Diagnosis of California La Crosse Virus Infection by Counterimmunoelectrophoresis

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La Crosse virus cell culture-derived soluble antigen was used in a counterimmunoelectrophoresis procedure to assess the presence or absence of La Crosse antibodies in 79 paired acute- and convalescent-phase human sera. The counterimmunoelectrophoresis test appeared to measure the same antibody as the complement fixation test but was more sensitive and rapid.

La Crosse virus, an arbovirus in the California group of the *Bunyaviridae* family, causes disease ranging from a mild undifferentiated febrile illness to an acute central nervous system involvement with severe encephalitis (6). Surveillance records have established that La Crosse virus is second only to St. Louis encephalitis virus as the causative agent of mosquito-borne disease in the United States (8). Its importance has become increasingly recognized since it was first associated with a fatal illness in 1964 (7). Most laboratories rely on the complement fixation (CF) test for diagnosing La Crosse infections, although the hemagglutination-inhibition (HI) and serum dilution neutralization (SDN) tests may be more definitive (3). An alternative to the CF test is the counterimmunoelectrophoresis (CEP) test (1, 2, 5), which is very rapid but has not been widely used because the infected suckling mouse brain used as antigen was crude and often gave confusing results. Recently we reported on a soluble cell culture-derived antigen for La Crosse virus that was clearly superior to ISMB when compared by several serological tests (4). As a result of that study, we decided to re-evaluate the CEP method as a rapid diagnostic procedure for identifying human La Crosse virus infections.

La Crosse virus soluble antigen was precipitated from virion-free BHK-21 cell culture fluids with ammonium sulfate. As described previously, the CEP tests were performed on glass slides in 0.7% agarose and veronal buffer with 0.1 M NaCl and 0.002 M ethylenediaminetetraacetic acid (4). In each CEP test, appropriate positive and negative controls were electrophoresed together with "unknown" coded human sera for 1.5 h at a constant current of 35 mA/slide. The densities of the immunoprecipitin arcs were graded as follows: strong dense

arcs, 4+; strong but slightly less dense, 3+; distinct but moderate density, 2+; distinct but without density, 1+; and faint weak precipitation, $\pm$. These criteria for grading the intensities of the CEP reactions are based on the reactions shown in Fig. 1. In addition, the HI, CF, and SDN results of these selected acute (S_1) and convalescent (S_2) sera are included. Only those sera which clearly reacted at 1+ or greater were considered positive; $\pm$ reactions were classified as suspect. Frequently, when sera collected early in the illness reacted at $\pm$, a positive reaction could be confirmed in later sera (Fig. 1).

For test result comparisons, acute and convalescent-phase sera were collected from 79 patients with clinical encephalitis and were grouped as follows: (Table 1) sera from serologically confirmed infections with a demonstrated fourfold antibody titer rise or fall by CF, HI, or SDN; and (Table 2) sera with a stable CF, HI, or SDN antibody titer high enough to suggest recent infection or serologically negative sera from patients with a clinical syndrome compatible with La Crosse virus infection.

The sera from 57 patients which gave positive serological reactions (Table 1) all gave CEP immunoprecipitation reactions compatible with the other serological data (i.e., CF, HI, and SDN tests). Sera showing a CF antibody titer usually had a strong CEP reaction; however, a few of the sera with low CF titers (1:4 or 1:8) occasionally gave a weak ($\pm$) CEP reaction. Of the 57 paired sera tested, none was CF positive without a reaction by CEP. We frequently observed positive CEP results with sera which were collected during the acute phase when CF antibody was absent and HI and SDN antibody titers suggested infection (See Fig. 1 and Table 1, patient no. W-3, W-4, W-7, W-9, W-16, W-17, Pa-1, O-2, O-6, O-10, O-11, O-13, O-16, O-22, O-27, I-7, I-9,

TABLE 1. CEP test results of diagnostically positive human La Crosse virus infections

Patient no.	Age (yr), sex ^a of patient	Days postonset	CEP	Serological titers ^b		
				CF	HI	SDN
W-1	9, f	4	- ^c	<4	20	160
		36	4+	16	40	320
W-2	2, f	52	4+	64	320	80
		78	4+	16	160	40
W-3	2, f	2	1+	<4	10	<10
		19	2+	16	160	160
W-4	7, m	9	-	<8	10	<10
		19	3+	<8	80	160
W-6	1, f	4	±	<4	10	<10
		19	1+	8	80	40
W-7	4, f	0	±	<8	40	40
		6	2+	<8	160	320
W-8	6, m	1	-	<4	<10	<10
		20	2+	8	160	640
W-9	6, f	2	-	<4	<10	<10
		16	3+	<4	40	20
W-11	3, m	18	4+	32	320	160
		84	4+	128	80	80
W-13	4, m	1	-	<4	20	20
		13	±	<4	160	80
W-16	12, f	S ₁	1+	<4	40	160
		18	2+	<4	160	320
W-17	5, f	3	-	<4	10	20
		25	3+	4	80	640
W-18	5, f	8	2+	<4	80	80
		27	4+	16	160	NT ^d
W-20	3, m	3	-	<4	<10	<10
		26	3+	32	640	320
W-22	2, m	3	-	<8	<10	<10
		28	3+	64	320	160
W-23	8, f	4	2+	4	80	20
		32	3+	≥256	320	20
W-25	8, m	0	-	<4	<10	<20
		33	3+	32	320	3,200
W-26	?, m	4	-	<4	<10	<20
		47	3+	32	320	12,800
W-27	5, f	1	-	<8	<10	100
		85	2+	16	≥160	400
W-28	1, f	14	-	<4	80	20
		24	2+	<4	320	12,800
W-29	9, f	3	-	4	NT	50
		43	2+	32	NT	1,600
W-30	5, m	2	-	<8	<10	50
		18	-	<8	160	1,600
Pa-1	3, m	9	2+	<8	NT	800
		29	4+	64	NT	800
Pa-2	12, f	170	4+	NT	NT	400
		3	-	<8	NT	400
O-1	4, f	26	±	16	NT	800
		1	-	<8	NT	100
O-2	16, m	17	3+	32	NT	3,200
		0	1+	<8	20	100
O-3	6, f	28	2+	16	40	6,400
		3	-	<8	<10	<20
O-4	10, f	35	-	<8	40	400
		7	-	<8	<10	20
O-5	7, m	17	-	<8	80	3,200
		42	-	<8	80	400
		5	-	<8	10	50
		26	1+	8	640	1,280

TABLE 1—Continued

Patient no.	Age (yr), sex ^a of patient	Days postonset	CEP	Serological titers ^b		
				CF	HI	SDN
O-6	8, f	4	-	<8	<10	<20
		18	1+	<8	320	1600
O-7	8, m	5	-	<8	20	50
		18	1+	16	1,280	12,800
O-8	7, m	3	-	AC ^c	<10	<20
		29	±	8	80	800
O-10		5	3+	<8	320	NT
		38	4+	64	80	NT
O-11		S ₁	1+	<8	20	NT
		S ₂	3+	16	80	NT
O-13		5	2+	<8	640	NT
		22	3+	16	640	NT
O-15		9	-	8	20	NT
		11	±	8	40	NT
O-16		S ₃	3+	32	20	NT
		3	1+	<8	40	NT
		6	2+	<8	80	NT
		28	4+	16	40	NT
O-17		5	-	8	20	NT
		26	3+	32	10	NT
O-18		3	-	<8	<10	NT
		55	4+	64	80	NT
O-22		2	±	<8	10	NT
		14	1+	8	80	NT
O-24		S ₁	1+	8	80	NT
		S ₂	4+	32	80	NT
		S ₃	2+	16	80	NT
		2	-	<8	10	NT
O-25		43	3+	16	160	NT
		1	±	<8	<10	NT
O-27		14	±	<8	320	NT
		33	3+	32	160	NT
		64	2+	64	80	NT
		4	-	<8	10	NT
O-28		7	-	<8	80	NT
		S ₁	±	<8	320	NT
O-29		S ₂	1+	16	320	NT
		2	-	<8	80	NT
O-30		27	±	8	80	NT
		S ₁	-	<8	<10	NT
O-34		S ₂	4+	128	160	NT
		1	-	<2	NT	320
I-1	26, m	32	±	16	NT	1,280
I-2		3	±	<8	NT	NT
		20	1+	16	NT	NT
I-3	4, m	3	-	<2	NT	NT
		18	1+	16	NT	NT
I-4	6, m	4	±	<2	NT	640
		18	1+	16	NT	640
I-5	1, m	7	±	<2	NT	NT
		21	1+	16	NT	NT
I-6	6, f	1	-	<2	NT	40
		17	±	8	NT	1,280
I-7	4, f	8	1+	<8	NT	640
		15	1+	<8	NT	1,280
		32	3+	32	NT	2,560
		12	-	<8	10	100
I-8	9, f	23	±	16	80	1,600

TABLE 1—Continued

Patient no.	Age (yr), sex ^a of patient	Days postonset	CEP	Serological titers ^b		
				CF	HI	SDN
I-9		5	1+	<8	20	400
		48	4+	256	160	3,200
NC-1	11, m	4	1+	<8	20	2,560
		47	2+	8	40	2,560

^a Blanks indicate information not available. m, Male; f, female; ?, age unknown.^b Titers expressed as reciprocals of the serum dilution.^c -, No reaction.^d NT, Not tested.^e AC, Anticomplementary.

TABLE 2. CEP test results of suspect human La Crosse virus cases

Patient no.	Age (yr), sex ^a of patient	Days postonset	CEP	Serological titers ^b		
				CF	HI	SDN
W-5	<1, f	25	1+	8	80	320
		52	3+	8	40	160
W-10	5, f	41	4+	16	40	80
		83	4+	16	10	80
W-15	11, m	1	1+	<4	160	40
		21	3+	8	160	40
W-19	26, f	S ₁	- ^c	<8	<10	<10
		S ₂	-	<8	<10	<10
W-21	16, m	6	2+	<4	320	160
		12	3+	8	160	160
W-24	33, f	S ₁	-	<8	<10	<10
		S ₂	-	<8	<10	<10
O-9		9	-	<8	40	NT ^d
		11	-	<8	40	NT
O-12		S ₁	1+	8	320	NT
		S ₂	2+	16	640	NT
O-14		10	2+	<8	20	NT
		24	2+	8	40	NT
O-19		S ₁	-	<8	80	NT
		S ₂	-	<8	80	NT
O-20		S ₁	-	<8	10	NT
		S ₂	-	<8	10	NT
O-21		9	-	<8	320	NT
		19	±	8	160	NT
O-23		5	-	<8	10	NT
		32	-	<8	10	NT
O-26		S ₁	±	<8	40	NT
		S ₂	±	<8	80	NT
O-31		3	-	<8	160	NT
		27	-	<8	160	NT
O-32		11	-	<8	40	NT
		25	-	<8	40	NT
O-33		35	4+	16	160	NT
		61	4+	16	80	NT
O-35		S ₁	-	<8	20	NT
		S ₂	±	8	40	NT
O-36		S ₁	2+	32	40	NT
		S ₂	3+	32	40	NT
O-37		S ₁	1+	<8	80	NT
		S ₂	1+	<8	80	NT
A-1	4, f	9	-	<8	20	320
		48	1+	<8	20	320

^a Blanks indicate information not available. m, Male; f, female.^b Titers expressed as reciprocals of the serum dilution.^c -, No reaction.^d NT, Not tested.

and NC-1). These results suggest that CEP was more sensitive than CF for detecting early La Crosse antibody.

Four patients (Table 1, patients no. W-30, O-3, O-4, and O-28) which were serologically positive by HI or SDN tests did not have CF antibody and were negative by CEP. These unexpected results may indicate that the patients were infected with a California virus subtype other than La Crosse. Other possibilities are that they may not have developed CF antibody despite being infected with La Crosse virus, or the serum samples may have been collected too early after onset of infection.

The CEP test results shown in Table 2 were with sera from suspect cases that did not have at least a fourfold rise or fall in antibody titer but were otherwise suggestive of a La Crosse virus infection. The CEP results on several patients' sera (i.e., W-5, W-15, W-21, O-21, O-35, O-36, and A-1, Table 2) showed higher immunoprecipitin levels in convalescent- than in acute-phase specimens, lending support to the conclusion that the CF, HI, or SDN antibody titers were probably the result of a current infection. In addition, Table 2 includes data on two patients with clinical infections (no. W-19 and W-24) whose sera were serologically negative and on six (no. O-9, O-19, O-20, O-23, O-31, and O-32) whose sera indicated a prior infection not related to the current illness (i.e., stable HI antibody without CF antibody). CEP results were clearly negative on these specimens.

This study using paired sera from actual and suspect infections has demonstrated that CEP is useful for rapid diagnosis of La Crosse infections. Balfour et al. (1) have earlier presented data showing some success with this method. In their investigations they used infected suckling mouse brain tissue as a test antigen and observed single,

double, and sometimes triple precipitation arcs with some human sera. Our use of a soluble cell culture-derived antigen resolved this problem (4), since we observed only a single major precipitation line by CEP. In addition, our antigen was noninfectious. Also important for the diagnostic laboratory is the small quantity of reagents required (0.03 ml) and the short time needed to obtain a positive result (1.5 h).

In our initial characterization of La Crosse soluble antigen, we found that this antigen was the major nucleocapsid protein, N, of the virus (4, 6). Thus it is apparent that the CF and CEP test procedures measure antibody against the internal component of the virus.

LITERATURE CITED

1. Balfour, H. H., Jr., R. J. Majerle, and C. K. Edelman. 1973. California arbovirus (La Crosse) infections. II. Precipitin antibody tests for the diagnosis of California encephalitis. *Infect. Immun.* 8:947-951.
2. Calisher, C. H., and K. C. S. Maness. 1970. Arbovirus identification by an agar-gel diffusion technique. *Appl. Microbiol.* 19:557-564.
3. Lindsey, H. S., C. H. Calisher, and J. H. Mathews. 1976. Serum dilution neutralization test for California group virus identification and serology. *J. Clin. Microbiol.* 4:503-510.
4. Lindsey, H. S., R. A. Klimas, and J. F. Obijeski. 1977. La Crosse virus soluble cell culture antigen. *J. Clin. Micro.* 6:618-626.
5. Murphy, F. A., and P. H. Coleman. 1967. California group arboviruses: immunodiffusion studies. *J. Immunol.* 99:276-284.
6. Obijeski, J. F., and F. A. Murphy. 1977. Bunyaviridae: recent biochemical developments. *J. Gen. Virol.* 37:1-14.
7. Thompson, W. H., B. Kalfayen, and R. O. Anslow. 1965. Isolation of California encephalitis group virus from a fatal human illness. *Am. J. Epidemiol.* 81:245-253.
8. U. S. Public Health Service. 1976. Neurotropic viral diseases surveillance, annual encephalitis summary (Center for Disease Control Publication), 1974, p. 9. U.S. Public Health Service, Atlanta, Ga.