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Citation	Japanese Journal of Veterinary Research, 48(1), 87-87
Issue Date	2000-05-31
Doc URL	<a href="http://hdl.handle.net/2115/2843">http://hdl.handle.net/2115/2843</a>
Type	bulletin (article)
File Information	KJ00003408177.pdf



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## Generation of hantavirus recombinant nucleocapsid proteins and the application of ELISA antigens for serotyping

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Hantaan (HTN), Seoul (SEO), Dobrava-Belgrade (DOB), and Puumala (PUU) viruses are different serotypes of the genus Hantavirus in the family *Bunyaviridae* and are causative agents of hemorrhagic fever with renal syndrome (HFRS). Focus reduction neutralization test (FRNT) is the most specific serodiagnostic technique for differentiating HTN, SEO, DOB, and PUU virus infections. However, FRNT takes more than 1 week to obtain results and requires a biological safety containment facilities for virus manipulation. Therefore, a simple, safe, and rapid diagnostic method which is able to distinguish HTN, SEO, DOB, and PUU virus infections is required. In this thesis, by using a recombinant baculovirus system, the complete nucleocapsid proteins (cNP) and two kinds of the truncated nucleocapsid proteins (trNP) lacking a common antigenic region of the N terminus were prepared. One trNP was lacking 49 amino acids of the N terminus (50trNP) and the other was lacking 154 amino acids of the N terminus (155trNP). A new ELISA system for the specific serodiagnostic procedure using these antigens was established. This new method was applied for serological survey among sera of patients with hepatitis of unknown etiology.

When cNPs were used as ELISA antigens, cNPs of HTN, SEO, and DOB viruses had strong cross-reactivities against sera of HFRS patients or rabbits infected with HTN, SEO,

or DOB viruses. However cNP of PUU virus reacted specifically to sera of HFRS patients or rabbits infected with PUU virus. Therefore, ELISA using cNPs could distinguish PUU virus infection from HTN, SEO, and DOB virus infections. The 50trNPs of HTN, SEO, and DOB viruses had strong specific reactivities to sera of patients or rabbits infected with the homologous virus. However, 155trNPs were antigenically unstable for ELISA. Especially HTN 155 trNP had low reactivity to rabbit immune sera. Therefore, 50trNPs of HTN, SEO, DOB virus were useful as serotyping antigens.

Serological survey among sera of patients with hepatitis of unknown etiology was performed by using cNPs and 50trNPs as ELISA antigens. As a result, 4 samples in a total of 165 samples had anti-hantavirus antibodies. All of the 4 samples revealed positive reactions in the Western blotting analysis. Three among the 4 samples strongly reacted against 50trNP of SEO virus in the ELISA and the 3 samples had highest neutralization antibody titers to SEO virus in FRNT. This indicates that these 3 patients were infected with SEO virus.

This ELISA takes only 1 day to perform and does not require biosafety containment. The ELISA had remarkable advantages to the current FRNT and is very useful for large scale serological survey and clinical diagnosis of HFRS.