Pak J Med Res
Vol. 57, No. 3, 2018

Review Article

Occupational Zoonosis and Laboratory Animals-Based Research in Pakistan: A Strategic Vision for the Welfare of Laboratory Animals

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Abstract

Worldwide history shows, zoonosis has resulted in number of deaths and has a negative impact on economies. Zoonosis is not only limited to the wild or domestic animals but there is also a risk of zoonosis for researchers working with the laboratory animals in research institutes/universities. In the laboratory animal facilities, exposure of zoonotic agents from laboratory animals to humans has occupational and public health importance. In addition to the public health importance, the presence of zoonotic agents and other infections also affect the quality and reproducibly of biomedical research. According to international biomedical laboratory animal-based research data, the primary laboratory animal species being used mostly are rodents especially mice. Rodents including mice are the sources of various zoonotic agents. So, working with laboratory rodents, various diseases can be transmitted to researchers, technical and managerial staff. These diseases are usually asymptomatic in laboratory animals but can cause serious illness in humans and may also affect the reproducibility and quality of research. This review article mainly focuses on common zoonotic diseases of rats and mice, their mode of transmission and prevention. Most importantly, authors in this review article, are proposing a strategic vision focusing on making legislation, councils/associations to regulate laboratory animal care and use program for laboratory animal-based research in Pakistan. This strategic vision will not only help to prevent zoonosis but also help to improve the quality and reproducibility of animal-based research.
Key words: Zoonosis, laboratory rodents, mouse, rat, animal research, reproducibility, occupational health, laboratory animal research, Pakistan.

Introduction

The word zoonosis originates from Greek words zoion (animal) and noses (disease) and is defined as the diseases transmitted from animals to humans are called as zoonotic diseases and the phenomena is called as zoonosis.[1] The phenomena of zoonosis have been classified into direct and cyclozoonosis.[2] Diseases transmitted either by infected vertebrate or with a fomite or any mechanical vector is called direct zoonosis. Examples are leptospirosis, lymphocytic choriomeningitis etc.

Outbreaks of zoonotic diseases have resulted in economic losses of billions of dollars and the concept of one health has been implicated in the reduction of zoonosis risk.[3] Outbreaks of zoonotic infections have also been reported in laboratory animals.[4] However, zoonosis incidence from laboratory animals have not been reported yet in Pakistan but studies have indicated that leptospirosis like infections are emerging zoonosis in Pakistan.[5] Laboratory animals are being used in research for years and have been resulted in many inventions in applied as well as basic biomedical researches.[6] Infections in laboratory animals have been lined to alter physiological parameters including immune functions leading to impact quality and reproducibility of research.[7] So, all laboratory animals based experiment should be conducted in a way to minimize the risk of zoonosis leading to quality and valid research.

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Received: 01 January 2018, **Accepted:** 06 September 2018, **Published:** 29 September 2018

Authors Contribution

MR conceptualized the project. MR & JH did the literature search, drafting, revision and writing of manuscript.

In recent decades, efforts have been made to minimize the risk of laboratory animal zoonosis and improving the quality and reproducibility of animal-based research. Internationally, researchers are using laboratory animals complying 3Rs principle of Russell and Burch. Russell and Burch originated the concepts of 3Rs, replacement, reduction, and refinement, which they described in their book, The Principles of Humane Experimental Technique in 1959.[8] Russell and Burch proposed this model which is aimed to improve humane care and use of laboratory animals, quality of research and reducing the risk of zoonosis. Internationally, to ensure humane care and use of laboratory animals and minimize the risk of zoonosis, one health concept is being used. Different stakeholders in laboratory animal research are active, including accrediting agencies, animal welfare organization, animal biosafety organizations and laboratory animal training centers and specific species training programs.

The concept of 3Rs in the use and care of laboratory animals in Pakistan is still relatively less practiced and there is a self-regulated system by individual research universities.[9] However, researchers are getting exposure to this concept for humane care and use through increasing international collaborations. At federal and provincial government levels, the efforts in the form of legislation for humane care and use is lacking. However, a few universities have developed their own guidelines to comply minimum international standards.[9]

So, in Pakistan, there is a high need to establish a laboratory animal care and use program that not only ensure to minimize the risk of zoonosis but also improve the quality of research. This approach can be accomplished through a strategic vision involving different stakeholders including, the Federal Government, higher education agencies, veterinary council and institutes, animal welfare organizations, research institutes and researchers.

This review can play an important role in understanding laboratory animal zoonotic diseases, the importance of laboratory animal zoonosis, principals of animal care, use and establishing a comprehensive laboratory animal care program. This review will enable students, researcher, technical and managerial staff working and associated with the laboratory animal facilities about the common zoonotic diseases from rats and mice. This review will also include clinical signs, their mode of transmission and prevention from zoonotic diseases associated with laboratory rats and mice. This is especially more focused to enlighten students, researchers, higher education authorities about modeling an international laboratory animal care and use program. This will also help authorities to prevent any risk of an outbreak as there may be chances of the outbreak in laboratory animals' facilities.[4]

The first part of the review highlights the common zoonotic pathogens present in the laboratory rat and mouse, as almost 95% of the studies have been conducted on these species. The second part of the review gives a strategic vision for modeling a comprehensive animal care and use program.

Common zoonotic pathogens present in the laboratory rat and mouse

Lymphocytic choriomeningitis virus (LCMV), as the name indicates, LCMV is a viral zoonotic disease. LCMV belongs to Arenaviridae family of viruses. Mice and hamsters are known to transmit the LCMV infections, however, rats and few other mammals are also susceptible.[10] LCMV is transmitted among the rodents through different routes including mucous membranes, infected urine and through the oral route. Humans are infected with LCMV when exposed to urine, droppings, saliva, or nesting materials.[11]

LCMV is usually not deadly and mortality is reported less than 1%. All the people infected with LCMV do not show clinical signs and symptoms; however, the persons who become ill, symptoms may occur one to two weeks after exposure of the virus. Characteristic symptoms include; anorexia, muscle aches, headache, nausea, vomiting and sometimes fever as well. The less frequent symptoms may be joint pain, sore throat, chest pain, coughing and testicular pain.[11] Infected people with LCMV need medical and supportive treatment depending on condition and anti-inflammatory drugs are also indicated. In vitro studies have shown the effectiveness of ribavirin; however, its routine use in human has not been established.[12]

Hantavirus belongs to the Bunyaviridae family of viruses. Hantaviruses are further divided into different genotypes including the Hantaan virus and the Seoul virus. The first Hantavirus was reported in 1978 from Korea. Hantavirus associated diseases have been reported throughout Eurasia, especially in Scandinavia and Northeastern Asia.[13] In the United States, Hantaviruses have been isolated and serological studies have shown the presence of Hantaviruses in human as well. The outbreak and severity of infection in human is determined by the rodent host. In the laboratory animal facilities, the laboratory rats have been reported as a reservoir of Hantavirus and cause infections among the laboratory animal facility personals including researchers in Asia and Europe.[13]

In rodents, the virus is isolated mainly from the kidney and lungs and virus is shed in saliva,

urine, and feces of the infected rodents. Aerosol is reported the main mode of transmission of the virus however, the role of arthropods and biting is also reported as an important mode of transmission.[14]

Hantavirus in humans causes Hantavirus Pulmonary Syndrome (HPS). Symptoms may develop between 1 and 5 weeks after exposure and persons with HPS feel fatigue, fever and muscle aches, on early stages. Other symptoms may include headaches, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain. Around one week after the early phase, late symptoms include coughing and shortness of the breath.[12]

Rat bite fever, also called Haverhill fever, is a human illness that can be caused by *Streptobacillus moniliformis* (S. moniliformis) from asymptomatic rodents.[15] S. moniliformis is usually transmitted through rat saliva, via a bite. It may also be transmitted through ocular or nasal secretions. Generally no any symptoms in carrier rats; rarely, opportunistic pulmonary infections or abscesses are seen. In human, the symptoms usually occur 3-10 days after exposure to an infected rodent. The illness is characterized by chills, fever, headache, and arthritis, skin rashes with reddish brown or purple plaques, vomiting or sore throat.[16] Diagnosis for its prevention and control is very important, as this be responsible in affecting the quality and reproducibility of research. S. moniliformis may be diagnosed by culturing on blood agar from the nasopharynx of carrier rats or infected mice.

Salmonellosis is caused by a bacteria called Salmonella and has been reported in all the rodents including rat and mice. The infection is transmitted mainly through oral route by taking contaminated food and water. Salmonellosis in rat and mice is usually subclinical. However, when clinical, the symptoms reported include anorexia, ruffled coat, hunched posture, diarrhea, weight loss, conjunctivitis and porphyrin staining at the external nares in the rats. Diagnosis is usually made by direct culturing the feces and or intestinal contents.[17]

The personals working with infected laboratory rodents may develop diarrhea, fever, and abdominal cramps 12-72 hours after infection and the infection last for 4 to 7 days. The infection can be diagnosed by culturing the stool sample. Infected personals must visit the medical doctor for the treatment. If it is not treated, severe infections may occur and the bacteria may spread from intestines to the bloodstream and develop systemic infection leading to death. Salmonella infection in the rodent colonies may be responsible for heavy losses in terms of interference with research and health of the workers.[18]

Leptospirosis is caused by spirochetes belonging to the genus Leptospira, and around 17 species have been identified.[19] Several animals have been recognized as reservoirs for this organism including laboratory as well as pet rodents. Humans are at risk when exposed to contaminated urine through mucosal membranes. Infection may also develop when there is direct contact with urine, water and soil. Leptospira infection produces a wide spectrum of clinical signs in human depending on the severity of the infection. A mild form of the disease may cause chill, headache, fever. However, myalgias are also observed in addition to multisystem complications such as jaundice, renal insufficiency and hemorrhagic pneumonitis, meningitis and even death in severe forms.[20] In laboratory animal facilities, Leptospira infection is a serious zoonosis risk for the facility staff and is unacceptable in experimental animals.

Dermatophytosis or dermatomycosis is a fungal disease commonly called ringworm and is reported in a variety of animals including laboratory rodents. It is caused by *Microsporum spp*. or *Trichophyton spp*. and has zoonotic importance.[21] It is transmitted to human from a variety of animals including rodents via direct or indirect contact. Laboratory animals can easily transmit dermatomycosis during handling, changing the bedding while remaining asymptomatic.[22] Control and prevention of zoonotic dermatophytosis involves the early recognition and quarantine of infected animals, personal hygiene, and disinfection of the facility environment. The contaminated areas should be vacuumed to remove rodent's fur and then thorough disinfection with chlorine dioxide disinfectants or bleach solutions. The infected animals should be handled with personal protective equipment like gloves, gowns and masks.[23] In addition to zoonotic importance, infected mice and rats may develop severe pneumocystosis with/ without immune-suppression. So, such animals may compromise research data, if used for experimental purposes.[7]

Tropical rat mite, Ornithonyssus bacoti also called the tropical rat mite has been reported from many parts of the world including India and have been reported as a vector in transmitting rickettsial pathogens.[24] Tropical rat mite has been found in various animals, however, rats, mice and other rodent species are known to be the preferred hosts. Personals working in laboratory animal facilities including technicians, researchers and husbandry staff were infested while handling laboratory mice. Infestation occurs while handling the rodents or direct contact with the rodents while changing the bedding and transporting those to new cages.[25] People

infested with this mite usually develop pruritic cutaneous lesions and at the time of medical visits, the patients are usually unaware of the cause.

In addition to all above mentioned bacterial, viral, fungal and mite zoonosis, it has been also studied that certain types of allergies may be transmitted to from rodents to personals working with laboratory animals. After the laboratory animal contact with the working personals, the allergic symptoms are; rashes on the skin, nasal congestion and sneezing, nasal discharge leading to severe respiratory problems including asthma etc.[26]

In order to eliminate the risk for zoonosis, a routine examination and diagnosis of the infection, quarantine measures and use of protective equipment should be used while working in the animal facilities. In addition to these measures, a standardized laboratory animal care and use program is required which comply with the international standards and norms. For this, there should be a strategic approach following current situation laboratory animal use in Pakistan that may regulate an international animal care and use program in biomedical research.

Laboratory animal care and use in Pakistan

In Pakistan, there are universities, institutes and medical research centers where laboratory animals are being used for biomedical research. According to the Gazette of Pakistan, extra Sept 17, 1979 Ordinance, there are laws for the import, export and quarantine procedures for the animals like birds, reptiles, mammals other than humans and fish. However, there is no legislation on animal use in research especially laboratory animals including rodents. Veterinary colleges and universities in Pakistan are regulated by Pakistan Veterinary Medical Council (PVMC). These institutes offer inadequate courses on laboratory animal care and use for veterinarians i.e. only a course of one credit hour, “Lab and Zoo Animals Management” during Doctor of Veterinary Medicine (DVM) program. Indeed the specialty of Laboratory Animal Medicine is important that can improve the animal care and use program in Pakistani universities.

According to authors’ knowledge, there are laboratory animal facilities in various biomedical institutes of Pakistan. However, no laboratory animal facility is accredited by an international organization like the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Due to all these issues, universities in Pakistan are unable to regulate animal care and use program that comply with international standards. Such issues of noncompliance may have public health importance and affect the reproducibility and quality of the research. However, a scientific and good strategic vision can guide the scientific community, higher education authorities and legislators for the development of animal care and use program in biomedical research that fulfils international norms, ethics and standards.

Strategic vision for laboratory animal use and care program

In order to establish an animal use and care program that complies with international norms and standards, primarily there should be legislation for animal use and care in research. Higher education authorities like the Higher Education Commission (HEC) in collaboration with PVMC, Pakistan Medical and Dental Council (PMDC) should establish guidelines to ensure the quality and reproducibility of animal-based research. Furthermore, universities and research institutes will design a comprehensive animal use program that comply with the guidelines recommended by HEC, PVMC and PMDC. Each university’s research council should establish laboratory animal facility and constitute Animal Care Committee (ACC) to execute the animal use program in accordance with HEC guidelines. The ACC must include a laboratory animal veterinarian as a member and a senior scientist as chair of the ACC. ACC should work according to the Term of Reference and establish guidelines for animal care and use in research to ensure Russel and Burch 3Rs.

The laboratory animal facility should be designed and constructed to ensure the welfare of the laboratory animals as well as to prevent the risk of zoonosis. PVMC should direct veterinary colleges and universities to offer satisfactory courses on laboratory animal medicine and management in veterinary medicine programs. A laboratory animal veterinarian must be present in each laboratory animal facility to provide veterinary treatment to the sick animals as and when required. For personals working in laboratory animal facilities should be screened for any infections annually or as needed. There should be a vaccination program for those who have access to laboratory animal facilities.

Physical facilities like labs should be constructed according to the international guidelines. Biosecurity, biosafety levels (BSL) and the quarantine periods for different animals should be designed according to the international standards for the better reproducibility of the research. Strict implementation of the given instructions is necessary according to the BSL of the respective lab. For better guidelines and awareness, different international organizations working for laboratory animals’ care and use should be consulted as they have developed standardized practices to handle the lab animals in order to

minimize the risks associated with zoonosis. Refresher courses should be offered to the lab's staff in order to get them to familiarize with the emerging challenges and thus getting them ready to plan better strategies to cope with the risks associated with zoonosis.

Every lab animal facility should follow the principle of 3Rs i.e. Replacement, Refinement and Reduction. There must be educational seminars and workshops for the awareness about zoonosis and their coping strategies for researchers, laboratory animal workers and managerial staff.

Conflict of interest: None declared.

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