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NOTE

Public Health

Evidence of infection with *Leptospira interrogans* and spotted fever group rickettsiae among rodents in an urban area of Osaka City, Japan

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ABSTRACT. We examined 33 rodents captured in an urban area of Osaka City, Japan for IgG antibodies against Seoul virus, severe fever with thrombocytopenia syndrome virus, hepatitis E virus, *Leptospira interrogans*, *Yersinia pestis*, spotted fever, typhus and scrub typhus group rickettsiae. We found that 3 (9.1%) and 1 (3.0%) of the 33 rodents had antibodies against *L. interrogans* and spotted fever group rickettsiae, respectively. DNAs of leptospires were detected from 2 of the 3 seropositive rodents, but DNA of rickettsia was not detected. Phylogenetic analysis and multiple locus sequence typing revealed that the 2 leptospires were *L. interrogans* belonging to a novel sequence type. There is a potential risk for acquiring rodent-borne zoonotic pathogens even in cities in developed countries.

KEY WORDS: leptospire, rickettsia, rodent, spotted fever, zoonosis

Rodents possess many pathogens including zoonotic pathogens. *Rattus* species live near habitats of humans worldwide not only in rural areas but also in urban areas. Therefore, there is a potential risk for acquiring rodent-borne zoonotic pathogens even in cities in developed countries. The aim of this study was to determine the infection status of some zoonotic pathogens in rodents captured in Osaka City, Japan.

Serum, spleen and kidney samples were collected from 33 rodents (*Rattus* spp.) captured in an urban area of Osaka City, Japan in 2012 and 2014. Serum samples were examined for IgG antibodies against hantavirus (Seoul virus), severe fever with thrombocytopenia syndrome virus (SFTSV), human and rat hepatitis E virus (HEV-1 and HEV-C1), *Yersinia pestis*, *Leptospira interrogans*, *Rickettsia japonica*, *R. typhi*, and *Orientia tsutsugamushi* by an immunofluorescence assay (IFA) or an enzyme-linked immunosorbent assay (ELISA) as shown in Table 1. *R. japonica*, *R. typhi* and *O. tsutsugamushi* were representatives for spotted fever, typhus and scrub typhus group rickettsiae, respectively.

Antibodies to *L. interrogans* and spotted fever group rickettsiae were detected from 3 (9.1%) and 1 (3.0%) of the 33 rodents, respectively (Table 2). Antibodies to other pathogens were not detected. Because of the small amount of serum samples, we could not conduct a microscopic agglutination test, a standard method for serodiagnosis of leptospirosis. There were no apparent clinical manifestation or pathological lesion in the antibody-positive rodents.

In order to identify the species of leptospires, DNA was extracted from kidneys of the seropositive rodents by using DNAzol Reagent (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and subjected to nested PCR by using Platinum Taq DNA Polymerase (Thermo Fisher Scientific) according to the manufacturer's instructions. Primers for the 16S rRNA (*rrs2*) gene [4] were used for the PCR. As a result, 547 bp DNA fragments of the *rrs2* gene were amplified from kidneys of 2 of the 3 seropositive rodents. Nucleotide sequences of the DNA fragments amplified from the 2 rodents were identical. The sequences showed high homology (99.8%) to strains of *L. interrogans*. To isolate leptospires, pieces of kidneys were minced with a 5 m*l* syringe and cultured in modified Korthof's medium (Denka Seiken, Tokyo, Japan) containing 100 μg/m*l* 5-fluorouracil. However, no leptospires were isolated from culture of kidney tissue. Because of unavailability of urine samples, we could not try to isolate or

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Table 1. Pathogens examined in this study

Pathogen	Method	Antigen	Reference
Virus			
Hantavirus	IFA	Seoul virus-infected cells	[12, 20]
SFTSV[a)]	IFA	Strain YG1-infected cells	[13]
HEV[b)]	ELISA	Virus-like particles of HEV-1[c)] and HEV-C1[d)]	[15]
Bacteria			
Leptospira interrogans	ELISA	Recombinant LipL32	[18]
Yersinia pestis	ELISA	Fraction 1 antigen	[10]
Rickettsia			
Rickettsia japonica	IFA	Strain YH-infected cells	[7]
Rickettsia typhi	IFA	Strain Wilmington-infected cells	[7]
Orientia tsutsugamushi	IFA	Strain Gilliam and Karp-infected cells	[7]

a) Severe fever with thrombocytopenia syndrome virus. b) Hepatitis E virus. c) Human HEV. d) Rat HEV.

Table 2. Prevalence of antibodies against each pathogen

Pathogen	No. positive/No. tested	Positive rate (%)
Virus		
Hantavirus	0/33	0
SFTSV	0/33	0
HEV-1	0/33	0
HEV-C1	0/33	0
Bacteria		
L. interrogans	3/33	9.1
Y. pestis	0/33	0
Rickettsia		
R. japonica	1/33	3.0
R. typhi	0/33	0
O. tsu. strain Gilliam	0/33	0
O. tsu. strain Karp	0/33	0

detect leptospires from urine. In order to genetically characterize the leptospires in more detail, multiple locus sequence typing (MLST) scheme 3 was applied [3]. DNA fragments of adenylate kinase (*adk*), isocitrate dehydrogenase (*icdA*), lipoprotein L32 (*lipL32*), lipoprotein L41 (*lipL41*), *rrs2* and pre-protein translocase secY subunit (*secY*) genes were amplified by using reported primers [2, 3, 5]. As for *icdA* and *secY* genes, the following inner primers were additionally designed and used: icdA-inner-F (5′-GAACTGGAYTATTATGATTTAGG-3′), icdA-inner-R (5′-TCYTTTGTIGCRAACCAAAGATC-3′), secY-inner-F (5′-CARACGATYATTCARTGGTTRTC-3′) and secY-inner-R (5′-TTTCTCATYAARAGTTGRGACTC-3′). The nucleotide sequences of each of the DNA fragments amplified from the 2 rodents were identical. The sequences were not exactly matched with any reference allele sequences in the *Leptospira spp.* MLST database (http://pubmlst.org/leptospira/). Allele IDs of each locus with the nearest match were 5 for *adk*, 2 for *icdA*, 4 for *lipL32*, 3 for *lipL41*, 14 for *rrs2* and 8 for *secY*. A sequence type with this combination of allele IDs was not found in the database. Phylogenetic analysis of each gene confirmed that the 2 leptospires formed a cluster with strains of *L. interrogans* (data not shown). These results indicate that the 2 leptospires detected in this study were *L. interrogans* belonging to a novel sequence type. Many leptospires have been detected and/or isolated in Japan. However, genetic information is limited. Further studies are needed to determine whether the leptospires detected in this study is really a novel type in Japan.

As stated above, we detected an antibody against spotted fever group rickettsiae. However, because of the serological cross reactivity among spotted fever group rickettsiae, we could not identify the species of rickettsia that the seropositive rodent had been infected with. In order to identify the species of rickettsia, DNA was extracted from the spleen of the seropositive rodent as described above. PCR was conducted by using primers R1 and R2, which are common to spotted fever and typhus group rickettsiae, as well as primers Rj5 and Rj10, which are specific to *R. japonica* [6, 9]. However, DNA of rickettsia was not detected. We also examined spleens of seronegative rodents, but no DNA of rickettsia was detected. In Japan, several species of tick-borne spotted fever group rickettsiae such as *R. japonica* [11, 19], *R. helvetica* [8, 14] and *R. tamurae* [7] have been found. However, ticks are less frequently parasitic on rodents of the genus *Rattus* in the urban area of Osaka City. Therefore, there is a possibility that the rodent had been infected with flea-borne spotted fever group rickettsia, *R. felis*, which causes febrile illness in humans [16, 17]. *R. felis*-infected fleas have been recovered from various mammals including rodents [1]. Further study is needed to identify the species of rickettsia distributed in the study area.

This study revealed that rodents in an urban area of Osaka City, Japan had been infected with *L. interrogans* and spotted fever

group rickettsiae. The number of patients with leptospirosis in Osaka Prefecture in the period from 2006 to 2016 was reported to be 10, but there have probably been many undiagnosed cases. In addition, there is a possibility that unidentified spotted fever group rickettsiae may cause disease in humans. It is important to control rodents, conduct an extensive survey and provide information to residents and physicians.

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