THE JOURNAL OF INFECTIOUS DISEASES • VOL. 153, NO. 1 • JANUARY 1986
 0022-1899/86/5301-0019$01.00

Detection of Antibodies to *Rickettsia conorii* with a Latex Agglutination Test in Patients with Mediterranean Spotted Fever

Mediterranean spotted fever (MSF) is prevalent during the summer in southern France [1] and along the Mediterranean coast, as well as in other parts of the world, e.g., South Africa. The causative agent is *Rickettsia conorii*. Of methods currently used for serodiagnosis of MSF, the microimmunofluorescence (micro-IF) test for detection of antibodies to *R. conorii* is the test of choice. However, this test is not readily available, is time consuming, and requires personnel experienced in fluorescence microscopy. With this in mind we have developed and conducted a preliminary evaluation of a simple, rapid latex test (latex–*R. conorii*) for detection of antibodies to *R. conorii*. The new test could serve as a first line of testing for MSF, similar to the latex–*Rickettsia rickettsii* test [2] for Rocky Mountain spotted fever, which is currently used in some laboratories in the United States.

Materials and Methods

All sera from patients (476 sera from 422 patients) submitted to the Marseille laboratory for MSF testing during the 1983 season were tested by both micro-IF and latex-*R. conorii* at Marseille for this study. The patients were classified according to their clinical diagnosis [1] into four groups: definite, probable, possible, and not MSF (table 1). Selected sera from 135 patients with other diseases, some of which may be clinically confused with MSF, were also tested. This group comprised 62 patients with syphilis, 33 with measles, 29 with rubella, 6 with infectious mononucleosis, and 5 with cytomegalovirus infection.

Of the 476 sera, 276 (from 222 patients) were also tested by both micro-IF and latex–*R. conorii* at the New York Laboratory. Each laboratory withheld its results until all testing had been completed; the results were then exchanged.

The antigen used to coat the latex particles was erythrocyte-sensitizing substance (ESS [3]) obtained from *R. conorii* (Malish strain) grown in egg yolk sac and purified with renograffin [4]. The latex reagent was prepared by forcibly adsorbing ESS onto the particles [2]. Whenever needed, ESS was also passively adsorbed onto sheep red blood cells [3] for use in the micro-IHA. The antigen used for micro-IF was whole *R. conorii* grown in L-929 cells, harvested by centrifugation at 12,000 *g* for 30 min, and resuspended in Hanks' balanced salt solution to a dilution at which many organisms were seen by microscopy to be clearly separated. The latex–*R. conorii* and micro-IF tests were performed as previously described for the *R. rickettsii* tests [2].

Cross-reactivity of the micro-IF–reactive sera from patients with MSF with antigens of *R. rickettsii* and *Rickettsia typhi* was determined by the micro-IF and latex tests [2]. We also used SDS-PAGE and immunoblotting to confirm the cross-reactivity of three sera.

The CF antigens from *R. conorii* (provided by Dr. J. Vruolgyi, Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslavakia) and from *R. rickettsii* and *R. typhi* (both obtained from the Centers for Disease Control, Atlanta), all grown in egg yolk sac, and egg yolk sac as a control, were analyzed by SDS-PAGE by the procedure of Laemmli [5], as previously described. Low-molecular-weight markers (Bio-Rad Laboratories, Richmond, Calif) were used as standards. Each antigen was run in seven lanes. Electroblotting was done with 25 m*M* phosphate buffer (pH 7.4), and the transfer conditions were 250 mA for 12 hr at 5 C. The lane of molecular weight standards and one lane of each antigen were stained with amido black [6]. The remaining lanes were blocked in 50 m*M* Tris buffer plus 3% bovine serum albumin [6].

One lane of each antigen was incubated at 37 C overnight with one of five human antisera to rickettsia and a rabbit antibody to egg yolk sac (EYS 1). EYS 1 was prepared by injecting a rabbit im for a total of three injections at weekly intervals with 1 ml of 10% egg yolk. One week after the last injection, the rabbit was injected im with Ketaset® (Veterinary Products, Bristol Laboratory, Syracuse, NY) and exsanguinated by cardiac puncture. Three of the five sera were from patients clinically diagnosed as having MSF (figure 1; Rcl, Rc2, Rc3), one with Rocky Mountain spotted fever (figure 1, Rrl), and one with endemic typhus (figure 1, Rtl; provided by Dr. L. Bruce Elliott, Texas Department of Health, Austin, Tex). All lanes were washed in the blocking buffer repeatedly for 30 min. Horseradish peroxidase–labeled protein A (Bio-Rad) was used to identify antibody. The enzyme substrate for color development was 4-chloro-1-naphthol [7].

Results and Discussion

The minimum significant level of reactivity (MSLR), i.e., the minimum antibody titer considered significant for diagnosis of MSF (active or at an undetermined time) for

Received for publication 1 April 1985, and in revised form 28 June 1985.

D. Raoult was supported in part by a grant from DRASS, Marseille, France.

This work was presented in part at the Third International Congress on Rickettsiae and Rickettsial Diseases on 10 September 1984 in Smolenice, Czechoslavakia.

We thank D. Jensen and G. Vestris for their excellent assistance.

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Table 1. Classification of 422 patients on the basis of clinical diagnosis and correlation of test results.

Diagnosis	No. of patients	Criteria				Test results*			
		Occurred June–November	Fever	Tache noir	Rash	Latex		Micro-IF	
						NY	MAR	NY	MAR
Definite	36	Y	Y	Y	Y	36/0	36/0	36/0	36/0
Probable	25	Y	Y	N	Y	23/2	24/1	24/1	25/0
Possible†									
a	92	Y	Y	N	N	10/82	14/78	16/76	17/75
b		N	Y	N	Y				
Not MSF‡	69	N	Y	N	N	0/69	0/69	2/67	2/67
	200					ND	1/199	ND	0/200

NOTE. Y, yes; N, no; NY, New York; MAR, Marseille; ND, Not done.
* No. of patients with sera reactive/no. of patients with sera nonreactive.
† Alternative criteria.
‡ Two groups.

each test was determined by a logistic function [8] from paired sera that were analyzed separately. Results of the micro-IF test were assumed to be true results. The MSLR was determined by maximizing the probability of correct allocations [8]. The results showed that the MSLR was 32 for latex–*R. conorii* and 128 for micro-IF.

Micro-IF results for sera tested in both laboratories were in qualitative agreement—whether MSF cases or not—for 212 of 222 patients (95.5%). Results of latex–*R. conorii* were in agreement with each other for 215 patients (96.9%). The discrepancy might be ascribable to deterioration of the specimens during shipment and to the lapse of two

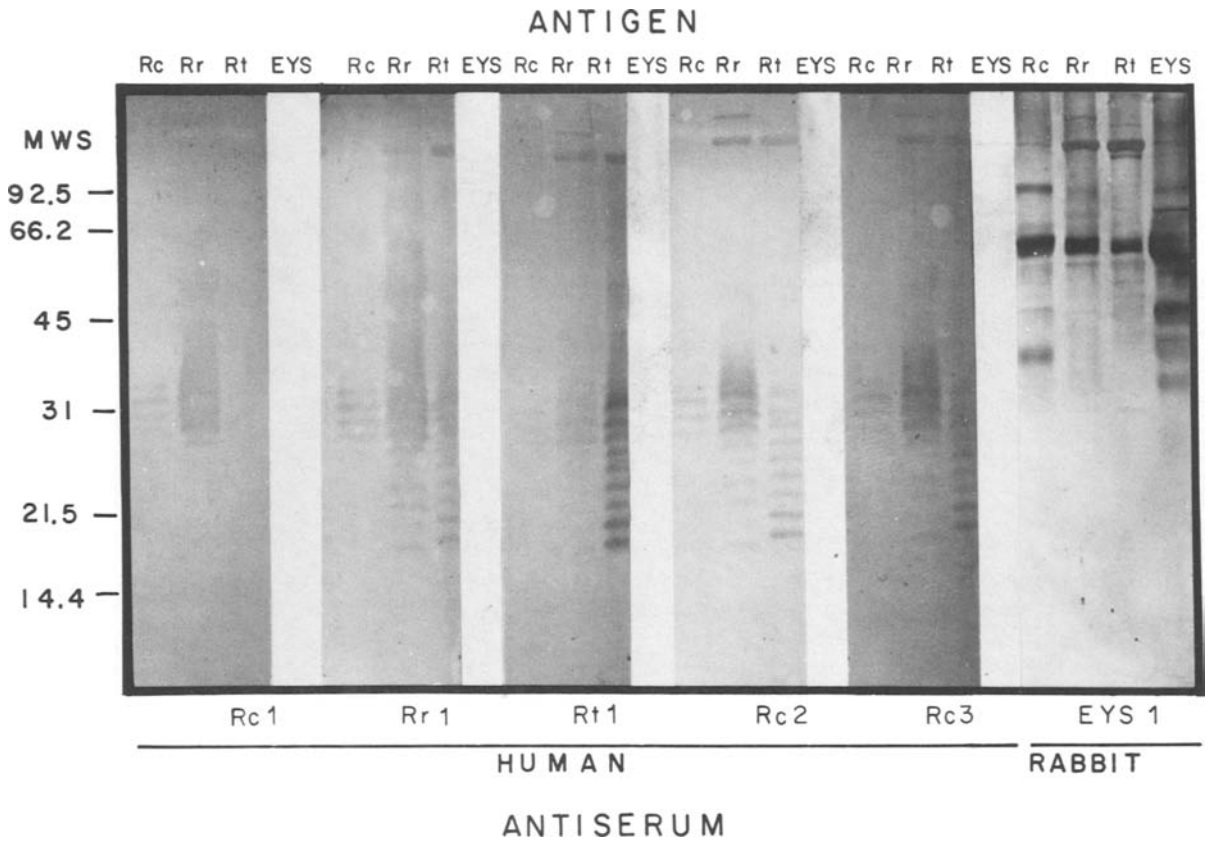

Figure 1. Presence of antibodies to *R. conorii* (Rc), *R. rickettsii* (Rr), *R. typhi* (Rt), and egg yolk sac (EYS) in three sera from MSF patients (Rc1, Rc2, and Rc3). Sera from patients with RMSF (Rr1) and *R. typhi* infection (Rt1) and from rabbit injected with EYS (EYS 1) were used as controls. MWS are the molecular weight standards.