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Antibody detection from Middendorf's vole (*Microtus middendorffii*) against Tula virus captured in Mongolia

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Abstract

Seroepizootiological surveys among wild rodents were carried out on the east side of Lake Khovsgol in Mongolia in 2010 and 2011. A total of 76 voles belonging to the genera *Myodes* and *Microtus* were captured. Most of the voles that were seropositive to Tula virus antigen were Middendorf's voles (*Microtus middendorffii* (6/31)). Two of the 18 *Myodes* voles were also seropositive to Tula virus antigen. On the other hand, only one vole was seropositive to Puumala virus antigen. The results suggest that Tula virus was maintained in Middendorf's vole. This is the first report of detection of anti-Tula virus antibody in the central part of the Eurasia continent.

Key Words: hantavirus, bunyavirus, rodent-borne

The hantavirus belongs to the genus *Hantavirus* of the family *Bunyaviridae*. Various hantaviruses have been isolated from rodents, shrews, moles and bats²⁷. Each hantavirus appears to have a single predominant species of mammal as a natural reservoir. Among rodent-

borne hantaviruses, subfamily Sigmodontinae-borne viruses and subfamily Neotominae-borne viruses are causative agents of hantavirus pulmonary syndrome (HPS) and subfamily Murinae-borne viruses are causative agents of hemorrhagic fever with renal syndrome (HFRS)².

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In addition to those viruses, various hantaviruses have been detected from rodents belonging to the subfamily Microtinae, most of which are voles. Among those viruses, Puumala virus (PUUV) carried by the bank vole, (*Myodes glareolus*) is the most important causative agent of HFRS in Europe¹. On the other hand, Tula virus (TULV) was originally isolated from the European common vole (*Microtus arvalis*)¹². TULV has been widely found from voles in Russia²², Germany¹⁷ and other European countries^{10,13,18} is thought to be apathogenic for human^{8,24}.

Voies inhabit North America, Europe, and Asia. Distributions of various vole-borne hantaviruses in North America^{11,20} European countries^{15,18,22} and eastern parts of the Eurasia continent^{3,4,7,23,19} have been reported. However, there have been few studies on hantaviruses and their reservoir animals in the central region of the Eurasia continent^{14,25}.

In this study, rodents were captured in Mongolia and serologically screened with hantavirus antigens, after species identifications were confirmed with complete cytochrome b sequences. The captured rodents included various species of voles and the occurrences of the Middendorf's vole (*Microtus middendorffii*) in Mongolia was confirmed with molecular evidence. Throughout the screening, Middendorf's voles showed anti-Tula virus antibody. This is the first report about hantavirus infection in Mongolia and in Middendorf's voles.

A total of 77 rodents were captured on the east side of Lake Hövsgöl in Mongolia in 2010 and 2011 by using Sherman traps baited with raw seeds of millet (Table 1). Blood from the rodents was absorbed on filter papers (Advantech TOYO, type-1 filter paper). After collection of sera, the filter papers were stocked in the dark and 4°C until use, approximately for 3 years. Sera were extracted from the filter paper to 1 : 50 dilutions with Dulbecco's phosphate buffered saline pH 7.3 (PBS) according to the manufacturer's instruction. Then the extracted sera were treated for 30 min at 56°C for inactivation. PUUV and TULV

immune vole sera (*Microtus monteberlli*) prepared in a previous study were used as positive controls in serological tests²¹.

PUUV strain CG1820 and TULV strain Morabia were originally provided by Dr. A. Plyusnin (Helsinki, Finland) and were cultivated with Vero E6 cells as previously described²¹. The indirect immunofluorescent antibody (IFA) assay was performed as described previously²⁶. Briefly, acetone-fixed smears of Vero E6 cells infected with PUUV or TULV were used as antigens. Alexa Fluor 488-conjugated Protein A (Thermo Fisher Scientific, Waltham, MA, USA) was used for sera of voles (*Microtus* spp. and *Myodes* spp.) and sera of control voles²¹. The IFA antibody titer was regarded as the reciprocal of the highest serum dilution that displayed characteristic fluorescence in the infected Vero cells.

To verify the identity of the host species, total DNA was extracted from lung tissues using the GC series MagDEA[®] DNA 200 (GC) Kit (Precision System Science, Matsudo, Chiba, Japan). The entire 1,140-nucleotide cytochrome *b* gene of mtDNA was amplified by PCR using modified universal primers that were previously described⁵. PCR was performed in 50- μ L reaction mixtures containing 250 μ M dNTP, 1 U of AmpliTaq 360 Gold polymerase (Thermo Fisher Scientific) and 0.25 μ M of each primer. Cycling conditions consisted of initial denaturation at 95°C for 10 min followed by 35 cycles of denaturation at 94°C for 20 sec, annealing at 55°C for 1 min, and elongation at 72°C for 2 min in a Veriti thermal cycler (Life Technologies, Foster City, CA, USA).

As shown in Table 1, 77 rodent sera were screened by the IFA test against both PUUV and TULV antigens. Among the 77 rodents, 76 were voles belonging to the genus *Myodes* or *Microtus*. The IFA patterns of sera from six Middendorf's voles (MG349R36, MG1411RR7, MG1429RR25, MG1436RR32, MG1443RR39, and MG1445RR41) were similar to the IFA pattern of the positive control serum against TULV, which was characteristic granular and perinuclear

Table 1. List of rodents used in this study and serum antibody detection against PUUV and TULV antigens in IFA

Genus	Species	No. of tested	IFA against*			
			PUUV		TULV	
			±	+	±	+
<i>Myodes</i>	<i>rufocanus</i>	4	0	0	0	1
	<i>rutilus</i>	14	0	0	1	0
<i>Microtus</i>	<i>gregalis</i>	12	0	0	0	0
	<i>middendorffii</i>	31	1	0	3	3
	<i>oeconomus</i>	5	0	0	0	0
<i>Apodemus</i>	<i>peninsulae</i>	1**	0	0	0	0
Total		77	1	0	4	4

*+, IFA titers over 100; ±, IFA titers was 50. IFA patterns of + and ± were identical. All sera did not show granular and diffused fluorescence in IFA antigen of Vero cells without infection.

**This serum was also negative to Hantaan virus antigen.

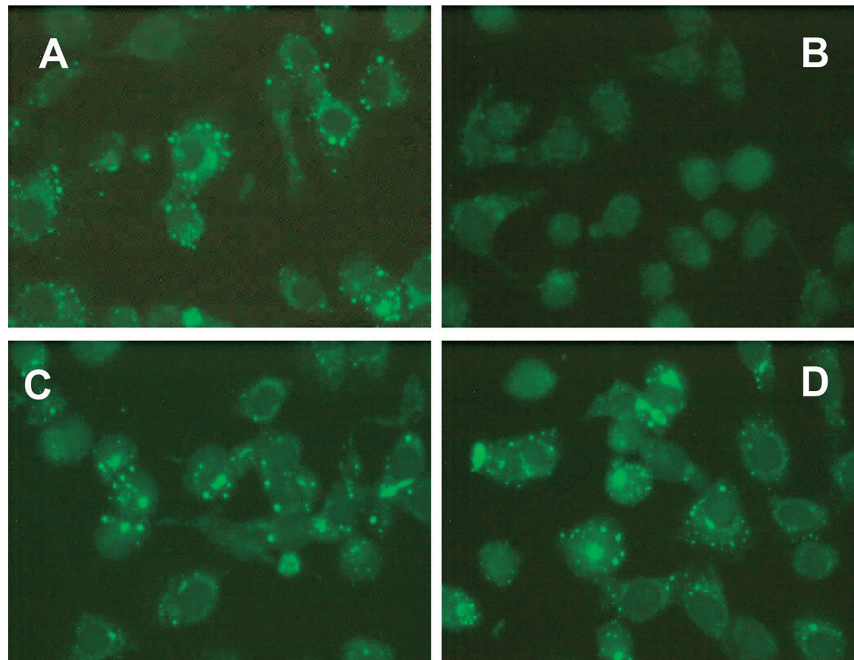


Fig. 1. IFA profiles of Middendorff's vole sera against TULV antigens. A: Positive IFA pattern of antiserum obtained from experimentally inoculated *Microtus montebelli* (Tegshduuren *et al.*). B: Typical negative IFA pattern of Middendorff's vole serum (MG1437RR33). C, D: Typical positive IFA patterns of Middendorff's vole serum (MG1429RR25 and MG1436RR32).

fluorescence in cytoplasm. Typical IFA antibody-positive patterns obtained with MG1429RR25 and MG1436RR32 are shown in Fig. 1. A typical antibody-negative IFA pattern from Middendorff's voles is also shown in the lower left panel of Fig. 1 (MG1437RR33). Six (19.3%) of the Middendorff's voles showed IFA titers that were positive (≥ 100) or weakly positive (50) to TULV

antigen. On the other hand, 1 (3.2%) of the 31 Middendorff's voles showed a weak positive IFA profile to PUUV antigen. This vole was antibody negative to TULV antigen. No antibody-positive voles against PUUV and/or TULV antigens was not found from *M. gregalis* and *M. oeconomus*. Antibody-positive voles were captured in both 2010 and 2011. The results indicated that

Middendorf's voles in northern Mongolia might be reservoir rodents for TULV or TULV-related hantavirus.

Eighteen *Myodes* rodents were captured in the same area in Mongolia. One *Myodes rufocanus* rodent and one *Myodes rutilus* rodent showed positive and weakly positive IFA profiles against only TULV, respectively. One field mouse (*A. peninsulae*) showed negative IFA reactions to PUUV, TULV and Hantaan virus antigen in the IFA (data not shown).

Sympatric habitats of voles belonging to the genera *Myodes* and *Microtus* have been reported in European countries and the Far East^{6,16}. In Europe, *Myodes* voles with PUUV and *Microtus* voles with TULAV have been captured at the same time. In Far Eastern part of Russia, *Myodes* voles with Hokkaido virus (HOKV) and *Microtus* voles with Khabarovsk virus (KBRV) or Vladivostok virus (VLAV) have been captured in the same area. In this study, a sympatric habitat of *Myodes* voles and *Microtus* voles was found in eastern shore of Lake Hövsgöl in Mongolia.

Myodes rufocanus was reported to be a reservoir rodent for HOKV, a PUUV-related virus, in Japan and Far Eastern part of Russia. Yashina *et al.* reported HOKV from *My. rufocanus* in the central area of Russia near Lake Baikal²⁵. Plyusnina *et al.* also reported HOKV *My. rufocanus* and VLAV from *Mi. fortis* which was also PUUV-related virus¹⁴ near Lake Baikal in Buryatia. Although *My. rufocanus* was captured in Mongolia, voles with antibodies positive to PUUV antigen were not found. On the other hand, one Middendorf's vole showed an anti-PUUV antibody in serum. HOKV might be maintained in a minor population of reservoir voles in Mongolia.

In addition to these observations, two *Myodes* voles showed anti-TULV antibody in their sera. The prevalence of antibodies in Middendorf's voles against TULV was 19% (6/31), that was a higher prevalence than other species of voles. TULV-like virus maintained among Middendorf's voles in the area might be source of spillover infection from Middendorf's voles to other voles.

Similar spillover infection of PUUV was reported in Europe from *Myodes* rodents to *Microtus* rodents⁹.

Previously, we examined antibody cross-reactivities to TULV and PUUV²¹. Little cross-reactivity of antibodies was detected in the sera of experimentally inoculated mice and voles. Similarly, sera from Middendorf's voles did not show any cross-reactivity against PUUV antigen in this study. These observations indicated that both TULV and PUUV antigens should be used for serological screening of voles.

In this study, a virus genome was not detected from preserved organs of Middendorf's voles (data not shown). However, the existence of a TULV-like virus in the central region of the Eurasia continent was serologically shown (Supplement Fig. 1). The results obtained in this study might contribute to studies on radiation and evolution of *Microtus* rodents with their hantaviruses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.14943/jjvr.65.1.39>

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