Challenges in Maintaining Microbial Status in Specific Pathogen-Free (SPF) and Conventional Animal Housing

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ABSTRACT

Routine microbiology, virology and parasitology monitoring of rodent colonies in animal facilities is essential for evaluating the health status of animals used in research. Over a five-year period, we examined the presence of selected microbial infections and parasitology contaminations in various types of animal facilities at Tel Aviv University, including specific pathogen-free (SPF), conventional and quarantine facilities. Animal health monitoring followed the Federation of European Laboratory Animal Science Associations (FELASA) recommendations. A total of 955 rodents (mice and rats) were monitored during the study. The most common bacterial strains found in both conventional and SPF units were *Pasteurella spp.*, followed by *Staphylococcus aureus*, *Klebsiella* (*pneumoniae, oxytoca*) and *Pseudomonas aeruginosa*. Other isolated bacteria, not included in FELASA recommended panels, such as *Proteus spp.*, *Enterobacter cloacae and Morganella morganii* were less prevalent. Pinworms and mites were not found in SPF rodents and showed a prevalence of 0.5-8% in the conventional facilities. The rodents housed in the SPF unit had a statistically significant lower prevalence of specific pathogens compared to those in conventional units, emphasizing the critical role of microbiological barriers established by SPF health monitoring standards. This study demonstrates that despite the physical proximity of the SPF and conventional facilities, their distinct microbiological status can be maintained long-term through rigorous health monitoring, strict management and well-designed facility infrastructure.

Keywords: Animal Facility; Health Monitoring; Infectious Diseases; Parasites Rodents

INTRODUCTION

The presence of contaminated pathogens in research animals, even in cases of subclinical microbial outbreaks, may compromise research outcomes, as animals that are sick or stressed do not yield results as reliable as those obtained from healthy and unstressed animals (1). Therefore, ensuring animal health by minimizing microbial variability is vital for research integrity, supporting the reduction in animal use and advancing the ethical principles of the 3Rs: replacement, reduction, and refinement (2).

Health monitoring and continuous diagnosis of infectious pathogens, in experimental research animals and breeding colonies, are essential for assessing the prevalence of infections and for maintaining the sanitary and environmental conditions of the facilities. Environmental and rodent microbiological monitoring programs have been published in different countries (3-6). Institutional Health Monitoring programs and testing laboratories can be accredited by the Federation of European Laboratory Animal Science Associations (FELASA) (7) or by the National Research Council (US) Committee on Infectious Diseases of mice and rats (8).

Classical health surveillance includes serologic tests, bacterial cultures and parasitology examinations. Molecular diagnostics have been developed for precise bacteria and pinworms identification using real-time polymerase chain reaction (PCR), DNA sequencing, 16S ribosomal DNA (rDNA) and 18S ribosomal RNA (rRNA) sequencing. The latest development in the veterinary field is the use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) as a reliable tool for identifying anaerobic bacteria (9-16). This technology enables the rapid identification of these microorganisms using well-characterized isolates in animal facilities. However, gram positive anaerobic cocci remain under-represented in available databases (15). Environmental monitoring of areas in the facilities is also considered to be part of a routine including frequency of sampling, time, duration and sample size per surface area controlled by different techniques as contact and settle plates, swabbing, active monitoring of air volume and ATP-based methods to detect the presence of live or dead organic material (17, 18).

Direct examination for parasites, including pinworms and mites, remains classical in animal facilities. Oxyurina order pinworms, *Syphacia obvelata* and *Aspiculuris tetraptera* are the most common parasites found in laboratory mice, transmitted through the ingestion of embryonated eggs (19-21). Helminths are opportunistic pathogens, and are generally expected at low levels in laboratory mice, rarely causing clinical signs, unless there is a heavy infection. The prevalence of helminths infection is associated by factors such as host age, strain, health status, stocking density and environmental conditions (22). *Syphacia muris* is commonly detected in rats (23, 24).

The two most frequently observed ectoparasites are fur mites, *Myocoptes musculinus* and *Myobia musculi*. While low infestations are typically subclinical, heavy infestations can cause irritation, pruritus, hair loss and scabs (22). In addition to detecting mites through microscopic examination of fur and skin and PCR assays, a metagenome of *Myocoptes musculinus* was derived by sequencing fur plucks of an infected mouse. *Myobia spp*. and *Demodex spp*. are particularly found in immunodeficient or transgenic laboratory mice (25).

Specific pathogen-free (SPF) colonies are integral to modern biomedical research, ensuring the reliability and reproducibility of experimental outcomes by providing a consistent and controlled baseline for experiments. SPF animals are housed in barrier facilities to prevent exposure to pathogens. These facilities include features such as filtered air, sterilized food and bedding, and strict protocols for cleaning and staff access. Entry of new animals or materials is closely monitored, often requiring quarantine and testing. Regular health monitoring and testing are performed to confirm the SPF status of the colony (26).

While research mice are commonly maintained as SPF colonies to ensure they are free of defined infectious agents, many academic and research institutions continue to house laboratory animals in conventional units (11). The specific pathogens in conventional animal facilities compared to SPF units, as well as imported animals in quarantine, have been poorly investigated. In this study we aimed to identify and describe the pathogens in our animal facilities and to compare them across units.

MATERIAL AND METHODS

Study design. This observational study analyzed pathogens identified in rodents housed in various facilities at Tel Aviv University based on comprehensive health monitoring tests during a five-year period (2019-2023). Our center functions according to the FELASA recommendations (7). The study was approved by the Institutional Animal Care and Use Committee of Tel Aviv University (approval number TAU-MD-IL-2307-150-2) (27).

Animals. The animals included in two-month-old sentinel female ICR strain mice (CD-1 outbred) and Sprague-Dawley (SD) rats both purchased from Harlan Laboratories (Jerusalem, Israel), male and female quarantine mice aged 1-3 months, which were C57BL/6-based transgenic mice from various institutes and universities in Israel and other countries. The animals were confined in designated rooms with restricted personal access for four weeks, followed by health monitoring tests and allocation to SPF or conventional units. Only imported quarantined mice, negative for endoparasites and ectoparasites tested by PCR enter the facilities. SPF and conventional sentinel animals were from the same strain, age, sex and originated from the same distributor.

Housing facilities. The animals were housed in pairs in individually ventilated cages provided with filtered and acidified sterile reverse osmose water, sterilized food ad libitum (Irradiated Rodent Diet, Cat. # 1318, Altromin, Lage,

Germany) and animal sterilized bedding (Sani-Chips 7090, Harlan-Teklad, Madison, WI, USA).

The facilities in which the rodents were housed included: 1) an SPF unit (494 sentinel mice), which was built according to practices and guidelines for animal housing and husbandry of the National Institutes of Health.[1] This facility is located at Tel Aviv University. 2) The conventional housing of the Faculty of Medicine (CM, 94 sentinel mice and rats), Faculty of Life Sciences (LS, 38 mice and rats) and the School of Psychology Sciences (PS, 56 mice and rats), which were also located at Tel Aviv University campus. 3) The conventional housing of the School of Zoology (ZOO, 9 sentinel mice), Felsenshtein Medical Research Center (FEL, 36 sentinel mice), Sheba Medical Center (TEL, 10 sentinel mice) and biotechnology companies (Companies, 85 sentinel mice), which were all grouped as "Group 1" (G1) and included 140 rodents altogether. All G1 housing were external facilities to the Tel Aviv University main campus but are under the care of the university's Animal Facility staff. 4) Quarantine facilities (QUA) located at Tel Aviv University's Animal Facility, which included imported animals (133 mice). G1 and QUA were considered at high risk for pathogen contamination.

Health monitoring. Rodents housed in SPF facilities were monitored quarterly, while those in all other facilities were monitored biannually. Pathogens were tested according to FELASA guidelines and recommendations (See Supplementary Data) (7). Post-mortem sample collection was conducted following euthanasia of sentinel animals with carbon dioxide followed by a clinical examination of external and internal organs.

Serology. A total of 20 µl of blood was collected from a facial vein puncture using the HemaTIP Microsampler (Charles River Laboratories, Wilmington, MA, USA). Viruses were identified by serology antibodies using multiplexed fluorometric immunoassays and immunofluorescence assays at Charles River Research Animal Laboratory (Diagnostic Service, Wilmington, MA, USA).

Bacteriology. Standard microbiological procedures were applied under Biosafety Level 2. These procedures included a systematic inoculation of the nasopharynx by a gel swab introduced into the respiratory tract, and collection of fecal specimens from different sources from the rodents' gut duodenum, cecum and colon. The specimens were then plated in differential agar plates (HyLaboratories, Rehovot, Israel) followed by analysis with a series of chromogenic media and biochemical differentiation for enteropathogenic bacteria (HyLaboratories, Rehovot, Israel) (28, 29). FELASA bacterial panels were used according to FELASA recommendations (7). Other isolated bacteria not included in the FELASA recommendations were included in the analyses as output microbiology results followed by selective and differential media. *Pasteurella spp.* (30) identification was confirmed by oxidase strip test (HyLaboratories, Rehovot, Israel). *Helicobacter spp.* was identified by PCR analysis using the gene target 16S rRNA (31) (detailed in Supplementary Data). Bacterial characterization was confirmed by MALDI-TOF (32) by the Authority for Biological and Biomedical Models at the Hebrew University of Jerusalem (Jerusalem, Israel).

Gross pathology. Clinical examination following euthanization of sentinel animals included external and internal inspection to identify abnormalities. Externally, the skin, eyes, ears, teeth and genital area were observed for lesions, discoloration, or deformities. Internally, organs such as the heart, lungs, liver, spleen, pancreas, kidneys, uterus, ovaries and gastrointestinal tract were examined for signs of disease.

Parasitology. Direct exams and PCR tests were applied for parasite screening. Feces from the duodenum, cecal and proximal colon and fur hair tape were collected with forceps, mounted on slides and inspected for pinworms, mites and eggs under a microscope (Nikon TS-2-S-SM, Nikon Instruments Inc., Melville, NY, USA). When pinworms, mites and eggs were observed, they were counted and classified by morphology parameters (33, 34). Fresh feces and environmental cage swabs of quarantined imported animals, were tested by real-time PCR at Charles River Laboratory Research Animal Laboratory (Diagnostic Service, Wilmington, Massachusetts, USA). Detection of pinworms and mites by PCR is described in the Supplementary Data.

Monitoring of environmental microorganisms. Water and working surfaces were monitored for the presence of microorganisms using the Lumitester-ATP System (Kikkoman Biochemifa Company, Tokyo, Japan). The normal range values were 0-10 relative light units (RLU) for

water, up to 200 RLU for smooth and hard surfaces, and up to 500 RLU for fragile surfaces. If the read exceeded 500 RLU, a disinfection protocol with ethanol, Virusolve (Amity International, South Yorkshire, United Kingdom), hydrogen peroxide and Biocide solution (Airsurdis, Robaix, France) was applied until RLU levels reached the normal ranges.

Statistical analysis. Statistical analysis was performed using Excel (Microsoft, Redmond, WA, USA). Mouse and rat data were combined for simplification, as previously analyzed by Albers *et al*. (35).The number and frequency of each pathogen tested were summarized per housing unit and in total. To understand if there is a correlation between the presence of specific pathogens and the husbandry type, the prevalence of identified pathogens was compared by *t*-test between pairs of housing types (SPF versus all conventional facilities [CM, LS and PS]; G1 versus QUA; SPF versus G1; and SPF versus QUA). The samples were not normalized since there was no control group. P-values <0.05 were considered statistically significant.

RESULTS

Pathogen prevalence in animal facility units. A total of 955 mice and rats were tested over the five-year study period (Table 1).

Bacteria. The most commonly isolated bacterial strains using the FELASA panel were *Pasteurella spp*. (22%) and *Staphylococcus aureus* (8%). The prevalence of *Klebsiella pneumoniae* and *Klebsiella oxytoca* was 0.73% and 0.52%, respectively. *Mycoplasma pulmonis*, showed very low prevalence, in 0.52% of tested animals – all of them rat samples from the PS unit. *Mycoplasma genus* was found in only one quarantined mouse, indicating an overall prevalence of 0.1%. Other isolated bacteria not listed in the FELASA recommended panel, such as *Enterobacter cloacae, Morganella morganii*, and *Staphylococcus saprophyticus* showed prevalence rates of 2.2%, 1.47%, and 1.47%, respectively. The prevalence of *Proteus mirabilis* and *Proteus vulgaris* was low at 0.42%. *Helicobacter spp.,* which was tested exclusively in animals from companies, showed a frequency of 11.76% (10/85 rodents) (Table 1).

Viruses In mice, the most prominent pathogens were mouse hepatitis virus (MHV) and Theiler's murine encephalomyelitis virus (TMEV-GDII) with a prevalence of 0.63% and 0.42%, respectively. In rats, the highest prevalence was observed for rat theilovirus (46.81%) and *Pneumocystis carinii* rat respiratory virus (14.89%). Murine norovirus (MNV) was considered positive in all units, since its prevalence was very high in previous findings in our facilities (Table 1).

Gross pathology. During sentinel mice necropsy health monitoring, abnormal signs were observed and considered as gross pathology in 4.61% of cases (Table 1). The range of signs included ovarian cysts, hydrometra, bilateral hemorrhagic ovaries, alopecia, skin lesions, internal hemorrhagic organs or tissues, abscesses and abnormal mass of tissue representing potential incidental tumors, necrotic tumors or blocked ducts. The most common sign was ovarian cysts in sentinel female mice, which could be attributed to the age of the tested females (8-9 weeks old).

Alopecia was found in patches around the face, concentrated in one facial area, whereas the skin was healthy and in a few cases the whiskers or eyelashes might be missing. This "barbering" sign was caused by overgrooming by animal cage mates or the mice themselves, which can represent a compulsive grooming disorder. No medical treatment was applied; however, a benefit was observed when environmental enrichment was increased. Abscesses were rare; however, they were associated with bite wounds.

Parasitology. Among the 955 tested rodents, 26 (2.7%) had pinworms in feces samples, including *Syphacia obvelata* adult specimens, gravid females and eggs. *Syphacia sp*. was identified (33) based on the presence of a muscular oesophagus ending within an oesophageal bulb (Figure 1A, B) and distinctive ellipsoidal eggs. These eggs were found embryonated in uteri and measured 0.120–0.139 mm in length (mean 0.129±0.001 mm). The eggs were asymmetrical with one flattened banana-shaped side, and operculated on the convex side. (Figure 1C, D, E). PCR tests confirmed the presence of nine cases of *Syphacia obvelata* and one case of *Aspiculuris tetraptera* (Table 1). Figure 1F shows a characteristic egg from *Aspiculuris tetraptera* featuring a symmetrical ovoid ellipsoid shape resembling a "football". Pinworms were found in all conventional units but not in animals housed in the SPF unit.

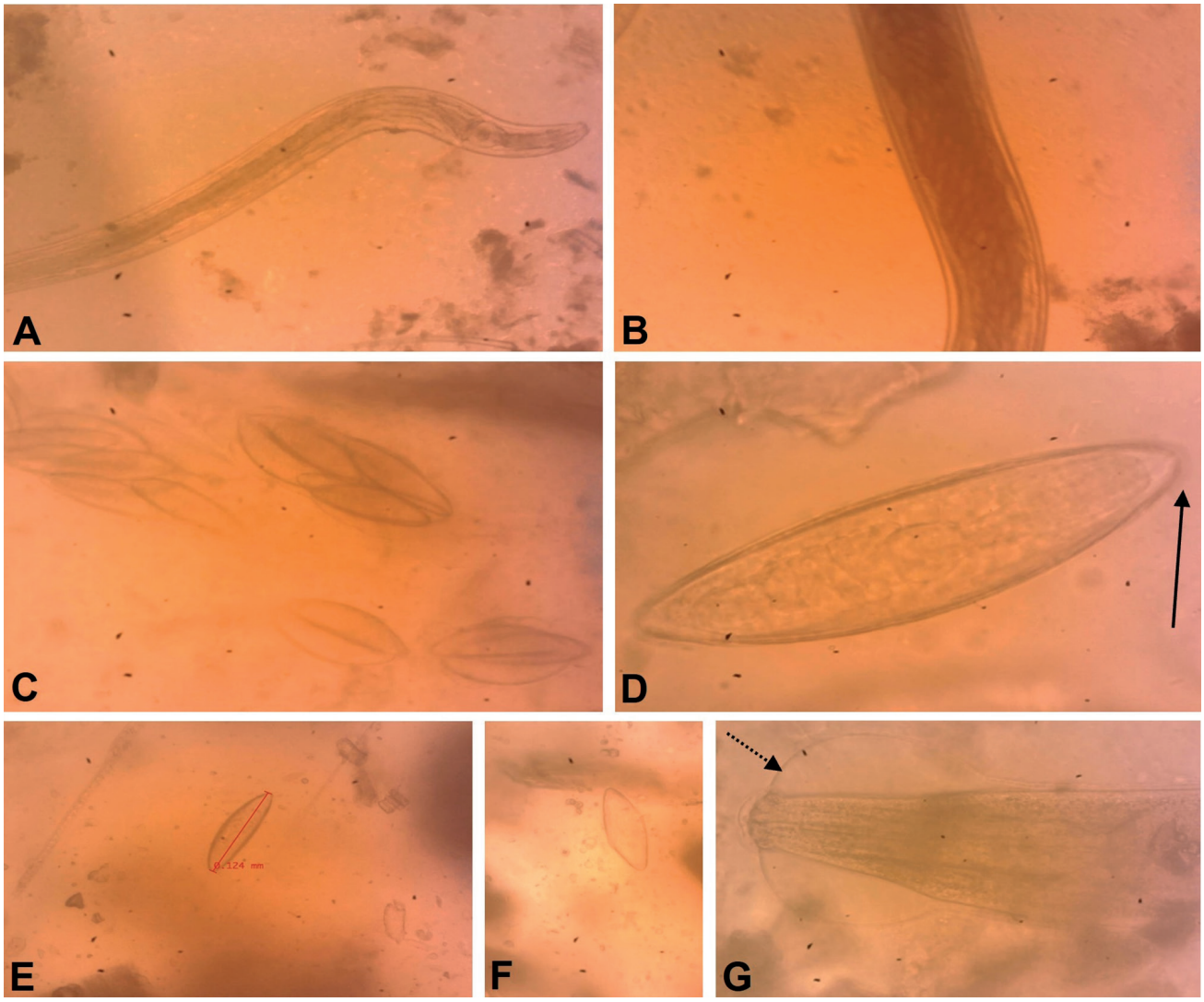

Figure 1. Nematodes identified in rodents housed in conventional facilities images. (**A**) Adult *Syphacia obvelata*, identified by morphology showing a cephalic end with lips followed by pharynx esophagus, bulb and intestine. (**B**) Gravid *Syphacia obvelata* female carrying eggs embryonated in uteri. (**C**) "Banana-shaped" eggs. (**D**) Egg overview with operculum (arrow). (**E**) Egg measurement 0.124 mm length. (**F**) *Aspiculuris tetraptera* with round football-shaped ova. (**G**) Female head halo (dashed arrow). Magnification 40X (A, B); 100X (C, E, F, G); 400X (D).

Ectoparasites (mites) were found in 3 out of 955 cases (0.31%), detected only in two conventional units (LS, ZOO) (Table 1). Figure 2 illustrates a *Myocoptes musculinus* adult (Figure 2A) and Acariformes mites spp. (Figure 2B) according to morphology parameters (36). Treatment was applied in units in which parasites were detected.

Comparison of pathogen prevalence among husbandry types. Comparison of the prevalence of pathogens between SPF and conventional units (Table 2) showed that among the bacteria tested, *Pasteurella spp.* was the most common bacteria, with a prevalence of 22.06% among SPF animals and 18.62% among rodents housed in conventional units, with no statistically significant difference between the two husbandry types. *Staphylococcus aureus* was also a very common pathogen in both SPF and conventional units (7.1% and 9.6%, respectively) with no statistically significant differences between the two husbandry types. *Klebsiella oxytoca* was found in two of 494 mice in SPF (0.41%) but not in animals housed in the conventional units (P=0.04 for the prevalence difference between the units). *Proteus mirabilis* (an opportunistic isolated bacteria not listed in FELASA recommended panel), was also found in one SPF mouse (0.20%) but not in animals housed in the conventional units (P=0.041). Endoparasites

Table 1. Pathogen prevalence by health monitoring program

	Number of positive-tested mice/rats by unit (n)										
	SPF	Conventional facilities								Total	
	N=494	Medicine N=94	Life Sciences N=38	Psychology N=56	Zoology[a] N=9	Felsenshtein[a] N=36	Sheba[a] N=10	Quarantine N=133	Companies[a] N=85	n/N	(%)
Number of health monitoring/year	4	2	2	2	2	2	2	1	1		
Bacteria FELASA panel N=955											
Bordetella bronchiseptica	0	0	0	0	0	0	0	0	0	0/955	(0)
Citrobacter rodentium	0	0	0	0	0	0	0	0	0	0/955	(0)
Corynebacterium kutcheri	0	0	0	0	0	0	0	0	0	0/955	(0)
Klebsiella pneumoniae	0	0	0	0	0	0	0	7	0	7/955	(0.73%)
Klebsiella oxytoca	2	0	0	0	1	0	0	1	1	5/955	(0.52%)
Pasteurella spp.	109	12	7	16	0	7	2	25	31	209/955	(21.88%)
Pseudomonas aeruginosa	0	0	0	0	0	1	1	0	3	5/955	(0.52%)
Salmonella spp.	0	0	0	0	0	0	0	0	0	0/955	(0)
Staphylococcus aureus	35	7	1	10	1	2	1	12	10	79/955	(8.27%)
Streptococci b-haemolytic (not group D)	0	0	0	0	0	0	0	0	0	0/955	(0)
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0/955	(0)
Streptobacillus moniliformis	0	0	0	0	0	0	0	0	0	0/955	(0)
Dermatophytes (skin)	0	0	0	0	0	0	0	0	0	0/955	(0)
Corynebacterium bovis	0	0	0	0	0	0	0	0	0	0/955	(0)
Pneumocystis carinii (Nude lung)	0	0	0	0	0	0	0	0	0	0/955	(0)
Helicobacter spp.[b]	NT	NT	NT	NT	NT	NT	NT	NT	10	10/85	(11.76)
Other isolated bacteria not listed in FELASA recommended panel N=955											
Proteus mirabilis	1	0	0	0	1	0	0	2	0	4/955	(0.42%)
Proteus vulgaris	2	2	0	0	0	0	0	0	0	4/955	(0.42%)
Morganella morganii	0	2	1	1	0	0	0	1	9	14/955	(1.47%)
Staphylococcus saprophyticus	3	0	0	1	0	0	0	8	2	14/955	(1.47%)
Staphylococcus epidermis	0	0	0	0	0	0	0	0	0	0/955	(0)
Providencia rettgeri	0	0	0	0	0	0	0	0	0	0/955	(0)
Bacillus spp.	0	0	0	0	0	0	0	3	0	3/955	(0.31%)
Pseudomonas stutzeri	0	0	0	0	0	0	0	0	0	0/955	(0)
Enterobacter cloacae	8	2	0	1	0	2	0	4	4	21/955	(2.20%)
Enterobacter hormaechei	0	0	0	0	0	0	0	0	1	1/955	(0.10%)
Escherichia Coli "shigella-like"	0	0	0	0	0	0	0	1	0	1/955	(0.10%)
Enterococcus faecalis	0	0	0	0	0	0	0	0	0	0/955	(0)
Serratia marcescens	0	0	0	0	0	0	0	0	0	0/955	(0)
Staphylococcus xylosus	0	0	0	0	0	0	0	0	0	0/955	(0)
Mycoplasma genus	0	0	0	0	0	0	0	1	0	1/955	(0.10%)
Virology/other pathogens FELASA panel N=955 *(cont. on next page)*											
Mouse hepatitis virus (MHV)	0	1	5	0	0	0	0	0	0	6/955	(0.63%)

	Number of positive-tested mice/rats by unit (n)										
	SPF	Conventional facilities								Total	
	N=494	Medicine N=94	Life Sciences N=38	Psychology N=56	Zoology[a] N=9	Felsenshtein[a] N=36	Sheba[a] N=10	Quarantine N=133	Companies[a] N=85	n/N	(%)
Mouse rotavirus (EDIM-ROTA-A)	0	0	0	0	0	0	0	0	0	0/955	(0)
Minute virus of mice (MVM)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse parovirus (MPV)-1,-2,-5	0	0	0	0	0	0	0	0	0	0/955	(0)
Pneumonia virus of mice (PVM)	0	0	0	0	0	0	0	0	0	0/955	(0)
Sendai virus (SEND)	0	0	0	0	0	0	0	0	0	0/955	(0)
Virology/other pathogens FELASA panel N=955											
Theiler's murine encephalomyelitis virus (TMEV) GDVII strain	0	0	0	0	2	2	0	0	0	4/955	(0.42%)
Ectromelia virus (ECTRO)	0	0	0	0	0	0	0	0	0	0/955	(0)
Lymphocytic choriomeningitis virus (LCMV)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse adenovirus type 1,2 (FL-MAV-1, K87-MAV-2)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse cytomegalovirus (MCMV)	0	0	0	0	0	0	0	0	0	0/955	(0)
Reovirus type 3 (REO)	0	0	0	0	0	0	0	0	0	0/955	(0)
Generic parvovirus (NS-1)	0	0	0	0	0	0	0	0	0	0/955	(0)
Murine norovirus (MNV)[c]	+	+	+	+	+	+	+	+	+		
Mycoplasma pulmonis (MPUL)	0	0	0	5	0	0	0	0	0	5/955	(0.52%)
Clostridium piliforme (CPIL)	0	0	0	0	0	0	0	0	0	0/955	(0)
Virology FELASA panel (Rat) N=47											
Rat zoonotic hantaan virus (HANT)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Toolan's H1-rat (H1)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat minute virus (RMV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Kilham's rat virus-parvovirus (KRV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat coronavirus (RCV/SDAV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat theilovirus (RTV)	NT[d]	2	6	10	NT[d]	NT[d]	NT[d]	NT[d]	4	22/47	(46.81%)
Pneumocystis carinii (PCAR, 'RRV')	NT[d]	0	2	9	NT[d]	NT[d]	NT[d]	NT[d]	0	7/47	(14.89%)
Rat cytomegalovirus (RCMV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47 (0)	(14.89%)
Pathology N=955											
Gross pathology	22	2	0	3	1	1	0	2	13	44/955	(4.61%)
Parasitology N=955											
Endoparasites (pinworms)	0	8[e]	6	1	1	1[e]	4	1[f]	4	26/955	(2.72%)
Ectoparasites (mites)	0	0	1	0	2	0	0	0	0	3/955	(0.31%)

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations; NT, not tested

[a] Included in Group 1 (G1)

[b] *Helicobacter spp.* was tested only for rodents from companies' facilities

[c] MNV was not tested in the current study and was considered positive (+) in all cases due to its high prevalence previously tested in our facilities

[d] Rat pathogens were not tested because there were no rats in these housing.

[e] Pinworms were diagnosed as *Syphacia obvelata* by PCR

[f] Pinworms were diagnosed as *Aspiculuris tetraptera* by PCR

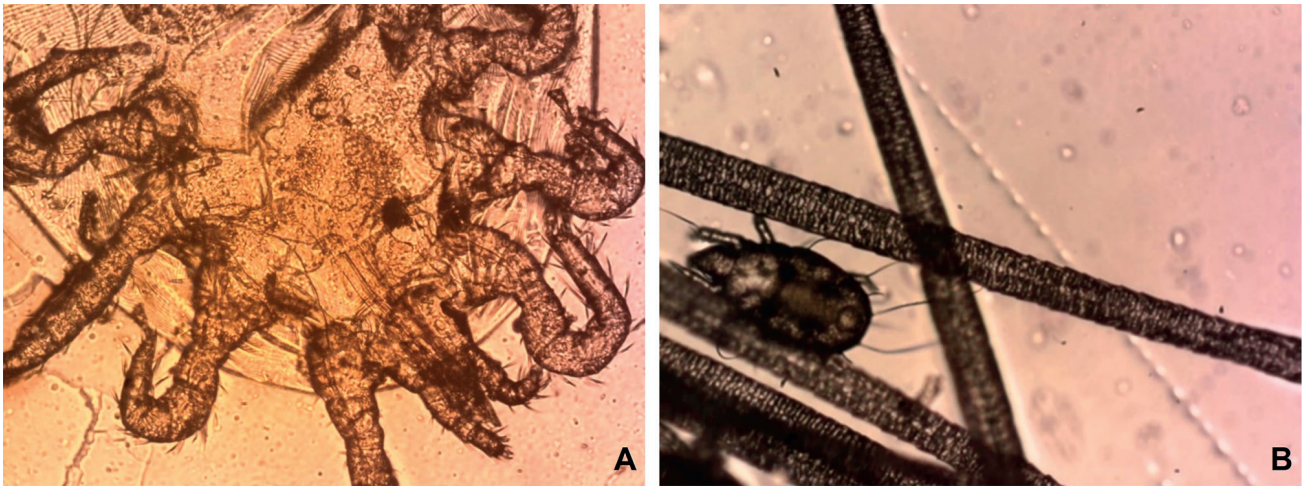

Figure 2. Acariasis images.
(**A)** Mites identified as *Myocoptes musculinis* by morphology; (**B**) Ectoparasite in fur hair. Magnification 100X (B); 400X (A).

and ectoparasites were detected only in conventional units at a prevalence of 8.0% and 0.53%, respectively; however no statistically significant difference was observed between husbandry types.

G1 and QUA were considered at high risk for pathogen contamination. Between these two groups, the prevalence of *Klebsiella pneumonia, Pseudomonas aeruginosa, Morganella morganii,* gross pathology findings and pinworms was

Table 2. The prevalence of selected pathogens in SPF versus conventional facilities

	SPF		Conventional units[a]		P-value (*t*-test)	Standard deviation
	N=494	n (%)	N=188	n (%)		
Pathogens bacteria FELASA panel						
Klebsiella oxytoca	2	(0.41)	0	(0)	0.04*	0.005
Pasteurella spp.	109	(22.06)	35	(18.62)	0.33	0.146
Staphylococcus aureus	35	(7.1)	18	(9.6)	0.34	0.095
Other isolated bacteria not listed in FELASA recommended panel						
Proteus mirabilis	1	(0.2)	0	(0)	0.041*	0.002
Proteus vulgaris	2	(0.4)	2	(1.06)	0.42	0.02
Morganella morganii	0	(0)	4	(2.13)	0.2	0.039
Staphylococcus saprophyticus	3	(0.61)	1	(0.53)	0.5	0.02
Enterobacter cloacae	8	(1.62)	3	(1.6)	0.28	0.023
Virology/other pathogens FELASA panel						
Mouse hepatitis virus (MHV)	0	(0)	6	(3.2)	0.14	0.071
Mycoplasma pulmonis (MPUL)	0	(0)	5	(2.66)	0.25	0.074
Gross pathology	22	(4.45)	5	(2.66)	2.24	0.044
Parasitology						
Endoparasites (pinworms)	0	(0)	15	(8)	0.065	0.105
Ectoparasites (mites)	0	(0)	1	(0.53)	0.289	0.036

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations
[a] Medicine, Life Sciences, Psychology
* P<0.05 (statistically significant)

Table 3. The prevalence of selected pathogens in Group 1 versus quarantine facilities

	Group 1[a]		Quarantine		P-value (*t*-Test)	Standard deviation
	N=140	n (%)	N=133	n (%)		
Pathogens bacteria FELASA panel						
Klebsiella pneumoniae	0	(0)	7	(5.26)	0.04*	0.05
Klebsiella oxytoca	2	(1.43)	1	(0.75)	0.4	0.02
Pasteurella spp.	40	(28.6)	25	(18.8)	0.3	0.17
Staphylococcus aureus	13	(9.3)	12	(9.02)	0.29	0.09
Pseudomonas aeruginosa	5	(3.57)	0	(0)	0.04*	0.03
Other isolated bacteria not listed in FELASA recommended panel						
Proteus mirabilis	1	(0.71)	2	(1.50)	0.24	0.02
Proteus vulgaris	0	(0)	0	(0)	0	0
Morganella morganii	10	(7.14)	1	(0.75)	0.02*	0.05
Staphylococcus saprophyticus	2	(1.43)	8	(6.01)	0.07	0.05
Enterobacter cloacae	6	(4.29)	5	(3.75)	0.45	0.05
Enterobacter hormaechei	1	(0.71)	0	(0)	0.17	0.007
Escherichia coli "Shigella-like"	0	(0)	1	(0.75)	0.15	0.016
Serratia marcescens	1	(0.71)	0	(0)	0.17	0.009
Virology/other pathogens FELASA panel						
Mouse hepatitis virus (MHV)	0	(0)	0	(0)	0	0
Mycoplasma pulmonis (MPUL)	0	(0)	0	(0)	0	0
Gross pathology	15	(10.71)	2	(1.5)	0.003**	0.05
Parasitology						
Endoparasites (pinworms)	13	(9.29)	1	(0.75)	0.04*	0.06
Ectoparasites (mites)	2	(1.43)	0	(0)	0.17	0.01

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations

[a] Group 1 included the conventional housing of the School of Zoology (9 sentinel mice), Felsenshtein Medical Research Center (36 sentinel mice), Sheba Medical Center (10 sentinel mice) and biotechnology companies (85 sentinel mice)

* P<0.05,

** P<0.005 (statistically significant)

statistically significantly higher in G1 compared to QUA (Table 3). Since G1 comprised external facilities, an expected significantly higher prevalence of endoparasites was found in 13 of 140 cases (9.29%), compared to one case among 133 rodents (0.75%) in QUA.

Next, we examined if the prevalence of pathogens in the SPF unit is lower compared to G1 and QUA (Table 4). The prevalence of *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* was statistically significantly higher in QUA compared to the SPF group (P=0.04 for both pathogens). The prevalence of *Pseudomonas aeruginosa* and pinworms was statistically significantly higher in G1 compared to the SPF group (P=0.04 and P=0.012, respectively).

Environmental monitoring of microorganisms. Analysis of the water showed that the pH was within normal range (2.8 to 3.2). The Lumitester-ATP average was at an acceptable level of 8.50 RLU. Surfaces showed an average level of 182.25 RLU. High levels of ATP-bioluminescence (1500-1800 RLU) were found in biological safety cabinets. These levels were reduced after protocol cleaning, until they reached less than 500 RLU.

DISCUSSION

Our five-year analysis showed that we were able to define safe and lower microbiological counts in our SPF veterinary service unit compared to the conventional husbandry unit,

Table 4. The prevalence of selected pathogens in SPF facilities compared to Group 1 facilities and quarantine facilities

Units (Groups)	SPF		Group 1[a]		Quarantine		P-value (*t*-test) SPF vs Group 1	SD SPF vs Group 1	P-value (*t*-test) SPF vs Quarantine	SD SPF vs Quarantine
	N=494	n (%)	N=140	n (%)	N=133	n (%)				
Pathogens bacteria FELASA panel										
Klebsiella pneumoniae	0	(0)	0	(0)	7	(5.26)	0	0	0.04*	0.05
Klebsiella oxytoca	2	(0.4)	2	(1.43)	1	(0.75)	0.22	0.01	0.25	0.02
Pasteurella spp.	109	(22.1)	40	(28.6)	25	(18.8)	0.18	0.16	0.39	0.12
Staphylococcus aureus	35	(7.09)	13	(9.28)	12	(9.02)	0.25	0.04	0.19	0.08
Pseudomonas aeruginosa	0	(0)	5	(3.57)	0	(0)	0.04*	0.03	0	0
Other isolated bacteria not listed in FELASA recommended panel										
Proteus mirabilis	1	(0.2)	1	(0.71)	2	(0.015)	0.28	0.01	0.11	0.01
Proteus vulgaris	2	(0.4)	0	(0)	0	(0)	0.17	0.006	0.17	0.007
Morganella morganii	0	(0)	10	(7.14)	1	(0.008)	0.006*	0.05	0.17	0.016
Staphylococcus saprophyticus	3	(0.61)	2	(1.43)	8	(6.01)	0.21	0.016	0.04*	0.06
Enterobacter cloacae	8	(1.62)	6	(4.29)	5	(0.034)	0.12	0.034	0.26	0.05
Enterobacter hormaechei	0	(0)	1	(0.71)	0	(0)	0.17	0.007	0	0
Escherichia coli "Shigella-like"	0	(0)	0	(0)	1	(0.008)	0	0	0.17	0.016
Serratia marcescens	0	(0)	1	(0.71)	0	(0)	0.17	0.009	0	0
Virology/other pathogens FELASA panel										
Mouse hepatitis virus (MHV)	0	(0)	0	(0)	0	(0)	0	0	0	0
Mycoplasma pulmonis (MPUL)	0	(0)	0	(0)	0	(0)	0	0	0	0
Gross pathology	22	(4.45)	15	(10.7)	2	(1.5)	0.06	0.05	0.1	0.04
Parasitology										
Endoparasites (pinworms)	0	(0)	13	(9.29)	1	(0.75)	0.012*	0.06	0.17	0.02
Ectoparasites (mites)	0	(0)	2	(1.43)	0	(0)	0.17	0.01	0	0

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations; SD, standard deviation

[a] Group 1 included the conventional housing of the School of Zoology (9 sentinel mice), Felsenshtein Medical Research Center (36 sentinel mice), Sheba Medical Center (10 sentinel mice) and biotechnology companies (85 sentinel mice)

* $P<0.05$ (statistically significant)

despite the proximity of both units, demonstrating the importance of a barrier facility. To reduce or eliminate the potential of introducing biological pathogens into the facility, it was essential to monitor critical control points that pose safety risks. Key factors to consider include the entry of animals, the use of biological materials (e.g., cells, parasites, viral stocks, proteins, antibodies, non-pathogenic bacteria), cleaning, disinfection, and sterilization processes, as well as housing and husbandry practices (water, food, air and bedding quality) and it must be ensured that personnel must be carefully trained and managed.(36) Additionally, facility construction and animal services must adhere to animal facility standards (2).

Pathogens are present in low levels in animal facilities worldwide and generally do not affect biomedical research. In this study, the prevalence of isolated pathogens was below the permitted ratio, ensuring our animal facilities met the FELASA standards.

It is well documented that the composition of gut microbiota is dynamic and influenced by factors such as host genetics, environment and geographical location. Wild mice exhibit significant differences in microbiome composition compared to laboratory animals (37). For example, in Argentina, the most frequently isolated bacteria in animal facilities were *Pseudomonas aeruginosa* and *Proteus spp.* (4),

whereas in Baghdad, almost a complete list of FELASA and other isolated bacteria not listed in the FELASA recommended panels were found in captured rats (38). Among these, the prevalence of *Escherichia coli* O157:H7, which requires Biosafety Level 3 laboratory working conditions, was 6.7%, as is typical for wild captured rodents. Similarly, in New York city, house mice (*Mus musculus*) were found to harbor high reservoirs of bacteria capable of causing gastrointestinal disease, including *Shigella spp*. and *Clostridium spp*. (10).

The most prevalent bacteria identified in our facilities were *Pasteurella spp.*, identified in 22% likely due to contamination from the supplier, followed by *Staphylococcus aureus* (8%). In the current study, the prevalence of *Klebsiella (pneumoniae, oxytoca)* and *Pseudomonas aeruginosa* was low. In comparison, a recent study conducted at Charles River Laboratories, reported higher prevalence rates for *Staphylococcus aureus* (38%), *Proteus mirabilis* (24%), *Klebsiella pneumonia* (5%), and *Klebsiella oxytoca* (3%) (35). These findings indicate that the pathogen levels in our facilities are relatively low. Furthermore, compared to the 9.4%, prevalence of *Klebsiella pneumoniae* among wild captured mice in New York City (10), the prevalence of this pathogen in our quarantined laboratory rodents was lower at 5.3%. Opportunistic isolated bacteria, such as *Proteus spp.*, *Morganella morganii*, and *Enterobacter cloacae*, were detected at even lower rates.

Helicobacter spp. is common both in wild rodents and laboratory animal facilities with a high prevalence ranging from 5% to 50% (35, 39) detected by PCR or multiplex DNA analysis for species identification, which is highly applicable in epidemiological studies (40). In our study, *Helicobacter spp*. was identified without species-specific identification and was routinely tested only in the companies' animal facilities, where a prevalence of 12% was observed.

Virology findings showed a low prevalence (less than 1%) of MHV and TMEV-GDVII in mice in our units compared to a prevalence of 2-3% reported for external clients in the Charles River Laboratories study (35). Rats in our conventional facilities, showed a high prevalence of rat Theilovirus (47%) and *Pneumocystis carinii* rat respiratory virus (15%), compared to a very low prevalence of 0.06% and 0%, respectively, reported in the Charles River Laboratories study (35).

Endoparasites attention is also essential for maintaining animal welfare. Animal facilities typically use direct microscopic examination for monitoring pinworms. Additionally, real-time PCR can be used to differentiate among *Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera* by analyzing rDNA sequences spanning the internal transcribed spacer 1, the 5.8S gene, and internal transcribed spacer 2. This data was the basis for applying real-time PCR tests by fluorogenic 5' nuclease and target probes of 28S rRNA sequences on lysates from filter top media, pooled swabs and fecal pellets at Charles River Research Animal Diagnostic Services (41).

In our facilities the prevalence of pinworms was around 3%, and they were detected only in conventional units. Microscopic observations allowed us to distinguish between *Syphacia obvelata* and *Aspiculuris tetraptera*. Additionally, PCR was used in some cases to differentiate pinworms species, with *Syphacia obvelata* being more prevalent. In the Charles River Laboratories study, pinworms were detected in 1% of animals from external clients (35). In Argentinian laboratories, *Syphacia muris* was found in 39% of rats and *Syphacia obvelata* was present in 34% of mice (4). In a conventional animal facility in Malaysia, helminth types were significantly associated with mice strains, with *Syphacia obvelata* and *Aspiculuris tetraptera* more prevalent in ICR mice compared to BALB/c mice (22). Pinworm infections were detected in 8-30% of cases. The study also revealed that the perianal tape test is optimal for identifying *Syphacia obvelata*, while the fecal flotation technique is more effective for detecting *Aspiculuris tetraptera* (22). Fecal samples from wild rodents captured in Chile showed an 90% prevalence of endoparasites with various helminth egg types. Among the detected parasites, *Syphacia sp*. accounted for 3.7% of cases in live rodents compared to 36.4% of cases in post-mortem examinations (42).

At our center, mites were detected in only two vivaria at a very low frequency (0.31%). Interestingly, a pilot study conducted in animal facilities in Finland revealed that mites were absent from the animal rooms but were present in 25% of samples taken from staff room chairs and storage areas. The spread was attributed to contamination via animal food or bedding (43).

The prevalence of pathogens is highly associated with the cleanliness standards maintained in the tested facility. In our units, high pathogen levels were initially detected in biological cabinets by the Lumitester-ATP technique, but these levels decreased following the implementation of a cleaning protocol.

In conclusion, we have successfully lowered microbiological counts in our SPF veterinary service compared to the conventional animal units, despite their close proximity

on campus, demonstrating the importance of a barrier facility. Modern animal health monitoring is a dynamic process system that must evolve with advancements in technology, such as utilizing databases, such as REDCap, which integrate data from investigators and veterinarians, to promote best practices for improving experimental animal health (44).

Our rodent health monitoring system has certain limitations, as it relies on classical in-house microbiology protocols, including bacterial detection through culture and biochemical assays. Parasites are detected by indirect microscopy, while virus detection is outsourced to an external laboratory service. Additionally, the microorganism detection method, which is based on sentinels' soiled-bedding depends on the pathogen load present in the bedding during exposure and the agent's ability to remain viable and infectious (45). To modernize our methodology and increase assay sensitivity, our service is transitioning to an alternative health monitoring method using collection media filters. The validated system enhances pathogen detection by recovering nucleic acids from the media using PCR, providing a more efficient and reliable management platform for animal facilities.

SUPPLEMENTARY DATA

Material and Methods

Pathogens tested according to the Federation of European Laboratory Animal Science Associations (FELASA) recommendations and isolated bacteria not listed in FELASA panel are as follows:

Virology/Serology mouse FELASA panel (material blood). MHV, EDIM-ROTA-A, MVM, MPV-1, -2, -5, PVM, SEND, TMEV-GDVII, ECTRO, LCMV, FL-MAV-1, K87-MAV-2, MCMV, REO, NS-1, MNV, MPUL, CPIL.

Virology/Serology rat FELASA panel (material blood). HANT, H1, MAV1, MAV2, RPV, RMV, KRV, PVM, RCV/SDAV, REO, RTV, SEND, NS-1, PCAR/RRV, MNV, RCMV, CARB, MPUL, CPIL.

Microbiology FELASA panel (material nasopharynx and feces swabs). MPUL, *Bordetella bronchiseptica*, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutcheri*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Pasteurella spp.*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Staphylococcus aureus*, *Streptococci* β-haemolytic, *Streptococcus pneumonia*, *Helicobacter spp.*, *Streptobacillus moniliformis*, Dermatophytes, *Corynebacterium bovis*, *Pneumocystis carinii.*

Microbiology isolated bacteria not listed in FELASA recommended panel (material nasopharynx and feces swabs). *Proteus mirabilis*, *Proteus vulgaris*, *Morganela morganii*, *Staphylococcus saprophyticus*, *Staphylococcus epidermis*, *Providencia rettgeri*, *Bacillus spp.*, *Pseudomonas stutzeri*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *E. coli* "Shigella-like", *Enterococcus faecalis*, *Serratia marcescens*, *Staphylococcus xylosus*, *Mycoplasma genus.*

Parasitology. *pinworms and fur mites* (material feces and environmental swab cages): in quarantine group animals real-time PCR was tested by Charles River Laboratory Research Animal Laboratory (Diagnostic Service, Wilmington, Massachusetts, USA). Pinworms specification for *Aspiculuris tetraptera*, *Syphacia muris* (rat), *Syphacia obvelata* and mites specification for *Myobia musculi*, *Radfordia affinis*, *Radfordia ensifera*, *Myocoptes musulinus*.

Abbreviations. For mice, MHV, mouse hepatitis virus; EDIM-ROTA-A, mouse rotavirus; MVM, minute virus of mice; MPV-1,-2,-5, mouse parvovirus; PVM, pneumonia virus of mice; SEND, Sendai virus; TMEV-GDVII, Theiler's murine encephalomyelitis virus; ECTRO, ectromelia virus; LCMV, lymphocytic choriomeningitis virus; FL-MAV-1, K87-MAV-2, mouse adenovirus type 1,2; MCMV, mouse cytomegalovirus; REO, reovirus type 3; NS-1, generic parvovirus; MNV, murine norovirus; MPUL, mycoplasma pulmonis; CPIL, *Clostridium piliforme*. For rats, HANT, zoonotic hantaan virus; H1, Toolan's H1-rat parvovirus; MAV1, MAV2, rodent adenovirus strain 1, 2; RPV, rat parvovirus; RMV, rat minute virus; KRV, Kilham's rat virus-parvovirus; PVM, rodent pneumovirus; RCV/SDAV, rat coronavirus; REO, rodent reovirus; RTV, Rat theilovirus; NS-1, PCAR/RRV, *Pneumocystis carinii*; MNV, RCMV, rat cytomegalovirus; CARB, cilia-associated respiratory bacillus.

Helicobacter PCR. DNA was extracted from stool feces (3-4 pellets) using EZ-DNA (Biological Industries, Beit Ha'emek, Israel). For *Helicobacter spp*. identification, PCR analysis was used with the gene target 16S rRNA. Primers as followed: forward primer: 5'-CTA TGA CGG GTA TCC

GGC-3'; reverse primer: 5'- ATT CCA CCT ACC TCT CCC A-3' [37]. Program: 94°C 5' one cycle; 94°C-30"; 60°C-45"; 72°C- 1' 30 cycles; 72°C-1' one cycle; keep 10°C. PCR SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Rhenium, Modiin, Israel). PCR products were separated in 1% gel agarose; the *Helicobacter spp*. band size was estimated at 400 bp (46).

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Author Contributions

D.R. conceptualization, formal analysis, investigation and methodology; M.L.A. resources and revision; M.H. resources, revision and supervision.

Declaration of conflicting interests

The authors declare no conflict of interest.

Data availability statement

Full health monitoring data and history can be accessed at https://med.tau.ac.il/Health-Reports. Interested parties can contact Dr. Debora Rapaport debirapa@tauex.tau.ac.il and Dr. Michael Harlev mickey@tauex.tau.ac.il.

Ethics statement

Ethics for sentinel animal health evaluation at Tel Aviv University Animal Facilities were under the Institutional Animal Care and Use Committee approval number TAU-MD-IL-2307-150-2.

REFERENCE:

1. Burkholder, T., Foltz, C., Karlsson, E., Linton, C. G. and Smith, J. M.: Health Evaluation of Experimental Laboratory Mice. Curr. Protoc. Mouse Biol. 2:145-165, 2012.
2. Hubrecht, R.: Guide for the Care and Use of Laboratory Animals, Eighth Edition Anim, Welf,. 20:455-456, 2011.
3. Schlapp, G., Fernández-Graña, G., Arévalo, A. P. and Crispo, M.: Establishment of an environmental microbiological monitoring program in a mice barrier facility. An. Acad. Bras. Cienc. 90:3155-3164, 2018.
4. Carriquiriborde, M., Milocco, S., Laborde, J. M., Gentil, F., Maschi, F., Principi, G., Rogers, E., Cagliada, M. D. P., Ayala, M. A. and Carbone, C.: Microbiological contaminations of laboratory mice and rats in conventional facilities in Argentina. Rev. Argent. Microbiol. 52:96-100, 2020.
5. Miller, M., Zorn, J. and Brielmeier, M.: High-Resolution Melting Curve Analysis for Identification of Pasteurellaceae Species in Experimental Animal Facilities. PLoS One. 10:e0142560, 2015.
6. Basic, M., Bolsega, S., Smoczek, A., Gläsner, J., Hiergeist, A., Eberl, C., Stecher, B., Gessner, A. and Bleich, A.: Monitoring and contamination incidence of gnotobiotic experiments performed in microisolator cages. Int. J. Med. Microbiol. 311:151482, 2021.
7. Mähler Convenor, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K. and Raspa, M.: FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab. Anim. 48:178-192, 2014.
8. National Research Council Committee on Infectious Diseases of Mice, R. The National Academies Collection: Reports funded by National Institutes of Health. Companion Guide to Infectious Diseases of Mice and Rats. Washington (DC): National Academies Press (US) Copyright © 1991 by the National Academy of Sciences CIP.; 1991.
9. Woo, P. C., Lau, S. K., Teng, J. L., Tse, H. and Yuen, K. Y.: Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Rev. 14:908-934, 2008.
10. Williams, S. H., Che, X., Paulick, A., Guo, C., Lee, B., Muller, D., Uhlemann, A. C., Lowy, F. D., Corrigan, R. M. and Lipkin, W. I.: New York City House Mice (*Mus musculus*) as Potential Reservoirs for Pathogenic Bacteria and Antimicrobial Resistance Determinants. mBio. 9, 2018.
11. Compton, S. R.: PCR and RT-PCR in the Diagnosis of Laboratory Animal Infections and in Health Monitoring. J. Am. Assoc. Lab. Anim. Sci. 59:458-468, 2020.
12. Dubourg, G., Baron, S., Cadoret, F., Couderc, C., Fournier, P. E., Lagier, J. C. and Raoult, D.: From Culturomics to Clinical Microbiology and Forward. Emerg. Infect. Dis. 24:1683-1690, 2018.
13. Goto, K., Yamamoto, M., Asahara, M., Tamura, T., Matsumura, M., Hayashimoto, N. and Makimura, K.: Rapid identification of Mycoplasma pulmonis isolated from laboratory mice and rats using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. J. Vet. Med. Sci. 74:1083-1086, 2012.
14. Randall, L. P., Lemma, F., Koylass, M., Rogers, J., Ayling, R. D., Worth, D., Klita, M., Steventon, A., Line, K., Wragg, P., Muchowski, J., Kostrzewa, M. and Whatmore, A. M.: Evaluation of MALDI-ToF as a method for the identification of bacteria in the veterinary diagnostic laboratory. Res. Vet. Sci. 101:42-49, 2015.
15. Alcalá, L., Marín, M., Ruiz, A., Quiroga, L., Zamora-Cintas, M., Fernández-Chico, M. A., Muñoz, P. and Rodríguez-Sánchez, B.: Identifying Anaerobic Bacteria Using MALDI-TOF Mass Spectrometry: A Four-Year Experience. Front. Cell. Infect. Microbiol. 11:521014, 2021.
16. Sastre, N., Calvete, O., Martínez-Vargas, J., Medarde, N., Casellas, J., Altet, L., Sánchez, A., Francino, O. and Ventura, J.: Skin mites in mice (*Mus musculus*): high prevalence of Myobia sp. (Acari, Arachnida) in Robertsonian mice. Parasitol. Res. 117:2139-2148, 2018.
17. Turner, D. E., Daugherity, E. K., Altier, C. and Maurer, K. J.: Efficacy and limitations of an ATP-based monitoring system. J. Am. Assoc. Lab. Anim. Sci. 49:190-195, 2010.
18. Ihssen, J., Jovanovic, N., Sirec, T. and Spitz, U.: Real-time monitoring of extracellular ATP in bacterial cultures using thermostable luciferase. PLoS One. 16:e0244200, 2021.

19. Eguíluz, C., Rossi, M. and Viguera, E.: Pinworm detection in mice with immunodeficient (NOD SCID) and immunocompetent (CD-1 and Swiss) soiled bedding sentinels in individually ventilated cage systems. Lab. Anim. 49:302-310, 2015.
20. Gerwin, P. M., Ricart Arbona, R. J., Riedel, E. R., Lepherd, M. L., Henderson, K. S. and Lipman, N. S.: Evaluation of Traditional and Contemporary Methods for Detecting Syphacia obvelata and Aspiculuris tetraptera in Laboratory Mice. J. Am. Assoc. Lab. Anim. Sci. 56:32-41, 2017.
21. Abdel-Gaber, R., Abdel-Ghaffar, F., Al Quraishy, S., Morsy, K., Saleh, R. and Mehlhorn, H.: Morphological Re-Description and 18 S rDNA Sequence Confirmation of the Pinworm Aspiculuris tetraptera (Nematoda, Heteroxynematidae) Infecting the Laboratory Mice Mus musculus. Journal of Nematolog. 50:117-132, 2018.
22. Ain-Fatin, R., Nur-Fazila, S. H., Nur-Mahiza, M. I., Yasmin, A. R., Losheni, S. and Najwa-Syaza, A.: Detection of pinworms in conventionally maintained laboratory mice. J. Anim. Plant Sci. 32:954-960, 2022.
23. Feldman, S. H. and Bowman, S. G.: Molecular phylogeny of the pinworms of mice, rats and rabbits, and its use to develop molecular beacon assays for the detection of pinworms in mice. Lab Animals (New York). 36:43-50, 2007.
24. Isayama, K., Watanabe, K., Okamoto, M., Murata, T. and Mizukami, Y.: Standardization of an LNA-based TaqMan assay qPCR analysis for Aspiculuris tetraptera DNA in mouse faeces. BMC Microbiol. 20:371, 2020.
25. Randall, T. A. and Kurtz, D. M.: Assembly of a Draft Genome for the Mouse Ectoparasite Myocoptes musculinus. J. Am. Assoc. Lab. Anim. Sci. 62:55-63, 2023.
26. Mohan, G. H., Shwetha Reddy, R. V. and Yogesh, C. Management of Specific Pathogen-Free (SPF) Mice and Rats. In: Nagarajan P, Gudde R, Srinivasan R, editors. Essentials of Laboratory Animal Science: Principles and Practices. Singapore: Springer Singapore; 2021. p. 633-653.
27. Laber, K., Newcomer, C. E., Decelle, T., Everitt, J. I., Guillen, J. and Brønstad, A.: Recommendations for Addressing Harm-Benefit Analysis and Implementation in Ethical Evaluation – Report from the AALAS-FELASA Working Group on Harm-Benefit Analysis - Part 2. Lab. Anim. 50:21-42, 2016.
28. Perry, J. D.: A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin. Microbiol. Rev. 30:449-479, 2017.
29. Martin, W. J., Yu, P. K. and Washington, J. A., 2nd: Evaluation of the enterotube system for identification of members of the family Enterobacteriaceae. Appl. Microbiol. 22:96-99, 1971.
30. Dafni, H., Greenfeld, L., Oren, R. and Harmelin, A.: The Likelihood of Misidentifying Rodent Pasteurellaceae by Using Results from a Single PCR Assay. J. Am. Assoc. Lab. Anim. Sci. 58:201-207, 2019.
31. Riley, L. K., Franklin, C. L., Hook, R. R., Jr. and Besch-Williford, C.: Identification of murine helicobacters by PCR and restriction enzyme analyses. J. Clin. Microbiol. 34:942-946, 1996.
32. Topić Popović, N., Kazazić, S. P., Bojanić, K., Strunjak-Perović, I. and Čož-Rakovac, R.: Sample preparation and culture condition effects on MALDI-TOF MS identification of bacteria: A review. Mass. Spectrom. Rev. 42:1589-1603, 2023.
33. Abdel-Gaber, R.: Syphacia obvelata (Nematode, Oxyuridae) infecting laboratory mice Mus musculus (Rodentia, Muridae): phylogeny and host-parasite relationship. Parasitol. Res. 115:975-985, 2016.
34. Whary, M. T., Baumgarth, N., Fox, J. G. and Barthold, S. W.: Chapter 3 – Biology and Diseases of Mice. In: Fox J.G., Anderson L.C., Otto G.M., Pritchett-Corning K.R., Whary M.T., editors. Laboratory Animal Medicine (Third Edition). Boston: Academic Press; 2015. p. 43-149.
35. Albers, T. M., Henderson, K. S., Mulder, G. B. and Shek, W. R.: Pathogen Prevalence Estimates and Diagnostic Methodology Trends in Laboratory Mice and Rats from 2003 to 2020. J. Am. Assoc. Lab. Anim. Sci. 62:229-242, 2023.
36. NRC. Companion Guide to Infectious Diseases of Mice and Rats. . Washington (DC): The National Academies Collection: Reports funded by National Institutes of Health. National Research Council Committee on Infectious Diseases of Mice and Rats; 1991.
37. Weldon, L., Abolins, S., Lenzi, L., Bourne, C., Riley, E. M. and Viney, M.: The Gut Microbiota of Wild Mice. PLoS One. 10:e0134643, 2015.
38. Ayyal, N. M., Abbas, Z. A., Karim, A. J., Abbas, Z. M., Al-Salihi, K. A., Khalaf, J. M., Mahmood, D. D., Mohammed, E. A., Jumaa, R. S. and Abdul-Majeed, D. I.: Bacterial isolation from internal organs of rats (Rattus rattus) captured in Baghdad city of Iraq. Vet. World. 12:119-125, 2019.
39. Duangchanchot, M., Inpunkaew, R., Thongsiri, P., Hayashimoto, N., Gemma, N., Nikaido, M., Takahashi, M. and Kengkoom, K.: Prevalence of helicobacter in laboratory mice in Thailand. Exp. Anim. 63:169-173, 2014.
40. Butt, J., Schmitz, M., Berkus, B., Schmidt, K. and Höfler, D.: Validation of Multiplex PCR and Serology Detecting Helicobacter Species in Mice. Microorganisms. 11, 2023.
41. Gerwin, P. M., Ricart Arbona, R. J., Riedel, E. R., Henderson, K. S. and Lipman, N. S.: PCR Testing of IVC Filter Tops as a Method for Detecting Murine Pinworms and Fur Mites. J. Am. Assoc. Lab. Anim. Sci. 56:752-761, 2017.
42. Carrera-Játiva, P. D., Torres, C., Figueroa-Sandoval, F., Beltrami, E., Verdugo, C., Landaeta-Aqueveque, C. and Acosta-Jamett, G.: Gastrointestinal parasites in wild rodents in Chiloé Island-Chile. Rev. Bras. Parasitol. Vet. 32:e017022, 2023.
43. Pennanen, S. M. and Harju, A. T.: Mites in facilities for laboratory animals. Scand. J. Work Environ. Health. 29:314-316, 2003.
44. Carvajal, C., Vallejos, C., Lemaitre, D., Ruiz, J., Guzmán, C., Aguilera, V., Baño, D. and Calligaris, S. D.: A REDCap application that links researchers, animal facility staff and members of the IACUC in animal health monitoring. Lab. Anim. 53:500-507, 2019.
45. Henderson, K. S., Perkins, C. L., Havens, R. B., Kelly, M. J., Francis, B. C., Dole, V. S. and Shek, W. R.: Efficacy of direct detection of pathogens in naturally infected mice by using a high-density PCR array. J. Am. Assoc. Lab. Anim. Sci. 52:763-772, 2013.
46. Riley, L.K., Franklin, C,L., Hook, R.R., Jr. and Besch-Williford, C. 1996. Identification of murine helicobacters by PCR and restriction enzyme analyses. J. Clin. Microbiol. 34:942-946, 1966.