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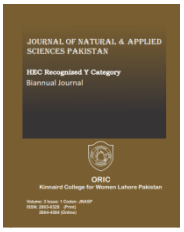

DERELICTION OF LABORATORY MICE HEALTH IN RESEARCH LABORATORY

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Abstract

Animal quality of life is based on psychological and physical state. It is commonly acknowledged that the animals involved in the experimental research should be housed in the best possible environment. Various laboratory mice modulations have been explored, but comprehensive study on environmental characteristics still lacks mice welfare. This study is based on the management and care of the laboratory mice to investigate the present conditions of the animal houses of Southern Punjab (Pakistan). We visit the animal houses of well-known universities in southern Punjab, and water samples were collected. Different phytoplankton species with various life stages and other undefined species were observed under the clear vision of microscope. Contaminated water can infect albino mice with various infections that can surely interfere with experimental research results. It was concluded that universities need to improve their animal house conditions and pay strict attention to this most important issue.

Keywords

Bioethics, Southern Punjab, Phytoplankton, Mice health, Contaminated water

1. Introduction

It is commonly acknowledged that the animals involved in the experimental research should be housed in the best possible environment. The care for laboratory animals is not a lawful and moral necessity, but it is also essential for obtaining excellent scientific quality. In the biomedical research laboratory, mice are one of the most commonly used vertebrates. Because of their short lifespan, reproductive cycle, low maintenance costs, and small size, they are considered the most suitable models for biomedical research. Mice are selected as the appropriate model in biomedical research, including

cancer, drug study, antibody and vaccine preparation, and effective pharmaceutical drugs. Mice is considered to be best laboratory model for experiments because of their small size and short generation time (Coombs, 2014). Laboratory mice origin, gender, age, and genetic manipulations are highly considered experimental manipulations. Physical factors like phenotype and body weight and some environmental factors like temperature, humidity, water, feed, light intensity, noise, and housing box are monitored and managed excellently, followed by the changing of the cell, bedding substrates, fluctuation in temperature, humidity and air changes within the enclosure are considered and appropriately managed daily. Adult mice range from about 28 to 40 g; moreover, this small size makes it difficult to accurately evaluate heart rate, body temperature, and respiration rate without using specialized apparatus. The water delivery system needs to be highly considered. The laboratory mice should be provided with pure sterilized water. The fineness of water usually depends upon the immune status and health of an animal. Two choices are mainly considered: either an automatic water system or the use of bottles. Recently, plastic pouches have been used as suppliers of water as a better option. Box changing and cleaning are essential to overcome the exposure of allergens to laboratory animals. The robots are used somewhere for changing and washing the box and remove the leftover substantial and transfer them to the courier of shaft workers. When the boxes became dry, robots take them off the conveyor and make them prepared for usage. These systems are pretty luxurious to build, but they can handle a lot of data and run practically continuously, and they provide the best protection against laboratory animal allergies. ICR albino mice are the most frequently used experimental animals in Pakistan for research purposes and scientific study. Pakistan is also following the criteria of international countries for the care and housing of experimental mice. Papers published in Pakistan regarding the management and maintenance of laboratory mice show that they entirely follow the international criteria for housing mice, but the reality is different. We planned a visit to animal houses in Southern Punjab's Universities to evaluate the conditions of these mice houses.

2. Material and Method

Permission letters were taken from the university to visit various Animal Houses of well-known universities of Pakistan (Southern Punjab). Water samples were collected from those universities, and present environmental considerations of animal houses were observed considering parameters like temperature, humidity, location of animal houses, boxes, cleanliness, water bottles, feeding pattern, bedding, number of mice (each box), conditions of mice, and biosafety measures. Water samples were collected from the bottles used in animal houses and tested in the laboratory to detect different bacterial colonies and color. We used petri film count plate, cultured and having violet red bile nutrients, cold-water-soluble gelling agent, and tetrazolium indicator. After that, the petri film was incubated at 35˚C for 48 hours. After 48 hours, the grid lines and colonies present in petri film are observed. Moreover, the water sample collected was observed under a microscope by preparing a slide. The water sample, which has to be observed under a microscope, was shaken in the bottle and then transferred to the Petri dish with the help of a dropper, the water drop was taken from the petri dish, applied on slide and placed cover slip. The extra water was removed with the help of a

blotting paper and observed under microscope. Pictures of the species were taken through the mobile phone and identified with the help of identification keys of authentic websites.

3. Results

3.1 Location

Most universities' animal house location was not appropriate. From all the directions, sunshine falls directly at the animal house, which can increase the temperature inside. Only one of the university's animal house locations was up to standard.

3.2 Unhygienic mice rooms

The animal house room condition was shockingly awful and fetid even though it was unbearable. Some mice were out of their box. In some universities, there was only one lab coat available for everyone to come and wear while working in animal house; on the other hand, most universities don't even facilitate lab coats and sterile gloves to enter the mice room. The students were unaware of the ethics of entering the animal house. These conditions could easily infect mice and even students as they kept on wearing same lab coat with all dissection stains and even sweating. Although the cage arrangement for mice has always different requirement according to number of mice, condition of room, temperature etc but still it is always preferred to keep mice boxes clean in order to protect them from being infected with different allergies (Scharmann, 1991).

3.3 Water quality

Some universities directly dispose of the killed mice after experiments outside of the animal house. Other universities wrap the killed mice in cotton paper and throw them as waste in the dustbin. The water given to the mice and rats in various universities of Southern Punjab has contaminated tap water taken from those particular universities for drinking purposes of mice and rats; on the other hand, only some universities provide clean water while housing. The study also reveals that the water supplied to the mice and Rats was yellowish-green compared to clean water. Here, the quality and color of the water are shown in Figure 1.

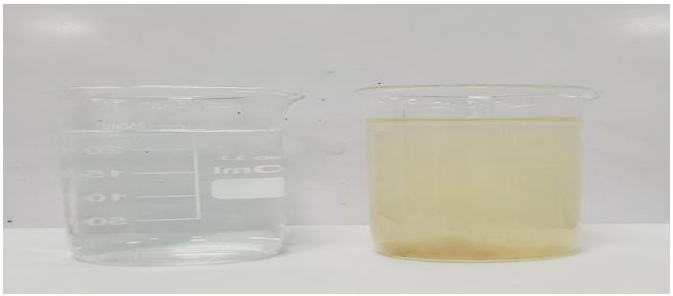

Figure 1: Quality of water: Jar on left carry control normal clean water while jar on right carry water from mice water bottles in animal house of Southern Punjab Universities

3.4 Phytoplankton

The water sample collected from different universities when observed under a microscope, the contaminated water shows various life forms of phytoplankton feeding on the green algae, i.e., Nematodes, Paramecium, Shelled amoeba, Daphnia Stylaria locusts, Spirostomium minus, and other unidentified planktons. Some planktons which are identified under the microscope are shown below in Figure 2.

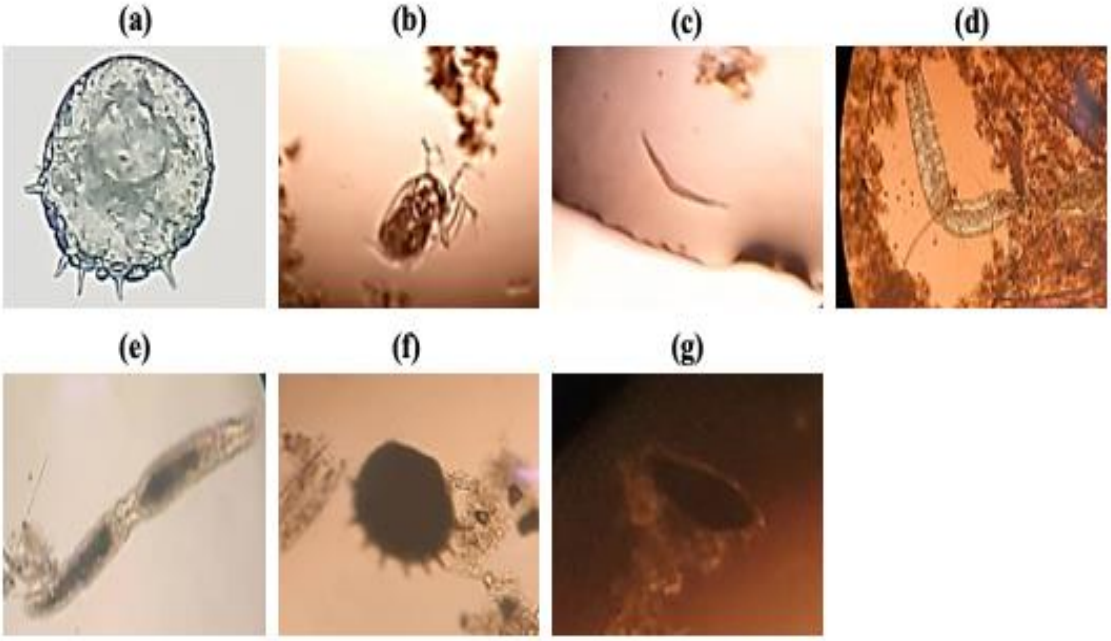

Figure 2: The water sample containing planktons (a) Shelled amoeba (b) Daphnia (c) Nematode (d) Stylaria locustris. (e) Chaetogaster diastrophus (f)Testate amoeba (g) Paramecium.

3.5 Coliform test

Water samples from different animal houses of Southern Punjab, resulting in the detection of 17 colonies of total coliform and nine colonies of fecal coliform from one sample- The other sample results in the detection of 19 colonies of total coliform and 13 colonies of fecal coliform. A normal drinking water sample was taken for the control, and a microbiology test was performed to ensure that no microorganisms were found in the purified water. The Microbiology Test results presented 0 total coliforms, 0 fecal coliforms, and no bacterial colonies in control. Moreover, an E. coli test was also performed, but E. coli was not detected in both water samples. The growth and numbers of coliform is shown in Figure 3 and Table 1-3, respectively. The results shown in diagram illustrates if red colonies with gas bubbles appear it will show count for total coliform, and fecal coliform and on the other hand, if blue colonies with gas bubbles appeared on the petri film grid lines it will show count for E. coli. Gridlines are present in the Petri film to count the number of bacteria in the sample. The number of bacteria can be calculated with the help of these gridlines. The temperature maintained in the rooms of the animal house was about 26-28 degrees Celsius (indoor), while at the same time, the outdoor temperature was about 45 degrees Celsius and the relative humidity was about 73-75%. According to the workers the temperature rises above 38°C when in summer electricity failure or power outage. This condition increases stress, aggression in mice which leads to different experimental failures. Sometime this stress can severely affect the reproduction of mice.

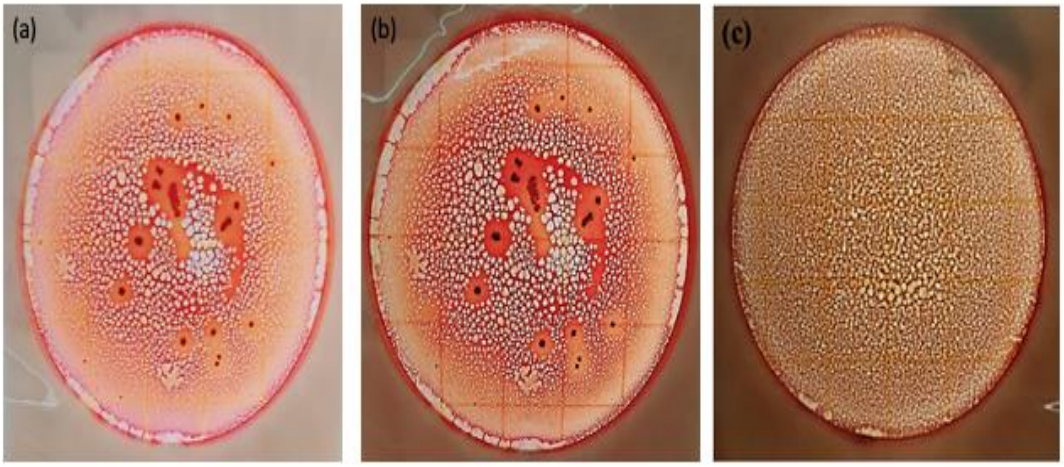

Figure 3. Coliform test: (a) Bacterial colonies (b) Grid lines shown in petri film (c) Control test:(No bacterial colonies)

Table 1: Showing colonies of total coliform, fecal coliform, and E. coli for a control.

Sr. #	Water quality parameters	Highest desirable level	Results
1	Total coliform	0/100 ml (PSQCA)	0
2	Fecal coliform	0/100 ml (PSQCA)	0
3	E. coli	-	-

Table 2: Colonies of total coliform, fecal coliform, and E. coli are shown below.

Sr. #	Water Quality Parameters	Highest Desirable Level	Results
1	Total coliform	0/100 ml (PSQCA)	17
2	Fecal coliform	0/100 ml (PSQCA)	9
3	E. coli	-	-

Table 3: Colonies of total coliform, fecal coliform, and E. coli are shown below.

Sr. #	Water Quality Parameters	Highest Desirable Level	Results
1	Total coliform	0/100 ml (PSQCA)	19
2	Fecal coliform	0/100 ml (PSQCA)	13
3	E. coli	-	-

3.6 Mice box and bottles

Boxes used in animal houses were polycarbonate and polypropylene and transparent. Box size was 290×220×140mm(L×B×H), and floor space was 388cm^2. Minimum 8 to 10 and maximum 20 to 25 mice were kept in each box. Different size of boxes was used. Moreover, the size of box remains almost identical in all university's animal house of Southern Punjab. The box was not tidy and seemed not rinse for days. Bedding was also stinky and wet. According to students and workers the bedding of mice was changed after 6 to 7 days.A single water bottle was placed for 10-25 mice. Bottles placed over the box became contaminated, green algae started growing in the bottles basement as they were not washed and sterilized since weeks or months. Moreover, the pH of the water was acidic. Various boxes with contaminated water bottles used in animal houses are shown in Figure 4.

Figure 4: Unwashed boxes and contaminated water bottles in animal house.

3.7 Food

The feed provided to the mice was unhygienic. Management prepares the food for mice with exposed hands, and the level of cleanliness was abysmal inside the animal house. While preparing food for the mice, the hands were in direct contact with the food. The management of animal houses of different universities uses another method to provide food to the mice. Some universities offer food in beads form and dry it by placing it in sunlight for some time, and when it is scorched, they put the feed over the box for the mice. On the other hand, some universities provide the food in ball shape by making the food wet and place it on the top of the box for mice to eat, which is unclean in both cases as shown in Figure 5 (a and b). Feed kept in open air can easily be contaminated with different bacteria present and even dust around. While the feed in form of a big ball can increase aggression in mice as less mice could eat at same time because of round shape. All the Universities of Southern Punjab must follow the standard condition and composition of mice feed.

Figure 5. (a) Mice feed in form of beads drying in open environment placed on dusty ground under sun. (b) Mice feed in ball shape with a lot of mice in same box and even dead mice kept above box which is against bioethics. (c) Clusters of noxious waste.

3.8 Waste material

In some universities, the bedding waste material is gathered and thrown outside the animal house, and it smells significantly worse outside the animal house. Clusters of waste bedding material are formed outside the animal house and look like it's been a long time throwing the waste in open environment (figure 5c) which was unhygienic for students and other people around in university even the canteens are also present near such dispose places.

4. Discussion

Unhygienic and contaminated feed is being provided to the laboratory mice in almost all the well-known universities (animal houses) of Southern Punjab. Most universities offer a meal to form beads of the food and let it dry by putting in the sun for a short period without covering it. Other universities use another way to facilitate the mice with the feed. They form a ball-shaped structure of food and make it wet by adding contaminated tap water, and, after that, they make it with food available to the mice. When the food is dry, mice cannot eat it properly as they find it difficult to eat food.Moreover, when food is placed on top of the box, mice will fight for food as there will only be a single ball available to eat for so many mice in a cell. This aggression level among mice increases and affects breeding, due to which results may become bias. But this data cannot be finalized similarly up to given standards. According to a previous study, commercially available standardized diets are in a pelleted meal or extruded form that meets the complete nutrient growth and development requirement. Natural, purified, or chemically defined ingredients create a healthy diet (Nih *et al*., 2011). During the experiment, there was a steady effect of contaminated food on the morphology of Swiss albino mice like gradual hair loss with exposed skin, loss of body weight, change in the posture of the tail, feet abnormalities, and excessive hair loss from the body (Prasad, 2011).Temperature and humidity factors play an essential role in keeping the laboratory mice in a controlled environment. In southern Punjab universities, both these factors are maintained in an animal house when electricity is available; the equipment shows the temperature and humidity as 28 degrees Celsius and 50 to 70%, respectively. Still, there is no electricity backup in most animal houses in the absence of electricity. However, it makes the conditions worst for the mice to survive under such circumstances, and the breeding cycle disturbs a lot, especially with these hectic conditions. There should

be proper electricity backup in generators and UPS so that mice get favorable conditions and a healthy environment. Temperature factor is itself a long-term study so, there is a need for focused and separate study for its consequences and outcomes. Previous research shows that when the mice are housed in cold environmental conditions, it can affect stress levels in the female mice. While the decrease in temperature can increase the aggression level of female mice, due to which she kills her pubs when they are born. So, it can harm mice below their thermoneutral range and in cold conditions (Marsteller & Lynch, 1987).Moreover, the water provided ad libitum to the laboratory mice was contaminated water having microbial species like a shelled amoeba, testate amoeba, nematode, daphnia, Stylaria locustris, Chaetogaster diastrophus, paramecium, and other unknown microbial species. All these species were feeding upon green algae present in the water bottles of the animal houses. The contaminated water samples were taken and tested in the laboratory for the microbiology test to check out various water parameters, including total coliform, fecal coliform, and E. coli. It was revealed that the drinking water was unfit for drinking as total coliform and fecal coliform were found in all the water samples. Contaminated water can infect albino mice with various infections. As documented before, infections caused in laboratory animals from various microorganisms can interfere with experimental research. Mostly, immunodeficient and perinatal laboratory animals may found disorders in clinical disease along with various pathological changes (Shek *et al*., 2015). The most common pollutants in laboratory animal drinking water are microorganisms, which might influence the physiological responses and perhaps induce experimental variability. Contaminated water increases the danger of water-borne infectious illnesses. E. coli, fecal coliform, and total coliform are among the water-borne microbes of more significant concern. Surface water is said to be more polluted with harmful microbes(Cabral, 2010). There are several techniques for purifying water, and animal research institutes frequently use a filtration method, including granular media filters, sand filters, carbon filters, deionization filters, and membrane filters. Arsenic in the drinking water of laboratory mice can impact female reproductive endpoints and increase body weight and glucose intolerance (Kurtz & Feeney, 2020). It is recognized that chemical pollutants in the drinking water of laboratory animals and other variable substances might be a source of experimental results and variability. A thorough description has been provided about the quality source of drinking water supplied to laboratory animals. Water has been a microbiome study to influence the physiological responses (Wolf *et al*., 2014), (Sofi *et al.*, 2014). A congested or inadequate water supply can rapidly result in a general failure to grow, desiccation, and even death. Moreover, it might induce box flooding, causing animal stress, hypothermia, and perhaps death, particularly in newly born pubs having poor thermoregulation (*British Library Cataloguing-in-Publication Data*, 2009).The uncontaminated water was being supplied to the laboratory mice of animal houses of southern Punjab. Total coliform and fecal coliform colonies were present in almost all the animal houses of Southern Punjab's water samples. Total coliform in water indicates the presence of microorganisms, which may lead to various diseases in the laboratory mice, and should bias the experimental

research results. Fecal coliform is the most frequent natural waterways contaminants found in the digestive tract of warm-blooded animals. Swiss albino mice were treated with fecal coliform contaminated drinking water at high and moderate doses for three months daily. The study reveals a significant increase and decreases in various enzymes compared to the control of pure drinking water, free from microbes. Increase concentrations of multiple enzymes in the plasma blood of mice indicates the effect on the permeability of the cell tissue of the liver and also shows the effect on the metabolism process in mice, which further causes the disturbance in the synthesis of the lipoprotein to enhance more secretion of the protein into the blood by the help of liver. An increase of enzymes in the blood concentrations results in the incapability of the liver to convert glucose into glycogen, and the pancreas may also be affected and not able to excrete out the insulin. Bacteria drinking water results in disrupting the activities of the liver, which is involved in the metabolism process and also results in increasing the level of plasm in the blood of the mice. The rise in the level of plasma in the blood of mice is mainly attributable to hemoglobin degradation due to the breakdown of erythrocytes due to cell death or duct thrombosis. Clean drinking water should be supplied to the laboratory mice, free from all types of microbes (Hasawi *et al*., 2016).

5. Conclusion

Various laboratory mice modulations have been explored, but comprehensive study on environmental characteristics still lacks mice welfare. Such research should be carried out in the housing of laboratory mice, and the impacts of such measures should be accessed. The current study reveals the poor animal house management in most of the universities of Southern Punjab. There was no check and balance and proper waste management in animal houses. Waste management is a significant issue in almost all the animal houses of Southern Punjab. The waste of animals directly discharge near the animal house should be disposed of at a far distance. These kinds of waste management can also affect the human environment and add detritus components to the environment. Hence, it is concluded that almost all animal houses found in Southern Punjab didn't follow the standard management international criteria for the housing of laboratory mice and needed to improve management for the laboratory mice to get the desired experimental results. Furthermore, knowing the social context in which laboratory animals are housed is essential for their well-being, experimental findings, and research quality.

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