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NOTE

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Prevalence of murine astrovirus in laboratory animal facilities in Japan

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ABSTRACT. To investigate the prevalence of murine astrovirus (MuAstV) in mice in laboratory animal facilities in Japan, a polymerase chain reaction (PCR) test targeting the RNA-dependent RNA polymerase (RdRP) gene was performed on the cecum contents of 1,212 mice (1,183 immunocompetent mice and 29 immunodeficient mice) from 226 facilities. The results showed that 118 (52.2%) of the 226 facilities were positive for MuAstV. Out of the 1,212 mice, 424 (35.0%) were positive. No gross lesions were observed in any of the mice examined. A phylogenetic analysis for 15 selected strains revealed that 13 strains formed one cluster, while two were genetically distant from that cluster. These results suggest that multiple strains are prevalent in laboratory mice in Japan.

KEY WORDS: laboratory mice, murine astrovirus, prevalence

An astrovirus is a non-enveloped, positive-sense, single stranded RNA virus that sometime causes gastrointestinal disease in mammals and poultry [4]. Astroviruses were first discovered in 1975 from fecal samples derived from a child with diarrhea by using electron microscopy [1]. Astroviruses are taxonomically divided into two groups: Mamastroviruses (MAstVs) that infect mammals and Avastroviruses (AAstVs) that infect birds [4]. Murine astrovirus (MuAstV)-like particle was first discovered in the gut contents of nude mice using electron microscopy when collective diarrhea occurred in a colony [5]. Although MuAstV has not been successfully cultured, the entire genome has recently been sequenced [7]. There are a few previous reports of the prevalence of MuAstV in mice in laboratory animal facilities [6, 8], but there is still a shortage of data to comprehensively understand the epidemic. We surveyed the prevalence of MuAstV in mice in laboratory animal facilities in Japan.

In total, 1,212 mice (immunocompetent mice: 1,183; immunodeficient mice: 29) from 226 facilities located in universities, institutes, pharmaceutical companies, and contract research organizations across Japan were surveyed. Mice were sent to the Central Institute for Experimental Animals (CIEA) for microbiological monitoring from October to December, 2016. Mice and samples from commercial breeders or other contract testing laboratories were not included in this study. Mice were euthanized by exsanguination from the axillary artery and vein under isoflurane anesthesia. Necropsy was performed on all mice and the cecum was collected. Cecum samples were stored at −80°C until the test were performed. The mice were also tested for routine microbiological monitoring tests after necropsy in CIEA, which included the following items: *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, and *Salmonella* spp. by culture; *Clostridium piliforme*, ectromelia virus, lymphocytic choriomeningitis virus, mouse hepatitis virus (MHV), *M. pulmonis*, and Sendai virus by serology except in immunodeficient mice; intestinal protozoa, pinworms (*Aspiculuris* sp., *Syphacia* sp.), and ectoparasites by microscopic observation; and *Helicobacter hepaticus* and *Helicobacter bilis* by polymerase chain reaction (PCR) test. The investigation protocol was based on that used in previous studies [3]. This protocol was approved by the CIEA Institutional Animal Care and Use Committee (approval number: 15068A).

RNA was extracted from cecum samples using RNAiso (Takara Bio Inc., Kusatsu, Japan). MuAstV was screened using the PCR test with previously reported primers, MuAstV-BF (5′ GAATTTGACTGGACACGCTTTGA 3′) and MuAstV-BR (5′ GGTTTAACCCACATGCCAAA 3′), which targeted RNA-dependent RNA polymerase (RdRP) gene. The described PCR product size of 328 bp in the cited document [6] is probably incorrect, and the amplified sequence was estimated to be 419 bp from the

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basic local alignment search tool (BLAST) search result of the corresponding primers. A reverse transcription (RT)-PCR was performed using the Prime Script One Step RT-PCR Kit Ver.2 (Takara Bio Inc.). The amplification program comprised 50°C for 30 min, 94°C for 2 min, and followed by 45 cycles of 94°C for 30 sec, 58°C for 30 sec (decreasing incrementally by 0.2°C per cycle), and 72°C for 1 min. The PCR product was analyzed by electrophoresis. All PCR positive samples of MuAstV were confirmed by testing the PCR products through direct sequencing and comparison with the data available in a public database (GenBank) by the BLAST search.

Fifteen samples were selected to disperse the BLAST search results. Sequence data of the 15 selected samples were deposited to the DDBJ/EMBL/GenBank database. The 15 selected sample data and other astrovirus data sequences in GenBank/EMBL/DDBJ were aligned using the Bio Edit Sequence Alignment Editor Ver. 7.2. The phylogenetic tree was constructed from a total of 389 bp of the RdRP gene sequence data for the selected samples and the other databank data using the neighbor-joining method. The branching pattern was tested with 1,000 bootstrap replications. MEGA-X 10.1.7 software was used for distance estimation.

PCR results for the 1,212 mice from 226 facilities showed that 424 mice (35.0%) from 118 facilities (52.2%) were positive for MuAstV (Table 1). No gross lesions were observed in either the immunocompetent or immunodeficient mice. For the immunocompetent mice, 413 of the 1,183 samples tested positive (34.9%), while in the immunodeficient mice 11 of the 29 samples tested positive (37.9%). Based on a χ^2 test, the two groups were found to be not significantly different in terms of separation ($P>0.05$). There was no significant difference in the percentage of mice that was positive for infections between the immunocompetent and immunodeficient mice ($P>0.05$).

The results of the microbiological monitoring test of the 1,212 mice showed that the serum and culture tests were all negative for pathogenic gastrointestinal bacteria and viruses. Microscopic examination results of the MuAstV-positive 424 mice confirmed the presence of nonpathogenic gastrointestinal protozoa in 27 samples (6.4%); *Aspiculuris tetraptera* was detected in four samples (0.9%), and *Syphacia obvelata* in two samples (0.5%). Microscopic examination results of the MuAstV-negative 788 mice confirmed the presence of nonpathogenic gastrointestinal protozoa in 34 samples (4.3%), *Aspiculuris tetraptera* was detected in one sample (0.1%), and *Syphacia obvelata* was detected in three samples (0.4%). Based on PCR test results *H. hepaticus* was detected in six samples (1.4%) of the MuAstV-positive 424 mice and six samples (0.8%) in the MuAstV-negative 788 mice (Table 2).

The results of the BLAST analysis for RdRP gene in 118 MuAstV-positive samples from 118 facilities revealed that 93 samples showed 92.8% to 98.7% similarity with BSRI1 (Accession No. KC609001), which accounted for 78.8% of the total. Eleven samples showed 93.1% to 97.7% similarity with STL1 (Accession No. JX544743), accounting for 9.3% of the total, and one sample showed 97.4% similarity to Y (Accession No. KX683863), accounting for 0.9% of the total. Six samples showed the same percentage of similarity to BSRI1 and STL1 (92.1% to 96.1%), accounting for 5.1% of the total. Five samples showed the same percentage of similarity to Y and STL1 (93.1% to 95.7%), accounting for 4.2% of the total, and two samples showed the same percentage of similarity to Y and BSRI1 (92.8% to 93.1%), accounting for 1.7% of the total (Table 3). The obtained sequences that showed similarity to multiple strains by the BLAST search is shown in Fig. 1. Phylogenetic tree analysis revealed that of the selected strains, 13 strains dispersed into two clusters and two strains were genetically distant (Fig. 2).

Hayashimoto *et al.* (2013) reported that the most common virus in laboratory mice in Japan was murine norovirus (MNV), followed by mouse hepatitis virus, with positive rates of 11.9% and 0.68%, respectively [3]. In the current study, we found that the percentage of individual mouse that were positive for MuAstV was 35.0%, while 52.2% of the facilities were positive (Table 1). These results suggested that the percentage of MuAstV-positive mice was relatively higher compared to other common murine viruses in mice in Japan. This result was presumed to be due in part to the fact that MuAstV has not been tested as an item of microbiological monitoring until now.

It is thought that this finding reflects the actual situation throughout the year since the laboratory mice is usually bred in a well-managed breeding room with temperature and humidity conditions controlled according to the SOP of each facility, without being affected by seasonal changes.

There was no significant difference in the percentage of MuAstV-positive mice between immunocompetent and immunodeficient mice in the present study. Previous studies have shown that adaptive immunity is involved in the control of MuAstV in mice [10]. Further studies are needed to investigate the difference in MuAstV-positive rates between immunocompetent and immunodeficient mice.

Among the MuAstV-positive mice (424) examined in the present study, 6.4% (27) were also positive for intestinal protozoa which was included in the routine testing at our center. However, in MuAstV negative mice (788), 4.3% (34) were positive for intestinal protozoa. MuAstV-positive mice also had a higher positive rate of other microorganisms than MuAstV-negative mice (Table 2). These intestinal protozoa are indicators of the microbiological quality of the animal facility or the animal itself, and it is known

Table 1. Percentage of murine astrovirus positive mice in animal laboratory facilities in Japan

		Sample	Positive	Positive rate (%)
Mice	Immunocompetent	1,183	413	34.9
	Immunodeficient	29	11	37.9
	Total	1,212	424	35.0
Facilities		226	118	52.2

Table 2. Microbiological monitoring test resuts of murine astrovirus positive and negative mice

Method	Items	Numbers of positive samples (n=424)	Numbers of negative samples (n=788)
Culture tests	*Citrobacter rodentium*	0	0
	Corynebacterium kutscheri	0	0
	Mycoplasma pulmonis	0	0
	Salmonella spp.	0	0
Serology	*Clostridium piliforme*	0	0
	Mycoplasma pulmonis	0	0
	Sendai virus	0	0
	Ectromelia virus	0	0
	LCM virus	0	0
	Mouse hepatitis virus	0	0
Microscopy	Intestinal protozoa	27 (6.4%)	34 (4.3%)
	Amoeba	12 (2.8%)	16 (2.0%)
	Chilomastix sp.	4 (0.9%)	2 (0.3%)
	Giardia sp.	0	0
	Octomitus sp.	13 (3.1%)	14 (1.8%)
	Spironucleus sp.	0	0
	Trichomonad	4 (0.9%)	4 (0.5%)
	Ectoparasite	0	0
	Pinworm	6 (1.4%)	4 (0.5%)
	Aspiculuris sp.	4 (0.9%)	1 (0.1%)
	Syphacia sp.	2 (0.5%)	3 (0.4%)
PCR	*Helicobacter bilis*	0	0
	Helicobacter hepaticus	6 (1.4%)	6 (0.8%)

Table 3. Basic local alignment search tool (BLAST) search results of 118 samples from 118 murine astrovirus positive facilities

Most similar strain[a)]	Similarity %	Number of strains
BSRI1	98.03–98.68	19
	97.03–97.87	50
	96.05–96.71	22
	95.63	1
	92.80	1
STL1	97.04–97.70	2
	96.05–96.71	3
	95.39	3
	94.41	1
	93.09	2
Y	97.37	1
BSRI1 / STL1[b)]	96.05	1
	92.11–92.88	5
Y / BSRI1[c)]	93.11	1
	92.76	1
Y / STL1[d)]	95.39–95.72	2
	94.74–94.75	2
	93.09	1

a) BSRI1 (Accession No. KC609001), STL1 (Accession No. JX544743), Y (Accession No. KX683863), b) Same percentage of similarity BSRI1 and STL1, c) Same percentage of similarity Y and BSRI1, d) Same percentage of similarity Y and STL1.

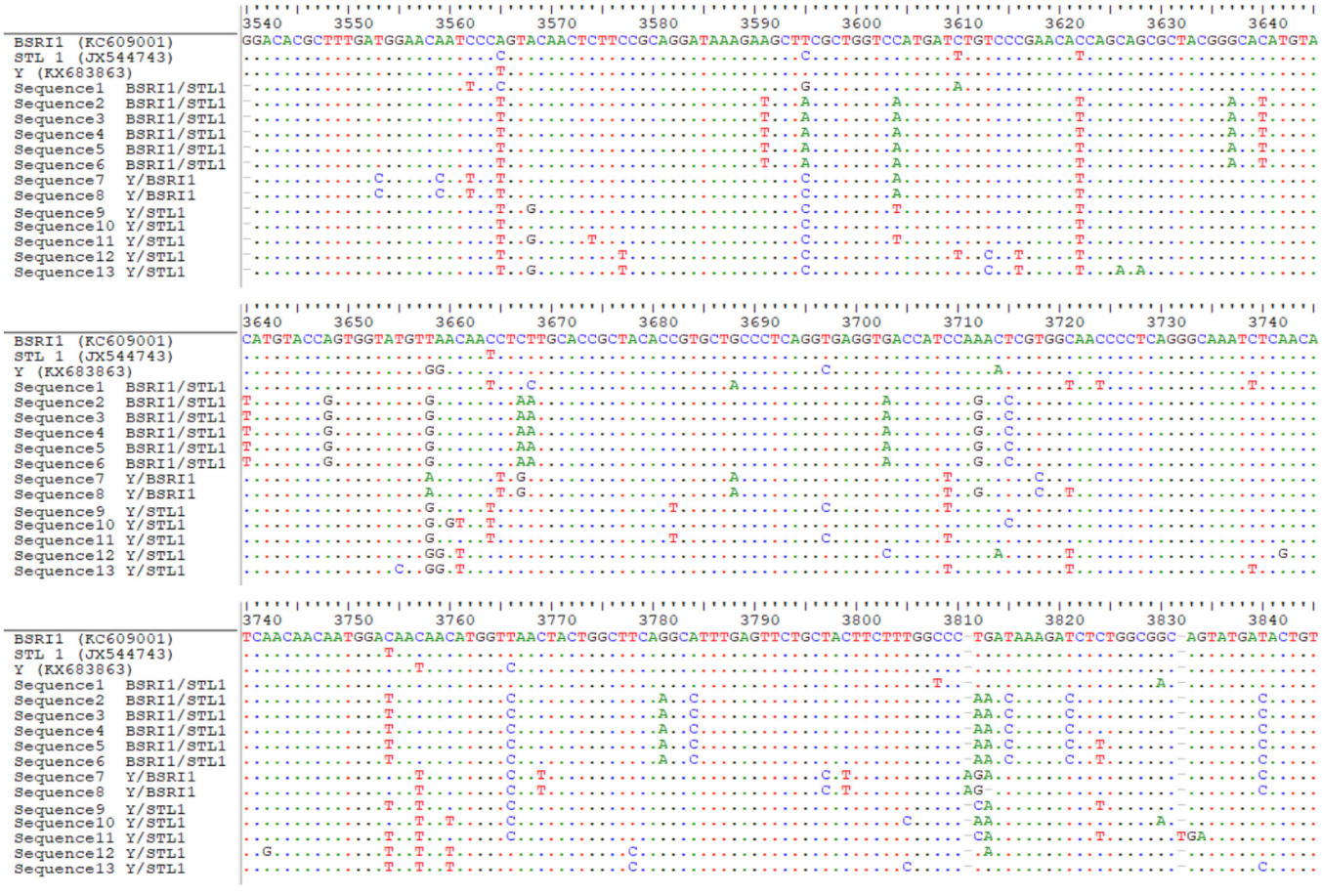

Fig. 1. The obtained sequences that showed similarity to multiple strains by the basic local alignment search tool (BLAST) search.

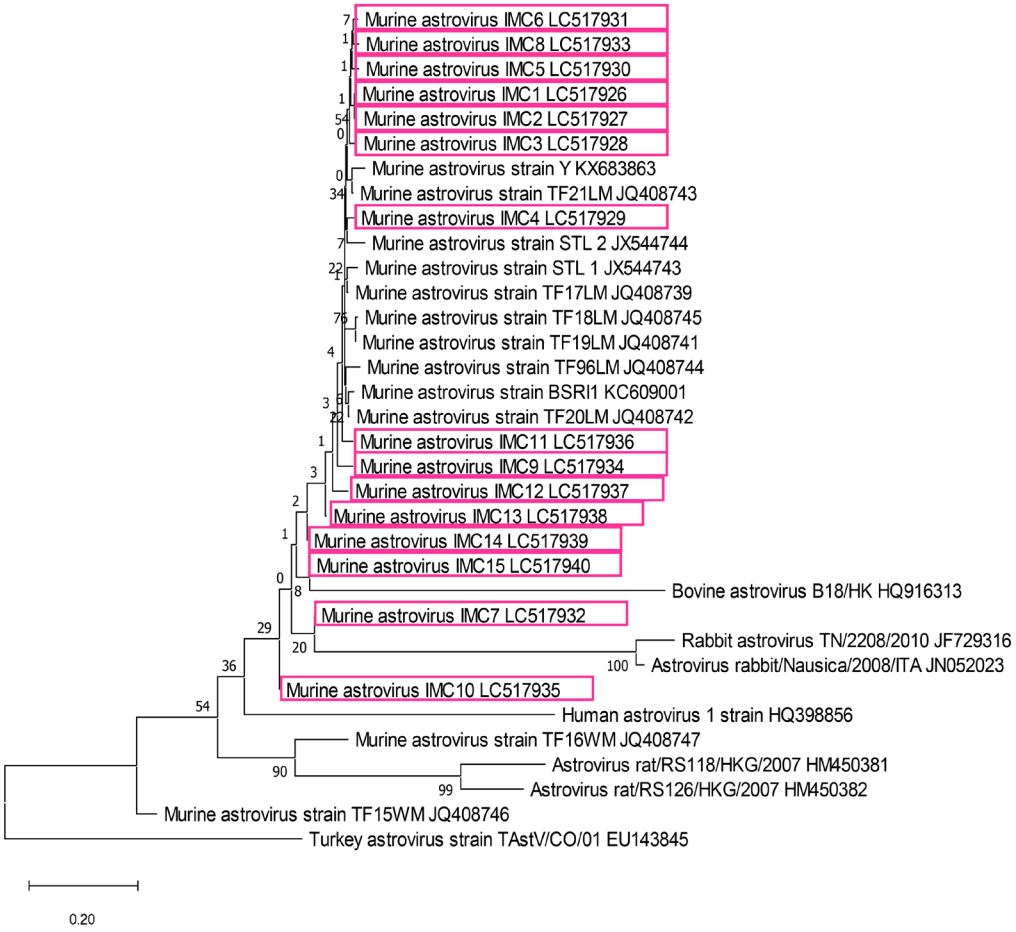

Fig. 2. Phylogenetic analysis of the RNA-dependent RNA polymerase (RdRP) nucleotide gene sequence of murine astrovirus (MuAstV) in laboratory mice from Japan (IMC1-IMC15), along with the MuAstV sequences from the Genbank database.

that intestinal protozoa positive facilities tend to have relatively higher rates of infection by other microorganisms in mice [9]. No information was available regarding the microbiological quality of mice examined in this study, but the MuAstV-positive mice may have relatively poor microbiological profile.

The RdRP gene analysis of 118 strains derived from different facilities showed a high similarity to various strains, including BSRI1 and STL1 from public databases (Table 3). Although the results are limited to the RdRP gene alone, these results suggested that the prevalence of different MuAstV strains in mice in Japan.

Although the occasional detection of MuAstV in laboratory animal facilities in Japan has been reported previously [6], in this report, we increased the number of facilities and samples, and included other microbial infections and individual MuAstV strains analysis as parts of this survey to elucidate the current MuAstV-positive status in Japan.

No gross lesions were observed in the MuAstV-positive mice in this study, including both immunocompetent and immunodeficient mice. Compton *et al.* also reported no gross lesion in MuAstV-positive mice [2]. To further investigate the pathogenicity of MuAstV, experimental infection studies using various immunodeficient mice of varying microbiological grades may be necessary.

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