Prevalence of Rabies Virus and Hantaan Virus Infections in Commensal Rodents and Shrews Trapped in Bangkok

WANNEE KANTAKAMALAKUL, PhD*,
PRASERT THONGCHAROEN, Drmed*,
PILAIPAN PUTHAVATHANA, PhD*
SONTANA SIRITANTIKORN, Drrernat*,
CHANTRA SINGCHAI, DVM**,

Abstract

Five hundred rodents and shrews (*Rattus norvegicus*: 458, *Rattus rattus*: 28, *Rattus exulans*: 5, *Mus musculus*: 4 and *Suncus murine*: 5) trapped from the fresh food markets around Bangkok area were investigated for rabies virus and Hantaan virus infections. No rabies viral antigens in the animals' brains were detected by direct immunofluorescence. On the other hand, antibodies to Hantaan virus were demonstrated in the sera of 7 (1.53%) *R. norvegicus* caught in various markets using a particle agglutination technique. Further determination of the viral genome in rat lung tissue was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR, 3 (0.66%) out of 7 were positive. *Hin*dIII and *Hif*I restriction enzyme analyses showed the pattern of the Hantaan virus genome in 2 samples and that of the Seoul virus genome in the other.

The results of the present study suggest that rodents from Bangkok's fresh food markets did not carry rabies. Thus, getting rid of rabies in dogs or cats in the Bangkok area may be easier than anticipated because there are no sources of asymptomatic reservoirs. This may result in the low incidence of rabies patients observed in Bangkok. On the contrary, the presence of antibodies and the Hantaan virus genome and Seoul virus genome in *R. norvegicus* will definitely provide evidence for physicians to be aware of hemorrhagic fever with renal syndrome (HFRS) and other clinical settings of Hantaan/ Seoul virus disease in patients with a history of having contact with rats or their excreta.

Key word : Rabies Virus, Hantaan Virus, Seoul Virus, Commensal Rodents and Shrews

KANTAKAMALAKUL W, SIRITANTIKORN S, THONGCHAROEN P, SINGCHAI C, PUTHAVATHANA P
J Med Assoc Thai 2003; 86: 1008-1014

* Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700,
** Veterinary Public Health Division, Department of Health, Bangkok Metropolitan Administration, Bangkok 10200, Thailand.

Various species of commensal rats and shrews exist in Thailand. They are as follows: *Rattus rattus, Rattus norvegicus, Rattus exulans, Rattus surifer, Mus musculus, Bandicota indica, Bandicota savilei* and *Suncus murinus*. As has been reported[1-4], they may transmit some viruses that cause diseases to human such as rabies virus and Hantaan virus.

Rabies is one of the oldest and most frightening diseases of man and animals. Rabies occurs throughout the world. The World Health Organization (WHO) has reported about 50,000 rabies deaths per year. Approximately 25,000 cases come from countries in Asia[5]. In Thailand, about 19 deaths from rabies were reported in 2002[6]. However, this number is generally accepted to be an underestimation. Rabies deaths are reported all year-round with a slight increase during the summer. As is true in almost every country, dogs are the most common source of rabies transmitted to man in Thailand[7,8]. However, other vectors including non-human primates, rodents and other animals have become increasingly important as a threat to humans in many countries. In Thailand, rabies virus was isolated from several species of wild rodents around small towns in central and south-eastern provinces by Smith et al in 1968[9]. The percentage of virus recovery varied from 0-9 per cent. The species shown to harbour rabies virus were *B. indica, B. bengalensis, S. murinus, R. rajah, R. norvegicus, R. rattus* and *R. exulans*. In addition, Phuangsab et al[10] also reported in 1967 that 14.7 per cent of 241 rats, 13 ground squirrels and a skunk-like animal trapped in Chiang Mai province, tested positive for rabies antigen in the brain using immunofluorescence technique.

Hantavirus is distributed worldwide. The genus hantavirus comprised at least 7 serotypes. The serotypes Hantaan virus, Seoul virus, and Belgrade (Dobrava) virus cause hemorrhagic fever with renal syndrome (HFRS), the serotype Sin Nombre virus causes fatal pulmonary syndrome, and the serotypes Prospect Hill virus and Thottapalayum virus have not been shown to cause any disease. All of these serotypes are maintained in nature by infecting a single rodent species[11-14]. These infections are asymptomatic and life-long. The virus is transmitted horizontally among rodents. The infected rodents shed the virus in saliva, urine, and feces for many weeks following infection, but the duration of shedding and the period of maximum infectivity are not known. Transmission to humans is thought to occur predominately after the infected excreta is inhaled, although infections after rodent bites have been reported. Outbreaks of HFRS have occurred among laboratory workers exposed to infected rodents. Person-to-person transmission has not been documented.

Hantaan virus does exist in Thailand in at least two different species of rodents[15]. The viruses were isolated from lung tissue of *R. norvegicus* captured in Si Racha and Chon Buri provinces, and from *B. indica* captured in Kanchanaburi province. Approximately, 0-24 per cent of the rodents captured in different parts of the country either from slum areas or from fields had hantavirus antibodies. Prevalence of the antibody in humans was also determined by other groups of investigators, and ranged from 2.3-30 per cent depending on the history of exposure to the rodents[15,16].

It is of interest to determine the prevalence of rabies virus and Hantaan virus infections in rodent and shrew populations of Bangkok as rabies infection in these animals has been reported in Thailand for more than 30 years, and Hantaan virus is considered to be a potential emerging pathogen in the country.

MATERIAL AND METHOD

Specimen collection

Five hundred brains and lungs were collected from trapped rodents and shrews for detecting rabies antigen and Hantavirus genome, respectively. In addition, blood was also collected from each animal to be used for determining serum antibody to Hantaan virus. Tissues and sera were kept at -70°C and -20°C, respectively until used.

Direct immunofluorescence staining for rabies antigen detection

Portions of Ammon's horn and brain stem from each brain were pressed between two slides in order to produce a smear on each slide. The slide was dried at room temperature and fixed with precooled acetone at -20°C for 30 minutes. The dried slide was then stained for rabies antigen with Rabies-FITC (fluorescent isothiocyanate) -labeled-monoclonal IgG antibody (Fujurebio Diagnostics, Centocor, USA) following the manufacturer's protocol. All slides were examined under a fluorescence microscope (Nikon, Japan). A positive control slide of rabies positive dog brain was included in each staining procedure.

Hantaan virus antibody detection by particle agglutination test

Serum from each rodent or shrew was tested for antibody to Hantaan virus antigen using a Hantadia particle agglutination kit (Korea Green Cross Corp,

Korea) following the manufacturer's protocol. Briefly, the serum was diluted at 1 : 40 and then mixed with Hantaan virus antigen sensitized high density composite particles (HDP) in a v-shaped 96 well plate. The plate was then incubated at room temperature for an hour. Complete agglutination indicated a positive result.

RT-PCR and Nested PCR for detection of Hantaan virus genome

Lung tissue from rodents/shrews that had Hantaan virus antibody in the sera were then processed for Hantaan virus genome detection. Initially, approximately 100 mg of lung tissue was homogenized in TRIzol reagent (GibcoBRL, USA) and total RNA extractions were performed according to the manufacturer's protocol. The RT-PCR and nested PCR were carried out using primers and conditions as described by Kim et al[17]. The published primer sets (deduced from the sequences of the S segment of Hantaan virus genome) for the first round RT-PCR and nested PCR were as follows: primer SK1 (5'-ATTGA TGAACCTACAGGAC-3') and primer SK4 (5'-TG TATCCCCATTGATTGTG-3') for 800 bp amplified product; and primer SK2 (5'-AGCATGAAGGCAG AAGAG-3') and primer SK3 (5'-ACAAGCATGTTG GTGGAC-3') for 403 bp amplified product. Briefly, 10 μg total RNA was added into 90 μl of reaction mixture, which contained 2.5 U *Taq* polymerase (Promega, USA), 20U RNase inhibitor (RNasin, Promega), 5 U avian myeloblastosis virus (AMV) reverse transcriptase (Promega), 100 μM dNTP, and 50 pmol of each primer (SK1 and SK4), in a manufacturer's buffered solution containing an additional 75 μM $MgSO_4$. The initial RT-PCR was carried out at 42°C for 1 h, and 35 cycles of 1 min at 94°C, 1 min at 55°C, and for 3 min at 72°C were followed in a DNA thermal cycler (Perkin-Elmer, USA).

Then nested PCR was performed with the second set of primer (SK2 and SK3) using 10 μl of the RT-PCR product. After 15 cycles under the same conditions an additional incubation for 5 min at 72°C was included to complete the primer extension. Amplified products were detected by electrophoresis in 1.2 per cent agarose gel, and stained with ethidium bromide and visualized under UV light.

Restriction enzyme analysis of PCR product

Amplified nested PCR product, purified by QIAquick Gel Extraction kit (QIAGEN, CA, USA) following the manufacturer's protocol, was mixed with 3 U of either *Hin*dIII or *Hin*fI enzyme and incubated at 37°C for 2 h. The enzyme *Hin*dIII could cleave nested PCR product of Hantaan virus but not Seoul virus into segments of 175 bp and 228 bp. On the other hand, *Hin*fI could digest nested PCR product from both Hantaan virus (280 bp and 60 bp) and Seoul virus (155 bp, 115 bp, 60 bp and 32/29 bp). Electrophoresis of DNA fragments was performed on 2 per cent agarose gel, and stained with ethidium bromide and visualized under UV light.

RESULTS

Five hundred trapped rodents and shrews including *R. norvegicus* (458), *R. rattus* (28), *R. exulans* (5), *M. musculus* (4) and *S. murinus* (5) were studied. To detect rabies virus infection, each sample of brain tissue was stained with monoclonal anti-rabies antibody-FITC and then examined under a fluorescence microscope. No rabies antigen was detected in any brain tissue sample as shown in Table 1.

Table 1. Detection of rabies antigen in brain tissue by direct immunofluorescence and Hantaan viral antibody in sera by particle agglutination in rodents and shrews in the Bangkok Metropolitan Area.

Rodents/shrews	Number tested	Rabies viral Ag detected	Hantaan viral Ab detected	%
Rattus norvegicus	458	0	7	1.53
Rattus rattus	28	0	0	
Rattus exulans	5	0	0	
Mus musculus	4	0	0	
Suncus murinus	5	0	0	
Total	500	0	7	1.40

For Hantaan virus infection, specific antibody to the virus was detected in the sera of 7 (1.53%) *R. norvegicus* (Table 1). These rodents were found in different areas around Bangkok as shown in Table 2. Lung tissue from these rodents was further investigated for Hantaan virus genome as shown in Table 3. Three out of seven rodents demonstrated the product of nested PCR. The amplified products were then analyzed with *Hin*dIII and *Hin*fI restriction enzyme. PCR products from two rodents showed the patterns of enzyme cleavage according to the Hantaan viral genome while the other showed a pattern that belonged to the Seoul viral genome.

Table 2. Names of districts in Bangkok Metropolitan Area where *R. norvegicus* carrying Hantaan viral antibody were trapped.

District	*R. norvegicus* with Hantaan viral antibody
Bangkae	1
Bang Rak	1
Chomthong	1
Pom Prap	1
Pasricharoen	1
Thon Buri	1
Yan Nawa	1

DISCUSSION

In the 1960s there were two reports of rabies found in rodents trapped in rural farm/forest areas and in the cities of other provinces outside Bangkok (9,10). Both reports showed that the percentage of positive cases were highest in *B. indica* (7-14%). Rabies was also detected in other rodent species. Interestingly, infection of *R. norvegicus* was detected in one study but not in the other. The authors did not detect any rabies virus antigen in the brain tissue of *R. norvegicus*, which were the majority of the rodent species studied here. No *Bandicota* were used in this study.

Rabies infection in rodents has occasionally been reported(1-6). Some experts suggest that rodents may be an asymptomatic reservoir of the rabies virus (2). Rodents contract the infection from exposure to stray dogs and cats in an area of high rabies incidence. Bats may be another possible source of infection. In Bangkok, most of the rabid animals are dogs. If there is a chance that rats would get rabies, it would most likely be from exposure to stray dogs. Results from the present study suggest that rodents from the fresh food markets around the Bangkok Metropolitan Area (BMA) do not carry rabies. Thus, elimination of rabies in dog or cat populations of the BMA may be easier than anticipated if there are no asymptomatic reservoirs in the rodent population of the city. Controlling rabies in the dog and cat population may result in a lower incidence of rabies patients in the BMA.

All seven rodents with a specific antibody to Hantaan virus belong to the species *R. norvegicus*. The per cent of Hantaan virus antibody found in 458 *R. norvegicus* was 1.53 per cent. Elwell et al, reported that 5.7 per cent (4/70) of these rodents trapped in Bangkok and other nearby provinces had antibodies to Hantaan virus(15). They also demonstrated that 24 per cent (12/50) of *B. indica* trapped in the same locations had positive antibodies. Unfortunately, no *Bandicota* was trapped in the present study.

Further investigation for the presence of Hantaan virus genome in lung tissue showed the virus in 3 (0.66%) rodents. Not all seven rodents were positive for viral genome detection in lung tissue. RT-PCR together with nested PCR used for detection of viral genome extracted from animal tissue may not work as well as that used for the detection of viral genome extracted from isolated virus(18-20). Some parameters should be taken into account, for example, the presence of inhibitors in lung tissue(21), the optimal

Table 3. Detection of Hantaan/Seoul viral genome in lung tissues of 7 *Rattus norvegicus* in Bangkok.

RT-PCR & nested PCR	Number	*Hin*dIII & *Hif*I analyses	
		Hantaan virus	Seoul virus
Positive	3	2	1
Negative	4	ND	ND
Total	7	2	1

*ND = not done

conditions for tissue treatment and for the assay. The confirmation of the RT-PCR and nested PCR product of Hantaan virus by *Hin*dIII and *Hin*fI restriction enzymes[15] used here could also be used to indicate the certain pattern in Seoul virus PCR product. Two out of three viral genomes belong to the Hantaan virus while the other belongs to the Seoul virus.

The serotypes of hantavirus found in Asia are both Hantaan virus and Seoul virus[22]. Cross reactivity among members of this genus have been reported[11,23]. Detection of the Seoul viral genome in lung tissue of rodents carrying antibody to Hantaan virus may indicate that the Hantaan virus antigen in this kit may be able to cross react with the antibody to the Seoul virus.

The presence of antibody and the detection of the viral genome indicate the prevalence of Hantaan virus and Seoul virus infection in these rodents, especially in the *R. norvegicus* population in various markets throughout the BMA. Although the role of hantavirus in human disease in Thailand has not yet been documented, the presented data will provide information to increase doctors' awareness of Hantaan viral disease in clinical settings other than HFRS in patients with a history of exposure to rats or their excreta. In addition, clinical symptoms other than HFRS will also support the finding that Hantaan virus could cause various clinical symptoms including pulmonary syndrome which has been reported in the USA[13,14].

ACKNOWLEDGEMENT

This work was supported by a grant from the World Health Organization (SE THA OCD 001 RB99).

(Received for publication on March 18, 2003)

REFERENCES

1. Moro ML, Horman JT, Fischman HR, Grigor JK, Israel E. The epidemilolgy of rodent and lagomorph rabies in Maryland, 1981 to 1986. J Wildlife Diseases 1991; 27: 452-6.
2. Von Reyn CF. Editorial: Rodent rabies. JAMA 1976; 235: 2423.
3. Dulta DJ. Treatment after rodent exposure necessary to avoid death from rabies. Public Health 2001; 115: 243.
4. Douron E, Moriniere B, Matheron S, et al. HFRS after a wild rodent bite in the Haute-Savoie and risk of exposure to Hantaan-like virus in a Paris laboratory. Lancet 1984; 1: 676-7.
5. Noah D, Drenzek CL, Smith JS, et al. Epidemiology of human rabies in the United States, 1980 to 1996. Ann Intern Med 1998; 128: 922-30.
6. Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. Disease Under Surveillance. Monthly Epidemiological Surveillance Report 2002; 33: 421.
7. Thongcharoen P. The problem of rabies control in Thailand. J Med Assoc Thai 1973; 56: 295-9.
8. Thongcharoen P, Wasi C, Chavanich L, Sirikawin S. Rabies in Thailand. In : Mackenzie JS, ed. Viral diseases in South-East Asia and The Western Pacific. Sydney: Academic Press; 1982: 606-30.
9. Smith PC, Lawhaswasdi K, Vick WE, Stanton JS. Enzootic rabies in rodents in Thailand. Nature 1968; 217: 954-5.
10. Phuangsab A, Panas-Ampol K, Lawhaswasdi K, Le Beau LJ. Rats as a reservoir of rabies in Chiang Mai. J Med Assoc Thai 1967; 50: 26-36.
11. Lee PW, Gibbs CJ Jr, Gajdusek DC, Yanagihara R. Serotyping classification of hantaviruses by indirect immunofluorescent antibody and plaque reduction neutralization tests. J Clin Microbiol 1985; 22: 940-4.
12. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. Prog Med Virol 1989; 36: 62-102.
13. Butler JC, Peters CJ. Hantaviruses and hantavirus pulmonary syndrome. Clin Infect Dis 1994; 19: 387-95.
14. Kahn AS, Ksiazek TG, Peters CJ. Hantavirus pulmonary syndrome. Lancet 1996; 347: 739-41.
15. Elwell MR, Ward GS, Tingpalapong M, Leduce JW. Serologic evidence of Hantaan-like virus in rodents and man in Thailand. Southeast Asian J Trop Med Public Health 1985; 16: 349-54.
16. Tantivanich S, Chongsa-nguan M, Impand P, Potha U, Imlarp S. Serological studies of Hantaan virus among Thai people and urban rats. J Parasitol Trop Med Assoc Thailand 1988; 11: 71.
17. Kim EC, Kim IS, Choi Y, Kim SG, Lee JS. Rapid differentiation between Hantaan and Seoul viruses by polymerase chain reaction and restriction enzyme analysis. J Med Virol 1994; 43: 245-8.
18. Puthavathana P, Lee HW, Kang CY. Typing of hantavirus from five continents by polymerase chain reaction. Virus Res 1992; 26: 1-14.
19. Puthavathana P, Dobbs M, Baek L, Chu Y, Lee HW, Kang C. Comparison of nucleotide sequences among hantaviruses belonging to the same serotype: An analysis of amplified DNA by thermal cycle sequencing. Virus Res 1993; 30: 161-9.
20. Tang YW, Ruo SL, Sanchez A, Fisher-Hoch SP, McCormick JB, Xu ZY. Hantavirus strains isolated from rodentia and insectivora in rural China differetiated by polymerase chain reaction assay. Arch Virol 1990; 115: 37-46.
21. Greenfield L, White TJ. Sample preparation methods. In : Persing DH, Smith TF, Tenover FC, White TJ, eds. Diagnostic molecular microbiology. Rochester: Mayo Foundation; 1993: 122-37.
22. Lam SK, Chua KB, Myshrall T, et al. Serological evidence of hantavirus infections in Malaysia. Southeast Asian J Trop Med Public Health 2001; 32: 809-13.
23. Sugiyama K, Morikawa S, Matsuura Y, et al. Four serotypes of hemorrhagic fever with renal syndrome viruses identified by polyclonal and monoclonal antibodies. J Gen Virol 1987; 68: 979-87.

ความชุกชุมของเชื้อพิษสุนัขบ้าและเชื้อไวรัส Hantaan ในสัตว์แทะที่ดักจับได้ในตลาดสดของกรุงเทพมหานคร†

วรรณี กัญฐกมาลากุล, PhD, สนทนา ศิริตันติกร, Drrernat*,*
ประเสริฐ ทองเจริญ, Drmed, จันทรา สิงห์ชัย, สพบ**, พิไลพันธ์ พุธวัฒนะ, ปรด**

การตรวจหาความชุกชุมของเชื้อพิษสุนัขบ้าและเชื้อไวรัส Hantaan ในสัตว์แทะชนิดต่าง ๆ 500 ตัว (*Rattus norvegicus*: 458, *Rattus rattus*: 28, *Rattus exulans*: 5, *Mus musculus*: 4, และ *Suncus murine*: 5) ซึ่งดักจับได้ในตลาดสดหลายแห่งของกรุงเทพ ฯ ปรากฏว่าไม่พบเชื้อพิษสุนัขบ้าในสมองสัตว์แทะเหล่านี้ ซึ่งตรวจด้วยวิธีย้อมหาแอนติเจนแบบ direct immunofluorescence แต่สามารถตรวจพบแอนติบอดีย์ต่อเชื้อไวรัส Hantaan ในซีรั่มสัตว์แทะ 7 ตัวโดยวิธี particle agglutination สัตว์แทะทั้งหมดเป็น *R. norvegicus* (หนูท่อ) คิดเป็น 1.53% เมื่อนำปอดของสัตว์ทั้ง 7 มาตรวจหายีโนมของไวรัส โดยวิธี RT–PCR, nested PCR พบผลบวก 3 ราย (0.66%) หลังจากวิเคราะห์ด้วยเอนไซม์ *Hin*dIII และ *Hif*I พบว่า 2 รายเป็นยีโนมของไวรัส Hantaan ส่วนอีก 1 รายเป็นยีโนมของไวรัส Seoul

การไม่พบสัตว์แทะที่ติดเชื้อพิษสุนัขบ้าในตลาดสดของกรุงเทพมหานครจากการศึกษาครั้งนี้ จะเป็นข้อมูลสำหรับการกำจัดสุนัขบ้าและแมวบ้าในเขตกรุงเทพ ฯ ว่าน่าจะกระทำได้รวดเร็วขึ้นเนื่องจากไม่พบสัตว์แทะซึ่งเป็นแหล่งแพร่เชื้อที่ไม่แสดงอาการ ส่วนการตรวจพบเชื้อไวรัส Hantaan และ Seoul ในหนูท่อน่าจะเป็นข้อมูลให้แพทย์ระวังโรคไข้เลือดออกที่มีไตวายร่วม (hemorrhagic fever with renal syndrome, HFRS) และโรคอื่น ๆ ที่อาจเกิดจากเชื้อนี้ในผู้ป่วยที่มีประวัติสัมผัสหนูท่อหรือ สิ่งขับถ่ายจากหนูท่อ

คำสำคัญ : เชื้อไวรัสพิษสุนัขบ้า, เชื้อไวรัสฮันทาน, เชื้อไวรัสโซล, สัตว์แทะ

วรรณี กัญฐกมาลากุล, สนทนา ศิริตันติกร, ประเสริฐ ทองเจริญ, จันทรา สิงห์ชัย, พิไลพันธ์ พุธวัฒนะ
จดหมายเหตุทางแพทย์ ฯ 2546; 86: 1008–1014

* ภาควิชาจุลชีววิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700
** สำนักอนามัย, กองสัตวแพทย์สาธารณสุข, กรุงเทพ ฯ 10200
† ทุนวิจัยจากองค์การอนามัยโลก