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Outbreak of Haemorrhagic Fever with Renal Syndrome in Greece

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An outbreak of eight cases of haemorrhagic fever with renal syndrome in North-West Greece is presented. The major clinical manifestation was fever and all patients subsequently developed decreased renal function and proteinuria. The disease was diagnosed by rising antibody titers to the Hantaan virus.

Haemorrhagic fever with renal syndrome (HFRS), also known as Korean haemorrhagic fever or nephropathia epidemica, is an acute febrile nephropathy caused by viruses closely related to the family *Bunyaviridae*. The Hantaan virus, isolated in 1978 from lung tissue of *Apodemus agrarius* rodents, is associated with Korean haemorrhagic fever, a severe form of HFRS disease seen in Korea (1, 2) and other parts of Asia. A related virus recovered from *Clethrionomys glariolus* voles in Scandinavia is the cause of nephropathia epidemica, which is usually less severe (3, 4, 5). In Greece, antibodies to the Hantaan virus have been found by indirect immunofluorescent antibody assay in sera of the healthy Greek population (6). This paper reports the first outbreak of the disease in Greece.

Outbreak

Eight male shepherds 23 to 66 years old were admitted to the Ioannina General Hospital in July and August 1983.

HFRS was suspected on the basis of clinical and laboratory findings, and was finally confirmed by rising antibody titers to Hantaan virus determined by indirect immunofluorescent antibody IFA assays on spot slides. To prepare the slides, Hantaan virus was passaged 15 times in A 549 cells and three times in Vero cells. Infected Vero cells were fixed to the slides which were then exposed to ultraviolet irradiation to inactivate the virus. Sera were tested in two-fold dilutions starting at 1:16 using an indirect immunofluorescence technique (7) with goat antihuman fluorescent immunoglobulin (Gibco Diagnostic, Madison, WI). Sera were considered positive if characteristic fluorescence was present at a dilution of 1:32. Positive sera were re-examined and filtrated. Titers were recorded as the highest serum dilution yielding characteristic Hantaan virus fluorescence. Complement fixation tests for leptospirosis were also done. Liver biopsy was performed in one patient and percutaneous kidney biopsy in another patient on the tenth day of illness.

The predominant symptom in all patients was fever. Headache, nausea, vomiting and abdominal pain were also common symptoms and four patients had conjunctivitis. Hypertension and oliguria were not frequently observed. The main laboratory findings are shown in Table 1. Proteinuria with microscopic haematuria and increased serum creatinine were present in all patients tested. Only one patient had gross haematuria. A whitish gelatinous material was observed in the urine of three patients. Histologic examination of this material revealed fibrinoid and nectoric tissues. Hypocalcemia was observed in five patients, while four out of six patients developed hypoproteinemia. Serum C_3 and C_4, rheumatoid factor titers, direct and indirect Coombs tests and platelet counts were all within normal limits or negative. The complement fixation test for leptospirosis was negative, as were blood cultures and tests for antibodies to *Salmonella typhi*. Chest x-rays were normal in seven patients, while in one patient the chest film revealed discoid atelectasis of the right lower lung field. Liver biopsy revealed non-specific reactive hepatitis and renal biopsy degenerative lesions of renal tubules with evidence of regeneration.

One patient died on the day of admission with signs of shock and a partial autopsy was performed. Kidney histology revealed the characteristic picture of haemorrhagic interstitial nephritis. Two patients developed severe acute renal failure with serum creatinine levels of 9.0 and 11.9 mg% respectively. One patient required a long period of hospitalization while the remaining six patients were discharged with normal serum creatinine levels after a mean period of hospitalization of 12 days. Two patients were followed up for five months after discharge. Both felt well and their laboratory tests were normal. Antibody titers to Hantaan virus are shown in Table 2.

Convalescent serum samples were taken from only three patients two to three months after the acute episode. Study of the antibody titers to Hantaan virus in these sera showed reduction or disappearance of the IgM antibody titers with persistence of high IgG antibody titers.

Discussion

HFRS or muroid virus nephropathies (8) came to the attention of the Western medical world during the Korean war (1950–1953). According to Nystrom (5), however, nephropathia epidemica, which is a similar viral disease, has been known in Sweden since 1934.

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Table 1: Laboratory findings in eight Greek patients with haemorrhagic fever with renal syndrome.

Finding	No. of patients
Anemia (hematocrit < 38%)	2/8
Leucocytosis (> 10,000/mm^3)	3/8
Serum creatinine (> 1.5 mg%)	8/8
Hypocalcemia (< 8.5 mg%)	5/5
Hypoproteinemia (< 6.5 g%)	4/6
C-reactive protein (> 16 mg%)	5/7
Microscopic haematuria (> 10 RBC/HPF)[a]	8/8
Urine casts (hyaline, granular)	5/8
Proteinuria (> 0.5 g/24 h)[b]	7/7

[a] HPF = high power field.
[b] Six patients had 24-hour urine protein levels > 2.5 g.

In this report we describe an outbreak of HFRS in eight patients in Greece. The disease showed wide variations in clinical course and severity. Three patients had a severe form of the disease and five patients a mild form. Epidemic haemorrhagic fever is characterized by five clinical phases: fever, hypotension, oliguria, diuresis and convalescence (2, 9).

Since only two out of eight patients developed severe hypotension and only one oliguria, we think that the clinical manifestation of the disease in our patients is more consistent with the mild forms of the disease seen in Finland (4). Involvement of the central nervous system, gastrointestinal tract and myocardium have also been described (4, 9, 10). Six of the eight patients complained of mild to severe headache and more than half had gastrointestinal symptoms. However, liver biopsy performed in one patient showed non-specific lesions. Bradycardia, which has been described as a common finding, was present in only one patient.

Abnormal findings on urinalysis are the charateristic signs of the disease, and include proteinuria and microscopic and macroscopic haematuria. All of our patients had microscopic haematuria and six out of seven had 24-hour urine protein levels ranging from 2.0 to 7.3 g. In one patient the 24-hour urine protein level was 0.5 g. This patient had the mildest form of the disease.

The passage of tubular membrane material in the urine has been observed in patients with HFRS and is considered a very poor prognostic sign (9). We observed this phenomenon in the three patients who were most severely affected. Histologically the renal lesion in HFRS is characterized by haemorrhagic interstitial nephritis (11). Renal biopsy in one of our patient revealed only mild tubular abnormalities, however the biopsy was performed during the diuretic phase of the disease when serum creatinine was almost normal. On the other hand, the kidney histology on autopsy was compatible with haemorrhagic interstitial nephritis.

In all our patients the diagnosis was confirmed by detection of high antibody titers to Hantaan virus. Lee et al. (1) report high antibody titers to Hantaan virus determined by immunofluorescence assay in sera of patients in the acute phase of the disease. The presence of Hantaan virus-specific IgM antibodies in all our patients is additional evidence that the infection was recent and caused by the Hantaan virus or a closely related virus.

Epidemiological studies are underway and attempts are being made to isolate the virus from humans and rodents in order to elucidate further this interesting disease.

Table 2: Antibody titers to Hantaan virus in eight patients with haemorrhagic fever with renal syndrome.

Patient no.	Day of illness[a]	Acute phase serum IgM	Acute phase serum IgG	Convalescent phase serum IgM	Convalescent phase serum IgG
1	8	2,048	32,000	64	16,000
2	18	512	4,096		
3	5	2,048	2,048	0	32
4	3	32	128		
5	8	2,048	512		
6	14	64	8,192	0	4,096
7	5	2,048	512		
8	8	2,048	8,192		

[a] Applies to acute phase sera only.

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