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Microbiological survey of mice (*Mus musculus*) purchased from commercial pet shops in Kanagawa and Tokyo, Japan

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Abstract: Information regarding the prevalence of infectious agents in mice in pet shops in Japan is scarce. This information is particularly useful for minimizing the risk of potential transmission of infections to laboratory mice. Therefore, we surveyed infectious agents in mice from pet shops in Kanagawa and Tokyo, Japan. The survey was conducted in 28 mice from 5 pet shops to screen for 47 items (17 viruses, 22 bacteria and fungi, 10 parasites) using culture tests, serology, PCR, and microscopy. The most common viral agent detected was murine norovirus (17 mice; 60.7%), followed by Theiler's murine encephalomyelitis virus (13 mice; 46.4%), and mouse hepatitis virus (12 mice; 42.8%). The most common agent amongst the bacteria and fungi was *Pasteurella pneumotropica* (10 mice; 35.7%), followed by *Helicobacter ganmani* and *Pneumocystis murina* (8 mice; 28.5%, for both). *Tritrichomonas muris* was the most common parasite (19 mice; 67.8%), followed by *Spironucleus muris* (13 mice; 46.4%), *Aspiculuris tetraptera*, and *Syphacia obvelata* (8 mice each; 28.5%). Remarkably, a zoonotic agent, *Hymenolepis nana*, was found in 7 mice (25%). Given these results, we suggest that the workers in laboratory animal facilities should recognize again the potential risks of mice outside of the laboratory animal facilities as an infectious source, and avoid keeping mice as pets or as feed for carnivorous reptiles as much as possible for risk management.

Key words: Infectious disease, Mice, Pet shop, Zoonosis

Introduction

Microbiological quality control of laboratory mice and rats are performed routinely to maintain high reproducibility in results of experiments utilizing laboratory animals, and to ensure safety of workers in animal facilities. Therefore, quarantining of rats and mice procured from other facilities, and microbiological monitoring is performed under various programs to ensure that the microbiological status of laboratory mice and rats remains largely unaffected.

The causes of infection of laboratory mice in animal facilities include direct contact from invading feral rodents and personnel who keep rodents as a pet and/or food for carnivorous reptiles. Roble *et al.* reported a high positive rate of various pathogens such as mouse hepatitis virus and mouse parvovirus in 18 mice derived from six pet stores in New York City, USA [13]. Dammann *et al.* also reported high positive rate of various pathogens such as *Helicobacter* species and Mouse parvovirus in 28 mice derived from six pet shops in North Rhine-Westphalia and Brandenburg, Germany and suggested such mice may play a role as a source of infection [4].

In contrast, the information on the prevalence of infec-

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Table 1. The pet shohps and mice tested in this survey

	Pet shops				
	A	B	C	D	E
Number of mice tested[a)]	6	6	4	6	6
Number of Male/ Female	6/ 0	4/ 2	2/ 2	2/ 4	3/ 3
Body weight range (g)	21.4-44.0	15.3-21.2	12.0-16.2	15.4-18.2	17.8-31.3
Location	Kanagawa	Kanagawa	Kanagawa	Kanagawa	Tokyo

a) The mice tested were of unknown genetic back ground, age, and health status.

tious agents in mice in pet shops in Japan is scarce. To minimize the risk of infection to laboratory mice, it is important to identify and characterize potential sources of infection. Therefore, we surveyed for infectious agents in mice procured from pet shops in Kanagawa and Tokyo, Japan.

Materials and Methods

Animals

Animal use was approved by the Central Institute for Experimental Animals' Institutional Animal Care and Use Committee.

Information on pet shops and mice tested is shown in Table 1. Mice (17 male; 11 female) of unknown genetic background, age, and health status were purchased from 5 pet shops (4 in Kanagawa, Japan, and 1 in Tokyo, Japan). The mice selected from each pet shop had been housed together in the same cage. Mice were transported in sealed, passive-ventilated containers from each pet shop to the ICLAS Monitoring Center, Central Institute for Experimental Animals. Mice were sacrificed immediately by exsanguination from the axillary artery and vein under isoflurane anesthesia, and blood was collected.

Autopsy

The major organs of all mice tested were examined macroscopically for the existence of gross lesions.

Serology and PCR test

The microorganisms being screened for, as well as the test used for identification are indicated in Table 2. Serologic diagnosis was performed by enzyme-linked immunosorbent assays (ELISA) and/or indirect immunofluorescence assays (IFA).

All antigen plates were prepared in our center except for 5 items (HantaV, MHV, Mp, Tyzzer, and SV) that have a commercially available ELISA kit, and LCMV. HantaV, MHV, Mp, Tyzzer and SV were tested using commercially available ELISA kits, MONILISA (Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan), according to manufacturer's instructions. The IFA plates of LCMV and HantaV were obtained from Nagasaki University and Hokkaido University, respectively. The ELISA and IFA procedures were done as described previously [5, 9]. PCR testing was performed using previously reported primers [2, 5–10, 14]. Spleen samples were used to detect DNA or RNA from LDHV, MPV, and MVM. Cecum samples were used to detect MNV and all *Helicobacter* species, and lung samples were used for detecting *Pneumocystis murina*. PCR-based detection was carried out as described previously [5]. The *Helicobacter* genus (except for *H. hepaticus* and *H. bilis*) was detected using *Helicobacter* genus–specific PCR and identified by the direct sequencing of PCR products using PCR primers [7].

Parasitology

Parasite identification was carried out using light microscopy. Wet smears of cecal and duodenal contents were examined immediately after sacrifice for intestinal protozoa and helminths. Rectal samples were dissected in saline in a petri dish and were examined for *Aspiculuris tetraptera*. Scotch tape tests were carried out to detect ectoparasites (pelt) and *Syphacia obvelata* (perianal area).

Microbiology

Bacterial isolation was performed using non-selective and selective agar media according to previously reported procedures [9]. Bacterial colonies suspected to contain any of the targeted microbes were harvested and organisms were identified using commercially available biochemical test kits (ID test series: HN-20 for *P. pneumotropica*, EB-20 for *Klebsiella pneumoniae* and *K. oxytoca*, SP-18 for *Staphylococcus. aureus*; Nissui Pharmaceutical). A pinch of hair from the back of each mouse was used to inoculate Potato dextrose agar (Eiken

Table 2. Test panel and results

Items (Category)[a)]	Screening test	Confirmation test	Pet shop A/ 6[b)]	Pet shop B/ 6	Pet shop C/4	Pet shop D/ 6	Pet shop E/ 6	Prevarence (%)
Ectromelia virus (B)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Hantavirus (A)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Lactate dehydrogenase elevating virus (C)	PCR	None	Neg.	Neg.	Neg.	Neg.	Neg.	0
Lymphocytic choriomeningitis virus (A)	IFA	None	Neg.	Neg.	Neg.	Neg.	Neg.	0
Minute virus of mice (C)	PCR	None	Neg.	Neg.	Neg.	Neg.	Neg.	0
Mouse adenovirus K87 (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	4	14.2
Mouse adenovirus FL (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Mouse cytomegalovirus (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Mouse hepatitis virus (B)	ELISA	IFA	6	Neg.	Neg.	Neg.	6	42.8
Mouse parvovirus (C)	PCR	None	3	Neg.	Neg.	Neg.	5	28.5
Mouse polyomavirus (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Murine norovirus (C)	PCR	None	5	Neg.	Neg.	6	6	60.7
Mouse rotavirus (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Pneumonia virus of mice (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Reovirus type 3 (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Sendai virus (B)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Theiler's murine encephalomyelitis virus (C)	ELISA	IFA	6	Neg.	1	5	1	46.4
Citrobacter rodentium (C)	Culture	BC[c)]	Neg.	Neg.	Neg.	Neg.	Neg.	0
Cilia-Associated Respiratory bacillus (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Clostridium piliforme (C)	ELISA	IFA	Neg.	Neg.	Neg.	Neg.	Neg.	0
Corynebacterium kutscheri (C)	Culture	BC	Neg.	Neg.	Neg.	Neg.	Neg.	0
Helicobacter hepaticus (C)	PCR	None	2	Neg.	Neg.	Neg.	Neg.	7.1
Helicobacter bilis (C)	PCR	None	Neg.	Neg.	Neg.	Neg.	Neg.	0
Helicobacter fennelliae (N)	PCR	Seq[d)]	4	Neg.	Neg.	Neg.	Neg.	14.2
Helicobacter ganmani (N)	PCR	Seq	Neg.	6	Neg.	2	Neg.	28.5
Helicobacter marmotae (N)	PCR	Seq	1	Neg.	Neg.	Neg.	Neg.	3.5
Helicobacter rodentium (N)	PCR	Seq	Neg.	Neg.	Neg.	Neg.	2	7.1
Helicobacter sp. MIT 01-6451 (N)	PCR	Seq	1	Neg.	Neg.	3	2	21.4
Klebsiella oxytoca (D)	Culture	BC	Neg.	Neg.	Neg.	Neg.	1	3.5
Klebsiella pneumoniae (D)	Culture	BC	Neg.	Neg.	1	Neg.	3	14.2
Mycoplasma pulmonis (B)	ELISA	IFA	5	Neg.	Neg.	Neg.	Neg.	17.8
Pasteurella pneumotropica (D)	Culture	BC	1	3	3	3	Neg.	35.7
Pseudomonas aeruginosa (D)	Culture	BC	Neg.	Neg.	Neg.	Neg.	Neg.	0
Salmonella spp. (A)	Culture	BC	Neg.	Neg.	Neg.	Neg.	Neg.	0
Staphylococcus aureus (D)	Culture	BC	1	Neg.	Neg.	Neg.	Neg.	3.5
Streptococcus pneumoniae (C)	Culture	BC	Neg.	Neg.	Neg.	Neg.	Neg.	0
β-haemolytic *Streptococcus* (D)	Culture	BC	Neg.	Neg.	Neg.	Neg.	Neg.	0
Dermatophytes (A)	Culture	Microscopy	Neg.	Neg.	Neg.	Neg.	Neg.	0
Pneumocystis murina (B)	PCR	None	6	Neg.	1	1	Neg.	28.5
Aspiculuris tetraptera (C)	Microscopy	None	3	Neg.	Neg.	1	4	28.5
Syphacia obvelata (E)	Microscopy	None	5	Neg.	Neg.	Neg.	3	28.5
Helminths (eggs)[e)] (N)	Microscopy	None	4	Neg.	Neg.	Neg.	3	25
Chilomastix bettencourti (E)	Microscopy	None	Neg.	Neg.	Neg.	Neg.	Neg.	0
Entamoeba muris (E)	Microscopy	None	4	Neg.	Neg.	Neg.	1	17.8
Giardia muris (C)	Microscopy	None	1	Neg.	3	2	Neg.	21.4
Octomitus pulcher (E)	Microscopy	None	1	Neg.	Neg.	Neg.	4	17.8
Spironucleus muris (C)	Microscopy	None	4	Neg.	3	Neg.	6	46.4
Tritrichomonas muris (E)	Microscopy	None	6	Neg.	2	6	5	67.8
Ectoparasites (mite body and/or eggs) (E)[f)]	Microscopy	None	2	3	2	Neg.	Neg.	25

a) The microbiological category in the ICLAS Monitoring Center, Central Institute for Experimental Animals. Category A: pathogens that migh infect humans, Category B: pathogens fatal to animals, Category C: pathogens not fatal, but can cause disease in animals and affect their physiological functions, Category D: opportunistic pathogens, Category E: indicators of the microbiological status of an animal colony, N: The category was not set up fpr these agents, The category of *Pneumocysitis murina* is only for immunodeficeint mice, b) Numbers of mice tested, c) Biochemical tests using commercially available test kits, d) Direct sequencing of PCR products using PCR primers, e) The eggs were identified as *Hymenolepis nana* by their morphology, f) The mites were identified as *Myobia muscli* by their morphology. Mite bodies were detected in 2 mice in pet shop A and 1 mouse in pet shop B.

Chemical) and incubated at 25°C for 14 days under aerobic conditions to detect Dermatophytes. The agar media were assessed for the presence of suspected fungal colonies after incubation.

Results

Autopsy

No gross lesions were observed on the major organs of the 28 mice tested.

Serology and PCR tests

Results of serology and PCR tests are indicated in Table 2. Serological diagnoses revealed the presence of antibodies to GDVII (13 mice), MHV (12 mice), *Mycoplasma pulmonis* (5 mice), and MAV K87 (4 mice). Six mice derived from pet shop B showed negative results to all serologic items tested. PCR tests revealed the presence of DNA or RNA from several *Helicobacter* species [21 mice; 2 mice for *H. hepaticus*, 4 mice for *H. fennelliae* (2 mice were co-infected with *H. hepaticus*), 8 mice for *H. ganmani*, one mouse for *H. marmotate*, 2 mice for *H. rodentium,* 6 mice for *Helicobacter* spp. MIT 01–6451], MNV (17 mice), MPV (8 mice), and *Pneumocystis murina* (8 mice). The direct sequencing of PCR products of the *Helicobacter* genus–specific PCR revealed over 99% similarity in 280 bp with the data in GenBank having the following accession nos.; GQ867167 (*H. fennelliae*), AY56183.1 (*H. ganmani*), GU902716 (*H. marmotae*), AY631957.1 (*H. rodentium*), EF373968.1 (*Helicobacter* spp. MIT 01–6451).

Parasitology

The results of microscopic examinartions for parasites are shown in Table 2. Direct examination of duodenal and cecal contents revealed eggs of helminths (7 mice), and various intestinal protozoa as follows; *Tritrichomonas muris* (19 mice), *Spironucleus muris* (13 mice), *Giardia muris* (6 mice), *Octomitus pulcher* (5 mice), *Entamoeba muris* (5 mice). The helminth eggs were identified as *Hymenolepis nana* by their morphology [3]. Direct examination of rectal samples revealed *A. tetraptera* (8 mice). Postmortem microscopic examination of the pelts revealed *Myobia musculi* (7 mice). Among these mice, eggs were detected in 4 mice only. Scotch tape tests on the perianal areas detected eggs of *Syphacia obvelata* in 8 mice.

Microbiology

The results of the microbiological analyses are indicated in Table 2. *Pasteurella pneumotropica* was isolated from tracheal and conjunctive swabs of 10 mice derived from 4 pet shops. *K. pneumoniae* was isolated from cecal contents of 4 mice, and *K. oxytoca* was isolated from the cecal content of one mouse. Other agents including dermatophytes were not detected in any of the mice tested.

Discussion

The results of this survey indicated the prevalence of various infectious agents in mice in pet shops in Kanagawa and Tokyo, Japan. Pet shops A and E specialized in selling reptiles, and the mice were sold as a feed for carnivorous reptiles. Conversely, pet shops B, C, and D were shops that sold small mammals and birds as pets, and the mice in these shops were suspected be sold as pets. The number of positive items in the mice from pet shops A and E was relatively higher (21 and 16 items, respectively) than that detected in the mice from pet shops B, C, and D (3, 8, and 9 items, respectively). Furthermore, the number of mice that tested positive for these items was markedly higher in the mice from pet shops A and E than in those from pet shops B, C, and D (Table 2). These results suggest that mice sold as feed for carnivorous reptiles may be more likely to be infected with pathogenic organisms than those sold as pets.

Remarkably, *Hymenolepis nana*, which is a zoonotic pathogen, was observed in the mice derived from pet shops A and E [15]. Although our survey is limited and further investigation might be necessary, these results suggested that the mice used as feed for carnivorous reptiles have a potential to be a source of zoonotic parasites such as *Hymenolepis nana*.

In the past, two comprehensive microbiological surveys in mice derived from pet shops have been performed in USA and Germany, and similar trends were observed in these two studies. Dammann *et al.* surveyed 28 mice derived from 6 pet shops in Germany. In their results, the most common viral agent in mice from pet shops was MPV (25 mice; 89.3%), followed by MHV (23 mice; 82.7%), and MVM (12 mice; 42.9%) [4]. Roble *et al.* surveyed 18 mice derived from 6 pet shops in New York City, USA and identified the most common viral agents as MHV (18 mice; 100%), MPV (14 mice; 80%), and MVM (10 mice; 60%) [13]. These results showed rela-

tively high positive rates of MPV in mice along with high prevalence in pet shops tested (all 6 pet shops tested were positive in Germany, and 5 out of 6 pet shops tested were positive in USA). On the other hand, the positive rate of MPV remained 28.5% in our results. Furthermore, the prevalence of MPV was limited to just 2 out of 5 pet shops tested. A similar trend was provided by the results of test for MHV in this study (2 pet shops were positive for MHV in this study, while 5, and 6 (of 6, for both) pet shops were positive in the study in Germany and USA , respectively). These results suggested that the microbiological quality of mice was greatly different in every pet shop in Japan.

In the results of our recent survey for more than 14,000 laboratory mice [9], the most common bacterial agents were *Staphylococcus aureus* (positive facilities/ facilities tested was 18.8%), and *Pasteurella pneumotropica* (5.3%), *H. hepaticus* (3.1%). The most common virus was MNV (11.9%) followed by MHV (0.6%). The most common parasites were *Entamoeba muris* (8.4%), *Octomitus intestinalis* (6.9%), and *Tritrichomonas muris* (4.9%). Among these agents, the prevalence of *Pasteurella pneumotropica*, *H. hepaticus*, MNV, MHV, *E. muris*, *O. intestinalis*, and *T. muris* were common between laboratory mice and mice derived from pet shops, although the degree of prevalence was different. On the other hand, the positive cases of MPV and GDVII were only observed for mice derived from pet shops, with a relatively high prevalence. These results suggested the potential risk of MPV and GDVII infection in laboratory mice arising from mice from pet shops.

Although several serological survey for viral agents in feral mice have been reported [1, 11, 12], the relative prevalence of infectious agents in feral mice and mice from pet shops is unknown. A large-scale comprehensive survey is necessary to examine the relative prevalence of infectious agents in both feral mice and mice sold in pet shops.

While we detected several respiratory and intestinal pathogens, including *M. pulmonis*, *Pneumocystis murina* and *H. hepaticus*, no gross lesions were observed in any of the mice tested. Because these mice were being sold either as pets or as feed for reptiles, it is likely that obviously sick or weak mice were artificially eliminated from the mice colonies. This would explain why each of the tested mice appeared clinically healthy and did not show any gross lesions.

In this survey, we revealed the presence of zoonoses such as *Hymenolepis nana* infection in mice derived from pet shops. We also revealed a potential risk of these mice as a source for infectious diseases peculiar to laboratory mice. From these results, we suggest that the workers in laboratory animal facilities should recognize again the potential risk of mice outside of laboratory animal facilities as an infectious source, and should not keep mice either as a pet or as feed for carnivorous reptiles as much as possible to mitigate the possibility of infection of laboratory mice.

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