

## HANTAVIRUS SPECIES IN INDIA: A RETROSPECTIVE STUDY

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### Abstract

Hantaviruses cause hemorrhagic fever with renal syndrome in Europe and Asia. There are about 20 documented hantavirus species and newer species are being described worldwide, especially in non-rodent reservoirs, i.e shrews. Focus reduction neutralization test is the classical serotyping technique for hantavirus. However, this study employs a previously established serotyping ELISA, to retrospectively analyze known hantavirus IgG reactive samples for infecting serotypes. The result suggests presence of *Thailand virus*-like and *Hantaan virus*-like strains in India.

**Key words:** *Hantavirus*, *India*, *serotypes*

### Introduction

Hantaviruses are rodent-borne viruses belonging to the *Bunyaviridae* family. They cause hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Hantaan virus (HTNV), Seoul virus (SEOV), Dobrava virus (DOBV) and Puumala virus (PUUV) are hantavirus serotypes circulating in Europe and Asia. HTNV and DOBV-related HFRS cases are severe while SEOV, which is hosted by rats, causes mild HFRS with a low mortality rate of 1-2%. Nephropathia epidemica (NE), a mild form of HFRS, is caused by PUUV in Scandinavia.<sup>[1]</sup> There have been some recent reports of Thailand virus (THAIV)-related HFRS.<sup>[2]</sup>

The only hantavirus serotype indigenous to India is the *Thottapalayam virus* (TPMV) which was isolated from a shrew, *Suncus murinus*, in 1964.<sup>[3]</sup> The lack of reactivity of the nucleocapsid (N) protein-specific monoclonal antibodies raised against HTNV and PUUV with recombinant TPMV N proteins produced in *E. coli* or yeast<sup>[4]</sup> (Mertens *et al.*, unpublished data) proves the phylogenetic and antigenic diversity of TPMV.

Serological evidence of hantavirus infections have been documented from India<sup>[5-7]</sup> but the circulating species are unknown. We report preliminary data on the serotyping of

hantavirus species in India by testing hantavirus reactive human sera using truncated nucleocapsid N protein derivatives of different Asian hantaviruses.

### Materials and Methods

Twelve samples were subjected to serotyping ELISA. Samples number 1, 3 and 12 were negative and the other nine (sample numbers 2, 4 to 11) were positive for anti hantavirus IgG antibodies. Criteria for anti hantavirus IgG reactivity were as per our previous publication.<sup>[6]</sup> All serotyped samples (n=12) were initially tested by two assays; a commercial anti hantavirus IgG ELISA (Focus Technologies Cypress, California, USA) and indirect immunofluorescence assay (IFA) using HTNV-infected Vero E6 cells. All nine seropositives were confirmed by Western blot (WB) analysis using a recombinant protein of HTNV (Fojnica strain). Except sample number 7, all others were part of a seroprevalence study on hantavirus infections in India and originated from patients with chronic renal disease (sample numbers 1 to 6) and healthy blood donors (sample numbers 8 to 12); (Fig. 1).<sup>[6]</sup> Sample number 7 was from a patient with suspected hantavirus-like disease. Samples were chosen for serotyping based on their availability and level of reactivity in the ELISA and IFA as shown in Table 1. Cumulative data on reactivity of these samples have been presented in our seroprevalence report.<sup>[6]</sup>

### Results

Results of 12 serotyped samples are represented in Fig. 1.

All samples were characterized using the entire recombinant N proteins of HTNV (HTNV antigen complex) and PUUV (PUUV antigen complex; Fig. 1). In addition, to differentiate between hantavirus serotypes, truncated and recombinant N proteins (trNPs) of HTNV, SEOV and THAIV lacking 49 amino acids in the amino-terminal region of the N protein and expressed by a baculovirus system were used as ELISA antigen for serotyping.<sup>[8,9]</sup> The cut-off OD of the serotyping ELISA was calculated using negative

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**Table 1: Summary of serological results (anti-hantavirus IgG reactivity) of samples used for hantavirus serotyping**

Sample ID	ELISA Index	IFA	WB	Serotyping
#1	1.6	N	N	N
#2	1.2	3+	P	P
#3	2.7	N	N	N
#4	3.5	3+	P	P
#5	1.12	2+	P	P
#6	2.0	>3+	P	P
#7	2.9	>3+	P	P
#8	2.2	4+	P	P
#9	2.6	>2+	P	N
#10	1.5	3+	P	N
#11	1.4	>2+	P	P
#12	1.3	N	N	*

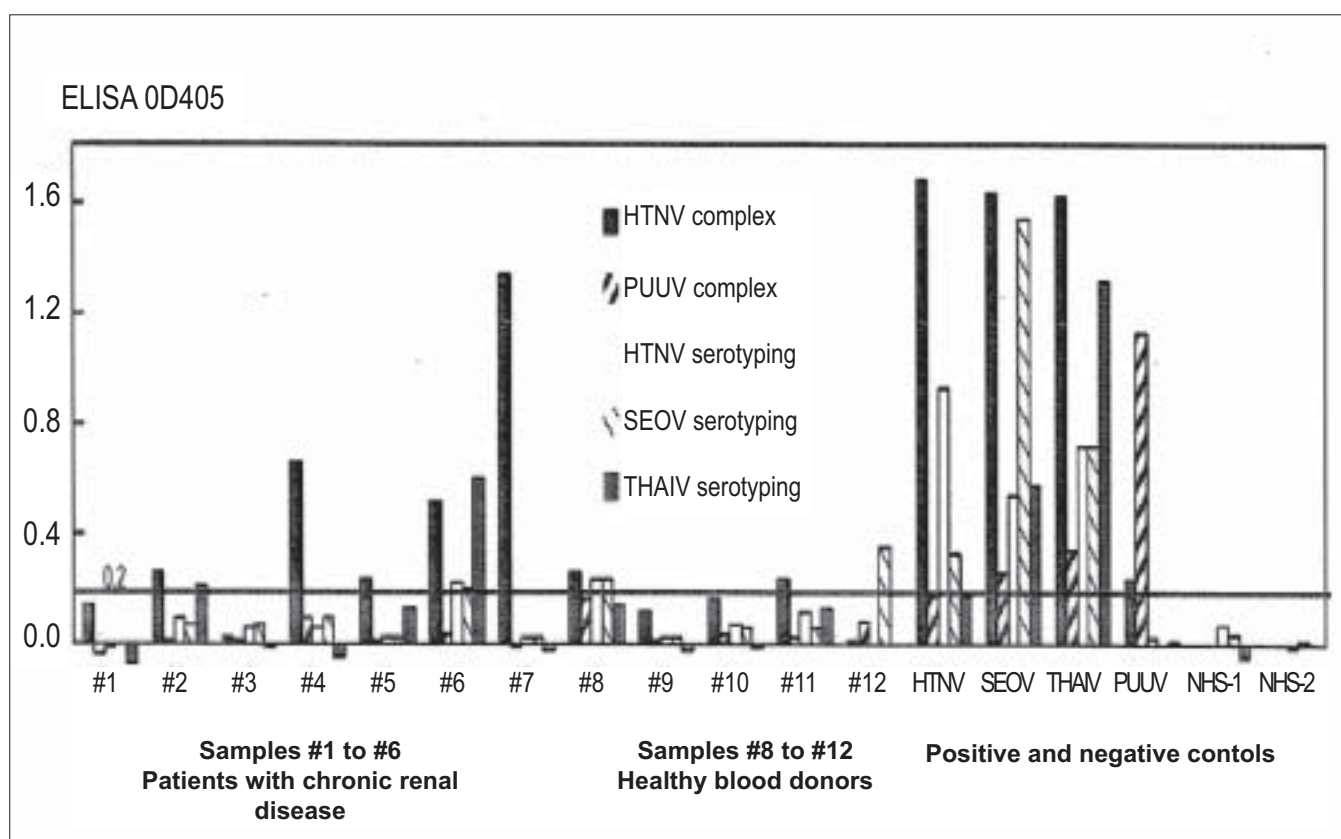
P- Positive, N-Negative. A sample was considered positive for anti-hantavirus IgG if reactive by both ELISA and IFA. An ELISA Index > 1.10 and an IFA reactivity  $\geq 2+$  were considered positive. \*This sample though non-reactive with HTNV complex antigen showed reactivity with SEOV serotyping antigen. Samples #1 to #6 Patients with chronic renal disease. Samples #8 to #12 Healthy blood donors

sera and was less than 0.1. However, to eliminate false positive reactions, 0.2 was taken as cut-off OD. Two human sera that were negative for hantavirus-specific antibodies

are represented in Fig. 1 as NHS-1 and NHS-2. Positive control sera from patients infected with HTNV, SEOV, THAIV and PUUV have been used as positive controls and demonstrated strongest reactivity with the corresponding homologous antigen (Fig. 1).

Seven of the nine screening positive sera, numbers 2, 4, 5, 6, 7, 8 and 11 were found reactive with the entire HTNV antigen complex. Sera numbers 9 and 10, although detected in the screening assays, were non-reactive with this antigen. None of the nine sera showed a significant reactivity with the entire PUUV N antigen complex. The causative hantavirus species that the seven individuals were exposed to belongs to the HTNV complex. Sera numbers 2 and 6 showed reactivity with trNP of THAIV antigen suggesting infection with a THAIV-like virus. Sera numbers 4 and 5 could not be serotyped using trNP of HTNV, SEOV and THAIV suggesting past infection with an unknown HTNV-like virus.

Sample number 7, from a patient with suspected hantavirus-induced disease, although strongly reactive with the entire HTNV antigen, showed no reactivity with any of the serotyping antigens suggesting an infection with a virus of the HTNV complex. This serum originated from a 13-year-old patient who presented with fever, headache, myalgia and cough at a peripheral hospital. However, virus-specific IgM was not detected (data not shown). Therefore,



**Figure 1:** Profiling of anti-hantavirus antibody in a serotyping ELISA Sample #7 was from a patient with hantavirus-like illness. Except samples #1, #3 and #12, all samples were anti-hantavirus IgG reactive in ELISA, IFA and WB. The positive control sera originated from HTNV- infected, SEOV- infected, THAIV-infected and PUUV-infected patients. The negative control sera NHS-1 and NHS-2 were non reactive to the antigens used

the individual may have been infected with hantavirus previously. Unfortunately, no information is available about the patient's living conditions or his exposure to rodents.

The blood donor samples reactive by the serotyping ELISA (numbers 8 and 11) showed only low levels of anti-hantavirus IgG antibodies. This low level of anti-hantavirus antibodies might be due to an infection long time ago, or perhaps by a distantly-related hantavirus. Sample number 8 showed similar OD values with entire HTNV N protein and trNP of HTNV and SEOV. The reactivity pattern of serum number 11 may indicate, similar to sera numbers 4 and 5, a previous infection with an unknown HTNV-like virus.

In general, the three negative sera; numbers 1, 3 and 12 did not react significantly with any of the N antigens used. The only exception was serum number 12 reacting with the trNP of SEOV. Similarly numbers 9 and 10, though detected in the screening assays, were non-reactive with the entire HTNV antigen complex. These results may indicate limitations in the sensitivity of the screening assays used and of the entire N protein of HTNV-based ELISA, also; alternatively a specificity problem of the SEOV serotyping ELISA.

## Discussion

Through this serological study it appears that at least two hantavirus species seem to circulate in India: the THAIV-like virus and perhaps one or more unknown HTNV-like viruses. However, we cannot exclude that the infections in subjects numbers 4 and 7 and perhaps numbers 5 and 11 are caused by a HTNV strain that contains amino acid exchanges in the N protein causing the unexpected lack of reactivity with trNP of HTNV. The reactivity pattern of the serum samples with the different serotyping antigens seems to clearly suggest the presence of more than a single novel hantavirus species in India.

Sample number 6 showed good reactivity with THAIV serotyping antigens and with HTNV complex antigen but low reactivity with HTNV and SEOV serotyping antigens. The difference in the OD is suggestive of infection with THAIV or THAIV-like serotype. Sample number 8 shows low reactivity with HTNV complex antigen, HTNV and SEOV serotyping antigens. This kind of reactivity could suggest presence of a non typeable HTNV-like serotype.

THAIV is pathogenic to humans and documented to cause HFRS in Thailand.<sup>[2]</sup> The known reservoir for the THAIV serotype, *Bandicota indica*, is also distributed in India. The reactivity pattern of numbers 2 and 6 may represent human THAIV infections in India.

The findings presented in this paper are preliminary. The differentiation of the etiologic species is relevant in

epidemiological terms as the disease severity of hantavirus infections depends on the hantavirus species/serotypes. Further studies including larger human serum panels as well as studies in rodent and shrew reservoirs are needed for more conclusive results on the circulating hantavirus species India. Future serotyping studies should include testing dilutions of sera. In future it is also important to conduct serological surveys using immunoassays that incorporate antigens of TPMV.

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