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Microbiological survey of Korean mouse facilities from 2014 to 2019

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Abstract: We surveyed mouse microbiological contamination rates by testing rates for common contaminants using serological, culture, and parasitological methods. A total of 21,292 experimentally housed mice from 206 animal facilities, including hospitals, universities, companies, and research institutes, were tested over a 6-year period from 2014 to 2019. The most commonly found contaminants were various species of nonpathogenic protozoa (47.2%). The most common pathogenic bacteria were *Staphylococcus aureus* (21.2%), *Pasteurella pneumotropica* (12.5%), and *Pseudomonas aeruginosa* (5.8%). Mouse hepatitis virus (6.1%) was detected, but no other viral or bacterial pathogens were found. These results establish that the main pathogens that currently contaminate mouse facilities in Korea are opportunistic pathogens and that contamination with important pathogens, such as those in Categories B or C, has decreased.
Key words: microbiological monitoring, mice, mouse hepatitis virus

Introduction

Confirming that animals are pathogen-free through regular and repeated testing of animal colonies for selected pathogens is important for obtaining reproducible and reliable animal test results. Such microbiological monitoring programs are essential to the operation of experimental animal facilities, not only to guarantee quality experimental results but also for general animal welfare and protection against zoonotic disease [1].

We previously reported on microbiological contamination of laboratory animal facilities in Korea from 1999 to 2003. That report revealed that the degree of contamination in conventional animal facilities was severe. Several such facilities were highly contaminated with fatal pathogens, with more than 40% of the facilities contaminated with mouse hepatitis virus (MHV), Sendai virus, and *Mycoplasma* (M) *pulmonis;* zoonotic pathogens, including hantavirus (23%) and lymphocytic choriomeningitis (LCM) virus (15%), were also detected at high levels. Specific pathogen free (SPF) barrier facilities were also contaminated with these pathogens, but contamination rates were lower compared with those in conventional facilities. Pathogenic bacteria were also highly detected in conventional and barrier animal facilities. *Pasteurella* (P) *pneumotropica* and *Pseudomonas* (Ps) *aeruginosa* were identified as the main bacteria contaminants; in particular, more than 50% of conventional and barrier animal facilities were contaminated with *P. pneumotropica*.

The number of SPF barrier facilities is increasing in Korea, and research trends have also resulted in increased use of genetically engineered mutant (GEM) mice in experiments. A previous study reported extensive contamination of GEM mice with several microorganisms, underscoring the increasing risk of pathogen transfer between animal facilities [2, 3].

As research changes, the types of animals used also

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change; accordingly, the types of contaminating microorganisms also change. Thus, identifying major contaminants under current conditions is necessary to create appropriate microbiological monitoring plans for protecting against future pathogen contamination in animal facilities. In this study, we investigated contamination in Korean mouse facilities from 2014 to 2019 and identified the major pathogens in mice during this period.

Materials and Methods

Specimens

We surveyed rodent pathogen contamination in Korean mouse facilities by performing microbiological monitoring, testing 21,291 mice from 206 animal facilities in Korea between 2014 and 2019 (Table 1). Animals from commercial breeders and the Korea Research Institute of Bioscience and Biotechnology were not included in this study. Animals were sacrificed by exsanguination under deep isoflurane anesthesia.

Table 1. Type of institution and number of animals tested in this survey

Type of Institution	No. of institutions	No. of animals
Hospital	16	570
Company	41	2,628
Research institute	35	3,173
University	114	14,921
Total	206	21,292

Selection of test items

Because the microbial items monitored by each animal facility were different, for the sake of accuracy, we limited our comparisons to the same contaminants tested in all animal facilities. A total of 21 test items for mice, chosen based on our previous contamination report [2], were investigated in this study (Table 2).

Serological tests

As the first serological test, we screened samples using enzyme-linked immunosorbent assays (ELISAs). Positive or suspected samples were confirmed by indirect immunofluorescent assay (IFA). Assays for LCM virus and ectromelia virus were performed as per previous reports [2, 4]. Briefly, serum samples were tested after diluting 1:40 for ELISAs and 1:10 for IFAs. LCM virus and ectromelia virus ELISA plates and all IFA slides were created by the International Council for Laboratory Animal Science (ICLAS) Monitoring Center (Kawasaki, Japan). Five items—MHV, *M. pulmonis, Clostridium* (C) *piliforme,* Sendai virus, and hantavirus—were tested using MONILISA IVa and MONILISA HANTA

Table 2. Test items and categories in this survey

Method	Test items	Category[c]
Serological test	Lymphocytic choriomeningitis (LCM) virus	A
	Ectromelia virus	*B*
	Hantavirus	*A*
	Sendai virus	*B*
	Mouse hepatitis virus (MHV)	*B*
	Clostridium piliforme (Tyzzer disease)	C
	Mycoplasma pulmonis	*B*
Culture test	*Corynebacterium*(C) *kutscheri*	*C*
	Pasteurella(P) *pneumotropica*	*D*
	Pseudomonas(Ps) *aeruginosa*	*D*
	Salmonella spp.[a]	*A*
	Staphylococcus(S) aureus	*D*
Microscopy	*Spironucleus*(S) *muris*	*C*
	Chilomastix(C) *bettencourti*	*E*
	Entamoeba spp.	*E*
	Tritrichomonas(T) *muris*	*E*
	Octomitus(O) *intestinalis*	*E*
	Gialdia(G) *muris*	C
	Pinworm[b]	E
	Ectoparasite	C

[a]*Salmonella* spp. in includes *Salmonella typhimurium* and *Salmonella enterica*. [b]Pinworm includes *Syphacia obvelata* and *Aspiculuris tetraptera*. [c]Microbiological categories according to the ICLAS Monitoring Center, Central Institute for Experimental Animals. Category: A, zoonotic and human pathogens carried by animals; B, pathogens fatal to animals; C, pathogens not fatal but capable of causing disease in animals and affecting their physiological functions; D, opportunistic pathogens for animals; and E, indicator of the microbiologic status of an animal.

kits (Wakamoto Pharmaceutical, Tokyo, Japan) according to the manufacturer's protocol.

Culture tests

Pathogenic bacteria were isolated by performing culture tests as described in our previous report [2]. Briefly, mucous membranes of the nasal cavity and trachea were wiped with a moistened fine cotton swab and streaked on Trypticase soy agar containing 5% sheep blood (BD Biosciences, Spark, MD, USA) for isolation of *Corynebacterium* (C) *kutscheri* and *P. pneumotropica*. Intestinal pathogens were isolated by streaking fecal contents onto DHL agar (Merck, Darmstadt, Germany) for *Salmonella* spp. Vogel- Johnson agar (Merck) for *Staphylococcus aureus,* and Pseudomonas-selective agar (Merck) for *Pseudomonas* spp. After incubating for 24 or 48 h at 37°C, candidate bacteria colonies were recovered and characterized biochemically using an Analytical Profile Index (API) series (BioMerieux, Marcy-l'Etoile, France). A MALDI Biotyper (MBT) system and MBT Compass reference library (Bruker Daltonics, Bremen, Germany) were also used for rapid confirmation of bacterial characterizations. To confirm *P. pneumotropica* identification results, we performed polymerase chain reaction (PCR) analyses as described in a previous report [5].

Parasite tests

Parasite tests were performed as described in previous studies [2, 4]. Briefly, *Chilomastix* (C) *bettencourti, Entamoeba* spp., *Giardia* (G) *muris, Spironucleus* (S) *muris, Tritrichomonas* (T) *muris, and Octomitus* (O) *intestinalis* were differentiated by morphological and movement analyses of duodenal and cecal contents using the direct smear method. To detect ectoparasites and *Syphacia* spp. ova, we used the cellophane tape method. *Aspiculuris* (A) *tetraptera* and *Syphacia* spp. were detected by floating colonic and cecal contents in saline in a Petri dish.

Data analysis

In this study, contamination found more than once in a single year at one animal facility was counted as one case of contamination. Type of institution was categorized as hospital, company, research institute, or university based on client information. Only government or international organizations were considered to be research institutes.

Results

Microbiological contamination of Korean mouse facilities based on serology (2014–2019)

In this study, we serologically tested for five viruses and two bacteria, as detailed in Table 2. MHV contamination was found in 6.1% of mouse facilities in Korea, but no other viruses were detected (Fig. 1). MHV contamination rates differed according to the type of institution. Companies (1.6%), hospitals (1.9%), and research institutes (3.6%) had relatively low contamination rates, but the MHV contamination rate at universities (9.4%) was relatively high (Fig. 2). MHV belongs to Category B, which includes pathogens fatal to animals. No other test items, including hantavirus, Sendai virus, LCM virus, ectromelia virus, *M. pulmonis,* and *C. piliforme* (Tyzzer's disease), were detected in this study.

Microbiological contamination of Korean mouse facilities with bacteria (2014–2019)

S. aureus (21.2%) was the most prevalent bacterial agent in mouse facilities (Fig. 1). The rate of contamination with this bacteria did not differ depending on the type of institution (Fig. 2). The second-most common bacterial contaminant was *P. pneumotropica* (12.5%).

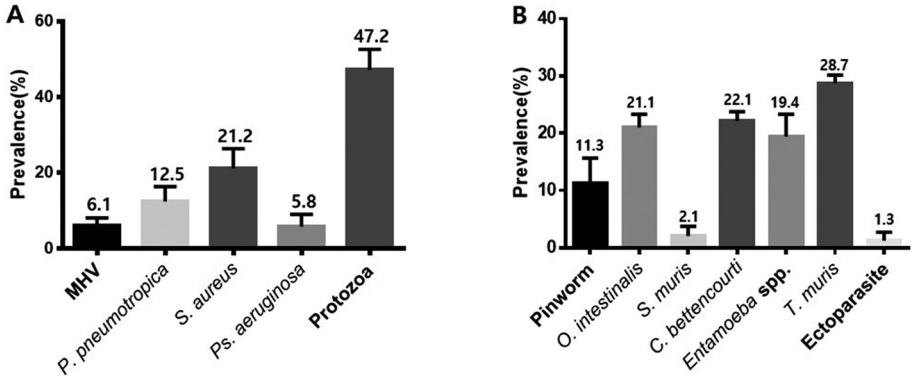

Fig. 1. Microbiological contamination of mouse facilities in Korea. Values shown are prevalence rates for mouse facilities. Several pathogens that were not detected are not shown in the figure A: Major pathogens contamination rates in Korean mouse facilities. B: Parasites contamination rates in Korean mouse facilities.

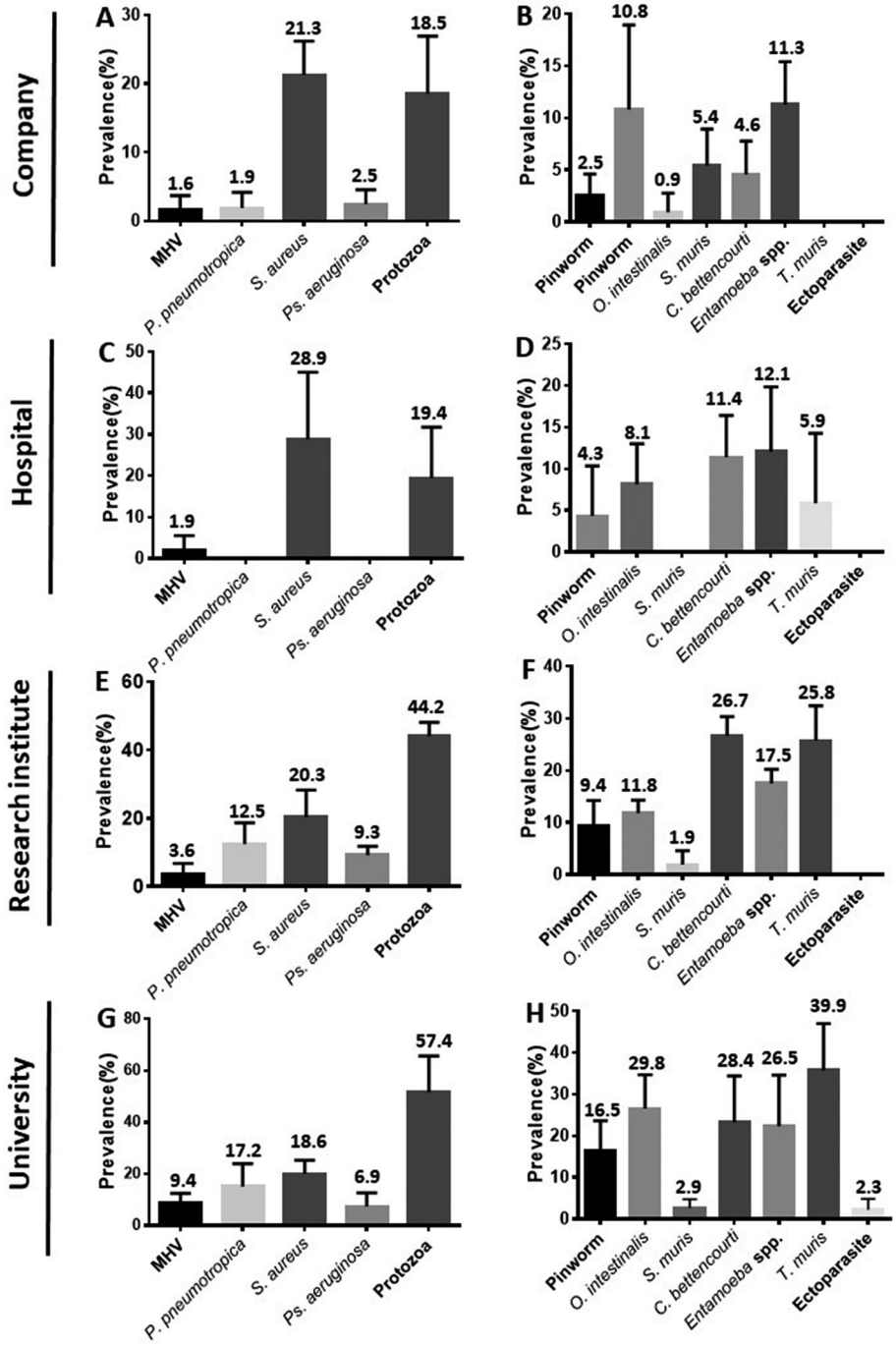

Fig. 2. Microbiological contamination of mouse facilities according to the type of institution. Values shown are prevalence rates for in mouse facilities. Several pathogens that were not detected are not shown in the figure. A and B: Contamination rates for major pathogens and parasites in hospital mouse facilities in Korea. C and D: Contamination rates for major pathogens and parasites in company mouse facilities in Korea. E and F: Contamination rates for major pathogens and parasites in research institute mouse facilities in Korea. G and H: Contamination rates for major pathogens and parasites in university mouse facilities in Korea. Data are presented as means ± SD (error bars).

The infection rate of this pathogen was low in hospitals (0%) and companies (1.9%) but high in universities (17.2%) and research institutes (12.5%). *P. aeruginosa* was also detected, but the total contamination rate was lower (5.8%); by institution type, the contamination rates were 2.5% for companies, 9.3% for research institutes, and 6.9% for universities, with no contamination by these bacteria detected in hospitals (Fig. 2). *Salmonella spp*. and *C. kutscheri* were not detected in this study.

Microbiological contamination of Korean mouse facilities with parasites (2014–2019)

Parasites were the most common contaminants in Korean mouse facilities between 2014 and 2019. The contamination rate of parasites differed depending on the type of institution. Hospital (19.4%) and company (18.5%) facilities showed relatively low contamination with these test items, but the research institute (44.2%) and university (57.4%) facility contamination rates were high (Fig. 2). The major characteristic feature of parasite contamination was the high rates of nonpathogenic parasites (Category E), including *O. intestinalis* (21.1%), *C. bettencourti* (22.1%), *Entamoeba* spp.(19.4%), and *T. muris* (28.7%); however, contamination with pathogenic parasites belonging to Category C, which are not fatal but cause disease and affect physiological functions, was remarkably low (Fig. 2). The total contamination rates for the pathogenic parasite *S. muris* (2.1%) and ectoparasite *Myobia musculi* (1.3%) were lower than those of other contaminants, with the latter found only in universities. The pathogenic parasites, *G. muris* and *A. tetraptera,* were not detected in any institutions.

Discussion

In this study, we characterized the current microbiological status of Korean mouse facilities. Microbiological monitoring is a basic control function for the operation of clean animal facilities and elimination of experimental "noise" in animal testing caused by pathogenic microorganism contamination. Thus, regular and repeated assessment of the contamination status of animal facilities is necessary to ensure a stable, reliable population of experimental animals [1].

In a previous study, we investigated the microbiological contamination status of Korean mouse facilities from 1999 to 2003 [2]. In this study, we investigated the contamination again 15 years later with a selected subset of pathogens, which reflected the very limited spectrum of microorganism contamination found in the facilities in our previous study [2]. The test items for the current study (Table 2) were selected based on the results of the previous study, with certain test items omitted because they were of interest to a limited number of institutions and inclusion of them night have affected the reliability of the results.

Our previous report included results covering 5 years based on 38 institutions housing 501 mice [2]. In the current study, we investigated 206 institutions housing 21,292 mice over 6 years. The main reason for this dramatic increase was the need to keep pace with expanded animal care infrastructures and the goal of improving animal care environments in Korea. Despite this increase in the number of institutions and size of the animal population investigated, contamination by important pathogens, namely those included in Categories A and B (Table 2), was not detected or was decreased compared with our previous study [2].

Contamination of laboratory rodent colonies with hantavirus, an important zoonotic disease with a mortality rate of ~0–15% among humans infected in Korea [6], represents a significant health hazard to individuals working in animal facilities. In our previous study, tests of mouse barrier facilities yielded positive results in 3% of cases [2]. By contrast, we found no zoonotic pathogen contamination in any facilities in the current study. A similar trend was found for the MHV contamination rate, which was 13% in barrier facilities in our previous study [2] but was only 6.1% in our current study. MHV is the most important pathogen affecting laboratory mice. Numerous reports have documented the effects of natural or experimental infection with MHV on host physiology and have demonstrated that MHV infections may seriously compromise the experimental value of these animals [7]. Companies and hospitals showed a low rate of contamination with this virus, whereas research institutes and universities showed high contamination rates (Fig. 2). This phenomenon may be the result of frequent movement and exchange of genetically modified mice among research institutes and universities as part of their research missions. Another possible reason is that these animals are kept in animal facilities for extended periods for breeding purposes. For the same reason, the infection rates of other pathogens, such as *P. pneumotropica* and nonpathogenic protozoa, were higher in these institutions. In particular, universities showed the highest MHV contamination rates and will thus require quarantine operations to reduce the source of this contaminant. On a positive note, other Category A or B contaminants, such as LCM virus, Sendai virus, and ectromelia virus, were not detected in either report. Categories A to E are defined in the footnote for Table 2.

From 2014 to 2019, the most common bacterial contaminant was *S. aureus.* However, the contamination rate for this bacterium was not investigated in 1999 and 2003, so comparisons were not possible. Although *S. aureus* causes opportunistic infection in immunocompetent mice, it has not been tested in many institutes because it does not affect experimental results. However, the recent increase in the use of immunodeficient animals has increased the need for testing for this bacterium. *P. pneumotropica* was identified as the second-most common contaminant in Korea, with 50% of mouse facilities showing contamination in our previous report [2]; the

rate was reduced to 12.5% among all facilities tested in the current study. The total *Ps. aeruginosa* contamination rate did not changed compared with our previous report. As was the case for MHV contamination, infection rates for bacterial contaminants were higher for universities and research institutes and lower for hospitals and companies. These bacteria belong to Category D: opportunistic animal pathogens [3]. Thus, even if animals are contaminated with these bacteria, animal test results should not be affected, provided the animals are in a carrier state.

Protozoa were the most common contaminants in this study, with 47.2% of facilities exhibiting contamination with these pathogens. Unlike the case for other pathogenic test items, the nonpathogenic protozoa contamination rate showed an increase, rather than a decrease, relative to our previous report. A more detailed investigation of each type of parasite revealed that contamination rates for the nonpathogenic protozoa, *O. intestinalis, C. bettencourti, T. muris*, and *Entamoeba* spp. were very high, but those for pathogenic Category C were low. Other regions, including Japan [3], Europe, and America [8], show similar high infection rates for nonpathogenic protozoa.

Cases of ectoparasite contamination were only found in universities, where all ectoparasite contaminations involved *Myobia musculi*. Mouse pinworms, including *Syphacia obvelata* and *A. tetraptera*, commonly infect laboratory rodents. The eggs of these pathogens are very light and can aerosolize, resulting in widespread environmental contamination. Most of the infections are subclinical, with reports of rectal prolapse, intussusception, fecal impaction, poor weight gain, and rough coat in heavily infected rodents [7]. The contamination rate for this test item in Korea in the present study (11.3%) represents a modest decrease compared with our previous report (18%).

Investigating contamination on an animal facility-wide basis, as we have done here, appears to yield higher rates of contamination compared with assessments of contamination rates of individual animals. Considered in this latter context, the contamination rates of the main contaminants, MHV (0.9%), *P. pneumotropica* (1.2%), and *S. aureus* (1.2%) in Korea were lower than those obtained in other surveys of mouse facility contamination. By comparison, the MHV contamination rates in Europe and North America were 1.6% and 3.3%, respectively; the *P. pneumotropica* contamination rate was 13.2% in North America and 4% in Europe; and the detection rates of *S. aureus* were 11.6% in Europe and 6.0% in North America [8]. Although these rates are higher than those in Korea, comparisons are problematic because the data from other regions were reported more than a decade ago and the survey results were not categorized according to institution.

Data obtained from the ICLAS Monitoring Center website (https://www.iclasmonic.jp) reporting results of the 2014–2019 Japanese laboratory mouse facility contamination survey for the above three major pathogens indicate that the average total contamination rates for MHV, *P. pneumotropica,* and *S. aureus* in Japan were 0.6%, 4.6%, and 14.9%, respectively. Compared with the results of this study, the rates for Japan are lower. In particular, the contamination rates for these pathogens in universities and research institutes were higher than those in other institutions, a pattern similar to that observed in the present Korean survey.

In conclusion, we investigated the major sources of contamination in mouse facilities in Korea between 2014 and 2019. The major sources of contamination were identified as *S. aureus, P. pneumotropica*, and nonpathogenic protozoa. Among the important pathogens, the MHV rates were high, but contamination rates for all pathogenic items (with the exception of nonpathogenic protozoa) were lower compared with those obtained in a survey performed 15 years ago. Continuous monitoring and quarantine will be required to prevent contamination by highly infectious and newly discovered pathogens.

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