

## DETECTION OF THE ANTIGEN AND ANTIBODIES TO THE EASTERN SUBTYPE OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS IN SMALL RODENTS IN SLOVAKIA

M. GREŠÍKOVÁ, O. KOŽUCH, M. SEKEYOVÁ, \*E. A. TKACHENKO,  
\*G. V. REZAPKIN, \*\*J. LYSÝ

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava; \*Institute of Poliomyelitis and Viral Encephalitis, Moscow, U.S.S.R., and \*\*Institute of Experimental Biology and Ecology, Slovak Academy of Sciences, Bratislava, Czechoslovakia

Received February 17, 1987

**Summary.** — Direct enzyme-linked immunosorbent assay (ELISA) was used for the demonstration of haemorrhagic fever with renal syndrome (HFRS) virus antigen in lung tissue of small rodents trapped in Eastern and Western Slovakia. The eastern subtype of HFRS virus antigen was demonstrated in the lungs of *Apodemus agrarius* and of the western subtype in the lungs of *Microtus arvalis*. Antibodies to HFRS virus antigen have been detected in *Apodemus species* (*A. agrarius* and *A. flavicollis*) in higher titres to the Eastern subtype.

**Key words:** *Haemorrhagic fever with renal syndrome virus; eastern subtype; small rodents; enzyme-linked immunosorbent assay*

### Introduction

In 1984 the antigen of haemorrhagic fever with renal syndrome (HFRS) virus was detected in the lungs of small rodents in Eastern Slovakia (Grešíková *et al.*, 1984). By complement-fixation this antigen was found closely related to the western subtype of HFRS virus. Antibodies to HFRS virus were detected in sera of *Clethrionomys glareolus*, *Apodemus agrarius*, *Pitymys subterraneus* collected in Eastern Slovakia and in the sera of *Clethrionomys glareolus*, *Apodemus sylvaticus*, *Microtus arvalis* and *Microtus oeconomus* collected in Western Slovakia (Grešíková *et al.*, 1986).

Different methods for detection of HFRS virus antigen in the lung of small rodents were described (Lee and Lee, 1976; Tkachenko *et al.*, 1981; Brummer-Korvenkontio *et al.*, 1982; Gavrilovskaya *et al.*, 1983; Daneš *et al.*, 1986). In the present study we used ELISA which is less time consuming than the immunofluorescence procedure.

### Materials and Methods

■ *Small rodents.* During 1984 and 1986 free living small rodents (*Apodemus agrarius*, *Apodemus flavicollis*, *A. sylvaticus*, *Clethrionomys glareolus* and *Microtus arvalis*) were captured alive using traps of Swedish type. The animals were autopsied and the lung specimens were collected.

**Table 1.** Detection of HFRS virus antigen in the lung suspension of small rodents by ELISA

Species	No. of examined species	No. of positives
<i>Apodemus flavicollis</i>	55	0
<i>Apodemus agrarius</i>	6	2*
<i>Apodemus sylvaticus</i>	50	0
<i>Clethrionomys glareolus</i>	56	0
<i>Microtus arvalis</i>	16	2**

\*The eastern subtype of HFRS

\*\*The western subtype of HFRS

**Immunofluorescence (IF) tests.** The sera of small rodents were examined as described (Brummer-Korvenkontio *et al.*, 1980).

**Enzyme-linked immunosorbent assay (ELISA) procedure.** Polystyren microtiter plates were coated with human IgG to HFRS virus and incubated overnight at +4 °C. The plates were washed 3 times with phosphate buffered saline containing (PBS) 0.5 % Tween 20. To each well 100 µl of antigen (10% small rodents lung suspensions) was added. After incubation for 18 hr at 4 °C, the wells were washed 3 times with PBS and Tween 20, then 100 µl of antihuman horse-radish peroxidase-conjugate was added. After 3 washes with phosphate-buffered saline, the substrate (o-phenyldiamine) was added and the enzyme reaction was stopped after 30 min at room temperature by the addition of 2 mol/l H<sub>2</sub>SO<sub>4</sub>. The absorbance was measured in Dynatech Minireader.

### Results

During 1985–1986 small rodents were captured alive in Eastern and Western Slovakia. The animals were autopsied and together 183 lung specimens were collected; 136 specimens were coming from Eastern Slovakia and 47 ones from Western Slovakia.

For detection of HFRS virus antigen in the lungs of small rodents, ELISA was used to select the positive specimens. The 183 samples of small mammals were as follows: *Apodemus flavicollis* (55 samples), *A. sylvaticus* (50 samples), *Clethrionomys glareolus* (56 samples), *Microtus arvalis* (16 samples), *Apodemus agrarius* (6 samples). Four positive antigens were found by ELISA (Table 1).

**Table 2.** Comparison of the HFRS antigens using antiserum of eastern and western subtype

Strain No./isolated from	HFRS antiserum of western type	HFRS antiserum of eastern type
317/ <i>Apodemus agrarius</i>	0	+
320/ <i>Apodemus agrarius</i>	0	+
141/ <i>Microtus arvalis</i>	+	0

\*One antigen detected in *M. arvalis* was not sterile, therefore, it was not used in further experiments

Table 3. Antibody titres to HFRS eastern and western subtype antigen in small rodents as detected by IF tests

Species No.	IF titres with	
	Eastern type of HFRS antigen	Western type of HFRS antigen
<i>Apodemus flavicollis</i> No. 291	64	16
<i>A. flavicollis</i> No. 294	128	16
<i>A. flavicollis</i> No. 313	32	< 16
<i>Apodemus agrarius</i> No. 317	64	< 16
<i>A. agrarius</i> No. 320	128	< 16

The HFRS virus antigen was detected in the lungs of *Apodemus agrarius*, collected in Eastern Slovakia and in lungs of *Microtus arvalis*, collected in Western Slovakia. A higher proportion of infected small rodents (4.2 %) was found in Eastern Slovakia than in Western Slovakia (1.4 %). The HFRS antigen detected in the lungs of *A. agrarius* was closely related to the eastern subtype of HFRS; the antigen detected in the lungs of *M. arvalis* was closely related to the western subtype of HFRS (Table 2). The results of serological survey on 47 sera of small rodents collected in Eastern Slovakia with the antigen of HFRS indicate the existence of natural focus of eastern subtype of HFRS in Eastern Slovakia. Antibodies were found in the following free-living rodents: *Apodemus flavicollis* and *A. agrarius*. A total 5 out of 47 small rodents trapped in investigated localities of Eastern Slovakia had antibodies to HFRS antigen (Table 3). Higher titres of antibodies were detected using the eastern subtype of HFRS antigen.

### Discussion

The HFRS antigen of the western subtype was demonstrated by the lungs of *Clethrionomys glareolus*, *Apodemus agrarius* and *A. flavicollis* captured in Eastern Slovakia (Grešíková *et al.*, 1984). The antibodies against the HFRS antigen were detected in Eastern and Western Slovakia (Grešíková *et al.*, 1986).

In 1984 and 1986 we collected small rodents in Eastern Slovakia to study the presence of antigen and antibody. We used solid-phase enzyme-immunoassay (Tkachenko *et al.*, 1981) for the detection of HFRS antigen in the lungs of small rodents. The demonstration of HFRS virus in lung tissues of *Apodemus agrarius* and *Microtus arvalis* had clarified the serological relationships of the strains isolated in Europe. It has been already proved that two types of HFRS exist: the eastern type in Far East Asia and the western type in Europe (Gajdušek, 1982; Baškircsev *et al.*, 1984). By the use of the ELISA tests, it has been demonstrated that the antigens detected in the lung tissues of *Apodemus agrarius* are related to the eastern subtype of HFRS; the antigens detected in the lungs of *M. arvalis* are related to the western subtype of HFRS.

It is of interest that HFRS virus antigen was detected in the lungs of *Apodemus agrarius* No. 317 and No. 320 simultaneously with antibodies. Our study also shows the presence of HFRS antigen in the lungs of males of *A. agrarius* and *M. arvalis* only.

## References

- Baškircsev, V. N., Tkačenko, E. A., Dzagurova, T. K. and Rylceva, E. V. (1984): Vydelenie štammov virusa gemorragičeskoj lichoradky s počočným syndromom v kulture kletok. *Vopr. virusol.* **29** (4), 497—502.
- Brummer-Korvenkontio, M., Vaheri, A., Hovi, T., von Bonsdorff, C. H., Vuorimies, J., Manni, T., Penttinen, K., Oker-Blom, N., and Lahdevirta, J. (1980): Nephropathia epidemica: Detection of antigen in bank voles and serologic diagnosis of human infection. *J. infect. Dis.* **140**, 131—134.
- Brummer-Korvenkontio, M., Henttonen, H., and Vaheri, A. (1982): Hemorrhagic fever with renal syndrome in Finland: ecology and virology of nephropathia epidemica. *Scand. J. infect. Dis. Suppl.* **36**, 88.
- Daneš, L., Tkachenko, E. A., Ivanov, A. P., Lím, D., Rezapkin, G. V., and Dzagurova, T. K. (1986): Hemorrhagic fever with renal syndrome in Czechoslovakia: Detection of antigen in small terrestrial mammals and specific serum antibodies in man. *J. Hyg. Epid. Microbiol. Immunol.* **30**, 79—85.
- Gajdusek, D. C. (1982): Rodent-borne viral nephropathy haemorrhagic fever with renal syndrome; nephropathia epidemica. Report by the World Health Organization Working Group on Haemorrhagic Fever with Renal Syndrome, Tokio, February 22—24, 3.
- Gavrilovskaya, I. N., Chumakov, M. P., Apekina, N. S., Ryltseva, E. V., Martynova, L., Gorbachkova, E. A., Bernstein, A. D., Zakharova, M. A., and Boiko, V. A. (1983): Adaptation to laboratory and wild animals of the hemorrhagic fever with renal syndrome virus in the foci of European U.S.S.R. *Arch. Virol.* **77**, 87.
- Grešíková, M., Rajčáni, J., Sekeyová, M., Brummer-Korvenkontio, M., Kožuch, O., Labuda, M., Turek, R., Weisman, P., Nosek, J., and Lysý, J. (1984): Haemorrhagic fever virus with renal syndrome (HFRS) in small rodents in Czechoslovakia. *Acta virol.* **28**, 416—421.
- Grešíková, M., Sekeyová, M., Brummer-Korvenkontio, M., Kožuch, O., Labuda, M., Rajčáni, J., and Lysý, J. (1986): Serological survey with antigen of haemorrhagic fever with renal syndrome in small rodents in Slovakia. *Acta virol.* **30**, 158—160.
- Lee, H. W., and Lee, P. W. (1976): Korean hemorrhagic fever. I. Demonstration of causative antigen and antibodies. *Korean J. int. Med.* (Seoul) **19**, 371—383.
- Tkachenko, E. A., Ivanov, A. P., Dzagurova, T. K., Donets, M. A., Rezapkin, G. V., and Leshchinskaya, E. V. (1981): Immunosorbent assay for diagnosis of hemorrhagic fever with renal syndrome. *Lancet* **2** (8240), 257—258.