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Author(s)	KAMIURA, Mizuho
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DETECTION OF HANTAVIRUS RNA IN THE TISSUES  
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NESTED PRIMER-BASED POLYMERASE CHAIN REACTION

Mizuho KAMIURA

*Department of Veterinary Public Health,  
Faculty of Veterinary Medicine,  
Hokkaido University, Sapporo 060, Japan*

A rapid and specific method is required to detect hantaviruses, the etiological agents of hemorrhagic fever with renal syndrome (HFRS), for epidemiological surveys and clinical diagnosis. We developed a reverse transcriptase-directed polymerase chain reaction (RT-PCR) and nested primer-based polymerase chain reaction (Nested-PCR) to detect hantavirus genomic sequences and compared their sensitivities with conventional virus isolation methods. RNA extracted from Hantaan Virus strain 76-118 (prototype) was initially reverse-transcribed. The complementary DNA (cDNA) was used as a template to amplify the region of the hantavirus S genome segment. Results with this method were summarized as follows.

1. cDNA of the Hantaan virus strain 76-118 S segment, corresponding to 0.6 FFU, was amplified by using Nested-PCR.
2. The clots and organs from infected mice were inoculated on Vero E-6 monolayer cells. The virus isolated from clots, lungs and kidneys at 3 to 21 days after inoculation. But it was not detected in the homogenized organs.
3. The course of the virus infection was monitored in mice inoculated with Hantaan virus strain 76-118 by antibody response, virus isolation and Nested-PCR.

We divided the course of 35-day infection into four stages. During the first stage, until 5 days after inoculation, antibody response was not detected but the virus in the clots and lungs was isolated by tissue culture and detected by Nested-PCR from 3 days after inoculation. In the second stage from 7 to 10 days, antibody was first detected and at the same time, the virus was isolated from the lungs. In the third stage from 10 to 21 days, the IgG antibody titer was considerably high but the virus was isolated from the lungs. In the fourth stage from 28 to 35 days, the antibody titer was as high as in the third stage, but the virus was no longer detected.

Collectively, the virus was detected in the clots and lungs from adult mice inoculated with Hantaan virus strain 76-118 at certain stages even in the presence of antibody with a high titer. However, the virus was not detected in the spleens and brains at any stage after virus inoculation. Similar results were obtained both by the virus isolation method using tissue culture and by genomic detection using Nested-PCR.