# Animal Bytes

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Animal Bytes examines biosafety challenges posed when conducting work with animals and provides solutions that promote both safe and responsible research. Good safety and animal husbandry are essential for good science. Learn about best practices when working with animals and applied safety information that can be used every day. Please e-mail your comments, questions, and insights to barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

## Laboratory Animal Zoonoses and Biosafety

The term "zoonosis" typically refers to a disease normally found in animals that can be transmitted to humans as a result of direct or indirect contact with naturally infected animal populations. While the emphasis of this column focuses on transmission of disease from animals to humans, it is also important to remember that some laboratory species are susceptible to diseases transmitted by humans (i.e., hepatitis A, measles, tuberculosis) and that research animals must be protected as well (Virginia Department of Health, 2008).

In the United States and countries where small laboratory animal species have been bred for decades in highly controlled clean environments, accounts of zoonotic infections acquired from laboratory animals have become relatively uncommon (Weigler et al., 2005). The ability to procure rodents that are tested and demonstrated to be free of adventitious pathogen contamination, including lymphocytic choriomeningitis virus (LCMV), hanta virus, *Mycoplasma* spp., parasites, pathogenic bacteria such as *Salmonella* and *Shigella* spp., etc., greatly reduces the risk that naturally occurring zoonotic infection will be transmitted to individuals working with animals.

As wild rodents can transmit these and other pathogens to disease-free animals, it is important that each institute working with laboratory animals implement a comprehensive pest control plan that is designed to prevent, control, or eliminate the presence of or infestation by pests in an animal environment. A regularly scheduled and documented program of control and monitoring should be implemented (ILAR, 2010). In addition, screening of animals should be performed periodically to identify infections in the colony. Dykewicz et al. (1992) describe a case where nude mice became infected with LCMV after hamsters had been inoculated with a cell line inadvertently harboring the pathogen. The screening program had lapsed and the seropositive status of hamsters and asymptomatic nude mice had not been identified until an animal care worker was hospitalized with meningitis and a test panel identified LCMV as the causative agent of the infection.

Harding and Byers (2006) have conducted an extensive search of literature pertaining to laboratory-acquired infections (LAIs) and report the "analysis of the viral LAIs associated with animal activities demonstrates how critical it is for laboratory staff to understand the potential for zoonotic infections which may be asymptomatic in the animal populations. Between 1979 and 2005, there were 171 overt infections, with one fatality, and 144 seroconversions associated with zoonotic viral infections that were not experimentally introduced to the research model. These zoonotic LAIs were caused by Hantavirus (155), CHV-1 (3), lymphocyticchoriomeningitis virus (LCMV) (9), Orf virus (2), and Ebola virus (1). In comparison, work with experimentally infected animals caused only 11 symptomatic infections and no asymptomatic infections. The LAIs from experimentally infected animals were caused by swine influenza (2), Vaccinia (2), Venezuelan equine encephalomyelitis (3), Marburg (1), West Nile (2), and Ebola (1)." This review of the literature underscores the risk associated with contracting an LAI even when conducting work with animals that have not been experimentally infected.

In instances where wild or free-range animals or laboratory animals obtained from less stringently controlled sources or from random-source procurement are used, zoonotic infections in humans can result in serious illnesses (Canadian Council on Animal Care, 2011; Suckow et al., 2002). Examples of infections acquired while working with naturally or experimentally infected animals include but are not limited to (adapted from NRC, 1997):

- Rodents: lymphocytic choriomeningitis virus, hantavirus, *Salmonella* spp., *Shigella* spp., and *Leptospira interogans*
- Nonhuman primates: herpes B virus, hepatitis A virus, Marburg virus, *Mycobacterium tuberculosis, Campylobacter jejuni,* and *Shigella* spp.
- Livestock: vesicular stomatitis virus, *Coxiella burnetii*, and *Brucella* spp.

• Other species (rabbits, cats, dogs): *Francisella tularenis*, *Salmonella* spp., *Campylobacter jejuni, Brucella* spp., and *Leptospira interogans*

Despite advances in animal husbandry and disease screening, zoonotic infections still represent a workplace hazard. Adhering to biosafety practices, use of primary containment, and personal protective equipment (PPE) can greatly reduce the risk of exposure to zoonotic pathogens. A thorough risk assessment includes input from the biosafety officer, veterinarian, occupational health provider, principal investigator, and other resources as applicable. A risk assessment that takes into account the available and applicable safety measures, animal species, potential or known zoonotic agent(s), other agents used in the research, practices and protocols, human factors and susceptibility, and ability to recognize early symptoms is needed to develop a strategy to eliminate or mitigate risk of infection and be prepared to medically treat injuries or illnesses should an accident occur.

## Transmission of Zoonotic Agents to the Workforce

As in the case of laboratory-acquired infections, zoonotic agents can be transmitted from the host animal to individuals who use or care for the animal by traditional routes of exposure including percutaneous, ocular/mucosal, oral, and respiratory routes. Sewel (1995) summarized two of the most comprehensive and recent surveys of laboratory-acquired infections (Miller, 1987; Pike, 1976), the first of which spanned 25 years of reporting at the National Animal Disease Center (NADC). Respectively, the studies indicate the majority of attributable LAIs are due to needle sticks/sharps injuries (41% and 15%), followed by: splashes (27% and 6%), animal or ectoparasite bites or scratches (14% and 6%), and mouth pipetting (13% and 0%). Instances where the cause of LAIs was unknown or due to other incidents made up approximately 6% and 74% of reported illnesses. The authors of the NADC study attribute the unknown exposures to transmission via the aerosol route with *Brucella* spp*., Mycobacterium* spp*., and Chlamydia* spp., which were responsible for 76% of the infections.

However, one must keep in mind the data in these studies have been generated from practices that were commonplace 10 to 30 years ago. Many countries have since made biosafety cabinets available when the risk of infection via aerosol exposure is relevant, replaced glassware with plastic, enacted sharps reduction programs and provided engineered safe sharps, and prohibited practices such as mouth pipetting. A more current literature review of LAIs would inform as to how these changes in biosafety practices and provision of equipment have impacted the types of LAIs and routes of transmission over the past decade.

## Animal Biosafety Practices, Primary Containment, and PPE

The use of biosafety practices, primary containment, and PPE sets the stage for prevention of infection inside areas where work with animals is conducted. Secondary containment and engineering are important to safeguard individuals outside containment and the environment and may be discussed in a future column. The publication, *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*) (U.S. Department of Health and Human Services, 2007) provides detailed safety recommendations for working with animals and infectious agents at all biosafety levels. The Safety Office, Laboratory Animal Resource Center, Institutional Biosafety Committee, and Institutional Animal Care and Use Committee are internal resources that can provide project-specific safety recommendations. Good animal biosafety practices for various animal biosafety levels (ABSL-1 through 4) include but are not limited to (Hau & Schapiro, 2011; NRC, 1997; U.S. Department of Health and Human Services, 2007):

• Conducting a risk assessment to determine the appropriate biosafety level
• Restricting access to animal rooms
• Posting hazard communication signage on the doors of rooms containing animals
• Adhering to Animal Biosafety Practices at all times, as described in the *BMBL*
• Training and demonstrating proficiency for all tasks associated with animal work; training should be done with uninfected animals
• Providing self-aid and first-aid training that includes bite and scratch treatment, and immediate accident reporting
• Raising staff awareness of the unique physical hazards posed by each species, as well as signs and symptoms of infection with zoonotic and research pathogens
• Concentrating on good hygiene (i.e., reducing face-hand contact and regular/proper hand washing)
• Using protective gloves, lab coat, and eye protection for work with uninfected animals and identifying the type(s) of PPE needed to mitigate the risk of exposure for work with potentially infected or known-to-be-infected animals
• Selecting animal caging (primary containment may include ventilated or HEPA filtered cages) commensurate with the transmission risk, biosafety level, and PPE available
• Using a biosafety cabinet or cage changing station for all manipulations that may result in generation of infectious aerosols to include cage changing (i.e., dumping of bedding)
• Reducing the use of sharps to the greatest degree possible
• Selecting and using appropriate animal restraints

(physical or chemical)
- Using aseptic techniques
- Using secondary containers when transporting infectious materials (waste, carcasses, specimens, etc.)
- Thoroughly decontaminating work areas and sterilizing waste
- Showering prior to exiting the containment area
- Investigating incidents/accidents, providing group lessons learned, and developing and training on new processes or procedures to prevent future occurrences

As well trained as a person may be and despite the availability of primary containment and PPE, bites, scratches, needle sticks, splashes, and inhalation of infectious agents can and do occur. Additional risks to people working with animals include the development of allergies to animal proteins and the inability to receive vaccines or prophylactic therapy due to changes in their health to include becoming pregnant or immunocompromised or immunosuppressed as a result of illness, medications, or other factors. Individuals may develop allergies to latex to include PPE such as gloves, and materials frequently used such as surgical tubing or drip-bags. In cases where the risk assessment calls for the use of a respirator, personnel are required to be medically evaluated prior to issuance and use of respiratory PPE (OSHA, 1998). For all of these reasons, a good Occupational Health Program is an important component of animal biosafety.

## Occupational Health Care

An occupational health care program is an indispensible part of an institution's commitment to the health and safety of its staff, students, and visitors. In the context of this topic, the focus will be on occupational health care available to individuals working with animals. The ILAR states that each institution must establish and maintain an occupational health and safety program as part of the overall animal care and use program. This is a requirement for all institutions that are Public Health Service-assured in the U.S. and globally to all institutions accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The program is site-specific in that it depends on the facility, research activities, hazards, and animal species involved (ILAR, 2010). The program would cover all employees who have contact with animals, although it provides for tiered coverage depending on the species, amount of time working with animals, and processes. The provisions should include at a minimum:
- Pre-placement medical evaluation
- Identification of hazards to personnel and safeguards appropriate to the risks
- Appropriate testing and vaccinations
- Training of personnel regarding their duties, hazards, and safeguards
- Policies and facilities that promote cleanliness
- Provisions for treating and documenting job-related injuries and illnesses

The *BMBL* requires that staff be provided a medical surveillance program as indicated by the risk assessment and administered appropriate immunizations for agents handled or potentially present before entry into animal rooms. Baseline serum samples would be taken at the discretion of the institute and securely stored. Many institutes offer immunization for tetanus and hepatitis B virus, and the purified protein derivative test, PCR test, or *in vitro* cytokine assays such as the QuantiFERON®-TB Gold In-Tube (Cellestis Limited, Melbourne, Australia) for tuberculosis screening for staff working in health care settings or with animals (CDC, 2005; Nyendak et al., 2009). Based on a risk assessment by Occupational Health and Safety personnel, staff may be offered other immunizations specific to the infectious agents worked with, animal species worked with, and the source of the animal (i.e., wild-caught vs. vivarium-raised and disease-free). In some instances Occupational Health programs provide periodic examinations, pulmonary function test, respirator fit testing, employee health counseling, and end of employment exit-exams. The program, like the biosafety program, should be site-specific and address the biosafety and health needs of individuals at the institute.

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