Acta virol. **29** : 87—90. 1985

DETECTION OF CRIMEAN HAEMORRHAGIC FEVER VIRUS ANTIGEN BY SOLID PHASE ENZYME IMMUNOSORBENT ASSAY

S. E. SMIRNOVA, A. S. KARAVANOV

Institute of Poliomyelitis and Viral Encephalitides, U.S.S.R. Academy of Medical Sciences, 142782 Moscow, U.S.S.R.

Received April 10, 1984

Summary. — Solid phase enzyme immunosorbent assay (SPEIA) is described for detection of the Crimean haemorrhagic fever (CHF) virus antigen in suspensions of ticks collected in the natural focus.

Key words: Crimean haemorrhagic fever virus; SPEIA; virus detection in vector ticks

Introduction

The presence of CHF agent in specimens collected in natural foci is commonly detected by inoculation of newborn white mice (bioassay); the isolates are identified by complement fixation (CF) test, indirect haemagglutination (IHA) test, and immunofluorescence (IF). These methods are highly specific but quite labour-consuming, and not all of them can be used for direct detection of the agent in original materials.

Recently developed SPEIA, which is highly sensitive and gives the results in a short time, has been recommended for diagnosis of arbovirus infections (Fraizer and Shope, 1979; Gavrilovskaya *et al.*, 1981; Tkachenko *et al.*, 1981; Donets *et al.*, 1982). This paper describes the use of SPEIA for detection of CHF virus antigen in tick suspensions.

Materials and Methods

Field specimens. Hyalomma plumbeum plumbeum (Hyalomma marginatum marginatum) ticks were collected in endemic areas of CHF in southern U.S.S.R.; 155 tick suspensions were stored at —20 °C.

Virus. Three CHF virus strains isolated from the blood of patients and 2 from Ixodid ticks collected in natural foci were used after 5 or more intracerebral (i.c.) passages in newborn white mice (NWM). Ten per cent suspensions of NWM brains and of Ixodid ticks were prepared in 0.15 mol/l sodium chloride solution (SCS). The borate-buffered and sucrose-acetone-extracted antigens were made by conventional methods. Control suspensions and antigens were prepared from uninfected NWM brains.

Antibody. Immune ascitic fluids (IAF) prepared in white mice were used as the source of antibody.

Bioassays. One-two-day-old mice were inoculated i.c. with 10% tick suspensions and observed for 14 days. Borate-buffered antigens from brains of sick and dead NWM were prepared and tested for specificity in CF test with IAF.

Table 1. Detection of CHF virus in different materials

Material tested	No. of specimens tested	Titres		
		SPEIA (P/n ⩾ 2.1)	CF test	Bioassay (log LD_{50}/ml)
Brain suspension of infected NWM prepared in:				
sodium chloride 10%	10	800—1500* (3.2—4.4)	4— 8*	6.8—7.3
borate buffer, 10%, pH 9.0	10	2000—4000 (4.6—5.4)	16—32	6.8—7.3
prepared by sucrose-acetone extraction, 25%	10	16000—32000 (5.6—6.4)	320—1280	0
10% suspension of Ixodid ticks in sodium chloride	18	10—50 (1.0—1.8)	0**	2.8—3.8

* reciprocals of dilutions (in parentheses: values expressed in $\log_{10}$ units)
** specimens with marked anticomplementary activity

Serological tests. CF test was performed by the micromethod with 2 units of complement at 4 °C.

SPEIA was run in flat-bottomed polystyrene microtitre plates of domestic origin as follows: Plates were coated with 200 µl IgG (IgG isolated from IAF, titre in CF test 1 : 256 to 1 : 512, by ammonium sulphate precipitation and subsequent gel filtration in a Sephadex G-200 column, 1.5 × 90 cm) were added into all the wells. The plates were incubated at 37 °C for 3—4 hr, then washed 3 times for 3 min with phosphate-buffered saline (PBS), pH 7.4, containing 0.5% twin-20 and 1 normal bovine serum (NBS). Then the plates were incubated with 100 µl antigen at 4 °C for 18 to 20 hr, and the wells were washed 4 times, 3 min each. Finally, 100 µl conjugate IgG conjugated with peroxidase, Sigma, type YI, RZ = 3.03, by the method of Nakane and Kawaoi (1974) modified by Mathiesen and Feinstone (1978) in dilution 1 : 100 prepared in PBS with 50% NSB was added. The plates were incubated at 20 °C for 2 hr, and the wells were washed 5 times, 4 min each. The substrate reagent contained 100 µl of freshly prepared orthophenylene diamine (0.4 mg/ml) in citrate-phosphate buffer, pH 5.0, and H_2O_2; after 50 min exposure in the dark at 20 °C the reaction was stopped by addition of 50 µl of 2 mol/l H_2SO_4.

The results were read in a Perkin spectrophotometer at 492 nm and calculated from the ratio of the data for experimental and control specimens (P/n); the result was considered to be positive at P/n ⩾ 2.1.

In each test, the specificity of the result was checked using the antigen from uninfected NWM brain or a negative specimen of tick suspension and a known positive antigen or tick suspension. The quality of the conjugate and the substrate was also controlled.

Results

The examinations of brain suspensions from the infected NWM revealed equal diagnostic potentials of SPEIA and CF test (equal number of positively reacting specimens), but the enzyme method gave higher titres than CF test. Most of the specimens tested possessed the infectious activity which was

Table 2. Results of testing tick suspensions by SPEIA and by bioassay

Period of storage	Suspension No.	SPEIA (P/n)			Bioassay
		1 : 10*	1 : 50*	1 : 100*	1 : 10*
2 years	1	2.1	0	0	80**
	2	3.0	2.8	not done	62
1 year	3	2.2	2.2	0	100
	4	2.4	0	0	45
	5	2.4	2.1	not done	87
	6	2.4	0	0	14
	7	2.1	0	0	72
	8	2.1	0	0	100
2 months	9	2.3	0	0	0***
	10	2.1	0	0	89
	11	2.8	0	0	100
	12	2.4	0	0	100
	13	2.1	0	0	100
	14	2.2	0	0	0***
	15	4.3	2.9	not done	0
	16	2.1	0	0	100
	17	2.2	0	not done	0***
	18	3.7	2.1	0	89

* dilutions of tick suspensions

** per cent of sick (or dead) mice

*** in cases No. 9 and No. 14 there were 10—30% and in case No. 17 up to 100% NWM eaten by mothers within 6—9 days after inoculation. In all other cases strains of CHF virus were isolated which were established by passages in i.c. inoculated NWM, with an exception of case No. 15 where we observed marked differences between the results of two methods used.

determined by bioassays. The data obtained by CF test, SPEIA, and bioassays are compared in Table 1.

The examinations of tick suspensions showed the enzyme method to be more advantageous than CF test. All the 155 tick suspension sepecimens prepared for CF test by the method recommended for brain suspensions had a marked anticomplementary activity which could not be eliminated by conventional treatments. Good correlations were found between the results of SPEIA and bioassay: among 155 specimens, 18 were positive by the former and 14 by the latter. In one case only were convincing differences between the results of these two tests; in other 3 cases the bioassay results were difficult to evaluate, because of the specific features of this test (Table 2).

Discussion

In investigations of CHF foci, one of the important aspects is determination of the circulation of causative agent in nature which requires methods that should not be too time-consuming, too laborous and would require no

specific laboratory equipment. Bioassay still remains the only method for isolation of virus strains, but it does not fulfil the above criteria and can be obtained within 4—7 days after animal inoculation. The CF test is a highly specific method but cannot be used for the detection of CHF virus antigen in tick suspensions because of their anticomplementary activity. The IF technique can be used for detection of CHF virus antigen in the vector ticks but it requires adequate training in opening the ticks and making the salivary gland preparations in which the specific antigen could be clearly distinguished. The enzyme method meets all the above-mentioned requirements (visual reading of the results is possible) and, according to our experience, may be used for the detection of CHF agent directly in tick suspensions. However, the sensitivity and, consequently, the quality of the test depends on the activity of conjugates; thus, in our studies conjugates used in dilutions of 1 : 100 or higher produced clear-cut specific results, which were highly reproducible. The SPEIA may be recommended for wide use in epidemiological studies of virus presence in Ixodid ticks, the vectors of CHF virus.

References

Donets, M. A., Rezapkin, G. V., Ivanov, A. P., and Tkachenko, E. A. (1982): Immunosorbent assays for diagnosis of Crimean-Congo hemorrhagic fever (CCHF). *Am. J. trop. Med. Hyg.* **31**, 156—162.

Fraizer, C. L., and Shope, R. E. (1979): Detection of antibodies to Alphaviruses by enzyme-linked immunosorbent assay. *J. clin. Microbiol.* **10**, 583—585.

Gavrilovskaya, I. N., Apekina, N. S., Gorbachkova, E. A., Myasnikov, Yu. A., Rylceva, E. V., and Chumakov, M. P. (1981): Detection by enzyme-linked immunosorbent assay of hemorrhagic fever with renal syndrome virus in lung tissue of rodents from European U.S.S.R. *Lancet* **8228**, 1050—1051.

Mathiesen, L. R., and Feinstone, S. M. (1979): Enzyme-linked immunosorbent assay for detection of hepatitis A antigen in stool and antibody to hepatitis antigen in sera: Comparison with solid-phase radioimmunoassay, immune electron microscopy and immune adherence hemagglutination assay. *J. clin. Microbiol.* **7**, 184—193.

Nakane, P. K., and Kawaoi, A. (1974): Peroxidase-labeled antibody: a new method of conjugation. *J. Histochem. Cytochem.* **22**, 1084—1091.

Tkachenko, E. A., Ivanov, A. P., Rezapkin, G. V., Donets, M. A., Dzagurova, T. K., and Drozdov, S. G. (1981): Immunosorbent assays for rapid diagnosis of viral hemorrhagic fevers, p. 203. *Abstr. of Fifth International Congress of Virology*, Strasbourg, France.