elevation within 2 weeks	20	_____
Total		_____

** Total score > 25 is consistent with a presumptive diagnosis of Mediterranean spotted fever (14)*

were investigated in sheeps and goats (12,4% and 4%). The data on rickettsial infections in vectors (ticks) are scarce. In a small group of ticks found on animals the rickettsia-infected Ixodes species prevailed (12).

As the isolations of rickettsia is a particularly hazardous for the laboratory personnel, serologic tests are mostly used for the confirmation of the clinical diagnosis. The tests currently used detect antibodies to rickettsial surface antigens. As the SFG rickettsias share common group antigens of the cell wall with other species, the tests like CF are less specific when compared to the tests detecting antibodies to species-specific S-protein or the rickettsial genome (13). The most commonly used serologic procedures are the enzyme-immuno assay and the indirect immunofluorescence test. The latter is the reference method. All rickettsial tests suffer from a lack of specificity due to the small amount of S-protein in antigen preparation as well as to cross-reactivity that may occur between SFG and typhus group of rickettsias (14).

IgM and IgG rickettsial antibodies appear between day 5 and 10 after the onset of fever. The IgG antibodies can persist for years. Detection of IgG and IgM antibodies in patients sera helps to prove a recent infection and to avoid false positive results due to cross-reactivity with antibodies (essentially IgM) to the lipopolysaccharides of other bacteria.

As expected, we were able to confirm the clinical diagnosis of MSF in both patients with a skin ulcer. In all other patients this rickettsiosis was rarely confirmed serologically. Out of 150 sera assayed for presumed Lyme borrelioses 10 turned to be positive for rickettsia (6,6%). This fact stresses the observation that in areas endemic for Lyme borreliosis infections with *Rickettsia conorii* are underdiagnosed.

Clinical manifestations of the MSF differs from RMSF in its less severe symptomatology and the presence of tache noire, an inoculation eschar at the site of the tick bite, but this occurs only in 70% of infected persons (15).

Beside this there are also subtypes of *Rickettisa conorii* causing Israelian tick typhus and Astrakhan fever in which the skin ulcer is uncommon. Therefore the diagnosis of rickettsioses is rather difficult and complex. There are several epidemiologic, clinical and other criteria to be considered for the correct diagnosis of infection. In this aspect we agree with Raoult and Brouqui (14) who proposed the diagnostic score for MSF (Table 3).

Lyme borrelisosis is endemic in Subalpine regions of Slovenia where during the 50s a serologic survey for arboviruses showed a high prevalence of antibodies to tick-born meningoencephalitis virus (TBE) (16). As rickettsia (according to our findings in the past), borrelia and TBE use the same vector for the transmission to the new host the agents must compete for their ecologic niche. This competition is reflected as a reduction of prevalence of TBE antibodies during the last decade in regions where borrelia has predominated (personal communication) and a very low prevalence of antibodies to *Rickettsia conorii.*

Coxiella burnetii, the etiologic agent of Q fever has a different way of spreading. Although the microbe can be transmitted from species to species (and also to men) by direct contact or through tick bite, however this occurs only very rarely. People become usually infected by inhaling the organism which is able to survive for months in the environment (10). It is obvious that the Q fever diagnosis is easier when epidemiologic data point to the disease (17).

Specific laboratory diagnosis is possible most often by serologic methods. Although *Coxiella burnetii* can be propagated in some laboratory animals or tissue cultures the isolation could be utilized in laboratories with P-3 level biohazard containment facilities. The risk of infection by handling specimen is high because as small an inoculum as one organism is capable of initiating infection.

Serologic methods currently used are complement fixation (CF) and indirect immunofluorescent antibody assay (IIF). *Coxiella burnetii* exhibits an antigenic variation with the appearing of antigens designated as antigens phase I and phase II. In the case of primary infection the patient develops both IgM and IgG antibodies to *Coxiella burnetii* phase II antigen which appear earlier than antibodies to phase I antigen. Antibodies to phase II antigen can be detected between the second and fourth weeks of infection. Later on the titers decrease. In the patients who develop chronic infection the IgG and IgA antibodies to *Coxiella burnetii* phase I antigen prevail and it can be provoked in high titers. Therefore chronic infection can be diagnosed with the single serum antibody titer to *Coxiella burnetii* phase I antigen (14). Out of 262 patients included in the study in cases with bronchopneumonia more than one half of sera (58%) appeared to have significant IgG titres. When the leading symptom was atypical pneumonia 25% of sera were reactive. The unpublished data on 146 cases of coxiella contacts during a zoonosis outbreak in Ilirska Bistrica (personal communication) showed that more than one half of persons (54%) had been infected with coxiella organism but only 24% of infected people developed respiratory disease.

CONCLUSION

Previous laboratory studies have demonstrated that reservoirs and vectors of *Rickettsia conorii do* exist in certain areas of Slovenia. In the present manuscript two typical cases of tache noire are mentioned. It has also to be stressed that in 10 out of 150 sera from patients initially diagnosed as Lyme borreliosis a diagnostic titer for antibodies to *Rickettsia*

conorii were detected. The same observation was made in 3 out of 15 cases diagnosed as erythema nodosum, in 4 cases of maculopapular rash, and in 2 with lymphadenopathy. These results should make dermatologists, infectologists as well as general practitioners aware of a possible Mediterranean spotted fever infection.

Similar conclusion is valid for infections with *Coxiella burnetii* causing the so-called Q fever.

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