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EVIDENCE FOR HANTAVIRUS DISEASE IN SLOVENIA, YUGOSLAVIA

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Summary. — In Slovenia, North-Western part of Yugoslavia, 17 clinically documented Hantavirus disease cases (HVD) were serologically confirmed so far. Previously HVD was reported in the Southern part of Yugoslavia. By the indirect fluorescent antibody test (IFA), the prevalence of IgG class antibodies against different Hantaviral antigens was demonstrated in human sera collected in Slovenia. Three different reactivity patterns were observed. Majority of the IFA-positive human sera were confirmed by the immunoblot method. The distribution of Hantaviral infections was examined in small mammals captured in two natural foci of HVD, where clinical documented cases were reported. Hantaviral antibodies and antigens were demonstrated in *C. glareolus*, *A. flavicollis*, *A. sylvaticus*, and *M. musculus*.

Key words: *Hantaviruses, antibody prevalence in human and animal sera, hantavirus disease*

Introduction

The syndrome known as Hantavirus disease (HVD) (Desmyter *et al.*, 1984) or Haemorrhagic Fever with Renal Syndrome (HFRS) is a collective name adopted for a viral disease in humans with a variety of clinical manifestations (Lee, 1982; Lähdevirta *et al.*, 1984). It is caused by viruses belonging to the Hantavirus group, a new genus in the *Bunyaviridae* family. The prototype of Hantavirus group, Hantaan virus, was first isolated from the lungs of the *Apodemus agrarius* corea (Korean striped field mouse) in 1976 by Lee (Lee *et al.*, 1978). Evidence for the infection of wild mammals and humans by Hantaviruses has now been reported worldwide. The number of small mammals known as a reservoir for Hantaviruses has dramatically increased (van der Groen, 1985).

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In Yugoslavia, the first HVD-case was reported in 1952 in a soldier who was infected in the forest of Fojnica (Bosnia). The first epidemic of HVD occured in 1962 in a military camp in Fruška Gora (Serbia) (Heneberg *et al.*, 1963). A total of 46 soldiers were ill with one fatal case. A second epidemic, in which 144 persons became ill and two died, occurred in 1967 in the central and Western Bosnia (Gaon, 1968). At the same time a third epidemic was around Plitvice Lakes in Croatia (Vesenjak-Hirjan, 1971). In 1986 the greatest outbreak of HVD occurred in almost all Yugoslavian republics with 161 serologically confirmed HVD cases and 11 deaths (Gligić *et al.*, 1987). Almost yearly sporadic cases with severe and mild clinical picture of HVD have been notified in Yugoslavia.

The first written data concerning HVD in Slovenia, North-Western part of Yugoslavia, dates back to the year 1952. Radoševič and Mohaček described four clinically well documented cases of acute nephritis with unknown aetiology — at that time called Nephropathia Epidemica, Myhrman-Zetterholm (Radoševič *et al.*, 1954). One of the cases was a seasonal timber worker who was infected in the forest of Pohorje in Slovenia. Since 1985 the virological and serological diagnosis of HVD became possible at the Institute of Microbiology, Medical Faculty, Ljubljana, Yugoslavia.

In this retrospective study we report clinical evidence as well as preliminary results on the prevalence of antibodies in human sera collected in Slovenia. In addition, antibody and antigen prevalence was investigated in populations of wild living mammals from a few natural foci of HVD in Yugoslavia.

Materials and Methods

Human sera. From 1985 until now, 120 human sera (acute and convalescent) were collected from 82 patients from different areas in Slovenia. Most of the patients were hospitalized at the department of Nephrology, University Medical Centre in Ljubljana because of acute renal failure (ARF). Their disease was suspected as: leptospirosis, rapidly progressive glomerulonephritis, interstitial nephritis, as HVD and acute renal failure of unknown aetiology. Sera were stored at −20 °C until use.

Animal sera. 180 animal sera were collected from 11 different species, 8 rodents and 3 insectivora. The sera were immediately diluted 1 : 16 with phosphate buffer saline (PBS) pH 7.2 and stored at −20 °C until use.

Trapping and processing of small mammals. Small mammals were captured by using live traps. All mammals were captured in July 1987. Trappings were conducted around houses and the surrounding fields and forests in Murska Sobota and Trebnje, two regions where HVD-cases have been reported. Captured animals were identified and dissected. Lung tissues were removed aseptically and stored in liquid nitrogen.

Indirect fluorescent antibody assay IFA . Human and animal sera were screened by the indirect fluorescent antibody test (IFA) using spot slides of Vero E6 cells infected with different hantaviruses as described previously (van der Groen *et al.*, 1983). Hantaviruses used in the study were: prototype *Hantaan* 76-118 (isolated from *Apodemus agrarius* corea in Korea), CG 18-20 (isolated from lung suspension of wild life *C. glareolus* in the Western part of the U.S.S.R.), Prospect Hill — Ph 1 (isolated from *M. pennsilvanicus* in U.S.A.), Fojnica (isolated from *A. flavicollis* captured in endemic area in Bosnia, Yugoslavia), Vranica (virus isolated from *C. glareolus* captured in endemic area in the Southern part of Yugoslavia) and FB 79-53 (virus recently isolated from *Apodemus* Sp. by P. W. Lee). Sera were screened at 1 : 16 dilution and considered positive if characteristic focal cytoplasmic staining was present in infected cells and absent

in non-infected cells. All positive sera were titrated. All sera were also tested for the presence of antibodies against leptospirosis by using the microscope agglutination test (Cole, 1973).

Western blot technique. After thawing Hantaan 76-118 virus-infected (2 ml containing $\pm$ 5.10^6 cells) and uninfected Vero E6 cell suspensions stored at -20 °C in growth medium containing 10 % dimethylsulphoxide (v/v), the cells were washed 3 times by centrifugation (5 min at 400 g 82/m type of centrifuge) with phosphate buffered saline pH 7.4. After resuspension of the cells in 0.01 mol/l Tris-HCl, pH 7.4, 1 % (v/v) Na-deoxycholate, 1 % (v/v) Triton X-100, 0.1 % SDS, 1 mmol/l Phenyl methyl-sulphonyl-fluoride (RIPA buffer) and sonication (two times 30 sec, 10 duty-cycle 50 %, Branson sonifier 250), the cell debris was centrifuged at 12 000 $\times$ g for 30 min at 4 °C. The supernatant was pelleted by centrifugation at 49 000 $\times$ g for 90 min at 4 °C. Pelleted virus was resuspended in 0.5 ml sample buffer (1 % SDS — 3 % β-mercapto-ethanol 0.125 mol/l Tris-HCl pH 8.2, 15 % glycerol). Bromophenol blue was added to a final concentration of 1 % (v/v) and the solution was boiled for 5 min, then electrophoresed on 10 % polyacrylamide gel (Maizel, 1971) and transferred electrophoretically to nitrocellulose according to the method of Towbin *et al.*, 1979). The electrophoretic blots were incubated with 3 % bovine serum albumin in 0.15 mol/l NaCl/0.01 mol/l Tris HCl, pH 7.4 and 0.1 mmol/l phenylmethyl-sulphonyl fluoride for 1 hr at 40 °C to saturate the remaining protein binding sites. Blots were stored at 4 °C until use. The blots were further incubated for 5 min at room temperature with 0.15 mol/l NaCl, 0.05 mol/l Tris-HCl, pH 7.4, 0.01 % merthiolate (TBS buffer) subsequently they were incubated 5 and 20 min respectively in 10 mmol/l Tris-HCl; pH 7.4, 0.1 mol/l $MgCl_2$, 0.5 % Tween 20, 1 % bovine serum albumin, 5 % foetal calf serum (Zeller buffer). After removal of the Zeller buffer, the blots were incubated for 2.5 hr with the antibody containing serum appropriately diluted in Zeller buffer. After washing 3 times (10 min each) with Zeller buffer and 1 time (5 min) with PBS 0.05 % Triton, the strips were overlaid 2 hr with alkaline phosphatase labelled anti-human IgG (H+L) conjugate (Promega 606 — 274 — 4330 protoblot) diluted 1/7500 in PBS 0.05 % Triton. The blots were washed 2 times (10 min each) with PBS 0.05 % Triton and 2 times (5 min each) with 0.1 mol/l Tris pH 9.5, 0.1 mol/l NaCl, 50 mmol/l $MgCl_2$ (substrate buffer) and substrate (264 µl) nitroblue tetrazolium (Sigma N. 6876) 75 mg/ml in 70 % dimethyl formamide + 132 µl 5-bromo 4-chloro 3-indolyl phosphate (Sigma B-8503), 50 mg/ml in 100 % dimethyl formamide diluted with Tris buffer to a final volume of 40 ml was added to the strips and incubated at room temperature for $\pm$3 min. To stop the reaction, the strips were washed two times with distilled water, dried inbetween filter paper and read.

For the detection of the bounded mouse monoclonal anti-50 KD antibody (ECO2/BDO1) the procedure was modified as follows: after washing of the strips with PBS 0.3 % (v/v) Tween-20 (2 times 10 min each), the strips were overlaid with mouse monoclonal ECO2/BDO1 diluted 1/50 in PBS 0.3 % (v/v) Tween-20 and incubated overnight at room temperature while shaking. The blots were washed with PBS 0.3 % (v/v) Tween-20 (3 times 5 min each) and incubated with biotine coupled anti-mouse horse IgG (Vectastain) diluted 1/1000 in PBS 0.3 % (v/v) Tween-20 at room temperature for 1 hr. Afterwards, the strips were washed 3 times for 5 min each with PBS — 0.3 % Tween-20 and avidin-labelled with peroxidase, 1/700 in PBS — 0.3 % Tween-20 (Dupont) was incubated for 1 hr at room temperature. The strips were washed 3 times (5 min each) with PBS 0.3 % (v/v) Tween-20, overlaid with substrate solution (60 microliter 30 % (v/v) H_2O_2 in 100 ml PBS was mixed with 60 mg 4-chloronaphtol in 20 ml ice-cold methanol just before use) and incubated maximum 5 min at room temperature. To stop the reaction, the strips were washed two times with distilled water, dried in between filter paper and read.

Sera were considered positive when a protein band of 50 kD (nucleoprotein) was observed on the Hantavirus immuno blot strips and a similar band was absent on immuno blot strips prepared with non-infected Vero E6 cells.

Detection of Hantavirus antigen. Cryostat sections (4 µm thick) of small mammal lung tissues were examined for the presence of Hantavirus antigen by the indirect immunofluorescence (Chumakov *et al.*, 1981), using four different convalescent sera from HVD patients (coming from the Southern part of Yugoslavia and from Slovenia). Hantavirus antibody-free human serum was used as a negative control.

In a comparative study, a modification of the enzyme-linked immunosorbent assay (ELISA) of Tkachenko (described in detail by Verhagen *et al.*, 1986) was used to detect the Hantavirus antigen in the lungs of small mammals. Purified human polyclonal anti-Hantavirus IgG from a convalescent patient (Western part of the U.S.S.R.) was used to capture the Hantavirus antigen.

Table 1. Indirect immunofluorescent antibody titres to different Hantavirus antigens in sera from HVD-patients from Slovenia, Yugoslavia

No.	HTN**	Fojnica	FB 79-53	TCH	CG18-20	Vra-nica	PH	xxx	Clinical**** course of HVD	Severity of ARF
1	256*	128	256	256	32	16	64		S	S
2	512	512	2048	512	32	256	256		MO	MO
3	1024	512	256	1024	128	32	256	I	S	S
4	512	256	512	64	64	256			MO	S
4	512	512	256	512	64	64	256		MO	S
5	1024	512	2048	512	32	32	<16		MI	MO
6	1024	1024	1024	512	64	64	64		MO	MO
7	64	128	<16	250	256	256			MI	MI
8	64	<16	<16	32	512	1024	256		MO	MO
9	64	256	32	64	2048	1024	128		MO	MI
10	64	<16	<16	32	1024	256	64	II	MI	MI
11	32	64	<16	16	2048	1024	128		S	S
12	64	32	<16	64	2048	1024	256		MI	MI
13	32	32	<16	16	2048	1024	256		MI	MO
14	32	16	<16	32	2048	1024	256		MI	MI
15	64	<16	16	64	64	<16	32		MO	S
16	64	<16	<16	256	128	16	128	III	NA	NA
17	256	512	32	256	512	512	256		MI	MI

* Reciprocal of the highest dilution for which still a characteristic fluorescent pattern can be observed by IFA.

** Acetone fixed and gamma irradiated Vero E6 cells infected with the following Hantaviruses were used in the IFA:
- HTN 76-118: prototype Hantaan — isolated from *A. agrarius corea* in Korea
- Fojnica: virus isolated from *A. flavicollis* in Southern Yugoslavia
- FB 79-53: virus isolated from *Apodemus Sp.* in Korea (kindly supplied by Dr. P. W. Lee)
- TCH: Tchoupitoulas virus isolated from *Rattus norvegicus* in the U.S.A.
- CG 18-20: virus isolated from *Clethrionomys glareolus* in the Western part of the U.S.S.R.
- Vranica: virus isolated from *Clethrionomys glareolus* in Southern part of Yugoslavia
- PH: Prospect Hill — virus isolated from *Microtus pennsilvanicus* in the U.S.A.
- NT: Not Tested

**** Clinical course of HVD

S — Severe: febrile phase > 5 days, more than 3 haemorrhagic manifestations, hypotension, CNS involvement; frequent secondary infections

MO — Moderate: febrile phase 3—5 days, hypertension or normal blood pressure, two or three haemorrhagic manifestations secondary infections rare

MI — Mild: febrile phase <3 days, normal blood pressure, and neurological disturbances, one or no haemorrhagic manifestations, no secondary infections.

Severity of ARF

S = serum creatinine > 1000 μmol/l + haemodialysis

MO = serum creatinine 700—1000 μmol/l + haemodialysis in case of hypervolaemia

MI = serum creatinine <700 μmol/l + no haemodialysis

Table 2. Clinical characteristics of Hantavirus disease in Slovenia compared to West European (WE), Scandinavian (NE) and Hantavirus disease as described in Greece

Signs and Symptoms	Percentage of cases			
	Slovenia (n=16)	WE* (n=43)	NE** (n=76)	Greece*** (n=23)
Fever	100	100	100	100
Headache	87	84	90	100
Nausea — vomiting	75	73	70	83
Red throat	50	20	67	ND*
Loin pain	87	100	82	93
Diarrhea	19	7	12	17
Visual disturbances	31	19	12	ND
Hypotension	19	7	40	65
Proteinuria	100	100	100	100
Microhaematuria	94	73	74	100
Oliguria	63	27	54	ND

*ND: no data
*WE: West European Nephropathia as described by van Ypersele de Strihou *et al.*, 1986
**NE: *Nephropathia epidemica* as described by Lähdevirta, 1982
***: HVD as described in Greece by Antoniadis *et al.*, 1987

Results

Human sera

Sera from seventeen patients out of eightytwo tested by IFA contained Hantavirus antibodies. As seen in Table 1 three different reactivity patterns could be recognized: 1. Sera (No's 1-6) with higher titres for HTN, Fojnica, FB 79-53 and Tchoupitoulas (TCH) antigens; 2. Sera (Nos 7-14) with higher titres for CG 18-20, Vranica and Prospect Hill antigens; 3. Sera with equal high titres for all Hantaviral antigens studied.

Five sera with reaction pattern one were from patients with a moderate or severe form of HVD concerning clinical course and severity of ARF (Table 2). Four of them needed haemodialysis treatment. However, serum No. 11 which corresponds to the second reactivity pattern belongs to patients with a rather severe form of the disease. Most of the patients with reaction pattern two and three had a milder, and few of them, a moderate form of HVD. Patient No. 16 from the third reactivity group had no clinical picture compatible with HVD.

Eighty sera from other patients tested in our study were serologically negative for Hantaviruses. All these sera were also tested for the presence of antibodies against leptospirosis. Five of them were positive. One of them, No. 9 (*Leptospirosis saxhelbing*) was also Hantavirus antibody-positive (Table 1). With the exception of sera No. 7, 10, and 15 all IFA positive/sera were confirmed by immunoblotting (IB) on strips prepared with Hantaan 76-188. Sera tested with the IB-method were diluted 1 : 50. They were

Table 3. Prevalence of Hantavirus-antibody and Hantavirus-antigen in different species of wild living small mammals in Slovenia

Order/Species	ANTIBODY			ANTIGEN			
	total tested	HTN 76-118 %pos	CG 18-20 %pos	total tested	IFA %pos	total tested	ELISA %pos
RODENTIA							
Apodemus flavicollis	80	10	12.5	66	18.2	82	6.1
Apodemus sylvaticus	46	2.2	2.2	37	10.8	45	0
Chlethrionomys glareolus	39	20.5	25.6	31	22.6	40	20
M. agrestris panonicus	4	0	0	3	0	4	0
Apodemus microps	3	0	0	3	0	3	0
Apodemus agrarius	2	0	0	2	0	2	0
Mus musculus	3	0	66.6	3	66.6	3	0
Pytimis subterraneus	1	0	0	—	—	1	0
INSECTIVORA							
Crocidura suaveolens	2	0	0	2	0	2	0
Sorex araneus	—	—	—	2	0	2	0
Sorex Sp.	—	—	—	1	0	1	0
TOTAL	180	9.4	11.6	150	16.6	185	7

considered positive when a clear nucleoprotein band of 50 kD was observed on the strips prepared with HTN 76-118 infected Vero E6 cells, and no band was found on IB-strips prepared with noninfected Vero E6 cells (Fig. 1). The specificity of the IB was confirmed by the positive reaction of a mouse monoclonal antibody (ECO2/BDO1) specifically directed against the nucleoprotein of Hantaan 76-118.

Three IB-Hantaan 76-118 negative but IFA-positive sera were further tested on IB strips prepared with CG 18-20 antigen. No specific protein band of 50 kD could be observed (data not shown).

Animal sera

In 180 animal sera the prevalence of IgG type of antibodies against Hantaan 76-118 and CG 18-20 antigens by IFA were found in 23 (12.7 %) rodent sera (Table 3). The highest positive rate was found in the bank vole (*C. glareolus*). Out of the 39 sera, 10 (25.6 %) were positive with titres ranging from 16 up to 2048. Higher (2 to 7 fold) titres were observed when CG 18-20 antigen was used. Seropositive house mice (*M. musculus*), wood mice (*A. sylvaticus*) and yellow-necked field mice (*A. flavicollis*) were also found.

Out of 150 lungs tested 25 (16.6 %) contained antigen when IFA-lung cryosection method was used (Table 4). However, with the ELISA-antigen capturing method 8 out of 150 (5.3 %) rodent lungs were positive.

Table 4. Prevalence of Hantaviral antigen and antibodies in free living mammals in Slovenia, Yugoslavia

	Ab+***	Ag+	Ab+Ag+	Ab−Ag+	Ab+Ag−
150*	(12) %	(5.3) %	(4) %	(1.3) %	(8) %
150**	(12) %	(16.6) %	(10) %	(6.6) %	(0) %

*: ELISA — antigen capture method
**: IFA — lung cryosection method
***: IFA — antibody against HNT 76-118 when screened at 1 : 16 dilution

The antigen prevalence was lower than the seroprevalence when tested by ELISA-antigen capture method. The opposite was true when the antigen was detected with IFA in cryostat sections (Table 4). Using lung cryosections Hantavirus antigen was also detected in *M. musculus* and *A. sylvaticus* (Table 3).

When the average coded IFA antibody titres on Hantaan 76-118 were compared for *Apodemus* and *C. glareolus* sera, no difference could be observed (Table 5). However, when the average coded IFA antibody titres on CG 18-20 were compared for *Apodemus* and *C. glareolus* sera, a significant higher average of coded titres was observed with the *C. glareolus* sera. In addition, when the average of coded ELISA-antigen titres was compared for *Apodemus* and *C. glareolus* lung suspensions, a significantly higher average of coded titres for *C. glareolus* was observed. These data are an indiciation for the existence of at least two different Hantavirus types in free living rodents in Slovenia.

Discussion

The results of sero-epidemiological survey presented here, provide substantial evidence for the occurrence of Hantavirus disease (HVD) in Slovenia. When sera from HVD patients have been analysed by IFA, three different

Table 5. Average coded indirect immunofluorescent antibody and ELISA - Hantavirus antigen titres in different rodents captured in Slovenia

Host	Number	Average of the coded (a) IFA titres		Average of the coded ELISA antigen titre
		Hantaan 76-118	CG 18-20	
Apodemus Sp.	13	5.1	3.5	0.9
C. glareolus	11	5.6	8.7	5.9
		NOT SIGNIFICANT	$P < 0.001$	$P < 0.001$

(a): Coded titre = Log_2 of the reciprocal of the highest dilution for which still a characteristic fluorescence can be observed.

Table 6. Comparison of haemorrhagic manifestations in HVD patients in Slovenia, Finland, Korea, and Greece

Haemorrhagic manifestations	Percentage of cases			
	Slovenia (n=16)	Finland* (n=76)	Korea** (n=125)	Greece** (n=23)
Conjunctival injection	44	18	97	65
Petechiae	31***	36	98	15
Haematoma	13	0	5	5
Gastrointestinal tract involvement	13	0	5	5

*: From Lähdevirta, 1982
**: From Antoniadis, 1987
***: data for only 10 patients

reaction patterns on different Hantaviral antigens have been observed. The reaction pattern with higher titres on HTN 76-118, Fojnica, FB 79-53 and Tchoupitoulas antigens, was correlated more frequently with a somewhat more severe clinical form of HVD in that more pronounced haemorrhagic manifestations and more severe acute renal failure was observed. The clinical characteristics of Hantavirus disease in Slovenia as summarized in Table 2 did not differ significantly with the West European, Scandinavian and Greek form of HVD. However, when haemorrhagic manifestations were compared (Table 6) the Slovenian form of HVD did show the closest resemblance with the HVD cases reported in Greece. These data correspond well with findings of P. W. Lee and A. Gligić who have demonstrated specific high plaque reduction neutralization titres of sera from patients with a rather severe form of HVD in the Southern part of Yugoslavia, when tested on the Fojnica strain (P. W. Lee, 1985, A. Gligić-personal communication) — a strain isolated from *A. flavicollis* captured in endemic area of HVD in Yugoslavia. Also by IFA a higher affinity of patients sera with a more severe form of HVD was observed on FB 79-53, a Hantavirus, most probably isolated from *Apodemus* species in Korea (P. W. Lee—personal communication). Moreover, similar findings were observed by Antoniadis (Antoniadis *et al.*, 1987) in the Northern part of Greece. These data suggest that HVD with more prounced haemorrhagic manifestations in Europe could be related to a Hantavirus isolated from *Apodemus flavicollis* species.

Majority of the sera from patients with HVD in Slovenia had a higher reactivity in IFA with CG 18-20 and Vranica antigens (reaction pattern II), both viruses isolated from bank voles (*C. glareolus*). This corresponds well with the epidemiological data observed in other West European countries and Scandinavia where a mild form of HVD called Nephropathia Epidemica (van Ypersele de Strihou *et al.*, 1986; Lähdevirta, 1982; van der Groen *et al.*, 1983) was described. These findings suggest the simultaneous circulation of at least two different serotypes of Hantaviruses in Slovenia which corresponded well with the epidemiological data reported so far for the rest of Yugoslavia (A. Gligić, 1987).

The preliminary results of the mammal survey presented here, provide substantial evidence that the bank vole *C. glareolus* and *Apodemus flavicollis* are the main hosts for Hantaviruses in the endemic areas of HVD in Slovenia. The observation that some animals showed only evidence for the presence of antibodies or antigen means that serological screening or screening for antigen alone, does not give the real prevalence of infection in rodent populations. Similar observations were obtained by Verhagen and co-workers (Verhagen *et al.*, 1986).

A larger number of HVD patients in Slovenia should be studied clinically and serologically, in order to establish a correlation between IFA reaction pattern one (high titres on HTN and Fojnica) and a more severe form of HVD with more pronounced haemorrhagic manifestations and IFA reaction pattern two (high titres on CG 18-20, Vranica, low on HTN and Fojnica) and a mild form of HVD. Further investigations are necessary to elucidate the true nature of the aetiologic agent(s) causing HVD in Slovenia as well as in the other parts of Yugoslavia.

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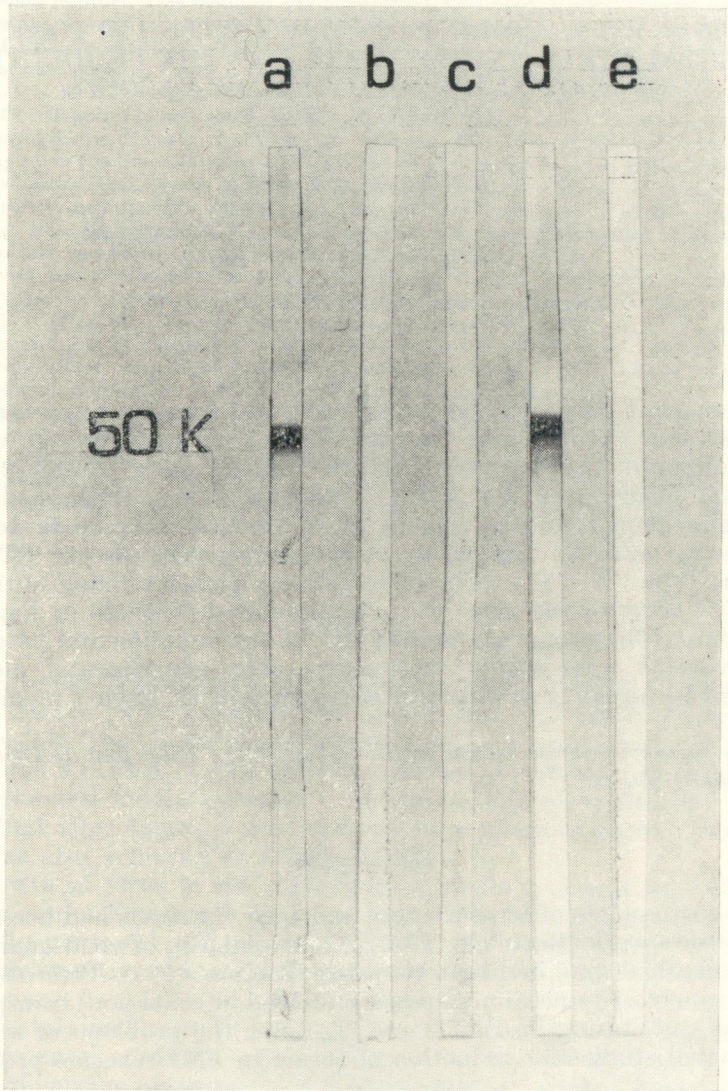

Fig. 1.

Lane a: Reaction of a serum of a convalescent patient with HVD in Slovenia with the electrophoretically blotted 50KD nucleoprotein of Hantaan 76-118 on nitrocellulose strips. Serum dilution used was 1/50.

Lane b: Reacton of an anti-Hantaan 76-118 antibody negative human serum.

Lane c: Reaction of a serum of a convalescent patient with HVD in Slovenia with the electrophoretically blotted proteins of uninfected lysed Vero E6 cells on nitrocellulose strips. Serum dilution used was 1/50.

Lane d: Reaction of a specific mouse monoclonal antibody ECO2/BD01 anti-Hantaan 76-118 with the electrophoretically blotted 50 kD nucleoprotein of Hantaan 76-118 on nitrocellulose strips. Tested at 1/50 dilution.

Lane e: Reaction of the specific mouse monoclonal antibody ECO2/BDO1 with electrophoretically blotted proteins of uninfected lysed Vero E6 cells on nitrocellulose strips.