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What happened to laboratory animals during COVID-19 lockdown? India answers.

A survey from India on Laboratory animal care during COVID 19 lockdown

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Summary

Publishing the practical problems that occurred during a local or global disaster can help to plan improved future logistic and personnel management, and this can ultimately result in better welfare of laboratory animals. COVID-19 necessitated euthanasia of research animals, and partial or complete closure of laboratory animal facilities, throughout the world. In an attempt to find out the impact of COVID-19 lockdown on laboratory animals in India, a survey was conducted based on the voluntary participation of institutions from different regions of the country. A total of 56 facilities participated from 14 states and union territories. From their responses, 95% of the facilities continued their operations during lockdown. Among these facilities, 13% were completely operational. 63% had no interruption in receiving essential supplies and 16% operated with complete man-power. 20% paused breeding completely and 54% partially. In spite of the strict lockdown, 91% of the institutes were able to provide veterinary care. 60% of the institutions conducted online institutional animal ethical committee meetings indicative of continuing research activities. Many facilities reported animal morbidity (64%) and mortality (9%). To optimize resources 41% reported euthanasia of animals. Statistical analysis revealed a significant association of lockdown with increased animal morbidity, euthanasia and reduced animal breeding. In conclusion, even though lockdown had created a partial disruption of activities with some reduction in animal health and welfare, operations and research continued at most of the facilities surveyed in India.

Introduction

COVID-19 disrupted normal working patterns in all areas, including laboratory animal facilities, across the globe. Unlike many other organisations, animal research facilities require year-round human attention to facilitate husbandry and care of laboratory animals. Support of research activities is also of importance at an animal facility. The urgent mandate of COVID-19 vaccine development and other relevant products required uninterrupted functioning of research facilities (Kumar et al. 2020). The pandemic didn't only affect humans as it indirectly affected animals as well (De Briyne et al. 2020); the health and welfare of all animals including laboratory animals were affected. Several thousands of mice were reported by the press to have been euthanized at research laboratories in the United States and across the European Union, and many research laboratories were forced to shut down their operations. Ways had to be found to continue to care for the animals on site. The focus of biomedical research shifted towards specialised animal models including transgenic mice and ferrets to develop vaccines and treatments to mitigate COVID-19 (De Briyne et al. 2020). India played an important role in COVID-19 vaccine research and development and an account of the activities and functioning of animal facilities in India during the lockdown is needed. For this reason, we performed a survey among 56 organizations, consisting of academic/research institutions, medical, veterinary, pharmacy colleges and CROs, having animal facilities across India; confidentiality was assured to all the respondents. The survey included animal facilities from all over the Indian mainland. The data along with the lessons learned are important for planning of activities and welfare protocols to meet similar crises in the future.

Materials and Methods

The survey was prepared in English and questionnaires were sent to research animal facility managers via e-mail. Steps were taken to ensure that a questionnaire was sent to only one person per institution and assurance was given that respondent's anonymity would be maintained. The survey, which started on October 10th, 2020 and was closed by January 26th, 2021, consisted of eleven questions and a space for suggestions for tackling similar calamitous situations in future (Table 1). It was hypothesised that lockdown would have had an impact on animal health (Gortázar and de la Fuente 2020). The questionnaire specifically asked if mortality and morbidity of animals had increased compared with pre-Covid times. Indian statutory guidelines on the euthanasia of laboratory animals were developed by the Committee for the Control and Supervision of Experiments on Animals (CCSEA) based on the Prevention of Cruelty to Animals Act enacted by the Indian parliament in 1960 (amended in 2006). Under these guidelines, euthanasia of laboratory animals can be performed only if they pose a serious threat (like spreading a zoonosis) to other animals or humans, or when the animals experience irreversible pain and suffering, or if the animal is paralyzed and is not able to perform its natural functions or it becomes incapable of independent locomotion or it can no longer perceive the environment in an intelligible manner. However, when the unprecedented global pandemic hit, facilities across the globe had no other option than optimising the stock of animals to meet the demands with their available resources and manpower (Thurston et al. 2021). Furthermore, it is understood that the euthanasia performed was to prevent anticipated suffering of animals, as warranted by the existing rules. Since this survey was not using any animals for generating data, approval from the ethical committee was not sought by the authors.

Statistical Analysis

Microsoft Excel was used to determine percentages including the percentage of the total number of animals used which was represented by each species. GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com) was used for making graphs. Chi-squared test of independence was performed to assess the association between lock down (complete or partial) and increased morbidity, mortality and euthanasia (in comparison with pre-Covid times) or reduced breeding. $P < 0.05$ was considered as statistically significant. Percentages in the text are rounded off to the nearest whole number whereas actual values, up to two decimal points, are given in the Figures.

Results

From the survey of 56 institutions, it was found that a total of 438,564 animals were used for research over a 12-month period (2018-2019) comprising 84% mice, 10% guinea pigs, 5% rats, 0.5% of rabbits, 0.4% of large animals, 0.04% of non-human primates and 0.3% other rodent species and 0.05% birds (Fig 1A).

Table 1. The questionnaire used for survey

Question number	Question	Options given for answers
1.	What was the duration in months where lockdown conditions applied for operation of the facility?	1/2/3/4/mention if more
2.	From April 1st 2018 to March 31st 2019, what were the number of animals used species wise (all strains/stock together) for research and education in your establishment?	Mice/Rats/Guinea Pigs/Rabbits/ Large animals/Any other species
3.	Did the lockdown lead to a total shutdown of animal facility?	Yes/No/Partial shutdown of operations
4.	Did the lockdown lead to cessation of essential supplies/critical items (feed/bedding/animals/drugs/cleaning agents) to the animal facility?	Yes/No/Local alternatives were available that were never relied upon before the pandemic
5.	If the answer to the question number 4 was option- "yes", or option- "Were local alternatives available that were never relied upon before the pandemic", what supplies were affected?	Feed/Bedding/Animal purchase/ Drugs
6.	To avoid risk of being left unattended, were the animals euthanised?	Yes/No/Animal stock number reduced to optimize services
7.	Was daily attendance by animal caretaking staff (animal handlers and cleaners of animal facility) reduced during the lock down?	a. Reduced without lay off from employment b. No c. 50% shifts as compared with Govt. norms
8.	Was the Veterinarian/s available during the entire lockdown?	Yes/No/On call availability
9.	Was animal breeding and supply for research affected?	Completely stopped/Reduced breeding but continued
10.	Were online Ethical Committee meetings held during lockdown?	Yes/No
11.	Were health issues noticed in animals during lockdown that were increased compared with the pre-Covid times?	Noticed with mortality/Noticed without mortality/Not noticed
What are the suggestions to tackle similar conditions as that of a pandemic or natural calamity in future to minimize the repercussions to animal research? In two or three sentences.		Descriptive answers

Information regarding the use of zebra fishes was not received from any of the institutions that participated in the survey. Four institutions reported that they did not house any live animals during 2018-2019.

Operational status during lockdown:

During the lockdown period, 7 facilities (13%) were fully and 46 facilities (82%) were partially operational, whereas 3 (5%) were completely shut down (Fig 1B).

Essential supplies/critical items:

Thirty-five facilities (63%) had uninterrupted supplies of critical items. The remaining 21 (38%) of the facilities had no supplies, but they could manage with the available resources. To cope with the situation, 17 institutions started to procure locally available animal feed and 8 institutions found a local alternative for bedding material. Four facilities procured animals for research from local breeders. The availability of drugs for veterinary use was not a problem and only 3 institutions started to procure drugs available locally (Fig 1C, 1D).

Euthanasia of animals to manage resources:

Thirty-three institutions (59%) avoided euthanasia and were able to manage their daily operations with the available resources. However, 23 facilities (41%) reported euthanasia of some of their animals to optimize the available resources anticipating scarcity of supplies and reduced manpower (Fig 2A).

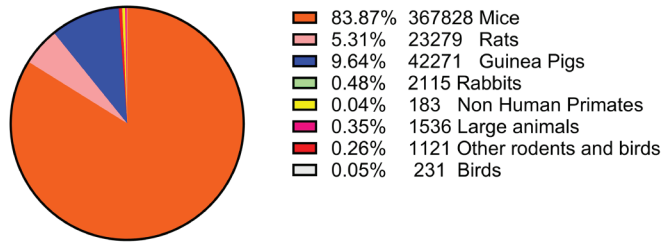

Fig 1A- Total number of animals used in 2018-2019, prior to lockdown, at the 56 institutions

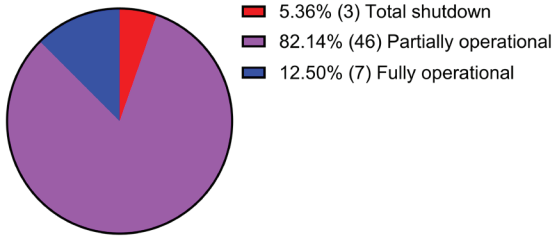

Fig 1B- Operational status of the institutions during lockdown

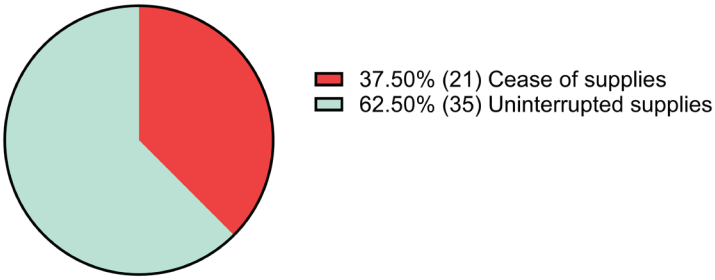

Fig 1C- Availability of essential supplies during lockdown

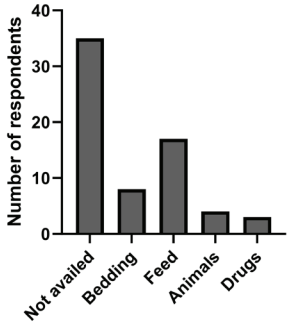

Fig 1D- Use of local alternative supplies during lockdown

Figure 1. 1A: Total number of animals used in 2018-2019, prior to lockdown, at the 56 institutions. 1B: Operational status of the institutions during lockdown. 1C: Availability of essential supplies during lockdown. 1D: Use of local alternative supplies during lockdown

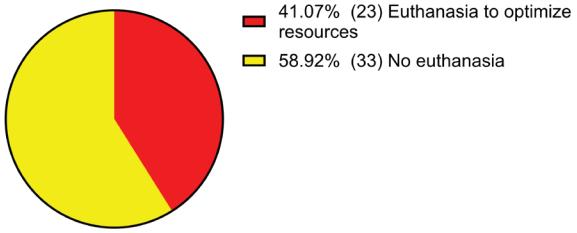

Fig 2A- Euthanasia to prevent suffering and manage resources during lockdown

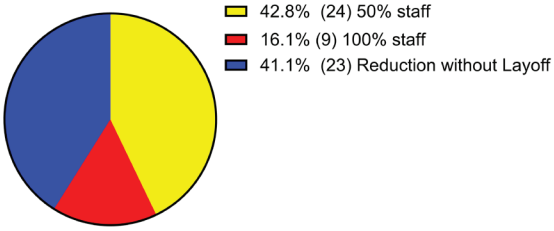

Fig 2B- Animal-care staff levels during lockdown

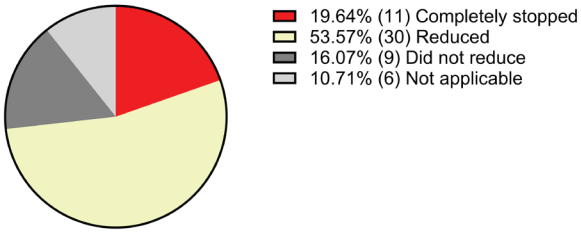

Fig 2C- Animal breeding during lockdown

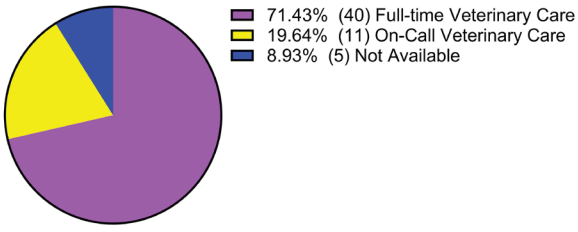

Fig 2D- Availability of veterinarians during lockdown

Figure 2. 2A: Euthanasia to prevent suffering and manage resources during lockdown. 2B: Animal-care staff levels during lockdown. 2C: Animal breeding during lockdown. 2D: Availability of veterinarians during lockdown.

Manpower available during lockdown to care for animals:

As per the Department of Personnel and Training, Govt. of India's order dated 23rd April 2020, institutions were allowed to operate with one-third staff strength. Twenty-four (43%) institutions had reduced the staff strength to 50% to minimize risks of exposing all the staff at the same time to a possible COVID-19 infection whereas 23 (41%) of the institutions retained over 50% of staff and did not lay off any employees. Nine (16%) facilities operated with full staff strength (Fig 2B).

Reduction in animal breeding program:

It was noted that 11 facilities (20%) stopped animal breeding completely and 30 (54%) partially, whereas 9 institutions (16%) did not change their breeding program and continued as they had done before the lockdown. The remaining 6 (10%) institutions mentioned that the question on breeding reduction did not apply to them (Fig 2C).

Veterinary care:

The health of the animals was given priority and 51 institutes (91%) provided veterinary care to animals. While 40 institutions (71%) provided full-time veterinarians, in 11 institutes (20%) veterinarians were available on-call and only 5 (9%) of the facilities were unable to provide veterinary care (Fig 2D).

Institutional Animal Ethics Committee (IAEC) meetings for scrutinizing protocols and ensuring animal welfare:

Indian research institutions continued to perform research and hence 33 (60%) institutions made use of the CCSEA order to conduct online IAEC meetings. The rest of the institutes (40%) did not conduct IAEC meetings during lockdown (Fig 3A).

Morbidity and mortality during lockdown period:

Animal morbidities and mortalities were reported in 36 (64%) and 5 (9%) facilities respectively (Fig 3B and 3C).

Statistical analysis (Chi-squared test)

A significant association of morbidity with lockdown was found ($\chi2(1, N=56) = 14.4$, $P = 0.0001$). Mortality was found not to have an association with lockdown ($\chi2(1, N=56) = 0.8$, $P = 0.37$). Euthanasia was found to have an association with the lockdown ($\chi2(1, N=56) = 5.58$, $P = 0.0182$). Breeding was reduced which was associated with COVID-19 lockdown ($\chi2(1, N=54) = 20$, $P <0.0001$).

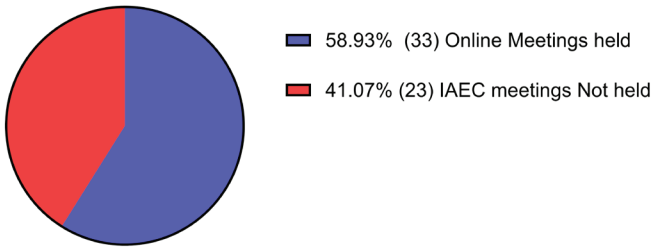

Fig 3A- Online IAEC meetings held during lockdown

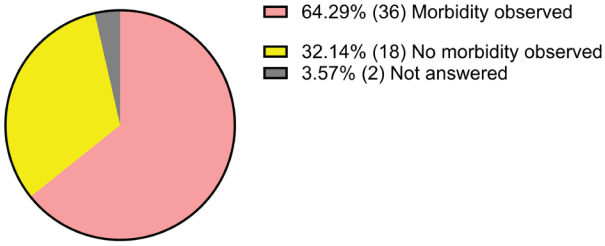

Fig 3B- Animal morbidity during lockdown

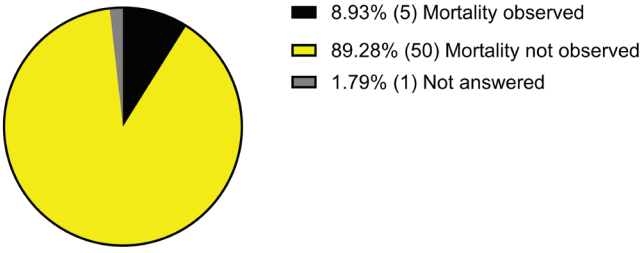

Fig 3C- Animal mortality during lockdown

Figure 3. 3A: Online IAEC meetings held during lockdown. 3B: Animal morbidity during lockdown. 3C: Animal mortality during lockdown.

Suggestions for dealing with future crises

Most of the institutions opined that employment of animal-care staff from the local area would make it easier to maintain animal care should similar crises occur in future. Respondents also opined that to deal with a future crisis, there should be surplus stock of critical supplies such as feed and bedding in the institutions. Other suggestions were the creation of common animal facilities in Indian cities that can be used by all institutions in the region and the cryopreservation of embryos.

Discussion

The European Union periodically publishes data on experimental animal usage (Busquet et al. 2020) and from the 3R's point of view, keeping a track on animals used is very important. An estimate of numbers of animals used in India for research purposes is extremely hard to find. This survey provides data on animal usage from the surveyed institutions for the financial year (2018-2019) just before the pandemic struck. Estimates are also made on global usage of animals for scientific purposes (Taylor and Alvarez 2019), which are a calculated guesses, and in a similar way efforts should be made to estimate the total usage of animals in India, as India is now emerging as a global leader in pharmaceutical research and is also a fast growing economy.

CCSEA, the statutory authority that controls research using animals in India made several modifications to its requirements for animal wellbeing during the COVID lockdown, notably encouraging online IAEC meetings and including animal feed and bedding as essential commodities and thereby prioritising their transport across India. Veterinary and animal facility staff were considered as frontline healthcare workers and the Indian government excluded them from curfews and lockdowns. Based on an order issued by CCSEA, the implementation of appointing veterinarians in all the animal research facilities in India is progressing. These amendments made by the CCSEA and Govt. of India ensured that research and testing continued during the COVID-19 pandemic.

Despite these positive efforts by the government to overcome the situation, a few hurdles still remained owing to quarantine rules, travel restrictions, difficulty in obtaining raw materials to manufacture feed, and finding transport to deliver feed, bedding material and animals due to restrictions imposed on entry and exit between states. It was hypothesised that the supply chain would have been affected leading to euthanasia of laboratory animals (citing potential suffering due to uncertainties in food and manpower availability posed by the pandemic), a reduction in research activities and an increased incidence of morbidities and mortalities. It was also hypothesised that most of the institutions would have euthanized a part of their colony to optimize available resources and manpower. COVID-19 especially at its onset posed a question globally on captive animal well-being. The road/rail/air connectivity between different regions in India varies considerably due to geographical diversity, and in turn, the accessibility varies between Indian states as well. Hence, the data on receipt of essential supplies during the lockdown within India will have high variability. It is impossible to determine the supply situation for each area in the Indian mainland and so a general picture of what happened is being attempted. Most of the affected facilities were dependent on local alternatives. Among the supplies that were disrupted, animal feed was procured by most of these facilities, followed by bedding, animals and drugs, from local sources. The respondents' answer to the question on the association between lockdown and increased rates of morbidity and mortality in comparison to pre-COVID times was not based on any statistical tests but on the general feeling and experience of animal facility managers, and hence can have a high level of variability. However, the statistical test done based on the respondents' answers reinforces the association between lockdown, leading to reduced staff strength, and morbidity, euthanasia and reduced breeding.

Despite the financial slow down caused by the COVID-19 pandemic on the Indian economy (Dev and Sengupta 2020), scientific research in the country continued. India was employee-friendly, providing salaries and avoiding layoffs during this global crisis and this was empowered through a national government order. The responses from the survey indicated that the use of local products, together with surplus storage of critical items like feed and bedding and employment of staff who live near to the facilities could be the key to handling similar crises in the future.

Publishing the practical problems that occurred during a local or global disaster can help to plan logistic and personnel management in a better way in future. This can ultimately result in better welfare of laboratory animals. Be it in a localised case of isolation as observed in the floods or in similar disease outbreaks or wars that cuts off man and material movement, being prepared is the key to tide over

adverse events that can affect the care and welfare of laboratory animals. In conclusion, even though the COVID-19 lockdown had created a partial disruption of activities with some reduction in animal health and welfare, operations and research continued at most of the surveyed facilities in India.

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Conflict of Interest

All authors declare no conflict of Interests.

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