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# Pathogen Prevalence Estimates and Diagnostic Methodology Trends in Laboratory Mice and Rats from 2003 to 2020

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**Rodents used in biomedical research are maintained as specific pathogen-free (SPF) by employing biosecurity measures that eliminate and exclude adventitious infectious agents known to confound research. The efficacy of these practices is assessed by routine laboratory testing referred to as health monitoring (HM). This study summarizes the results of HM performed at Charles River Research Animal Diagnostic Services (CR-RADS) on samples submitted by external (non-Charles River) clients between 2003 and 2020. Summarizing this vast amount of data has been made practicable by the recent introduction of end-user business intelligence tools to Excel. HM summaries include the number of samples tested and the percent positive by diagnostic methodology, including direct examination for parasites, cultural isolation and identification for bacteria, serology for antibodies to viruses and fastidious microorganisms, and polymerase chain reaction (PCR) assays for pathogen-specific genomic sequences. Consistent with comparable studies, the percentages of pathogen-positive samples by diagnostic methodology and year interval are referred to as period prevalence estimates (%$P_E$). These %$P_E$ substantiate the elimination of once common respiratory pathogens, such as Sendai virus, and reductions in the prevalence of other agents considered common, such as the rodent coronaviruses and parvoviruses. Conversely, the %$P_E$ of certain pathogens, for example, murine norovirus (MNV), *Helicobacter*, *Rodentibacter*, and parasites remain high, perhaps due to the increasing exchange of genetically engineered mutant (GEM) rodents among researchers and the challenges and high cost of eliminating these agents from rodent housing facilities. Study results also document the growing role of PCR in HM because of its applicability to all pathogen types and its high specificity and sensitivity; moreover, PCR can detect pathogens in samples collected antemortem directly from colony animals and from the environment, thereby improving the detection of host-adapted, environmentally unstable pathogens that are not efficiently transmitted to sentinels by soiled bedding.**

**Abbreviations and Acronyms:** %$P_E$, percent prevalence estimate; DAX, data analysis expression language; GEM, genetically engineered mutant; HM, health monitoring; LIMS, Laboratory Information Management System; MFIA, Multiplexed Fluorometric Immunoassay; MHV, Mouse Hepatitis Virus; MNV, Murine Norovirus

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## Introduction

Starting in the last century, numerous studies documented that contamination of research animals and reagents with infectious pathogens could confound research findings and imperil the health of staff.[38,39,53] Consequently, a long-held tenet is that mice, rats and other animals used for biomedical research should be specific-pathogen-free (SPF).

Approaches for producing SPF rodents were developed after World War II by pioneers in the field of laboratory animal medicine. These approaches included rederivation by hysterectomy (and by embryo transfer today) to eliminate horizontally transmitted pathogens, maintenance of rederived rodents as gnotobiotic (that is, either germfree or having a defined commensal microbiome) in otherwise sterile isolators, and the transfer of gnotobiotic rodent colonies to barrier rooms for large-scale production to supply biomedical research. To assure the exclusion of pathogens from rederived isolator- and barrier-reared rodent colonies, their supplies were disinfected by chemical and physical means; in addition, air was HEPA-filtered, and technicians wore disinfected personal protective equipment (PPE).[11,52]

Notwithstanding the advent and widespread adoption of modern biosecurity practices by rodent vendors by the 1970s, a previous study[5] found a considerable percentage of barrier-reared breeder colonies were still contaminated with common rodent viruses and parasites in the early 1980s. This finding underscored the need to substantiate the efficacy of modern biosecurity practices through routine testing, commonly referred to as health monitoring (HM). The need for HM has been reinforced more recently by the expanding development and exchange of genetically engineered mutant (GEM) animals, mostly mice, that are frequently found to harbor traditional and newly discovered pathogens.[4,20] Moreover, adventitious infections of these often-immunocompromised animals, can alter or obscure the effects of genetic modifications, or cause severe and sometimes atypical disease.[12] Finally, advances in molecular diagnostics have led to both the detection of traditional pathogens, notably parasites, that were thought to have been eliminated and to the discovery of prevalent pathogens in rodent colonies.[23,44,45]

Reports on the frequency with which pathogens are found in rodent facilities have been based on surveys of research institutions[4,20,32] as well as the test results from

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individual HM laboratories.[30,33,42] One study[42] reported rodent pathogen-prevalence levels at research facilities in North America and Europe over a 5-y period in the early 2000 s based on results of HM performed at Charles River-Research Animal Diagnostic Services (CR-RADS) laboratories in North America and Europe. A serologic survey of viral agents and *Mycoplasma pulmonis* for mice and rats in Western Europe[30] has been published. In addition, a report was published on the prevalence of viral, bacterial, and parasitological pathogens of mice and rats used in research in Australasia over a 5-y period.[33]

In the current report, we extend the data presented previously[42] to include CR-RADS results for mouse and rat samples submitted by external clients from 2003 to 2020. Summarization of this large dataset, comprising millions of result records, was made practicable by automated, standardized categorization of results as positive (or not) by the CR-RADS laboratory information management system (LIMS), and by the Microsoft Power Query and Power Pivot Excel add-ins that have permitted highly efficient storage and accurate summarization of the dataset.[35] Each LIMS test (that is, the LIMS unit for which a sample result was reported to clients) was assigned a microbial taxonomy and one of the diagnostic methodologies described in the Materials and Methods. DAX (for Data Analysis Expression language) measures,[34] as defined in Power Pivot, calculated the percentages of samples, per year or multiyear interval, that tested pathogen-positive by a diagnostic methodology. Because our data were derived by testing client-selected rather than randomly selected samples, the pathogen positive percentages reported here do not strictly meet the definition of prevalence. On the other hand, as the pathogen-positive percentages reported here were based on large numbers of samples from many institutions, we believe these samples provide a useful estimate of prevalence and are consistent with the use of "prevalence" in the studies comparable to ours. Therefore, these positive percentages are presented as pathogen period prevalence estimates ($\%P_E$).

The conventional methodologies on which HM has traditionally relied have included serology, consisting of immunoassays for antibodies to viruses and several fastidious nonviral microorganisms, direct exams of animal specimens for parasites, and cultural isolation and identification of bacteria and fungi from animal and other specimens. More recently, however, the molecular diagnostics polymerase chain reaction technique (PCR) for amplification of microbial genomic nucleic acid sequences has augmented and in some instances supplanted traditional methodologies for several reasons.[51] First, PCR is suitable for detection of all pathogen types and sample sites, including specimens such as feces and antemortem swabs collected directly from colony and study animals, and environmental samples, notably the dust that accumulates on exhaust ducts and filters. Moreover, PCR of environmental samples, like antibody serology, can reveal both active and past infections from which a colony has recovered.[17] In this way, PCR overcomes the insensitivity of sentinel surveillance, particularly for host-adapted and environmentally labile pathogens not readily transmitted in soiled bedding.[7-9,26,27,31,36,37,41,56] Because of these advantages, molecular diagnostics by PCR has become an increasingly prominent rodent HM methodology over the past decade. In addition, ongoing advances in molecular genetic techniques, particularly next-generation sequencing, have expedited the rate at which pathogens, mostly viruses, have been identified, such as murine astrovirus,[43] mouse kidney parvovirus,[45] and others[54] However, the prevalence of recently discovered pathogens is not covered in this report.

## Materials and Methods

**Samples.** The pathogen prevalence data presented in this report were derived from the results of testing performed at CR-RADS in North America, Europe, and Japan on animal and environmental specimens from external client mouse and rat colonies and biologics, specifically excluding Charles River commercial rodent production colonies. Typical sample types and the diagnostic methodologies by which they were tested are shown in Table 1.

**Sample processing.** Orders for HM were recorded in the CR-RADS LIMS. All rats and mice submitted live were euthanized with carbon dioxide and a gross necropsy was performed. Samples for serology and PCR, including those collected from animals at necropsy, were processed in batches determined by sample host species and type, and the panel of tests ordered by the client. Direct parasitologic exams and microbiologic cultures of animal specimens collected at necropsy or submitted by the client were processed one LIMS order at a time. All animal procedures were performed in AAALAC International-accredited facilities in accordance with Charles River IACUC-approved protocols. Animal procedures adhered to the then-available AVMA guidelines on euthanasia and followed all applicable local and national animal welfare regulations.

**Diagnostic methodologies.** Table 2 shows the diagnostic methodologies and the types of pathogens to which they apply.

***Direct exams for parasites.*** Most direct examinations for parasites were collected at CR-RADS from euthanized animals at necropsy. Screening for ectoparasites was conducted by examination of the pelt under a stereoscopic microscope. Helminth infestations were diagnosed by the examination of macerated cecum and colon with stereoscopic microscopy. Intestinal protozoa were primarily detected by high-magnification phase-contrast microscopy of wet mounts of duodenal and cecal mucosal scrapings. Encysted protozoan and helminth ova in fecal specimens were concentrated by centrifugation and

**Table 1.** Diagnostic methodology sample types

| Methodology | Blood [a] | Resp Tract [b] | GI Tract [c] | Skin [d] | Env [e] | Biologics [f] |
|---|---|---|---|---|---|---|
| Direct Examination for Parasites | | | √ | √ | | |
| Microbial Cultural Isolation and Identification | | √ | √ | √ | | |
| Serologic Pathogen Antibody Immunoassay | √ | | | | | |
| PCR for Pathogen Genomic Sequences | √ | √ | √ | √ | √ | √ |

[a]Serum, dried blood spot or HemaTIP microsampler; PCR for LDV.
[b]Swab or lavage of upper and/or low respiratory tract
[c]Direct exam, swab of cecal or colon contents, and feces
[d]Direct exam or swab
[e]Swab (for example, cage, equipment, HVAC) or exhaust filter
[f]Murine passaged cell lines and reagents


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**Table 2.** Pathogens monitored by HM methodology

| Methodology | Viruses | Parasites | Bacteria/Fungi |
|---|---|---|---|
| Direct Examination (of animal specimens) | | X | |
| Cultural Isolation and Identification[a] | | | X |
| Serology (that is, Pathogen Antibody Immunoassays) | X | | X |
| PCR (assays for Pathogen Genomic Sequences) | X | X | X |

[a]Bacterial isolates were identified based on colonial and cellular morphology, biochemical analysis, PCR and/or MALDI-TOF spectrometry.

**Table 3.** Study DAX [a] measures to determine prevalence by pathogen and methodology

| Measure | DAX Calculation of Measure |
|---|---|
| Samples Tested | Distinct Count of Samples with Test Results |
| Samples Positive | Distinct Count of Samples with Positive Test Results |
| % Prevalence (%$P_E$) | Samples Positive / Samples Tested[b] |
| Results Reported | Count of Results Reported |
| Positive Results | Count of Results Interpreted Positive |
| % Results Reported Positive | Positive Results / Results Reported |
| Results per Sample | Results Reported / Samples Tested |

[a]Microsoft Excel Data Analysis Expression language
[b]By Pathogen Taxonomy and Diagnostic Methodology

flotation in a $ZnSO_4$ solution (specific gravity 1.18), followed by morphologic identification by light microscopy.[40]

***Culture and identification of pathogenic bacteria.*** Specimens collected at necropsy from the nasopharynx, large intestines, and other tissues (for example, skin for *C. bovis*) of euthanized animals or submitted by clients were cultured using media and conditions that favored the isolation of specific opportunistic and primary bacterial pathogens. Prior to being released for diagnostic use, culture media lots were confirmed to support the growth of relevant bacteria and to be sterile. Colony morphology consistent with suspected pathogens were further characterized by microscopic examination, biochemical tests, MALDI-TOF spectrometry, and/or PCR.[10]

***Serology immunoassays for pathogen-specific antibodies.*** Serum and, more recently, dried blood samples were assayed for antibodies to viruses and several fastidious microorganisms such as *Mycoplasma pulmonis* and *Pneumocystis carinii*. Most of these samples were submitted by clients directly to CR-RADS. The primary screening technique for most agents had been the enzyme-linked immunosorbent assay (ELISA); however, starting in 2007, the ELISA was supplanted by the multiplexed fluorometric immunoassay (MFIA) described in detail elsewhere.[55] Samples that gave inconclusive, nonspecific, or otherwise unexpected results were typically retested, often by the indirect immunofluorescent assay (IFA). Infrequently employed serologic techniques have included hemagglutination inhibition, the Western immunoblot, and a lactate dehydrogenase (LDH) assay for LDV infection.

Controls for the MFIA, ELISA, and IFA included positive, negative, and diluent system suitability controls to substantiate assay analytic sensitivity and specificity. Sample suitability controls included the "tissue" control for nonspecific binding of sample immunoglobulin to the solid phase and, in the MFIA, an assay to corroborate that the immunoglobulin species and concentration were appropriate. Provided that suitability controls passed, results were classified as positive or not (that is, negative, or equivocal) by comparison to preestablished positive and negative cutoff signals or, in the case of IFA, the positive and negative control reactions.

***PCR for pathogen-specific genomic sequences.*** The real-time TaqMan PCR and reverse transcription (RT-) PCR for detection of pathogen genomic DNA and RNA sequences, respectively, have been described in detail elsewhere.[17] Each PCR run included positive- and negative-template system-suitability controls to confirm analytical sensitivity and specificity; in addition, nucleic acid recovery sample-suitability controls monitored for insufficient nucleic acid, reverse-transcription for RNA viruses, and sample-mediated inhibition of the PCR. Provided that suitability controls passed, PCR results were classified as either positive or negative; positive results were confirmed by repeat PCR testing.

Real time PCR (including RT-PCR) results were read as cycle threshold levels (Ct), which are the number of cycles required for the fluorescent reporter dye signal to cross a threshold (or background) level. Ct positive cutoffs were assigned by assay and sample type. Ct levels are inversely proportional to the amount of target genomic sequence. Thus, a sample Ct level at or below the assay's cutoff was called positive; if there was no amplification or the Ct was above the cutoff, the result was interpreted as negative.[14,16]

**Data analysis.** CR-RADS LIMS test results, along with their interpretations as positive or not, were uploaded to Excel usingPower Query (Microsoft, Redmond, WA). Key metadata includedwith the results were the sample ID, receipt date and species, and whether the client that submitted the samples was external to Charles River Rodent Production. Results for Charles River Rodent Production colonies were excluded from this summary. External client results were summarized anonymously.

For consistent and accurate results summarization, each LIMS test was assigned a microbial taxonomy and one of the diagnostic methodologies described in the Introduction. The summaries shown in Table 3 were calculated as DAX measures in Excel Power Pivot (Microsoft, Redmond, WA).[34,35] Data were summarized by diagnostic methodology, pathogen taxonomy, and year or multiyear interval for mouse and rat samples.

## Results

**Samples tested and results reported by methodology.** As shown in Table 4, the data presented in this study were derived from CR-RADS testing of just over 3.4 million murine samples from external clients from 2003 through 2020, with 3.1 million, or 91%, being from mice. In Table 4, the samples tested are summarized by individual methodology and as an overall total for all methodologies. The sum of the methodology sample totals is greater than the overall total because a sample (for example, animal submitted for HM) tested by multiple methodologies still counts as a single sample in the overall total. A total of 62 million results were reported for the 3.4 million samples, with an average of 18 results per sample. We deliberately use the term "Results Reported" instead of tests or assays because an individual result might be derived from more than one PCR or serologic antibody assay; alternatively, multiple results may be obtained for some single diagnostic procedures, such as a parasite examination.


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**Table 4.** CR-RADS 2003–2020 external client murine sample and result totals by methodology

| Methodology | Murine Species | Samples [a] | | Results Reported [b] | | Results/ Sample |
|---|---|---|---|---|---|---|
| | | Total # | % [c] | Total # | % [c] | |
| Direct Exam for Parasites | Mouse | 644,424 | 19% | 7,404,043 | 12% | 12 |
| | Rat | 56,104 | 2% | 693,031 | 1% | 12 |
| | **Total** | **700,528** | **20%** | **8,097,074** | **13%** | **12** |
| Cultural Isolation and Identification of Bacteria | Mouse | 511,399 | 15% | 10,676,546 | 17% | 21 |
| | Rat | 54,287 | 2% | 883,201 | 1% | 16 |
| | **Total** | **565,686** | **16%** | **11,559,747** | **19%** | **20** |
| Serology for Pathogen Antibodies | Mouse | 2,410,470 | 70% | 30,608,547 | 49% | 13 |
| | Rat | 282,747 | 8% | 3,273,005 | 5% | 12 |
| | **Total** | **2,693,217** | **78%** | **33,881,552** | **55%** | **13** |
| PCR for Pathogen Genomic Sequences | Mouse | 983,367 | 29% | 8,056,203 | 13% | 8 |
| | Rat | 77,268 | 2% | 507,197 | 1% | 7 |
| | **Total** | **1,060,635** | **31%** | **8,563,400** | **14%** | **8** |
| **Overall Total** | | **3,438,279** | | **62,101,773** | | **18** |

[a]Total # = count of distinct samples (that is, unique LIMS sample identification #s). A sample (for example, animal submitted for HM) tested by different methodologies is counted as a single sample in the Overall Total. Therefore, the Overall Total may be less than the sum of the Methodology sample subtotals.
[b]Results Reported is used instead of tests or assays because an individual result may be derived from more than one PCR or serologic antibody assay; alternatively, multiple results may be derived for a single diagnostic procedure, such as a parasite examination.
[c]Percentage of the sample or result Overall Total.

**Table 5.** CR-RADS 2003–2020 external client murine sample and result totals by pathogen type

| Pathogen Kingdom | Murine Species | Samples Tested [a] | | Results Reported [b] | | Results/ Sample |
|---|---|---|---|---|---|---|
| | | Number | % [c] | Number | % [c] | |
| Viruses | Mouse | 2,693,254 | 78% | 29,535,524 | 48% | 11 |
| | Rat | 296,045 | 9% | 2,795,622 | 5% | 9 |
| | **Total** | **2,989,299** | **87%** | **32,331,146** | **52%** | **11** |
| Parasites | Mouse | 940,128 | 27% | 8,547,616 | 14% | 9 |
| | Rat | 76,556 | 2% | 766,769 | 1% | 10 |
| | **Total** | **1,016,684** | **30%** | **9,314,385** | **15%** | **9** |
| Bacteria and Fungi | Mouse | 2,148,164 | 62% | 18,662,199 | 30% | 9 |
| | Rat | 287,981 | 8% | 1,794,043 | 3% | 6 |
| | **Total** | **2,436,145** | **71%** | **20,456,242** | **33%** | **8** |
| **Overall Total** | | **3,438,279** | | **62,101,773** | | **18** |

[a]Samples Tested = count of distinct samples (that is, unique LIMS sample identification #s). A sample (for example, animal submitted for HM) tested by different methodologies is counted as a single sample in the Overall Total. Therefore, the Overall Total may be less than the sum of the Methodology totals.
[b]Results Reported is used instead of tests or assays because an individual result may be derived from more than one PCR or serologic antibody assay; alternatively, multiple results may be derived for a single diagnostic procedure, such as a parasite examination.
[c]Percentage of the sample or result Overall Total

As shown in Table 4, the percentages of murine (that is, both mouse and rat) samples tested were 20% by direct examination for parasites, 16% by cultural isolation for bacteria, 78% by serology for antibodies to viruses and selected fastidious, invasive micro-organisms, and 31% by PCR for pathogen genomic sequences; result percentages by methodology were 13% by direct examination for parasites, 19% by cultural isolation and identification of bacteria, 55% by serology, and 14% by PCR. The percentages of murine samples tested by pathogen type, as presented in Table 5, were for 87% for viruses, 30% for parasites, and 71% for bacteria and fungi; result percentages by pathogen type were 52% for viruses, 15% for parasites, and 33% for bacteria and fungi.

Figure 1 plots murine samples tested, and results reported annually by diagnostic methodology. The analysis showed a substantial decrease in samples tested by serology from a high of 196,000 in 2011 to 78,000 in 2020, with a concomitant decrease in serologic results reported from 2.6 to 1.0 million. In contrast, the samples tested by PCR increased from 25,000 in 2003 to 71,000 in 2020, with results reported over that same time frame increasing from 65 thousand to 1.0 million.

**Pathogen prevalence by conventional methodologies compared with PCR.** The $\%P_E$ of murine rodent pathogens by PCR vis-à-vis complementary conventional diagnostic methodologies were calculated for multiyear intervals 2003 to 2005, 2006 to 2010, 2011 to 2015, and 2016 to 2020 to keep the presentation and viewing of data summaries manageable. Samples tested by PCR and conventional methodologies, and $\%P_E$ for the most recent 2016 to 2020 interval are given for viruses in Table 6, parasite families and speciesin Table 7, and Table 8, and bacteria and fungi in Table 9. The $\%P_E$ of *Helicobacter* species during the 2016 to 2020 interval are given in Table 10. Samples tested by PCR and conventional methodologies and $\%P_E$ trends across all 4 multiyear intervals are plotted for viruses in Figure 2, for parasites in Figure 3, and for bacteria and fungi in Figure 4 and Figure 5.


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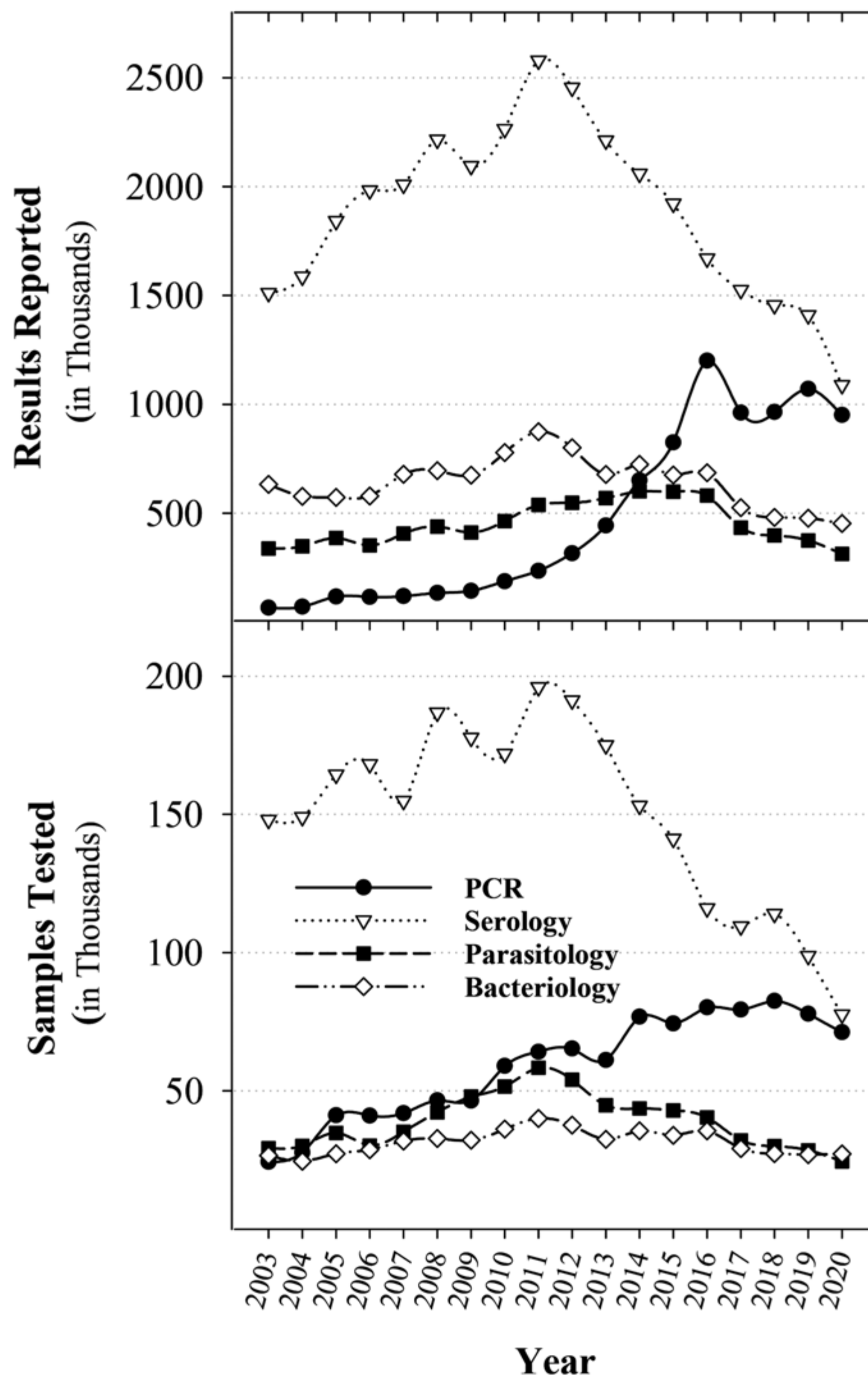


**Figure 1.** CR-RADS 2003-2020 Annual External Client Murine Sample and Result Totals by Diagnostic Methodology.

***Viruses.*** Of the viruses in Table 6, MNV has been by far the most prevalent, with $\%P_E$ for the 2016 to 2020 interval of 32% by serology and 20% by PCR. As shown in Figure 2, MNV $\%P_E$ by serology have remained above 30% since monitoring for this virus began in the early 2000 s, suggesting that MNV infection is tolerated at many research institutions. By comparison, the $\%P_E$ of viruses traditionally considered to be common contaminants of rodent colonies (including the coronaviruses, parvoviruses, and mouse rotaviruses) has steadily declined. For instance, as shown in Figure 2, the $\%P_E$ of MHV by serology decreased from 1.6% for the 2003 to 2005 interval to 0.2% during the 2016 to 2020 interval; over these same intervals, the PCR $\%P_E$ of MHV decreased from 3.8% to 0.3%. The $\%P_E$ of LDV is notably much lower by serology (0.02%) than by PCR (1.08%), perhaps because PCR is used to test biologics, including transplantable cell lines, that often contain LDV as a common contaminant.[2,49] Serology, on the other hand, is used to screen SPF mouse colonies, in which LDV is rare.

***Parasites.*** Parasite $\%P_E$ by direct examination and PCR were compared, with the proviso that PCR were not performed for lice and were used selectively for protozoa, primarily for those considered pathogenic, such as *Cryptosporidium*, *Entamoeba*, *Giardia*, and *Spironucleus*. Conversely, *Demodex* surveillance was by PCR alone as standard examination of the skin does not reliably reveal this mite. Table 7 shows $\%P_E$ of parasites, during the 2016 to 2020 interval, separately for mouse and rat


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**Table 6.** CR-RADS 2016–2020 estimated prevalence of viruses in murine samples from external clients

| | Mouse | | | | Rat | | | |
|---|---|---|---|---|---|---|---|---|
| | Serology | | PCR | | Serology | | PCR | |
| Virus [a] | Samples [b] | $\%P_E$ [c] | Samples | $\%P_E$ | Samples | $\%P_E$ | Samples | $\%P_E$ |
| Coronavirus | 438,107 | 0.22% | 136,436 | 0.28% | 51,376 | 0.05% | 9,395 | 0.00% |
| Hantavirus | 129,789 | 0.00% | 14,376 | 0.00% | 18,336 | 0.00% | 4,722 | 0.00% |
| CMV | 140,754 | 0.03% | 14,266 | 0.06% | 56 | 0.00% | 117 | 0.00% |
| MTLV | 127,413 | 0.09% | 6,724 | 0.12% | — | — | — | — |
| LCMV | 207,844 | 0.00% | 89,383 | 0.02% | 18,598 | 0.00% | 699 | 0.00% |
| LDV | 119,544 | 0.02% | 12,083 | 1.08% | — | — | — | — |
| MAV | 187,973 | 0.00% | 88,569 | 0.08% | 17,588 | 0.01% | 5,073 | 0.02% |
| MNV | 370,652 | 32.05% | 130,140 | 19.95% | — | — | — | — |
| Parvovirus | 415,382 | 0.16% | 152,005 | 0.25% | 47,854 | 0.23% | 9,632 | 0.26% |
| PIV-1 (Sendai) | 330,841 | 0.00% | 68,647 | 0.00% | 36,865 | 0.00% | 4,649 | 0.00% |
| PIV-3 | — | — | — | — | 697 | 1.72% | 16 | 0.00% |
| PVM | 305,068 | 0.01% | 64,854 | 0.00% | 34,667 | 0.08% | 4,246 | 0.00% |
| Polyoma | 177,640 | 0.00% | 14,662 | 0.07% | 5,276 | 5.31% | 2,230 | 2.83% |
| Polyoma K virus | 164,919 | 0.00% | 7,282 | 0.00% | — | — | — | — |
| Poxvirus | 211,669 | 0.00% | 88,434 | 0.00% | — | — | — | — |
| Reovirus | 296,816 | 0.02% | 88,559 | 0.05% | 30,945 | 0.01% | 5,121 | 0.00% |
| Rotavirus | 405,210 | 0.04% | 128,919 | 0.05% | 11,464 | 0.00% | 194 | 0.00% |
| Theilovirus | 395,516 | 0.05% | 125,706 | 0.14% | 45,335 | 0.75% | 8,925 | 0.06% |

[a] **Abbreviations**: CMV= cytomegalovirus, MTLV = mouse thymic virus, LCMV = lymphocytic choriomeningitis virus, LDV = lactate dehydrogenase elevating virus, MAV = mouse adenovirus, MNV = murine norovirus, Parvovirus = for mice: minute virus of mice, mouse parvoviruses 1-5; for rats: Kilham rat virus (KRV, H-1 virus, rat minute virus (RMV) and rat parvovirus (RPV-1); PIV = parainfluenza virus; PVM pneumonia virus of mice; Polyomavirus including mouse polyomavirus and rat polyomavirus-2; K = mouse pneumonitis virus; Rotavirus group A for mice and group B for rats; Theilovirus comprises Theiler mouse encephalomyelitis virus (TMEV) and rat Theilovirus (RTV). Coronaviruses refer to mouse hepatitis virus (MHV) and rat coronavirus (RCV).
[b] Total number of mouse or rat samples tested by diagnostic methodology over 2016–2020 (5-y) interval.
[c] Estimated Percent Prevalence ($\%P_E$) = # of Positive Samples/# of Samples Tested formatted as a percentage

**Table 7.** CR-RADS 2016–2020 prevalence of parasites in murine samples from external clients

| | | Direct Exam | | PCR | |
|---|---|---|---|---|---|
| Host | Family | Samples [a] | $\%P_E$ [b] | Samples | $\%P_E$ |
| **Mouse** | Lice | 117,099 | 0.00% | — | — |
| | Mites | 117,584 | 0.02% | 157,855 | 1.77% |
| | Pinworms | 129,615 | 0.27% | 171,215 | 1.01% |
| | Protozoa | 117,039 | 10.10% | 112,812 | 13.01% |
| | **Mouse Total** | **136,299** | **8.80%** | **200,212** | **8.72%** |
| **Rat** | Lice | 14,612 | 0.00% | — | — |
| | Mites | 14,627 | 0.03% | 11,914 | 0.18% |
| | Pinworms | 14,938 | 1.04% | 13,025 | 2.24% |
| | Protozoa | 15,538 | 5.08% | 7,665 | 13.01% |
| | **Rat Total** | **15,890** | **5.64%** | **14,314** | **8.51%** |
| **Overall Total** | | **152,189** | **8.47%** | **214,526** | **8.71%** |

[a] Total number of mouse or rat samples tested by diagnostic methodology over 2016–2020 (5-y) interval.
[b] Estimated Percent Prevalence ($\%P_E$) = # of Positive Samples/# of Samples Tested formatted as a percentage

samples. The $\%P_E$ for lice by direct examination was 0.00% for both mice and rats. Combining mouse and rat results (for simplification), the $\%P_E$ by direct examination as compared with PCR were, respectively, 0.02% and 1.66% for mites, 0.40% and 1.09% for pinworms, and 9.5% and 13.0% for enteric protozoa. Compared with the $\%P_E$ by direct examination, PCR values were 84-fold higher for mites and 3-fold higher for pinworms. As illustrated in Figure 3, parasite surveillance by PCR grew during 2011 to 2015 and increased further during 2016 to 2020, when for the first time, more samples were screened for mites and pinworms by PCR than by direct examination. Over these same year intervals, the PCR $\%P_E$ declined for mites from 3.1% to 1.7% and for pinworms from 2.4% to 1.1%.

Table 8 shows the $\%P_E$ of selected parasite genera and species during the 2016 to 2020 interval by direct examination and/or PCR. Because the genus-species of parasites were not always determined or reported, the sample numbers for calculating the genus-species $\%P_E$ were lower than those shown in Table 7 for corresponding parasite families (that is, mites, pinworms, or protozoa).

The most prevalent mite genus was *Demodex*, with PCR $\%P_E$ of 3.5% in mice and 4.2% in rats, with the rat prevalence was


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**Table 8.** CR-RADS 2016–2020 prevalence of parasite species in murine samples from external clients

| | | Mouse | | | | Rat | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Direct Exam | | PCR Positive | | Direct Exam | | PCR | |
| **Type** | **Genus-Species** | **Samples** [a] | **%$P_E$** [b] | **Samples** | **%$P_E$** | **Samples** | **%$P_E$** | **Samples** | **%$P_E$** |
| Mites | *Myobia musculi* | 63,901 | 0.02% | 155,506 | 0.26% | 4,706 | 0.00% | 11,769 | 0.00% |
| | *Myocoptes musculinus* | 63,901 | 0.00% | 155,506 | 0.08% | 4,706 | 0.00% | 11,769 | 0.00% |
| | *Radfordia* spp. | 63,901 | 0.01% | 155,506 | 0.44% | 4,735 | 0.00% | 11,769 | 0.08% |
| | *Demodex* spp. | — | — | 45,076 | 3.52% | — | — | 214 | 4.21% |
| Pinworms | *Aspiculuris tetraptera* | 106,941 | 0.20% | 169,348 | 0.76% | 11,397 | 0.00% | 12,848 | 0.02% |
| | *Syphacia muris* | 106,941 | 0.00% | 169,348 | 0.04% | 11,397 | 1.34% | 12,848 | 2.14% |
| | *Syphacia obvelata* | 106,941 | 0.12% | 169,348 | 0.20% | 11,397 | 0.00% | 12,848 | 0.02% |
| Intestinal Protozoa | *Chilomastix* spp. | 15,977 | 2.75% | — | — | 1,094 | 0.09% | — | — |
| | *Cryptosporidium* spp. | 7,030 | 0.00% | 60,239 | 0.28% | 822 | 0.00% | 4,311 | 0.09% |
| | *Entamoeba* spp. | 15,977 | 5.81% | 75,934 | 9.48% | 1,094 | 8.87% | 5,915 | 14.74% |
| | *Giardia* spp. | 15,977 | 0.00% | 66,828 | 0.05% | 1,094 | 0.00% | 4,358 | 0.02% |
| | *Hexamastix* spp. | 15,977 | 1.32% | — | — | 1,094 | 0.37% | — | — |
| | *Retortamonas* spp. | 15,977 | 0.00% | — | — | 1,094 | 0.00% | — | — |
| | *Spironucleus* spp. | 15,977 | 0.02% | 86,517 | 1.66% | 1,094 | 0.00% | 6,666 | 1.14% |
| | *Tritrichomonas* spp. | 15,977 | 2.44% | 56,747 | 15.42% | 1,094 | 0.91% | 301 | 9.30% |

[a]Total number of mouse or rat samples tested by diagnostic methodology over 2016–2020 (5-y) interval. NB: Because the genus-species of parasites were not always reported, the sample totals on which the genus-species %P in this table were lower than those shown in Table 7 for corresponding parasite families (that is, mites, pinworms, or protozoa).
[b]Percent Prevalence Estimate (%$P_E$) = # of Positive Samples/# of Samples Tested formatted as a percent

**Table 9.** CR-RADS 2016–2020 prevalence of pathogenic bacteria and fungi in murine samples from external clients

| | | Mouse | | | | Rat | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Conventional | | PCR | | Conventional | | PCR | |
| **Conventional Methodology** | **Genus-Species** | **Samples** [a] | **%$P_E$** [b] | **Samples** | **%$P_E$** | **Samples** | **%$P_E$** | **Samples** | **%$P_E$** |
| **Culture and ID** | *Bordetella bronchiseptica* | 94,173 | 0.00% | 53,975 | 0.00% | 14,772 | 0.01% | 4,016 | 0.00% |
| | *Bordetella pseudohinzii* | 1,714 | 2.80% | 71,143 | 0.46% | — | — | — | — |
| | *Campylobacter* spp. | — | — | 70,371 | 0.37% | — | — | 4,169 | 1.13% |
| | *Citrobacter rodentium* | 103,990 | 0.00% | 86,376 | 0.06% | — | — | — | — |
| | *Corynebacterium bovis* | 4,815 | 2.93% | 107,079 | 2.26% | — | — | — | — |
| | *Corynebacterium kutscheri* | 119,667 | 0.00% | 83,958 | 0.01% | 15,235 | 0.00% | 5,295 | 0.19% |
| | *Klebsiella oxytoca* | 94,722 | 0.47% | 86,048 | 3.27% | 9,672 | 0.12% | 4,505 | 2.53% |
| | *Klebsiella pneumoniae* | 96,223 | 0.37% | 85,779 | 1.36% | 9,945 | 3.14% | 4,530 | 4.83% |
| | *Rodentibacter heylii* | 43,916 | 1.02% | 124,467 | 15.77% | 3,580 | 3.30% | 8,953 | 4.08% |
| | *R. pneumotropicus* | 80,209 | 0.62% | 124,501 | 10.17% | 9,561 | 0.28% | 8,936 | 3.27% |
| | *Proteus mirabilis* | 9,419 | 0.25% | 76,120 | 6.83% | 834 | 3.24% | 4,264 | 23.85% |
| | *Pseudomonas aeruginosa* | 102,805 | 1.97% | 74,968 | 1.75% | 10,777 | 1.44% | 4,327 | 3.54% |
| | *Salmonella* spp. | 115,134 | 0.05% | 86,296 | 0.00% | 15,092 | 0.07% | 5,512 | 0.00% |
| | *Staphylococcus aureus* | 98,979 | 2.18% | 78,411 | 5.04% | 10,413 | 23.63% | 4,698 | 37.97% |
| | *Staphylococcus xylosus* | — | — | 1,426 | 51.82% | — | — | — | — |
| | *B hemolytic Strep Group B* | 98,797 | 0.16% | 86,037 | 1.10% | 10,273 | 2.17% | 4,904 | 20.35% |
| | *B hemolytic Strep Group G* | 98,741 | 0.01% | 83,623 | 0.00% | 10,340 | 0.00% | 4,858 | 0.10% |
| | *B hemolytic Strep* spp. | 79,706 | 0.03% | — | — | 8,829 | 0.19% | — | — |
| | *Streptococcus pneumoniae* | 100,282 | 0.00% | 87,478 | 0.01% | 12,264 | 0.01% | 5,227 | 0.17% |
| | *Streptobacillus moniliformis* | 3,153 | 0.00% | 101,455 | 0.02% | — | — | 6,204 | 0.03% |
| **Serology** | *Clostridium piliforme* | 53,471 | 0.00% | 84,445 | 0.01% | 12,196 | 0.41% | 5,606 | 0.12% |
| | *Encephalitozoon cuniculi* | 125,561 | 0.00% | 5.263 | 0.00% | 14,596 | 0.15% | 300 | 0.00% |
| | *Filobacterium rodentium* | 134,935 | 0.00% | 59.759 | 0.00% | 17,132 | 0.13% | 3,898 | 0.05% |
| | *Mycoplasma pulmonis* | 328,731 | 0.01% | 82,346 | 0.09% | 35,736 | 0.05% | 8,200 | 0.04% |
| | *Pneumocystis* spp. | — | — | 82,150 | 0.17% | 42,833 | 4.07% | 7,005 | 0.71% |
| **None** | *Helicobacter* spp. | — | — | 214,846 | 13.79% | — | — | 17,697 | 5.00% |

[a]Total number of mouse or rat samples tested by diagnostic methodology over 2016–2020 (5-y) interval.
[b]Percent Prevalence Estimate (%$P_E$) = # of Positive Samples/# of Samples Tested formatted as a percent.


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**Table 10.** CR-RADS 2016–2020 prevalence of enterohepatic *Helicobacter* species in murine samples from external clients

| | Mouse | | Rat | |
|---|---|---|---|---|
| ***Helicobacter sp.*** | **Tested** [a] | **%P$_E$** [b] | **Tested** | **%P$_E$** |
| *H. bilis* | 105,100 | 2.09% | 9,468 | 0.18% |
| *H. ganmani* | 74,908 | 23.02% | 5,096 | 5.44% |
| *H. hepaticus* | 109,387 | 15.74% | 9,468 | 0.49% |
| *H. mastomyrinus* | 74,903 | 18.21% | 5,096 | 1.06% |
| *H. rodentium* | 74,897 | 1.15% | 5,096 | 6.71% |
| *H. typhlonius* | 74,908 | 23.51% | 5,096 | 1.20% |

[a]Total number of mouse or rat samples tested by diagnostic methodology over 2016–2020 (5-y) interval. NB:
[b]Percent Prevalence Estimate (%P$_E$) = # of Positive Samples/# of Samples Tested formatted as a percent

based on just 214 samples. The PCR %P$_E$ of *Radfordia* were 0.44% in mice and 0.08% in rats; 99% of the *Radfordia* in mice were *R. affinis*, whereas in rats, 89% were *R. ensifera*.

By both direct examination and PCR, the pinworm species found most often in mice was *Aspiculuris tetraptera* followed by *Syphacia obvelata*, with PCR %P$_E$ of 0.8% and 0.2%, respectively. Practically all pinworms identified in rats were *Syphacia muris*, with a PCR %P$_E$ of 2.1%.

The protozoan endoparasites identified by direct examination in more than 1% of mice were *Chilomastix*, *Entamoeba*, *Hexamastix*, and *Tritrichomonas* (with %P$_E$ of 2.8%, 5.8% 1.3%, and 2.4%, respectively). Those found in more than 1.0% of mice by PCR were *Entamoeba*, *Spironucleus*, and *Tritrichomonas* (with %P$_E$ of 9.5%, 1.7%, and 15.4%, respectively). In rats, while only *Entamoeba* was found in more than 1% of animals by direct

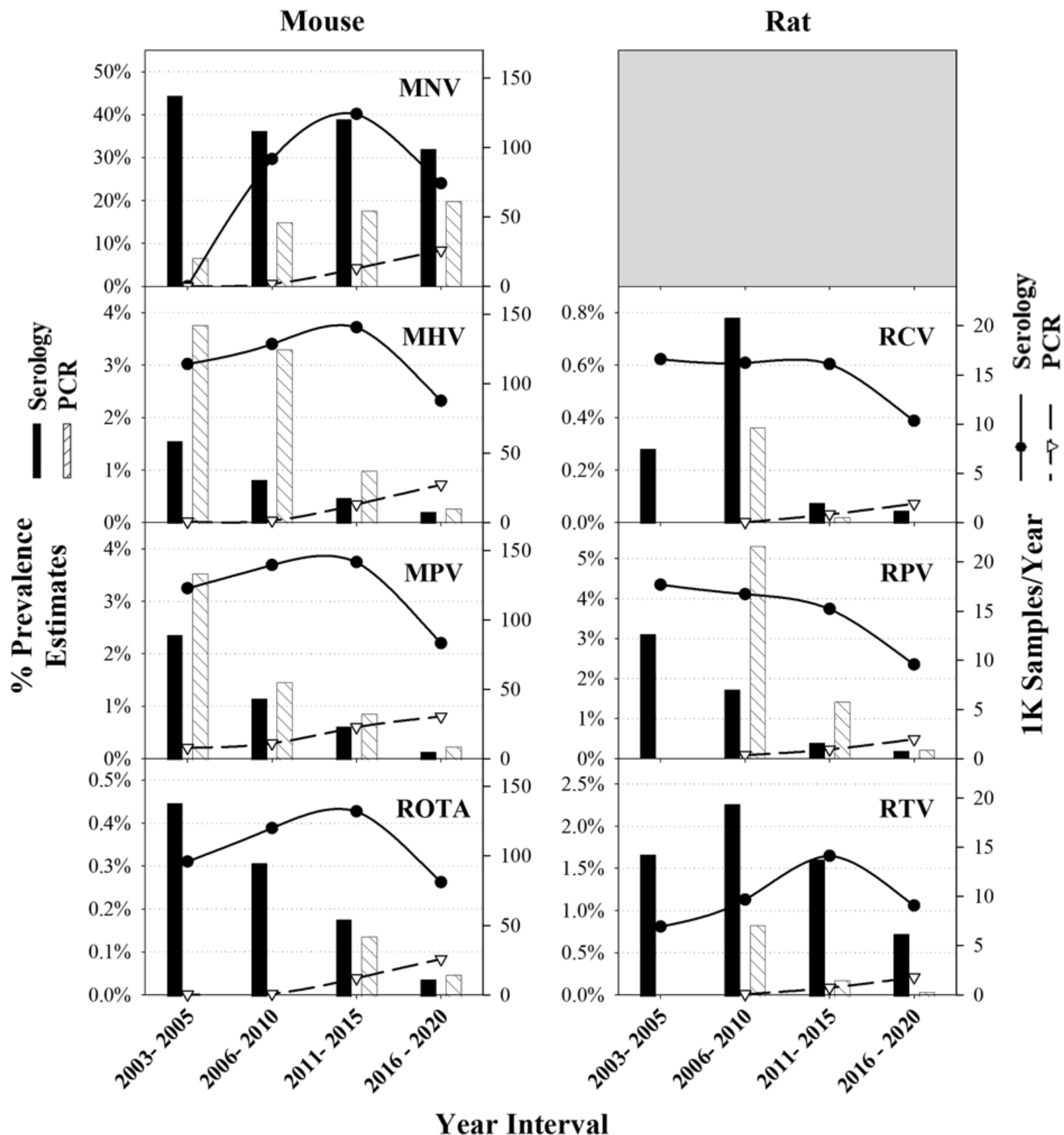


**Figure 2.** CR-RADS Assessment of HM for Viruses in Murine Samples from External Clients. Data were plotted by virus, HM methodology (that is, serology compared with PCR) and multiyear interval, with % prevalence estimates represented by bar graphs and samples tested annually by line graphs. Coronaviruses of rodents include mouse hepatitis virus (MHV) and rat coronavirus (RCV).


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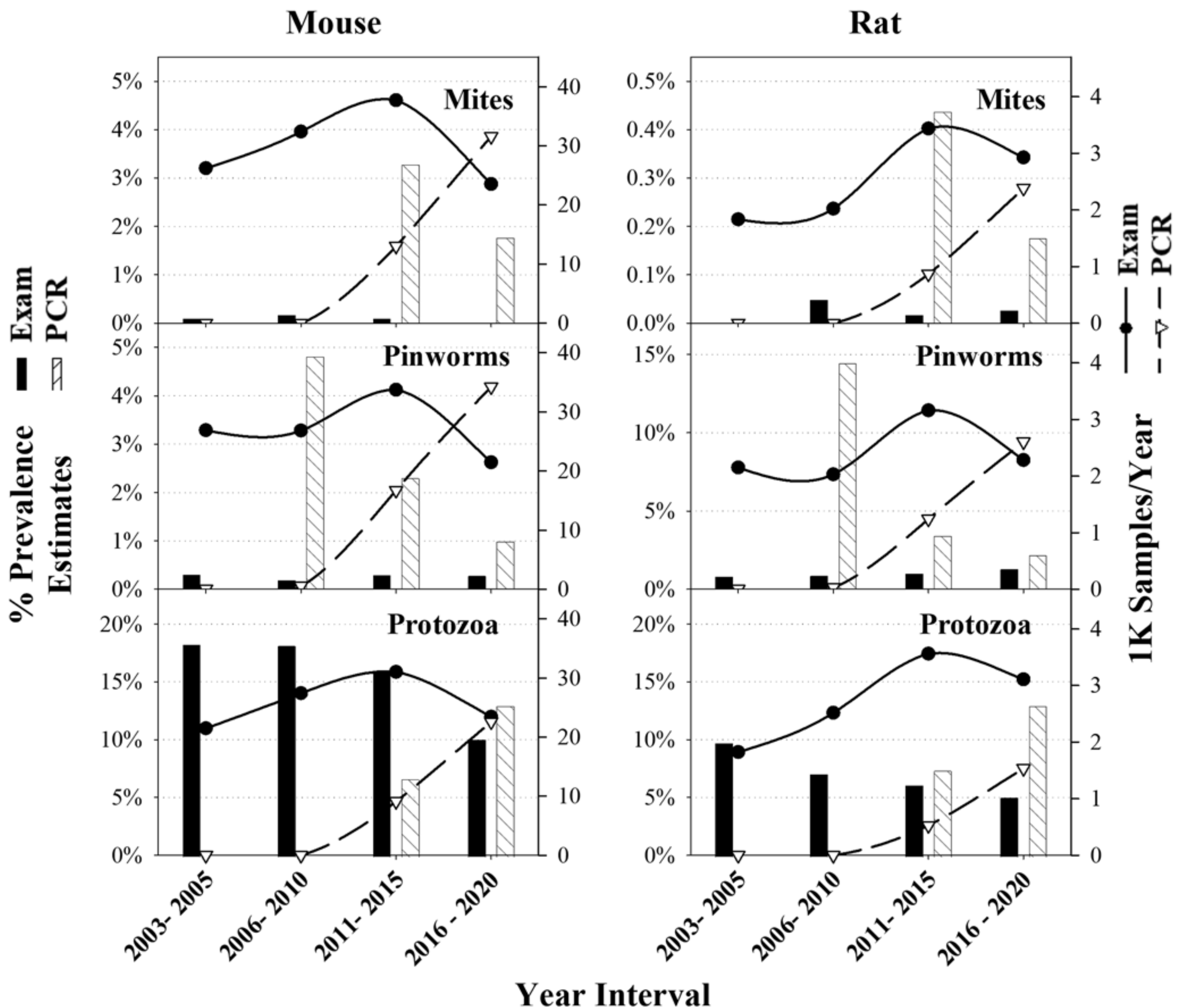


**Figure 3.** CR-RADS Assessment of HM for Parasites in Murine Samples from External Clients. Data were plotted by parasite family, HM methodology (that is, direct examination compared with PCR) and multiyear interval, with % prevalence estimates represented by bar graphs and sample tested annually by line graphs.

examination (with a $\%P_E$ of 8.9%), by PCR, the $\%P_E$ exceeded 1.0% for *Entamoeba* (14.7%), *Spironucleus* (1.1%), and *Tritrichomonas* (9.3% of just 312 samples). PCR results are not reported for *Chilomastix*, *Hexamastix*, and *Retortamonas* because assays for these agents were not in routine use or had not yet been developed during the study period. For protozoa monitored by both methodologies, however, $\%P_E$ by PCR were consistently higher than by direct examination, particularly for the pathogenic protozoa *Cryptosporidium*, *Giardia*, and *Spironucleus*.

***Bacteria and fungi.*** Table 9 shows the $\%P_E$ for bacterial pathogens and the fungi *Encephalitozoon cuniculi* and *Pneumocystis* during the 2016 to 2020 interval. The conventional diagnostic methodology for most bacteria was cultural isolation and identification, but serology was the primary conventional approach to monitor for invasive, fastidious bacterial and fungal pathogens that elicit a strong humoral immune response. Only PCR was used to screen mice for *Pneumocystis*, and mice and rats for *Campylobacter* and *Helicobacter*.

For bacterial pathogens that were routinely monitored by cultural isolation and PCR, the ones isolated most frequently from mice were *Bordetella pseudohinzii*, *Corynebacterium bovis*, *Rodentibacter heylii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (with $\%P_E$ of 2.8%, 2.9%, 1.0%, 2.0%, and 2.2%, respectively); those isolated most often from rats were *Klebsiella pneumoniae*, *Proteus mirabilis*, *Rodentibacter heylii*, *Staphylococcus aureus*, and β hemolytic *Streptococcus* Group B (with $\%P_E$ of 3.1%, 3.2%, 3.3%, 23.6%, and 2.2%, respectively). Although the $\%P_E$ by PCR and cultural isolation were often comparable, PCR $\%P_E$ were markedly higher for certain bacteria. For instance, *Rodentibacter heylii* and *Rodentibacter pneumotropicus* were detected in 15.8% and 10.2% of mice by PCR, compared with just 1.0% and 0.6% by cultural isolation.

For bacteria and fungi that were identified for HM by serology and PCR, the $\%P_E$ in mice were 0.01% or less for *Clostridium piliforme* (the etiology of Tyzzer's disease), *Encephalitozoon cuniculi*, *Filobacterium rodentium* (a.k.a., cilia-associated respiratory bacillus), and *Mycoplasma pulmonis*, regardless of methodology. For rats, the $\%P_E$ for *Clostridium piliforme* and *Pneumocystis carinii* were, respectively, 0.4% and 4.1% by serology compared with 0.1% and 0.7% by PCR.


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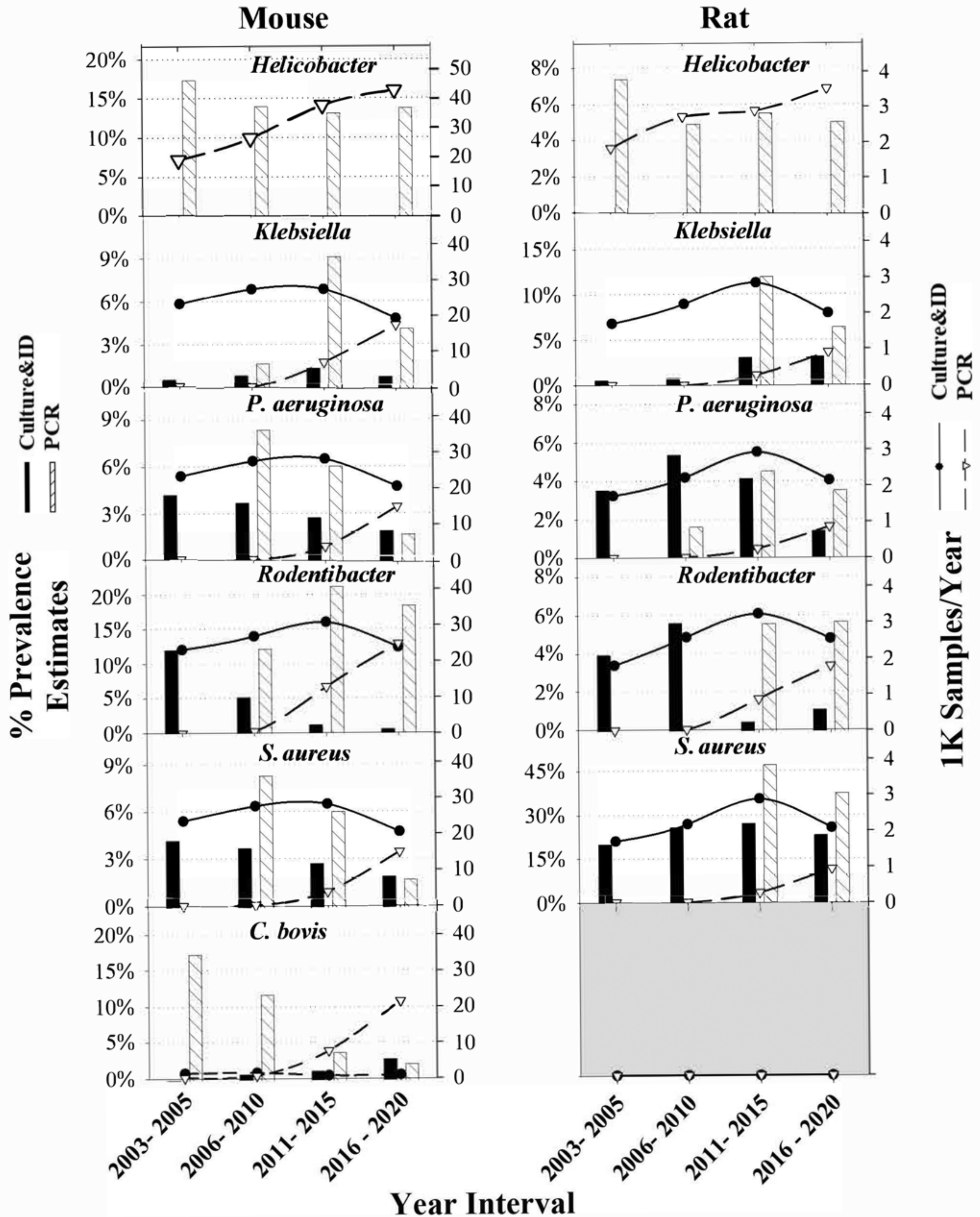


**Figure 4.** CR-RADS Assessment of HM for Bacteria in Murine Samples from External Clients. Data were plotted by bacterial genus-species, HM methodology (that is, conventional compared with PCR) and multiyear interval, with % prevalence estimates represented by bar graphs and samples tested annually by line graphs.



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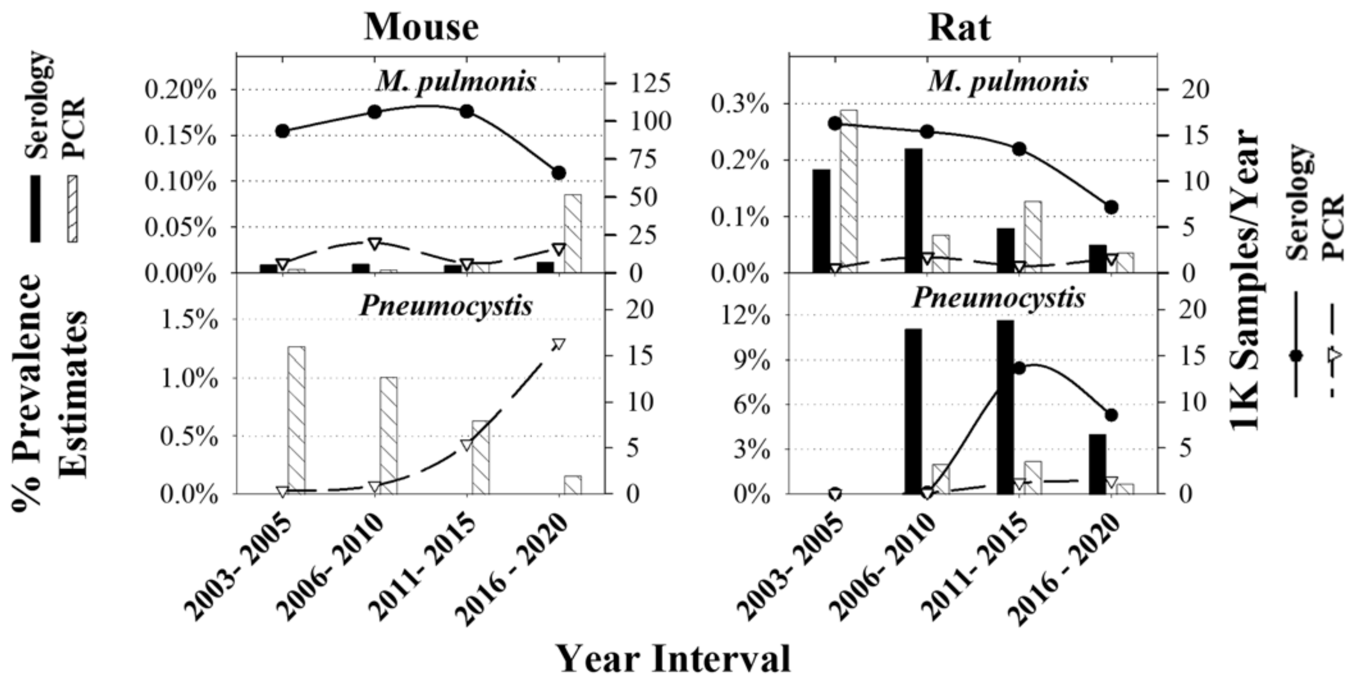


**Figure 5.** Comparison of CR-RADS Assessment of Murine Samples submitted by External Clients for *M. pulmonis* and *Pneumocystis* spp. by Serology compared with PCR. Data were plotted by parasite family, HM methodology (that is, serology or PCR) and multiyear interval, with % prevalence estimates represented by bar graphs and the sample tested annually by line graphs.

The PCR $\%P_E$ for *Pneumocystis* was 0.2% in mice and for *Helicobacter spp*. were 13.8% in mice and 5.0% in rats. Of the 6 rodent enterohepatic *Helicobacter* species in Table 10, those with $\%P_E$ exceeding 15% in mice were *H. ganmani*, *H. hepaticus*, *H. mastomyrinus*, *and H. typhlonius*. In rats, the most prevalent species were *H. ganmani* and *H. rodentium*, identified in 5.4% and 6.7% of samples, respectively. The higher $\%P_E$ of individual species compared with the overall prevalence of *Helicobacter* occurred because species-specific assays were performed on a subset of samples, typically those first shown to be positive by the generic *Helicobacter* spp. PCR. Thus, the $\%P_E$ of 23% for *H. ganmani* represents just 8.0% of the 214,846 samples tested by PCR for *Helicobacter* spp.

## Discussion

This report summarizes the results of CR-RADS HM performed between 2003 and 2020 on over 3 million samples from external (non-Charles River) mouse and rat research colonies in North America, Europe, and Japan. We did not summarize results by geographic region because of the complexity of presenting results by region in tables and figures. Charles River Rodent Production colonies were not included because the prevalence of infections of these colonies for several agents is atypically low due to rigorous biosecurity and rapid depopulation of contaminated colonies.

As noted in the Introduction, each LIMS test was assigned a microbial taxonomy and a diagnostic methodology. Table 2 shows that the conventional methodologies (those other than PCR) typically apply to one or 2 pathogen kingdoms (direct examination for parasites, cultural isolation for bacteria, and serology for viruses and certain invasive fungal and bacterial pathogens). By contrast, PCR is broadly applicable to all microbial taxonomic kingdoms.

The percentages of samples, per year or multiyear interval, that were pathogen-positive by a diagnostic methodology are reported as period prevalence estimates, abbreviated as $\%P_E$. However, because our data were derived by testing client-selected rather than randomly selected samples, the $\%P_E$ do not strictly meet the definition of period prevalence.[15] On the other hand, as the reported $\%P_E$ were derived from large numbers of samples from many institutions, we believe they provide a reasonable estimate of prevalence and are consistent with the use of "prevalence" in studies comparable to ours.[30,33,42]

A limitation inherent to diagnostic testing is that results may be inaccurate because of sampling and laboratory errors or due to the limits of an assay's diagnostic sensitivity and specificity. The ability of popular cage-level barrier systems to impede the spread of infection can keep the prevalence of infection low, decreasing the predictive value of positive results (that is, the likelihood that positive results are true positives).[25,28,57]

The samples tested and $\%P_E$ by methodology reported in this study support the growing reliance of HM on PCR surveillance and the more frequent detection by PCR assays of certain pathogens, particularly those not readily transmitted to sentinels. The advantages of PCR that have led to its increased use relative to other diagnostic methodologies include its applicability to all types of pathogens and its ability to specifically detect even minute levels of pathogen genomic sequences in samples collected from the environment or antemortem directly from colony rodents. By contrast, HM by conventional methodologies is typically reliant on the testing sentinels that are exposed to infectious agents in a colony through routine transfers of soiled bedding. This approach is problematic for several reasons. First, fomite transmission to sentinels is not effective for important host-adapted and environmentally labile pathogens.[17,24,26,37,46] Moreover, soiled bedding might not transfer infection, even of an environmentally stable pathogen, if the dose to which sentinels are exposed is subinfectious because: 1) the prevalence of colony infection is low, as is common for cage-level barrier systems, or 2) sentinels are resistant to infection due to their age


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or genetic background.[1,6,18,19,21,27] Finally, the sentinels may test positive after infections from sources other than the colony being monitored. For instance, adventitious infection of sentinels could have occurred prior to their placement while in transit or quarantine.[50]

The findings reported here also show that the $\%P_E$ for many pathogens, including once common adventitious agents such as Sendai virus, PVM, and the rodent coronaviruses MHV and SDAV, have fallen below one percent, This decrease in prevalence reflects advances in HM and stricter adherence to biosecurity practices that include disinfection of supplies, the widespread adoption of cage-levels barrier systems, and the elimination of infected colonies by depopulation or rederivation. Notwithstanding these quality control (QC) enhancements, the $\%P_E$ for some rodent pathogens have remained high, approximately 5% or above. For example, mouse infections with MNV, *Helicobacter*, *Rodentibacter,* and parasites have become prevalent in association with the decentralized production and frequent exchange of GEM mice by investigators at institutions whose pathogen QC practices and exclusion requirements vary. These infections were overlooked when GEM rodents first gained popularity in the 1990 s for several reasons. For example, MNV had yet to be discovered. Furthermore, HM relied largely on using conventional diagnostic methodologies to surveil soiled-bedding sentinels This approach is considerably less effective at detecting colony infections than is PCR assessment of colony animal and environmental samples, particularly with host-adapted, environmentally labile microorganisms like *Helicobacter* and *Rodentibacter* and with parasites transmitted most efficiently by contact. Because MNV and the latter bacteria were so widespread, eliminating them from colonies has been considered out of reach at many research institutions. The same situation will also likely arise for prevalent rodent viruses and other infectious agents that are now being discovered at an accelerated pace through the application of advanced molecular genetic techniques, in particular next-generation sequencing. Opportunistic bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Klebsiella spp.* have also remained prevalent, as they are ubiquitous and are viable in the environment; consequently, they have not been consistently excluded from barrier rooms and research colonies.[3,47]

With the introduction of parasite PCR to HM programs starting circa 2010, rodent colonies thought to be free of mites and pinworms based on conventional, soiled bedding sentinel surveillance were unexpectedly shown by PCR to be infested.[22,36] This finding once again highlights the benefits of PCR surveillance, given its ability to find parasites in samples collected directly from colony animals and the environment.[13,22] Improved detection and antiparasite medications have promoted a decrease in the $\%P_E$ of mites and pinworms in research colonies.

Testing frequency is mainly affected by incidence (rate) of new infections;[48] however, reliably determining the incidence of adventitious infections is problematic because clients often do not provide to the testing laboratory with the identity of the rodent colonies or rooms sampled. High prevalence can be used instead of incidence as the basis for frequent monitoring of SPF colonies for pathogens found elsewhere in the facility, for rodents intended for import and in quarantine; and for colonies after biosecurity breaches such as the incursion of feral or wild rodents. Still, frequent monitoring should also be performed for certain low prevalence pathogens that nonetheless commonly cause adventitious infections. Examples include rodent coronaviruses and parvoviruses that are found here to have $\%P_E$ of less than 0.5%.

Notwithstanding the clear advantages of nonsentinel surveillance by PCR, using multiple diagnostic methodologies has important benefits for a robust HM program. Confirming the pathogen status by complementary methodologies is especially important for confirming diagnoses and for managing murine breeding colonies that supply animals for research. Sole reliance on PCR may miss an adventitious infection if the PCR is not performed frequently, the standard sample types are not appropriate, or the active infection is short-lived. As an example, the $\%P_E$ in rats reported herein for MNV, *Clostridium piliforme* and *Pneumocystis carinii,* were higher by serology than by PCR. Finally, designing PCR that are sufficiently inclusive to detect all variants of a pathogen but will still exclude nonpathogens such as commensal bacteria is a complex and ongoing task that may be driven by the results of alternative methodologies.

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