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Title	Serological detection of antibodies against bat-borne and shrew-borne hantaviruses in Sri Lanka
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Citation	Japanese Journal of Veterinary Research, 73(2), 51-58 https://doi.org/10.57494/jjvr.73.2_51
Issue Date	2025-06-30
Doc URL	https://hdl.handle.net/2115/95637
Type	departmental bulletin paper
File Information	JJVR73-2_51-58_RakiiyaSikatariiSarii.pdf



SHORT COMMUNICATION

Regional Study

Serological detection of antibodies against bat-borne and shrew-borne hantaviruses in Sri Lanka

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Received for publication, April 7, 2025; accepted, May 19, 2025

Abstract

Using serological tools for bat- and shrew-borne hantaviruses, we investigated the seroprevalence of these viruses in 336 human sera from Sri Lanka, consisting of 168 healthy individuals and 168 febrile patients. Twelve and 31 sera were positive for Mix antigen-bat (recombinant N antigens of Brno, Dakrong, Quezon, Robina, and Xuan Son viruses) and Mix antigen-shrew (recombinant N antigens of Altai, Asama, Cao Bang, Nova, Seewis, and Thottapalayam viruses), respectively. 2 of 168 control sera and 39 of 168 febrile patient sera were positive for Mix antigen-bat or Mix antigen-shrew. Individual antigen reactions were positive for Quezon, Robina, Altai, Asama, Cao Bang, Seewis, and Thottapalayam viruses. These findings suggest that bat- and shrew-derived hantaviruses may infect humans in Sri Lanka.

Key Words: recombinant N, serology, Zoonosis

Hantaviruses belong to the family *Hantaviridae* and are the causative agents of two rodent-borne zoonotic diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)^{13,18)}. Many species of rodents carry their specific hantaviruses, so rodents and hantaviruses seem to have coevolved²⁰⁾. However, hantaviruses have also been found in shrews, moles, and bats²⁶⁾. Thus, the acquisition of hantavirus diversity remains controversial²⁷⁾.

From the aspect of public health, the infectivity and pathogenicity of these novel

hantaviruses should be clarified. In Sri Lanka, we examined serological screening of 375 human sera for 4 shrew-borne hantaviruses, Asama (*Orthohantavirus asamaense*, ASAV)¹⁾, Altai (ALTV)¹²⁾, Seewis (*Orthohantavirus seewisense*, SWSV)¹⁵⁾, and Thottapalayam (*Thottimvirus thottapalayamense*, TPMV)⁵⁾ by using recombinant antigens. Although three seropositive human sera, two to ALTV and one to TPMV, were found in the Girandurukotte area of Sri Lanka, the relation between seropositivity and diseases was not found²⁵⁾.

Recently, numerous novel hantaviruses have

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doi: 10.57494/jjvr.73.2_51

Table 1. Indirect immunofluorescence antibody test screening of sera from Sri Lanka by Mix antigen-bat and -shrew

Intensity of IFAT*	Mix antigen-bat		Mix antigen-shrew	
	Febrile	Control	Febrile	Control
+	1	0	7	0
++	5	2	9	0
+++	4	0	15	0
Total	10**	2	31**	0

*IFAT, indirect immunofluorescence antibody test

* Criteria of intensity was determined as previous²¹⁾.

** Two specimens, 173F and 305E, were positive to both Mix antigen-bat and -shrew.

been identified from various shrews, moles, and bats species. We developed a serological detection system to identify antibodies against bat-borne hantaviruses, including Brno (*Loanvirus brnaense*, BRNV)^{7,23)}, Dakrong (*Mobatvirus dakrongense*, DKGV)⁴⁾, Quezon (*Mobatvirus quezonense*, QZNV)³⁾, Xuan Son (*Mobatvirus xuansonense*, XNV)²⁾, Robina (*Mobatvirus robinaense*, ROBV)²²⁾, shrew-borne hantaviruses, ASAV, ALTV, Cao Bang (*Orthohantavirus caobangense*, CBNV)⁸⁾, Nova (*Mobatvirus novaense*, NVAV)¹⁰⁾, SWSV, and TPMV. This study aimed to determine the presence of antibodies against bat- and shrew-borne hantavirus in human sera from Sri Lanka. We attempted to elucidate the potential for human infections by these novel hantaviruses in Sri Lanka.

Following a previously reported protocol, serum samples were screened for bat-borne and shrew-borne hantavirus antigens using an indirect immunofluorescent antibody test (IFAT)²¹⁾. Fixed Vero E6 cell antigen-expressing recombinant N antigens for bat-borne hantaviruses, a Mix antigen-bat (BRNV, DKGV, QZNV, ROBV, and XSV), and a Mix antigen-shrew (comprising ASAV, ALTV, CBNV, NVAV, SWSV, and TPMV) were tested using an IFAT with Alexa488-conjugated goat anti-human IgG (H + L) (A11013; Invitrogen, Thermofisher Scientific) as the secondary antibody. According to the previous procedure, Mix antigen-bat and -shrew samples were used for the initial screening antigen in IFAT at a serum dilution of 1:200 with phosphate-buffered saline

(PBS). IFAT determined the binding specificity with individual antigens, and positive reactions were further validated by Western blott assay as previously reported.^{21,25)}

Serum samples of hospitalized acute febrile patients (n = 168) from several hospitals collected by the Department of Microbiology, University of Peradeniya, until 2016 for the confirmatory diagnosis of hantavirus infection were used for the study. These patients experienced episodes of acute febrile illness of unknown origin. Serum samples collected from the community of Girandurukotte region (n = 168) were also used in this study. These control sera were the same as those of the previous study, but the number of sera was reduced in this current study because of availability.

Ethical approval for this study was obtained from the Institutional Ethical Review Committee (ERC), Faculty of Medicine, University of Peradeniya, Sri Lanka (Project No. 2015EC/SP/15, and Project No. 2016/EC/64 for the National Science Foundation – Sri Lanka [NSF-Sri Lanka] study) and written consents from all participants were preserved in the University of Peradeniya, Faculty of Medicine, Department of Microbiology. The ERC of the Institute for Genetic Medicine, Hokkaido University, Japan (21-001) was approved to treat Sri Lankan human materials based on G0313-5, Project No. 2015EC/SP/15, Project No. 2016/EC/64, and 2021/EC/44.

As shown in Table 1, 12 sera were positive for Mix antigen-bat, while 31 sera tested positive for Mix antigen-shrew. In this study, +, ++, and +++ represent the intensity of IFAT-positivity as earlier reported²¹⁾. The number of seropositive sera for shrew-borne antigens was significantly higher than the number of positives to bat-borne antigens ($P < 0.01$ in Fisher's exact test), performed using R software (R Foundation for Statistical Computing, Vienna, Austria²⁴⁾). Among them, two febrile specimens showed positive for Mix antigen-bat and -shrew. The seropositivity in febrile patients (39/168) was higher ($P < 0.01$ in Fisher's exact test) than in the control group (2/168). Control sera were collected from the Girandurukotte community, but the area of residence of the febrile cases was not identified. Therefore, the difference

Table 2. Indirect immunofluorescence antibody test results of individual antigens for sera positive in screening by Mix antigen-bat

Serum ID	IFAT intensity to Mix antigen-bat	IFAT intensity to individual antigen of					No. of positive antigens
		BRNV	DKGV	QZNV	ROBV	XSV	
40F	+++	-	-	++	-	+++	2
100F	++	-	-	-	++	-	1
164F	++	-	-	-	-	-	0
173F	+++	-	-	++	+++	++	3
227F	++	-	-	++	-	-	1
305F	++	-	-	-	++	-	1
416F	+++	-	-	++	+++	-	2
448F	++	-	-	-	-	++	1
569F	+	-	-	-	-	-	0
AIN 71F	+++	-	-	++	+++	-	2
G/Con/01	++	-	-	++	-	-	1
G/Con/09	++	-	-	++	-	-	1
Total	12	0	0	7	5	3	15

BRNV (Brno virus), DKGv (Dakrong virus), QZNV (Quezon virus), ROBV (Robina virus) XNV (Xuan Son virus).

IFAT, indirect immunofluorescence antibody test

Table 3. Indirect immunofluorescence antibody test results of individual antigens for sera positive in screening by Mix antigen-shrew

Serum ID	IFAT intensity to Mix antigen-shrew	IFAT intensity to individual antigen of						No. of positive antigens
		ASAV	ALTV	CBNV	NVAV	SWSV	TPMV	
169F	++	++	-	-	-	++	-	2
173F	++	-	-	++	-	-	-	1
174F	++	-	++	-	-	-	-	1
175F	+++	-	+++	-	-	-	-	1
223F	+	-	-	-	-	-	-	0
233F	+++	-	++	-	-	-	-	1
236F	+	-	-	-	-	-	-	0
250F	+++	-	+++	-	-	++	-	2
253F	+	-	-	-	-	-	-	0
305F	+++	-	-	+++	-	+++	-	2
311F	+++	+++	-	-	-	-	-	1
313F	+	-	-	-	-	-	-	0
317F	+++	-	-	-	-	-	+++	1
328F	+++	-	-	+++	-	++	-	2
329F	+++	+++	-	-	-	-	-	1
333F	++	++	-	-	-	-	-	1
337F	+++	-	-	++	-	-	-	1
357F	+++	+++	-	+++	-	-	-	2
358F	+++	+++	-	-	-	-	-	1
413F	++	-	++	-	-	-	-	1
433F	+++	+++	-	++	-	-	+++	3
480F	+++	+++	-	-	-	-	-	1
501F	++	-	-	-	-	-	++	1
505F	+++	+++	-	-	-	-	-	1
507F	++	-	-	++	-	-	-	1
522F	+	-	-	-	-	-	-	0
545F	+	-	-	-	-	-	-	0
554F	+++	+++	-	-	-	-	+++	2
560F	++	-	-	++	-	-	-	1
578F	++	-	-	++	-	-	-	1
587F	+	-	-	-	-	-	-	0
Total	31	10	5	9	0	4	4	32

ASAV (Asama virus), ALTV (Altai virus), CBNV (Cao Bang virus), NVAV (Nova virus), SWSV (Seewis virus), TPMV (Thottapalayam virus)

IFAT, indirect immunofluorescence antibody test

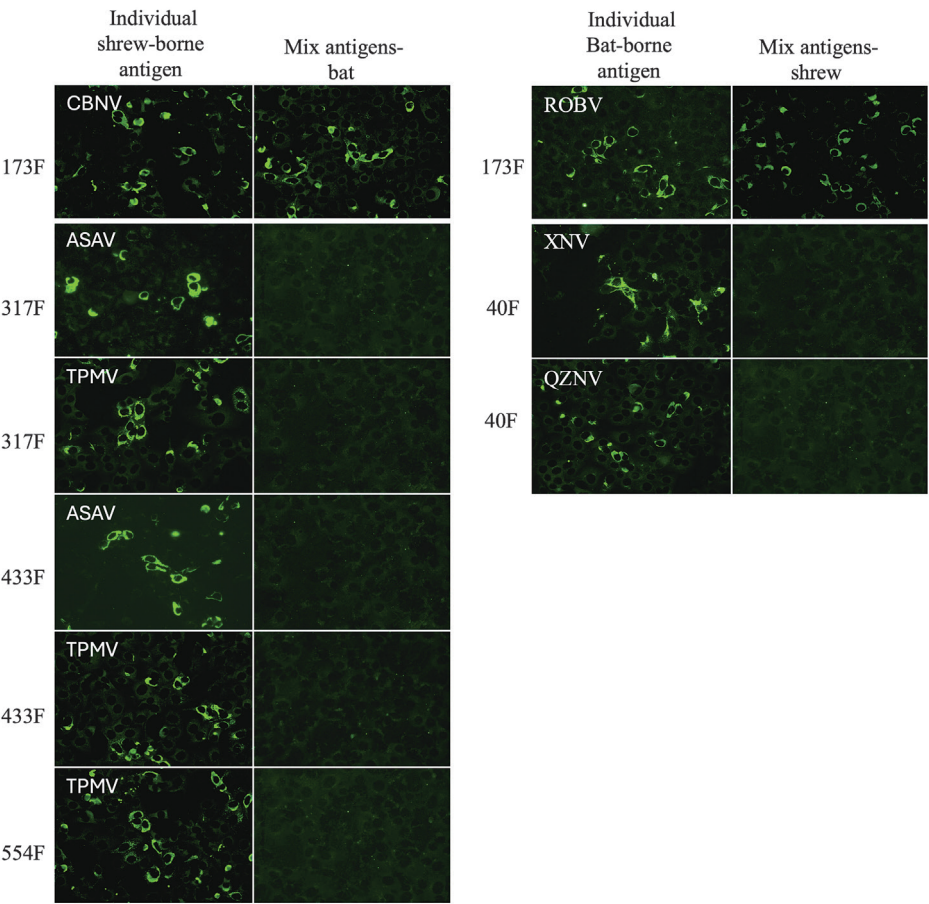


Fig. 1. Indirect immunofluorescence antibody test profiles of positive human sera to recombinant N antigens of bat- and shrew-borne hantaviruses
Indirect immunofluorescence antibody test (IFAT) showing results from Western blot positive human sera at 1:200 dilution. The left panel shows an individual shrew-borne antigen against its respective positive human serum, as well as its reaction on a mixed-antigen bat. On the right is a bat-borne positive human serum against the respective antigen and mixed-antigen shrew. While 173F showed double positive to Robina (ROBV) and Cao Bang (CBNV), with a corresponding reaction in the respective mixed-antigen system, the rest showed no such corresponding reaction. Hence, serum 173F reacted to both CBNV and ROBV antigens.

between the two groups, healthy controls and febrile patients, could not be compared directly.

The outcome of the individual antigen assays is shown in Tables 2 and 3. Table 2 shows the results for individual antigen-bat assays of control and febrile specimens, while Table 3 shows the individual antigen-shrew assays. Though 12 seropositives were recorded in the mix antigen-bat, 15 seropositives were seen in the individual bat-borne antigen assay due to multiple positives. Similarly, 31 seropositives for the mix antigen-shrew; however, 32 positives were recorded in the individual assay. No seropositivity was observed for BRNV and NVAV; this might be because

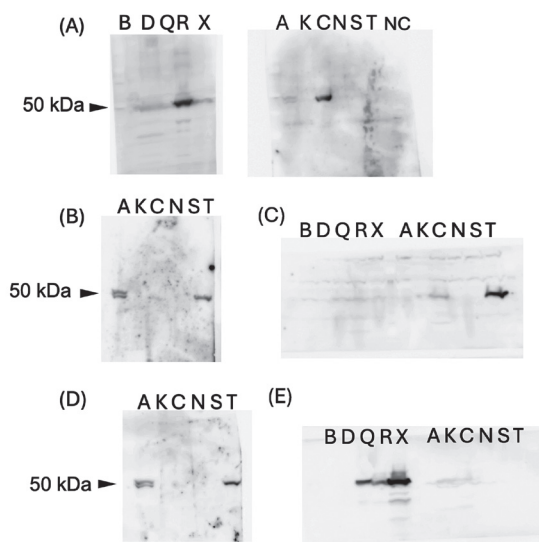
these viruses were originally found in Europe. The geographical distribution of viruses might be related to some seropositivity in Sri Lanka. Sera with mixed-antigen reactivity (+) produced no positive reaction in the individual antigen assay. This may be due to the presence of very low IgG for binding.

In the previous study, we examined anti-ALTV, ASAV, SWSV, and TPMV antibodies in controls in Girandurukotte and found positives to TPMV antigens in 2/271²⁵⁾. Although not all control sera, including those previously positive, were available, the results of these sera were consistent with previous studies that were

Table 4. Host and virus distribution of individual antigens

Individual antigen of		Original host	Virus distribution	No. of positive	
				/168 Control	/168 Febrile
Brno	(BRNV)*	<i>Nyctalus noctula</i>	Czech Republic	0	0
Dakrong	(DKGV)*	<i>Aselliscus stoliczkanus</i>	Vietnam	0	0
Quezon	(QZNV)*	<i>Rousettus amplexicaudatus</i>	Philippines	2	7
Robina	(ROBV)*	<i>Pteropus alecto</i>	Australia	0	5
Xuan Song	(XSV)*	<i>Hipposideros pomona</i>	Vietnam/ China	0	3
Asama	(ASAV)**	<i>Urotrichus talpoides</i>	Japan	0	10
Altai	(ALTV)**	<i>Sorex caecutiens</i>	Russia	0	5
Cao Bang	(CBNV)**	<i>Anourosorex squamipes</i>	Vietnam/ China	0	9
Nova	(NVAV)**	<i>Talpa europaea</i>	Europe	0	0
Seewis	(SWSV)**	<i>Sorex araneus</i>	Sweden, Russia, Japan, Americas	0	4
Thottapalayam	(TPMV)**	<i>Suncus murinus</i>	India, China, Indonesia, Vietnam, Nepal	0	4

Bat-borne*, Shrew-borne**

**Fig. 2. Western blotting profiles of positive human sera to recombinant N antigens of bat- and shrew-borne hantaviruses**

Recombinant N proteins expressed in 293T cell lysates were used as an antigen. B, Brno; D, Dakrong; Q, Quezon; R, Robina; X, Xuan Song; A, Asama; K, Altai; C, Cao Bang; N, Nova; S, Seewis; T, Thottapalayam; NC, 293T cell lysate without expression. Western blotting profiles of febrile (F) patient serum IDs 173F (A), 433F (B), 554F (C), 317F (D) and 40F (E) were shown. A: 173F showed a positive reaction to Robina (left) and Cao Bang (right). B and D: 433F and 317F showed double positives to Asama and Thottapalayam, C: 554F was positive to only Thottapalayam. E: 40F showed a positive reaction to Quezon, Robina, and Xuan Song antigens.

negative for ASAV, ALTV, SWSV, and TPMV. In addition, these were negative to other novel shrew-borne hantavirus antigens, CBNV and NVAV.

However, this study found two positives to the QZNV antigen, which was a bat-borne hantavirus.

The high seropositivity for ASAV among febrile patients raises a question regarding the host species. Because original host animal for ASAV is the Japanese shrew mole (*Urotrichus talpoides*), that is endemic to Japan⁶⁾. However, ASAV seropositive bats have also been found in Zambia²¹⁾. Since Japanese shrew mole cannot be a reservoir of ASAV in Zambia and Sri Lanka, ASAV-related viruses must be carried by different species. It will be necessary to identify the host animal species in these areas.

TPMV was carried by an Asian house shrew (*Suncus murinus*) and distributed widely in Asian countries, India, China, Nepal, Vietnam and Indonesia^{5,9,11,14,16,19)}. Since Asian house shrew is widespread in Sri Lanka, and antibody-positive humans with TPMV were detected in both this study and previous studies²⁵⁾. These observations suggest that a virus antigenically similar to TPMV is circulating in Sri Lanka. Without information on the genetic makeup of these suspected circulating viruses, we cannot definitively state that they are Asama and Thottapalayam viruses. These viruses may share a cross-reactive epitope.

Most of the seropositive reactions to bat-borne antigens were to QZNV and ROBV, which were originally found in fruit bats (Table 4). Sri Lanka is home to at least two species of fruit bats,

Pteropus medius and *Rousettus leschenaulti*. Sri Lankan fruit bats may be reservoirs for QZNV and ROBV-related viruses. Further investigations are necessary to identify the reservoir species for hantaviruses.

As shown in Tables 2 and 3, individual antigen reactions were positive for QZNV, ROBV, ALTV, ASAV, CBNV, SWSV, and TPMV viruses. These positive reactions to QZNV and XNV (40F), ROBV and CBNV (173F), ASAV (317F), and TPMV (317F, 433F, and 554F) in IFAT (Figure 1) were also confirmed in the Western blot assay (Figure 2). On the other hand, four IFAT-positive sera to SWSV were not confirmed in the Western blot. It is unclear why responses to SWSV were not confirmed by Western blot, but not all antigens induce antibodies to linear epitopes detected in Western blot. Other methods are needed to confirm SWSV infection.

Sri Lanka is an island country at the intersection of the Indian Ocean and Asia intersect and, therefore, has a rich and distinctive fauna. Two types of hantaviruses have been detected in rodents to date: the Lanka virus carried by *Mus booduga* and the Anjozorobe virus carried by *Rattus rattus*¹⁷⁾. There are many types and species of small mammals belonging to the order Euonymus, commonly called shrews and moles. Additionally, Sri Lanka is home to numerous types and populations of bats. However, no studies have been conducted to investigate the presence of hantavirus in these animals. Our findings suggest that bat- and shrew-derived hantaviruses could transmit to humans in Sri Lanka. Careful planning and implementation of studies to assess the pathogenicity of these viruses in Sri Lanka will be required.

Acknowledgments

This study was partially supported by the Science and Technology Research Partnership for Sustainable Development (SATREPS) (JP23jm0110019), and A grant provided by the National Science Foundation of Sri Lanka (RPHS/2016/CKDu/06) was received

by Professor Rohana Chandrajith (Study 03 PI: Chandika D. Gamage). We sincerely thank Dr. Lishanata Gunarathne, MBBS, and Dr. Nishantha Nanayakkara, MBBS, MD, for their continuous help with the human sample collection arrangements in Girandurukotte, Sri Lanka.

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