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# Microbiological assessment of laboratory rodents: Perspectives from laboratory animal facility of ACTREC

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## Abstract

Detection of pathogenic organisms in any module of the conventionally bred animal facility is suggestive of possible infection in other strains/ modules of the facility as well. Timely diagnosis of the microbes prevailing in the conventional animal facility is possible if well planned screening program is in place. Laboratory Animal Facility of ACTREC breed and maintains 22 different strains of mice, two strains of hamsters and one rat strain. This paper report the microbiological screening results of rodent pathogens prevailing in the Laboratory animal Facility, ACTREC, India. Microbiological status of these strains was assessed by conventional microbiology using agar media and biochemical tests; readymade ELISA kits and PCR methods. Results of the conventional microbiology revealed that the rodents in ACTREC were reported positive for *Klebsiella pneumoniae*, *Staphylococci aureus, Escherichia coli* and Proteus sp. Recent ELISA based results revealed presence of *Mycoplasma pulmonis,* CAR bacilli, Sendai, Tyzzer's disease, MHV, Helicobacter hapaticus, Polyoma, Minute virus of mice, *Pneumocystis carinii, Pasturella pneumotropica* and Mouse Parvovirus; whereas PCR based methods revealed presence of *Mycoplasma pulmonis*, Sendai, *Helicobacter hapaticus*, *Pneumocystis carinii, Helicobacter bilis*, MNV, MHV and CAR bacilli. Effective quarantine, barrier maintenance, frequent surveillance for all recommended pathogens and eradication of the existing pathogens is the challenge to be accepted.

**Key words: ACTREC, microbial status, pathogen, rodents**

## Introduction

Reproducibility of the animal experimentation is primarily based on the use of quality animals. Use of quality animals is also one of the pre-requirement to adopt and practice the 3R principles of Russel and Burch (1959). Number of variations are responsible for altered results of animal studies; these could be genetic, physical, chemical and microbial. Since the adventitious infections of pathogenic bacteria, viruses and parasites in laboratory animals may end up with pathological changes, use of such animals may not be suitable for biomedical research (Scavizzi and Raspa, 2006; Pritchett-Corning *et al.* 2009). Highly defined animals may be easy to procure from the reputed sources. However, maintaining

in the same conditions in which they were procured is a challenge. Therefore, in addition to procuring quality animals, environmental conditions, barrier maintenance, and effective health surveillance program of animals plays a crucial role in maintenance of pathogen free animals. Since all the above variations cannot be eliminated at once, one has to focus on variations that directly affect the research data. Pathogens being a variable, do not always cause the overt clinical disease and therefore identification of such variable is always a challenge and need variety of specialized tests.

With the advent of animal production under barriers conditions, it is possible to produce quality animals if a system for monitoring the microbial health status of animals is adopted along with other standard breeding practices. Elimination of the rodent pathogens from the rodent colony involves concerted efforts and enormous cost. Similar to the bacterial rodent pathogens, some of the parasitic diseases such as *Syphacia* spp. (pin worms) and *Klossiella muris* are difficult to eradicate. However, concerted efforts of monitoring and treatment, help to maintain the colony free from these parasites too (Ingle, 1999; Ingle and Shinde, 2011). Animal Facilities across the globe have their own program for microbiological screening of their laboratory animals. Such program yields the information of the prevailing infections in the areas and helps to decide other facilities to formulate the list of rodent pathogens to be screened. In absence of such published information from India, this paper reports the screening results of rodent pathogens prevalent in Animal Facility of ACTREC.

# Materials and methods

### *Animals and maintenance*

The proposal to screen the strains of rodents maintained in the ACTREC Animal Facility was approved by the Institutional Animal Ethics Committee of the ACTREC which is duly endorsed by the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, New Delhi vide registration no. 65/1999/CPCSEA. Breeder animals were maintained either 1:1 or 1:2 as the case may be in a cage. Experimental animals were maintained in groups of 5-6 animals per cage. All breeder as well as experimental Nude and SCID mice were maintained in individually ventilated polysulfone cages. All Nude and SCID mice were handled for checking, exchanging or experimentation in a cage changing station using sterile technique. Breeder animals of BALB/c, C57BL/6, DBA/2, Swiss, Swiss bare as well as all experimental animals other than mentioned above were maintained in conventional polypropylene cages. All animals were maintained under controlled conditions of 55 ± 5% humidity, 23 ± 2°C temperature, and 12-hr light/12-hr dark cycle under specific pathogen-free conditions. All mice were maintained on sterilized corn cob bedding material and in-house pelleted animal feed and UV treated water from the commercial water purifier *ad libitum*.

## Specimen collections

### *Conventional microbiology*

Generally, freshly collected stool samples were screened for the presence of gastro-intestinal tract infections during Jan. 2010 and Dec. 2011. However, in some cases irrespective of their age group, weak or moribund animals in the colony were used for screening of samples system-wise. In short, samples were collected aseptically from respiratory, gastro-intestinal, lymphatic and integumentary system as the case may be. In case of stools samples, they were collected in sterile petri dishes from individual animals in the respective rooms and were labeled accordingly. For conventional microbiology, 279 stool samples, 32 lungs, 41 cecum, 17 spleen and 8 skin samples were processed during this period.

### *Media preparation and quality control*

Nutrient agar (NA), blood agar (BA) base, MacConkey's agar, Salmonella & Shigella agar (SSA), Baired & Parker agar (BPA), Pleuropneumonia like organism (PPLO) agar, Sabroud's agar (SA) and Potato dextrose agar (PDA) were procured from HiMedia Laboratories, Mumbai, India. Media were reconstituted as per the manufacturer's instructions and liquid media was poured in each 90 mm diameter disposable Petri dishes (Tarsons, Kolkata, India). All media used were tested for the internal quality control using the standard bacterial cultures received from the ATCC, USA.

### *Antibiotic sensitivity test*

Antibiotic sensitivity discs for tetracycline, ofloxacin, ciprofloxacin, enrofloxacin, norfloxacin, gatifloxacin, cephalexin, doxicyclin and oxytetracycline were procured from HiMedia Laboratories, Mumbai, India.

### *ELISA kits*

Ready to use 96-well plate ELISA kits targeted for diagnosis of 15 rodent pathogens such as *Mycoplasma pulmonis (*MP), Ectromelia virus, *Cilia Associated Respiratory* (CAR) bacilli, Sendai virus, Pneumonia virus of mice (PVM), Tyzzer's disease, Mouse hepatitis virus (MHV), Lymphocytic choriomeningitis virus (LCMV), *Helicobacter hepaticus* (Hh), Hantavirus, Polyoma virus *(*PV), Minute virus of mice (MVM), *Pneumocystis carinii (*PC), *Pasturella pneumotrophica (*PP) and Mouse parvovirus (MPV) were procured from the XpressBio Life Science Products, USA.

### *ELISA based microbiology*

Serum samples were separated from the blood samples collected from the orbital plexus of the animals between Jan. 2008 and June 2012 and were stored in refrigerator till further use. For MP- 275, Ectromelia- 54, CAR bacilli- 54, Sendai- 86, PVM- 86, Tyzzer's disease- 56, MHV- 24, LCMV- 82, Hh- 58, Hantavirus- 185, PV- 95, MVM- 95, PC- 70, PP- 70 and for MPV- 70 serum samples were tested.

### *PCR based microbiology*

Depending on the predilection of site of the organisms in the animal body, samples were collected during Jan. 2010 and Dec. 2011. For MP, CAR bacilli, Sendai, Hantaan and PC 142, 141, 138, 21 and 87 lungs samples were collected in 1.8 ml capacity pre-cooled eppendorf tubes respectively and were stored at -80 °C after snap freezing in liquid nitrogen. For MHV, *Helicobacter bilis* (Hb) and Hh, 136 cecum samples with cecal content each; and for Murine norovirus (MNV), 106 cecum samples with cecal content were collected. For Lactate dehydrogenase-elevating *virus* (LDV) 54 liver samples were collected.

### *Disease specific primers*

Disease specific primers for Mycoplasma (Kazuo *et al.* 1994), Hantavirus (Simmons and Riley, 2002), Sendai (Bootz *et al.* 2003), Hh (Jeong *et al.* 2011), PC (Yeom *et al.* 2009; Yabuuchi *et al.* 2010), LDV (Kazuo *et al.*1998), Hb (Kazuo *et al.* 2000), MNV (Kazuo *et al.* 2009), MHV (Matthaei *et al.* 1998) and CAR (Kazuo *et al.* 1995) were selected from the respective reported literature. The oligo primers were commercially synthesized from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India. The primers were reconstituted and diluted as per the instructions of the manufacturer.

### *Disease specific positive controls*

Disease specific control DNA/ cDNA's were procured for these diseases from the Central Institute of Experimental Animals, ICLAS Asia Monitoring Centre, Kanagawa, Japan.

### *Routine microbiology*

Small piece of freshly collected stool sample was taken on a sterile nichrome wire loop and was directly streaked on different agar media plates. The plates were incubated in incubator for at least 24 h and colonies grown were used for further identification by colony characters, microscopy, and biochemical tests and also for conducting antibiotic sensitivity tests.

### *Antibiotic sensitivity test*

Discrete colonies of the microbes grown on the agar plates were picked up with the use of moist ear buds and were uniformly streaked on the Nutrient agar plates. Antibiotic sensitivity discs of tetracycline, ofloxaciin, ciprofloxacin, enrofloxacin, gatifloxacin, norfloxacin, cephalexin, doxicyclin and oxytetracyclin were then placed on these plates and were incubated in the incubator at 37ºC for 24 h.

### *ELISA and PCR based methodology*

Selection of rodent pathogens screened in the present study was based on the Federation of European Laboratory Animal Association (FELASA) guidelines (Nicklas *et al.* 2002). As per the FELASA guidelines, more than 10 samples were collected from the animal colony representing the entire breeder as well as experimental stock. The only deviation from FELASA guidelines was that for ELISA and PCR method samples were collected and tested six monthly.

Serum samples were tested for presence of antibodies of rodent pathogens as per the methodology given by the manufacturers. In short, 2 ml of test serum sample diluted to 100 ml was added in each well, 100 µl of positive control sample provided by the manufacturer was added to one well, the plate was covered with aluminum foil and incubated for 45 min at 37°C, washed 5-6 times with wash solution, the wells were dried and 100 µl peroxidase conjugate solution was added in each well, the plate was covered with aluminum foil and incubated for 45 min at 37°C, washed 5-6 times with wash solution, the wells were dried and 100 µl ABTS substrate solution was added in each well, the plate was incubated at room temperature for 30 min, 25 ml of stopping solution was added in each well and reaction in the plates were read at 405 nm with the help of ELISA reader. Sample readings with values of greater than or equal to $\geq 0.300$ were considered positive.

DNA's were extracted from the collected samples using routine method of phenol chloroform extraction and ethanol precipitation; the precipitates were suspended in TE buffer, pH 8.4. RNA's were extracted from the suspected tissues using RNA Sure mini kit, Nuceo-pore (Genetix Biotech Asia Pvt. Ltd., New Delhi, India) as per the manufacturer's instructions. cDNA's were synthesized from these RNA's using RevertAid First Strand cDNA synthesis kit (MBI Fermentas, USA) as per the manufacturer's instructions. These DNA's/ cDNA's were used for the PCR amplification using disease specific primer sequences for MP, Hantavirus, Sendai, Hh, PC, LDV, Hb, MNV, MHV and CAR bacilli as per the sequences available from the published literature (Bootz *et al.* 2003; Jeong *et al.* 2011; Kazuo *et al. 1994;* Kazuo *et al.* 1995 ; Kazuo *et al.* 1998 ; Kazuo *et al.* 2000 ; Kazuo *et al.* 2009; Matthaei *et al.* 1998; Simmons *et al.* 2002; Yeom *et al.* 2009; Yabuuchi *et al.* 2010). In short, 100 ng of target DNA/ cDNA was amplified in 15 µl reaction volume containing 5 U of TaqDNA polymerase (Invitrogen, USA), 0.4 pM each oligonucleotide primers (Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India), 10 mM Tris buffer (Sigma Chemicals, USA), 3.5 mM $MgCl_2$, 0.4 mM each of DNTP (Fermentas, USA). PCR consisted of 5 m of denaturation at 94°C followed by 34 cycles of 20 s denaturation at 94°C, 20 s annealing at 58°C, and 30 s of elongation at 72°C in a thermal cycler (Eppendorf, India). Labeled PCR product (15 µl) was electrophoretically separated using 2% molecular biology grade agarose gel (cat. no. N605, Ambresco, USA) and visualized under UV light. Standard DNA marker (Fermentas, USA) of 100 bp size DNA was run every time for easy determination of the size of the reaction product developed after electrophoresis.

## Results

### *Conventional microbiology test*

Conventional microbiology revealed that the rodents in ACTREC were tested positive for *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Proteus* spp. as diagnosed by use of media culture, microscopy and biochemical tests. Summary of the results of the rodent pathogens testing by conventional method of microbiology testing are shown in table 1. *Escherichia coli* was predominantly detected in cecum and stool samples only and not in lungs, spleen or skin. *Klebsiella pneumonia* was detected in lungs, cecum, spleen and stool samples but not in skin. *Staphylococcus aureus* were detected in lungs, cecum and skin and not in spleen and stool. Proteus was detected in cecum and stool and not in lungs, spleen or skin. Lungs showed presence of *Klebsiella pneumonia* and *Staphylococcus aureus* only. Cecum samples showed presence of *Escherichia coli*, *Klebsiella pneumonia* as well as *Staphylococcius aureus*. Spleen samples showed presence of *Klebsiella pneumonia* only. Stool samples showed presence of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus* spp. Skin samples showed presence of *Staphylococcus aureus* only.

**Table 1: Results of conventional microbiology testing of rodent pathogens**

| | Samples and numbers tested by conventional microbiology | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lungs | | Cecum | | Spleen | | Stool | | Skin | |
| Year | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| No. of samples | 21 | 11 | 27 | 14 | 14 | 3 | 198 | 81 | 2 | 6 |
| *Escherichia coli* | 0 | 0 | 9 | 2 | 0 | 0 | 15 | 1 | 0 | 0 |
| *Klebsiella pneumonia* | 2 | 1 | 9 | 2 | 2 | 2 | 6 | 4 | 0 | 0 |
| *Staphylococcius aureus* | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Proteus spp.* | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |

### *Antibiotic sensitivity test*

Antibiotic sensitivity results revealed that the *Klebsiella pneumonia* most responded to enrofloxacin, norfloxacin, ofloxacin and ciprofloxacin. *Klebsiella pneumonia* was consistently found resistant to oxytetracycline, cephalexin and doxycycline but was also found intermittently resistant to ciprofloxacin, norfloxacin, and ofloxacin. *Escherichia coli* were found sensitive to gatifloxacin, enrofloxacin, ofloxacin, ciprofloxacin and norfloxacin. *Escherichia coli* were found to be consistently resistant to doxycycline, oxytetracycline but intermittently resistant to ofloxacin, enrofloxacin, norfloxacin as well as ciprofloxacin. *Staphylococcus aureus* were found to be consistently sensitive to gatifloxacin, norfloxacin and ciprofloxacin and consistently resistant to enrofloxacin, doxycycline, ofloxacin and oxytetracycline but intermittently resistant to ciprofloxacin. Proteus were found to be consistently sensitive to ofloxacin, ciprofloxacin, oxytetracycline, enrofloxacin and norfloxacin whereas consistently resistant to doxycycline.

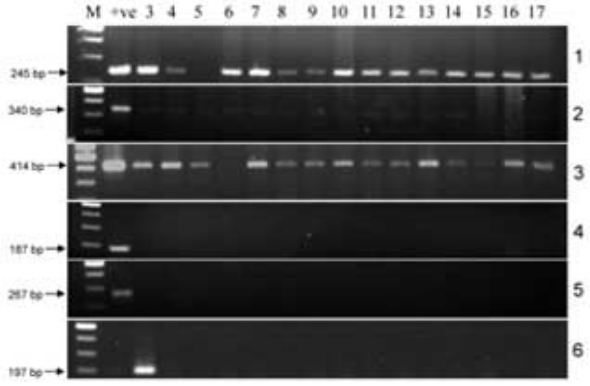
### *Serology tests*

Recent ELISA based results revealed presence of *Mycoplasma pulmonis*, CAR bacilli, Sendai virus, Tyzzer's disease, MHV, *Helicobacter hepaticus*, Polyoma virus, MVM, PC, PP and MPV. All animals were found negative for Ectromelia, PVM and LCMV. All samples tested during Jan. 2011- June 2012 were found negative for Hantavirus. *Mycoplasma pulmonis*, CAR bacilli, Sendai virus, Tyzzer's disease, Hantavirus and Polyoma virus revealed positive rate of less than 4%. Helicobacter, MVM and PP incidence ranged from 10-20% whereas MHV and MPV was 41% each.

### *PCR tests*

PCR based methods revealed presence of MP, Sendai, Hh, PC, Hb, MNV, MHV and CAR bacilli. All animals were found negative for Hantavirus and LDV. For MNV and MHV incidence was reported below 5% and single sample was found positive for CAR bacilli which accounted to less than 1% incidence. MP, Sendai, Hh, PC and Hb showed the incidence ranging from 27- 59%. Results of the PCR testing of the representative rodent pathogen is shown in Fig. 1 A and B. Summary of the results of the rodent pathogens detected by ELISA and PCR method are shown in table 2 and 3, respectively.

**Fig. 1.A. PCR gel demonstrating positive results of rodent pathogens.**




Lane 1- Standard marker; lane 2- positive control; lane 3 to 17- test samples

**Panel 1- Mycoplasma pulmonis.**
Lane 3 to 4 and 6 to 17- positive test samples. Lane 5- negative test sample.

**Panel 2- Sendai virus.**
Lane 3 to 14- positive test samples.
Lane 15 to 17- negative test samples.

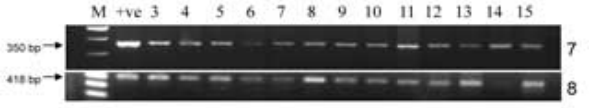
**Panel 3- Helicobacter hepaticus.**
Lane 3 to 5 and 7 to 17- all positive test samples.
Lane 6- negative test sample.

**Panel 4- Murine Norovirus.**
Lane 3 to 17- all negative test samples.

**Panel 5- Cilia Associated Respiratory bacilli.**
Lane 3 to 17- all negative test samples.

**Panel 6- Mouse hepatitis virus.**
Lane 3- positive test sample.
Lane 4 to 17- negative test samples.

**Fig.1.B. PCR gel demonstrating positive results of rodent pathogens.**




Lane 1- Standard marker; lane 2- positive control; lane 3 to 15- test samples.

**Panel 7- Pneumocystis carinii.**
Lane 3 to 15- all positive test samples.

**Panel 8- Helicobacter bilis.**
Lane 3 to 15- all positive test samples.

**Table 2. Results of ELISA testing of rodent pathogens.**

| | Rodent pathogen tested | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | Ectromelia | CAR | Sendai | PVM | Tyzzers | MHV | LCMV | Hh | Hantaan | PV | MVM | PC | PP | MPV |
| | Number tested / positive samples | | | | | | | | | | | | | | |
| 2008 | 54/2 | 54/0 | 54/1 | 54/1 | 54/0 | 24/0 | 24/10 | 24/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| 2009 | 58/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 58/0 | 58/10 | 58/1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| 2010 | 61/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 61/3 | 61/0 | 61/12 | 0/0 | 0/0 | 0/0 |
| 2011 | 70/3 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 34/0 | 34/2 | 34/13 | 70/7 | 70/10 | 70/29 |
| 2012 | 32/1 | 0/0 | 0/0 | 32/1 | 32/0 | 32/2 | 0/0 | 0/0 | 0/0 | 32/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| **Total** | **275/6** | **54/0** | **54/1** | **86/2** | **86/0** | **56/2** | **24/10** | **82/0** | **58/10** | **185/4** | **95/2** | **95/25** | **70/7** | **70/10** | **70/29** |
| Percent positive | 2.18 | 0 | 1.85 | 2.33 | 0 | 3.57 | 41.67 | 0 | 17.24 | 2.16 | 2.11 | 26.32 | 10 | 14.29 | 41.43 |

**Table 3. Results of PCR testing of rodent pathogens.**

| | Samples and numbers tested by PCR method | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | | Hantaan | | Sendai | | Hh | | PC | | LDV | | Hb | | MNV | | MHV | | CAR | |
| Year | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| No. of samples tested | 54 | 87 | 21 | 0 | 51 | 87 | 52 | 84 | 0 | 87 | 54 | 0 | 52 | 84 | 22 | 84 | 52 | 84 | 54 | 87 |
| No. of samples positive | 10 | 72 | 0 | 0 | 21 | 16 | 20 | 45 | 0 | 46 | 0 | 0 | 41 | 39 | 1 | 4 | 4 | 0 | 0 | 1 |
| Year wise positive percentage | 18.52 | 82.76 | 0 | 0 | 41.18 | 18.39 | 42.31 | 53.57 | 0 | 52.87 | 0 | 0 | 78.85 | 46.42 | 4.55 | 4.76 | 7.69 | 0 | 0 | 1.15 |
| Total positive percentage (2010-11) | 58.16 | | 0 | | 26.81 | | 47.79 | | 52.87 | | 0 | | 58.82 | | 4.72 | | 2.94 | | 0.71 | |

## Discussions

Almost all strains maintained in ACTREC are imported either from The Jackson Laboratory, USA; Charles River Laboratories, USA; National Cancer Institute, USA or other well known Universities from the USA. The microbiology reports received at the time of import of the breeding pairs indicates the negative status of the positive results reported in this study.

Isolated efforts have been made to detect the presence of rodent pathogens in India. However, in the absence of established reference laboratories for microbiological monitoring of laboratory animals, published interpretive data and evaluative standards are lacking in this country. We do not have even indigenously developed serological kits to diagnose the rodent pathogens. Lack of surveillance data and structured health monitoring program for the regular screening of the species specific organisms drives the laboratory animal scientists in India to depend on and to follow the health monitoring program laid by the western countries.

The purpose of this work is to screen and publish the results of the presence of rodent pathogens so as to make the guidelines for screening of the probable presence of organisms. Results of the present study indicates that the rodent population in ACTREC has acquired variety of pathogens such as *Mycoplasma pulmonis*, CAR bacilli, Sendai virus, Tyzzer's disease, MHV, *Helicobacter hepaticus*, Hantavirus, Polyoma virus, MVM, PC, PP, MPV, *Helicobacter bilis*, MNV and CAR bacilli.

Conventional method of culture and isolation of rodent bacterial pathogens is important for treating the animals with antibacterial agents. This is only possible if the antibiotic sensitivity tests are conducted on the isolated organisms (Yeom *et al.* 2009; Burr *et al.* 2011). However, this method is laborious and expensive for culture of rodent viruses and therefore is least practiced. Combination of ELISA and PCR is the best choice for detection of rodent pathogens when antibacterial treatment of bacterial diseases is not required or recommended. Immuno-histochemistry is the other choice which helps to detect the presence of the organism before serologic response is detectable. Accepted eradication methods for bacterial pathogens are complete destruction of colony in the module and disinfection of those housing rooms. Re-establishment of the colony is then possible through import of new strain or re-derivation of embryos from the cryo-preserved stock (Scavizzi and Raspa, 2006; Yeom *et al.* 2009: Thorat and Ingle, 2012).

Since many pathogens can not be cultured easily with conventional microbiology, PCR method assumes importance in such cases. ELISA is the second choice of method for detection of the rodent pathogens. ELISA method is useful after the formation of antibodies in the body. However, it may take few days to weeks to form the detectable antibodies in the body. Since the antibodies formed in the body may persist for months, serology provides insight to the past exposure to the infection but fails to provide the information of active or current infection status. PCR assumes importance in such cases where the presence of pathogens can be detected as soon as the pathogens infect the organs and settle in the organ of predilection (Bauer and Riley, 2006). The only disadvantage may be that the self limiting or inactivated viruses may also be detected by PCR. However, such information may also be important for extracting the first hand information of the exposure of the laboratory animals to the pathogens. PCR method also assumes importance in detection of rodent pathogens in immuno-compromised mice where sufficient antibody titer may not be present to detect by ELISA. In Indian scenario where sentinel animal testing is seldom practiced, PCR is the method of choice for detection of rodent pathogens in immuno-compromised mice. Even in cases where sentinel program is used for screening, longer period of exposure of sentinel animals may be required to acquire the infection.

MHV infection was consistently prevalent as compared to other infections. Presence of MHV was consistently observed by ELISA method since 2005 till 2008 (unpublished data). Since the samples were consistently found positive for MHV, they were not included for screening by ELISA during Jan. 2009 to June 2012. The PCR method was included in 2010 which again confirmed the presence of MHV in the colony (Fig. 1.A.6). MHV is a category B pathogen and is a widespread in laboratory and wild mice worldwide (Yamada *et al.* 2001). All samples in 2011 were found negative by PCR may be because of the self limiting nature of the infection (Nicklas *et al.* 2002). Results of the first half of the year 2012 also showed absence of MHV in the colony (unpublished data). MHV positive percentage by ELISA was found to be 41.67% for the year 2008 which is highest of any other pathogen found in the current study. MHV positive percentage by PCR was found to be less than 3%. Such a significant variation in ELISA and PCR positive percentage may be because of the cross reactive antibody response amongst various MHV strains infecting the laboratory rodents as well as wild type isolates (Yamada *et al.* 2001).

Helicobacter infection, the most frequently diagnosed infection in the Animal Facilities is often reported as subclinical but may have potential to induce clinical disease or may even interfere with the results in experimental animals (Martino-Cardina *et al.* 2010). Therefore, FELASA recommends screening of *Helicobacter*. *Helicobacter hepaticus* is reported to induce persistent hepatitis in certain mouse strains and the species is also reported to be associated with liver cancer in A/J mice. *Helicobacter bilis* is identified as a novel *Helicobacter* spp. colonizing the bile, liver and intestine of the adult mice (Fox *et al.* 2004). In the present study *Helicobacter* testing was carried out by ELISA as well as PCR. In this study, we observed 17% incidence of Hh by ELISA in 2009. We also observed 48% of the samples infected

with Hh (Fig. 1.A.3) and 59% samples positive for Hb by PCR (Fig.1.B.8). Possible reason may be that the sequences used for amplification of Hh and Hb may also be amplifying the other species of the *Helicobacter* genus (Poynter *et al.* 2009). Other possibility is that the open-air cage housing has high possibility of spilling the dirty bedding on the floor and thereby transferring the infection to the other cages. Housing the animals in filter top cages or IVC's could be of any help in reducing the spread of infection. *Helicobacter* spp. is also reported in the sex organs of the animals. However, the infection was not associated with the vertical transmission of the infection to the offspring (Scavizzi and Raspa, 2006).

Murine respiratory mycoplasmosis is a common and significant disease of laboratory rat and mice caused by *Mycoplasma pulmonis*. In addition, this organism is also reported to produce genital infection, endometritis, salphingitis and perioophoritis and thereby reduced fecundity (Reyes *et al.* 2000). Culture and serological methods are generally used for diagnosis of MP. Since culture is time consuming as well as difficult, we have used serological as well as PCR method in this study. However, serological method has a drawback of interspecies cross-reaction (Kim *et al.* 2005). In the present study, we observed only 6 MP positive cases out of 275 which accounted to 2%. However, PCR method showed 56% incidence (Fig. 1.A.1). *Mycoplasma* species most commonly found in the serum are bovine, porcine or human origin. They may render the colony sterile and therefore should not be present in the animal colonies. However, *Mycoplasma* species pathogenic to rodent is a frequent pathogen found in laboratory rodents.

Mouse parvovirus is the most common pathogen of laboratory rodents and biological materials but has not been associated with disease in either natural or experimental infection (Bauer and Riley, 2006). Diagnosis of MPV relies mostly on serologic detection of anti-viral antibodies in the host because of high sensitivity and specificity. In the present study, we observed 41% and 26 % incidence of MPV and MVM by ELISA method in the laboratory rodents maintained at ACTREC. Due to non-availability of positive control of these diseases, PCR method was not adopted.

Sendai virus induces acute respiratory tract disease in laboratory mice and is also a common contaminant of the research biologics. Very low incidence (2%) of Sendai virus positive samples is reported in this by ELISA whereas PCR revealed 27% incidence (Fig. 1.A.2).

*Pneumocystis carinii* is one of the pathogens which are reported to be pathogenic only to immuno-deficient animals (Yeom *et al.* 2009; Yabuuchi *et al.* 2010). In the present study, we observed the presence of PC (10%) in immuno-competent as well as immuno-deficient animals by ELISA method. However, the incidence was seen significantly high (53%) by PCR method (Fig. 1.B.7).

Mouse polyoma virus is reported to induce persistent infection and tumors in neonatal mice (Carty *et al.* 2001). Very low incidence of PV (3%) was observed in the present study by ELISA method. Due to non-availability of positive control of this disease, PCR method was not adopted.

*Pasturella pneumotropica* is a ubiquitous opportunistic pathogen in the animal colonies and is associated with abscesses of periorbital and preputial region in immuno-deficient rodents (Kawamoto *et al.* 2011). We observed 14% of incidence in immuno-deficient as well as immuno-competent animals.

MNV is recently recognized as pathogen that causes lethal infection in immuno-compromised mice. In the present study, MNV was found positive in less than 5% cases by PCR method (Fig. 1.A.4) and that too in the immuno-competent mice strains.

Hanta viruses are newly emerged rodent pathogen which are transmitted by aerosol or bite and pose significant health hazard to personnel exposed to the infection (LeDuc, 1994; Simmons and Riley, 2002). In the present study, out of 119 samples, 4 samples were found positive by ELISA method in the year 2009-2010. However, in 2011 and first half of 2012, all samples were negative by ELISA method. Hanta viruses were found negative by PCR method in 2010.

In the present study, Ectromelia, Pneumonia virus of mice and LCMV were tested by ELISA method and all samples tested were found negative for these pathogens. Similarly CAR was tested by ELISA as well as PCR but both the method identified very negligible incidence of less than 2%. Cross reactivity with antibodies to other bacterial species is a limiting factor for the specificity of CAR bacilli diagnosis by ELISA. As against the notion that the CAR may be relatively common in conventionally housed rodents (Waggie *et al.* 1994), the incidence reported here is very negligible. Testing of Tyzzer's disease by ELISA method in this study also identified small incidence of 3%. Again cross reactivity is reported between strains of isolates from various animal species.

Infection of the laboratory rodents is reported world wide and cause of entry of the infection is seldom known (Scoondenmark-van *et al.* 2006). Certain species/ strains are more sensitive to certain infections than other strains which produce altogether different symptom or mild or may even remain as asymptomatic. These differences have also been linked to be genetically controlled (Itoh *et al.* 1988; Bauer and Riley, 2006). Nude and SCID mice are more sensitive to the infections because of their immuno-compromised status.

Use of infected animals which do not have implications on the experiments may harbor the infection in body fluids, tumors or cells and may persist in them for a long time and may even affect the *in vitro* experiments (Bootz *et al.*

2003). Therefore, microbiological quality control must be considered as an integral part of the animal experiments. The results of the microbiological assessment of animals therefore assume importance in publication of results using such animals. Implications of pathogenic infections are several times mistaken as experimental results and may be falsely interpreted. This may be the reason for the lack of reproducibility of the experiments (Scoondenmark-van *et al.* 2006).

Even though we procure animals from the reputed international vendors who provide the microbiology quality assurance reports, standard practice of screening the random samples from the external party is highly recommended. It is the general practice of every reputed international vendor to provide the microbiological quality assurance certificate along with the animal supply. However, local vendors in India rarely follow this practice for variety of reasons. Main reason could be the lack of such organized microbiological screening program or no demand from the customer. This observation is highlighted from the fact that there are hardly any data published on incidences of microbiological screening in pubmed indexed journals from the organized animal facilities/ laboratories in India. With more and more such efforts are made, many more pathogens will be evident in the country. Presence of reported rodent pathogens in ACTREC Animal Facility could be a result of maintenance of animals under conventional Animal Facility with absence of positive pressure and HEPA filtered air for the animal rooms during the report period. The result highlights the importance of maintenance of research animals under positive pressure and HEPA filtered air.

Microbiological monitoring program is either based on randomly selected animals from the colony or sentinel animals exposed to the animals in question or to their soiled bedding. Use of sentinel animals is seen adopted in developed countries because it contributes standardized health monitoring program. However, it may not always provide reliable information as some of the pathogens like *Helicobacter* are not easily transferred to the sentinel animals (Jacobsen *et al.* 2005). In the present study, we have not used sentinel animals for the screening and rather used randomly selected animals for one more reasons of the non-availability of clean animals to be introduced as sentinel animal.

Although there are guidelines worldwide to screen the rodent pathogens, there are no authentic documented literature/ guidelines in India for surveillance of rodent pathogens. There also lack the document for the action to be taken when animals are found positive for the pathogens. We need to establish the guidelines for this purpose suited for Indian scenario. Results of the present study highlight the probable presence of rodent pathogens in Indian scenario although animals of better microbiological quality are the need of the researchers. Locally, prevalence of rodent pathogen may vary between research institutes. Publishing and compiling such data will be of great help to the Indian laboratory animal science fraternity. We do not advocate here comparing our positive rodent pathogen incidence with any of the reports elsewhere. Our aim was just to report the findings as incidence or prevalence of these organisms under Indian situation.

More organized efforts are required for early diagnosis of the prevailing/ persistent infection and strategies for prevention and treatment of these pathogens. Frequent screening schedule with recommended and accurate diagnostic program will help to timely detect and prevent the prevailing infection. In addition to the moribund animals, it is recommended to routinely screen the asymptomatic animals as well as research biologics used in animal experiments. Moreover, quarantined animals must be screened for rodent pathogens before entering the main Animal Facility. We recommend monthly screening by conventional culture method and at least six monthly ELISA and PCR method of testing of rodent pathogens in the laboratory rodents. In order to execute the microbiological quality control program, appointment of qualified specialist with relevant education and experience with laboratory animals is key factor for the successful program.

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