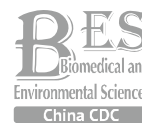


Policy Forum



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

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