SEROLOGICAL EVIDENCE OF HANTAAN VIRUS IN SLOVAKIA DURING 1989 – 1991

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Summary. – *Nephropathia epidemica* was supossed to be an European form of haemorrhagic fever with renal syndrome (HFRS). However, in 1989 – 1991 we found serological evidence of infection with Hantaan virus in patients' sera from Slovakia. Higher titers of antibodies against Hantaan virus were detected in sera of 6 out of 75 patients with febrile disease, renal failure and clinical diagnosis of HFRS. The higher titers of antibodies against Puumala virus were detected in sera of 6 out of 75 patients. Serologic differentiation between Hantaan and Puumala infection in patients was based on the detection of higher antibody titers (4-fold or greater differences).

Key words: hantaviruses; serology; patients' sera; Slovakia

Serotypic characterization of hantaviruses showed that their isolates fall into four distinct groups or serotypes (Lee *et al.*, 1985). Serotype 1 includes *Apodemus*-derived strains (prototype strain Hantaan), serotype 2 includes *Rattus*-derived strains (Seoul virus), serotype 3 includes *Clethrionomys*-derived strains (Puumala virus), and serotype 4 includes *Microtus*-derived strains (Prospect Hill virus).

It has been already proved that the hantavirus serotype 1 (Hantaan) occurs in Far Eastern Asia and the hantavirus serotype 3 (Puumala) occurs in Europe (Gajdusek, 1983).

In 1988, by use of direct enzyme-linked immunosorbent assay (ELISA) the Hantaan antigen was demonstrated in lungs of *Apodemus agrarius* and the Puumala antigen was detected in lungs of *Microtus arvalis,* both trapped in Slovakia. Antibodies to Hantaan virus were also detected in *A. agrarius* and *A. flavicollis* (Grešíková *et al.*, 1988).

Human sera examined for HFRS-specific antibodies were 75 specimens obtained from patients with clinical diagnosis of HFRS. For determination of antibody titers by indirect immunofluorescence, antigens were inoculated into Vero cells. As HFRS-specific antigens, Hantaan and Puumala viruses were used.

In the period of 1989 – 1991, 12 cases were clinically diagnosed as suspected HFRS. By the method of indirect immunofluorescence, the antibodies to Hantaan and Puumala viruses (genus *Hantavirus*) were found in 6 patients each (Table 1). Antibody titers varied from 1:16 to 1:1024. From 12 confirmed HFRS patients 11 had mild form of

Table 1. Antibody titers against Hantaan and Puumala viruses in HFRS patients' sera

No. of patient	Antibody titer	
	Hantaan	Puumala
1	n.t.	1:32
2	<1:16	1:128
3	1:128	1:512
4	1:32	<1:10
5	1:32	<1:16
6	1:160	1:40
7	1:16	<1:16
8	<1:16	1:64
9	1:160	1:20
10	1:640	1:20
11	1:20	1:1024
12	<1:16	1:40

n.t. = not tested

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Table 2. Clinical picture of HFRS in Slovakia

Serotype	Form of disease		
	Mild		Severe
	Virus serotype		
	Puumala	Hantaan	Hantaan
Fever	6	5	1
Somnolence	–	–	1
Headache	5	2	1
Meningism	3	1	1
Backache	1	2	–
Abdominal pains	3	1	–
Nausea	2	1	1
Petechiae	–	1	–
Flush over the face	1	–	–
Read throat	3	2	1
Diarrhoea	3	1	1
Conjuctival injection	2	2	1
Visual disturbances	1	1	–
Cough	3	3	–
Hypotension	–	1	–
Hypertension	–	2	–
Haematuria	2	5	–
Proteinuria	4	5	1
Creatinine (over 115)[a]	–	5	1
Urea (over 7.0)[b]	–	3	1
Acute renal insufficiency	2	3	1
Brain oedema	–	–	1
No. of patients	6	5	1

[a]Creatinine elevated to 115 – 1273 mmol/l.
[b]Serum urea nitrogen elevated to 7.1 – 46.6 mmol/l.

disease (5 with antibodies to Hantaan virus and 6 with antibodies to Puumala virus) and one patient had severe form of HFRS (antibodies to Hantaan virus) with wide range of clinical symptoms (Table 2).

Patients were 10 males and two females, 17–56 years of age, 6 professionals in forestry and agriculture and the other 6 also entering into field and forest frequently. Eleven patients admitted direct or indirect contacts with rodents during the incubation period which was 4 – 42 days. Geographically, 7 cases were from Eastern Slovakia, 1 case from Central Slovakia and 4 cases from Western Slovakia.

First fatal cases of serologically non-confirmed HFRS have been reported in Eastern Slovakia by Plank *et al.* (1955). The clinical symptoms of the disease were high fever, rash, renal failure (albuminuria, haematuria, uraemia). Morphologically, a typical picture of nephroso-nephritis was found.However, these cases were not serologically proved. Incidence and clinical symptoms of serologically proved HFRS in Eastern Slovakia have been already described (Bilčíková *et al.,* 1988). Hantavirus disease in Slovakia as well as in Europe was suppossed to be a mild disease.

Recently, antibodies to hantavirus were detected in a patient with clinical symptoms of high fever and acute renal faillure with proteinuria, haematuria and anuria (Palanová *et al.,* 1993).

Previously, in accordance with findings of others, the importance of rodents in the epidemiology of the disease in Slovakia has been documented. Antigen of Puumala virus was demonstrated in lung tissues of *C. glareolus* and *M. arvalis*; antigen of Hantaan virus was detected in *A. agrarius, A. flavicollis* and *M. arvalis* (Grešíková *et al.,* 1988).

The results of serological survey on 47 sera of small rodents collected in Eastern Slovakia with the antigen of HFRS indicated also the existence of natural focus of eastern type of HFRS in Eastern Slovakia (Grešíková *et al.*, 1988). HFRS-specific antibodies have been detected in *Apodemus* species (*A. agrarius* and *A. flavicollis*) in higher titers to Hantaan virus.

C. glareolus and *M. arvalis* have been the common hosts for Puumala virus and correlated with the mild form of the disease. *Apodemus* species seems to be the host for the Hantaan virus and related with severe form of HFRS.

Apodemus species in the natural focus of infection ensures the circulation of hantavirus in Slovakia and the presence of *C. glareolus* species ensures the circulation of Puumala virus in foci in Slovakia.

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