*Paper*

# Contemporary prevalence of infectious agents in laboratory mice and rats

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## Abstract

**Periodic health screening of rodents used in research is necessary due to the consequences of unwanted infections. One determinant of the risk of infection for any given agent is its prevalence; other factors being equal, a prevalent agent is more likely than a rare one to be introduced to a research facility and result in infection. As an indicator of contemporary prevalence in laboratory populations of rats and mice, the rate of positive results in the samples received at a major commercial rodent diagnostic laboratory was compiled for this paper. Although samples from laboratory rodent vendors have been excluded, results are tabulated from samples from more than 500,000 mice and 80,000 rats submitted over several years from pharmaceutical, biotechnology, academic, and governmental institutions in North America and Europe, allowing meaningful determination of which agents are common in the research environment versus which agents are rare. In mice, commonly detected infectious agents include mouse norovirus, the parvoviruses, mouse hepatitis virus, rotavirus, Theiler's murine encephalomyelitis virus, *Helicobacter* spp., *Pasteurella pneumotropica*, and pinworms. In rats, commonly detected infectious agents include 'rat respiratory virus', the parvoviruses, rat theilovirus, *Helicobacter* spp., *P. pneumotropica*, and pinworms. A risk-based allocation of health-monitoring resources should concentrate frequency and/or sample size on these high-risk agents, and monitor less frequently for the remaining, lower-risk, infectious agents.**

**Keywords:** Animals, laboratory, seroepidemiologic studies, mice, rats, rodent diseases

*Laboratory Animals* 2009; **43**: 165–173. DOI: 10.1258/la.2008.008009

Health surveillance of laboratory animals is conducted because adventitious infection is a realistic possibility and has deleterious effects on animal welfare and research; there is both significant risk and significant consequence. However, health monitoring of laboratory animals can be a substantial drain on finite institutional funds. To best allocate limited health-monitoring budgets, consideration should be given to the species, strain or stock, and immune status of the animals in a facility when one designs a health-monitoring scheme. In addition, the likelihood of these animals actually encountering any individual infectious agent should also be considered. The best use of limited health surveillance resources is to correlate monitoring frequency with the risk of contamination, frequently testing a representative number of appropriately chosen animals for the most prevalent agents in an area and infrequently testing for rare agents.[1–3] This risk-based approach requires knowledge of the general prevalence of infectious agents, which allows one to predict which agents are likely to contaminate a facility. Prevalence is defined as the total number of cases of a given disease in a specified population at a specified time. Simply put, 'prevalence' means 'proportion' (typically expressed as a percentage of diseased animals over a total number of animals). In this paper, prevalence is used as the proportion of positive samples in the 'population' of samples passing through a large commercial rodent diagnostic laboratory. For these purposes, a positive sample is defined as antibody-positive for agents detected by serology, whereas for other agents, a positive result indicates the agent was directly detected by polymerase chain reaction (PCR), culture or microscopic observation.

Other factors being equal, prevalent agents pose a higher risk than rare agents. Prevalence data also help in the estimation of the predictive value of surveillance results, which in turn can assist in determining the course of action when results are received. The predictive value of a negative result is the proportion of all negative results, i.e. the sum of true negatives and false negatives, which are true negatives. The predictive value of a positive result on an assay is more often of concern, and is the proportion of

all positive results, true positives plus false positives, that are true positives. Thus, the closer the rate of true positive results, i.e. prevalence, is to 0, the lower the predictive value of a positive result. In reality, the rate of false positives (1-specificity) and false negatives (1-sensitivity) is never known for an assay. Predictive value is nonetheless an important concept as it indicates that a positive assay result for an agent of low prevalence, such as reovirus, is likely to be a false positive and must be confirmed prior to taking any action which could disrupt a research programme. Knowledge of the general prevalence of infectious agents in laboratory animal populations, therefore, is helpful in knowing how much weight to accord a positive assay result.

Previous reports of laboratory rodent disease prevalence have incorporated surveys and self-reporting of positive findings by institutions as well as the compilation of data by individual institutions, reference laboratories and commercial laboratories.[4,5] Charles River's Research Animal Diagnostic Services (Charles River) is in the uncommon position of being able to compile, anonymize and report data for a large number of individual animal samples from North America and Europe, over a five-year period. These results, which include the largest series of samples yet reported, confirm the reported changes in the prevalence of many infectious agents in laboratory rats and mice over the last few decades, with some emerging while others have virtually disappeared, and provide a current basis for modifying health surveillance schemes to target contemporary agent prevalence profiles.

## Materials and methods

Data were compiled using software (Internet Laboratory Information Management System; ILIMS) developed at Charles River. Data comprised all samples submitted for the reporting period, excluding those from production colonies owned by Charles River. The reporting period was approximately five years for the North American laboratory (Wilmington, MA, USA) and approximately three years for the European laboratory (Les Oncins, France). Both laboratories used the ILIMS software and reagents produced at the Wilmington laboratory. Together, the two laboratories received more than 100,000 serum samples and more than 30,000 animals for necropsy each year from external (non-Charles River) sources. In the sample stream, mice outnumber rats approximately 5:1. The client base of the laboratories included more than 500 clients, predominantly from North America, followed by Western Europe, with a relatively small number of clients submitting samples from Asia and elsewhere. Most clients were in the pharmaceutical and biotechnology industries, with much smaller numbers being submitted from academia and various governmental institutions. A few samples were also received from the pet industry. This study was a survey of all samples and animals submitted. No clients were excluded from reporting. Animals and samples were assigned accession numbers on receipt at the laboratory. Samples were then labelled and managed using these accession numbers. Laboratory workers were unaware of the provenance of the samples and animals, i.e. where the animals were bred or raised.

### Serology

For all viral agents, except mouse thymic virus (MTV), rat respiratory virus (RRV), and lactate dehydrogenase elevating virus (LDHV), the primary assay for the early part of the compilation period was an enzyme-linked immunoassay, confirmed by either an indirect fluorescent antibody assay (IFA) or haemagglutination inhibition assay (HAI). During the last two years of the compilation period, the primary assay changed to another solid-phase immunoassay, the Multiplexed Fluorometric ImmunoAssay™ (MFIA™), which uses antigen-coated beads placed in microtiter plates rather than antigen-coated microtiter plates. For MTV, an IFA was the primary test method. For RRV, histopathology was the primary test method. For LDHV, a chemical assay of the quantity of LDH in the serum was the primary test method. Tables 1 and 2 list the agents routinely tested in mice and rats, the primary assay and confirmatory test method used.

### Bacteriology

While most serology samples were shipped to the laboratories separately, the majority of bacteriology samples were collected at necropsy of animals shipped to the laboratories. All routine microbiologic cultures were aerobic. Sites sampled, media, and target organisms were given in Table 3. These cultures of the respiratory and gastrointestinal tract were considered primary cultures by our laboratories. Target organisms were those listed on our exclusion list, or reported on our health-monitoring reports. The ILIMS software compiled assay numbers by agent, based on all primary cultures for which that agent was a target organism. Thus, the total test numbers for some bacteria were roughly double those for others, since those bacteria were targeted by more than one primary culture per animal. For example, *Pasteurella pneumotropica* was targeted in both respiratory and intestinal cultures. Although it is beyond the scope of this paper to detail the identification of each bacterium, identification of *P. pneumotropica* merits brief mention. Suspect colonies on the primary culture media, usually blood agar, were further identified using API 20NE strips. We have recently become aware that a minority of colonies identified as *P. pneumotropica* by biochemical methods are negative by PCR assay for the two biotypes, Jawetz and Heyl (unpublished data). Thus, the percentage of positive samples for *P. pneumotropica* may overestimate the actual prevalence in our sample stream. In addition to routine samples collected at necropsy, these results included cultures taken directly from abscesses and other lesions, as well as culture swabs shipped to the laboratory. Not all bacterial colonies that grow on primary culture were identified; generally only those with characteristics compatible with the target organisms were further characterized. It should also be noted that ILIMS only compiles data for those bacteria that were

**Table 1** Mouse serology: agents and methods

| Agent (abbreviation) | Method | |
|---|---|---|
| | Primary | Confirmation |
| Cilia-associated respiratory bacillus (CARB) | MFIA/ELISA | IFA |
| Ectromelia virus (ECTRO) | MFIA/ELISA | IFA |
| *Encephalitozoon cuniculi* (ECUN) | MFIA/ELISA | IFA |
| Epizootic diarrhoea of infant mice virus (EDIM) | MFIA/ELISA | IFA |
| Hantavirus (HNT) | MFIA/ELISA | IFA |
| Lactate dehydrogenase-elevating virus (LDHV) | Enzyme | PCR |
| Lymphocytic choriomeningitis virus (LCMV) | MFIA/ELISA | IFA |
| Minute virus of mice (MVM, MMV) | MFIA/ELISA | IFA, HAI |
| Mouse adenovirus FL/K87 (MAV, MAV 1 and 2) | MFIA/ELISA | IFA |
| Mouse cytomegalovirus (MCMV) | MFIA/ELISA | IFA |
| Mouse hepatitis virus (MHV) | MFIA/ELISA | IFA |
| Mouse parvovirus (MPV 1 and 2) | MFIA/ELISA | IFA |
| Mouse pneumonitis virus (K) | MFIA/ELISA | IFA |
| Mouse thymic virus (MTV, MTLV) | IFA | PCR |
| Murine norovirus (MNV) | MFIA/ELISA | IFA |
| *Mycoplasma pulmonis* (MPUL) | MFIA/ELISA | IFA, PCR |
| Pneumonia virus of mice (PVM) | MFIA/ELISA | IFA, HAI |
| Polyoma virus (POLY) | MFIA/ELISA | IFA |
| Reovirus (REO, REO-3) | MFIA/ELISA | IFA, HAI |
| Sendai virus (SEND) | MFIA/ELISA | IFA, HAI |
| Theiler's murine encephalomyelitis virus (TMEV, GD-VII) | MFIA/ELISA | IFA |

Agents tested by screening of dilute serum for antibodies are listed in the left column; both the common name and the common abbreviation are given. The primary assay method is listed in the centre column. For most assays, the multiplexed fluorometric immunoassay (MFIA) is the current method, although the compilation period extends back into the time when the enzyme-linked immunosorbent assay (ELISA) was predominant. LDV is screened by assessing lactate dehydrogenase (LDH) activity in whole serum. For MTV, the primary assay is the indirect fluorescent antibody (IFA) assay. Any time a positive or equivocal result is noted in the primary assay, the sample is retested by the confirmatory assay, listed for each agent in the right column. PCR: polymerase chain reaction; HAI: haemagglutination inhibition assay

**Table 2** Rat serology: agents and methods

| Agent (abbreviation) | Method | |
|---|---|---|
| | Primary | Confirmation |
| Cilia-associated respiratory bacillus (CARB) | MFIA/ELISA | IFA |
| *Encephalitozoon cuniculi* (ECUN) | MFIA/ELISA | IFA |
| Hantavirus (HNT) | MFIA/ELISA | IFA |
| Kilham rat virus (RV, KRV) | MFIA/ELISA | IFA, HAI |
| Lymphocytic choriomeningitis virus (LCMV) | MFIA/ELISA | IFA |
| Mouse adenovirus FL/K87 (MAV, MAV 1 and 2) | MFIA/ELISA | IFA |
| *Mycoplasma pulmonis* (MPUL) | MFIA/ELISA | IFA, PCR |
| Pneumonia virus of mice (PVM) | MFIA/ELISA | IFA, HAI |
| Rat minute virus (RMV) | MFIA/ELISA | IFA |
| Rat parvovirus (RPV) | MFIA/ELISA | IFA |
| Rat theilovirus (RTV; formerly GD-VII assay) | MFIA/ELISA | IFA |
| Reovirus (REO, REO-3) | MFIA/ELISA | IFA, HAI |
| Sendai virus (SEND) | MFIA/ELISA | IFA, HAI |
| Sialodacryoadenitis/rat coronavirus (SDAV, RCV) | MFIA/ELISA | IFA |
| Toolan's H-1 virus (H-1) | MFIA/ELISA | IFA, HAI |

Agents tested by screening of dilute serum for antibodies are listed in the left column; both the common name and the common abbreviation are given. The primary assay method is listed in the centre column. For most assays, the multiplexed fluorometric immunoassay (MFIA) is the current method, although the compilation period extends back into the time when the enzyme-linked immunosorbent assay (ELISA) was predominant. Any time a positive or equivocal result is noted in the primary assay, the sample is retested by the confirmatory assay, listed for each agent in the right column. IFA: indirect fluorescent antibody assay; HAI: haemagglutination inhibition assay; PCR: polymerase chain reaction

routinely targeted in particular assays. All other identifications were simply tabulated under 'other', even if a bacterium with potential health or research implications, but not listed as a targeted organism, were detected (data not shown).

### Molecular diagnostics

For *Pneumocystis* spp. and *Helicobacter* spp., PCR was used as the primary assay, using samples of lung and faecal pellets, respectively. *Pneumocystis* was assessed routinely only in immunodeficient rats and mice. In addition, PCR was used as both a primary and confirmatory test for *Corynebacterium bovis*, as well as a confirmatory test for the agents listed in Tables 1 and 2, and sometimes for *P. pneumotropica*.

### Parasitology

The majority of parasitology samples were collected at necropsy. Screening for ectoparasites was conducted by the examination of the pelt under a stereoscopic microscope, with particular emphasis on the dorsum.[6] The primary evaluation for helminth infestation was by the examination of macerated cecum and colon with stereoscopic microscopy. Intestinal protozoa were assessed primarily by high-magnification phase-contrast microscopy of a wet mount of duodenal and cecal mucosal scrapings. Confirmation of protozoa was by the evaluation of the sample by a second technician, as well as by a faecal concentration and centrifugation technique.

### Necropsy and histopathology

All rats and mice submitted live were euthanized with carbon dioxide and a gross necropsy was performed. Lungs from rats were gently inflated with 10% neutral buffered formalin for histopathological screening of haematoxylin and eosin-stained four micron sections for lesions characteristic of the tentatively named, uncharacterized, RRV. All slides were examined by a board-certified (American College of Veterinary Pathologists) veterinary pathologist, and all positive RRV findings were confirmed by a second board-certified veterinary pathologist.

## Results

### Viruses

The prevalence of viral antibodies in laboratory mice and rats in Europe and North America is given in Tables 4 and 5. The most prevalent viruses in Europe were also most prevalent in North America, and *vice versa*. Charles River's North American laboratory has been in operation longer than its European laboratory, and has contributed

**Table 3** Rat and mouse routine microbiologic culture

| Topography | Media | Target agents |
|---|---|---|
| Respiratory tract (nasopharyngeal lavage) | Mycoplasma media enriched with horse serum | *Streptobacillus moniliformis* (currently tested by PCR) |
| | Sheep blood agar, Tergitol-7, Mycoplasma media | *Bordetella bronchiseptica*, $\beta$-haemolytic *Streptococcus* spp. (Serologic groups A, B and G), *Streptococcus pneumoniae*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, *Klebsiella pneumoniae* and *oxytoca*, *Pasteurella pneumotropica* and other *Pasteurella* spp., *Pseudomonas aeruginosa* and other *Pseudomonas* spp., *Staphylococcus aureus* |
| Gastrointestinal tract (cecum) | Selenite enrichment broth, then incubation in Hektoen | *Salmonella* spp. |
| | Pseudomonas isolation agar | *Pseudomonas aeruginosa* and *Pseudomonas* other spp. |
| Gastrointestinal tract (colon, mice only) | Selenite enrichment broth, then incubation in Hektoen | *Salmonella* spp. |
| | Pseudomonas isolation agar | *Pseudomonas aeruginosa* and other *Pseudomonas* spp. |
| | Tergitol-7 | *Citrobacter rodentium* and other *Citrobacter* spp., *K. pneumoniae* and *oxytoca* |

The site from which routine screening samples are collected from immunocompetent animals are listed in the left column. *Topography*: From each site, samples are cultivated onto multiple media, listed in the centre column, to target specific agents of concern or interest, listed in the right column. It is these agents for which results are compiled, although any bacterial colonies that grow on the media may also be identified

**Table 4** Mouse surveillance results: viruses

| Agent (assay abbreviation) | Method | *N* | Prevalence (%) NA | Europe | Total |
|---|---|---|---|---|---|
| Ectromelia (ECTRO) | Serology | 246,857 | 0.02 | 0.00 | 0.02 |
| Hantavirus (HANT) | Serology | 144,946 | 0.00 | 0.00 | 0.00 |
| K virus (K) | Serology | 225,353 | 0.00 | 0.00 | 0.00 |
| Lymphocytic choriomeningitis virus (LCMV) | Serology | 241,453 | 0.01 | 0.02 | 0.01 |
| Mouse adenovirus 1 and 2 (MAV) | Serology | 230,351 | 0.02 | 0.22 | 0.02 |
| Mouse cytomegalovirus (MCMV) | Serology | 146,511 | 0.04 | 0.00 | 0.04 |
| Mouse hepatitis virus (MHV) | Serology | 558,673 | 1.57 | 3.25 | 1.59 |
| Mouse norovirus (MNV) | Serology | 44,876 | 32.64 | 24.03 | 32.37 |
| Parvovirus generic assay (NS-1) | Serology | 578,464 | 1.65 | 1.92 | 1.65 |
| Mouse parvovirus 1 and 2 (MPV) | Serology | 594,539 | 1.83 | 3.64 | 1.86 |
| Mouse minute virus (MMV, MVM) | Serology | 595,903 | 0.33 | 0.46 | 0.33 |
| Pneumonia virus of mice (PVM) | Serology | 447,656 | 0.01 | 0.01 | 0.01 |
| Polyoma virus (POLY) | Serology | 225,868 | 0.02 | 0.20 | 0.02 |
| Reovirus 3 (REO, REO-3) | Serology | 428,821 | 0.01 | 0.05 | 0.01 |
| Rotavirus (EDIM) | Serology | 466,572 | 0.56 | 0.35 | 0.56 |
| Sendai virus (SEND) | Serology | 462,209 | 0.00 | 0.00 | 0.00 |
| Theiler's murine encephalomyelitis virus (TMEV, GD-VII) | Serology | 435,772 | 0.26 | 0.27 | 0.26 |

Viruses for which screening is conducted are listed in the left column; both the common name and the common abbreviation are given. The next column to the right gives the primary method of screening for this agent. For all mouse viruses, serology is the primary method. The next column to the right gives the total number of samples included in this compilation. For each agent, the percent of positive results is given in the next two columns for North America (NA) and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each agent. It is this number that is used as an estimate of prevalence

more data to this data-set. This explains the skew of the total prevalence towards the North American results. In mice, the recently discovered mouse norovirus (MNV) is the most prevalent agent, with a total prevalence (32%) more than 10-fold greater than any other virus. Other prevalent viruses were mouse parvovirus (MPV; 1.86%), mouse hepatitis virus (MHV; 1.59%), rotavirus, also known as epizootic diarrhoea of infant mice, (0.56%), and Theiler's murine encephalitis virus (TMEV; 0.26%). Antibodies against the conserved parvoviral non-structural protein, NS1, were also assayed in mice and had prevalence slightly less than that of MPV. The MPV assay in use at Charles River uses the VP2 antigen. These findings are consistent with literature reports indicating that not all mice with antibodies against MPV VP2 may also have antibodies against the NS1 protein.[7,8] The prevalence of positive samples for all other viruses was less than 10% of the prevalence of any of the above agents. In addition to the antibody serology, approximately 6000 serum enzyme activity assays for LDHV were performed by chemically testing for elevated LDH levels. The initial positive rate in the LDH enzyme activity assay was almost 10%, virtually all of which were determined to be false positives by PCR on the remaining serum (data not shown). LDH is found in red blood cells, and falsely elevated LDH levels may be produced by hemolyzed serum.

In rats, as with mice, the prevalent viruses in Europe were also prevalent in North America. In rats, the most prevalent virus was RRV, with greater than 6% of the samples examined having histopathologic lesions consistent with RRV infection. Parvoviral infection of rats was also prevalent, including rat minute virus (RMV; 1.46%), rat virus (RV; 1.30%), rat parvovirus (RPV; 1.21%), and Toolan's H-1 virus (H-1; 1.41%). In addition to parvoviruses detected using the serotype-specific antigens, the generic parvovirus

**Table 5** Rat surveillance results: viruses

| Agent (assay abbreviation) | Method | *N* | Prevalence (%) | | |
|---|---|---|---|---|---|
| | | | NA | Europe | Total |
| Adenovirus (MAV 1 and 2) | Serology | 35,084 | 0.06 | 0.16 | 0.07 |
| Hantaviruses (HANT) | Serology | 23,248 | 0.01 | 1.09 | 0.07 |
| Lymphocytic choriomeningitis virus (LCMV) | Serology | 37,709 | 0.00 | 0.00 | 0.00 |
| Parvovirus generic assay (NS-1) | Serology | 63,808 | 2.00 | 3.34 | 2.05 |
| H-1 (Toolan's) | Serology | 79,451 | 1.37 | 2.85 | 1.41 |
| Rat virus (RV, KRV) | Serology | 86,764 | 1.23 | 4.05 | 1.30 |
| Rat minute virus (RMV) | Serology | 47,596 | 1.39 | 2.99 | 1.46 |
| Rat parvovirus (RPV) | Serology | 85,901 | 1.19 | 1.86 | 1.21 |
| Pneumonia virus of mice (PVM) | Serology | 79,954 | 0.06 | 1.24 | 0.10 |
| Rat coronavirus (RCV/SDAV) | Serology | 82,733 | 0.28 | 0.61 | 0.29 |
| Rat respiratory virus (RRV) | Histopathology | 3901 | 6.36 | na | 6.36 |
| Rat theilovirus (RTV, GD-VII) | Serology | 35,920 | 1.43 | 1.32 | 1.43 |
| Reovirus-3 (REO-3) | Serology | 72,886 | 0.01 | 0.00 | 0.01 |
| Sendai virus (SEND) | Serology | 80,851 | 0.01 | 0.51 | 0.02 |

Viruses for which screening is conducted are listed in the left column; both the common name and the common abbreviation are given. The next column to the right gives the primary method of screening for this agent. For rat viruses, serology is the primary method except for RRV. All evaluation for RRV is conducted by histopathology at the laboratory in North America (NA). The next column to the right gives the total number of samples included in this compilation. For each agent, the percent of positive results is given in the next two columns for NA and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each agent. It is this number that is used as an estimate of prevalence. na: not available

**Table 6** Mouse surveillance results: bacteria

| Bacterium | Method | *N* | Prevalence (%) | | |
|---|---|---|---|---|---|
| | | | NA | Europe | Total |
| *Bordetella bronchiseptica* | Culture | 109,802 | 0.00 | 0.00 | 0.00 |
| Cilia-associated respiratory bacillus | Serology | 158,741 | 0.01 | 0.00 | 0.01 |
| *Citrobacter rodentium* | Culture | 82,337 | 0.00 | 0.00 | 0.00 |
| *Corynebacterium kutscheri* | Culture | 109,804 | 0.00 | 0.00 | 0.00 |
| *Helicobacter* genus (any sp.)* | PCR | 91,119 | 15.88 | 21.28 | 16.08 |
| *Helicobacter hepaticus* | PCR | 91,463 | 12.45 | 10.23 | 12.37 |
| *Helicobacter bilis* | PCR | 91,386 | 2.20 | 1.49 | 2.17 |
| *Klebsiella oxytoca* | Culture | 185,937 | 0.38 | 1.32 | 0.38 |
| *Klebsiella pneumoniae* | Culture | 186,667 | 0.10 | 0.85 | 0.10 |
| *Mycoplasma pulmonis* | Culture | 61,592 | 0.00 | nt | 0.00 |
| | Serology | 455,102 | 0.01 | 0.16 | 0.01 |
| | PCR | 43,777 | 0.00 | nt | 0.00 |
| *Pasteurella multocida* | Culture | 109,376 | 0.00 | 0.00 | 0.00 |
| *Pasteurella pneumotropica* | Culture | 109,403 | 13.20 | 4.00 | 12.90 |
| Other *Pasteurella* species | Culture | 106,232 | 0.31 | 0.00 | 0.31 |
| Any *Salmonella* species | Culture | 109,655 | 0.00 | 0.00 | 0.00 |
| *Staphylococcus aureus* | Culture | 107,002 | 6.03 | 11.56 | 6.07 |
| *Streptobacillus moniliformis* | Culture | 2842 | 0.00 | 0.00 | 0.00 |
| *Streptococcus pneumoniae* | Culture | 109,804 | 0.00 | 0.00 | 0.00 |
| *Streptococcus* sp. – $\beta$-haemolytic, Group B | Culture | 106,971 | 0.24 | 0.00 | 0.24 |
| *Streptococcus* sp. – $\beta$-haemolytic, Group G | Culture | 109,733 | 0.00 | 0.11 | 0.00 |

Bacteria specifically targeted by screening are listed in the left column. The next column to the right gives the primary method of screening for this bacterial species. The next column to the right gives the total number of samples included in this compilation. For each bacterium, the percent of positive results is given in the next two columns for North America (NA) and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each bacterium. It is this number that is used as an estimate of prevalence. nt: not tested; PCR: polymerase chain reaction

*The results of this generic assay indicate the total number of animals infected with one or more *Helicobacter* spp., including *H. hepaticus* and *H. bilis*

assay using the NS1 protein detected antibodies in 2.05% of rats, higher than the prevalence detected for any of the serotype-specific assays. This was expected since, in theory, all rats infected with any of the parvoviruses should have antibodies to NS1, although the NS1 antibody prevalence should not be expected to simply equal the sum of the serotype-specific antibodies since some rats may be infected with more than one serotype. Also, the potential for some rats to seroconvert to the VP2 proteins but not non-structural proteins was not well investigated. Because the NS1 is highly conserved among parvoviruses, some of the positive results could also be the result of infection with currently unknown parvoviruses. The only other virus frequently detected was the picornavirus, rat theilovirus (RTV; 1.43%), which was assayed for using GD-VII antigen.

## Bacteria

The prevalence of bacteria identified in mice and rats in Europe and North America is given in Tables 6 and 7. In these tables, agents are listed in alphabetical order and no distinction is made between primary pathogens and opportunists, nor is their inclusion intended to imply that all of the listed bacteria are unacceptable in laboratory rodents. As with the viruses, the prevalence profile for bacteria, i.e. which agents are common and which are rare, is similar in Europe and North America for both rats and mice.

In mice, the most prevalent of the listed bacteria were *P. pneumotropica* (12.9%) and the helicobacters (16.08%), with *Helicobacter hepaticus* the most frequent at 12.37%. *Staphylococcus aureus* was also prevalent at 6%. As described in the materials and methods section, the *P. pneumotropica* results were based on biochemical identification of organisms which may overestimate (unpublished data) the actual prevalence in our sample stream relative to the PCR

**Table 7** Rat surveillance results: bacteria

| Bacterium | Method | *N* | Prevalence (%) NA | Europe | Total |
|---|---|---|---|---|---|
| *Bordetella bronchiseptica* | Culture | 8282 | 0.00 | 0.00 | 0.00 |
| Cilia-associated respiratory bacillus | Serology | 26,057 | 0.27 | 4.63 | 0.48 |
| *Corynebacterium kutscheri* | Culture | 8289 | 0.00 | 0.00 | 0.00 |
| *Helicobacter* genus (any sp.)* | PCR | 8852 | 6.59 | 7.14 | 6.63 |
| *Helicobacter hepaticus* | PCR | 8915 | 0.46 | 0.31 | 0.45 |
| *Helicobacter bilis* | PCR | 8915 | 0.42 | 0.31 | 0.42 |
| *Klebsiella oxytoca* | Culture | 7315 | 0.37 | 0.00 | 0.37 |
| *Klebsiella pneumoniae* | Culture | 7513 | 0.55 | 0.00 | 0.53 |
| *Mycoplasma pulmonis* | Culture | 3433 | 0.06 | nt | 0.06 |
| | Serology | 81,524 | 0.16 | 2.57 | 0.23 |
| | PCR | 3734 | 0.29 | nt | 0.29 |
| *Pasteurella multocida* | Culture | 8223 | 0.00 | 0.00 | 0.00 |
| *Pasteurella pneumotropica* | Culture | 8241 | 4.92 | 3.99 | 4.81 |
| Other *Pasteurella* spp. | Culture | 7346 | 0.45 | 0.00 | 0.44 |
| *Salmonella* sp. | Culture | 8235 | 0.00 | 0.00 | 0.00 |
| *Staphylococcus aureus* | Culture | 7365 | 23.50 | 30.63 | 23.61 |
| *Streptobacillus moniliformis* | Culture | 797 | 0.00 | 0.00 | 0.00 |
| *Streptococcus pneumoniae* | Culture | 8289 | 0.00 | 0.00 | 0.00 |
| *Streptococcus* sp. – $\beta$-haemolytic, Group B | Culture | 7503 | 3.74 | 1.34 | 3.67 |
| *Streptococcus* sp. – $\beta$-haemolytic, Group G | Culture | 8314 | 0.01 | 2.71 | 0.35 |

Bacteria specifically targeted by screening are listed in the left column. The next column to the right gives the primary method of screening for this bacterial species. The next column to the right gives the total number of samples included in this compilation. For each bacterium, the percent of positive results is given in the next two columns for North America (NA) and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each bacterium. It is this number that is used as an estimate of prevalence. nt: not tested; PCR: polymerase chain reaction

*The results of this generic assay indicate the total number of animals infected with one or more *Helicobacter* spp., including *H. hepaticus* and *H. bilis*

assay. Nonetheless, as with other agents often detected in our laboratory, the important finding remains simply that *P. pneumotropica* is prevalent. *C. bovis* was also monitored in immunodeficient mice, but not included in the table as it was felt that many of the cultures were taken from mice with lesions suggestive of 'scaly skin disease'. However, 2% of cultures and 9% of PCR assays were positive for *C. bovis* (data not shown), indicating that this bacterium continues to be found in immunodeficient mice. PCR results may include environmental monitoring as well as screening of animals (data not shown), so the data should not be construed to indicate that one method was more or less sensitive than the other.

In rats, the most frequently detected of the listed bacteria were *S. aureus* (23.61%), the helicobacters (6.61%), and *P. pneumotropica* (4.81%). Comments relative to the identification of *P. pneumotropica* in mice also apply to its identification in rats. While *H. hepaticus* and *H. bilis* were the most common of the helicobacters in mice, most helicobacters in rats were other, most likely rat-specific, species. Nude rats were also monitored for *C. bovis*, but no samples were found to be positive (data not shown).

## Other agents

This category includes protozoan and metazoan parasites, as well as the few fungi which were routinely monitored. The prevalence of selected agents in North America and Europe may be found in Tables 8 and 9. Protozoan parasites of mice and rats included the pathogenic *Giardia muris*, which was not found, and *Spironucleus*, which was detected at a low prevalence (0.08% in mice and 0.19% in rats), as well as numerous non-pathogenic or commensal protozoa, which were found at varying frequencies. These include *Entamoeba*, *Chilomastix*, *Hexamastix*, *Monocercomonoides*, *Retortamonas,* and trichomonads. The prevalence of these agents was similar in Europe and North America.

The fungus, *Encephalitozoon cuniculi*, was monitored by serology. No positives were detected in mice, although in rats a low prevalence of positive results was detected in both Europe and North America. As this assay is more prone than most viral assays to false positives, presumably because of the greater antigenic complexity of *E. cuniculi*,

**Table 8** Mouse surveillance results: *Eukaryota*

| Agent | Method | *N* | Prevalence (%) NA | Europe | Total |
|---|---|---|---|---|---|
| *Encephalitozoon cuniculi* | Serology | 145,053 | 0.00 | 0.00 | 0.00 |
| Lice | Direct | 126,482 | 0.00 | nr | 0.00 |
| Mites | Direct | 130,976 | 0.11 | 0.43 | 0.12 |
| Oxyurids* | | | | | |
| *Aspiculuris tetraptera* | Direct | 135,860 | 0.19 | | |
| *Syphacia muris* | Direct | 128,657 | 0.01 | 1.31 | 0.25 |
| *Syphacia obvelata* | Direct | 128,657 | 0.11 | | |
| Protozoa | | | | | |
| *Chilomastix* sp. | Wet mount | 94,890 | 3.74 | nr | 3.74 |
| *Entamoeba* sp. | Wet mount | 94,890 | 8.08 | nr | 8.08 |
| *Giardia* sp. | Wet mount | 102,093 | 0.00 | 0.00 | 0.00 |
| *Hexamastix* sp. | Wet mount | 94,890 | 4.45 | nr | 4.45 |
| *Monocercomonoides* sp. | Wet mount | 94,890 | 0.04 | nr | 0.04 |
| *Retortamonas* sp. | Wet mount | 94,890 | 0.03 | nr | 0.03 |
| *Spironucleus* sp. | Wet mount | 102,093 | 0.08 | 0.00 | 0.08 |
| Trichomonads | Wet mount | 94,890 | 8.88 | nr | 8.88 |

Eukaryote species or genera specifically targeted by screening are listed in the left column. The next column to the right gives the primary method of screening for this agent. The next column to the right gives the total number of samples included in this compilation. For each agent, the percent of positive results is given in the next two columns for North America (NA) and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each agent. It is this number that is used as an estimate of prevalence. nr: not reported

*In Europe, oxyurid nematodes were reported as pinworms, but the species were not recorded. All are included in this table as *A. tetraptera*, the most common pinworm of mice, although some were probably *Syphacia*

**Table 9** Rat surveillance results: *Eukaryota*

| Agent | Method | *N* | Prevalence (%) | | |
|---|---|---|---|---|---|
| | | | NA | Europe | Total |
| *Encephalitozoon cuniculi* | Serology | 22,486 | 0.08 | 1.55 | 0.16 |
| Lice | Direct | 8202 | 0.00 | nr | 0.00 |
| Mites | Direct | 9241 | 0.00 | 0.00 | 0.00 |
| Oxyurids* | | | | | |
| *Aspiculuris tetraptera* | Direct | 9233 | 0.00 | | |
| *Syphacia muris* | Direct | 9233 | 1.10 | 3.58 | 1.10 |
| *Syphacia obvelata* | Direct | 9233 | 0.10 | | |
| Protozoa | | | | | |
| *Chilomastix* sp. | Wet mount | 7741 | 1.65 | nr | 1.65 |
| *Entamoeba* sp. | Wet mount | 7741 | 3.18 | nr | 3.18 |
| *Giardia* sp. | Wet mount | 9810 | 0.00 | 0.00 | 0.00 |
| *Hexamastix* sp. | Wet mount | 7741 | 4.60 | nr | 4.60 |
| *Monocercomonoides* sp. | Wet mount | 7741 | 0.09 | nr | 0.09 |
| *Retortamonas* sp. | Wet mount | 7741 | 0.17 | nr | 0.17 |
| *Spironucleus* sp. | Wet mount | 7741 | 0.19 | nr | 0.19 |
| Trichomonads | Wet mount | 7741 | 1.58 | nr | 1.58 |

Eukaryote species or genera specifically targeted by screening are listed in the left column. The next column to the right gives the primary method of screening for this agent. The next column to the right gives the total number of samples included in this compilation. For each agent, the percent of positive results is given in the next two columns for North America (NA) and Europe, respectively. The column on the right gives the percentage of the total samples that tested positive for each agent. It is this number that is used as an estimate of prevalence. nr: not reported

*In Europe, oxyurid nematodes were reported as pinworms, but the species was not recorded. All are included in this table as *Syphacia muris*, the common pinworm of rats, although some may have been other species

the significance of the positive results in rats is unclear. No dermatophytic fungi were detected in the animals tested (gross examination, with skin scraping and culture where indicated or by special request; data not shown). Generic PCR to detect *Pneumocystis* species, primarily conducted in immunodeficient rats and mice, was positive in approximately 1% of samples (data not shown).

Metazoan parasites in mice and rats included various species of lice, mites, and helminths. No lice were detected in either mice or rats. Mites were found on mice (0.11% in North America and 0.43% in Europe), but not on rats. The only helminths detected were pinworms (oxyurids). When the pinworms were speciated, mice had *Aspiculuris tetraptera* (0.19%) more often than *Syphacia obvelata* (0.11%), but the rat pinworm, *S. muris*, was also occasionally detected in mice. In rats, as expected, *S. muris* was by far the most common pinworm (1.10%), but *S. obvelata* was also occasionally identified (0.10%).

## Discussion

The limitations of the data presented here should be acknowledged. Although the large number of samples provides a strong basis for recognizing highly prevalent versus less prevalent agents, sample stream 'prevalence' does not necessarily correlate exactly with prevalence of any particular agent in a geographic area, or with the percentage of affected institutions. We acknowledge the presence of sampling error in our data; these data are merely compilations of results from the sample stream passing through our laboratories, and not selected as representative samples from entire populations. To reiterate, these data should only be used to help guide surveillance to focus on the more prevalent agents. There are several reasons that data compiled in this manner from ILIMS have the potential either to overestimate or to underestimate prevalence. Overestimation of prevalence may occur via several routes. Prevalence may be overestimated by the inclusion of undetected false positives among true positives. Although all positive findings are confirmed by a second and sometimes third assay, this may not rule out all false positives. Also, when facilities experience outbreaks of infectious agents, there may be increased sampling of animals, resulting in an upward skewing of prevalence for that particular agent. Institutions may intentionally submit known positive animals, either as quality control for the diagnostic laboratory, or for completeness' sake in their monitoring programme. In addition, our laboratory also tests a small number of pet and wild or feral rodents each year. These have an entirely different disease profile than a standard laboratory rodent, and may skew results for some of the rarer agents.[9–11] However, the effect of these animals on prevalence numbers is greatly diluted by the far greater number of laboratory animals tested. We estimate the percentage of feral, wild and 'conventional' (feeder, pet) rodents tested at less than 0.1% of the total samples.

Conversely, these data may underestimate prevalence. Institutions with populations previously recognized as positive, e.g. for *Helicobacter*, may choose to omit those areas or agents from their monitoring programmes. Prevalence may also be underestimated because, although clients of Charles River's diagnostic laboratory constitute a large sample size, they are not necessarily a representative sample of all laboratory rodent users. However, even if a global prevalence could be accurately calculated, it would have limited value because it is unlikely that the true prevalence of a disease is geographically uniform. There are differences seen when Europe is considered separately from North America; even within a geographic region, the movement of a desirable, but infected, population of animals from institution to institution will affect the prevalence in that area. Additionally, because these data are derived almost exclusively from testing of laboratory rodents, typically of a specific pathogen-free (SPF) health status, the data cannot be construed to indicate or imply that rodents raised for pets or to feed raptors, reptiles, etc. are of similar health status as SPF rodents.

A final caveat is that these data reflect prevalence in individual samples, not institutions. The prevalence of affected institutions is much higher than the prevalence of positive animals from those institutions. The true prevalence of these diseases for laboratory mice and rats is probably unknowable, unless it is for a single institution, or perhaps a single room within an institution. In spite of

these caveats, these data are useful indicators of which infectious agents pose high levels of ongoing risk of facility contamination, thereby warranting the highest levels of vigilance, including the most frequent surveillance.

A review of the literature indicates that few agents have been removed from surveillance lists over the past 50 years, although the lists have expanded with the discovery of new agents, such as RRV, *Helicobacter*, and MNV. Eradication of agents that caused clinical disease, such as ectromelia, in laboratory rodent colonies was in the best interest of colony management and animal welfare and this was often accomplished relatively early, as was eradication of agents which were only mildly contagious.[12] Axenic, i.e. germ-free (GF), animals were also in use.[13]

Early reports on prevalence originate primarily from North America and Western Europe and concentrate on viral agents. In literature reviewed from 1963 to 1986, which primarily emphasizes serology, polyoma and K virus were frequently found in institutional mouse colonies, even ones designated as GF.[14,15] MHV, pneumonia virus of mice (PVM), reovirus, and Sendai virus are also reported as commonly detected in mice, and PVM, Sendai, sialodacryoadenitis/rat coronavirus (SDAV), RV, and H-1 are reported as common in rats.[16–21] Results reported in 1988 indicated that many commercial sources of rodents were serologically positive for MHV, SDAV, and Sendai,[22] surely a concern for those colony managers trying to exclude such agents. A retrospective report of health monitoring in 69 mouse and 36 rat colonies from 1988 to 1997 in France correlates well with our findings that the prevalence of certain viruses in mice and rats has declined with time, while others have become more prominent.[23] For example, in 1988–1990, 30% of rat colonies tested were positive for Sendai virus, but by 1996–1997, only 5.9% of colonies were positive.[23] In contrast, the prevalence of parvoviruses in rats remained steady or increased. In mice, a similar story unfolds, with the prevalence of a contagious agent, such as MHV, remaining relatively steady, while agents that are more difficult to transmit, such as PVM, decline precipitously.[23]

Livingston and Riley[24] present a more recent collection of data from the diagnostic laboratory at the University of Missouri in a 2003 paper. Their data span a one-year period from 2001 to 2002. The most prevalent bacterium reported in mice is *H. hepaticus*, and the most prevalent virus was MPV, with MHV and rotavirus following closely. In rats, parvoviruses were the most prevalent viruses, with PVM and TMEV (RTV) coming in second and third. For bacteria of concern in rats, the *Helicobacter* genus is the most prevalent, and *P. pneumotropica* is the second most prevalent agent. In 2006, Schoondermark-van de Ven *et al.*[25] presented work compiling their laboratory's three decades of health monitoring in Western Europe. The most prevalent mouse virus reported is MHV, with the parvoviruses of mice also remaining prevalent; whereas in rats, the group of parvoviruses is the most prevalent, with TMEV (RTV) listed as the next most prevalent.[25] Although some of the older sample sizes are small, these data are valuable for illustrating changes over three decades and show striking declines in the prevalence of almost all agents. For example, 52% of samples were seropositive for reovirus in mice in 1981–1984 but only 0.6% in 2000–2003.[25]

When areas other than North America and Western Europe are considered, some differences in agent prevalence are revealed, although many of the common agents remain problematic. These areas are not well-represented in Charles River's sample stream, and these reports emphasize the commonalities in prevalence, despite geographical diversity. Current and historical reports from Korea, Brazil and Japan show a similar pattern of prevalent agents as North American and Western European reports. In Brazil, in 1996, Gilioli *et al.*[26] found MHV as the most common contaminant of mouse colonies, and SDAV as the most common contaminant of rat colonies. Nakagawa *et al.*[27] examined the prevalence of 11 pathogens, both bacterial and viral in both SPF and conventional colonies of mice and rats in Japan from 1972 to 1981. Conventional mice were most likely to be contaminated with *Syphacia*, *P. pneumotropica* and Sendai, whereas conventional rats were likely to be contaminated with *P. pneumotropica*, *Syphacia*, and *M. pulmonis*.[27] More recent results include those from Won *et al.*[28] who discuss current prevalence of agents in Korea. *P. pneumotropica*, MHV, pinworms and protozoa were found in the reported Korean mouse facilities, while parvoviruses appear less prevalent, but only minute virus of mice was monitored in this study.[28] For rats, antibodies to Sendai virus were detected in both barrier and conventional facilities, as was *P. pneumotropica*.[28]

Some infectious agents are still detected frequently in laboratory mouse and rat populations, even those maintained under strict barrier conditions. Notably, though, few of the currently prevalent agents are likely to cause clinical disease in immunocompetent animals. Several factors seemingly contribute to the continued prevalence of certain adventitious infections in laboratory rodents. It is possible that some of the prevalence in a diagnostic sample stream may actually be due to more widespread testing, in which more and more institutions have initiated disease surveillance programmes. The prevalence of some agents may have been boosted by the rise of the genetically-modified animal, as many of these animals are developed by, and shipped from, research institutions where the level of biosecurity may be unavoidably less than in a vendor setting. In addition, some of these genetically-modified animals have derangements of host defence mechanisms that allow viruses that would otherwise be cleared to be shed long term, increasing the risk of transmission to other animals. However, many of the prevalent infectious agents, including parvoviruses, norovirus, pinworms, and possibly, RRV, are shed for long periods, even by immunocompetent animals. Prevalent agents include those such as MHV, which is highly contagious, even though it does not remain active for long periods in the environment. Other prevalent agents, including parvoviruses, rotaviruses, noroviruses and pinworms, persist in the environment unless decontamination is thorough. Finally, some agents may be prevalent simply because they are newly discovered (RRV, MNV).

Many of the classical laboratory rodent infectious agents are vanishingly rare, or perhaps even absent from modern

laboratory populations. However, testing for these agents should not be discontinued entirely. These agents may be present in archived material in freezers, may contaminate tumour lines[29] or other biologicals.[30] They may be used as models for other disease states,[31] or may contaminate a facility through exposure to wild, pet or feeder rodents[32] or through acquisition of rodents from other institutions.[33] Health surveillance programme design for a facility should also consider the different activities undertaken at that facility. The most frequent serologic surveillance of laboratory rodents should concentrate on the most common viruses. Surveys such as this and those undertaken by other laboratories provide valuable information to organizations making testing recommendations, and contemporary disease prevalence should be a primary consideration in designing efficient and effective health-monitoring schemes.

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*(Accepted 30 May 2008)*