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RESEARCH ARTICLE

Some specific biological risks of using the Syrian hamsters infected with SARS-CoV-2 virus in the animal biosafety level 3 laboratory

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Abstract

The emergence of novel severe acute respiratory syndrome 2 (SARS-CoV-2) has prompted extensive research to identify its pathogenesis, transmission, and reservoir entry in animals. Syrian hamsters (Mesocricetus auratus) are being identified as an ideal animal model for the study of SARS-CoV-2 infection due to their susceptibility and respiratory symptoms similar to that observed in humans. This study focused on the specific biological risks associated with the use of infected Syrian hamsters in animal biosafety level 3 (ABSL-3) laboratories.

The professional and psychological training of professionals working with laboratory animals should be a priority. Biological safety requires the involvement of all parties from technical staff to top management. The COVID-19 pandemic has shown a limitation in experimental studies when obtaining the right strain of mice proved difficult. A rare experimental model of Syrian hamsters started to be used in the work, which Syrian hamsters have several distinctive characteristics associated with increased aggressiveness of females, leading to a requirement to keep them isolated and some technical difficulties during manipulations in the biological safety cabinet. Therefore, a risk-based approach must be introduced to reduce the additional risks to workers to an acceptable level. This includes enhanced accuracy in the use of personal protective equipment (PPE), rigorously following biosecurity protocols, and monitoring staff health to detect potential risk of zoonotic transmission. The implementation of international standards and regulations on biological safety and biosecurity is of paramount importance.

Introduction

Epidemiologically significant infections have the potential to impact the public health system, both globally and within countries. Pandemic-potential influenza viruses [1] and coronaviruses [2], which are researched in laboratories, are characterized by high genetic variability and potential for human-to-human spread. Global changes in climate, logistics and demography further increase the risk of the spread of other infectious diseases, as well as their severity [3]. Therefore, special attention is required for the investigation of (re)emerging infectious diseases (EIDs) - these are either re-emerging or completely new infections [4]. Working with many such causative agents, especially with exotic infectious agents that cause severe and/or fatal diseases and those transmitted by aerosols, requires compliance with high biological safety standards, including biosafety level 3 (BSL-3) and biosafety level 4 (BSL-4) laboratories [5]. All procedures with such agents, including sorting of samples, nucleic acids extraction from vectors (ticks, fleas and other arthropods) and their storage require BSL 3 or 4 [6]. For many infectious agents, isolation of the pathogen by direct culture is difficult or models of human infection may be required. In such cases, genetically modified laboratory animals are used, such as mice, guinea pigs or rabbits and less often non-human primates [7].

When working with experimental animals, biological risks such as aerosol transmission to lab staff or needlestick injury increase significantly, due to human error and accidents. Therefore, the requirements for biosafety, when working in a BSL-3 facility, are significant [8]. Infected animals should be housed in individually ventilated cages (IVCs) or micro-isolators with a negative air pressure gradient relative to the environment [9]. Laboratory personnel require personal protective equipment, which varies depending on the pathogen and the specific nature of the laboratory work. The staff should undergo training and lab skills testing on a regular basis [10]. BSL3 laboratories investigate a range of EIDs, with the exception of the especially virulent viral infections as Ebola, Marburg, Congo-Crimean hemorrhagic fever, Junin, Lassa, and Hendra viruses, which are usually handled in the highly containment BSL-4 facilities [11].

One of the major work health and safety concerns in the laboratory is the infection of personnel, with many of such cases described globally [12]. Laboratory-acquired infections (LAI) have a long history [13], with cases of brucellosis [14], tuberculosis [15] and other infections are registered annually among laboratory staff [16]. LAIs are predominantly of a bacterial origin; with viral infections less common [17]. Many viral infections lack effective therapy and specific vaccines or prophylaxis. For example, in the case of exposure to the Dengue virus as a result of the needle stick injury, only acetaminophen and antihistamines are used for symptomatic treatment [18]. Therefore, when infected with laboratory-associated infections of viral etiology, there is a significant risk for personnel. It is extremely important to prevent infection in the laboratory, which is facilitated by the development and implementation of the most effective biological risk assessment. The most important risk factor for laboratory associated infection is human infection during manipulations with laboratory animals. For example, a case of Zika virus infection of a PhD student transmitted from the bite of a laboratory mouse was reported in Brazil [19]. Another example is the case of a laboratory worker infected via a needle stick injury in the thumb of the left hand when inoculating the mice with wild type vaccinia virus (VACV) [20].

The COVID-19 pandemic, which started in December 2019, has shown the relevance of the availability of laboratories with enhanced elements of biological safety and security in the public health system [21].

The investigation of hazardous pathogens and, in particular, SARS-CoV-2 at the M. Aikimbayev's National Scientific Center for Especially Dangerous Infections (NSCEDI) is conducted in high containment BSL-3 and ABSL-3 facilities [22]. The purpose of this research was to identify, assess and manage biological risks in the handling of laboratory animals in high containment laboratories (ABSL-3), including for the study of SARS-CoV-2 performed on Syrian hamsters (*Mesocricetus auratus*). When collecting information for risk assessment, attention should be paid to the professional skills and knowledge of researchers, the behavioral characteristics of animals, and the technique of the procedures performed. It is important to carry out regular handling for taming laboratory animals before the experiment, to work slowly and confidently inside the biological safety cabinet (BSC).

Approaches to biorisk assessment when working with laboratory animals in the ABSL-3 laboratory

The World Health Organization (WHO) manual uses data collection rather than prescriptive requirements for the identification and reduction of biological risks [23]. As part of risk management, the risk assessment framework is presented as a process consisting of five steps [24]:

- gather information
- evaluate the risks
- develop a risk control strategy
- select and implement risk control measures
- review risks and risk control measures

Information should be collected, as fully and efficiently as possible, prior to commencing research work, and include the following main parameters [24]:

- Planned laboratory work or procedures
- Availability and sufficiency of laboratory equipment
- The type of equipment used in the work
- Competence of personnel performing the work
- The concentration and volume of the biological agent and potentially infectious material for the procedure
- Potential pathways of pathogen transmission.
- Infectious dose of biological material
- Interaction of biological agents
- Severity in case of infection with a biological agent
- Availability of effective prevention and/or treatment
- Stability (resistance) of a biological agent in the laboratory and in the environment
- Collective and personal protective equipment
- Susceptibility of laboratory personnel to a biological agent
- Host range of a biological agent
- The frequency of failures of equipment and life support systems of the building (power supply, construction, infrastructure and utility systems)
- Existing control measures in the organization.
- Endemicity of a biological agent among the local population
- Historical data on biosafety events
- Characteristics of the territories and buildings of the laboratory
- Biological waste management procedures

The described parameters for identifying hazards, as well as their assessment, development of a strategy, measures and analysis of the effectiveness of biorisk treatment are legislated in Kazakhstan in the form of an order of the Minister of Health *"On approval of a biological risk management methodology"*, 2022 [25].

Concurrently, the implementation of the international standard ISO 35001:2019 "Biorisk management for laboratories and other related organizations" in institutions such as NSCEDI is important for the effective organization and management of processes associated with hazardous biological materials. In Kazakhstan, this standard was adopted and approved in 2021.

A plan for research using laboratory animals undergoes bioethical examination and evaluation by the Institutional Animal Care and Use Committee (IACUC). The bioethical 3R-concept recommends that alternative approaches be considered when handling laboratory animals, and if possible, replace them with other research models, for example in vitro. It recommends that reducing the number of laboratory animals in the experiment may be beneficial not only in terms of bioethics, but also biosafety [26-29].

Following approval of the research plan by the IACUC, it is submitted for approval to the Institutional Biosafety Committee (IBC), along with the developed standard operating procedure (SOP) for the investigation and a risk assessment carried out by head of project and the biorisk manager. The research design and SOP are approved if the risk is assessed as "low".

The process of passing the examination of the research design for compliance with bioethical norms and the requirements of biological safety and biosecurity adopted in NSCEDI is shown in Figure 1.

It is important that each member of the IACUC and IBC participate in the inspection control, discussion of documents (study plans, SOP), in the investigation of cases related to work in the BSL-3/ABSL-3 laboratories. All information must be open and accessible.

When gathering information about potential risks in the handling of laboratory animals, in addition to the standard biorisks encountered in BSL-3 laboratories, such as the generation of aerosols from centrifugation and pipetting, other specific risks emerge. These include the use of sharp and cutting objects (injections, necropsy), bites and scratches by animals [29]. In addition, a pathogenic agent may become more virulent after several passages in animals [30]. These residual biorisks are mostly managed through in-depth staff training and enhanced use of personal protective equipment. Regular testing of laboratory animal handling skills is also recommended, especially during extended work breaks. In such cases, the research design may include first working with non-infected animals (control groups), and then with infected animals. At the same time, an additional biorisk check can be carried out at the first stage.

Typically, laboratory mice are used in *in vivo* studies. Firstly, these are well-known experimental models, and secondly, there are many genetic strains, including transgenic ones. However, the COVID-19 pandemic disrupted logistics, and the need for transgenic mice susceptible to the SARS-CoV-2 virus has increased dramatically. The only transgenic hACE 2 mouse strain that existed at the beginning of the pandemic turned out to be rare and poorly studied [31]. Among the existing animal models, only hamsters, ferrets, cats and non-human primates have been found to be sensitive to SARS-CoV-2 [31]. In this case, the Syrian hamster proved to be a reproducible and optimal, albeit poorly accessible, experimental model for COVID-19 disease [34]. It is much more difficult and costly to work with larger animals, and many laboratories in a short time had to reorganize their work on the use of Syrian hamsters, which have a number of features in keeping and handling them. Syrian hamsters are solitary animals, with high intra-group aggressiveness in females [32,33]. Thus, the behavioral characteristics of female Syrian hamsters have influenced biorisk and its management.

Biorisk management measures related to Syrian hamster aggressiveness.

The aggressiveness of laboratory animals when working in laboratories is a consideration, as it can result in a bite or scratch of a lab worker. For example, for mice, the aggressiveness index is 15 per 1000. Mice aggressiveness can be caused by various factors, such as the type of genetic strain, the frequency of cage replacement, the number of animals per cage, and other environmental parameters [34]. A high level of aggressiveness of females, but not males, has been shown in the Syrian hamster [35]. This animal species is of interest because the *Mesocricetus auratus* has been shown to be susceptible to SARS-CoV-2 and can be used to simulate COVID-19 infection [31]. In addition, viral infections such as influenza [35], Ebola [36] and Marburg [37] hemorrhagic fevers, are reproduced on the Syrian hamster. In contrast, the Syrian hamster is relatively resistant to Mpox [38]. The relatively large size of hamsters, in comparison with mice, makes it possible to obtain a larger volume of blood for various diagnostic tests [39]. These and other characteristics have made the Syrian hamster a good model for studying EIDs. Since it

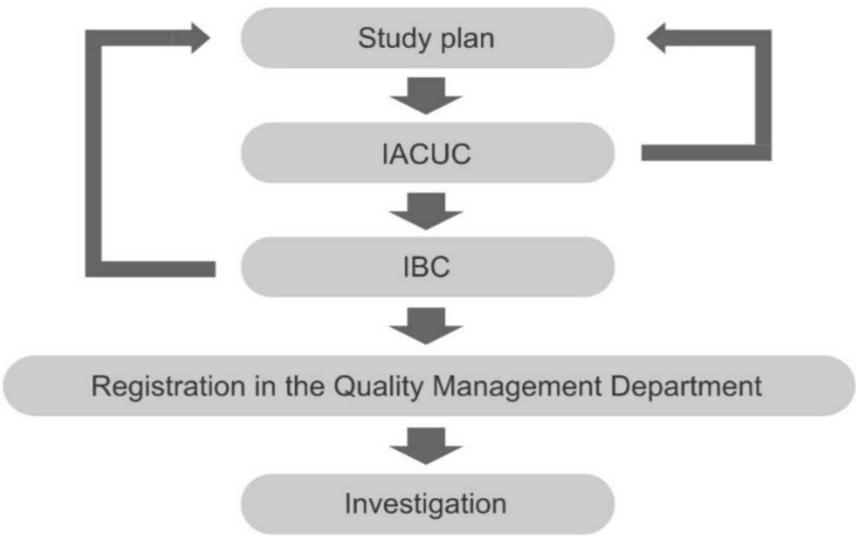

Figure 1. Scheme for the examination of the study plan using laboratory animals in the ABSL-3 laboratory

is not always possible to exclude Syrian hamsters from research without compromising quality, for example, by replacing them with *in vitro* models, we propose a number of primary measures for managing biorisks related to their aggressiveness:

- Rejection of animals with increased aggressiveness, both in relation to their species and the personnel caring for them. Our studies culled 3% of highly aggressive females of Syrian hamsters. Female hamsters showed greater aggressiveness and mobility than males, when handling inside the BSC.
- Unhurried work and more careful fixation of the animal by hand. The best way to capture the animal was to use an opaque cup. The hamsters calmly moved from the cage to the cup, and were then fixed by hand on the working surface of the BSC.
- According to the design of the study, the work should be started with control uninfected hamsters. This allows the researcher to adapt psychologically and once again practice the skills of handling animals.
- Conducting manipulations by an employee who already had experience with these animals (grooming, weighing and clinical examination). The hamsters become accustomed to handling and behave more calmly.

Conclusion

The professional and psychological training of professionals working with laboratory animals should be a priority. Biological safety requires the involvement of all parties from technical staff to top management. The COVID-19 pandemic has shown a limitation in experimental studies when obtaining the right strain of mice proved difficult. A rare experimental model of Syrian hamsters started to be used in the work, which have several distinctive characteristics associated with increased aggressiveness of females, the need to keep them alone and some technical difficulties during manipulations in the biological safety cabinet. Therefore, there are additional risks that need to be considered and reduced to an acceptable level. To do this, a risk-based approach should be introduced into the work, as well as the implementation of international standards and regulations on biological safety and biosecurity.

Ethics statement

All study protocols of in vivo was approved by IACUC by the Ethics Committee of the Aikimbayev's National Scientific Center for Especially Dangerous Infections.

Conflict of interest

The authors confirm that there is no conflict of financial/non-financial interests associated with writing the article.

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Author contributions

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Data Availability Statement

The article is written based on literature data, as well as our own research. Additional questions can be directed to the corresponding author.

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