

SEROEPIDEMIOLOGY OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME IN BULGARIA

*M. CHUMAKOV, L. SHINDAROV, I. GAVRILOVSKAYA, SV. VASILENKO,
E. GORBACHKOVA, G. KATZAROV

*Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences of the U.S.S.R., 142782 Moscow, and Institute of Infectious and Parasitic Diseases, Chair of Virology, 1233 Sofia, Bulgaria

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Summary. — During the period of 1954—1986, 399 cases of haemorrhagic fever with renal syndrome (HFRS) were registered in Bulgaria with 63 (15.7%) deaths. Three hundred serum samples from 214 patients who had contracted the disease from 1957 to 1986 were investigated by indirect fluorescent antibody test (IF-AT). As antigen Vero-E6 cells infected with the Asian Hantaan virus were used, as well as lung sections from bank voles (*Clethrionomys glareolus*) infected with strains Udmurt and Kazan 6-Cg from the European part of the U.S.S.R. Specific antibodies were detected in 194 sera, i.e. in 90.6% of persons investigated: in 131 single serum samples and in 63 paired sera. The results of the serological studies which covered 53.6% of all known local cases showed the territorial distribution of the natural foci in this country and the aetiological relationship with the HFRS virus from the European part of the U.S.S.R.

Key words: haemorrhagic fever with renal syndrome; seroepidemiology; immunofluorescence

Introduction

Cases of HFRS were diagnosed clinically in Bulgaria for the first time in 1955 (Kirov, 1956; Matinchev, 1956). Afterwards, many reports were published which described observations of sporadic cases and small epidemic outbreaks in different parts of the country. These papers were listed in the bibliography on HFRS edited by Gajdusek *et al.* (1983). Detailed data on the epidemiology, clinical and pathoanatomical picture of the local cases were published in a monograph by Bulgarian authors (Radev *et al.*, 1980).

In a previous paper we reported the first evidence of HFRS in Bulgaria — 42 persons were discovered who had caught the disease during the period of 1959—1983, and were found to be seropositive against strains Udmurt and Kazan when tested by IFAT with antigen-containing lung sections of bank voles *Clethrionomys glareolus* (Gavrilovskaya *et al.*, 1984).

Recently we have extended our seroepidemiological studies investigating all sera collected from patients in the last 30 years; we included in the testing antigens of the European and Asian HFRS viruses. In this report the results of the investigations are presented, performed by IFAT simultaneously using the European strains Udmurt and Kazan 6-Cg and the Far-East Hantaan virus which is the aetiological agent of Korean HFRS.

Materials and Methods

Antigens. As antigens of the European virus were used cryostat lung sections of *Clethrionomys glareolus*: spontaneous virus carriers caught in natural foci of HFRS in Udmurt A.S.S.R., as well as laboratory animals experimentally infected with strains Kazan 6-Cg. The 4 μ m thick sections were airdried, fixed for 15 min with cold acetone and stored at -20°C . The control antigen was prepared by the same procedure from normal bank voles held in vivarium. The Asian virus Hantaan 76-118 received from D. C. Gajdusek was maintained in Vero-E6 cells. The cell monolayer was removed with versen 7 days after infection, resuspended in 199 medium, dropped on slides and after drying fixed with acetone as the cryostat sections.

Sera. All sera collected in the National HFRS Serum Bank were investigated — a total of 300 samples from 214 patients of the period 1957–1986. All 151 paired samples from 65 patients taken during the acute phase and the convalescent phase of the disease, the 149 single serum samples were taken 1 to 16 years after illness. The sera were kept at -20°C as follows: 40 sera less than a year; 16 sera — 1 to 5 years; 183 sera — 6 to 10 years; 61 sera — 11 to 12 years. Two-step dilutions were prepared from each sample starting from 1:10, and the samples were investigated simultaneously with the European strains Udmurt and Kazan 6-Cg, and with the Far-East Hantaan virus.

Indirect fluorescent antibody test (IFAT). The method used was described earlier (Gavrilovskaya *et al.*, 1982). FITC-labelled rabbit anti-human-gammaglobulin was used as an antispecies serum. A rhodamine-labelled albumin was utilized for contrasting the background and for elimination of nonspecific staining. Reference sera were used for control of the antigens: rat anti-Hantaan serum (titre 1:1280 against Hantaan antigen and 1:80 against Udmurt and Kazan 6-Cg antigens) and human serum "LIM" against the European virus (titre 1:1280 against Udmurt and Kazan 6-Cg antigens and 1:80 against Hantaan antigen). The human sera tested were referred to as positive when the titre was $\geq 1:10$.

Results

Cases registered

According to the data gathered from the hospital documents, 380 HFRS cases were registered during the period of 1954–1986. The real number, however, was 399 because in a retrospective study of the clinically suspicious cases 19 new seropositive persons were discovered.

Some cases were detected each year, this number varied from 1–3 to 30–40 or more (Table 1). Usually they represent sporadic small epidemic outbreaks with 10–17 cases in one focus. The seasonal maximum was 54.6% during the warmest months of June – July – August (peak 25.8% in July), and the lowest level 11.2% during the cold months of November – December – January with the minimum of 2% in January (Table 2). Persons of the active age of 21–50 years were mostly affected (72.5%), and for the remaining age groups the percentages were as follows: up to 10 years — 0.3%, 11–20 years — 10.6%, 51–60 years — 10.8%, over 60 years — 5.8%. The disease was found mainly among rural population, as well as among

Table 1. HFRS cases in Bulgaria registered during the period of 1954—1986

Year	Cases (deaths)	Year	Cases (deaths)	Year	Cases (deaths)
1954	2 (2)	1965	12 (2)	1976	13 (0)
1955	13 (3)	1966	17 (0)	1977	14 (0)
1956	7 (2)	1967	33 (4)	1978	8 (0)
1957	15 (1)	1968	18 (2)	1979	9 (2)
1958	8 (0)	1969	4 (0)	1980	1 (0)
1959	43 (11)	1970	10 (2)	1981	1 (0)
1960	9 (1)	1971	24 (3)	1982	6 (1)
1961	8 (2)	1972	17 (1)	1983	7 (1)
1962	3 (2)	1973	6 (0)	1984	4 (0)
1963	6 (3)	1974	28 (2)	1985	5 (4)
1964	15 (5)	1975	13 (0)	1986	20 (7)

Cases (deaths) = 399 (63)

The data for the period of 1954 — 1967 are according to Verbev *et al.*, 1968.

workers in the forests and in construction projects in the mountains. Among the patients there were 11.7 times more men than women. The lethality was high — an average of 15.7%.

Serologic data

Out of 214 selected persons tested for specific antibodies, 194 (90.6%) were found to be seropositive: 131 (87.9%) out of 149 persons from which a single serum sample was tested and 63 (96.9%) out of 65 persons tested more than once. As it is shown in Table 3, the predominant part (70.2%) of the group of 131 seropositive persons diagnosed retrospectively on the basis of a single serum sample had antibodies against the strains of the European virus only.

Table 2. Season incidence of HFRS in Bulgaria, 1954—1986

Years	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1954—1958	0	3	2	12	5	3	7	4	3	2	3	2
1959—1963	2	4	0	1	3	6	29	10	8	0	3	3
1964—1968	0	5	5	2	5	14	21	17	6	8	5	6
1969—1973	2	1	2	7	2	10	11	7	9	5	2	3
1974—1978	3	6	2	7	1	9	24	13	3	2	3	3
1979—1983	1	0	4	0	2	5	6	2	2	2	0	0
1984—1986	0	1	0	1	1	8	5	7	1	1	0	4
Total	8	20	15	30	19	55	103	60	32	20	16	21

Table 3. Antibody detection in single serum samples by IFAT

Result	No. of sera	Serum titre reciprocal									
		10	20	40	80	160	320	640	1280	2560	5120
Positive against Udmurt strain only ⁺	92	14	5	10	8	19	14	15	3	3	1
Positive against both Udmurt strain and Hantaan virus ⁺⁺	20	$\frac{8}{27}$	$\frac{3}{0}$	$\frac{5}{1}$	$\frac{3}{1}$	$\frac{6}{0}$	$\frac{2}{1}$	$\frac{1}{0}$	$\frac{1}{0}$	$\frac{1}{0}$	0
Positive against Hantaan virus only	9	8	1								
Total	131										

⁺ Antibody titres against Udmurt strain only are given in the Table, because they are one dilution step higher than those against Kazan strain.

⁺⁺ Numerator — number of sera with the respective titre against Udmurt strain, denominator — number of sera with the respective titre against Hantaan virus.

Antibodies against both the European and the Far-East viruses were detected in 30 (22.9%) persons, but only in two of them titres against Hantaan virus were higher than those against strain Udmurt (1 : 320 against Hantaan and 1 : 40 against Udmurt; 1 : 80 against Hantaan and 1 : 10 against Udmurt). In 9 persons (6.9%) antibodies were found against Hantaan virus only: in 5 the serum samples were taken before the 9th day; in 3 between 1 and 3 months and in one 16 years after the onset of the disease. Specific antibodies were found in samples taken 10—16 years after the onset of the disease and in some cases with titres as high as 1 : 640—1 : 2560.

The analysis of the second group of 63 seropositive persons based on two and more serum samples tested showed the following: in 32 individuals (50.8%) antibody titres increased only against the European strains (Udmurt, Kazan), in 26 persons (41.3%) the parallel increase of antibody against the European strain was higher than against Hantaan strain, in 5 persons (7.9%) antibodies occurred only against the Hantaan strain (3 cases with a titre of 1 : 10 in the convalescence sample) or they appeared simultaneously against Hantaan and against the European strains in lower titre to the latter (one case with 64-fold increase against Hantaan and only 4-fold against the European strains in the convalescence sample, and a second case with 16-fold increase against Hantaan and only 4-fold against the European strains in the convalescence sample). The specific antibodies appeared as early as within 3—5 days (titres 1 : 10—1 : 40); at the end of the second week antibodies were

detected in 70% of the cases (titres 1 : 10—1 : 60), and during the third week the titres usually were between 1 : 20—1 : 1280 (mean geometric titre 1 : 380).

Geographic epidemiology

The disease cases were connected epidemiologically with 158 HFRS natural foci, situated on the territory of 19 out of 28 districts of the country. The greatest number of active foci was found in the Southern part of the country, including the areas of Rila-Rodopa mountains (West Rodopa — 65.1% of the seropositive cases), as well as the mountains Pirin, Vlahina, Belassitza, Osogowska, Ograjden. On the second place were the northern and southern slopes of the Balkan (Stara Planina), predominantly in its central part, and with fewer foci were represented East Rodopa and the plains of North and South Bulgaria. In the endemic zone, the natural foci were predominantly in forestlands with mountaneous-hilly relief up to 1700 m above sea-level, covered with high-stemmed coniferous and broad-leaved vegetation. Rarely they were found in locations covered with mountainous pastures and field meadows. These peculiarities determined the rural type of diseases and the lack of foci on the Black Sea coast.

Discussion

The investigations performed gave information about distribution of HFRS on the territory of Bulgaria which is part of the Carpathian-Balkan geographic area of the disease (Lebedev, 1964).

Their importance is mainly due to the aetiological evidence for the presence of diseases even 30 years ago in the South-West part of the Euro-Asian continental region, where until recently HFRS cases were documented only on the basis of clinical data.

The epidemiological analysis of the IFAT seropositive patients established mostly indirect contact with rodents, usually by contaminated food or environment or during a stay in some natural places. It is noteworthy that the characteristics of the local natural foci was typical for biotopes of *Cl. glareolus* and some species of *Apodemus* and *Microtus*, known as natural reservoirs of HFRS. A significant point in this respect was the examination of 41 natural foci of HFRS (33 in Rodopa, 5 in Rila, 3 in Stara Planina), on the basis of which 6 kinds of biotopes were described characteristic for these representatives of Rodentia (Mateva *et al.*, 1985).¹

An important question at the present time is the aetiology of HFRS in the South-West part of the Euro-Asian continental region — whether the local cases are connected with virus strains of European origin only or there are some cases caused by strains antigenically related to the Asian virus. As far as our serological investigations are concerned, we used a great number of sera and covered a very long period of time; the results proved that the causative agent was antigenically related to the Udmurt and Kazan strains from the European part of the U.S.S.R. This is an evidence for aetiological unity of close antigenic relationship between the viruses from Bulgaria

and the European virus strains. It should be mentioned, however, that in some cases with single serum samples the antibody titres against the heterologous (Hantaan) antigen were several fold higher than against the homologous (European) antigen and also that in two cases there was a clear seroconversion against the Hantaan virus antigen as well. For the present time, the interpretation of these data remains open and could receive a clear-cut answer only after the comparison of the isolated by us local strains from humans and wild rodents with European strains of HFRS and with the Asian Hantaan virus.

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