AD_____

INVESTIGATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER AND HEMORRHAGIC FEVER WITH RENAL SYNDROME IN GREECE

Final Report

Antonios Antoniadis, M.D.

August 19, 1991

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21702-5012

Grant No. DAMD17-87-G-7019

Aristotelian University of Thessaloniki

School of Medicine

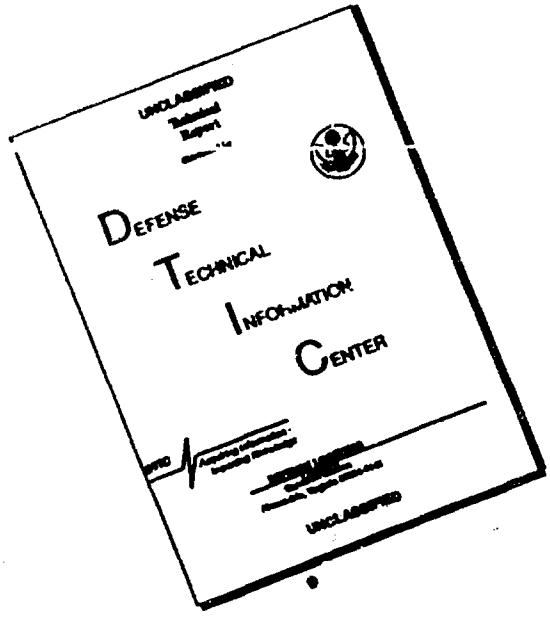
Thessaloniki, 54006 Greece

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release, distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Aristotelian University of Thessaloniki; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Thessaloniki 54006, Greece	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S.Army Medical Research and Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-87-C-7019
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1- 61102BS13; TASK NO. AA; WORK UNIT ACCESSION NO 021

11. TITLE (Include Security Classification)
Investgation of Crimean-Congo Hemorrhagic Fever and Hemorrhagic Fever with Renal Syndrome in Greece

12. PERSONAL AUTHOR(S)
Antoniadis Antonios, M.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 4.15.87 TO 6.15.90	1991/8/19	19

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	07		Hemorrhagic fever:; Epidemiology; Rodent; CCHF; Bu.
06	12		Congo-Crimean; Foreign; Risk Assessments; RA I

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

During a 3 year and 2 month period, from April 1987 to June 1990, 3,067 human sere were tested for antibody to Hantaan and C-CHF viruses. The overall antibody prevelance rate was found to be 3,0% and 1,8% respectively. Animal serosurveys were conducted for antibody to C-CHF virus (goats and sheep) and for antibody to Hantaan virus (small mammals). The overall antibody prevelance rate was found 3,8% and 2,8% in goats and sheep respectively. High antibody titers to Hantaan virus were detected only in Apodemus flavicollis sera suggesting that Ap. flavicollis is the possible host of the virus. A Hantavirus was isolated from a severely HFRS ill patient. Serological comparison of the isolated virus to other Hantaviruses established it as a member of this group. Twenty HFRS cases have been serologically diagnosed. The disease was a severe form of HFRS with a mortality rate 15%. For C-CHF blood samples were obtained from patients with disease resembling C-CHF (60 patients) and from patients with other diseases. None of the patients was found to be infected with C-CHF virus. During May 1990, an inapparent C-CHF infection was serologically diagnosed. The infected individual had no recollection of an illness clinically resembling a serious type of Crimean-Congo hemorrhagic fever. This fact and the

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Virginia M. Miller	22b. TELEPHONE (Include Area Code): 301-663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

19.and also the high percentage of seropositive individuals in certain surveyed areas suggest that the C-CHF virus which exists in Greece may not cause severe form of Crimean Congo hemorrhagic fever or the antibody to C-CHF virus found in humans and animals are due to the infection with another virus antigenically close related to C-CHF virus.

TABLE OF CONTENTS

FOREWORD

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

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NHI Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

A. INTRODUCTION

During a 38 month period, from April 1987 through June 1990,research has been carrying out to identify Hemorrhagic Fever with Renal Syndrome (HFRS) and Crimean-Congo Hemorrhagic Fever (C-CHF) endemic areas, isolate viruses and diagnose diseases caused by Hantaan and C-CHF viruses. Thus, new HFRS and C-CHF endemic areas have been identified in Greece, the clinical course as well as the main laboratory findings (hematology-chemistry) of the severe form of the HFRS as it occurs in Greece has been described, a Hantaan virus has been isolated from a severely ill HFRS patient, the probable rodent host of the virus has been described and the technique ELISA IgM captured has been applied for the rapid HFRS serological diagnosis. Attempts have been made to diagnose human C-CHF infections, to isolate C-CHF viruses from collected ticks and serosurveys have been conducted among domestic animals.

B. MATERIALS AND METHODS

B1. Materials

B1.1 Serological diagnosis of human disease caused by HFRS and C-CHF viruses.

One thousand and twenty-two acute and convalescent sera were collected between April 1987 to June 1990 from patients whose illnesses had been clinically diagnosed as hemorrhagic fever with renal syndrome, leptospirosis, acute nephritis, or acute renal insufficiency, and from patients with influenza- like disease, with pyrexia of unknown origin, pyrexia with elevated liver enzymes and from patients suspected for Crimean-Congo hemorrhagic fever. The patients were residents of various parts of northern and central Greece. The patient's serum, single or paired, was examined on the day of arrival in the laboratory, or stored at -20 C until they could be tested by the indirect immunofluorescence and ELISA IgM capture tests. In positive HFRS diagnosis, blood and urine samples were collected from the patients for virus isolation in Vero E-6 cells.

B1.2. Human serosurvey

Sera from 3067 apparently healthy individuals, mainly farmers, wood cutters and shepherds were obtained in 16 of 54 counties of Greece: 13 in northern Greece (Thrace, Macedonia and Epirus), and 3 islands of Aegean sea. Additionally, 81 sere were obtained from soldiers camping in an HFRS endemic area. Sera were collected annually from April 1987 to June 1990 and stored at -20 C until they could be tested for antibodies to Hantaan and C-CHF viruses. Individuals were identified by age, sex, occupation, previous travel history (mainly abroad) and location of residence.

B1.3. Animal serosurvey.

Sera from 350 sheep and 883 goats were obtained mainly from herds pastured in areas where antibodies to C-CHF virus had been previously found in humans and from herds pastured in areas close to Bulgarian and Albanian borders. Sera were collected annually and stored at -20 C until they could be tested for antibodies to C-CHF virus. Sheep and goats were identified by sex and location of the herd.

B1.4. Small mammal collection and serosurvey.

One hundred and seventy-seven small mammals were live-trapped at various sites of northern Greece where human HFRS cases had been previously serologically diagnosed. The captured animals were identified as to species and exsanguinated by cardiac puncture. Sera were kept at -20 C until tested for antibodies to Hantaan virus. Lungs, spleen and kidneys were taken and after treatment were used for virus isolation in Vero E-6 cells.

B1.5. Tick collection

A total of 1233 ticks were collected from goats and sheep for C-CHF virus isolation. Ticks were identified as to species and sex and the locality of the herd was also identified. Pools of 10 to 15 ticks of the same species were treated for C-CHF virus isolation in Vero E-6 cells.

B2. Methods

B2.1. Serological diagnosis and serosurveys.

Patients sera, and apparently healthy individual's sera collected for serosurvey, were examined by IFA tests with goat anti-human fluorescence immunoglobulin. Patient's sera were examined by both IgG and IgM anti-human fluorescence immunoglobulin whereas the apparently healthy individual's sera only with IgG. Small mammal's sera were tested by the same method with rabbit anti-mouse IgG fluorescence immunoglobulin. Spot-slides were prepared in the laboratory and contained Vero E-6 cells, approximately 50% infected with the 76-118 strain of

Hantaan virus. Patient's sera were further tested by μ-capture enzyme-linked immunosorbent assay (ELISA). The antigen as well as the control positive and negative sera were supplied be the USAMRIID.

Concerning Crimean-Congo hemorrhagic fever, patient's sera, and apparently individual's sera collected for serosurvey were examined by IFA tests with goat anti-human fluorescence immunoglobulin. Patient's sera were examined by both IgG and IgM anti-human fluorescence immunoglobulin whereas the apparently healthy individual's sera only with IgG. Animal's sera (goat and sheep) were test by the same method with rabbit anti-goat IgG fluorescence immunoglobulin. Spot-slides were prepared in the laboratory and contained Vero E-6 cells, approximately 50% infected with the 10200 Ibr strain of C-CHF virus. Patient's sera were further tested by enzyme-linked immunosorbent assay (ELISA). The antigen was a crude one prepared from Vero E-6 cells infected by 10200 Ibr strain of C-CHF virus.

B2.2. Hantaan and C-CHF virus isolation.

a. Hantaan virus isolation from rodents and from HFRS patients.

Lung tissues from 9 seropositive and 78 seronegative rodents were dissociated with a mechanical blender. The dissociated lung tissues were inoculated into Vero E-6 cells grown in 25-cm2 plastic flasks. Inoculated flasks were incubated at 37o C for 15 days, then cells were suspended with trypsin and passed to fresh flasks. While suspended, some cells were used to prepare 10 well spot slides, which were then fixed in cold acetone and examined for characteristic hantavirus antigen by IFA assays with human antibody to Hantaan virus. The same procedure was followed for another two passages in order the inoculum to be characterized as negative.

Whole blood, serum and urine samples were collected from 14 HFRS patient's during the first week of their disease. One of blood and serum samples were inoculated into separate 25-cm2 flasks of Vero E-6 cells in the presence of 8 ml of growth medium. Urine samples were

immediately alkalized with sodium bicarbonate and aliquot of 0.25, 0.5, 1.0 and 1.5 ml of the urine were inoculated into separate 25-cm2 flasks of Vero E-6 cells in the presence of 8 ml growth medium. For the detection of Hantavirus antigen, was attempted following the procedures described above (b2.2).

b. Crimean-Congo hemorrhagic fever virus isolation from ticks.

One thousand and six hundred twenty-two collected ticks were identified into species and were separated in 150 pools. Pooled ticks were ground in a mortar in PBS buffer (ph 7.2) with 1% bovine serum albumin (fraction V) and 1% penicillin/streptomycin to make a 10% suspension. After centrifugation at low speed, the supernatant was inoculated in 25-cm2 flasks containing Vero E-6 cells. Inoculated flasks were incubated at 37o C for six days, then the cells were suspended with trypsin and were used to prepare 10 well spot-slides, which were they fixed in cold acetone and examined for characteristic C-CHF antigen by IFA assays with human and mouse ascitic fluid antibodies to C-CHF virus. The supernatant as well as part of inoculated cells were stored at -70 C.

C. RESULTS

C.1. Hemorrhagic fever with renal syndrome

C1.1. Patients and disease

Seven hundred and eighty-nine male and female farmers, shepherds, woodcutters were admitted to various Hospitals of Thessaloniki and other General Hospitals located in the county capitals,with clinical diagnosis of leptospirosis, acute nephritis or acute renal insufficiency, with pyrexia of unknown origin, and with influenza-like disease. The diagnosis of HFRS was serologically confirmed in 20 of these patients by rising antibody titers (IgG and IgM) to Hantaan virus. In case where only single blood samples were available, the determination of specific IgM antibodies to Hantaan virus in high titers confirmed the diagnosis. In 19 out of

20 serologically diagnosed HFRS patients, the clinical diagnosis was suspected HFRS, leptospirosis, acute nephritis and in one patient the clinical diagnosis was influenza-like disease.None of the patients with pyrexia of unknown origin was found to be infected with Hantaan virus (Table 1).

Analysis of data concerning clinical signs and symptoms of the disease as reported in the patients medical records are shown in Table 2. Of 20 serologically diagnosed cases, 3 died (mortality 15%) and 16 (80%) developed severe symptoms including flushing over face and neck, conjunctival injection, pneumonic infiltration, pulmonary edema, confusion, shock, and hemorrhagic manifestations. Eleven of these severely ill patients (55%) required renal dialysis. The predominal symptoms in all patients were fever, headache, nausea, vomiting, and abdominal pain, while flushing of the face, conjunctival injection, pulmonary edema, shock and hemorrhagic manifestations were only common in the severely ill patients. Proteinurea with microscopic hematurea and increased serum urea and creatinine were present in all patients.

As in the case of HFRS in other parts of the world the clinical course of the disease in these patients could be separated into five distinct phases: febrile, hypotensive, oliguric., diuretic and convalescent. Most patients had entered the hypotensive phase by the time they were hospitalized (4-7 days after onset) and showed prominent skin flushing. During this phase, shock developed in 3 patients, of whom 1 died. During the oliguric phase (ninth to 12th days of illness), most patients developed marked nausea and vomiting. 2 developed pulmonary edema, and 11 exhibited hemorrhagic manifestations associated with coagulation abnormalities. Eleven patients required hemodialysis because of severely impaired renal function. 2 of these patients, both of whom survived, required more intensive treatment with respiratory support because of recurrent and persistent pulmonary edema. Two of the patient's with hemorrhagic manifestations died during the oliguric phase (two on ninth day of disease and one on the 12th). All patient's who survived the oliguric phase entered the diuretic phase, with a gradual improvement in renal function and clinical symptoms. In this phase the patient's had a urinary

output of 2-4 L/24 hours. After a further 1-2 weeks, patient's entered the convalescent phase and were discharged from the hospital. They were observed for up to 6 months, and none showed any signs of renal failure.

C1.2. Human serosurvey.

During the period April 1987 through June 1990, 3067 human sera collected from 16 out of 54 counties of Greece. All sera analyzed together and the results summarized according to region and the county of origin in which the serosurveys were conducted, as shown in Table 3. The overall antibody prevalence rate ~ 2.93% with a range from 0 to 16.6%. Moreover, seropositive were detected in 14 of 15 counties, indicating that the virus is widespread in Greece. From analysis of the age distribution of individuals with antibody to Hantaan virus, it is suggested that Hantaan virus is active in all investigated areas and the age groups which are at increased risk are 31-40 and 41-50 years (Table 4). It is difficult to interpret the significance of antibody prevalence by occupation because the residents of small villages are mainly farmers but sometimes work as shepherds and wood cutters. However, according to our data, it appears that farmers and wood cutters are at increased risk to infection with Hantaan virus (Table 5). From the clinical cases and serosurveys, the ratio of males to females infected is approximately 3:1. The first cases appeared in early May, and cases were observed until late October.

C1.3. Small mammal serosurvey.

During the period April 1987 through June 1990, 177 small mammals were collected mainly from HFRS endemic areas. High antibody titers to Hantaan virus were detected in 7 Apodemus flavicollis sera (Table 6). To date, neither Apodemus agrarius, the host of Hantaan virus, nor Clethrionomys glareolus, the host of Puumala virus, was captured at any site sampled.

C1.4. Hantaan virus isolation from HFRS patients and small mammals.

A Hantavirus was isolated from the urine of a severely ill HFRS patient. Serological

comparison of the isolated virus to other hantaviruses establishes it as a member of this group. Although the IFA test results revealed little difference between the isolate and Hantaan virus, the more specific PRN tests demonstrated sufficient differences to suggest that the isolated virus represents a unique strain of hantaviruses. A comparison of PRN titers to both Hantaan virus and the Greek isolate with titers of convalescent sera from patients previously diagnosed with severe HFRS found highest titers to the Greek virus, a result suggesting that this virus may have been responsible for many of HFRS cases in Greece.

So far, all attempts to isolate virus from lung tissues of Apodemus flavicollis have been unsuccessful.

C2. Crimean-Congo hemorrhagic fever.

C2.1. Patients and disease.

Blood samples were collected from patients hospitalized in General Hospitals where human and animal seropositive were found during serosurveys for antibody to C-CHF virus. Eight hundred and eight blood samples (single or paired) were examined by IFA and ELISA tests for detection of IgG and IgM antibodies to C-CHF virus. Blood samples were obtained from patients with disease resembling C-CHF, from patients with pyrexia of unknown origin and from patients with pyrexia and elevated liver enzymes (SGOT, SGPT, γGT) and from patients with influenza-like disease. None of these patients was found to be infected by the C-CHF virus (Table 1).

C2.2. Human serosurvey.

During the period April 1987 through June 1990, 3067 human sera were collected from 15 out of 54 counties of Greece. All sera analyzed together and the results summarized according to region and the county of origin in which the serosurveys were conducted, as shown in Table 3. The overall antibody prevalence rate was 1.79% with a range from 0 to 8.7%. During June 1989 a serosurvey was conducted in residents of Grammos (Kastoria county) and Theodoriana (Arta

county) villages. In one year interval (late May 1990), a second serosurvey was conducted in the same villages. In both villages the percentage of seropositivity remained almost the same between the first and the second serosurvey. In Grammos village which is very close to Albanian borders, the percentage of seropositive was found extremely high (15.1% in 1989 and 13.7% in 1990). Additionally, one individual who was seronegative in 1989 developed antibody to C-CHF virus in 1990. The IFA titer in 1989 was 1:4 whereas in 1990 was 1:64. The seropositive individuals of the Grammos village as well as the individual with seroconversion had no recollection of an illness clinically resembling a serious type of Crimean-Congo hemorrhagic fever. Additionally, the individual with seroconversion had no recollection of a mild illness such as influenza-like disease during the period between June 1989 and May 1990. Seropositive were detected in 13 of 16 counties, indicating that the virus is widespread in Greece. From analysis of the age distribution of individuals with antibody to C-CHF virus, it is suggested that C-CHF virus is active in Greece and the age groups which are more infected with the virus are 31-40 and 41-50 years (Table 4).As for Hantaan virus, it is difficult to interpret the significance of antibody prevalence by occupation. However, according to our data, it appears that shepherds are at higher risk to infections with C-CHF virus than the other examined groups.

C2.3. Animal serosurvey.

One thousand and two hundred thirty-three blood samples were obtained from goats and sheep whose herds were pastured in different counties of Greece (Table 7). All sera were examined by IFA and ELISA assays for antibody to C-CHF virus. Antibody to C-CHF virus were found in 7 herds which were pastured in 13 counties surveyed. (Table 7). The highest percentage of seropositive (5%) was found in Kastoria county where also the highest seropositivity in humans was found.

C2.4. Attempts for C-CHF virus isolation from ticks.

So far, all attempts to isolated C-CHF virus from ticks have been unsuccessful.

D. COMMENTS

D1. Hemorrhagic fever with renal syndrome.

In Greece (previous and present investigation) and surrounding Balkan countries, HFRS more closely resembles the severe form of disease. Even the severest cases of Nephropathia Epidemica in Scandinavia and Western European countries present a milder clinical picture in hospitalized patients compared with HFRS in our hospitalized patients. The clinical picture of the disease in Creece seem to be quite similar to those of the severe form of HFRS in Asia, including abrupt onset, conjunctival injection, and high incidence of severe systemic manifestations requiring aggressive treatment. HFRS in Greece progresses the same five phases seen in patients in Asia and is associated with a relatively high mortality rate (15%).

The isolation of a Hantavirus from the urine of a severely ill Greek patient further establishes the similarity between GreeK and severe Asian disease. This virus is antigenically more close related to prototype Hantaan virus than to either Puumala virus of Scandinavia or Seoul virus of Asia. From serosurveys conducted in Greece, it seems that the virus is widespread in the country infecting human population. Results obtained from serosurveys suggest that mild and inapparent cases without hospitalization may exist very often. The vertebrate host of the virus causing severe HFRS in Greece may be Apodemus flavicollis. Antibodies to Hantaan virus have been found only in this species, and Apodemus flavicollis is found throughout the Balkan Peninsula. Data obtained from serosurveys and from the occurrence of HFRS cases in several areas of Greece, suggest that a hyperendemic HFRS area exists in Greece. This area is located in northwest and central Greece. Mountain Pindos which is a continuation of the Albanian Karst, covers a large area from northwest to south west. More than 50% of HFRS cases as well areas with very high seropositivity, are located on the foothills of this mountain (Fig. 1).

D2. Crimean-Congo hemorrhagic fever.

Antibodies to C-CHF virus were found in humans as well as in animals, and in some areas

(close to Albanian borders) the percentage of seropositive had been found very high (15.1%). In contrast, the examination by IFA and ELISA assays of 808 blood samples obtained from patients with suspected C-CHF or from patients with pyrexia of unknown origin, influenza-like disease, and pyrexia with elevated liver enzymes showed that none of these patients was found to be infected with C-CHF virus. Facts such as seroconversion of an individual to antibody to C-CHF virus, high percentage of seropositive individuals in certain surveyed areas and no recollection of an illness clinically resembling a serious type of Crimean-Congo hemorrhagic fever, suggest that the Greek strain (AP92) which was isolated in 1976 from ticks, may be not pathogenic to cause the severe form of the disease or the antibodies to C-CHF virus found in humans and animals are due to the infection with another virus antigenically close related to C-CHF virus. Attempts to isolate C-CHF virus from ticks have been unsuccessful.May be it is due to the use of Vero E-6 cells for the isolation of the virus. Suckling mice have not been used for safety reasons.

E. PUBLICATIONS (April 1987 to June 1990)

1. Clinical and epidemiological aspects of hemorrhagic fever with renal syndrome (HFRS) in Greece (1987): A. Antoniadis, J.W. LeDuc and ST. Alexiou-Daniel. Eur. J. Epidemiol. 3,3:295-301.

2. Isolation of a Hantavirus from a severelly ill patient with hemorrhagic fever with renal syndrome in Greece (1987): A. Antoniadis, D. Grekas, C.A Rossi and J.W. LeDuc. J. Infect. Dis. 156,6:1010-1013.

3. Hemorrhagic fever with renal syndrome in Greece: Clinical and laboratory characteristics (1989): A. Antoniadis, J.W. LeDuc, N. Acritidis, S. Alexiou-Daniel, A. Kyparissi and G. A. Saviolakis. Rev. Infect. Dis. II, [Suppl. 4]:891-895.

4. Seroepidemiological survey for antibodies to arboviruses in Greece (1990): A. Antoniadis, S. Alexiou-Daniel, N. Malissiovas, J. Doutsos, Th. Polyzoni, J. W. LeDuc, C.J. Peters, and G. Saviolakis. Arch Virol, [Suppl 1]:277-285.

List of Publications Under DAMD17-87-G-7019

1. Eur. J. Epidemiol. 3,3:295-301, 1987.

2. J. Infect. Dis. 156,6:1010-1013, 1987.

3. Rev. Infect. Dis. II, [Suppl. 4]:891-895, 1989.

4. Arch Virol, [Suppl 1]:277-285, 1990.

Table 1. Blood samples collected from hospitalized patients for HFRS and C-CHF serological diagnosis.

Disease	No of patients examined	HFRS cases	C-CHF cases
Pyrexia of unknown origin	374	0	0
HFRS-like disease	214	19	0
Influenza-like disease	201	1	0
C-CHF-like disease	60	0	0
Pyrexia with elevated liver enzymes	173	0	0
TOTAL	1022	20	0

Table 2. Clinical symptoms and signs in 20 HFRS Greek Patients.

Symptoms and signs	No. of Patients
Fever	20
Rigors	20
Headache	20
Abdominal pain	20
Myalgia	18
Arthralgia	18
Vomiting	17
Backache	17
Flush over the face and neck	16
Conjnctival injection	14
Hypotention	14
Confusion	10
Shock	3
Diarrhea	4
Pneumonic infiltration	3
Cough	3
Hemorrhagic manifestations	11
Pulmonary edema	2

Table 3. Antibody to Hantaan and C-CHF viruses in healthy residents of Greece.

Region, county	No of sera tested (% positives) Hantaan	C-CHF	No of HFRS cases
Thrace			
Evros	162 (3.0)	162 (1.2)	-
Rodopi	525 (1.3)	525 (1.1)	-
Xanthi	183 (0.5)	183 (1.0)	-
Macedonia			
Drama	416 (0.4)	416 (1.0)	1
Serres	35 (0.0)	35 (0.0)	-
Kilkis	102 (3.9)	102 (1.8)	
Pella	173 (1.1)	173 (1.6)	-
Halkidiki	123 (0.8)	123 (0.8)	-
Kozani	76 (3.9)	76 (2.6)	-
Kastoria	171 (12.2)	171 (8.7)	2
Florina	122 (5.7)	122 (2.4)	-
Epirus			
Ioannina	484 (3.0)	484 (1.6)	12
Arta	114 (16.6)	114 (4.3)	3
Islands			
Thasos	188 (0.5)	118 (0.5)	-
Chios	71 (1.4)	71 (0.0)	-
Krete	41 (2.4)	41 (0.0)	-
Others	81 (0.0)	81 (0.8)	-
TOTAL	3067 (2.93)	3067 (1.79)	18

Two HFRS cases were also diagnosed in Thessalia region (1 in Trikala and 1 in Karditsa). In these counties serosurveys were conducted in 1986.

Table 4. Antibody to Hantaan and Crimean-Congo hemorrhagic fever viruses in residents of Greece, by age.

Age (years)	No. of tested	Hantaan No. positive	CCHF No positive
0-10	149	2	0
11-20	320	1	1
21-30	458	5	2
31-40	533	19	6
41-50	472	25	18
51-60	534	24	21
61-70	519	9	4
70	82	5	3
TOTAL	3067	90	55

Table 5. Antibody to Hantaan and C-CHF viruses in residents of Greece, by occupational distribution.

Occupation	No of tested	Hantaan No Positive	C-CHF No positive
Farmers	1830	19	7
Woodcutters	257	29	5
Farmers-woodcutters	271	27	18
Shepherds	242	6	12
Farmers-shepherds	252	6	11
Others	215	3	2
TOTAL	3067	90	55

Table 6. Antibody to Hantaan virus in small mammals captured in endemic and nonendemic HFRS areas of Greece.

Species	No of tested	No of positive
Apedemus flavicollis	70	7
Apodemus sylvaticus	20	0
Mus musculus	16	0
Rattus r. alexandrinus	56	2
Rattus r. frugivorus	10	0
Unidentified	5	0
TOTAL	177	9

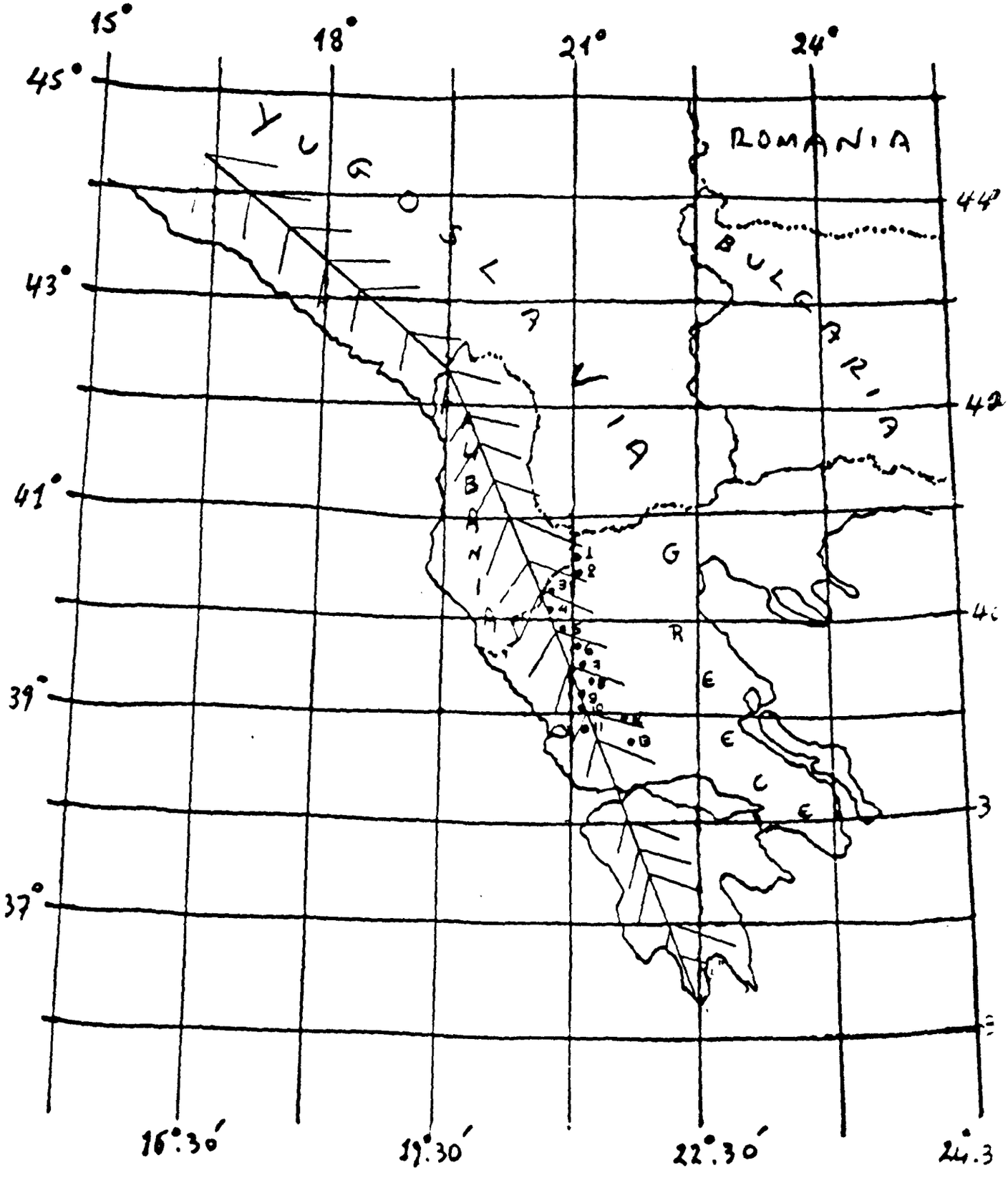
Table 7. Antibody to C-CHF virus in goats and sheep.

Region, county	No of goats	No positive	No of sheep		No positive		No o
			IFA	ELISA		IFA	ELISA
Thrace							
Evros	82		4	4	95	2	3
Rpdopi	116		5	5	103	2	4
Xanti	55		2	2	87	2	3
Macedonia							
Drama	154		4	5			
Kilkis	35		0	0			
Pella	46		0	0			
Thessaloniki	81		0	0			
Pieria	22		0	O			
Kozani	25		2	2			
Kastoria	112		6	8	65	4	5
Central Greece							
Attiki	34		1	1			
Islands							
Thasos	26		0	0			
Lesbos	38		8	10			
Krete	57		0	0			
TOTAL	883		32	40	350	10	15

Table 8. Ticks collected for C-CHF virus isolation during April 1987 through June 1990.

Species	No of ticks
Rhipicephalus sanguineus	59
Rhipicephalus bursa	246
Ixodes gibbosus	312
Hyalomma sp.	264
Unidendified	241
TOTAL	1622

Fig. 1. HFRS hyperendemic area of Greece, which covers north western and central Greece on mountain Pindos.



Numbers indicate villages where HFRS cases have been serologicaly diagnosed