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Microbial Contaminations of Laboratory Mice and Rats in Taiwan from 2004 to 2007

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Limited data are available on the pathogen status of contemporary rodent colonies in Taiwan. Here we summarized the rodent pathogen diagnostic records of the Taiwan National Laboratory Animal Center during a 4-y period that representing approximately 10% of the rodent colonies in Taiwan. Demand for pathogen diagnostic service increased continuously from 2004 to 2007, with a 20% increase each year. In 2007, more than 20% of the mouse colonies were positive for mouse parvovirus, mouse hepatitis virus, Theiler murine encephalomyelitis virus, and *Mycoplasma pulmonis*, with fewer colonies diagnosed as having infections of pneumonia virus of mice, mouse adenovirus, lymphocytic choriomeningitis virus, and reovirus. Almost 40% of tested rat colonies were positive for *Mycoplasma pulmonis* and rat parvovirus, with fewer colonies containing Kilham rat virus, sialodacryoadenitis virus, pneumonia virus of mice, Sendai virus, and *Syphacia* spp. These data provide a sound overall picture of the health status of mouse and rat colonies in Taiwan.

Abbreviations: IVC, individually ventilated cage; LCMV, lymphocytic choriomeningitis virus; MHV, mouse hepatitis virus; PVM, pneumonia virus of mice; TMEV, Theiler murine encephalomyelitis virus.

The demand for laboratory animal resources in Taiwan has increased dramatically during the past decades due to a boom in biomedical research and biotechnology. In addition, we have seen a huge concurrent increase in investment in building new vivaria and renovating facilities in the governmental, academic, and private sectors. The Chinese Society of Laboratory Animal Science, a scientific member of the International Council for Laboratory Animal Science, was founded in 1989 with an aim to promote the quality and wellbeing of laboratory animals. In addition, the Animal Protection Act, which governs the scientific application of animals, was approved by the Taiwanese government in 1998. Despite these advancements, the foundation of laboratory animal science and medicine in Taiwan is still very feeble in terms of education, training, and management. For example, in 2007, only 4 of the 207 institutions with functioning institutional animal care and use committees were fully AAALAC-accredited.

Routine health monitoring of rodent and rabbit colonies in breeding and experimental units is essential to maintaining high-quality animals.[10,14,20,28,33,34] However, most of the animal facilities in Taiwan lack monitoring programs because of limited resources. The Taiwan National Laboratory Animal Center, a nonprofit organization and an AAALAC-accredited facility, has established a quality-assurance program based on those used by American universities,[9,18,19] the Federation of European Laboratory Animal Science Association,[13,21] the Korea International Council for Laboratory Animal Science,[33] The Biosafety Committee of the Japanese Association of Laboratory Animal Facilities of National Universities,[34] and other recommendations[2,24,31,32] for health monitoring to ensure both the health and genetic integrity of the 250,000 rodents produced there annually. The diagnostic laboratory also has provided customer service as part of a countrywide effort to promote the quality of laboratory animals.

In the present study, we report the health status of rodent facilities that requested diagnostic services from the Taiwan National Laboratory Animal Center during 2004 to 2007. Our data show a steady increase in the demand for diagnostic services during this period and a high rate of pathogen infections in contemporary rodent populations in Taiwan.

Materials and Methods

Animals and sampling. This study was based on records of the Diagnostic Laboratory of National Laboratory Animal Center, a facility that is accredited by the Taiwan Accreditation Foundation. Both live animals and serum samples were accepted for health monitoring services. Live animals were inspected by veterinarians and then underwent a panel of tests including gross and histopathology, parasitology, bacteriology, and serology, as requested by clients. Briefly, veterinarians or certified technicians examined animals for ectoparasites before they were euthanized by CO_2, and serum samples were collected by cardiocentesis and stored at –20 °C. Animals were assessed for the presence of intestinal parasites before organs were removed for bacterial culture. All major organs and tissues collected were processed for histopathologic diagnosis. The experimental protocols were approved by the Institutional Animal Care and Use Committee at the Taiwan National Laboratory Animal Center.

Serology. According to the manufacturer's instructions, serum samples of mice were examined by ELISA (Charles River Laboratories, Wilmington, MA) for antibodies to the following 12 microorganisms: pneumonia virus of mice (PVM), reovirus 3, Sendai virus, lymphocytic choriomeningitis virus (LCMV), hantavirus, Theiler murine encephalomyelitis virus (TMEV),

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mouse adenovirus, minute virus of mice, mouse parvovirus, *Ectromelia*, *Mycoplasma pulmonis,* and mouse hepatitis virus (MHV). Serum samples of rats were examined by ELISA for antibodies to the following 9 microorganisms: PVM, Sendai virus, LCMV, hantavirus, TMEV, Kilham rat virus, rat parvovirus, sialodacryoadenitis virus, and *Mycoplasma pulmonis*. Briefly, 50 µL prediluted serum (diluted 1:60 in milk diluent; KPL, Gaithersburg, MD) was added to each of the appropriate antigen wells and adjacent tissue control wells. The plate was covered and incubated for 40 min at 37 °C. After several washes in washing solution (KPL), 50 µL horseradish peroxidase-conjugated, affinity-purified horse antirodent IgG (Charles River Laboratories) was added to each well. After a 40-min incubation at 37 °C, the plate was washed again; 100 µL 0.4 mM ABTS–2.0 mM H_2O_2 chromogenic substrate (Charles River Laboratories) was added to each well; and the plate was incubated at room temperature for 40 min. Reaction intensities of samples were determined colorimetrically at 405 nm by using an ELISA plate reader (ThermoMax, Molecular Devices, Sunnyvale, CA) and compared with negative and positive control wells. Absorbance values were converted to integer scores (1 to 10) by dividing by 0.13. A result was considered nonspecific and recorded as 'tissue control' when the scores of both sample and control wells were at least 2. Net scores were calculated and interpreted as follows: 0 to 1, negative; 2, borderline; 3 or greater, positive, providing that the score of the tissue control well was < 2 (absorbance < 0.26). All positive samples were verified by using the same ELISA protocol; infection was confirmed based on a second positive result. Indirect immunofluorescent assays were used as alternative confirmatory tests as requested by client institutions. A colony was defined as an animal facility with animals submitted for diagnostic service.

Indirect immunofluorescent assay. Indirect immunofluorescent assays (Charles River Laboratories) of samples and virus-infected and uninfected control cells were performed on Teflon-coated glass microscope slides according to the manufacturer's instructions. Briefly, antigen-coated slides each were blocked with 5 µL milk diluent (KPL). Then 5-µL aliquots of positive control sera, prediluted negative control sera and test sera (1:4 dilution in PBS) were added to the appropriate wells. After incubation at 37 °C in a humidified chamber for 30 min, the slides were washed with PBS and incubated with10 µL antimouse or antirat IgG FITC-labeled conjugate (1:50 dilution) depend on either mice or rats sample sera were tested. The slides were rinsed with PBS and deionized water, mounted, and examined microscopically (Olympus BX51, Shinjku-Ku, Tokyo, Japan).

Bacteriology. The presence of the following pathogens was identified by bacteriologic culture: *Bordetella bronchiseptica*, *Corynebacterium kutscheri, Pasteurella pneumotropica, Staphylococcus aureus*, *Streptococcus pneumoniae* (tracheal loop swab), *Mycoplasma pulmonis* (trachea PBS flush), *Citrobacter rodentium, Salmonella* spp. (cecal contents), and *Klebsiella pneumoniae* (both tracheal loop swab and cecal contents). For immunocompromised mice, the presence of *Pseudomonas aeruginosa* in cecal contents was tested also. Tracheal loop swabs were used to inoculate blood agar, phenylethylene alcohol agar, and MacConkey agar (Creative Microbiologicals, Taiwan) and incubated at 35 ± 2 °C aerobically. *Bordetella bronchiseptica* and *Pasteurella pneumotropica* were identified by using an automated system (API 20NE, bioMérieux, Marcy l'Etoile, France), as were *Klebsiella pneumoniae* (API 20N, bioMérieux), *Corynebacterium kutscheri* (API Coryne system, bioMérieux), and *Pseudomonas aeruginosa* (API 20NE system, bioMérieux), which also was identified through colonial morphology on blood agar, MacConkey agar, and *Pseudomonas* agar (Difco *Pseudomonas* agar F, Becton and Dickinson, Franklin Lakes, NJ). Segments of trachea were cut aseptically and inoculated in PPLO broth for *Mycoplasma pulmonis* isolation [per 100 mL: 2.1 g PPLO broth (Becton Dickinson), 60 mL distilled water, 2 mg phenol red (Sigma–Aldrich, St Louis, MO), 1% D-(+)-glucose (Merck, Germany), 10^5 U penicillin G (Sigma-Aldrich), 0.025% thallium(I) acetate (Sigma–Aldrich), 0.25% Bacto Yeast extract (Becton Dickinson), and 20% heat-inactivated equine serum (Hyclone, Logan, UT)] for 5 to 7 d at 35 ± 2 °C aerobically, followed by subculture on 0.8% PPLO agar (Becton Dickinson) for 5 to 7 d at 35 ± 2 °C in 5% CO_2. Cecal contents were inoculated into GN broth (Hajina, Becton Dickinson) and aerobically incubated at 35 ± 2 °C. *Salmonella* spp. were isolated on Hectoen enteric agar (Creative Microbiologicals) and identified by using an automated system (API 20E system, bioMérieux) and *Salmonella* O antiserum Poly A-I and Vi (Becton Dickinson).

Parasitology. Before euthanasia, the ears, neck, and surrounding pelage of each animal were examined directly under a dissecting microscope for ectoparasites (*Myobia* spp.). Endoparasites (*Aspiculuris tetraptera*) were examined by fecal flotation with saturated NaCl solution for demonstration of distinctive eggs, and intestinal protozoa (*Spironucleus* spp., *Giardia* spp.) by direct examination of wet mounts of duodenal and cecal contents. Briefly, approximately 1 g duodenal or cecal contents was mixed with 2 to 3 mL 0.9% saline. A few drops of the suspension then were examined under light microscopy. The cellophane tape technique was used to detect *Syphacia obvelata* and *Syphacia muris*. Merthiolate iodine formaldehyde was used according to the manufacturer's instructions (Shih-Yung Medical Instrument, Taipei, Taiwan) to examine eggs and protozoa. Briefly, MIF working solution was prepared by mixing 9.4 mL solution I [50 mL distilled water, 5 mL formaldehyde, 40 mL thimerosal (tincture of merthiolate, 1:1000), 1 mL glycerin] with 0.6 mL solution II (100 mL distilled water, 10 g potassium iodide, 5 g iodine crystals) before use. Fresh duodenal or cecal contents (1 g) were mixed with 10 mL MIF working solution and incubated for 12 to 24 h. The interface and bottom layer were collected and mixed with 3 mL ethyl acetate (Bona Pure Chemical, Taipei, Taiwan). After homogenization and centrifugation at 666 × *g* for 2 min, the supernatant was discarded, and the sediment was smeared on glass slides for examination.

Pathologic examination. The lungs, trachea, lymph nodes, heart, liver, spleen, small intestine, stomach, kidneys, urinary bladder, adrenal glands, testis, ovary, thymus, salivary gland, Harderian gland, skin, and brain from animals were fixed in 10% neutral buffered formalin. The tissue samples were processed by routine methods to form paraffin wax-embedded blocks. Sections (6 µm) were stained with hematoxylin and eosin and examined by certified veterinary pathologists for detection of subclinical infections of various pathogens including MHV, *Mycoplasma pulmonis,* or *Pneumocystis* spp, endoparasite, ectoparasite and intestinal protozoa. Immunohistochemical staining with nonbiotin polymerized horseradish-peroxidase (Mouse on Mouse kit, Vector Laboratories, Burlingame, CA) were performed in cases suspected with infections of MHV, *Mycoplasma pulmonis,* or *Pneumocystis murina*.[15,17]

Organisms excluded from SPF mice and rats in the Taiwan National Laboratory Animal Center. Excluded pathogens for SPF mice maintained at the National Laboratory Animal Center are: PVM, reovirus 3, Sendai virus, LCMV, hantavirus, TMEV, mouse adenovirus, minute virus of mice, *Ectromelia*, MHV, *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Clostridium piliforme, Coryne-*

bacterium kutscheri, Salmonella spp., *Myobia musculi*, *Aspiculuris tetraptera*, *Syphacia obvelata*, *Syphacia muris, Rodentolepsis nana and Rodentolepsis diminuta.* SPF rats maintained at the National Laboratory Animal Center are free of the following organisms: PVM, Sendai virus, LCMV, hantavirus, TMEV, Kilham rat virus, sialodacryoadenitis virus, *Mycoplasma pulmonis, Bordetella bronchiseptica*, *Clostridium piliforme, Corynebacterium kutscheri, Salmonella* spp., *Myobia musculi, Aspiculuris tetraptera*, *Syphacia obvelata*, *Syphacia muris, Rodentolepsis nana and Rodentolepsis diminuta.* In addition, *Pneumocystis murina* (mice), *Pneumocystis carinii* (rats), and *Pseudomonas aeruginosa* are monitored only in immunocompromised animals.

Results

Increasing demand for diagnostic services. The number of institutes requesting diagnostic service for rodent pathogens increased 2.6-fold from 2004 to 2007 (Table 1). Most of this increase was due to submissions from government-funded organizations such as research institutions, universities, and hospitals. Despite these substantial increases, these figures represented only approximately 10% of the 200 institutes with animal experimentation in Taiwan. The numbers of both requests submitted and animals tested increased 4- to 5-fold from 2004 to 2007. Among the 3 diagnostic categories, requests for serology (37,575 tests) outnumbered those for parasitology (3911 tests) and bacteriology (3412 tests). In general, more than 50% of customers submitted samples for diagnostic service more than once annually (data not shown).

Prevalent contaminations of rodent facilities. Serologic tests showed that more than 20% of the mouse colonies tested were positive for mouse parvovirus (9 colonies positive of 24 colonies tested), MHV (9 of 26), TMEV (6 of 25), and *Mycoplasma pulmonis* (5 of 24) in 2007 (Table 2). Infection with PVM (2 of 25), mouse adenovirus (1 of 23), LCMV (1 of 23), and reovirus 3 (2 of 24) were also noted. In 2007, mouse parvovirus was the most common infection in mice (112 specimens positive of 961 specimens tested), followed by MHV (45 of 1341), TMEV (20 of 1126), *Mycoplasma pulmonis* (14 of 1258), and PVM (2 of 1253). Among rat colonies, almost 40% were positive for *Mycoplasma pulmonis* (6 colonies positive of 16 colonies tested) and rat parvovirus (3 of 8) in 2007 (Table 3). Infection with Kilham rat virus (5 of 16), sialodacryoadenitis virus (2 of 16), PVM (2 of 16), and Sendai virus (1 of 16) were noted also. Sialodacryoadenitis virus was the most common infection in rats (64 specimens positive of 571 specimens tested), followed by PVM (63 of 573), Kilham rat virus (45 of 488), rat parvovirus (17 of 249), and *Mycoplasma pulmonis* (31 of 573). However, the infection with Sendai virus was low (2 of 571). Among the viral pathogens, hantavirus, Sendai virus, LCMV, and minute virus of mice were not found in mice, and LCMV and hantavirus were not found in rats from 2004 to 2007.

Bacterial infections in the laboratory mouse and rat populations were uncommon except for *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which frequently were isolated from immunocompromised mice (Table 2 and 3). Among parasitic infestations, *Syphacia* spp. were found most frequently in mice and *Syphacia* spp. in rats (Table 2 and 3). However, *Giardia* spp. and *Aspiculuris tetraptera* were not found in either species from 2004 to 2007.

Discussion

Although this study is not a comprehensive survey of the prevalence or incidence of rodent infectious agents, this retrospective analysis of the 4-y diagnostic service data is the first report concerning pathogen contamination in Taiwan. This study reveals an increasing awareness of animal quality among animal users, as shown by the steady increase in demand for diagnostic services from the National Laboratory Animal Center: a 20% increase in 2005, 54% in 2006, and 61% in 2007 compared with the number of requests for services during 2004.

Our data clearly show that the laboratory rodents in Taiwan are contaminated with numerous infectious agents. Specifically, mouse colonies are affected by mouse parvovirus, MHV, TMEV, *Mycoplasma pulmonis*, and PVM, and rat colonies carry sialodacryoadenitis virus, PVM, Kilham rat virus, rat parvovirus, *Mycoplasma pulmonis*, or *Syphacia* spp. This situation is very similar to those in the United States, Europe, and Korea.[3,14,22,25,33,36] High prevalence of MHV,[3,14,22,25,33,36] TMEV,[8,22,25,36] Sendai virus,[3,14,36] minute virus of mice,[14,36] *Mycoplasma pulmonis*,[25] *Entamoeba* spp.,[22] *Hexamastix* spp.,[22] *Trichomonas* spp.,[22,33] reovirus[3,14] PVM,[8,36] and *Pasteurella pneumotropica*[22] in mice colonies and Kilham rat virus,[14,22,36] Toolan H1 virus,[14,22,36] TMEV-GD7,[22,25,36] sialodacryoadenitis virus,[3,14,36] Sendai virus,[3,36] *Clostridium piliforme*,[14,25] *Mycoplasma pulmonis*,[3,14,25,33] PVM,[25,36] *Staphylococcus aureus*,[22] *Entamoeba* spp.,[22] and *Hexamastix* spp.[22] in rat colonies was reported.

Mycoplasma pulmonis infection used to be very common in mouse (35% to 91%) and rat (8% to 78%) colonies in North America in the 1990s,[3,14,33] but its prevalence and incidence has declined since then. Similarly, Sendai virus also has become rare in American and European rodent facilities recently.[4,22,25] However, some agents, including parvoviruses of rats and mice, TMEV, MHV, mouse rotavirus, and pinworms (*Syphacia* spp.) remain threats to research facilities in the United States[4,22] and Taiwan alike.

Due to the likely inclusion of false-positive results, the ELISA data presented here may overestimate the actual situation in Taiwan. According to the manufacturer, the false-positive rates of the ELISAs used in the current study are approximately 0.5% to 1%. In addition, institutions may intentionally submit additional specimens in the face of previous positive results of infectious agents, resulting in an upward skewing of prevalence for particular pathogens. Despite its low sensitivity, the specificity of indirect immunofluorescent assay is high,[22] and it remains the primary confirmatory method for ELISA in our monitoring program.

Although pinworms are common in both mouse and rat populations in Taiwan, the infestation is more prevalent in rat colonies than in mouse colonies. The likely reason is that laboratory rats typically are housed in open, unfiltered cages, whereas mice are more likely kept in individually ventilated cage system or filtered-top cages, thus limiting parasite spread between and worm numbers in individual mice.[4] Furthermore, the choice of method for health monitoring is related to the type of infection and the manner in which the infection is spread in the environment. Therefore, in some cases direct contact and soiled bedding are the best sampling method to detect infection, whereas in other cases exhaust air is better for diagnosis.[5,6,26]

Discrepancy between the histopathologic results and cellophane tape test on pinworms (*Syphacia* spp.) were noted in this study, perhaps due to the higher detection rate of histologic examination (75%) than the tape test (38%).[8]

In the current study examination of cecal samples failed to identify any infection by *Aspiculuris tetraptera*. Examining both the cecum and colon may increase the likelihood of detecting the agent.[8] In addition, the low prevalence of bacterial agents was probably due to the small sample size; therefore the true

Table 1. Increased demand for rodent pathogen diagnostic service in Taiwan from 2004 to 2007

	No. of clients (% of total no. with IACUC)[a]	No. of requests	No. of animals tested	No. of tests		
				Serology	Bacteriology	Parasitology
2004	12 (6.1)	50	464	2534	198	218
2005	18 (9.6)	76	927	7476	355	401
2006	24 (11.9)	130	1442	10859	1595	1864
2007	31 (15.0)	264	1914	16706	1264	1428

[a]Number of institutes with IACUCs: 198 (2004), 201 (2005), 202 (2006), 207 (2007).[7]

Table 2. Infectious agents in mouse colonies in Taiwan from 2004 to 2007.

	No. of positive colonies / No. of colonies tested (%)				No. of positive samples/ No. of samples tested (%)			
Agent	2004	2005	2006	2007	2004	2005	2006	2007
MHV	2/9 (22.2)	3/17 (17.7)	8/21 (38.1)	9/26 (34.6)	32/297 (10.8)	87/725 (12.0)	76/962 (7.9)	45/1341 (3.4)
Mycoplasma pulmonis	3/9 (33.3)	1/16 (6.3)	4/21 (19.1)	5/24 (20.8)	4/318 (1.3)	3/695 (0.4)	19/972 (2.0)	14/1258 (1.1)
PVM	1/8 (12.5)	1/16 (6.3)	1/20 (5.0)	2/25 (8.0)	3/293 (1.0)	5/695 (0.7)	16/936 (1.7)	2/1253 (0.2)
TMEV	0/8(0)	3/16 (18.8)	6/21 (28.6)	6/25 (24.0)	0/100 (0)	36/522 (6.9)	28/790 (3.5)	20/1126 (1.8)
Mouse parvovirus	No data available	0/2 (0)	3/4 (75.0)	9/24 (37.5)	No data available	0/54 (0)	14/96 (14.6)	112/961 (11.7)
Mouse adenovirus	0/6 (0)	0/11 (0)	2/19 (10.5)	1/23 (4.4)	0/94 (0)	0/390 (0)	2/566 (0.4)	9/1070 (0.84)
Hantavirus	0/1 (0)	0/8 (0)	0/16 (0)	0/20 (0)	0/10 (0)	0/374 (0)	0/304 (0)	0/444 (0)
Reovirus 3	0/6 (0)	0/13 (0)	0/19 (0)	2/24 (8.3)	0/92 (0)	0/480 (0)	0/743 (0)	2/1095 (0.2)
Sendai virus	0/9 (0)	0/15 (0)	0/21 (0)	0/23 (0)	0/295 (0)	0/717 (0)	0/978 (0)	0/1267 (0)
LCMV	0/8 (0)	0/15 (0)	0/20 (0)	1/23 (4.4)	0/293 (0)	0/714 (0)	0/939 (0)	1/1123 (0.1)
Minute virus of mice	0/7 (0)	0/14 (0)	0/20 (0)	0/24 (0)	0/98 (0)	0/485 (0)	0/811 (0)	0/1221 (0)
Ectromelia	0/6 (0)	0/11 (0)	1/19 (5.3)	0/22 (0)	0/92 (0)	0/385 (0)	1/467 (0.2)	0/556 (0)
Syphacia spp.	0/3 (0)	2/6 (33.3)	3/12 (25.0)	0/14 (0)	0/43 (0)	5/47 (10.6)	14/257 (5.5)	0/211 (0)
Spironucleus spp.	0/2 (0)	0/6 (0)	1/10 (10.0)	0/12 (0)	0/33 (0)	0/50 (0)	1/137 (0.7)	0/190 (0)
Ectoparasites	0/2 (0)	0/4 (0)	0/11 (0)	0/10 (0)	0/33 (0)	0/42 (0)	0/231 (0)	0/116 (0)
Giardia spp.	0/2 (0)	0/6 (0)	0/10 (0)	0/12 (0)	0/33 (0)	0/50 (0)	0/237 (0)	0/190 (0)
Aspiculuris tetraptera	0/3 (0)	0/5 (0)	0/10 (0)	0/14 (0)	0/43 (0)	0/46 (0)	0/235 (0)	0/211 (0)
Bordetella bronchiseptica	0/1 (0)	0/5 (0)	0/11 (0)	0/11 (0)	0/10 (0)	0/53 (0)	0/252 (0)	0/173 (0)
Corynebacterium kutscheri	0/4 (0)	0/8 (0)	0/13 (0)	0/10 (0)	0/41 (0)	0/66 (0)	0/258 (0)	0/169 (0)
Mycoplasma pulmonis (culture)	No data available	0/1 (0)	0/6 (0)	No data available	No data available	0/34 (0)	0/200 (0)	No data available
Pseudomonas aeruginosa	1/3 (33.3)	2/6 (33.3)	2/11 (18.2)	0/7 (0)	1/42 (2.4)	2/24 (8.3)	5/195 (2.6)	0/151 (0)
Salmonella spp.	0/4 (0)	0/8 (0)	0/14 (0)	0/11 (0)	0/44 (0)	0/65 (0)	0/281 (0)	0/188 (0)
Streptococcus spp.	0/1 (0)	No data available	0/5 (0)	0/2 (0)	0/2 (0)	No data available	0/41 (0)	0/70 (0)
Citrobacter rodentium	No data available	0/2 (0)	0/5 (0)	0/4 (0)	No data available	0/7 (0)	0/45 (0)	0/82 (0)
Pasteurella pneumotropica	0/1 (0)	No data available	0/1 (0)	1/3 (33.3)	0/2 (0)	No data available	0/3 (0)	2/67 (3)
Staphylococcus aureus	1/1 (100)	2/2 (100)	1/4 (25.0)	0/3 (0)	12/22 (54.6)	3/6 (50.0)	1/27 (3.7)	0/78 (0)
Klebsiella pneumoniae	0/1 (0)	No data available	0/2 (0)	0/2 (0)	0/5 (0)	No data available	0/19 (0)	0/61 (0)

prevalence of these bacterial infections is likely greater than that reported here.

Although this 4-y monitoring data may provide an accurate overall picture of the health status of mouse and rat colonies in Taiwan, the test profiles included only 27 agents for mice and 24 agents for rats. This panel should be expanded to cover newly emerging murine pathogens, such as murine norovirus,[11,12,22] rat respiratory virus,[22] rat minute virus,[22] and *Helicobacter* spp.,[2,22,23,30,31,35] which have become new threats to contemporary rodent colonies. A preliminary study using PCR has indicated that the infection rate of MNV and *Helicobacter* spp. is very high in Taiwan (both 100%) and that *Pneumocystis murina* was present in 50% of the animal facilities with immunocompromised mice.[27] In addition, immunohistochemical techniques using murine sera containing specific antibody have been developed for detecting MHV and *Mycoplasma pulmonis* infections in immunodeficient mice.[17] In addition, we recently have detected both *Pneumocystis murina* and MNV[16] in immunodeficient mice by using a novel nonbiotin polymerized horseradish-peroxidase method.[15]

Our results are not unexpected. Among the 1 million laboratory rodents used annually during 2004 to 2007 in Taiwan,[7] only 25% were produced under SPF conditions by various research animal centers.[7] Furthermore, many of the end-users

Table 3. Infectious agents in rat colonies from 2004 to 2007.

	No. of positive colonies / No. of colonies tested (%)				No. of samples positive / No. of samples tested (%)			
Agent	2004	2005	2006	2007	2004	2005	2006	2007
Mycoplasma pulmonis	4/9 (44.4)	3/11 (27.3)	4/13 (30.8)	6/16 (37.5)	52/130 (40.0)	56/187 (30.0)	26/395 (6.6)	31/573 (5.4)
Sialodacryoadenitis virus	3/8 (37.5)	2/11 (18.2)	1/13 (7.7)	2/16 (12.5)	3/118 (2.5)	3/187 (1.6)	1/382 (0.3)	64/571 (11.2)
PVM	2/8 (25.0)	1/11 (9.1)	1/13 (7.7)	2/16 (12.5)	31/120 (25.8)	12/187 (6.4)	63/386 (16.3)	63/573 (11)
Kilham rat virus	0/6 (0)	1/10 (10.0)	1/9 (11.1)	5/16 (31.3)	0/16 (0)	3/135 (2.2)	4/208 (1.9)	45/488 (9.2)
TMEV	0/6 (0)	0/8 (0)	1/8 (12.8)	0/13 (0)	0/18 (0)	0/71 (0)	2/234 (0.9)	0/452 (0)
Sendai	0/8 (0)	0/11 (0)	1/13 (7.7)	1/16 (6.3)	0/120 (0)	0/187 (0)	10/384 (2.6)	2/571 (0.4)
LCMV	0/6 (0)	0/10 (0)	0/10 (0)	0/12 (0)	0/18 (0)	0/139 (0)	0/201 (0)	0/382 (0)
Hantavirus	0/4 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/12 (0)	0/126 (0)	0/131 (0)	0/132 (0)
Rat parvovirus	No data available	0/1 (0)	1/2 (50.0)	3/8 (37.5)	No data available	0/27 (0)	2/65 (3.1)	17/249 (6.8)
Ectoparasite	No data available	0/3 (0)	0/5 (0)	0/7 (0)	No data available	0/15 (0)	0/64 (0)	0/44 (0)
Spironucleus spp.	No data available	0/4 (0)	1/6 (16.7)	1/9 (11.1)	No data available	0/20 (0)	1/78 (1.3)	3/56(5.3)
Giardia spp.	No data available	0/4 (0)	0/6 (0)	0/9 (0)	No data available	0/20 (0)	0/78 (0)	0/56 (0)
Aspiculuris tetraptera	No data available	0/4 (0)	0/6 (0)	0/9 (0)	No data available	0/20 (0)	0/70 (0)	0/54 (0)
Syphacia spp.	No data available	2/4 (50.0)	3/6 (50.0)	3/9 (33.3)	No data available	4/20 (20.0)	21/70 (30.0)	6/54 (11.1)
Bordetella bronchiseptica	0/1 (0)	0/4 (0)	0/5 (0)	0/9 (0)	0/2 (0)	0/18 (0)	0/75 (0)	0/57 (0)
Corynebacterium kutscheri	0/2 (0)	0/5 (0)	0/6 (0)	1/8 (12.5)	0/14 (0)	0/28 (0)	0/77 (0)	1/57 (1.9)
Mycoplasma pulmonis (culture)	No data available	No data available	1/1 (100)	No data available	No data available	No data available	7/39 (18.0)	No data available
Pseudomonas aeruginosa	0/1 (0)	0/3 (0)	0/2 (0)	0/5 (0)	0/2 (0)	0/13 (0)	0/4 (0)	0/37 (0)
Salmonella spp.	0/2 (0)	0/5 (0)	0/7 (0)	0/8 (0)	0/12 (0)	0/28 (0)	0/87 (0)	0/37 (0)
Streptococcus pneumoniae	No data available	No data available	0/1 (0)	0/1 (0)	No data available	No data available	0/16 (0)	0/2 (0)
Citrobacter rodentium	No data available	0/2 (0)	0/1 (0)	0/2 (0)	No data available	0/12 (0)	0/2 (0)	0/13 (0)
Pasteurella pneumotropica	No data available	No data available	No data available	0/1 (0)	No data available	No data available	No data available	0/2 (0)
Staphylococcus aureus	No data available	No data available	0/1 (0)	1/2 (50.0)	No data available	No data available	0/2 (0)	2/15 (13.3)
Klebsiella pneumoniae	No data available	No data available	No data available	0/1 (0)	No data available	No data available	No data available	0/5 (0)

who obtained SPF animals did not maintain them in the same conditions in which they were produced, due to the lack of animal housing systems that effectively excluded adventitious agents. Lack of good colony management practice was noted also. In addition, most of the animal facilities did not have a diagnostic laboratory with designated personnel or an inhouse health quality assurance program to ensure the health and genetic integrity of animals.[7]

Overall, we recommend that facilities should comply with the following practices to ensure animal quality. First, obtain animals from reputable venders who maintain their animals with good management practice and regular testing. Second, quarantine all newly arrived animals. Third, maintain animals according to standard operating procedures implemented by adequately trained personnel. Fourth, upgrade housing conditions to cage systems that exclude undesired pathogens. Fifth, regularly test colonies and research animals to determine the disease status. Facilities with infection(s) must take actions to eliminate the contamination to ensure the quality of research data from animal models. Although depopulation and repopulation may be impractical for contaminated strains or stocks, assisted reproductive technologies such as caesarian section and embryo transfer may be the methods of choice for eradicating the contamination. Alternately, neonatal transfer with iodine or chlorine dioxide immersion within 24 to 48 h of birth was effective during efforts to rederive immunocompetent mice that tested positive during monitoring for MHV, MNV, mouse parvovirus, EDIM, TMEV, and *Helicobacter* spp.[1,29]

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