

RATS (*RATTUS NORVEGICUS* AND *RATTUS LOSEA*) HARBORING SEOUL HANTAVIRUS IN QINGYUAN, SOUTHERN CHINA: A SURVEY DURING 2011-2013

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Abstract. Hemorrhagic fever with renal syndrome (HFRS) is a zoonotic disease, which threatens public health and its incidence has increased sharply up to the present time in southern China. A survey of HFRS including in both the natural hosts and humans conducted in Qingyuan, southern China, during 2011-2013 revealed that one, two and seven confirmed cases of HFRS occurred in 2011, 2012 and 2013, respectively. Rodent densities ranged 1.73%-12.2% and Seoul hantavirus was detected by RT-PCR only in *Rattus norvegicus* and *Rattus losea*. The positive rate in humans was 0.95% serologically and 0.94% with IIFA in 2011 and 2013, respectively. DNA fragments detected in *Rattus norvegicus* and *Rattus losea* were highly homologous with those of Seoul hantavirus HB55 (96.2%) and L99 (95.1%), respectively. Thus HFRS is becoming an emerging and dangerous disease in southern China and it is necessary to further perform molecular characterization of strains isolated from rodents and humans.

Keywords: *Rattus* spp, hantavirus, hemorrhagic fever with renal syndrome (HFRS), survey, southern China

INTRODUCTION

Hantaviruses in the family Bunyaviridae are rodent-borne viruses, which cause two kinds of human diseases, namely, hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia and hantavirus pulmonary syndrome (HPS) in the Western hemisphere (Jonsson *et al*, 2010).

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Both species of *Hantavirus* genus, *Hantaan virus* (HTNV) and *Seoul virus* (SEOV), are recognized as the causative agents of HFRS in China, being associated with *Apodemus agrarius* and *Rattus norvegicus*, respectively (Wei *et al*, 2011). Guangdong located in southern China usually has a low incidence of HFRS (Zhang *et al*, 2010). According to a survey of HFRS in Guangdong during 1984 -1993, positive rates of IFA-IgG in clinical suspected cases are 29.7% (121/408) in males and 23% (45/195) in females (Wen *et al*, 1995).

Hantavirus antigens were identified in striped field mice (*Apodemus agrarius*)

from northeastern Inner Mongolia and in Norway rats (*Rattus norvegicus*) from middle and western Inner Mongolia (Zhang *et al*, 2009). According to the relationship of the migration of Norway rats with the distribution of Seoul hantavirus, it was hypothesized that an ancestor of phylogroup A SEOV variant was first exported from China to Europe and then spread through the New World following the migration of Norway rats (Lin *et al*, 2012).

But so far, there has been no detailed information of HFRS in both human and rodents in southern China. This study surveyed the epidemiological features of HFRS in humans and rodents in Qingyuan, in the north-central part of Guangdong.

MATERIALS AND METHODS

Geographic site

Qingyuan located nearby northern Guangzhou (the capital of Guangdong Province) is a mountainous region, covering 19,208 km² (Fig 1) and has a population of 3,913,000. It is characterized by a subtropical climate with short winter season. The mean annual temperature is 12.5°C and 28.8°C in winter and summer, respectively, with precipitation of 2,215 mm/year.

Samples collection

According to the presumed sites of HFRS cases and natural rodent habitats (Ertek and Buzgan, 2009), sampling sites were selected in rural and peripheral urban areas during 2011-2013, and 120-180 serum samples were collected from the general population and 120-180 rodents were captured. Permits for field collection were provided by the Guangdong Center for Disease Control and Prevention (protocol no. 015/2011). All the captured

rodents were processed in the field following established biosafety guidelines. Blood samples were drawn from the retro-orbital sinus using heparinized capillary tubes and stored at -30°C until analyzed.

Case definition

A clinical suspected case of HFRS is defined by typical symptoms and a confirmed case defined using an IgM test (at dilution \geq 1:100) (Ertek and Buzgan, 2009).

Assay

Both an indirect immunofluorescence assay (IIFA) and an enzyme linked immunosorbent assay (ELISA) (Limongi *et al*, 2013) were performed to detect specific IgM/IgG antibodies in serum, using HFRS antigens produced by the EUROIMMUN Medizinische labordiagnostika AG (Lübeck, Germany) and the Beijing Northern Biotechnological Institute (Beijing, China). ELISA and IIFA were considered as a primarily screening assay and a confirmatory assay, respectively, for clinical suspected cases.

Hantavirus detection in rodent was by RT-PCR of RNA extracted from lung as previously described (Zhong *et al*, 2013) using with primers SeoM F (TGTA-ATGGTCAGAAAAGAC) and SeoM R (TAGAATGGCTTTGAATCGGT). The amplicon (287 bp) was sequenced using an ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequence Kit in an ABI PRISM 3100 Genetic Analyzer (Hitachi, Tokyo, Japan). The DNA sequences were analyzed using DNASTAR Lasergene 7.1 (DNASTAR, Madison, WI).

Phylogenetic analysis

Nucleotide sequences were aligned and dendrograms constructed using the neighbor-joining (NJ) approach implemented in MEGA 6.06 (Tamura *et al*, 2013). The reliability of the tree was estimated

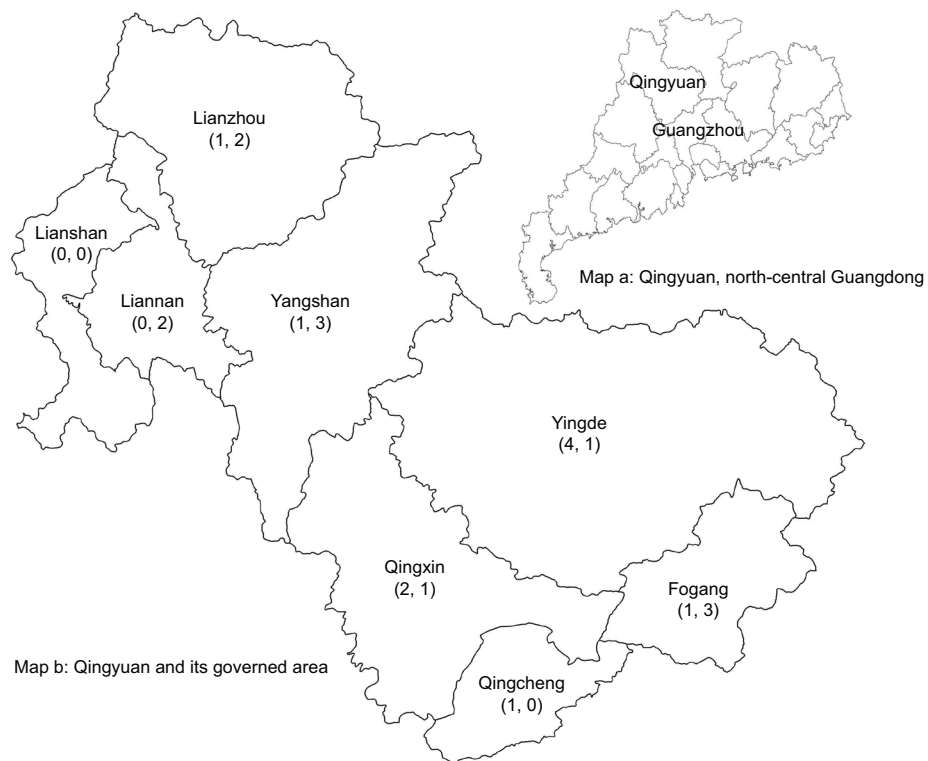


Fig 1–HFRS cases in Qingyuan of Guangdong, 2011-2013. Numbers in parenthesis refer to clinical and confirmed HFRS cases.

using 1,000 bootstrap replications. Genetic distances based on NJ phylogenetic trees were calculated by applying Kimura's two parameter method in MEGA 6.06.

Statistical analysis

Sera data were analyzed using Excel 2007 and SPSS version 19.0 (Huang *et al*, 2014). Rodent density is equal to the numbers of captured rats per 15 m².

RESULTS

HFRS cases

There were 1, 2 and 7 cases of HFRS confirmed by laboratory assays in 2011, 2012 and 2013, respectively, in addition

to 3, 3 and 6 suspected clinical cases of HFRS, respectively. The 10 confirmed cases occurred in Yingde (4), Qingxin (2), Qingcheng (1), Fogang (1), Yangshan (1) and Lianzhou (1) (Fig 1). The average age of the confirmed cases were 41.3 ± 12.4 years old with a male to female ratio of 1:9. Six subjects were farmers, 3 food or commercial workers and 1 job-waiting. All cases had history of contacting with local natural water or vegetables.

Rodent density and species

Rodent density (number of trapped animals/number of traps in 15 m²) ranged 1.73% - 12.2%, with density in outskirts areas being higher than that in housing areas

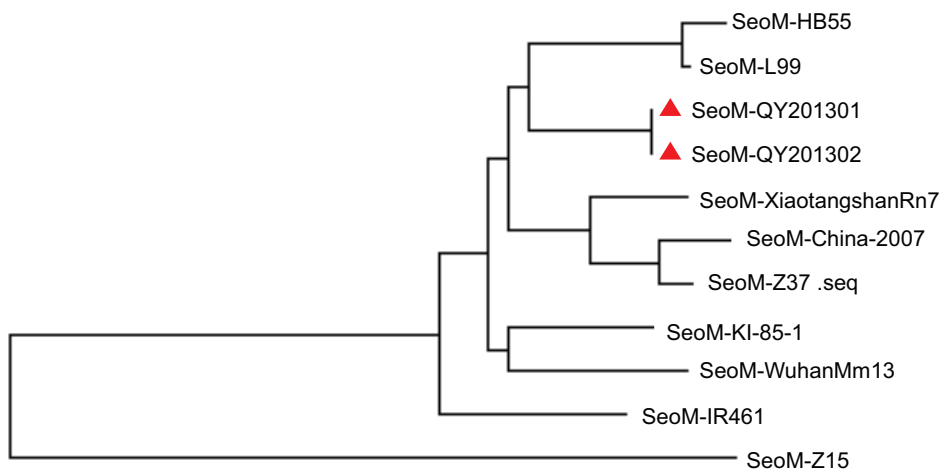


Fig 2–Phylogenetic tree of Hantavirus Seoul strain M gene. GenBank accession number of SeoM-HB55, -L99, -QY201301, -QY201302, -XiaotangshanRn7, -China-2007, -Z37, -KI-85-1, -WuhanMm13, -IR461 and Z15 is AF035832.1, AF035833.1, KM016895, KM016896, GU592929.1, EU163437.1, AF190119.1, D17593.1, JQ665886.1, AF458104.1 and FJ811839.1, respectively. ▲ Strain from this study.

Table 1
Rodent densities in Qingyuan, 2011-2013.

Year	Housing area		Outskirt area	
	No.	Density (%)	No.	Density (%)
2011	300	23 (7.7)	296	36 (12.2)
2012	347	6 (1.7)	700	43 (6.1)
2013	594	37 (6.2)	619	49 (7.9)
Total	1,241	66 (5.3)	1,615	128 (7.9)

Rodent density = no. of captured rats/no. of traps in 15 m².

(7.9% vs 5.3%) (Table 1). The difference of rodent densities might depend on the selection of capture sites. The capture sites in 2011 included Qingxin, Qingcheng, Yangshan and Lianzhou and those during 2012-2013 were located in Qingxin but in different villages. The low rodent density (1.73%) in housing area in 2012 might be related to the previous rat extermination campaigns.

Five rodent species were captured: *Rattus norvegicus* (187, 41.1%), *Rattus losea* (200, 44.0%), *Rattus flavipectus* (8, 1.8%), *Bandicota indica* (47, 10.3%), *Mus musculus* (8, 1.8%), and *Suncus murinus* (5, 1.1%) (Table 2). Only in *Rattus norvegicus* and *Rattus losea* were hantaviruses detected by RT-PCR. In the 2011 survey, positive samples were from lung tissues of *Rattus norvegicus* captured in Yangshan and of

Table 2
Rodent species in Qingyuan, 2011-2013.

Species	Year			
	2011 No. (+ve)	2012 No. (+ve)	2013 No. (+ve)	Total No. (+ve)
<i>Rattus norvegicus</i>	58 (1)	11 (0)	118 (2)	187 (3)
<i>Rattus losea</i>	57 (1)	132 (1)	11 (0)	200 (2)
<i>Rattus flavipectus</i>	4 (0)	1 (0)	3 (0)	8 (0)
<i>Bandicota indica</i>	13 (0)	23 (0)	11 (0)	47 (0)
<i>Mus musculus</i>	8 (0)	0 (0)	0 (0)	8 (0)
<i>Suncus murinus</i>	5 (0)	0 (0)	0 (0)	5 (0)
Total	145 (2)	167 (1)	143 (2)	455 (5)

+ve, infected with hantavirus.

Table 3
Surveillance of HFRS in Qingyuan, 2011-2013.

Year	Rodents and others						Humans	
	RT-PCR		ELISA		IIFA		IIFA	
	No.	+ve (%)	No.	+ve (%)	No.	+ve (%)	No.	+ve (%)
2011	145	2 (1)	145	4 (3)	145	4 (3)	211	2 (1)
2012	167	1 (0.60)	167	0 (0)	ND		228	0 (0)
2013	141	2 (1.42)	138	0 (0)	ND		106	1 (1)

ND, not done.

Rattus losea captured in Qingxin. During the 2012-2013 survey, one positive sample was detected in *Rattus losea* and two positive samples were from *Rattus norvegicus* captured in Qingxin.

Detection in human sera

Of 211 sera (male:female of 108:103) collected in 2011, two were IIFA positive, a 37 year-old male and a 43 year-old female in Yangshan (Table 3). Of 106 sera (male: female of 56:50) collected in 2013, only a female from Qingxin was IIFA positive.

Homology of Seoul hantavirus

Two samples of Seoul hantavirus

envelope polyprotein (M) gene sampled from *Rattus norvegicus* lungs using RT-PCR assay were sequenced. Both sequences, named QY201301 and QY201302, of 287 nucleotides were entirely the same (GenBank accession no. KM016895-6) and were highly homologous with Seoul hantavirus HB55 (96.2%) and L99 (95.1%) (Fig 2). Both rodents had been captured about 300 meters apart.

DISCUSSION

HFRS has been recognized as a neglected public health problem in south-

ern China for years. It has been reported that SEOV causes a milder form of HFRS than hantavirus and Dobrava-Belgrade virus and is responsible for 25% of cases of HFRS in Asia (Kariwa *et al*, 2007). However, there have been some reports of hantavirus infections in Thailand and Malaysia (Lam *et al*, 2001; Suputthamongkol *et al*, 2005). In this study, HFRS cases sharply increased during 2011-2013, an alarming trend indicating that the HFRS is becoming an important zoonotic and dangerous disease in southern China. The fact that clinical suspected cases were not confirmed because the samples collected from the suspected cases were not subsequently shown to be positive by assay IIFA or RT-PCR.

The severity of HFRS as well as the clinical symptoms can vary from sub-clinical to lethal, apart from the causative agent. In general, HFRS caused by SEOV is more moderate and is associated with a mortality rate of < 1% (Jonsson *et al*, 2010). In 2011 two sera and in 2013 one serum collected from a general population with specific antibodies against hantavirus using IIFA were detected. This suggested that new infections of HFRS occur occasionally in endemic regions, although each infected patient does not always present the typical clinical symptoms of HFRS. The low positive ratio of rat lung specimens in 2012 might have resulted from two causes: (i) selection of capture sites in 2012, and (ii) *Rattus losea* (79.0%, 132/167), although the major species, might not be a prevalent host of hantavirus in this captured field.

Despite quite a few of rodent species in this study, only *Rattus norvegicus* and *Rattus losea* were detected as the rodent carriers of hantavirus. Jonsson *et al* (2010) have reported that *Apodemus agrarius* and *Rattus* spp were the rodent hosts of HFRS

in China. However, as *Rattus* spp extends over the rural area in southern China, farmers working in cultivated land will be prone to catch the disease from this rodent species.

The two identical fragments of SEOV in this study might be due to the same pathogen as the capture traps were located near each other and/or that the fragments were too short to demonstrate any polymorphism. These two 287 bp fragments of SEOV were more homologous to those of SeoM L99 (GenBank No. AF035833) and HB55 (AF035832) than those of SeoM Z37 (AF190119) and WuhanMm13 (JQ665886). According to information in GenBank, SeoM L99 and HB55 are found in eastern China and SeoM Z37 and WuhanMm13 from Zhejiang and Hubei Provinces, respectively. As the strains from southern China are genetically close to those from eastern China, but more different from others to some degree, diversities of SEOV could be a remarkable feature in China.

It is necessary to obtain a more complete statistical analysis of the seroepidemiology of HFRS in southern China. This should include inventories of the local rodent species, identification of circulating hantavirus serotypes in rodents, molecular characterization of strains isolated from rodents and humans and comparison with strains circulating in the rest of China and in neighboring countries. Being a zoonotic disease, HFRS prevention requires identification of rodent reservoirs, as well as early diagnosis, prompt treatment and vaccination.

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