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NOTES

Rapid Diagnosis of Human Crimean-Congo Hemorrhagic Fever and Detection of the Virus in Naturally Infected Ticks

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An enzyme-linked immunosorbent assay (ELISA) was developed to detect Crimean-Congo hemorrhagic fever (CCHF) virus-specific immunoglobulin M (IgM) in human serum samples. For this test, a heat-inactivated antigen was prepared from the brains of suckling mice infected with CCHF virus. The IgM-capture ELISA proved more sensitive than indirect fluorescence tests for IgM to this virus. A human serum containing high-titer IgM to CCHF virus was used for an antigen-capture ELISA to detect this virus in heat-inactivated suspensions of virus-infected ticks. The antigen-capture ELISA appeared to be as sensitive as virus isolation in suckling mice. The studies described suggest that the IgM-capture ELISA and the antigen-detection ELISA should provide a rapid and sensitive diagnosis of human CCHF virus infection and should be useful in ecologic studies of this virus.

Crimean-Congo hemorrhagic fever (CCHF) is widely endemic in Africa (5). Most recently, human cases of hemorrhagic fever attributed to CCHF virus (family *Bunyaviridae*, genus *Nairovirus*) have been reported in West Africa (7, 9). In South Africa, the disease was first recognized in 1981, and a nosocomial outbreak of CCHF occurred at Tygerberg hospital, near Cape Town, in September 1984. In this outbreak, clinical diagnosis was often difficult in the early stages of illness, and there was a delay of up to 8 days before CCHF viremia could be confirmed (12). Because this is such a serious illness and because nosocomial outbreaks are so frequently associated with individuals hospitalized for CCHF, methods for the rapid diagnosis of infection with CCHF virus are urgently needed. Early recognition allows therapeutic intervention and provides the time necessary to trigger quarantine procedures that minimize the risk of secondary spread.

We describe the use of an enzyme-linked immunosorbent assay (ELISA) for detecting CCHF virus-specific immunoglobulin M (IgM) in serum samples of patients and for detecting CCHF virus antigens in naturally infected ticks.

Four sequential serum samples were obtained from a patient with serologically confirmed CCHF (7). In addition, 180 serum samples were collected in a CCHF-endemic area in southern Mauritania (8).

Mature ticks (males and females) collected in northern Senegal for viral assay were picked by hand from domestic animals, including cattle, sheep, goats, and camels. The ticks were pooled by species, usually 20 per pool, macerated with a mortar and pestle, and taken into suspension in 3 ml of Hanks balanced salt solution containing 10% heat-inactivated (30 min, 56°C) fetal bovine serum (GIBCO Ltd.) and 100 U of penicillin and 100 mg of streptomycin per ml. After centrifugation, each tick suspension was inoculated intracranially into suckling mice. Identification of Congo virus isolates was made in complement fixation (CF) and neutralization tests by the World Health Organization Collaborating Centre for Arbovirus Reference and Research at the Pasteur Institute in Dakar, Senegal.

CF was performed by the micro method (3) with heat-inactivated (1 h, 60°C) sucrose-acetone-extracted antigen prepared by the method of Clarke and Casals (4). The indirect fluorescent-antibody (IFA) test was described previously (6).

CCHF virus (strain Ib Ar 10200) was inoculated into suckling mice by the intracranial route. Crude antigen was prepared in Veronal gelatin buffer (3) as a 50% infected suckling mouse brain suspension. Antigen was heat inactivated (1 h, 60°C) and centrifuged at 10,000 × *g* for 5 min. No residual infectivity remained in treated antigen, as determined by inoculation of suckling mice.

ELISAs were performed using flat-bottom 96-well plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.). The plates were coated with 100 μl of pretitrated goat anti-human IgM μ-chain-specific antibody (Biosys, Compiegne, France) diluted 1:500 in carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed four times with phosphate-buffered saline (pH 7.2) containing 0.05% Tween 20, and 100 μl of test serum, serially diluted in ELISA buffer (phosphate-buffered saline containing 0.05% Tween 20 and 1% bovine serum albumin) was added. The plates were incubated an additional 1 h at 37°C. After the wells were washed, 100 μl of heat-inactivated CCHF antigen diluted 1:100 in ELISA buffer was added to each well. After overnight incubation at 4°C and washing, monoclonal antibody (84001-01; kindly provided by J. McCormick, Centers for Disease Control, Atlanta, Ga.) to CCHF virus was diluted 1:100 in ELISA buffer, and 100 μl was added to each well. The plates were then incubated for 1 h at 37°C and washed, and 100 μl of pretitrated sheep anti-mouse IgG conjugated with horse radish peroxidase (Biosys) was added to each well. After further incubation (1 h, 37°C) and washing, 100 μl of substrate (hydrogen peroxide plus *o*-tolidine; Sigma Chemical Co., St. Louis, Mo.) diluted

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TABLE 1. Titers of antibody to CCHF virus in serial serum samples from a patient with clinical evidence of infection

Test	Titer at the following no. of days after onset:			
	9	13	27	122
IgM IFA	10	80	40	<10
IgG IFA	160	320	640	320
CF	8	NT[a]	32	32
IgM ELISA	1,600	12,000	3,200	200

[a] NT, Not tested.

in citrate buffer (pH 4.0) was added, and the plates were incubated for 30 min at room temperature. The enzymatic reaction was stopped by the addition of 100 μl of 4 N H_2SO_4. The plates were read at 450 nm by using an automated ELISA reader (Titertek Multiskan; Flow Laboratories, Inc., McLean, Va.). Controls included known seropositive human serum, negative human serum, and antigen prepared from uninfected mice. Results were expressed as the ratio of absorbance values of test serum to negative control (P/N). Ratios of 2.0 were considered positive.

For detection of CCHF virus, plates were coated with anti-human IgM as described above. A known positive human serum was diluted 1:100 in ELISA buffer and added to each well, and the plates were incubated for 1 h at 37°C. Samples of centrifuged suspensions of ticks were diluted in an equal volume of Veronal gelatin buffer and heat inactivated (1 h, 60°C). To each well was added 100 μl of dilution of inactivated tick suspension, and the plates were incubated for 1 h at 37°C. With intervening washes, 100 μl of a pretitrated (1:100) dilution of monoclonal antibody to CCHF virus and substrate was added, and the test was read and recorded as described above.

In preliminary tests, optimal concentrations of reagents for use in the ELISA were determined by box titrations. Dilutions selected for use were the highest dilutions giving maximal absorbance values in the ELISA.

Results of the CCHF-specific IgM-capture ELISA were compared with results of CF and the IFA test by testing serially collected serum samples from a single patient (Table 1). Samples from days 9, 13, 27, and 122 did not contain antibody to Dugbe or Bandia virus, as determined by ELISA and by CF. Dugbe and Bandia viruses are nairoviruses antigenically unrelated to CCHF virus or to each other. Of 180 serum samples tested by IFA and IgM capture ELISA, 10 were positive by IFA for IgG (titers ranged from 16 to 256), and none was positive by ELISA.

The ELISA was compared with virus isolation (intracranial inoculation of suckling mice) for detecting CCHF virus in ticks. All those positive by virus isolation also were positive by antigen-capture ELISA (Table 2). Selected nairoviruses were tested by ELISA to determine cross-reactivity among members of this genus. Suspensions of ticks naturally infected with Dugbe, Bandia, and Bakel ArD 41258 (a new nairovirus of the Qualyub group isolated in Senegal from the tick *Alectorobius sonrai*) were negative by antigen-capture ELISA using the human serum mentioned above (Table 2). The specificity of the test also was determined by using sucrose-acetone–extracted antigens of these three nairoviruses. Sixty pools of *Hyalomma* species and *Amblyomma variegatum* ticks collected in western Senegal were negative by both antigen-capture ELISA and virus isolation.

Recent nosocomial outbreaks of CCHF in Pakistan (1), Dubai (10), and South Africa (12) demonstrated the need for rapid diagnostic tests to identify CCHF virus and the antibody it elicits. Once identified as infected, patients should be isolated from other patients and from hospital staff and visitors. Given a timely diagnosis of CCHF virus infection, relevant therapy could be instituted with the antiviral drug ribavirin augmented with plasma from CCHF-immune humans (12).

TABLE 2. Results of tests for CCHF virus in ticks

Suspension no.	Species	Bio-assay[a]	Virus isolated[b]	ELISA P/N[c]
1	*Hyalomma marginatum rufipes*	100	CCHF	3.0
2	*H. marginatum rufipes*	100	CCHF	4.9
3	*H. marginatum rufipes*	100	CCHF	5.7
4	*H. marginatum rufipes*	100	CCHF	6.4
5	*H. marginatum rufipes*	100	CCHF	7.6
6	*H. marginatum rufipes*	78	CCHF	4.0
7	*H. marginatum rufipes*	100	CCHF	6.6
8	*H. marginatum rufipes*	100	CCHF	3.8
9	*H. marginatum rufipes*	89	CCHF	6.8
10	*H. marginatum rufipes*	38	CCHF	6.8
11	*H. marginatum rufipes*	100	CCHF	4.5
12	*Amblyomma variegatum*	100	Dugbe	<1.5
13	*Alectorobius sonrai*	100	Bandia	<1.5
14	*A. sonrai*	13	Bakel	<1.5

[a] Results are expressed as percent sick or dead suckling mice.
[b] Virus identified by CF and neutralization tests.
[c] Results are expressed as the ratio of CCHF-positive to CCHF-negative control (P/N) absorbance values. Negative control was a suspension of noninfected ticks.

Several methods are available for detecting antibodies to CCHF virus, including CF, hemagglutination inhibition, IFA, reverse passive hemagglutination, and serum dilution-plaque reduction neutralization tests. Problems encountered in performing tests for antibody to CCHF virus were reviewed previously (11). In our study, an ELISA for detecting IgM to CCHF virus was developed with a heat-inactivated CCHF virus-infected mouse brain antigen, used because of its high titer. This test proved more sensitive than the IFA test in detecting IgM to CCHF virus (Table 1). The IFA assay for IgM may be of reduced sensitivity because of the presence of IgG. However, the IgM-capture ELISA was positive 122 days after infection, when IgM was not detected by the IFA assay. This persistence of virus-specific IgM suggests that finding such antibody in a single serum sample tested by ELISA may not indicate a very recent infection.

Serological cross-reactions between CCHF virus and other viruses was the basis for the establishment of the genus *Nairovirus* (2). Sequential serum samples from one individual were negative by IgM ELISA and by CF for antibody to two other nairoviruses, Dugbe and Bandia.

Thus, the IgM-capture ELISA, as described, is sensitive, specific, and rapid and may be used for early diagnosis of CCHF virus infection in humans. Further investigations are needed to determine the cause of the apparent persistence of IgM to this virus.

The ELISA also proved useful in detecting CCHF virus in ticks. Human serum containing high-titer IgM to CCHF virus was useful for antigen capture, and no false-positive results were found among 60 suspensions of noninfected ticks. Improvements in the antigen-capture ELISA described might be obtained by replacing the human serum with suitably selected monoclonal antibodies.

Now that sensitive, specific, and rapidly performed diag-

nostic tests are available for CCHF virus, intensive efforts should be made to apply them in the field, in areas in which CCHF virus is known to occur and when such a diagnosis is suspected in epidemic and potentially epidemic situations.

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LITERATURE CITED

1. **Burney, M. I., A. Ghafoor, M. Saleen, P. A. Webb, and J. Casals.** 1980. Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever-Congo virus in Pakistan, January 1976. Am. J. Trop. Med. Hyg. **29**:941–947.
2. **Casals, J., and G. H. Tignor.** 1980. The nairovirus genus. Serological relationships. Intervirology **14**:144–147.
3. **Casey, H. L.** 1965. Standard diagnostic complement-fixation method and adaptation to microtest. Public Health Monogr. **74**:1–34.
4. **Clarke, D., and J. Casals.** 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. **7**:561–573.
5. **Hoogstraal, H.** 1979. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe and Africa. J. Med. Entomol. **15**:304–417.
6. **Johnson, K. M., L. H. Elliott, and D. L. Heymann.** 1981. Preparation of polyvalent viral immunofluorescent intracellular antigens and use in human serosurveys. J. Clin. Microbiol. **14**:527–529.
7. **Saluzzo, J. F., P. Aubry, J. McCormick, and J. P. Digoutte.** 1985. Hemorrhagic fever caused by Crimean-Congo hemorrhagic fever virus in Mauritania. Trans. R. Soc. Trop. Med. Hyg. **79**:268.
8. **Saluzzo, J. F., J. P. Digoutte, J. L. Camicas, and G. Chauvancy.** 1985. Crimean-Congo hemorrhagic fever and Rift Valley fever in south-eastern Mauritania. Lancet **i**:116.
9. **Saluzzo, J. F., J. P. Digoutte, M. Cornet, D. Baudon, J. Roux, and V. Robert.** 1984. Isolation of Crimean-Congo hemorrhagic fever and Rift Valley fever viruses in Upper Volta. Lancet **i**:1179.
10. **Suleiman, M. N. H., J. M. Muscat-Baron, J. R. Harries, A. G. O. Satti, G. S. Platt, E. T. W. Bowen, and D. I. H. Simpson.** 1980. Congo/Crimean hemorrhagic fever in Dubai. An outbreak at the Rashid hospital. Lancet **ii**:939–941.
11. **Swanepoel, R., J. K. Struthers, and G. M. McGillivray.** 1983. Reversed passive hemagglutination and inhibition with Rift Valley fever and Crimean-Congo hemorrhagic fever viruses. Am. J. Trop. Med. Hyg. **32**:610–617.
12. **Van Eeden, P. J., S. F. Van Eeden, J. R. Joubert, J. B. King, B. W. Van de Wal, and W. L. Michell.** 1985. A nosocomial outbreak of Crimean-Congo hemorrhagic fever at Tygerberg hospital. Part II. Management of patients. S. Afr. Med. J. **68**:718–721.