Policy Forum

The Main Biological Hazards in Animal Biosafety Level 2 Facilities and Strategies for Control*

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Concern about the biological hazards involved in microbiological research, especially research involving laboratory animals, has increased in recent years. Working in an animal biosafety level 2 facility (ABSL-2), commonly used for research on infectious diseases, poses various biological hazards. Here, the regulations and standards related to laboratory biosafety in China are introduced, the potential biological hazards present in ABSL-2 facilities are analyzed, and a series of strategies to control the hazards are presented.

A series of laboratory-acquired infections were reported in recent years, in Singapore (September, 2003), Taipei (December, 2003), and Beijing (December 2003-January 2004)[1]. In addition, 28 teachers and students in the Northeast Agricultural University of China were infected with *Brucella* spp. in 2010[2]. In addition, the bacterium that causes anthrax escaped from a laboratory in USA in 2014[3]. These laboratory-associated infections have increased global concern for laboratory biosafety[4], and have prompted many countries, including China, to reexamine and revise the relevant laws and regulations for laboratory biosafety, to facilitate the effort to propose appropriate countermeasures.

Overview of Regulations and Standards for Animal Laboratory Biosafety in China Although *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) (published jointly by the Centers for Disease Control and Prevention and the National Institutes of Health 1999, USA) is the gold standard for laboratory biosafety, the actual biosafety programs applied to control biological hazards in individual facilities depend on numerous factors, including the agents being used, the source of funding, and local codes, among others. Here, we summarize the regulations and standards currently in place in China, and propose control strategies. The Regulation of Pathogenic Microorganism Laboratory Biological Safety (issued by the State Council of the People's Republic of China)[5] is the primary mandated regulation in China for the management of laboratory biosafety and pathogenic microorganisms. The Directory of Pathogenic Microorganisms Transmitted in Humans, issued by the Ministry of Health in China in 2006, specify the grade of laboratory in which specific pathogens and animals should be housed[6]. The Standards include the general laboratory requirements for biosafety[7] and the architectural and technical code for laboratory biosafety[8]. These provide national guidelines and standards for the construction, operation, and management of biosafety laboratories in China.

Based on a combination of our own practical experience and consultation of these references[9], here we consider aerosols, zoonoses, and laboratory-associated infections as the main biological hazards in ABSL-2 facilities.

Aerosols Aerosols are classified as small solid particles or liquid droplets, with a diameter of 0.001 to 100 μm, that form relatively stable dispersions in gaseous media[7]. Here, aerosols refer to both bio-aerosols and aerosols originating from laboratory animals.

Rodent allergens, which can cause anaphylaxis in animal care staff, show a wide range of particle sizes, and both small and large allergen-laden particulates have been shown to migrate throughout facilities[10]. Rat and mouse allergens have been shown to be carried mainly on particles 6 μm or larger[11], and another study found that rat allergens could be carried on smaller particles of 2-5 μm in size[12]. Therefore, aerosols originating from mice or rats may carry rodent allergens, and thus cause harm to staff through inhalation, skin contact, and eye

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contact, among others. Some studies on rodents have indicated that increased levels of room allergens are correlated with decreased humidity[13-14], increased animal density[15-17], and activities such as cage changing, room cleaning, and animal handling[15-19]. In order to reduce levels of animal aerosols, we should first reduce animal allergen levels, and thus relative humidity and staff activities should be taken into consideration.

Bio-aerosols are another type of biohazard. Bio-aerosols are classified as airborne particles that are living (bacteria, viruses, and fungi) or that originate from living organisms. Bio-aerosols are ubiquitous, highly variable, complex, and natural or man-made in origin[20]. Infected animals can release biohazardous materials through respiration or excretion. When staff handle these animals during activities such as feeding, cage changing, blood collection, and anatomical examinations, bio-aerosols may be generated. In order to reduce bio-aerosol loads in indoor environments, certain control measures should be followed[21]. These include proper identification and elimination of the microbial source in occupational settings, maintenance of equipment, humidity control, use of filters in ventilation, and air cleaning using disinfectants and biocides. The air in operating rooms and other critical areas, such as isolation rooms, can be disinfected by fumigation. In addition, an adequate air change rate and installation of filtration equipment are necessary[22].

Zoonoses Zoonoses are another important source of laboratory-acquired infections. Zoonotic infections, such as cases of infection by *Brucella* spp. and the bacteria that cause anthrax, have previously been reported, and here we will use human *B. canis* as an example. From 1968-2010, 52 individuals were infected by *B. canis*; most had close contact with dogs. Cases of *Brucella* infection have also been reported in laboratory workers[23]. These incidents indicate that zoonoses are an important biological hazard during animal experiments that can lead to serious infections in human.

Laboratory-associated Infections Pike[24] reported that only 18% of laboratory-associated infections could be traced to a known cause, whereas unexplained laboratory-associated infections account for 82% of all infections. Research on unexplained laboratory-associated infections has shown that most unexplained infections are caused by inhalation of aerosols containing infectious pathogens. Wedum[25] reported that more than 65% of all laboratory-associated infections are caused by aerosols containing microbes. Pedrosa[26-27] showed that 84% of laboratory arbovirus infections are caused by aerosols.

Zhanbo[28] and colleagues measured the strength of microbial aerosol sources in different situations, including both normal operation and accidents. They found that the strength of the aerosol source caused by accidental breakage of a bottle is higher than that caused by appropriate operation of a centrifuge[28]. Thus, it is very important to regulate activities and operations in animal laboratories to reduce the risk of biological hazards.

According to the Directory of Pathogenic Microorganisms Transmitted in Humans, most (277 of 374) species of pathogenic microbes should be contained in ABSL2 facilities. Thus, most experiments on infected animals are conducted in ABSL-2 facilities. From these figures, we can infer that ABSL-2 facilities are more likely to generate harmful biohazards than animal facilities of other grades. It is therefore necessary to implement methods to control transmission of harmful factors[6]. Based on our own experience and the relevant regulations and standards in China, we provide the following summary of control strategies.

Design and Construction of ABSL-2 Animal Facilities

The design and construction of the animal facility should be reasonable and correspond with the national standards.

The facility should be divided into several relatively independent functional units according to the rule that different species and different levels of laboratory animals should be housed separately[29]. The housing area and the experimental area within the containment barrier should be equipped with a controlled access system that only allows authorized staff and visitors to enter. The pressure in the core experimental area must be negative[7]. In addition, there should be a pressure gradient between the housing room and any 'dirty' corridors[8]. Housing rooms should contain a buffer unit with biosafety cabinets, in which researchers can perform experiments, in order to control aerosols efficiently. The main devices in ABSL-2 facilities should include individual ventilated cages (IVC) for rats and mice, negative housing cabinets for rabbits and dogs, and isolators for poultry. The animal experiment equipment should include a negative pressure autopsy table, a disinfection sterilizer, biosafety cabinets, centrifuges, independent ventilation

animal transport cages, and independent ventilation recovery cages. Other devices may include a cage changing work bench, cleaning equipment, and a hydrogen peroxide generator[30].

Heating, Ventilation, and Air Conditioning (HVAC) The HVAC system is a complex but very important part of an animal facility. A properly designed and functional HVAC system is essential to provide adequate pressure, temperature, and humidity. Pressurization contributes to controlling airborne contamination by providing directional airflow. Areas for quarantine, housing, and use of animals exposed to pathogenic microorganisms, and areas for housing nonhuman primates should be kept at negative pressure, whereas areas for clean equipment storage should be kept at positive pressure. The HVAC system should be designed for reliability (and redundancy, if applicable), ease of maintenance, and energy conservation and be able to meet the requirements of all animals housed. The system should also be adjustable and ideally maintain temperatures of ±1 °C. Relative humidity should generally be maintained within a range of 40%-70% throughout the year[29]. Although maintenance of humidification within a limited range over extended periods is extremely difficult, daily fluctuations (recognizing the effects of routine husbandry especially when caring for large animal species) in relative humidity should be minimized. Ideally, relative humidity should be maintained within ±10% of the set point. However, this may not be achievable under some circumstances. The type and efficiency of supply and exhaust air treatment should be matched to the quantity and type of contaminants and to the risks they pose. Overall, the principles for selection of the HVAC should be reliability, longevity, and minimal energy consumption.

Testing and Validation of HEPA Filters A set of strict assessment criteria for testing or verification has been established to ensure the reliability of the biosafety protective performance of facilities. The focuses of the assessment are verification of the airflow pattern and the capacity of the HEPA filter to remove contaminants. It should be noted that routine physical examinations are inadequate for effective evaluation, and that microbial aerosol technology is the most direct and specific technique for biological detection of airflow and HEPA filters[31-32]. While the HAVC system runs, the HEPA filter will collect particulates. The capacity of the building supply and the exhaust fan determine the life of a HEPA filter. When the HAVC can no longer maintain a proper airflow balance due to filter loading, the filters need to be replaced. If any filter shows visible signs of damage or leakage, it should be repaired or replaced immediately.

Management of Experimental Activities in the Animal Facility Before the experiments begin, researchers are required to detail the protocol for each animal experiment. The biosafety officer and the attending veterinarian then judge the feasibility of the experiment. The Directory of Pathogenic Microorganisms Transmitted in Humans is used to determine the laboratory grade of the animal experiments. During experiments, the status of the animals and the surroundings should be recorded in detail by the staff. For the experiments involving infectious agents, all handlings, such as cage changing, injection, and sampling, must be performed in biosafety cabinets. When sharps, such as needles or glass, must be used, operations should be performed carefully to avoid any injury. Researchers must strictly obey the standard operation procedures when working with infectious agents. The protective performance of the biosafety cabinets should be assessed regularly. Experiments should be performed on the cleanest laboratory animals firstly, then dirtier animals. At the conclusion of the experiments, the feeding rooms and cages should be sterilized thoroughly. The waste from experiments involving infectious agents should be decontaminated by autoclave sterilization before removal from the facility.

Depositing Bacterial Concentration Testing and Sentinel Animals The running conditions and the cleanliness of the animal facility should be monitored by the quality control officer. The depositing bacterial concentration is a required inspection item for every laboratory in the facility each term. Blood agar plates should be placed in each corner and in the center of the laboratory for 30 min and then cultivated in a 37 °C incubator for 48 hours, at which point the bacterial colonies should be counted.

Surveillance using sentinel animals is also essential. Sentinel animals are externally sourced animals that are introduced into a population, exposed to animals or soiled bedding from the population, and sampled in lieu of the principal animals. Most commonly, sentinel animals are indirectly exposed to infectious agents by using dirty bedding. The use of direct contact sentinels is a valuable complement, particularly in certain

sensitive situations such as quarantine. Bedding sentinel animals are the current standard practice for surveillance of isolated ventilated cages or when animals to be monitored are immunodeficient and there are no immunocompetent resident animals available. If sentinel animals are bred in an ABSL-2 facility, they should be monitored at an increased frequency, perhaps monthly, as they are a potential source of infection for the entire facility. Sentinel animals should be chosen and placed with a variety of criteria in mind. Sentinels should be immunocompetent so that they are suitable subjects for serology, and should have a mature immune system when sampled. Sentinels should be introduced to the colony at 3-5 weeks of age. Sentinels should be female, which will decrease fighting and lessen the chance of genetic contamination of the resident animals. Sentinel animals should also be demonstrably free of all infectious agents that would be of concern in the area that they are chosen to monitor.

Occupational Health and Safety Program The components of an occupational health and safety program for animal handlers include screening, training, work practices, effective use of engineering controls, selection and use of personal protective equipment, and emergency response protocols[33].

An effective occupational health program screens those with animal contact in order to identify individuals who may be particularly susceptible to animal allergens or the infectious disease under study, or who may present an elevated risk to the animals. This medical evaluation, which is part of the individual's medical record, must be private and confidential. Training should be tailored to the group in question. It is also prudent to provide additional training to cover risks or hazards faced by animal handlers even if it is not mandated by regulation. Examples of training programs in this category include ergonomics and slips, trips or falls, effective use of the biosafety cabinet or chemical fume hood, safe handling of sharps, sterilization/disinfection, and an emergency response program. Employees must also receive training for the use of any equipment that may involve risk or present a hazard to the employee, such as autoclaves, rack washers, containment equipments, ventilated caging systems, and precision vaporizers used by researchers. An individual who has never worked with infected animals should demonstrate proficiency with the required biosafety work practices and the institution's peer-review standard operating procedures (SOP) with noninfectious animals before proceeding to practice sessions with infected animals. Selection and use of personal protective equipment (PPE) is essential to avoid infections in an animal laboratory. PPE appropriate for the work environment, including clean institution-issued protective clothing, should be provided as often as necessary. Emergency protocols should be established so that employees can protect themselves and the animals.

Physical injures such as animal bites, skin punctures by needles, as well as other potentially hazardous injuries, should be reported immediately. In this situation, the director of the facility who will take responsibility should be informed as soon as possible. At the same time, the injured person should receive first aid treatment, and emergency procedures should be initiated. Finally, regular health examinations help to assure not only the occupational health and welfare of the staff but also the quality of the laboratory animals, and employees should be required to undergo an annual health examination.

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