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Survey of health monitoring programmes from laboratory animal facilities in India

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Abstract

Health monitoring in laboratory animals provides health status of the colony and helps in providing clean animals which plays a pivotal role in the outcome of the experimental results. The assessment of microbial status of the laboratory animals is considered as part of the animal care program. The primary objectives of the survey included were as follows: a) To understand the health status of the animals and health monitoring programs in India, b) To create a platform to share the ideas and practices followed among the laboratory animal care professionals, c) To provide solution to uplift the standards of these programs. The institutions that participated in this program were approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and some of them certified by Good Laboratory Practice (GLP) of the National GLP Compliance Monitoring Authority of Dept.of Science and Technology, Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and National Accreditation Board for Testing and Calibration Laboratories (NABL). The response from each institution was obtained unanimously and the compiled results were maintained confidentially prior to disclosure in an appropriate forum. The responses showed that most of the institutions were following health monitoring programs and are required to harmonize testing methods and frequency based on the duration of experiments as well as the integrity of the facility.

Key words: Health monitoring survey, sentinel program, rodent diseases, diagnostic methods, sanitation practices

Introduction

Laboratory animal health monitoring is considered as an integral part of the quality assurance system by Good Laboratory Practice (GLP), Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), International Standards Organization (ISO) (Nicklas *et al.*, 2002) and for other regulatory purposes (Weisbroth and Emily, 2000). Microbiological standardization is an important prerequisite for reproducible animal research because infections may influence the outcome of the experimental results. However, it is necessary to establish the health monitoring programme in breeding and experimental facilities as part of the quality assurance system (Mahler, 2014).

The importance of viral infections as complicating factors in biomedical research programmes are recognized widely and infections may alter biological parameters and animal responses. The microbiological contamination in laboratory animals implies the following problems a) Occupational health and safety problems for the laboratory animal care personnel against zoonoses such as hemorrhagic fever with renal syndrome (HFRS), (Chandy *et al.,* 2008; Pritchett-Corning *et al.*, 2009; Nitatpattana *et al.*, 2000) and lymphocytic choriomeningitis virus (LCMV), (Bhatt *et al.*, 1986; CDC, 2012; Knust *et al.,* 2013). b) The risk of spread of infectious diseases in the facility. c) Confounding of research results due to infection in animal facilities caused by the invasion of laboratory animal-specific pathogens (Bhatt *et al.*, 1986;

Baker 1998). Innumerable case reports and epidemiologic studies have documented the occupational health hazards of zoonotic diseases from laboratory animals or their tissues in the conduct of biomedical research, teaching, and testing (Weigler *et al.*, 2005). The risk factors are associated with transmission of pathogens leading to potential outbreak and disease occurrence in the laboratory animals and zoonotic to animal care personnel. Zoonoses are diseases and infections that are naturally transmitted between vertebrate animals and human beings (WHO, 1967). The pathogenicity may be due to favorable factors including host, microbial load, environmental or combination of all the factors (Berard *et al.,* 2009). The increasing demand of special strains for biomedical research in various therapeutic areas necessitated in-house breeding, transferring within institutions and importing from local and/or international vendors. The health monitoring programme depends on institution-wide implementation and is essential because of the collective risk associated with interactive animal use (Jacoby and Lindsay 1998). Nevertheless, disease free animals play a pivotal role and provide clean status of the facility, animal health assurance to the researchers and in turn ensure meaningful research data (Devan *et al.*, 2011). In addition, FELASA advocates accreditation of diagnostic laboratories and health monitoring schemes according to the FELASA guidelines (Nicklas *et al.*, 2010) and published recommendations for the health monitoring of laboratory animals for breeding colonies and experimental units. In the past, health monitoring workshops and seminars were organized related to laboratory animals in India but there was no previous epidemiological information documented on the health status of laboratory animals on pathogenic organisms as well as health monitoring practices in India except few like Harikrishnan *et al*, 2011; Ingle and Shinde, 2011; Ingle and Shinde, 2014. Considering all the above factors into account, the questionnaire was framed and the survey was conducted among laboratory animal care professionals from various organizations in India to understand the current health monitoring practices and prevalence of pathogens, if any, in their facilities.

Materials and methods

Survey Questionnaire

The present survey was conducted by creating 31 questions (Appendix 1) on laboratory animal health monitoring program. The online survey was conducted by creating unique web link for each institution and the responses were invited to complete the survey by providing choices based on their institutional policy. The questionnaire was validated prior to the survey and ensured that the program was created in such a way that no further edits options upon survey completion. The survey was conducted during the year 2012-13 and the participants were identified from established research institutions (central/state government), academic institutions/university, contract research organizations (CRO) and pharmaceutical/biotech firms. A non disclosure agreement was signed prior to the survey and to avoid any further conflicts of interest to disclose the results in the appropriate forum. The response from each institution was obtained unanimously and compiled results were maintained confidentially.

Results

The survey response from 107 participant organizations was received. Apart from this, serology survey showed positives in tested samples for some of the murine pathogens (data not shown). The respondents were research institutions (28%), academic institutions/university (22%), CRO (22%) and pharmaceutical/biotech (28%) (Fig. 1) and the population of laboratory animals maintained by each participating facilities were ranging from 1000 to 10000 laboratory animals. The institutions were approved by CPCSEA (97%) and some of them were GLP (23%) certified, AAALAC (21%) and NABL (7%) (Fig. 2).

Figure 1 : Type of Organizations

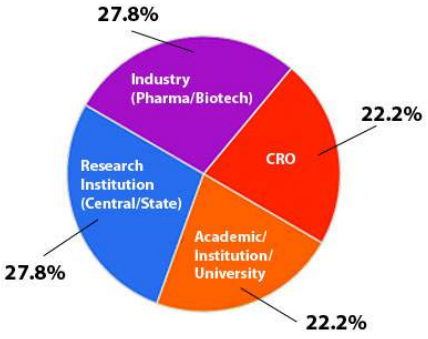
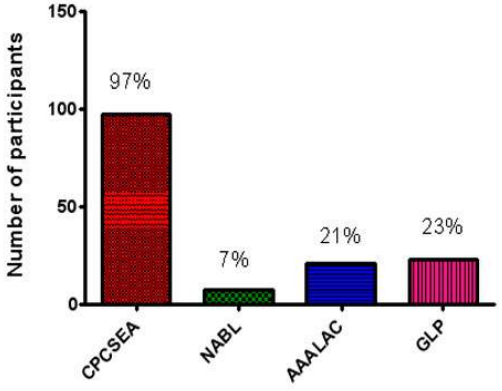


Figure 2: Type of Registration/Accreditation/Certification



The data was presented from mice, rats, hamsters, Guinea pig and rabbit facilities, but the respondents were also maintaining canine as well as primates (data not shown due to limited sample size). The systems for housing animals were conventional cages (77%) along with individually ventilated cages - IVC (47%) and usage of isolators (16%) depending upon integrity of the facility. Many institutions were following in-house breeding (74%) and some of them import animals from defined sources (54%) either from abroad (33%) or local procurement (41%) (Fig. 3). Most of the facilities participating in the survey were housing rodents (96%) based on their experimental need along with rabbits (66%) in conventional facilities (71%), specific pathogen

free (SPF) (24%), Germ free/Axenic (19%), and Gnotobiotic (2%) colony (Fig. 4). The institutions were using autoclaving (83%) for animal accessories/material sterilization, usage of disinfectant agents (79%) for floor mopping, fumigation (73%) of animal rooms and cage/rack washer or equivalent mechanical equipments (47%) as sanitation methods.

Figure 3: Source of Lab animals maintained in India

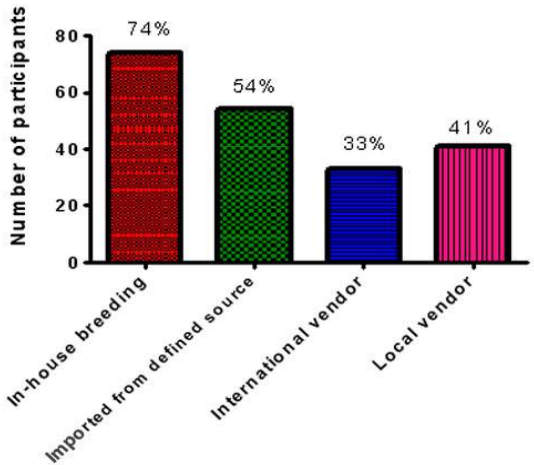
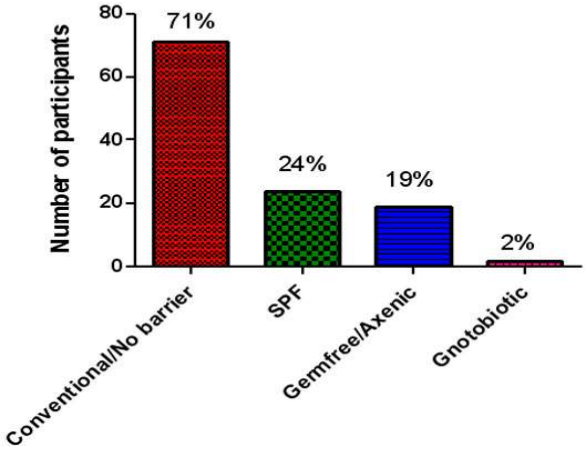


Figure 4: Type of Barrier Systems maintained



The quarantine procedure (94%) was followed by most institutions upon receiving animals from external sources and/or vendors and the period was maintained between 7 to 21 days for rodents, non rodents, rabbits and up to 42 days for dogs and non human primates depending upon the source of procurement. The survey responses showed that majority of them were performing limited and/or extended health monitoring program (87%) and maintaining historical data (62%) for their colonies. The pattern of health monitoring includes sentinel program (42%), random sampling from the colony (76%) and study based animals screening (26%) based on their institutional policy. The testing methods and frequency was varied and depends on their nature of work and duration of the animals housed for their experiment. The results showed that most of the institutions were screening organisms quarterly (61%) and some of them screening for selective organisms on monthly basis (21%); few institutions were screening organisms at less frequent intervals i.e. half-yearly (19%) and annually (15%). Most of the respondents opted for testing samples at in-house laboratory (83%) whereas some institutes were outsourcing (47%) in India and/or abroad. The sample size used for testing at each interval was <5 (46%), 5 to 10 (43%) and >20 (11%) samples. The test methods adopted were enzyme-linked immunosorbent assay (ELISA) (61%), polymerase chain reaction (PCR) (28%), immunofluorescence antibody assay (IFA) (2%), blood profile includes hematology and biochemistry (68%), microbiological (culture plates) and parasitological (74%) technique which includes tape test, fecal flotation and skin scrapping etc.

The organisms listed in the screening panel include viral (Fig. 5), bacteria and parasites (Fig. 6) from rats and mice. In addition, pathogens from other species viz., hamster (Fig. 7), Guinea pig (Fig. 8) and rabbit (Fig. 9) were screened as per the list given. In addition, pest control program was practiced in some of the organizations (76%). Policies for outbreak management and dealing with either of the following methods such as a) Reconfirmation of pathogens by different tests and/or laboratory, b) Treatment and eradication (mostly of parasitic infestations), c) Depopulation and rederivation, d) Contained with appropriate method during the experiment e) Quarantine procedure with restricted traffic pattern was also reported as practice.

Figure 5: List of Viruses screened from Rat & Mice

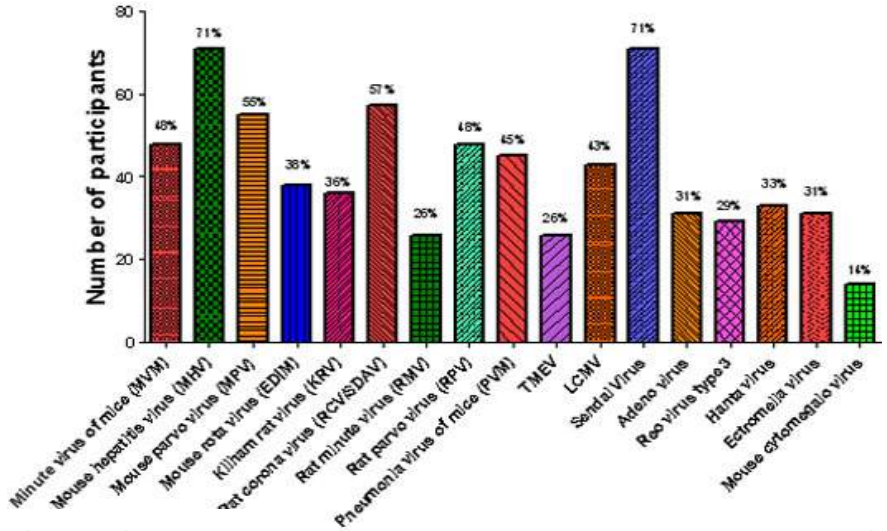


Figure 6: List of Bacteria and Parasite screened from Rat & Mice

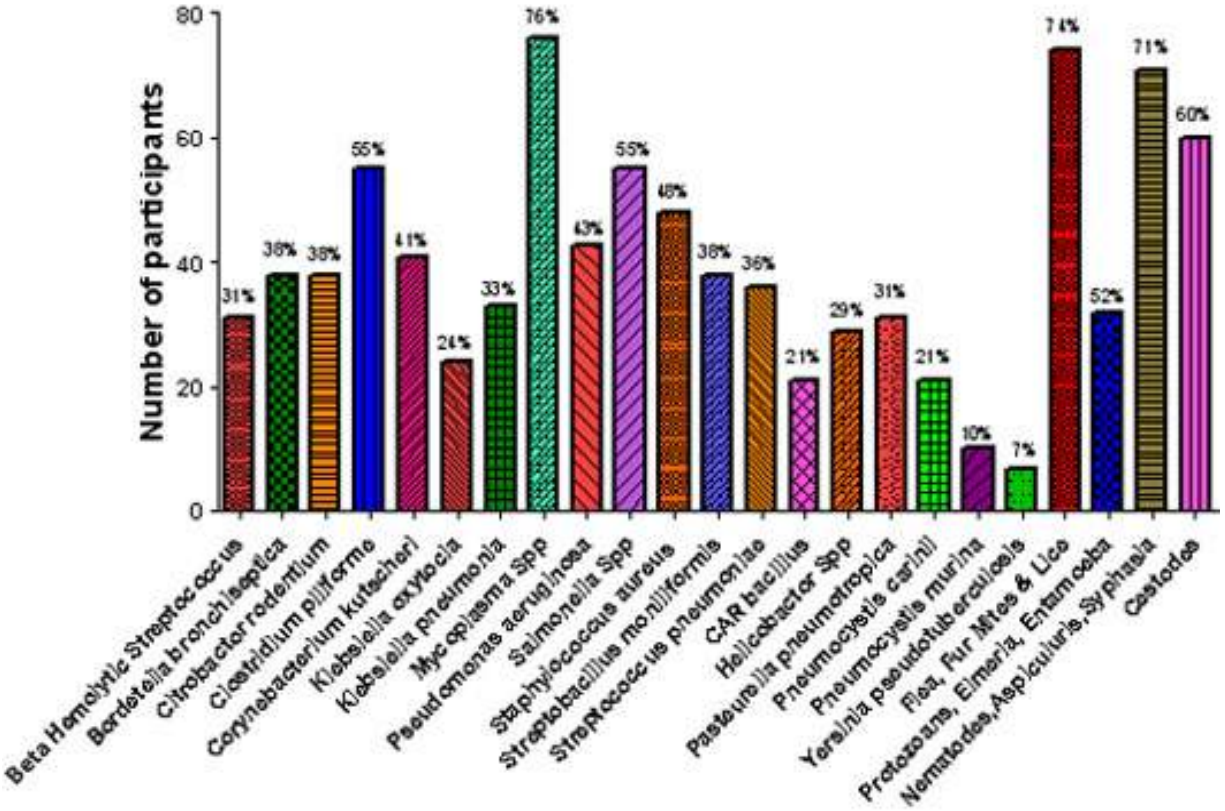


Figure 7: List of Virus, Bacteria and Parasite screened from Hamster

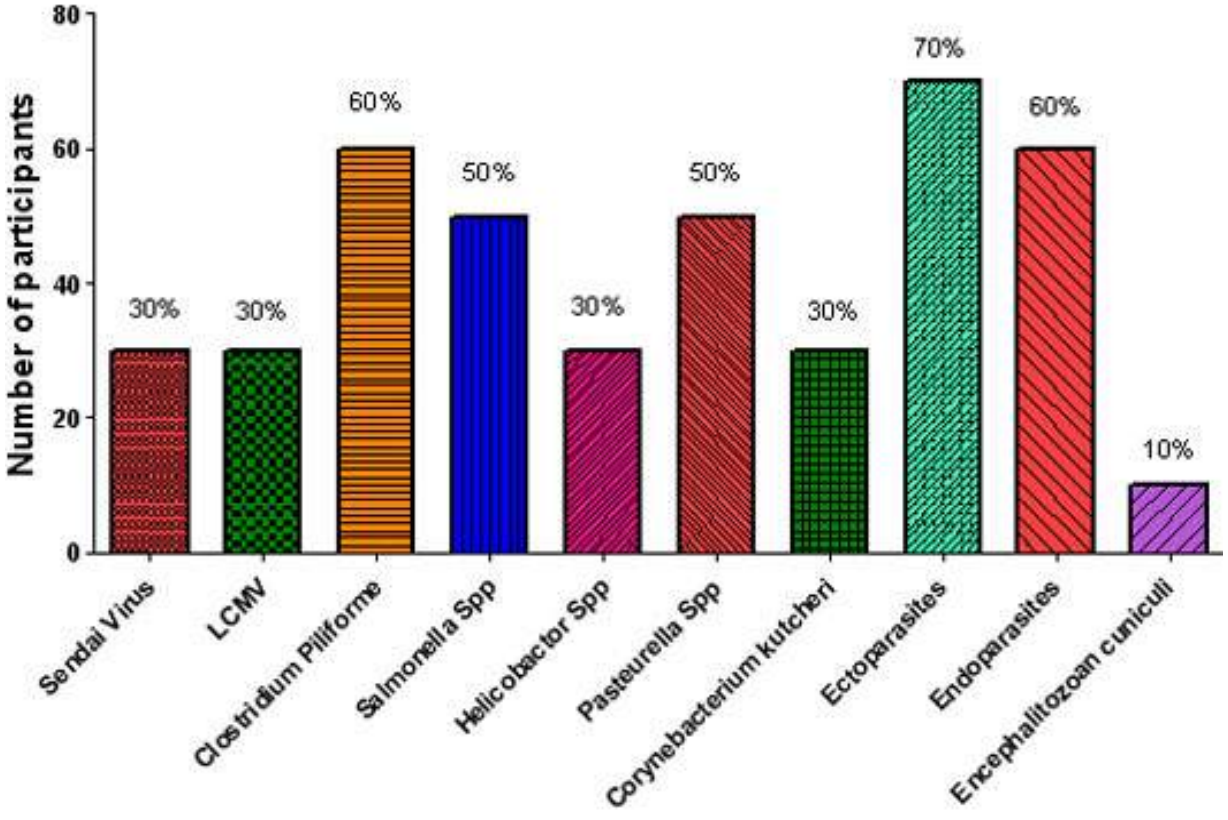


Figure 8: List of Virus, Bacteria and Parasite screened from Guinea pig

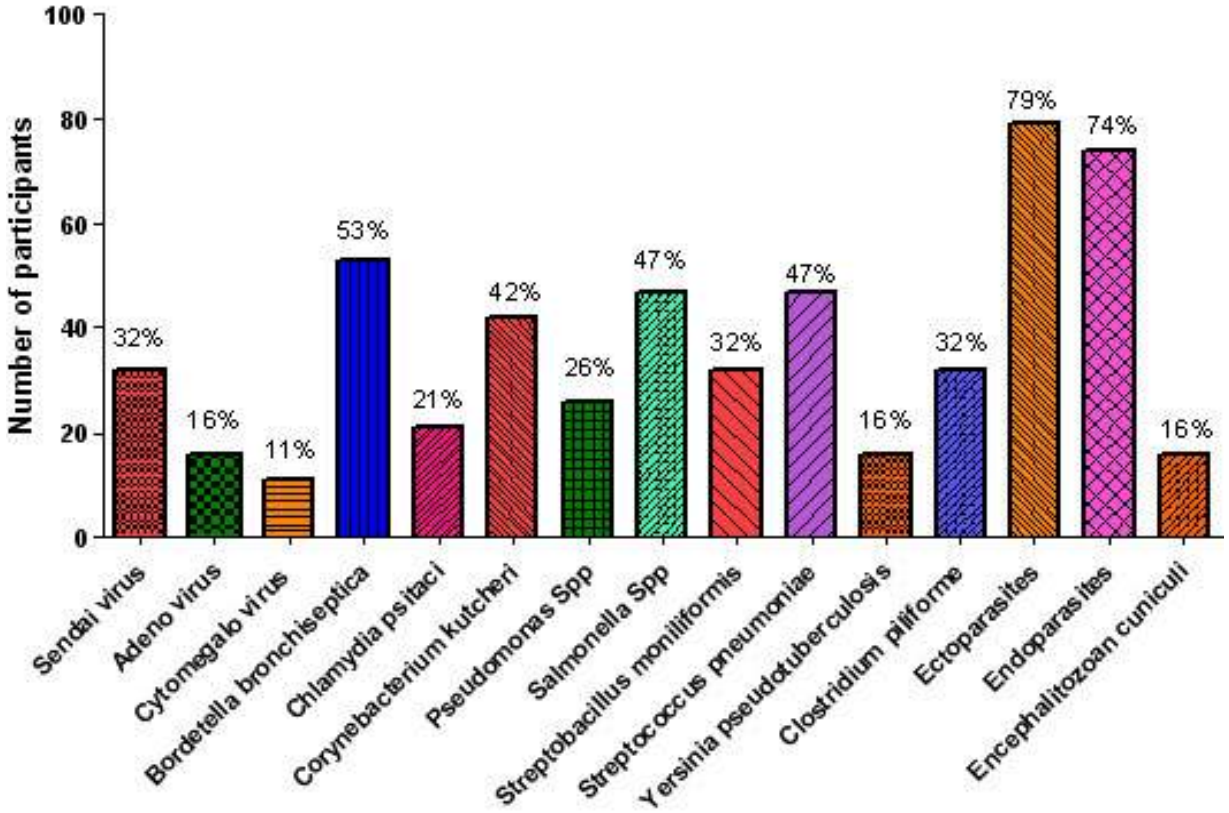
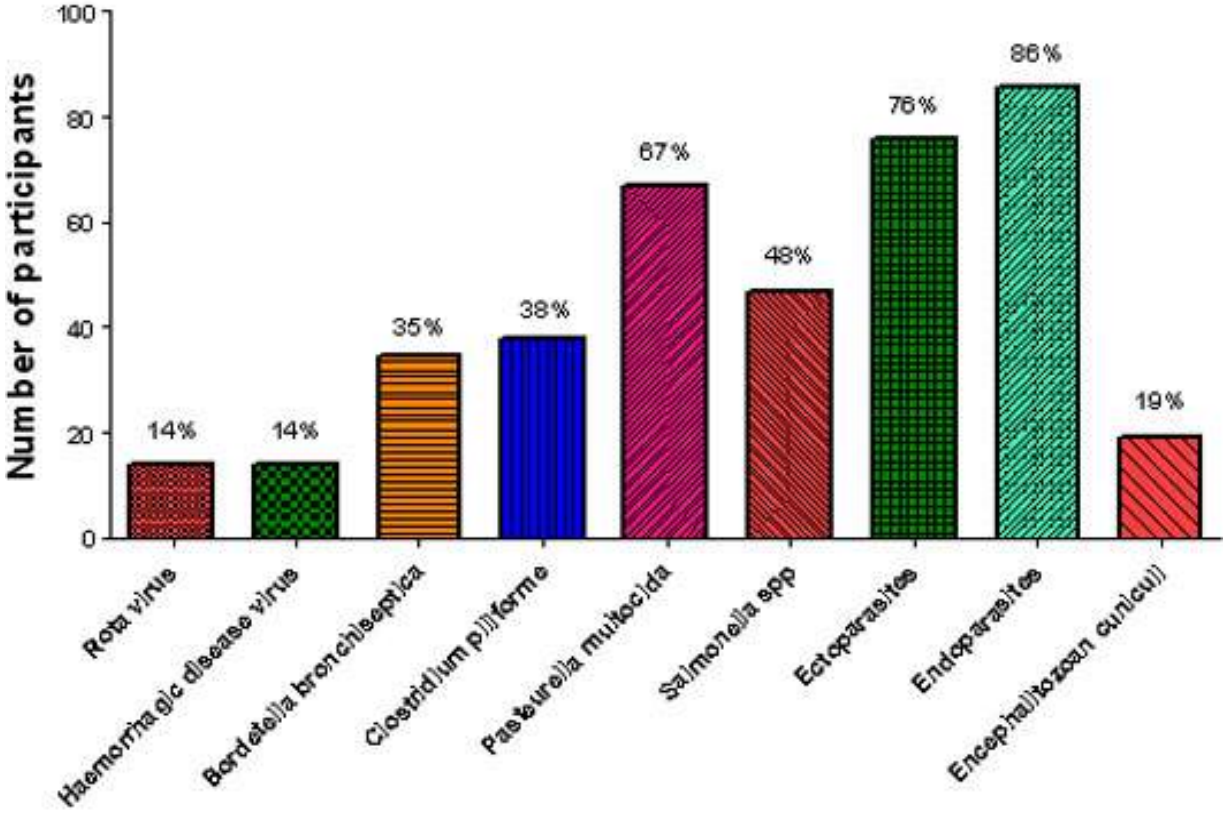


Figure 9: List of Virus, Bacteria and Parasite screened from Rabbit



Discussion

The survey responses showed the status on health monitoring systems in India and the participated organizations (Pharma/Biotech, CRO, Central/State Governmental Research Institutes and few academic universities) were screening the organisms at periodic interval either limited (few selected organisms) and/or extended (most of the organisms) profiles based on their research needs. In fact, the survey was initiated to collect the serum samples and test them at Veterinary Sciences Serology Laboratory at Bristol-Myers Squibb, Wallingford, CT, USA. Samples were received from few institutions and were screened for viral agents at the above laboratory. In addition, some of the institutions showed concerns to provide samples due to their institutional policies and process involved including shipping, testing at distant place and confidentiality. On the other hand, dispatching samples from unknown origin was considered risk at the testing laboratory. Meantime, it was decided to send an online survey to understand the current status. The health monitoring is considered as an important program especially when exchanges of animals and animal products occur among neighboring institutes. The organizations responded were maintained animals in the barriers as well as conventional setup and assumed that a risk of spread of infection within the facility considering the integrity and/or different species housed. There is a risk of cross-infection between SPF and non SPF facilities which may be a potential contamination in laboratory animals that can confound the research (Jacoby and Lindsey, 1998). The infection of laboratory rodent colonies occurs worldwide and the mode of pathogen introduction is often unknown (Baker, 1998; Livingston and Riley, 2003; Easterbrook, 2007; Gaertner, 2004). The transmission of infection may occur through animal transportation between the organizations, entry of wild rodents, crawling insects into the facility or contact during the transit. Biological materials such as cell lines and antibodies exchange will eventually leads to risk for animal care professionals who are dealing with conventional facilities of unknown status. The prevalence of various diseases may have increased recently as institutes are handling large numbers of transgenic mice and rats that are often immunocompromised (Pritchett-Corning *et al*, 2009). The survey revealed that import of animals from local and international vendors have increased. At the same time, the recipient institutions did not maintain them in the same conditions in which the animals were procured due to constraint of their facilities and practices. However, attention should be given to upgrade the conventional facilities as well as periodic monitoring of microbial agents to minimize the potential contamination.

The quarantine programs may be costly in terms of effort and time but these can be justified against potential outbreaks that could invalidate long term studies (Rehg and Toth, 1998). Consideration should be given to quarantine for newly arrived animals based on source as the health status is unknown because of surrounding environment where the shipper boxes being handled as well as mode of transportation. In cases where suspected positives samples for any organisms are tested during the quarantine period, in-house colony and sentinels may be retested with existing samples collected or again sampled for verification by any other established laboratory. If the suspected positive samples are reconfirmed then necessary measures should be taken for eradication *viz* rederivation, containment in the designated rooms for experimental infection or rejection of the consignment (Yamamoto, 2001). The survey revealed that ELISA was preferred as primary method and followed by PCR for viral and few microbial agents. In addition, culture techniques and tape tests were predominantly used for microbial and parasitological screening, respectively. However, the screening strategies varies among the organizations and most of them adopted to test the samples in-house and some of them preferred third party laboratory; few organizations were screening in-house as well as outsourcing. Although, some of the institutes did not have a diagnostic laboratory, they preferred to use the animals upon receipt with limited quarantine period for short term experiments. Health monitoring may be expensive, and the knowledge of which diseases are common or rare in local settings is very useful so that sample size and testing frequency can be adjusted (McInnes, 2011). Hence, it is important to test the samples with any established laboratory apart from in-house diagnostics; this will provide confidence on the results and better assurance for their own laboratory even though validated procedure followed to screen the samples. However, the comprehensive health monitoring before and during experimentation is the ideal way to demonstrate the presence or absence of unwanted microorganisms and suitability of the colony for specific experiment (Nicklas, 2008).

The survey is not comprehensive but this result is the first report based on the current practices on laboratory animal health monitoring programs in India. In addition to the existing results screened from serum samples; additional testing is required with more samples which will be obtained from different species along with representative samples for viral, bacterial, parasitic infections from various organizations that will provide complete prevalence of pathogens in laboratory animals. However, these survey results helps as guidance to understand the health monitoring systems in laboratory animals including the type of organisms to be screened as well as necessary steps required to focus towards improving current practices on laboratory animal health standards.

Conclusion

The compilation of survey responses showed that most of the institutions were following health monitoring programs and required to harmonize the testing methods and frequency based on the integrity of facilities, research needs as well as duration of the experiments. The survey provides an idea on the health status and practices followed in India which will be extended by obtaining additional samples to screen further using various methods to understand the prevalence of organisms from laboratory animal facilities. The existing practices should be improved towards health monitoring standards of those institutions that are monitoring with limited panel of screening and/or not part of the survey. It is evident that attention has been provided by most institutions towards quality animal research and welfare. The following options may be necessary to consider as precautionary measure and prevent the entry of any pathogens from outside sources as well as within the facility to avoid potential contamination in the vivarium.

1. Upgradation of caging systems, housing conditions including HVAC to enhance the containment of the facility,
2. Establishing or revising the institutional standard operating procedures and/or guidelines to improve diagnostic process,
3. Having an institutional policy on procurement of animals from defined sources,
4. Establishing quarantine practices and screening strategies for newly arrived animals prior to the release,
5. Sentinel program to detect early infection if any from the colony.

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Appendix 1 - Survey Questionnaire

Note: *Please type/ tick on your answers to complete the survey.*

1. **Demographic details of your organization**
   City/Town, State/Province

2. **Type of organization**
   - Academic Institution/ University
   - Research Institutions (Central/State)
   - Industry (Pharmaceutical/Biotech)
   - Contract Research Organizations
   - Others (please specify)

3. **Name of the organization (optional)**

4. **Type of registration/certification/accreditation of the facility**
   - CPCSEA
   - NABL
   - GLP
   - AAALAC
   - Others
   - None

5. **Laboratory animal species maintained at your facility**
   - Rodent (Rat, Mice, Hamster & Guinea Pig)
   - Rabbit
   - Canine
   - Non Human Primates.
   - Others (please specify)

6. **Type of barrier system maintained at the facility?**
   - Germ free (Axenic)
   - SPF
   - Gnotobiotic
   - Conventional/No barrier
   - Others (please specify)

7. **Caging systems used for housing the laboratory animals**
   - Conventional/Open top cages
   - Individually Ventilated Cage (IVC)
   - Isolators
   - Others (please specify)

8. **Types of sanitation practices followed at the facility**
   - Autoclaving
   - Fumigation
   - Cage/rack washer
   - Periodical mopping
   - Others (please specify)

9. **Source of animals maintained at the facility**
   - In-house breeding
   - Imported from defined source
   - International vendors
   - Local vendors
   - Others (please specify)

10. **Population size of the facility (including all the laboratory animals)**
    - Less than 1000
    - 1000 to 5000
    - 5000 to 10,000
    - More than 10,000

11. **Quarantine procedure followed at your facility**
    - Yes, Always Quarantined
    - Animals directly used for the experiment upon receipt.
    - No quarantine
    - Others (please specify)

12. **Quarantine period maintained for rodents/non-rodent/ large animals**
    - 7 Days
    - 14-21 days
    - 28-42 days
    - Others (please specify)

13. **Do you have historical data base for the health monitoring**
    - Yes
    - No
    - If yes, How many years (please specify)
    - Others (please specify)

14. **Do you perform health monitoring program at your facility (rodent/ non-rodent/large animals)**
    - Yes (rodent/ non-rodent/Large animals)
    - No
    - Others (please specify)

15. **If yes, what type of health monitoring program followed at your facility**
    - Sentinels
    - Random screening of samples at periodic interval
    - Health monitoring of study based animals
    - Others (please specify)

16. **Frequency of monitoring interval by screening the organisms**
    - Monthly
    - Quarterly
    - Half yearly
    - Annually
    - Others (please specify)

17. **Diagnostic methods adopted for screening the samples**
    - Serology :ELISA
    - PCR/LAMP
    - IFA/HI
    - Microbial & parasitological screening
    - Blood profile screening(hematology & Biochemistry)
    - Others (please specify)

18. **Diagnostic laboratory used for samples testing**
    - In-house laboratory
    - Out sourcing/Contract labs (Indian/International Labs)
    - Name of the laboratory
    - Others (please specify)

19. **Type of organisms screened at the facility**
    - Bacteria
    - Virus
    - Parasite, Protozoan
    - All of the above
    - None
    - Others (please specify)

20. **Number of animals tested for health screening from the colony (%)**
    - < 5
    - 5 – 20
    - > 20
    - Others (%) (please specify)

21. **List the organisms (viral/bacterial/parasitological) screened at your facility for rats and mice**

Viruses
- *Minute virus of mice (MVM)*
- *Mouse Hepatitis virus (MHV)*
- *Mouse Parvovirus (MPV)*
- *Mouse Rotavirus (EDIM)*
- *Kilham Rat virus (KRV)*
- *Rat corona Virus (RCV)*
- *Rat minute virus (RMV)*
- *Rat parvovirus (RPV)*
- *Pneumonia virus of mice (PVM)*
- *Theiler's murine encephalomyelitis virus (TMEV)*
- *Lymphocytic choriomeningitis virus (LCM)*
- *Sendai virus*
- *Adeno virus*
- *Reovirus type 3*
- *Hantaan virus*
- *Ectromelia virus*
- *Mouse cytomegalo virus.*
- *Pneumonia virus of mice (PVM)*
- *Murine noro virus(MNV)*
- *Mouse thymic virus(MTV)*

Bacteria
- β Hemolytic Streptococcus
- *Bordetella bronchiseptica*
- *Citrobacter rodentium*
- *Clostridium piliforme*
- *Corynebacterium kutscheri*
- *Klebsiella oxytoca*
- *Klebsiella pneumonia*
- *Mycoplasma*
- *Pseudomonas aeruginosa*
- *Salmonella sp.*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus pneumoniae*
- *Cilia Associated Respiratory Bacillus (CARB)*
- *Corynebacterium bovis*
- *Helicobacter sp.*
- *Pasteurella pneumotropica*
- *Pneumocystis carinii*
- *Pneumocystis murina*
- *Yersinia pseudotuberculosis*

Parasites
- Flea, Fur mites, lice
- Protozoans- *Eimeria spp. Entamoeba muris, Giardia muris, spironucleus muris, cryptosporidium muris*
- Nematodes *Aspiculuris* spp., *Syphacia* spp**.**
- Cestodes - *Hymenolepis* spp.
- Others ……..

22. **List the organisms (viral/bacterial/parasitological) screened at your facility for hamster**

Viruses
- *Lymphocytic choriomeningitis virus (LCM)*
- *Sendai virus*

Bacteria:
- *Clostridium piliforme*
- *Salmonella sp.*
- *Helicobacter sp.*
- *Pasteurellacea*
- *Corynebacterium kutscheri*

Parasites
- *Endoparasites*
- *Ectoparasites*
- *Encephalitozoan cuniculi*
- Others.....

23. **List the organisms (viral/bacterial/parasitological) screened at your facility for guinea pig**

Viruses
- *Guinea pig Adenovirus*
- *Sendai Virus*
- *Guinea pig cytomegalovirus*

Bacteria
- *Bordetella bronchiseptica*
- *Chlamydia Psittaci*
- *Corynebacterium kutscheri*
- *Pasteurella sp*
- *Salmonella Sp*
- *Streptobacillus moniliformis*
- *Streptococci pneumonia*
- *Yersinia pseudotuberculosis*
- *Closteridium piliforme*

Parasites
- *Ectoparasites*
- *Endoparasites*
- *Encephalitozoan cuniculi*
- Others.....

24. **List the organisms (viral/bacterial/parasitological) screened at your facility for rabbit**

Viruses
- *Rabbit haemorrhagic disease virus*
- *Rabbit rotavirus*

Bacteria
- *Bordetella bronchiseptica*
- *Clostridium piliforme*
- *Pasteurella multocida*
- *Salmonella*

Parasites
- *Ectoparasites*
- *Endoparasites*
- *Encephalitozoan cuniculi, Eimeria*
- Others.....

25. **List the organisms (viral/bacterial/parasitological) screened at your facility for dog**

Viruses
- *Canine adeno virus type I*
- *Canine distemper virus*
- *Canine parainfluenza virus*
- *Canine Parvo virus*
- *Corona virus*
- *Rota virus*

Bacteria
- *Bordetella bronchiseptica*
- *Borrelia spp*
- *Brucella spp*
- *Leptospira spp*
- *Salmonella spp*
- *Streptococci beta-hemolytic group G*
- *Campylobacter*
- *Ehrlichia canis*
- *Escherichia canis*
- *Escherichia coli*
- *Microsporum spp*
- *Pasteurella spp*
- *Staphylococcus spp*
- *Trichophyton spp*
- *Yersinia enterocolitica*

Parasites
- Ectoparasites
- Endoparasites
- Others.....

26. **List the organisms (viral/bacterial/parasitological) screened at your facility for primates**

Viruses
- *Herpes B*
- *Hepatitis A virus*
- *Simian immunodeficiency virus*
- *Measles*
- *Simian T-cell lymphocytic virus*
- *Simian retrovirus type D*
- *Filiovirus*

Bacteria
- *Mycobacteria spp*
- *Salmonella spp*
- *Shigella spp*
- *Pseudomonas spp*

Parasites
- *Entamoeba histolytica*
- *Toxaplasma gondii*
- Ectoparasites
- Endoparasites
- Dermatophytes
- Others.....

27. **Type of procedure followed to detect the parasites**
- Tape test method
- Faecal sedimentation/ floatation method
- Skin scraping
- Blood smears
- Others

28. **Do you have pest control program at your facility**
- Yes
- No
- If Yes, describe the method of control

29. **Any prior incidence of outbreak in your facility. If yes, please specify the pathogens, year of outbreak and duration**

Year ……. Duration …… Not Known ……
- Virus: ……
- Bacteria: ……
- Parasite: ……
- Protozoa: ……..
- Others ……

30. **Type of action taken/procedure followed during Outbreak**
- Reconfirmation of pathogens by different tests & labs
- Treatment & Eradication
- Depopulation & Rederivation
- Contained with appropriate method during experiment
- Quarantine procedure with restricted traffic & improved sanitization practices.
- Others (please specify)

31. **Please type your additional comments and/or informations if any about your facility to add value to this survey**

Yes,
Not applicable
Other (please specify)