A Portable Diagnostic Assay, Genetic Diversity, and Isolation of Seoul Virus from *Rattus novegicus* Collected in Gangwon Province, Republic of Korea

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Introduction

Hantaviruses are enveloped, single-stranded, negative-sense RNA viruses, consisting of large (L), medium (M), and small (S) genome segments.

The natural reservoirs of hantaviruses include rodents (order *Rodentia*), bats (order *Chiroptera*), and insectivores (Order *Lipotyphla* sub-order*Soricomorpha*).

Orthohantaviruses are zoonotic pathogens that causa hemorrhagic fever with renal syndrome (HFRS).

SEOV-related HFRS corresponds to approximately 20% of clinical cases with mortality rate of <1%.

The Franklin system (Biomeme, Philadelphia, PA, USA) is a handheld portable real-time polymerase chain reaction (PCR) device.

Introduction

The qPCR assay with Biomeme technologies offers a rapid and sensitive molecular diagnosis of infectious agents.

In this paper the authors assessed whether the handheld Biomeme platform could be used for a SEOV molecular diagnostic to facilitate rapid decision making in point-of-care testing (POCT).

Highlights the use of molecular and genomic tools to understand the epidemiology of various hantaviruses, including PUUV, HTNV, and SEOV, with a focus on phylogeography and virus-host dynamics.

Introduced a one-step qPCR assay for SEOV detection and a TaqMan probe-based one-step qPCR assay for real-time identification of SEOV.

Materials & Methods

Sample Collection

Small mammals were collected using Sherman live traps from multiple sites including Seoul Metropolitan City and Gangwon Province (Cheorwon-gun and Chuncheon-si, Korea from 2016 to 2020.

The positive traps were sequentially numbered, rodents were identified by morphological characteristics, placed in a sealed container, and then transported to Korea University.

Two *R. norvegicus*, two *A. agrarius*, two *Crocidura lasiura*, and two *Mus musculus* captured from Gangneung-si in Gangwon Province, were provided by the HFRS vector surveillance program of the ROK Army in 2019.

The serum samples and tissues (lung, liver, kidney, and spleen) of captured animals were collected aseptically and stored at −80 °C until processing.

Materials & Methods

Mitochondrial DNA Analysis

Total DNA was extracted from the liver tissues of rodents using a High Pure PCR template preparation kit (Roche, Basel, Switzerland).

Mitochondrial DNA cytochrome b gene was amplified using the conventional PCR method.

All this date were deposited in GenBank.

RT-PCR

Total RNA was extracted from lung tissues of rats using TRI Reagent Solution (Ambion, Austin, TX, USA).

cDNA was synthesized using a High Capacity RNA to cDNA kit Applied Biosystems, Foster City, CA, USA)

Materials & Methods

qPCR

Was performed using the TOPreal™ One-step RT qPCR Kit (Enzynomics, Daejeon, ROK).

The PCR was performed in triplicate on a Biomeme Franklin Three Real-Time PCR thermocycler system (Biomeme) and a Quantstudio 5 Flex Real-Time PCR System (Applied Biosystems)

The filtered reads were mapped onto the reference sequences of SEOV 80-39, and consensus sequences were extracted.

Virus Isolation

At each passage level, the cell culture was tested for the presence of SEOV antigen and RNA using the IFA and RT-PCR, respectively.

Statistical Analysis

Student's t-test was conducted to determine significant differences in performance between Franklin (Biomeme) and QuantStudio 5 (Applied Biosystems) platforms.

Results

Serological and Molecular Prevalence of SEOV

A total of 23 *R. norvegicus* were collected from multiple locations, including Seoul Metropolitan City, Cheorwon-gum, Chuncheon-si, and Gangneung-si.

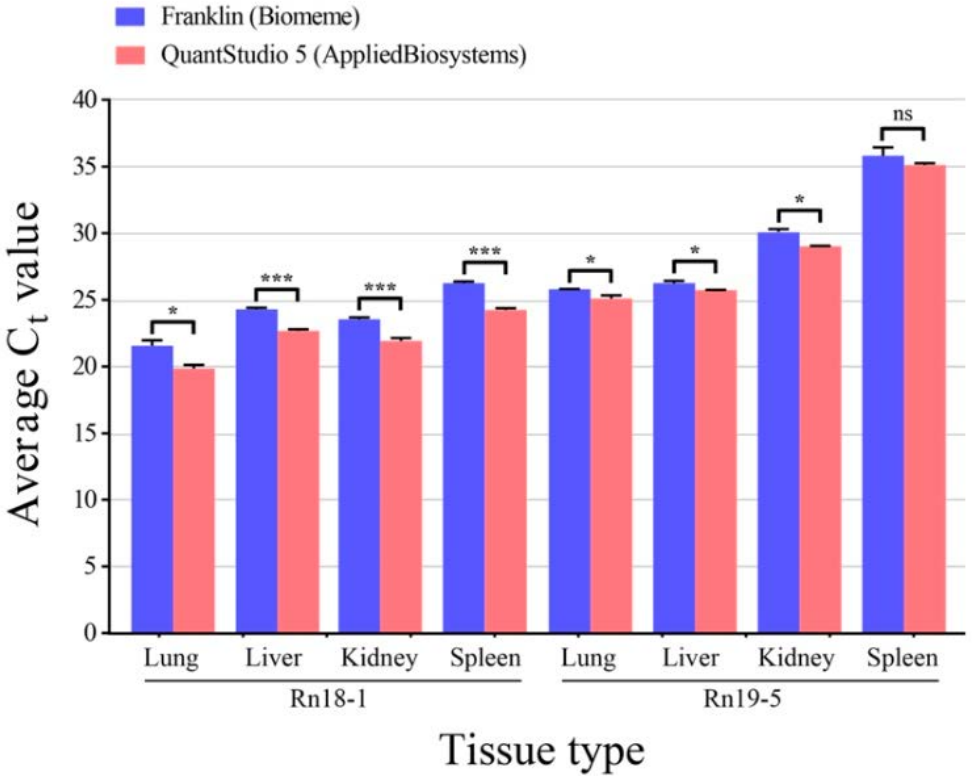
Characteristic	Number of Captured *R. norvegicus*	Seropositivity for Anti-SEOV IgG (%)	SEOV RNA Positivity (%)
Region (n = 27)			
Seoul Metropolitan City	23	3/23 (13.0)	0/23
Cheorwon-gun	1	0/1	0/1
Chuncheon-si	1	1/1 (100)	1/1 (100)
Gangneung-si	2	1/2 (50)	1/2 (50)
Sex (n = 27)			
Male	19	4/19 (21.1)	2/19 (10.5)
Female	8	1/8 (12.5)	0/8
Weight (n = 27)			
<50	10	1/10 (10)	0/10
51–100	11	2/11 (18.2)	0/11
101–150	3	0/3	0/3
151–200	3	2/3 (66.7)	2/3 (66.7)
Total	27	5/27 (18.5)	2/27 (7.4)

SEOV, Seoul virus; IgG, immunoglobulin G.

Results

To compare the relative sensitivity between handheld and benchtop qPCR systems for SEOV analysis, qPCR was performed on the lung, liver, kidney, and spleen tissues.

SEOV RNA was detected in all tissues of rodents, Rn18-1 and Rn19-5, using two qPCR machines.



Results

Whole-Genome Sequencing of SEOV

Using multiplex PCR-based NGS, nearly whole-genome sequences of SEOV were obtained from lung tissues of Rn18-1 and Rn-19-5 captured in Gangwon Province, ROK.

The mean numbers of mapped viral reads and depth are shown in Table S2. The 3′ and 5′ termini sequences were obtained by RACE PCR.

Sample	Region	Origin	Anti-SEOV IgG Titer	Ct Value [a]	SEOV Genomes, % Coverage [b]		
					L Segment	M Segment	S Segment
Rn18-1	Chuncheon-si	Lung	1:256	21.6	97.3	96.5	90.0
Rn19-5	Gangneung-si	Lung	1:256	25.8	99.5	98.9	97.3

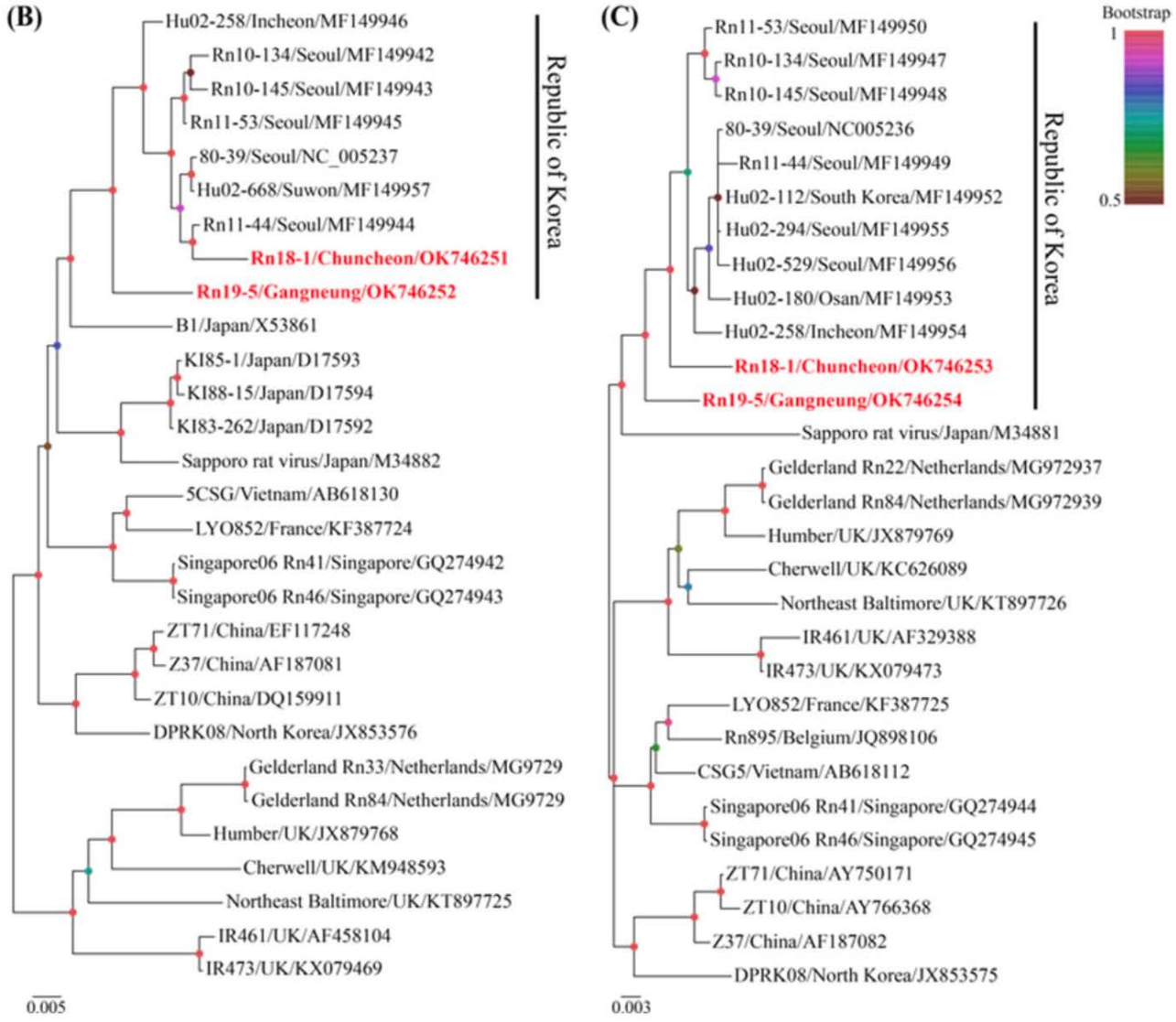
IgG, immunoglobulin G; Ct, cycle threshold; Rn, *Rattus norvegicus*; L, large; M, medium; S, small. [a]; determined using Biomeme system-based quantitative PCR for the S segment of SEOV. [b]; genome coverage was calculated using consensus sequence matching to the genome positions of the SEOV 80-39 strain.

Results

Phylogenetic Analysis of SEOV

The phylogenies of the L and M segments of SEOV Rn18-1 from Chuncheon-si shared a common ancestor SEOV Rn11-44 collected in Seoul Metropolitan City, whereas the S segment formed an independent genetic group in SEOV, ROK.

Results



Conclusion

Characterization of viral genomic sequences plays an important role in clinical diagnosis, patient precision management, epidemiological surveillance, and risk mitigation of virus outbreaks.

NGS is a robust tool for monitoring and tracking emerging viral infections.

NGS genomic surveillance has been applied to understand the characteristics and transmission dynamics of zoonotic viruses including EBOV, Zika virus, and SARS-CoV-2

The molecular evidence of SEOV improved the resolution of the phylogeographic map of orthohantaviruses for prevention and tracking of hantavirus outbreaks in the ROK.

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