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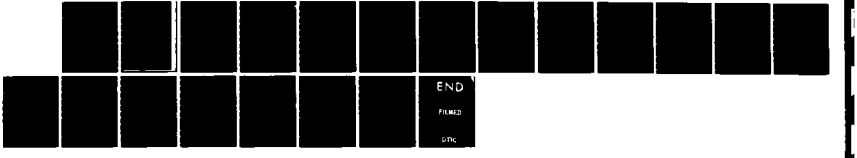

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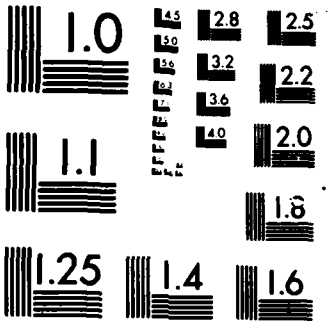

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Crimean-Congo Hemorrhagic Fever and Hemorrhagic Fever with Renal Syndrome in Greece

Final Report

Antonios Antoniadis, M.D.

December 1, 1984

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

CCHF virus or a virus closely related to it exists in Greece, infects humans (0.87% and 1.74% by IFA and ELISA tests respectively) but the clinical manifestations and signs of the disease are still unknown. For serosurveys and diagnosis, a more sensitive and specific test must be developed. HFRS also exists in Greece (5.7% seropositives) and the disease occuring in Greece is rather similar to Korean hemorrhagic fever and not to Nephropathia epidemica (two fatal out of thirteen serologically diagnosed cases). Hantaan or a Hantaan-like virus occuring in the country is probably antigenically closer to Hantaan

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20. virus (Korean type) than to Puumala virus. The reservoir of the virus is possibly the rodent Apodemus flavicollis. Keywords → to field 19

SUMMARY

Hemorrhagic fever with renal syndrome (HFRS) and Congo-Crimean hemorrhagic fever (CCHF) are two diseases existing in neighboring to Greece countries (Bulgaria, Yugoslavia). The purpose of this study was the identification of HFRS and CCHF foci in Greece, the serological diagnosis of HFRS and CCHF human cases, the description of the clinical manifestations and signs of the disease and the comparison of Indirect Immunofluorescence (IFA), Enzyme-Linked Immunoadsorbent (ELISA) and Agar Gel Diffusion (AGD) assays used for serosurveys and diagnosis of CCHF, all the aforementioned supported our attempts for virus isolation from patients and reservoirs. The methods used in this study were: IFA, ELISA, AGD and PRNT tests. For Hantaan virus isolation, Vero E-6 cells were used. According to our results related to CCHF virus, in a total number of 549 examined individuals, 4 were positive by IFA test (0.87%) and 8 by ELISA test (1,74%). Four hundred and twenty five human sera (single or paired), taken from patients, were examined for CCHF diagnosis and none of the patients was found to be infected by CCHF virus. Using goat sera obtained from herds of Central Macedonia, it was observed that ELISA test is four times more sensitive than AGD test and twice as sensitive than IFA test. As for HFRS, in a total number of 459 people examined by IFA test, 27 were positive (5,8%). This percentage of positives seems to be dependent on the area of serosurvey, ranging from 0% to 12%. During the period of the study, 81 human sera (paired or single) were collected from patients suspected for HFRS, Leptospirosis, acute renal syndrome and Pyrexia of unknown origin. The serological diagnosis was succeeded in 11 patients. Comparing the clinical manifestations and signs of the Greek patients to those of patients from Korea and Scandinavia it seems that the disease is more closely resembling to Korean hemorrhagic fever than to Nephropathia epidemica (two deaths out of 13 patients, 3 patients with hemorrhagic manifestations and 6 patients undertook renal dialysis). Additionally, the examination of the patients' sera by PRNT showed that the the virus existing in Greece is serologically closer to Hantaan virus than to Puumala virus. Finally, the examination of 86 trapped small mammals from endemic areas showed that two of them had antibodies against Hantaan virus and the reservoir of the virus is probably the rodent Apodemus flavicollis.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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Tables

A. Crimean-Congo hemorrhagic fever (CCHF) virus

A.1. Serosurvey

Four hundred and fifty nine sera were collected from people living in a rice growing area near Salonica: 299 sera from children under 14 years old and 160 from adults. Using the IFA test, two children and two adults were found to have antibodies to CCHF virus (table I).
In parallel the above sera were examined by the ELISA test. Two children and 6 adults were found to have antibodies to CCHF virus (table II).

A.2. Attempts to refine the ELISA technique for CCHF virus

During 1982 at YARU, preliminary work on ELISA by Drs. J. Meegan and A. Antoniadis showed that this test could be used for seroepidemiological surveys. In order to detect the sensitivity of the ELISA test, the latter was compared to the IFA and AGD tests. From previous studies done in Greece, it was revealed that a high percentage of goats examined by AGD test had antibodies to CCHF virus. For this reason we used goat sera instead of human sera in our work. According to our results the ELISA test is twice as sensitive than the IFA test (table III and IV).

The readings of the ELISA tests were done by naked eye (there is no ELISA reader and recorder in our laboratory). To improve the reading sensitivity we employed, as a stopping buffer, .005 % Evans blue in 1N HCl acid and the positives were identifiable within a clear cut margin.

A.3. Attempts to diagnose CCHF virus infections in humans

Five trips to Veria have taken place and we collected 360 human sera from patients (264 single and 96 paired). 75 other sera (paired) were collected from the "Infectious Diseases Hospital" of Salonica. None of the patients was found to be infected by CCHF virus.

B. Hemorrhagic fever with renal syndrome (Hantaan virus serosurvey)

B.1. Using the IFA test on the 459 sera mentioned above, 22 out of 299 (children sera) and 5 out of 160 (adult sera) were positive (table V). The titers were > 1:32.

B.2. During August of 1983, an HFRS outbreak occurred in Epirus, an area of N.W. Greece close to Albanian borders. Nine individuals were infected and one of them died. They were members of three families who resided in three different villages (Tsepelovo, Elatohori, Mikri Votista).

Theses villages are in the same locality and are situated at an altitude of approximately 1.000 m. The laboratory diagnosis of the disease was obtained by IFA test, and the results are shown in table VI. This outbreak was a stimulus to further investigate the epidemiology of the disease. Hence Dr. James LeDuc from U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) came to Greece in May of 1984 and a fieldwork was undertaken for 15 days. The village of Tsepelovo was selected for this purpose. 184, out of 330, residents were bled and 12 (7%) were found to have antibodies to Hantaan virus (table VII).

B.3. Attempts to diagnose HFRS cases. 81 human sera (20 single, 61 paired) were taken from patients suspected of HFRS and leptospirosis. These patients were hospitalized in Thessaloniki. During June and December of 1984, 4 cases of HFRS were diagnosed. One of them was very severe and this patient died (A). The results of the serological diagnosis and the clinical history of the patient who died are illustrated in tables VIII and VIII (I) respectively. All these patients were residents of Thessaloniki close to the Yugoslavian borders. It must be mentioned that another case of HFRS occurred 2 years ago in the same area.

B.4. Attempts to isolate Hantaan virus. During the course of fieldwork, rodents were trapped. Our aim was to trap rodents from a hut at an altitude of 2.000m where the infected family tended goats. However, owing to a severe winter, this area was still covered by snow and no rodents were trapped. Hence traps were set at decreasing altitudes and rodents were finally caught in and around the village. In the village the rodents were of the Rattus rattus species only. Surrounding the village, only the Apodemus species was found.

A small laboratory was set-up in the village. Blood, spleen, lung and kidney samples were taken and kept into liquid nitrogen until further examination.

As a result of IFA test, 2 rodents were found to have antibodies to Hantaan virus (table IX). The spleen, lung and kidney samples of the 2 positive rodents were transported to the USAMRIID. During my visit there, they were inoculated in Vero E-6 cells for virus isolation. This virus isolation is still in progress. Continuing this work in the USA, a comparison of Immunofluorescent antibody (IFA) and Plaque reduction neutralization test (PRNT) was made. According to our preliminary results (table X), the virus existing in Greece appears to be similar to Hantaan virus which exists in Korea.

C. Conclusions and future plans

C.1. CCHF virus

CCHF virus must exist in Greece. In the future we intend to extend our collection of human sera to several areas within Greece in an attempt to find other foci of CCHF. In parallel, we shall try to diagnose CCHF human infections. To this date we don't know the forms of the CCHF human infection. Maybe the infection is milder than that of Bulgaria or Russia. For this reason, a large number of sera taken patients with pyrexia of unknown origin will be examined in the future.

C.2. ELISA tests seems to work perfectly so this test will be used in the serosurvey and for the diagnosis of the disease, in parallel with IFA tests.

CCHF spot slides for IFA tests are already prepared in our laboratory and CCHF antigens for ELISA and HI test have also been prepared. Hence, we believe that our laboratory will be self-sufficient for the future.

C.3. HFRS

Our preliminary study indicates that Hantaan or Hantaan-like virus

exists in Greece. It seems that the virus which exists in Greece is more closely related to Hantaan than to Nephropathia epidemica virus (two deaths out of thirteen diagnosed cases) (table X) and the reservoir of the virus is the rodent Apodemus flavicollis. Virus isolation from rodents and humans is in progress. However, we believe that it is necessary during 1985 to undertake further fieldwork in HFRS epidemic areas. Attempts also need to be made to isolate the virus in humans.

In addition we shall collect a large number of samples form different areas of Greece in order to identify new endemic areas of HFRS.

For better progress of this project an increase has been made in its budget.

This extension covers the costs of:

i) A low temperature refrigerator (-70°C).

ii) ELISA recorder.

iii) Travel expenses for fieldwork in the HFRS endemic areas.

iv) Travel expenses of the PI to the USA.

v) 10% overheads for the University.

Table I

CCHF study: Serosurvey of humans using IFA test

	No. of sera examined	Positives
Children (14 yrs)	299	2
Adults	160	2
Total	459	4

Table II

CCHF study: Serosurvey of humans using ELISA test

	No. of sera examined	Positives
Children (<14 yrs)	299	2
Adults	160	6
Total	459	8

(Anti-human sera Peroxidase conjugate now available)

Table III

CCHF study: Comparison of IFA to ELISA for detection of antibodies to CCHF virus in 69 gost sera from Greece

Number of sera	IFA	ELISA
18	+	+
29	-	-
16	-	+
2	+?	+
1	+?	-
3	-	+?

Table IV

CCHF study: Comparison of IFA, ELISA and AGD for detection of antibodies to CCHF virus in goat sera from Greece

	IFA	ELISA	AGD
Positives	18	36	8
? Positives	3	1	2

Table V

Hantaan virus study: Serosurvey of humans using IFA test

	No. of sera examined	Positives
Children (<14 yrs)	299	22
Adults	160	5
Total	459	27

Table VI

Summary of Serological Results on HFRS Patients from Epirus, Greece, July-August, 1983

		1st Sample			2nd Sample			3rd Sample			4th Sample		
Location	Name	Day	IgG	IgM	Day	IgG	IgM	Day	IgG	IgM	Day	IgG	IgM
Tsepelovo	Ntagas E.	18	1:4096	1:512	300	1:16000							
	Ntagas K.	5	1:2048	1:2048	88	1:32	---						
	Ntagas N.	?	1:128	1:32	300	1:2048	---						
	Ntagas P.	8	1:512	1:2048	300	1:2048	---						
Elatohori	Pashos Th.	14	1:8192	1:64	24	1:4096	1:64	79	1:4096	---	300	1:2048	---
	Pashos Chr.	7	1:512	1:2048	DIED								
Mikri Votista	Dimos D.	8	1:32000	1:2048	39	1:16000	1:128	64	1:16000	1:64	101	1:16000	---
	Sioulas I.	8	1:8192	1:2048									
	Sioulas E.	NA	1:2048	1:512	Inapparent infection								

Table VII

Survey of Residents of Tsepelovo, Epirus, Greece for HFRS Antibody or Disease; June, 1984

Age	Male	Female	Disease	Abv	Total
6-9	1	6			7
10-19	35	24		1 F	59
20-29	21	10	1 M	1 M	31
30-39	12	6	2 M		18
40-49	9	15		1 F;1 M	24
50-59	18	12		2 F;1 M	30
60-69	5	5	1 M	1 F	10
70-79	5	--			50
TOTAL	106	77	4	8	184

12 (7%)

Table VIII

Serological Findings for one Fatal and one non Fatal Case of HFRS in Greece, 1984

Onset: A. June 4, 1984
B. August 2, 1984
C. September 14, 1984
D. August 13, 1984

		Hantaan Virus (IFA)		
Day of illness		IgG	IgM	Leptospirosis
	7	1:4096 1:2048	1:1024 ND	< 1:8
Patient A	8	1:4096 1:2048	ND 1:1024	< 1:8
	10	1:2048 1:1024	ND 1:512	< 1:8
	14	1:2048	1:1024	< 1:8
Patient B	18	1:2048	1:1024	< 1:8
	20	1:4096	1:512	< 1:8
Patient C	20	1:8192	1:1024	< 1:8
		1:8192	<1:16	< 1:8
Patient D	10	1:8192	1:4096	< 1:8
	13	1:8192	1:1024	< 1:8

Table VIII(2)

Clinical History of a Fatal Case of HFRS in Greece, 1984 (Patient A)

Patient: 54 year old male

Profession: Taxi driver

Possible exposure: Two weeks before onset had been fishing at a nearby river

Previous history: None

Onset of disease: 4 June 1984

Admission history:

High fever (39°C) of 4 days duration accompanied by rigors and muscle pain, then temperature fell and the patient developed renal incompetence.Patient admitted on day 6 of illness with anuria, conjuctivitis and large ecchymosis on left arm, but his general condition was satisfactory.

Management:

Peritoneal dialysis was begun and ascetic fluid obtained;However, the presence of ascitis was not clinically apparent.
On the 7th day the patient developed consolidation of right lung with no fever, followed by gradual confusion and stupor with meningism. Apart from passing 50 ml of blood stained urine, anuria persisted. There were no other hemorrhagic manifestations and blood clotting check showed commencing intravascular coagulation.
Gradually jaundice of the skin and sclerae appeared with increase of indirect biliburin and transaminase. On the 9th day cyanosis was present (PO_2 70 Hg) and the patient died on the 10th day of illness.

Table IX

Summary of Smalls Mammals Captured In and Near Tsepelovo, Epirus, Greece and Tested for IFA Antibody to Hantaan Virus; June, 1984

Species	No. Trapped	No. Positive	IFA Titer
Rattus rattus alexandrinus	41	---	
Rattus rattus frugivorus	10	---	
Apodemus flavicollis	33	2	1:256,1:2048
Apodemus sylvaticus	0	0	
Crocidura	1	---	
TOTALS	86	2	

Table X

Comparison of Immunofluorescence Antibody and Plaque reduction Neutralization Test (PRNT) Results on Hemorrhagic Fever with Renal Syndrome Patients' Sera from Greece and Korea

Serum	Day of Post-Onset Illness	IFA	PRN * 50%	PRN * 80%
Greece				
1	10	2048	2048	512
2	38	4096	2048	128-512
3	300	4096	512	32
4	300	1024	128	32
5	300	4096	128	32
6	Unknown	256-512	32-128	<8
7	64	2048	512	32
8	24	4096	2048	128
Korea				
Douglas	Unknown	2048	2048	512
7002	Unknown	>2048	2048	512

* Hantaan virus strain 76-118

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