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Introduction

Foreword and Introduction to This Issue on Contemporary Safety Topics in Animal Research

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Abstract

Institutions with animal care and use programs are obligated to provide for the health and well-being of the animals, but are equally obligated to provide for safety of individuals associated with the program. The topics in this issue of the ILAR Journal, in association with those within the complimentary issue of the Journal of Applied Biosafety, provide a variety of contemporary occupational health and safety considerations in today's animal research programs. Each article addresses key or emerging occupational health and safety topics in institutional animal care and use programs, where the status of the topic, contemporary challenges, and future directions are provided.

Key words: animals; occupational health and safety; personnel protection; research

Occupational health and safety (OHS) is an integral component of animal care and use programs (ACUP). Institutions must provide, and individuals should expect, a safe work environment that identifies the multiple and varied hazards and then mitigates the risk associated with hazards to acceptable levels. The *Guide for the Care and Use of Laboratory Animals*[1] recognizes the importance of ensuring a safe work environment for individuals working in the ACUP. Distinct elements of the OHS program include: hazard identification and risk assessment, personnel training and protection, written procedures and policies regarding hazard use and monitoring, and medical evaluation and preventive medicine.

There are many challenges to effectively orchestrate a comprehensive OHS program that considers all hazards and the health profiles of individuals. Programs are often decentralized and involve many scientific disciplines and a variety of animal species, which presents additional challenges in ensuring hazard awareness and the procedures to mitigate risks. Furthermore, each individual working in an ACUP has a unique personal health profile that should be assessed prior to their animal exposure. The hallmark of a successful OHS program is coordination among medical and safety personnel, researchers, veterinarians, and the Institutional Animal Care and Use Committee as well as the institutional administration and management.

The *Occupational Health and Safety in the Care and Use of Research Animals*[2] provides invaluable guidance on structuring an effective OHS program and the text is still widely used in most ACUPs. However, this text has not been updated to reflect advances in the field or information on new types of hazards, such as nanomaterials. Advances in technology drive research methods at a rapidly changing pace, requiring that workers and health care and safety professionals keep abreast of emerging risks and practices to mitigate them. Many publications have addressed specific OHS topics within ACUPs over the last 20 years, but consolidating the broad range of topics into one or two general reference texts is lacking. Therefore, it is the editors' attempt to capture the diversity of topics related to safety in the animal research laboratory and to consolidate the resources in complementary issues of the *ILAR Journal* and *Applied Biosafety Journal*. Each article examines salient features

of key or emerging OHS topics in institutions with an ACUP. Experts in the field address the existing status of the OHS topic, review contemporary challenges, and identify future directions.

In this special issue of the *ILAR Journal*, Swearengen provides information about the common gaps in OHS programs identified on site visits by AAALAC International.[3] Colby et al. provide an overview of relevant zoonotic risks found in contemporary animal care and use programs,[4] while safety considerations related to research animal specimens are covered by Asfaw and colleagues.[5] Lester et al cover the safety issues and concerns associated with transgenic animal work,[6] and Edwards et al address safety considerations in agricultural research settings.[7] O'Rourke et al discuss risks associated with nontraditional animal species.[8] Kendal et al examine the challenges related to the consideration of the 3Rs in studies involving hazards.[9] Lastly, Roble and colleagues discuss how disaster planning and personnel safety are interrelated.[10]

This array of topics in animal research programs, coupled with those included in the complimentary issue of the *Journal of Applied Biosafety*, provides an overview of contemporary occupational health and safety considerations in today's animal research programs. Undoubtedly, advances in technology and science will continue to influence the evolution of research-related safety issues. The information provided here helps strengthen the foundation of OHS programs relevant to those engaged in animal based research.

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Review Article

Common Challenges in Safety: A Review and Analysis of AAALAC Findings

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Abstract

The 8th edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*) is clear in its requirement for each institution to establish and maintain an occupational health and safety (OHS) program as an essential part of the overall program of animal care and use. For over 30 years, AAALAC International has utilized a variety of methods to evaluate this component of OHS programs as part of the accreditation process. AAALAC International began collecting data on site visit findings over 20 years ago using the *Guide* as a template for establishing the categories and subcategories to which findings are assigned. Data from 1656 findings associated with OHS were identified during calendar years 2014 through 2016. This information was used to provide an overview of the most frequently observed OHS findings that occurred during this time span. The 5 categories representing key OHS areas and the combined percentage of both mandatory findings and suggestions for improvement in each category included: workplace risk/safety assessment (37.3%); personnel protection (36.3%); personnel risk assessment (14.4%); hazard containment (9.4%); and medical services (2.6%). Information on the most commonly observed OHS findings and associated trends may be helpful to animal care and use programs when conducting internal reviews of their own OHS programs.

Key words: Occupational health; Safety; Animal; AAALAC International

Background

In the first 2 decades following the establishment of AAALAC International in 1965, site visits primarily involved the evaluation of animal care and the physical plant. As time progressed, more emphasis on the use of animals became apparent, and the December 3, 1985 version of the AAALAC International bylaws declared that the purpose of AAALAC is "to accredit animal care and use programs." This was shortly before the time that amendments to the Animal Welfare and Public Health Service Acts provided for broader institution-wide responsibilities in the areas of organizational lines of authority, Institutional Animal Care and Use Committee (IACUC) roles and responsibilities, training of personnel, and occupational health and safety (OHS) programs.[1]

One of the primary standards utilized by AAALAC International in the implementation of the accreditation program is the *Guide for the Care and Use of Laboratory Animals* (*Guide*).[2] The continued progression of both interest and information related to OHS issues is evident through the amount of emphasis placed on this subject over the last 20+ years. Chapter one (Institutional Policies) of the 1985 edition of the *Guide* included 4 sections related to OHS: the topics of special qualifications for personnel using hazardous agents; personal hygiene; occupational health; and animal experimentation involving hazardous agents. These 4 sections encompassed a total of 11 paragraphs in just over 2 pages. In contrast, Chapter 2 (Animal Care and Use Program) of the 2011 edition of the *Guide* contains 9 sections, including occupational health and safety of personnel; control and prevention strategies; hazard identification and risk assessment; facilities, equipment, and monitoring; personnel training; personal hygiene; animal experimentation involving hazards; personal protection; and medical evaluation and preventive medicine for personnel. These 9 sections embody 21

paragraphs over 6 pages. In response to changing regulations and guidelines affecting animal research, there has been a clear evolution from evaluating the housing, husbandry, and veterinary care of research animals to a much broader programmatic approach that also includes research animal use and the safety of personnel involved with the care and use of animals.

Responsibility for OHS Programs

The *Guide* requires that each institution must establish and maintain an OHS program as an essential part of the overall program of animal care and use that is consistent with federal, state, and local regulations. Components of an effective OHS program often include strong administrative support, adequate resources, a well-planned implementation strategy, and appropriate detail for the level of risks involved. Evidence of a well-implemented OHS program includes the identification of all at-risk employees who are fully informed of risks and appropriate safety precautions and are encouraged to participate in the OHS program. Although most countries have laws and regulations that require certain protections for workers, the *Guide* provides information more specific to the activities and hazards associated with the care and use of animals in research, teaching, and testing. In addition, the AAALAC International Council on Accreditation (Council) has adopted other relevant guidelines as reference resources that provide guidance for accredited programs as well as the AAALAC International representatives conducting the accreditation site visits (https://www.aaalac.org/accreditation/resources.cfm). The publication *Occupational Health and Safety in the Care and Use of Research Animals* (*OHS Guide*) is one of the key reference resources included in the subject area of OHS and is designed to assist institutions with the unique OHS challenges associated with the care and use of research animals.[3] Both guidelines recognize that the primary responsibility for developing and maintaining an effective OHS program is the responsibility of the institution and that the IACUC has an indirect role through its coordination with the research program, Attending Veterinarian (AV), Institutional Official (IO), environmental health and safety program, occupational health services, and administration.

Elements of an OHS Program

The key elements of an OHS program, as described in the *Guide*, include control and prevention strategies; hazard identification and risk assessment; facilities, equipment and monitoring; personnel training; personal hygiene; personal protection; and medical evaluation and preventive medicine. The purpose of establishing control and prevention strategies is to have an overarching and coordinated approach to managing the other key functional elements of the OHS program. Implementing a hierarchy of control and prevention strategies establishes a baseline approach that serves as the starting point for managing risk mitigation. The *Guide* clearly delineates the prioritized order of managing risks, which include: ensuring that facilities are designed to minimize risks, that those applicable design features function appropriately, and that appropriate safety equipment is used (collectively known as engineering controls); the establishment of administrative controls such as the development of processes and standard operating procedures (SOPs); and the provision of appropriate personal protective equipment (PPE). Once control and prevention strategies are established, the mitigation of identified hazards can be conducted more effectively.

Hazard identification should be a coordinated and well-defined process that occurs in an ongoing manner to identify new hazards that are commonly introduced in the constantly evolving research setting. Also, ongoing evaluation of the workplace may be useful in identifying new information that can be used to better mitigate, or understand, known hazards. The principal investigator is often the best source for identifying experimental hazards, but due to the presence of both experimental and nonexperimental hazards found in animal research settings, the involvement of health and safety professionals, husbandry staff, veterinarians, facility managers, veterinary technicians, and scientific staff is important for ensuring all aspects of the animal care and use program are evaluated.[4] Routine walk-throughs of animal holding, support areas, and investigator laboratories by safety professionals are a very useful tool for identifying hazards.[3] One-on-one conversations with employees during these walk-throughs can be helpful in understanding the hazards to which they may be exposed in their work environment. Once hazards have been identified, the risk assessment can be performed. There are multiple approaches to performing a risk assessment. Common elements include ensuring that the people assessing the risk are knowledgeable of the risks and procedures to manage them, determining the importance of the hazard, developing risk reduction strategies that can be effectively implemented, and successfully training the workforce. The risk assessment should include an evaluation of both the risks to which personnel are exposed in the course of carrying out their job-related responsibilities and the risks that may be associated with their health status, the latter being an important reason for performing initial and routine periodic medical evaluations. Not only should individuals be aware of existing medical issues that could affect their susceptibility to hazards in the workplace, but they should also be periodically reminded of the health hazards in their work environment and actions they should take in the event of a possible occupational injury or illness.[5] Underreporting of occupational injuries and exposures is problematic among laboratory animal workers, with only 35.7% of the alleged zoonotic disease case-episodes reported to the employee's supervisor, thus indicating the need for continuing emphasis on occupational injury and illness and timely reporting.[3] Although reporting of occupational-related illness or injury is obviously important to the health and safety of individual personnel, the information obtained from these reports can also be very useful for the institutional review process, which can use the information to identify patterns, high-risk activities or vulnerabilities within the OHS program.

Although there is an obvious importance to ensuring there are adequate facilities and equipment to support the safety of personnel for the specific type of activities being performed, it is equally important to ensure they are properly maintained and periodically validated.[2] Because engineering controls are considered the first level of protection for personnel, the requirement for their proper operation is even further enhanced, including such items as facility heating, ventilation and air conditioning systems, biosafety cabinets, fume hoods, isolation cubicles, bedding dump stations, ventilated animal housing systems, ultraviolet bulbs, eyewash stations, emergency showers, autoclaves, and fire protection systems to name a few.

The development and implementation of a proactive and responsive training program is critical to equip individuals with the knowledge and skills needed to work safely.[6] The concept of proficiency/competency becomes increasingly important

when it relates to personal safety, especially as the consequences of the hazards progress. There can be distinct differences between personnel who are trained and those who are competent in implementing the appropriate safeguards.[7] Competency is measurable, and although it includes the evidence of knowledge, skill, and abilities, it should also include judgment and self-criticism.[7] As mentioned previously, training personnel on the impact of personal health and the timely reporting of injury or illness are important concepts to be included in a safety training program.

There are many aspects of developing a sound safety training program for animal research programs that should be considered. The training program should recognize the wide differences in job tasks (workplace diversity) in animal research and, in many cases, cultural diversity that can result in different backgrounds and experiences in OHS issues such as hazard identification and safe work practices. Cultural diversity can also present language barriers that need to be overcome. In addition, the involvement of scientists in the development of health and safety training is particularly important to ensure that training materials contain all relevant information about risks associated with the experiments. Many times there are gaps in information that can occur between the scientist and animal care personnel because they frequently belong to different administrative lines of authority.

Providing effective training in the areas of both personal hygiene and the use of personal protection are paramount in establishing a solid cornerstone for the OHS program. The provision of clear guidance in policies and SOPs will assist personnel in understanding and complying with institutional expectations.

PPE is the last level of protection between personnel and the hazard(s) present; thus, making compliance a critical factor in ensuring it provides the appropriate protection. Personnel should be comfortable with the use of the required PPE and fully informed of why its consistent and proper use is important to their health and safety. Implementation of good personal hygiene and appropriate use of PPE will often reduce the possibility of occupational injury and cross contamination.[2] The selection of appropriate PPE requires specific knowledge of the hazard, sound professional judgment, and experience with the types of activities being performed. The experience component is crucial to ensure that decisions take into consideration the need for visual acuity and manual dexterity. PPE should be primarily selected based on a risk assessment; the level of protection required; and its material composition, which dictates the level of barrier performance it provides.[7]

Assessment of OHS Programs

AAALAC International evaluates OHS programs using a multilevel approach starting with the review of information provided in the AAALAC International Program Description (PD). The PD is an extremely valuable tool in the accreditation process for both AAALAC International and programs undergoing an accreditation site visit. Although the PD is necessary to provide the Council with sufficient information to make an objective judgment concerning accreditation of a program, it also requires institutions to perform a very detailed and comprehensive self-assessment of their animal care and use program. The requirement to provide an updated PD prior to each site visit provides a stimulus to relook at all animal care and use program components and activities on a regular basis and supports the need to engage in an ongoing process of review and continuous improvement. The level of detail required for inclusion in the PD may appear daunting, but once completed it provides an invaluable tool to help understand the complexity of interactions and wide variety of activities that make up an animal care and use program.

Approximately 7 pages of the PD template are dedicated to information regarding the OHS program. The first subsection of the OHS program section in the template requests details describing institutional oversight that include the institutional entities that are involved in the planning, oversight, and operation of the OHS program related to animal care and use and a description of their responsibilities and qualifications. In addition, this section requires information pertaining to the methods used to identify work-related hazards and the processes used to evaluate the significance of those hazards in the context of job-related duties and tasks. Differences in processes for identifying hazards and assessing the resultant risks for and between various categories of personnel are described including, but not limited to, researchers, veterinarians, husbandry staff, cage-washing staff, students, housekeeping, physical plant staff, security personnel, IACUC members (including nonaffiliated members), contractors, and visitors. Due to the need for ongoing identification and evaluation of hazards through periodic inspections and reporting of potential hazardous conditions and incidents, this section also requests information on the methods and frequency of reassessing work-related hazards and programs or methods used to track and evaluate safety-related workplace incidents.

The second subsection pertains to standard working conditions and baseline precautions associated with the animal care and use program. As part of the baseline precautions, a description of the medical evaluation and preventive medicine program for personnel must include information on who receives a personal medical evaluation as a component of the individual risk assessment process and those that are exempted from receiving a personal medical evaluation. This also requires a description of any provisions that allow an individual (following completion of individual health and job-related risk assessments) to decline participation in all or part(s) of subsequently available medical and preventive medicine components of the institutional OHS program (eg, vaccinations, physical examinations, respiratory protection). Detailed information is requested on the features of the medical evaluation and preventive medicine programs, within the context of work duties, including preemployment/preassignment health evaluation; medical evaluations (including periodicity); diagnostic tests (eg, tuberculosis); precautions for working with potentially hazardous species (eg, nonhuman primates, sheep, venomous species); immunization programs; and procedures for communicating health-related issues. Because each institution is responsible for ensuring that medical records and other personal health information are adequately protected in accordance with the Health Insurance Portability and Accountability Act, a description of provisions for assuring the confidentiality of medical information is requested.[8,9]

As previously mentioned, personnel training on OHS-related issues is vital to having a successful OHS program, and details regarding educational programs are requested on topics such as allergies, zoonoses, personal hygiene, physical injuries in animal facilities, and other general OHS educational programs. This includes listing the entities responsible for providing the training and the frequency of training or refresher training. Questions about personal hygiene involve the provision of routine PPE and work clothing to staff, including arrangements for laundering work clothing and expected practices for washing

hands, showering, and changing clothes when leaving animal areas. Several areas involving standard personnel protection request information regarding facility design features, equipment, and procedures employed to reduce the potential for physical injury inherent to animal facilities or species used; likely sources of allergens; facility design features, equipment, and procedures to reduce the potential for developing laboratory animal allergies or acquiring zoonotic diseases; procedures for the maintenance of protective equipment and how its function is periodically addressed; situations where respiratory protective equipment is available; programs for medical clearance, fit-testing, and training on the proper use and maintenance of respirators; how respiratory protective equipment is selected and its function periodically addressed; listing types of cage-processing equipment and other heavy equipment such as tractors and other farm equipment, and a description of their associated training programs, informational safety signage, and other program policies designed to ensure personnel safety when working with such equipment; the use of motorized vehicles for transporting animals and how drivers are protected from exposure to hazards such as allergens or zoonoses, and the decontamination methods employed; and safety procedures for using medical gases and volatile anesthetics, including how waste anesthetic gases are scavenged.

The final subsection covers the relatively specific area of using hazardous materials as part of experimental studies. In addition to listing hazardous or potentially hazardous agents approved for use in animals, the hazards are categorized according to major hazard type (eg, biological, chemical, and physical agents). Follow-up questions request information regarding the process to identify and evaluate experimental hazards; who is responsible for ensuring an appropriate safety review is conducted prior to study initiation; how the risks associated with the hazards are assessed and how procedures are developed to manage the risks; who is responsible for reviewing and implementing appropriate safety and containment procedures; how hazardous waste is handled, stored, and disposed; what are the aspects of the medical evaluation and preventive health that specifically apply to personnel potentially exposed to the hazardous agents; and what special qualifications and training are required of staff involved with the use of hazardous agents in animals. Finally, several follow-up questions request details on the facilities, practices, and procedures for housing animals exposed to hazardous agents, special equipment related to hazard containment, and husbandry practices and PPE used to ensure personnel safety. Although not exclusively related to the use of hazardous agents in experimental studies, the PD template also inquires as to the policies and procedures in place to protect humans when animals may be transported to human patient areas or through common use corridors or elevators.

The information included in the PD provides a comprehensive account of the OHS program and allows AAALAC International site visitors to arrive with an understanding of its organization and processes. Additional evaluation of the OHS program occurs during the onsite evaluation of the animal care and use program. Time is set aside during each site visit for a thorough review of the PD, while representatives from appropriate functional areas within the program are available to discuss what is written. Further enquiries help to clarify any questions the site visitors may have. Also, this discussion often reveals additional details about the program to assist the site visitors in better understanding both the program and its implementation. In addition, a common method used by AAALAC International site visitors to evaluate the effectiveness of an OHS training program is to use a performance standard approach at the implementation level. Personnel may be engaged in conversations during the site visit to determine if they understand the hazards of their job, are proficient in implementing the appropriate safeguards, and consistently follow established internal policies and SOPs throughout the animal care and use program. For example, site visitors may talk with animal caregivers working with immunodeficient mice inoculated with human tissue to determine if they understand the potential hazards and the importance of reporting animal-related injuries; ask personnel working with, or around, rodents if they can describe the symptoms associated with rodent allergies; ask cage wash personnel to describe the safety mechanisms and procedures for rack washers or bulk autoclaves; or have a research technician explain why they are wearing a particular form of PPE. Institutions can use this same technique to evaluate the effectiveness of their OHS training program when conducting routine safety visits to animal facilities and laboratories or during IACUC facility inspections.

AAALAC International Trends in OHS-Related Findings

AAALAC International began categorizing site visit findings over 20 years ago using the *Guide* as a template for establishing the categories and subcategories used to identify the type of each finding. Data from 1656 findings associated with OHS that were identified during calendar years 2014, 2015, and 2016 are used to provide this overview of the most frequently observed types of OHS findings that occurred during that time span. Each finding was further categorized as either a mandatory finding or a suggestion for improvement (SFI) based on the final determination made during the peer review process by the Council. Given the fundamental importance of the recommendations in the *Guide* that are prefaced with a "must," the Council generally categorizes site visit findings that do not conform with a "must" statement in the *Guide* as a mandatory item for correction. In AAALAC International's nomenclature, a mandatory item is a serious deviation from the recommendations of the *Guide*, and/or other AAALAC International standards, which requires correction in order to achieve or continue Full Accreditation. This judgment is based on the Council's assessment of the potential for the program deficiency to adversely affect the health, well-being or safety of animals or humans. An SFI is a recommendation that the Council feels would improve the program, but it is not a requirement for achieving or continuing Full Accreditation. AAALAC International considers the offering of SFIs to be an element of the peer review process that is designed to assist accredited programs by sharing the cumulative knowledge and experience of the Council. It should be noted that there is no obligation for institutions to make program changes based on SFIs; implementation of suggestions is, however, one means of promoting a high-quality animal care and use program.

The 5 major areas used to evaluate the 2014–2016 data, in the order of the most overall findings to the least, include animal environment; OHS; veterinary care; IACUC (or oversight body); institutional administration; and physical plant. The OHS findings were delineated into 5 categories, each with multiple subcategories to allow more granularity to the area of each finding (Table 1). The 5 OHS categories, in descending order from the greatest percentage of findings for both mandatory and SFIs to the least, include: workplace risk/safety

assessment (37.3%); personnel protection (36.3%); personnel risk assessment (14.4%); hazard containment (9.4%); and medical services (2.6%). Interestingly, although the workplace risk/safety assessment category contained the largest number of overall findings and the most mandatory findings of any category, the percentage of mandatory items within any single category was the greatest for Medical Services (30%) (Figure 1).

Workplace Safety/Risk Assessment

Performing thorough risk assessments and routinely evaluating workplace safety are integral to an effective OHS program. Deficiencies in cage wash safety were the most frequently observed findings related to the workplace risk/safety assessment category, comprising 56% of all mandatory findings and 27% of all SFIs within the category. A mandatory finding related to cage wash safety is illustrated by the following:

"*The walk-in rack washer lacked a de-energizing mechanism and instructional safety signage was not present on or near the rack washer. Entrapment inside a walk-in rack washer can result in serious injury or death. AAALAC International's position statement regarding safety requirements for walk-in cage washers and bulk autoclaves outlines features that help ensure safe operation of such machinery. The possibility of entrapment must be eliminated and ideally an emergency shut-off mechanism that is easily accessible from anywhere inside the machine and which de-energizes the washer when activated should be in place. Equipment operators must receive appropriate training and proper instructional safety signage should be posted where it is readily visible by operators. Sufficient evidence must be provided to demonstrate that rack washer safety has been addressed and will be periodically reviewed.*"

Concerns related to rodent allergies consisted of significantly fewer findings but was the second-most frequently cited issue, comprising 9% and 18% of the total mandatories and SFIs within the workplace risk/safety assessment category, respectively. An example of an SFI identified regarding this issue is provided.

"*Several personnel who were working in research laboratories that were designed in an open bay configuration, and in which rodents were brought to parts of the laboratory, were not aware of the associated risks. Also, no assessment of the risk regarding exposure of these personnel to animals had been performed. The exposure of personnel to animals and their allergens can result in adverse health effects. The institution should evaluate the risks of animal exposure for all personnel in these laboratories, ensure personnel are aware of the risks, and implement appropriate mitigations.*"

Although no mandatory findings were identified for concerns with scavenging of waste anesthetic gases, it did result in 15% of the SFIs for the workplace risk/safety assessment category.

Personnel Protection

The effective use of appropriate PPE is key to protecting personnel, but considerations also include the use of other types of safety equipment and protection of personnel from physical hazards associated with job-related tasks. The use of PPE was the most frequently observed finding in the personnel protection category, comprising 68% of the mandatory findings and 36% of the SFIs. The following is an example of a mandatory finding concerning the use of PPE.

Table 1 OHS Subcategories and Specific Descriptors

Workplace risk/safety assessment
Allergies
Autoclave safety
Biohazards
Bite/scratch kit
Cage wash safety
Chemicals
Ground fault interrupted outlets
Hazard usage
Husbandry equipment
Special facilities
Radiation
Safety procedures (eg, electrical, trips/falls, sharps, ergonomics, confined work environment)
Waste anesthetic gases
Zoonosis
Personnel protection
Personal protective equipment (i.e., masks, clothing, gloves)
Safety equipment (e.g., transport vehicles, biosafety cabinets, hoods, safety showers)
Safety procedures (e.g., electrical, trips/falls, sharps, ergonomics, confined work environment)
Personnel risk assessment
Initial evaluation
Periodic evaluation
Hazard containment
Air pressure differentials
Facilities
Procedures (e.g., signage, waste stream management, decontamination)
Medical service
Illness, accident, treatment
Immunizations/preventive medicine
Occupational health professional

"*Requirements for the use of PPE were posted at the entrance to the animal facility, including the use of eye protection when entering Old World nonhuman primate rooms. However, some staff were observed working in the nonhuman primate rooms without eye protection and site visitors were not provided or offered eye protection. Ocular exposure to Macacine Herpesvirus I has been reported, resulting in a fatal outcome. Proper use of PPE reduces the risk of transmission of zoonotic agents, and failure to wear appropriate PPE can compromise employee health. Established guidelines and internal operating procedures for the use of PPE when working with Old World nonhuman primates must be followed. Evidence that applicable personnel have been retrained and that established guidelines are being followed must be provided.*"

Although the evaluation of the areas of safety equipment and safety procedures resulted in relatively low percentages of mandatory findings (20% and 12%, respectively), the frequency of SFIs for these 2 subcategories was similar to that seen for the use of PPE (36% and 28%, respectively). The following is an example of an SFI for the subcategory of safety equipment.

"*Performance checks for the emergency eyewash stations located in the cage wash and chemical storage areas were performed annually. When checked by the site visitors, the water was rust colored and cloudy. Eyewash stations that are not properly maintained may increase the risk of injury to personnel should an ocular exposure occur. The frequency of monitoring and performing preventive maintenance for eyewash stations should be reviewed and practices augmented as necessary to conform with the manufacturer's recommendations and to ensure their safe and proper function.*"

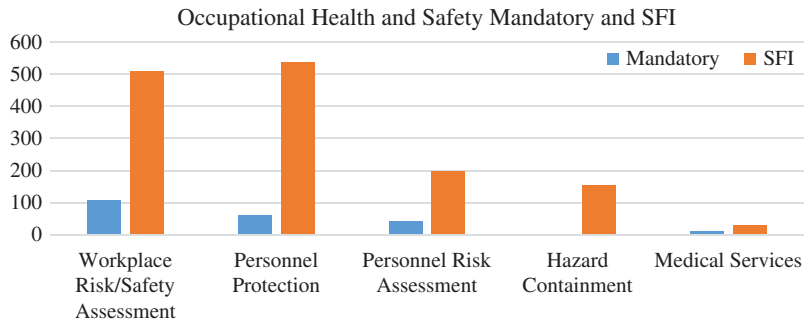

Figure 1: Categorization and frequency of OHS findings from AAALAC International site visits (2014–2016 data).

Personnel Risk Assessment

The initial evaluation component of the personnel risk assessment process comprised the clear majority of concerns for both mandatory findings (80%) and SFIs (80%). The differentiating factor in determining if a finding is a mandatory or SFI in this area often involves the extent to which the issue is seen throughout the program. The following example provides some insight to a situation in which the issue of an initial evaluation was considered a SFI due to the circumstances described.

"*Although animal facility and research staffs were assessed for risks and offered inclusion in the Occupational Health and Safety Program (OHSP), maintenance staff and the Institutional Animal Care and Use Committee non-affiliated member entering animal areas were not. The extent and level of participation in the OHS program should be based on the hazards posed by the animals and materials used; the exposure intensity, duration, and frequency; and the susceptibility of the personnel. A risk assessment by qualified personnel should be conducted to determine the potential risk and possible inclusion into the institutional OHSP for all persons with potential exposure to laboratory animals.*"

Hazard Containment

This category accounted for relatively few of the findings overall, with 81% of the findings within the category falling into the subcategory of procedures. The vast majority of findings for the category were considered SFIs (98.7%). The following is an example of a SFI for the subcategory of procedures.

"*In one research laboratory, research technicians were observed performing terminal cardiac perfusion on deeply anesthetized mice using 4% paraformaldehyde. The procedure was being performed on an open benchtop instead of inside the chemical fume hood located in the room. Exposure of personnel to paraformaldehyde vapor can result in adverse health effects. The conduct of this procedure on the open benchtop should be reviewed by the Environmental Health and Safety Office to ensure that appropriate safety procedures are in place to protect personnel.*"

Medical Services

With the least number of overall findings (2.4%) for all categories, the subcategory involving the role of the occupational health professional in the OHS program contained the most mandatory findings (58%) and SFIs (45%) within the medical services category. The primary issue for most findings in this subcategory were related to the level, or lack of involvement, of trained health professionals in the programs for medical evaluation and preventive medicine. An example of a SFI in this area is provided.

"*Although the basic structure of the occupational health and safety (OHS) program was in place, there was no input from a qualified health professional during risk assessment and enrollment of personnel into the OHS program. Consequently, a valid assessment of medical risk was not part of the OHS program enrollment process which may lead to health and safety issues for personnel working with animals. The AAALAC Reference Resource "Occupational Health and Safety in the Care and Use of Research Animals" (NRC 1997) suggests that a determination of employee risk associated with animal-related research should occur by medical evaluation and review of personal medical records. Also, as stated in the Guide for the Care and Use of Laboratory Animals (NRC, 2011), the development and implementation of a program of medical evaluation and preventive medicine should involve input from trained health professionals, such as occupational health physicians and nurses. Trained health professionals should be involved in the medical evaluation and preventive medicine program and the assessment of medical risk factors.*"

Although this finding was categorized as an SFI, at a different program that involved high consequence hazards a mandatory finding may have been the outcome.

Summary

The *Guide* is clear in its requirement for each institution to establish and maintain an OHS program as an essential part of the overall program of animal care and use. For over 30 years, AAALAC International has evaluated the component of OHS programs associated with the care and use of animals in research, teaching, and testing. The collection and analysis of information on findings cited over the last 2 decades have allowed AAALAC International to provide historical data to our accredited programs and the animal research community at-large, with the intent of providing an opportunity for introspection and benchmarking of their own animal care and use programs.

The categories of workplace risk/safety assessment and personnel protection were clearly the leading areas in which OHS program concerns were identified. Conducing risk assessments and identifying hazards should be an evolving process that is proactive, ongoing, and involving the appropriate expertise and not viewed as static processes.[4] Likewise, the determination of required PPE and safety equipment should involve an ongoing risk assessment approach. Their use should be risk based, taking into consideration specific knowledge of the potential hazards, activities being performed, engineering controls in place, experience, and sound professional judgment.[10]

With the most findings in 2011–2013 and the second-most findings from 2014–2016, OHS programs continue to be an area in which improvements can be made. This said, it must remain in context of the overall improvements made in this area over the

last 30 years and the excellent OHS programs that are observed during the majority of accreditation site visits. Only 13% of the findings involving OHS programs were considered mandatory issues, with the remaining findings being SFIs. In the opinion of the author, this indicates that the vast majority of programs have effective OHS programs and that concerns associated with the more subtle aspects of OHS programs are being identified.

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Review Article

Applied Institutional Approaches for the Evaluation and Management of Zoonoses in Contemporary Laboratory Animal Research Facilities

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Abstract

Zoonoses, diseases transmitted between animals and humans, have been a concern in laboratory animal medicine for decades. Exposure to zoonotic organisms not only poses health risks to personnel and research animals but may also affect research integrity. Early laboratory animal programs were ineffective at excluding and preventing transmission of zoonotic diseases: the health status of the animals were often unknown, endemic diseases occurred frequently, housing conditions were less controlled, and veterinary care programs were decentralized. Over time, these conditions improved, but despite this, zoonotic diseases remain a contemporary concern. To reduce the incidence of zoonoses, management should perform an accurate risk assessment that takes into account the type of research performed, animal species used, animal sources, and housing conditions. Specific research practices, such as the use of biological materials, can also affect the risk assessment and should be considered. Once identified, the characteristics of significant zoonotic organisms can be examined. In addition, personnel attitudes and training, facility design and management, equipment availability, personal protective equipment used, standard operating procedures, and the institution's vermin control program can impact the risk assessment. The effectiveness of the occupational health and safety program at managing risks of zoonoses should also be examined. Risk assessment, in the context of zoonotic disease prevention, is a complex exercise and is most effective when a team approach is used and includes research, husbandry, veterinary, and biosafety personnel.

Key words: animal research; anthropozoonosis; laboratory animal; reverse zoonosis; review; safety; zooanthroponosis; zoonoses

Introduction

The transmission of disease between humans and animals has occurred for thousands of years.[1] However, it was not until the 19th century that it was first formally recognized by the Russian physician, scientist, and statesman Rudolf Virchow when he coined the term "zoonosis" following his study of the life cycle of the nematode parasite, *Trichinella spiralis*.[2] Following Virchow's work, awareness of natural disease transmission between humans and animals has increased. Currently, it is recognized that zoonotic organisms are responsible for about 60% of known infectious diseases and approximately 75% of emerging infectious diseases.[3,4] Most recognized zoonotic disease transmission events occur between humans and agricultural animals, companion animals, and wildlife or through exposure to infected vectors (eg, arthropods and invertebrates) and fomites.[5] Zoonotic disease transmission has also been reported between humans and a variety of laboratory animals.[6,7] As a result of the recognized significance of zoonotic infections to both humans and animals, the

prevention and control of zoonotic diseases are the primary focuses of the One Health initiative.[8]

The vocabulary used to describe disease transmission between humans and animals has evolved over time. Initially, the terms zoonoses, anthropozoonoses, zooanthroponoses, and reverse zoonoses were used to denote the direction of disease transmission between humans and animals. However, due to confusion regarding the use and meaning of these words, the Joint WHO/FAO Expert Committee on Zoonoses defined zoonoses as "diseases and infections which are naturally transmitted between vertebrate animals and man," thus encompassing both directions of disease transmission. This definition of zoonoses has been adopted by others[9] with anthropozoonoses, zooanthroponoses, and reverse zoonoses largely falling out of use. Therefore, we are applying the WHO definition of zoonosis throughout this article.

Zoonotic disease transmission has been recognized in the research environment for decades.[10,11] The management and design of early animal research programs differed substantially from contemporary programs and allowed numerous opportunities for zoonotic disease exposure. For example, early programs frequently utilized animals of unknown health status and endemic diseases were common in animal colonies. Historically, environmental conditions in animal housing and use locations were less controlled, and veterinary care and occupational health and safety programs were minimal or nonexistent.

Over time, the nature and conduct of animal research has evolved to better safeguard human and animal health and minimize research variables. Today, the majority of research institutions strictly define and monitor the health status of their animal populations to exclude zoonotic organisms and other microorganisms of concern. Most animals are obtained from established animal colonies with rigorous animal health monitoring programs. Intensive veterinary care programs are employed to enhance animal health, and veterinary diagnostics are available with sufficient sensitivity and specificity to detect many zoonotic organisms prior to development of clinical signs or widespread dissemination within a colony. In addition, contemporary animal facilities are designed, built, and maintained to provide controlled environmental conditions for both animals and personnel; to segregate, contain, and facilitate sanitization of potentially contaminated areas (eg, animal quarantine rooms or soiled caging and waste processing areas); and to exclude pests and vermin. Specialized animal handling procedures and equipment are employed, such as housing rodents in enclosures with HEPA-filtered air supply and exhaust and handling them in laminar flow hoods with specialized microisolation techniques to prevent animal, personnel, and environmental contamination. Furthermore, institutional occupational health and safety programs have been enhanced[12] and contribute to the prevention and detection of disease conditions of personnel, including those that may present a zoonotic risk to animal subjects.

As the nature and conduct of animal research has evolved, so have the risks of zoonotic disease transmission to both humans and animals. In this article, we describe considerations pertinent to assessment and management of the zoonotic organisms most likely found in contemporary laboratory animal research facilities in the United States. Transmission of infectious organisms from research animals to personnel as well as from personnel to research animals is addressed. Emphasis is placed on organisms traditionally recognized as zoonotic agents in common laboratory animal species as well as opportunistic organisms normally regarded as nonpathogenic in healthy, immunocompetent humans or animals. Table 1 lists select organisms associated with known or potential zoonotic disease transmission events in contemporary laboratory animal research facilities. We do not present an exhaustive list of zoonotic organisms or an overview of zoonotic disease risks specific to wildlife field studies, agricultural research conducted in farm settings, experimental infection of animals, and infectious organisms that require intermediate hosts or vectors for transmission. Information regarding these is provided elsewhere.[7,12–17]

Risk Assessment

Risk assessment is necessary to define, quantify, and then determine how best to minimize a potential hazard. When assessing the relative risk of exposure to zoonotic organisms in contemporary animal research facilities, one should consider organism prevalence, virulence, communicability, routes of human and animal exposure, shedding patterns, environmental stability, and availability of prophylaxis and therapy.[6] In addition, risk assessments should reflect the unique characteristics of the environment in which the hazard may be present. Given the inherent variability between research institutions, assessments should be tailored to each individual situation and not be generalized across institutions or research projects.

When selecting appropriate methods to minimize and manage zoonotic organism exposure, it is critical that risk assessment findings accurately reflect true risks. Underestimating risk can endanger human and animal health, and overestimating risk can lead to unnecessary practices that result in unwarranted expense, unnecessary limitation of research endeavors, or the inadvertent introduction of additional risks to personnel and/or animals (eg, excessive and cumbersome personal protective equipment [PPE] usage resulting in personnel or animal injury). In addition, an overestimation of risk may lead to purposefully decreased animal handling with resultant diminished psychological well-being of animal species for which direct human interaction is generally a positive experience.

An inaccurate assessment of risk may result from a real or assumed lack of information or from a misunderstanding of a zoonotic organism's biologic characteristics in a particular animal species, including its shedding potential, potential modes of transmission, and pathogenicity. It may also result from an incomplete understanding of the protections provided by facility design, specialized equipment and practices, PPE, or from incorrect assumptions of how each is employed by personnel in the facility.

Fear of a zoonotic organism may inappropriately bias risk estimation and lead to excessive use of safety procedures and equipment. This in turn may reinforce misconceptions of risks and may foster overconfidence in unwarranted safety procedures or equipment. Implementation of excessive safeguards may also dilute personnel's attention to effectively utilizing essential safety procedures and equipment. Instead, educational resources and transparency in the risk assessment and hazard control process should be provided to support the use of appropriate safety procedures and equipment.

To conduct a thorough assessment of zoonotic disease risk within an animal research program, multiple components of the program must be considered including the animals, potential infectious organisms, personnel, and the physical structure of the animal facility. Given that this requires the attainment and critical evaluation of a diverse range of information, active collaboration with a team of individuals, including research, husbandry, veterinary, and biosafety professionals is highly

Table 1 Select Organisms with Known or Plausible Zoonotic Disease Transmission in Contemporary Laboratory Animal Research Facilities

Agent	Laboratory Animal Hosts	Comments	References[16,20,56]
Bartonella henselae	Cats, dogs	Fleas play a role in transmission to cats. No documented cases in laboratory animal workers.	
Chlamydia psittaci	Birds (especially those from order Psittacidae), cats, cattle, goats, sheep		
Coxiella burnetii	Cattle, sheep, goats, cats, dogs	Pregnant sheep pose significant risk in laboratory animal setting. Serology of individual animals is not a reliable indicator of shedding.	
Cryptosporidium parvum *C felis* *C canis*	Guinea pigs, mice, chickens, pigeons and other birds, cats, dogs, ferrets, macaques, baboons, squirrel monkeys and other nonhuman primates (NHP), fish, African clawed frogs, reptiles, sheep, pigs	Bovine isolate is especially zoonotic.	
Hantaviruses	Rats, mice, other wild rodents, cats, dogs, pigs, cattle, NHPs	Contamination of biological materials pose significant risk.	
Hepatitis E	Rats, rabbits, cats, dogs, NHPs, pigs, cattle, sheep, goats	No documented cases of laboratory acquired infections.	57
Lymphocytic choriomeningitis virus	Mice, hamsters, guinea pigs, rats, rabbits, dogs, NHPs, chickens, swine	Contamination of biological materials pose significant risk.	
Macacine herpesvirus-1 (MHV-1)	Macaques	<50 cases since originally identified, but high fatality rate if contracted. Serologic tests of individual animals unreliable. Cell cultures and autopsy materials derived from infected monkeys can present potential hazard.	58
Measles	NHPs exposed to humans	Humans serve as a reservoir for NHPs. Once infected, NHPs can transmit measles to humans.	46,59
Mycobacterium avium complex *M. abscessus* *M. bovis* *M. chelonae* *M. fortuitum* *M. haemophilum* *M. marinum* *M. peregrinum* *M. tuberculosis*	*M. avium*, *M. bovis*, *M. tuberculosis:* macaques, baboons, squirrel monkeys, other NHPs. *M. abcessus*, *M. chelonae*, *M. fortuitum*, *M. haemophilum*, *M marinum*, *M peregrinum:* zebrafish *M. bovis*, *M. tuberculosis*: dogs, cats, pigs *M. avium*—chickens, pigeons, pigs *M. bovis:* sheep, goats	Important concern in wildlife research programs or when wildlife are brought into the vivarium. High risk, especially in quarantine settings. *M. tuberculosis:* NHPs are usually infected by humans. Once infected, NHPs can infect humans.	60, 61
Orf virus	Sheep, goats	Highly contagious.	
Pasteurella multocida	Cats, dogs, birds	Commensal in oral flora of cats and dogs. 3–5% of people with intensive animal contact colonized (eg, vets, animal keepers).	
Rabies virus	Dogs, cats, ferrets, livestock, and NHPs especially those housed outdoors or obtained from random source vendors	Vaccination recommended for high-risk individuals. Wildlife housed in animal facility present significant risk. Contamination of biological materials pose risk.	
Spirillum minus and *Streptobacillus moniliformis*	Rats, guinea pigs, mice, gerbils, cats, dogs, ferrets, NHPs	Transmitted primarily through bites.	
Dermatophytes: *Microsporum canis* *Trichophyton equinum* *T. mentagrophytes* *T. verrucosum*	*M. canis*: cats, dogs, rodents, rabbits *T. mentagrophytes:* guinea pigs, rabbits *T. verrucosum* and *T. equinum*: sheep, goats		
Simian Foamy Viruses (SFV)	Old world primates including African green monkeys, baboons, macaques New world primates	High prevalence (>70%) in captive NHPs. Persistent infections possible. Infection of cell lines has been reported. Xenotransplantation concern.	62–65

Continued

Table 1 Continued

Agent	Laboratory Animal Hosts	Comments	References[16,20,56]
Simian Immunodeficiency Virus (SIV)	African NHPs	0.06% of samples tested from humans with occupational exposure to NHPs have antibodies that are cross-reactive to SIV.	64
Simian T-cell Leukemia Viruses (STLV)	>33 species of old world monkeys in Africa and Asia	No cases reported in laboratory animal workers, but there are many cases in humans exposed to wild NHPs.	
Simian Retroviruses (SRV)	Nonhuman primates		

recommended. Once risks are assessed, then procedures can be devised and implemented to mitigate the specific risks.

Research Animals

Institutional Research Focus and Animal Populations

An institution's research emphases dictate the types of procedures performed and the composition of its laboratory animal populations, which in turn directly influence the variety and prevalence of zoonotic organisms most likely associated with its animal research program. For example, risks associated with contaminated biological materials and risks inherent to severely immunocompromised animal models may predominate in human cancer research centers, while risks associated with rats and pigeons may be present in psychology research centers. Similarly, different types or levels of risk may be associated with distinctive animal populations. As an example, the use of privately owned animals (pets) in clinical research studies has increased over the past decade. Maintained outside of a controlled animal research environment, pet animals may be infected with zoonotic organisms from their environment[18,19] and have the potential to transmit disease not only to personnel but also to other research animals. Likewise, laboratory animal populations at most research institutions are highly unlikely to be infected with organisms actively excluded from that country. However, animals housed in international quarantine facilities are at appreciable risk of being exposed to these organisms. For instance, imported nonhuman primate species in designated international animal quarantine facilities are at risk of infection with zoonotic hemorrhagic fever viruses (eg, Marburg and Yellow Fever viruses), but this risk is virtually nonexistent in institutions that obtain animals exclusively from domestic sources.[20]

Populations of immunosuppressed animals may present unique risks within animal research programs. Their immune systems may be suppressed as a component of animal model development (eg, SCID mice used in cancer research or irradiated mice used in adoptive transfer experiments) or may be altered in response to disease or as a sequelae of other research activities (eg, transgenic animals with unintended disruption of immune system genes). Immunosuppressed animals may develop disease to organisms that are normally nonpathogenic, sustain prolonged or persistent infections, or be resistant to otherwise effective treatments. Organisms may also excessively proliferate in immunocompromised animals and be shed in unusually high quantities or by uncommon routes.

Humanized animals present other unique zoonotic disease risks as their native immune systems have been replaced with functioning human immune system components,[21,22] and they can support the maintenance or proliferation of implanted human tissues. During transplantation, zoonotic organisms can be transmitted from an infected human tissue donor to the humanized animal recipient. Growth and excretion of the organism is then strongly influenced by the humanized animal's immune system function. In addition, due to their modified immune system, humanized animals may be able to support a productive infection and/or persistent viremia not otherwise possible in the species. For example, humanized mice can support transmission and replication of laboratory-adapted and primary HIV isolates.[23]

The ability to reliably detect animals infected with zoonotic organisms should be considered in the risk assessment, as detection depends not only on the sensitivity and specificity of diagnostic tests, but also on the biologic behavior of infectious organisms, the host's immune response, and the prevalence of the organism. For instance, macaques infected with Herpes B (*Macacine herpesvirus 1*) can escape detection despite repeated testing, partially due to the virus' ability to persist latently in the trigeminal and lumbosacral ganglia.[24] Diagnostic tests that rely on detection of an immune system response, such as an indirect serologic ELISA, may be inadequate to identify infection in immunosuppressed animals. Additionally, diagnostic tests that utilize species-specific test reagents may not detect infectious contaminants in humanized animal models implanted with human-derived tissues as test reagents may not recognize elements of the stimulated immune response. Furthermore, the prevalence of infection within the respective population can directly impact the probability that a diagnostic test result accurately represents infection. For instance, in a population with low disease prevalence, as is common in most contemporary research rodent populations, the positive predictive value of a diagnostic test may be low.

Animal Sources

Animals may become infected with zoonotic organisms prior to their arrival at a research facility. When not bred at a research institution, many animals, including most laboratory mice and rats used in the United States, are purchased from commercial vendors that specialize in producing research animals. These vendors typically institute robust animal health monitoring and stringent infectious agent exclusion programs. As a result, undetected infection of these animals with zoonotic agents is highly unlikely. Animals obtained from other domestic or foreign sources present a greater risk of unidentified infection because screening of these animals for zoonotic diseases may be minimal or absent. This is especially true of animals maintained in agricultural settings, wild or feral animals, and unique or unusual species that are difficult to obtain. In addition, potential infection with zoonotic organisms may be assumed but not specifically communicated by a vendor. For instance, endemic infection of beef and dairy cattle with *Cryptococcus*

spp. is often presumed by vendors, but rarely specified in health-monitoring documentation provided to the research institutions purchasing these animals. Similarly, reptiles obtained from vendors that do not normally supply research institutions may be endemically infected with *Salmonella* spp., and client-owned dogs may be infected with the bacteria *Leptospira* or the parasite *Baylisascaris*.[25–27] Understanding the structure and limitations of a supplier's colony health-monitoring program is essential in estimating the risk that animals may introduce into a facility.

Research Environment

Research animals are also at risk of contracting zoonotic infections from the research environment. Although it is most common to maintain research animals exclusively indoors, some animals may be provided access to outdoor facilities, such as agricultural animals maintained on pastures, nonhuman primates housed in outdoor corrals, and dogs provided with outdoor play areas or exercise activities (eg, walks). Animals with access to outdoor enclosures may be exposed to zoonotic organisms when wildlife enter or otherwise contaminate an enclosure as well as through contact with infected disease vectors. Examples include pasture-reared sheep infected with skunk and raccoon variant rabies[28] and outdoor-housed marmosets and tamarins infected with lymphocytic choriomeningitis virus (LCMV), presumably from wild mice.[29,30] If infected, these animals may present a serious zoonotic health risk to personnel with direct or indirect exposure to them.

Zoonotic Organisms

Understanding the risks inherent to animal research populations helps in identifying the specific zoonotic organisms of concern within an institution's research program. Once these organisms have been identified, the risk assessment process should focus on evaluating their characteristics, including their transmission and exposure routes. Transmission of zoonotic organisms is uniquely dependent upon an organism's biologic behavior, which may vary between animal hosts. Animal species may differ in the pattern and quantity of organisms shed in their feces, urine, respiratory secretions, saliva, or blood. Personnel exposure may occur through direct contact with infected animals, their tissues, and their secretions as well as through indirect contact with contaminated environments or infectious vectors. Potential routes of exposure include inhalation, ingestion, contact with mucous membrane or breaks in the skin, or by percutaneous inoculation.

Percutaneous inoculation of zoonotic organisms may occur following accidental injury from sharps, animal scratches, or animal bites. Scratches and bites present distinctive risks. Both can cause punctures that heal quickly and entrap bacteria[31] to produce deep infections with secondary septic arthritis, osteomyelitis, tenosynovitis, or compartment syndrome.[32] Scratches can induce harm beyond the physical injury since claws and nails may have feces and urine embedded under them and may be contaminated with organisms from the animal's mouth. The mouths of common laboratory animals may contain a number of significant zoonotic organisms such as *Bartonella henselae*, *Capnocytophaga canimorsus*, methicillin-resistant *Staphylococcus aureus*, *Pasturella* species, *Salmonella*, Macacine herpes virus I, and Simian foamy viruses.[32–34] Table 2 lists examples of zoonotic organisms isolated from the oral cavity of common laboratory animal species that should be considered in the risk assessment process. Due to the diversity of organisms within the oral cavity, bite wounds are often polymicrobial, containing both aerobic and anaerobic microorganisms.[33]

Although it is valuable to recognize the zoonotic organisms most likely present in a research setting, it is also valuable to distinguish those that are highly unlikely to be present. Recognition of unlikely organisms allows institutions to apply limited resources where they are most needed to avoid unnecessary procedures and expense. For example, dogs derived from a closed research colony, maintained exclusively indoors and without known or suspected exposure to a source of rabies, are extremely unlikely to contract rabies infection. As a result, rabies vaccination and serum antibody testing of personnel who contact the dogs is likely unnecessary. However, when making decisions to reduce emphasis on specific zoonotic organisms, institutions must be cautious to still remain compliant with applicable local and regional regulations intended to protect public health and agriculture.

Biological Materials

Administration of biological materials, such as serum, cells, and tissues, can also serve as a mechanism for introducing zoonotic organisms to research animals. Mycoplasmas and viruses that produce minimal or undetectable cytopathy, such as LCMV[35] and hantaviruses,[36] are difficult to visually detect in contaminated biologics, and therefore these types of organisms are most likely to be inadvertently administered to animals. This is in contrast to most bacteria, fungi, and yeast as contamination with these organisms is usually detectable by visual observation of the material.[37]

Zoonotic contaminants of biological materials may originate from a variety of sources, including the animal or human from which the sample originated;[38] from media, reagents, additives, feeder cells, or extracellular matrices used to enhance cell growth;[39–41] or from experimental reagents. Also, researchers themselves may serve as a source of contamination if they do not follow appropriate aseptic technique.[37]

It is difficult to quantify the risk of exposure to zoonotic organisms from biological materials, but a risk does exist. For example, even with the use of PCR-based diagnostic testing, contamination of biological materials with LCMV remains a contemporary concern.[42] Patients from whom cells or tissues may be harvested for use in research experiments are not

Table 2 Zoonotic Organisms Isolated from Bite Wounds Inflicted by Select Laboratory Animal Species[17,32,33,66–69]

Species	Zoonotic Organisms
Dogs	*Pasteurella canis*, *Pasteurella multocida subsp multocida*, *Pasteurella multocida subsp septica*, *Streptococcus species*, *S. aureus*, *Staphylococcus pseudintermedius*, *Capnocytophaga canimorsus*
Nonhuman primates	*Haempohilus influenzae*, *Peptostreptococcus micros*, *Streptococci* species, *Capnocytophaga species*, Macacine Herpesvirus I, Simian Foamy Viruses, Cercopithecine Herpesvirus 8, Simian virus 40
Rodents	*Streptobacillus moniliformis*, *Spirillum minus*, *Salmonella spp*.

routinely screened for LCMV, and immunocompetent individuals infected with this virus remain asymptomatic or develop only a mild, self-limiting illness.[43,44] In fact, documented transmission of LCMV has occurred during organ transplantation when donor organs were harvested from infected patients. Given this, it is reasonable to believe that human cells and tissues implanted in animals could be a source of contaminated biological materials.

When planning to administer biological products to animals, it is important that the experimental animals themselves be considered during the risk assessment process, because their physiology may directly influence the risk of zoonotic organism growth. For example, the risk of an immunosuppressed or humanized animal contracting a zoonotic infection following the administration of contaminated biologic materials is greater than that of an immunocompetent or wild-type animal. In addition, the route of administration of biological materials may allow an infectious contaminant to circumvent an animal's defenses normally encountered following natural routes of exposure (eg, mucosal immunity for a pathogen transmitted by fecal-oral route).[45] The ability of an administered organism to replicate and be shed from the animal recipient would be heavily influenced by its host defenses.

Personnel

An individual's health status may influence the risk of infection with a zoonotic organism. Immunosuppression secondary to conditions such as pregnancy, disease, splenectomy, or medications can predispose personnel to disease. Personnel should be encouraged to report changes in their health to their personal physician and/or the institution's occupational health and safety professionals. In some cases, temporary or permanent reassignment of an immunosuppressed employee's work duties may be indicated to assure worker safety.

The attitudes of the personnel themselves can affect their risk of exposure to zoonotic organisms. Personnel must be willing to learn about the biology of the organisms, the practices used to mitigate exposure risk, and the rationale behind these practices. It is especially important that personnel understand how practices can minimize exposure risks. Without this understanding, personnel may be more likely to disregard or improperly implement practices. Finally, it may be advisable for temporarily ill personnel to avoid animal contact, both for their own protection and for the protection of the animals. Personnel who fail to closely observe safety practices and procedures may endanger themselves and other workers.

In addition to laboratory animals serving as a source of infection to personnel, personnel may serve as a source of infection to laboratory animals. Zoonotic organisms transmitted from humans to animals can cause significant disease and even fatalities in research animals (eg, measles infections in macaques[46,47]). They may also confound research studies. For example, transmission of circulating human strains of influenza to experimentally infected ferrets might result in viral reassortment, producing new viral strains with unique biologic behaviors,[48] and natural transmission of human adenovirus-36 to macaques can affect weight gain studies.[49,50] Furthermore, once these zoonotic organisms are established in laboratory animals, the animals may then serve as a source of infection to personnel.[51]

The risk of zoonotic disease transmission from humans to laboratory animals is difficult to gauge. Nonhuman primates are phylogenetically similar to humans, and for this reason, they may be more likely to become infected by organisms adapted to humans than are other species of laboratory animals. Unlike rodents that are often housed in microisolation cages or in individually ventilated caging systems with HEPA-filtered air supply, nonhuman primates tend to be housed in large open cages, making them vulnerable to exposure by respiratory droplets that may carry zoonotic organisms excreted by humans. Personnel use of appropriate PPE, such as surgical masks and gloves, can help to prevent exposure of animals to zoonotic agents.

Personnel performing clinical duties at human medical centers and hospitals may pose a noteworthy risk to animals due to their potential contact with high doses of infectious organisms shed from human patients. Medical professionals exposed to organisms such as *Clostridium difficile* or methicillin-resistant *S aureus* in the hospital setting may unwittingly carry zoonotic organisms into the vivarium. Hand washing when exiting patient areas, removing hospital attire prior to entering the vivarium, or changing into vivarium-dedicated clothing may help to prevent introduction of zoonotic agents by these means.

Facility Design and Building Management

A common strategy used to control spread of infectious organisms is the creation of functional adjacencies, the strategic and relative placement of functional areas within a facility. Functional adjacencies can entail separating rooms or areas that may contain a disease agent from those presumed free of disease or co-locating rooms to facilitate the containment of organisms within an area. For example, personnel breakrooms are normally isolated from animal housing areas, and anterooms containing PPE donning and doffing areas may be purposefully located adjacent to nonhuman primate housing rooms. Well-designed functional adjacencies can decrease the risk of organism dispersal during animal transportation by minimizing transportation distances and avoiding or limiting the movement of animals though areas where the risk of zoonotic transmission is increased. Maintaining effective functional adjacencies is especially important when established personnel or animal movement patterns are disrupted, such as during periods of facility construction or renovation and when facility animal populations exceed the maximum populations envisioned during facility design.

To minimize the spread of infectious organisms, animal research building heating, ventilation, and air conditioning systems should be designed to provide directional airflow and maintain relative air pressure differentials between adjacent rooms or areas.[52] Although relative room pressurization can be a useful tool, it is important to understand its limitations. For instance, unless continually monitored, fluctuations or reversals in relative room pressurizations may go unnoticed for extended periods. Also, the intensity of the pressure differential must be consistently large enough to maintain directional airflow across an opened doorway. Elevators can significantly impact building-wide airflow as elevator car movement within the elevator shaft simulates a piston, drawing and pushing air between floors. As a result, airborne contaminants can be drawn from one floor and forced to other, remote areas of a building.

Equipment, Personal Protective Equipment, and Standard Practices

Many of the strategies employed to prevent transmission of pathogens between animals, to protect staff from allergen

exposure, or to contain purposefully administered infectious agents are also effective in protecting staff from exposure to zoonotic organisms. Equipment designed to reduce exposure to allergens or experimental infectious agents, including individually ventilated cages, biological safety cabinets, or specially designed bedding disposal units, can significantly reduce the risk of exposure to zoonotic organisms, and their use should be encouraged.

The use of equipment that contacts both humans and animals is of special concern for zoonotic disease transmission. Equipment such as MRI units, CT-scanners, or diagnostic equipment used with both humans and animals can serve as fomites for infection. Practices must be employed to thoroughly disinfect these items between uses.

In addition to engineering controls, the use of select PPEs such as facility uniforms, lab coats, disposable gowns, jumpsuits, protective sleeves, gloves, bonnets, or respirators may offer additional protection against exposure to zoonotic organisms. Use of scratch- or puncture-resistant gloves and arm guards may be beneficial when working with animals prone to bite or scratch (eg, cotton rats, ferrets, nonhuman primates) or to protect workers from being scratched by potentially contaminated caging. Rubber boots that are easily disinfected or boot covers worn over facility shoes may be useful in containing zoonotic organisms to animal holding areas contaminated by feces and urine (eg, runs, pens, or stalls).

Observance of basic laboratory safety practices is also critical for reducing exposure to zoonotic organisms. Activities such as chewing gum; eating or drinking; storing food, cups, and eating utensils; applying cosmetics; or handling contact lenses should not be performed in laboratories or animal facilities.[7] In addition, hands should be washed immediately after working with animals or related equipment.

The importance of developing Standard Operating Procedures (SOPs) that document the correct use of equipment and PPE cannot be overstated. Failure to properly operate and decontaminate equipment or to properly don and doff PPE can result in exposure to zoonotic organisms. In addition, it is critical that staff be properly trained to follow SOPs precisely. Inattention to details described in the SOPs, such as failure to use appropriate eye protection or reliance on a tight-fitting respirator despite growth of facial hair, could expose workers to zoonotic organisms and have devastating consequences. Furthermore, management and staff should collaborate to assess established SOPs for their proper implementation and to ensure that they are achieving their desired outcomes. Periodic evaluation of risk management methods can help to identify ineffectual practices and devise methods for improvement.

Both animal care and research personnel should evaluate SOPs prior to implementation because each group can provide unique insights about the potential benefits and drawbacks from proposed safety equipment, PPE, and operational procedures. For example, research personnel should evaluate restraint devices designed to minimize the direct handling of animals to ensure that necessary experimental procedures can be safely and reliably performed without increased risk of accidental injury. Likewise, animal care personnel should assess PPE proposed for use when manipulating large animal caging to ensure that their vision is not dangerously obstructed by respiratory or face protection and that they are not at risk of injury from PPE entrapment in mechanical mechanisms. Evaluation by all groups involved in these processes will help to decrease procedural drift and ensure that personnel follow SOPs as directed.

Vermin

Intrusion of insects and other vermin into research facilities may introduce zoonotic organisms to resident animal populations. For example, multiple institutions have reported infestations of rodent colonies with *Ornythonyssus bacoti*, a mesostigmatid mite that has the potential to transmit a number of zoonotic organisms including murine typhus, scrub typhus, endemic typhus, plague, Q fever, rickettsial pox, tularemia, Coxsackie virus, eastern and western equine encephalitis viruses, and Langat virus.[53] Entry of vermin and control of their movement within facilities can be accomplished using integrated pest management programs. These programs encompass a combination of building design features (eg, sealed wall penetrations), equipment (eg, insect and animal traps placed near building entrances), and operational practices (eg, regular room sanitation). The design and implementation of integrated pest management programs should be evaluated when assessing the potential risk of insects and vermin introducing infectious organisms into animal research facilities.[54]

Occupational Health and Safety Programs

Institutional occupational health and safety programs serve a vital role in protecting employees from work-related injury and illness, including occupationally acquired diseases. They traditionally include 5 elements: hazard identification, risk assessment, personnel training and protection, formal procedures and policies for hazard use and monitoring, and medical programs.[55] For animal research programs, strong emphasis is placed on the prevention of zoonotic disease transmission to personnel as well as the reporting and tracking of injuries and illnesses. Emphasis is also placed on early detection of disease through the use of health surveillance as well as the use of effective and prompt treatment of illnesses should they occur.

Inclusion of individuals in an occupational health and safety program should be based on a risk assessment of potential exposure. All individuals with significant exposure to animals, their tissues, their environments, or their waste should be enrolled in the program. This may include not only animal care, veterinary, and research personnel but also students, volunteers, building maintenance personnel, janitorial staff, security personnel, and vendors. The level of services provided should be consistent across groups with similar levels of risk.

Review of Institutional Programs and Procedures

Knowledge of individual zoonotic organisms, including their prevalence in animal populations and their biologic behavior in human and animal hosts, continues to expand. Animal model systems are continually modified and refined over time, and these refinements may significantly impact zoonotic risks in an animal research environment. As a result, institutional programs and procedures should be periodically assessed to ensure they reflect current scientific knowledge, are responsive to the needs of personnel, and are correctly implemented. For instance, although the collection and storage of baseline and periodic serum samples was once common, the practice is now utilized with only a limited number of infectious organisms and experimental conditions for which the substantial cost and intensive efforts required for sample maintenance can be justified.[12,15] The effectiveness of safety training programs should also be periodically assessed to ensure that personnel have developed

necessary competencies (eg, animal handling methods to avoid animal bites and scratches, appropriate PPE donning and doffing procedures) and possess a clear understanding of zoonotic risks and management practices.[12] Failure to periodically review institutional programs and revise safety practices as needed may reduce their effectiveness and result in an increased risk of exposure to zoonotic organisms as well as the implementation or continuation of excessively burdensome practices.

Conclusions

Zoonotic diseases remain a notable risk to both research animals and personnel in contemporary animal research facilities despite their highly structured and controlled environments. Defining and estimating the level of risk is possible through the conduct of a risk assessment that evaluates features inherent to the research, animals, personnel, and the research environment. The risk assessment should also evaluate the zoonotic organisms that may be present, including their biologic behavior in susceptible animal subjects and personnel, and the potential means of transmissions between them. Experimental activities and physical features of the research environment that potentially influence zoonotic disease risk should also be identified. Once risks are identified and quantified, engineering methods, operating procedures, and equipment can then minimize risk.

However, assessing and managing zoonotic risks in an animal research program requires more than the conduct of a risk assessment and selection and implementation of a rigid risk management program. Rather, risk assessment and management should be dynamic processes that reflect current scientific knowledge of experimental systems, engage personnel, and include a periodic review of applicable institutional programs and procedures. The active collaboration of a diverse group of individuals is essential and should include biological safety officers, occupational health specialists, physicians, veterinarians, animal care personnel, and researchers. Building engineers and managers are also instrumental in devising and implementing components of the risk management program reliant on building system functions, such as heating, ventilation, and air conditioning systems. Without the involvement of all applicable groups, the estimation of risk or the level of protection provided by risk management measures may be considerably inaccurate. Furthermore, risk management procedures should be evaluated for their potential to introduce unintended and otherwise unrecognized risks to personnel or animals and should not conflict with efforts to minimize other risk factors present in an animal facility, such as exposure to environmental or animal allergens. When implemented, these practices can assure increased protection from zoonotic organisms for both personnel and laboratory animals and result in more consistent scientific results.

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Review Article

Managing Research Animal Specimens and Laboratory Safety

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Abstract

The procedures necessary to perform testing in a veterinary diagnostic laboratory have inherent associated risks to personnel in regard to exposure to infectious agents. In research institutions animals can be experimentally infected, acquire naturally occurring infections and can also be exposed to other hazards such as toxic chemicals or radiologic entities. A critical component of the use of animals in a research environment is the collaboration between the responsible researcher and the veterinary diagnostic laboratory with the institutional health and safety professionals to ensure that the proper engineering controls, personal protective equipment, laboratory procedures and training are in place for personnel working with the animals or their specimens. Unlike the typical researcher, the veterinary diagnostic laboratory generally has to be equipped to safely process and work with a wide range of potential hazards where the communication of pertinent information from the researcher to the diagnostic laboratory regarding the identity of the potential hazard is paramount. Diagnostic laboratory design, safety equipment, personal protective equipment, laboratory procedures, occupational health program and personnel training must be sufficient to address hazards based on a risk assessment performed in conjunction with safety professionals. This article will summarize safety considerations with the various areas of concern in the operation of a diagnostic laboratory for research animal specimens.

Key words: animal specimens; laboratory hazards; laboratory safety

Introduction

Laboratory workers face real risks of acquiring laboratory-associated infections based on various reports. Because reporting these infections is voluntary, acquiring accurate accounts of such infections can be difficult. According to Pike and Sulkin (1979), 4079 laboratory-associated infections (LAI) were reported between 1930 and 1978 with168 deaths.[1] Twenty-six years following this report, a global search by Harding and Byers[2] found that 1448 overt LAI with 36 deaths and 17 secondary infections were reported between the years.[2] Recent reports indicate a decline in the overall number of cases.[3]

Veterinary Laboratory personnel in research facilities handle and process specimens with potential hazards and are vulnerable to exposures and LAI. Safety procedures should address factors unique to each laboratory and program, and thus a risk assessment by the occupational safety office, or equivalent, is essential.

Institutional commitment to laboratory safety should be a high priority and is vital to creating a conducive environment for teaching, learning, and research. Institutions are also required to allocate appropriate resources for employee safety including a sound occupational safety program that promotes workplace safety through interdisciplinary programs and policies. Development of laboratory-specific standard operating procedures (SOP), training, and documentation must be part of the safety program.

The laboratory director and/or principal investigator is responsible for the safety of laboratory personnel including the provision of a safe work environment. However, each person working in the laboratory must assume responsibility for following all safety measures and compliance with set policies and SOPs. Employee training and provision of necessary information to protect personnel from hazards associated with the use of biological agents is crucial. Policies and guidelines, based on a thorough risk assessment, will serve as the basis for training in the use of proper engineering controls, laboratory procedures, and the use of personal protective equipment. A mechanism to provide information in regard to the biological agents that will be used as well as the route of transmission (eg, percutaneous injuries, inhalation/aerosol, mucous membrane, and/or ingestion) must be provided. Initial health screening and subsequent periodic health surveillance should be standard practice.

The purpose of this article is to render a comprehensive review and provide recommendations for laboratory safety in regard to the handling of research animal specimens, including laboratory design and safety equipment, as well as safety considerations for various hazards.

Laboratory Design and Safety Equipment [8, 5]

Pre-planning

A design team made up of individuals representing multiple disciplines (eg, engineering, architecture, biosafety, industrial hygiene) must be assembled well in advance of construction to ensure all safety and compliance issues are identified and properly addressed. Input from users of the new laboratory space during the pre-planning phase is critical, because they are intimately familiar with the planned use of the new facility. The following are examples of issues that must be considered during pre-planning:

- Research objectives
- Types of biological specimens to be manipulated
- Types and quantifies of chemicals to be used (eg, formalin, alcohols)
- Types of radiological materials used
- Technologies to be used
- Required diagnostic equipment
- Required laboratory equipment (eg, freezers, incubators)
- SOPs outlining workflow
- Relevant safety standards and guidelines
- Relevant security standards and guidelines
- Heating, ventilation, and air conditioning requirements

General Laboratory Design

Flexibility of laboratory set-up and configurations should be incorporated into the design to allow for evolving research needs over time.[4] One example is the use of modular workbenches and cabinetry that is locked into place but can be easily reconfigured in the future if needed.

In addition, and as noted in WHO guidelines,[5] the following features should be incorporated into all new research laboratories:

- Ample space for safe conduct of research activities and maintenance of laboratory equipment
- Laboratory finishes on walls, floors, and ceilings should be smooth and easy to clean. The surfaces should also be impermeable to liquids and disinfectants. Floors should be slip resistant
- Lab furniture should be sturdy and sanitizable
- Bench tops should be impervious to liquids and corrosion-resistant in regard to disinfectants
- Laboratory illumination should be adequate for all activities

Safety Equipment

A biological safety cabinet (BSC) should be installed for primary containment of infectious materials, especially if the manipulation of specimens is likely to create an aerosol. Examples of aerosol-generating procedures include centrifugation, grinding, blending, and sonication. In addition, simply opening a container of infectious material that may have an internal pressure that is different from the ambient pressure can result in the release of aerosol.[5] Safety centrifuges equipped with aerosol-tight features may be used on the open bench but should be loaded and unloaded in the BSC. The biosafety professional on the design team can determine the best type and configuration of BSC to install after performing a risk assessment. The BSC should be installed in low trafficked areas of the laboratory and away from the main entrance.

The biosafety professional may deem from the risk assessment that an autoclave should be installed in the laboratory to decontaminate biohazardous waste. The design plan should include a hood installed above the autoclave to capture steam and heat released from the unit. A pass-thru autoclave is sometimes chosen so that waste can be loaded on the laboratory side and treated waste removed from the "clean side," potentially a common corridor.

A chemical fume hood should be installed in the laboratory if significant quantities of hazardous volatile chemicals will be manipulated. The industrial hygienist on the design team can determine whether a fume hood is necessary based on the planned research activities. Like BSCs, fume hoods should be installed in areas of the laboratory where cross-drafts of air are not expected to occur. If hazardous chemicals are used in the laboratory, proper storage equipment might be deemed necessary. Flammable storage cabinets and acid cabinets can be used to ensure safe segregation of incompatible chemicals, and compliance with relevant standards. Cabinets should be placed in the low trafficked areas of the laboratory and away from the main doorway.

Handling and Processing of Specimens in a Veterinary Diagnostic Lab

All specimens to be submitted to a laboratory must be accompanied by submission forms listing potential hazards as well as other pertinent information in regard to clinical history, animal signalment, and principal investigator specific information. Handling and processing of the specimens should follow the appropriate SOP including the assignment of an accession

number and logging into the laboratory documentation system prior to starting any work. Laboratory hazard signs, emergency contact information, and safety requirements for entering the diagnostic laboratory should be maintained in a current state in regard to the particular hazards present. Laboratory chemical hygiene plans related to the particularly hazardous substances used in the laboratory including chemical waste disposal policies is important.

Personal protective equipment (PPE) serves as a primary barrier of protection. Risk assessment related to the functions and agents used in the laboratory is essential part of the process in recommending appropriate PPE. PPEs must be donned prior to handling specimens with potential hazard and removed and disposed of immediately after each use and before leaving the laboratory facility.

Examples of PPE commonly used in diagnostic laboratories:

- Laboratory coat: for protection of skin and street clothing
- Face shield: in cases of risk of splash exposure.
- Gloves: hand and skin exposure while handling hazardous material or specimens.
- Mask/PAPR/goggle: when handling agents that pose risk of aerosol exposure.

Engineering and Procedural Controls Related to Biological Agent Exposure

Biological agents that are either known to or could potentially pose risk to the health of humans and/or animals include, but are not limited to, bacteria, viruses, fungi, parasites, rickettsia, rDNA, toxins, human or animal blood and body fluid, and human or animal origin cell lines. Any laboratory samples suspected of containing these agents or use of these agents for any other purpose require oversight and approval of the safety office or a comparable responsible entity.

LAI can be contracted by various routes of exposure, and safety precautions must address each route depending on the respective hazard. The routes of laboratory exposure to infectious agents and considerations to prevent exposure are listed below.

Percutaneous

Exposure to biological agents due to percutaneous injury can result from needle sticks, cuts, or skin abrasions with potentially contaminated materials. Some precautions in regard to using sharps include the minimization of sharps used as much as possible, using sharps that include a safety feature, having a sharps container within arm's reach so it can be disposed of immediately after use, and avoiding recapping needles.

Ingestion

Mouth pipetting and accidental ingestion of contaminated food in the laboratory can lead to exposure with biological/infectious agents, and thus food and drink should be prohibited from the laboratory. Pipetting specimens can expose laboratory personnel to hazardous agents by way of ingestion, contact, or inhalation. Due to this risk, the use of mechanical pipetting devices to avoid mouth pipetting coupled with appropriate technique is highly recommended.

Inhalation

Some biological agents such as tuberculosis, vaccinia virus, and adenoviruses are known to be transmitted by the aerosol route. Laboratory procedures that use vortex, blenders, and sonicators can cause aerosolization. Proper work practice as well as the use of safety devices, such as handling of infections agents under the appropriate safety hood to prevent potential exposure, should be implemented in the laboratory.

Mucous Membrane

Exposure of mucous membranes such as eyes, nose, and mouth with infectious agents can lead to occupationally acquired infections. A face shield and other appropriate personal protective equipment to prevent splash exposure should always be used.

Laboratory Practices and Techniques

The safety considerations in regard to biological agents differ depending on the route of exposure and the severity of the LAI. There are 4 standard biosafety levels (BSLs) for the proper handling of biohazard materials, and they are described below.

Biosafety Level1 (BSL1)

Agents classified under this level are not known to cause diseases in immunocompetent adult humans and thus can be handled on the open benchtop without the use of safety equipment that is more than what is used for uninfected animals. Standard microbiological practice and availability of a sink are required.

Biosafety Level2 (BSL2)

Agents classified under level 2 are known to cause disease in humans, and potential exposure can occur due to percutaneous injury, mucous membrane exposure, ingestion, etc. In addition to BSL1 practices, the laboratory should have biohazard warning signs, established sharps precautions, and a laboratory manual defining appropriate waste decontamination procedures and medical surveillance policies. The use of primary barriers such as Class I and Class II biosafety cabinets or other physical containment devises are required for handling and manipulation of BSL2 agents to prevent aerosol and/or splash exposures. In addition, the use of laboratory coat, gloves, and face shields is highly recommended. Laboratory furniture should be non-fabric and can be sanitized easily. An autoclave to sterilize contaminated laboratory waste should be available. Alternatively, a third party vendor can be used for decontamination.

Biosafety Level3 (BSL3)

Agents under the BSL3 category can be indigenous or exotic with potential aerosol transmission, and the disease may have a serious or lethal consequence. In addition to all BSL2 practices, this category requires decontamination of all potentially contaminated materials including laboratory clothing before laundering. Baseline serum for all laboratory workers is also required, and access to the laboratory should be controlled. Primary barriers include Class I or II biosafety cabinets or other physical containment devices used for handling of agents. Laboratory coat, gloves, and respiratory protections as

Table 1. Example of Agent Classification According to Risk[7]

	Agents			
	Bacterial/Rickettsial	Viral	Fungal	Parasitic
BSL-1	*Bacillus subtilis* *Eschercia coli*-K12	Baculovirus Murine Leukemia Virus (ecotropic)		
BSL-2	Campylobacter: C. fetus subsp fetus, C. coli, C. jejuni subsp jejuni. and C. upsaliensis. *Chlamydia psittaci*, *trachomatis* *Clostridium botulinum*, *tetani* *Corynebacterium diphtheriae* *Legionella* spp *Neisseria gonorrhoeae Neisseria meningitidis Pseudomonas pseudomallei Salmonella* spp *Shigella* spp *Treponema pallidum* *Staphylococcus aureus* *Streptococcus* spp *Vibrio cholera* *Vibrio parahemolyticus Vibrio vulnificus* *Klebsiella* spp	Adenovirus Creutzfeld-Jacob agent Cytomegalovirus Epstein-Barr virus Hepatitis A, B, C, D, E Herpes simplex viruses HIV Respiratory syncytial virus Sindbis Virus Monkeypox virus SIV Spongiform encephalopathies Vaccinia virus VSV (lab adapted strains) HTLV types I and II	*Candida albicans* *Cryptococcus neoformans* *Microsporum* spp *Exophiala dermatitidis* (wangiella) *Fonsecaea pedrosoi* *Sporothrix schenkii* *Trichophyton* spp	*Entomeoeba histolytia* *Crytosporidium* spp *Giardia* spp *Naegleria fowleri Plasmodium* spp *Strongyloides* spp *Tania solium Toxoplasma* spp *Trypanosoma* spp
BSL-3	*Bacillus anthracis* *Francisella tularensis* *Mycobacterium tuberculosis* *Mycobacterium bovis* (non-BCG strain) *Rickettsia ricettsii* *Yersinia pestis* (resistant strain)	Rift Valley Fever (Zinga) VSV exotic strains (PIRY) Yellow Fever (wild type) West Nile Virus	*Coccidiodes immitis* *Histoplasma capsulatum* Strongyloid Tania soliu Toxoplasm Trypanoso	
BSL-4		Marburg Virus Ebola Virus Lassa Virus Crimean-Congo Hemorrhagic Virus		

appropriate should be used. Secondary barriers required for the BSL3 laboratory are the same as those required for the BSL2 considerations, except that the laboratory must be designed in such a way that there is physical separation from access corridors, self-closing doors, negative airflow into the laboratory, hands-free handwashing stations, and eye wash stations.

Biosafety Level4 (BSL4)

Agents under the BSL4 category are considered dangerous and exotic and pose high risk of serious or life-threatening diseases with risk of aerosol transmission or an unknown route of transmission. All BSL3 practices must be employed with the addition of the requirement to change street clothes before entering the laboratory followed by showering upon exit. All materials must be decontaminated and/or autoclaved before leaving the facility. Class III biosafety cabinets are required. Full body air-supplied positive pressure suits are required if Class I and II cabinets are used. A separate isolated zone or building with dedicated supply and exhaust system, vacuum, and decontamination system in place is required in addition to the BSL3 specifications (Table 1).

Cell Lines

Human patient-derived xenografts and animal cell lines must be screened for potential pathogens prior to injecting the cells into rodents to prevent devastating disease outbreaks and potential human exposures. PCR-based testing is available commercially with relatively faster turnaround time.

Common pathogens to consider are as follows:

- Lymphocytic Choriomeningitis Virus (LCMV)
- Human Immunodeficiency Virus (1 and 2)
- Human T-lymphotropic Virus 1 and 2 (HTLV 1 and 2)
- Hepatitis Virus (A, B, C)
- Human Herpes Virus 6 and 8 (HHV 6 and 8)
- Treponema pallidum (Syphilis)
- Hantaviruses
- Varicella Virus (VZV)

Engineering and Procedural Controls Related to Chemical Agent Exposure

A Globally Harmonized System of Classification and Labeling of Chemicals has been developed by the United Nations that places chemicals into categories according to the nature of the hazard they present. These include physical, health, and environmental hazards. Physical hazards include chemicals such as flammables, explosives, and oxidizers. Chemicals that are considered health and environmental hazards are categorized by their harmful effects and include acute toxicity, skin corrosion, skin irritation, eye effects, sensitization of the immune system,

germ cell mutagenicity, carcinogenicity, reproductive toxicity, target organ systemic toxicity (single exposure/repeated exposure), and aspiration toxicity. Exposure to some of the more common chemicals found in laboratories that work with research animal specimens are considered carcinogens (eg, formaldehyde), mutagens (eg, ethidium bromide), and teratogens (eg, xylene and toluene). In addition to chemicals used to process specimens, another potential source of exposure are the tissues or fluids from animals treated with potentially hazardous chemicals. For example, exposure to tamoxifen while working with tissues from animals treated with this chemical would be a potential concern for a pregnant worker due to its teratogenic effects. Laboratory precautions regarding chemical exposure should address the potential for ingestion, absorption, and inhalational exposure.[4]

Laboratories handling specimens or using hazardous chemicals are required to comply with 29 CFR 1910.1450, occupational exposure to hazardous chemicals in laboratories.[6] The standard requires that laboratories develop a written chemical hygiene plan to protect laboratory personnel from health risks associated with the use of hazardous chemicals in laboratories. Laboratory personnel should be trained in safe handling of chemicals as well as chemical hazard awareness. Chemicals should be labeled appropriately for identification, hazard information, and manufacturer's contact information, and material safety data sheets for each chemical must be readily available to laboratory personnel. Engineering controls such as the use of chemical fume hoods with acceptable lighting and plumbing are required. Chemical storage cabinets with ample space to segregate chemicals based on their chemical compatibility are another critical safety measure that should be available in the laboratory. Chemical disposal should follow the manufacturer's and laboratory safety office recommendation in a manner that is safe for laboratory personnel and the environment.

Work surfaces and countertops should be wiped down with an appropriate disinfectant before and after handling laboratory specimens. Disposable benchtop covers, if used, should be immediately disposed of after each use. Storing equipment in a safe and acceptable manner is required. The chemical hygiene plan should include procedures for managing spills as well as ready access to a medical facility in the event of accidental exposures.

Engineering and Procedural Controls Related to Radiologic Agents

Radiation exposure is another hazard in the laboratory that may be encountered when processing specimens. For example, radiation exposure could arise from the use of radionuclide or radioisotopes for labelling tissue for research or diagnostic purposes. Safety precautions must be employed as directed by the institutional radiation safety program for each radionuclide or radioisotope and can include the use of PPE, shielding, personnel dosimetry, proper waste disposal, and frequent monitoring of the laboratory facility for the presence radiation contamination.

Processing, Transport, and Disposal of Specimens

Laboratory-specific waste disposal should be designed to protect laboratory personnel as well as the environment. The development of a laboratory policy for the proper processing and potential decontamination, as appropriate, of specimens containing hazardous agents is essential to protect personnel such as laboratory, housekeeping, and facility maintenance staff who may be exposed to such material. All policies must be consistent with federal and state regulations regarding laboratory-generated waste disposal. Microbiological laboratory waste should be autoclaved, whereas animal carcass may be incinerated. Blood and body fluid can be autoclaved and/or treated with disinfectant prior to disposal. Contaminated sharps may be autoclaved prior to disposal. Chemical and/or radiologic wastes require disposal as directed by the institutional safety office.

Training Program of Personnel

A laboratory personnel training program is required for new employees, when new hazards are introduced into the laboratory, and annually or at a frequency set by the facility safety office. General safety training should include accessing information on emergency response and incidence reporting requirements. Laboratory SOP, locations of safety and emergency equipment, and methods of waste disposal including documentation of training are all important components of a safety training program.

Working With Institutional Biological, Chemical, and Radiation Departments

The laboratory director, in conjunction with guidance from the institutional safety office, should design a laboratory-specific safety plan. This requires documentation of potential laboratory safety hazards, risk assessment of individuals in the laboratory regarding any health concerns that would put them at a higher risk, and periodic safety inspections from a qualified, outside entity such as the institutional safety office. The laboratory personnel should receive training and information regarding the risk associated with each hazard and the safety measures to be taken in case of exposure. Introductory as well as periodic health review should be provided to laboratory personnel.

Conclusion

A sound safety program that meets national and international regulatory standards to protect veterinary diagnostic laboratory employees and the environment will reduce and possibly eliminate potential exposure to laboratory hazards. The concept of laboratory safety requires continuous evaluation of the program because exposure risks change with the introduction of new research protocols, chemicals, infectious agents, or other hazards. A program designed to ensure safety for laboratory employees and the public at large must address hazard identification, risk assessment, acceptable laboratory facilities and safety equipment, personnel training and monitoring, proper personal hygiene, appropriate PPE, record keeping, medical evaluation, and preventive measures such as vaccination for laboratory workers. Institutional commitment and support is imperative to implement an appropriate safety program.

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Review Article

Safety Considerations When Working with Humanized Animals

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Abstract

Research using laboratory animals has been revolutionized by the creation of humanized animal models, which are immunodeficient animals engrafted with human cells, tissues, or organs. These animal models provide the research community a unique and promising opportunity to mimic a wide variety of disease conditions in humans, from infectious disease to cancer. A vast majority of these models are humanized mice like those injected with human CD34+ hematopoietic stem cells and patient-derived xenografts. With this technology comes the need for the animal research enterprise to understand the inherent and potential risks, such as exposure to bloodborne pathogens, associated with the model development and research applications. Here, we review existing humanized animal models and provide recommendations for their safe use based on regulatory framework and literature. A risk assessment program—from handling the human material to its administration to animals and animal housing—is a necessary initial step in mitigating risks associated with the use of humanized animals in research. Ultimately, establishing institutional policies and guidelines to ensure personnel safety is a legal and ethical responsibility of the research institution as part of the occupational health and safety program and overall animal care and use program.

Key words: animal models; biosafety; bloodborne pathogens; humanized animals; occupational health and safety; regulations

Introduction

Humanized animal models have become increasingly important and useful scientific translational biomedical tools to understand and elucidate the pathophysiology and mechanisms of human disease, including cancer, infectious disease, hematology, immune-mediated pathology, regenerative medicine, and cellular functions of human disease. As animal models become more closely aligned with human disease, the risk to human personnel may also increase. As a result, additional safety factors including engineering, personal protective equipment (PPE) and standard guidance policies should be applied. This manuscript first describes the history of humanized animal model development, including commonly utilized models and their translational scientific applications. Relevant laws and regulations, guidance publications, and components of an occupational health and safety program (OHSP) will then be discussed. Lastly, a risk assessment program and recommendations to ensure

personnel safety when working with human materials and humanized animals will be presented.

Mouse Humanized Models

History

In general, humanized animal models can be described as immunodeficient animal models engrafted with human cells, tissues, or organs.[1–3] These models closely replicate human physiologic, cellular, and immune system functions. Most commonly, immunodeficient mice or those expressing human transgenes are engrafted with human progenitor cells, primary hematopoietic cells, tissues, or organs (eg, immune system, skin, reproductive, or digestive system tissues) that generate human functional tissues or systems (eg, immune system). The most common humanized models used in research include immunodeficient mice engrafted with a human immune system, which will be the primary focus of this article.

Historical discovery timelines and events surrounding the development and utilization of immunodeficient mice and their role in humanized research have been published.[1,2,4,5] The development of humanized animals first arose from the 1966 discovery of an immune deficient athymic nude mouse phenotype; the nude phenotype resulted from diminished keratin and brittle hair,[6] caused by a mutation in the transcription factor *Foxn1* gene, which affects multiple downstream targets.[7] Homozygous athymic nude mice lack a thymus and have severe defects in T cell maturation and function, including a reduced ability to mount a T-cell-dependent adaptive immune response. These characteristics make athymic nude mice a popular research tool for studying allografts, cancer biology, and xenografts, including patient-derived xenografts. However, athymic nude mice may develop T cell markers as they age and possess an intact and enhanced innate immune system including natural killer cells and macrophages.[8,9] These factors reduce the athymic nude mouse's ability to establish and engraft a fully functional humanized immune system model.[10] Approximately 20 years after the discovery and characterization of the athymic nude mouse, a severe combined immunodeficiency mutation (*scid*) was described in a C.B-17 mouse strain. The homozygous *scid* phenotype lacks both mature T and B lymphocytes with loss of cell-mediated and humoral immunity.[11,12] A nonsense mutation in the protein kinase, DNA activated, catalytic polypeptide *Prkdc*^{scid} gene was shown to disrupt the catalytic subunit of a DNA-dependent protein kinase responsible for V(D)J recombination of antigen receptors, leading to a severe combined immunodeficiency phenotype and enhanced sensitivity to ionizing radiation.[13–17] However, *scid* mice have demonstrated strain dependent antigen recombination within 3 to 9 months of age, secondary to a low frequency of V(D)J short section recombination described as "leaky" or "leakiness."[18,19] Similar to the athymic nude mouse, *scid* mice also possess an active innate immunity with natural killer cell, macrophage, and compliment activity.[20] Allografts and xenografts have been implanted in *scid* mice in the absence of severe cell-mediated rejection.[21–23] Conversely, due to antigen leakiness, innate immunity and sensitivity to radiation, *scid* mice humanized with peripheral blood leukocytes, bone marrow cells, or human tissues are associated with low engraftment rates.[24,25]

To overcome the inherent innate immunity in *scid* mice and to improve engraftment with human lymphoblastic hematopoietic cells, *scid* mice were backcrossed onto a non-obese-diabetic NOD mouse strain known for its immune system defects, including lack of complement C5 and reduced natural killer cell, dendritic, and myeloid function.[26] Due to improved engraftment rates, NOD/SCID mice have been used for decades to study human immune system function and related diseases, yet long-term studies with NOD/SCID mice are problematic due to incomplete human immune system development due to impaired T cell activation, a high incidence of thymic lymphoma, shorter lifespan of 6 to 9 months, and murine immune leakiness, although less than is observed with *scid* mice.[1,27,28] Xenogenic graft versus host disease (GVHD) secondary to activated human T cells against mouse tissues may also increase murine model morbidity and mortality.

The pivotal breakthrough for humanized mouse models was the targeted mutation of the murine interleukin-2 receptor subunit gamma chain, *Il2rγ*, (ie, Il2 receptor common gamma chain) encoding a signal transduction transmembrane receptor subunit shared by receptors IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.[29] Back-crossing the *Il2rγ*$^{-/-}$ mice with NOD-*scid* double homozygous mice established the NOD-*scid*/gamma null models, namely NOD/LtSz-*scid Il2rγ*$^{-/-}$ (NSG)[30] and NOD/Shi-*scid Il2rγ*$^{-/-}$ (NOG),[31] both devoid of mature T and B cells and natural killer cell activity, with very low antigenic leakiness with age and defective dendritic cells and macrophages.[2,29,30,32] The NSG mouse has established its utility as an efficacious humanized mouse model, in particular due to its longer average lifespan (approximately 2 years compared with 5–6 months with NOD-*scid* mice), most likely resulting from reduced incidence of IL-2-dependent thymic lymphoma.[30,31,33–35] Pearson et al. described the development of a NOD model crossed with targeted mutations in *Il2rγ*$^{-/-}$ and the recombination activating gene *Rag*$^{-/-}$ to create a NOD-*Rag*$^{-/-}$ *Il2rγ*$^{-/-}$ (NRG) model, which provided enhanced resistance to ionizing radiation versus the NSG mouse model.[36] In addition to the NSG and NOG models, the BRG model was developed by backcrossing *Il2rγ*$^{-/-}$ mice to BALB/c recombination activating gene *Rag2* deficient mice, BALB/c-*Rag2*$^{-/-}$ *Il2rγ*$^{-/-}$.[37] The NOG mouse model has a truncated cytoplasmic domain of the IL-2 receptor gamma chain that can bind cytokines, yet signaling is nullified compared with an absent gamma chain that cannot bind cytokines nor process signaling in NSG and BRG mice.[38] In general, *Il2rγ*$^{-/-}$ mice demonstrate minimal lymphatic tissue or node formation due to disruption in T cell maturation and signaling. Differences exist between the NOG, NSG, NRG, and BRG models in regards to engraftment using a humanized immune system. Ito et al. reported the following order for humanized tissue and cell engraftment with NSG = NOG > NRG > BRG > NOD-*scid*.[4,31,39] Furthermore, due to enhanced T cell support, engraftment rates may be higher in younger (up to 3-4 weeks) NSG mice compared with older NSG mice associated with limited lymphoid tissue or thymus development.[30,33,40] However, all of these immunodeficient models have been shown to be useful models for humanized immune system research.[35,41,42]

Model Development

There are 3 methodologies generally utilized for engrafting human immune systems into immunodeficient mice based upon current literature. With slight differences, the humanized-peripheral blood lymphocytes-SCID, humanized-scid-repopulating cell-SCID, and bone marrow, liver, thymus (BLT) humanized models allow for the engraftment of primary human immune system components including T cells, B cells, natural killer lymphocytes, dendritic cells, and macrophages, yet engraftment and development of erythroid cells, platelets, and granulocytes is usually less

efficient.[2] Immunodeficient mice used to create humanized models often receive sublethal doses of irradiation prior to injection of CD34+ cells to improve human immune system engraftment. The humanized-peripheral blood lymphocytes-SCID model is created via injection of human peripheral blood, spleen, or lymph node cells with rapid engraftment of CD34+ cells within 1 week, allowing for the study of mature T lymphocytes. The humanized-scid-repopulating cell-SCID model incorporates engraftment of intravenous or intraosseous injection of human CD34+ hematopoietic stem cells from multiple sources (eg, bone marrow, umbilical cord blood, or fetal liver), producing a functional immune system. The BLT model, which generates improved human adaptive and innate immune responses, utilizes fetal bone marrow, fetal liver, and fetal thymus. Fetal liver and thymus are implanted in the subrenal capsule, followed by sublethal radiation and intravenous injection of autologous fetal hematopoietic stem cells.[1,38,40,43] The BLT model allows for maturation of human T cells in human thymic tissue with subsequent improvements in T cell development, enhanced interactions between human T and B cells, and improved immunoglobin isotype switching.[2] The BLT model also demonstrates human mucosal immune system functionality, making it a useful tool for human immunodeficiency virus (HIV) research.

The development of humanized animal models is a complex process requiring immunocompromised animals, human cells or tissues, irradiation, surgical expertise, and potential lengthy intervals until engraftment.[41] Multiple safety processes and procedures are paramount when working with human tissue or disease agents, especially in models that can promote their dissemination or potentially expose personnel.

Limitations

The development of lymph node tissue architecture and germinal centers in common immunodeficient models used to create humanized immune model systems (eg, NSG, NOD, NGR, or BRG) is inconsistent due to lack of T cell development and B cell maturation secondary to a targeted mutation at the interleukin-2 receptor subunit gamma (cytokine receptor common gamma-chain) or *Il2rγ*$^{-/-}$.[40] Human and murine immune systems demonstrate multiple differences in major histocompatibility complexes (MHCs) (eg, murine MHC class I and II vs human leukocyte antigen, or HLA), which may limit recognition of antigenic peptides between host and species of engraftment. One approach to overcome these differences is to insert HLA transgenes autologous to HLA-matched human stem cells and/or create targeted null mutations in murine MHC molecules in immunodeficient models prior to humanization. Cytokine signaling normally involved with human T and B cell maturation, immune system functionality, and development of myeloid and erythroid lineages may be diminished and inconsistent unless human cytokines and growth factors (eg, IL-3, GM-CSF, or thrombopoietin) are genetically inserted or externally administered to humanized immunodeficient mouse models.[40] Due to mechanistic immune system and cell signaling differences between human and mouse species and their complexity, humanized mouse models may demonstrate deficiencies in primary T cell development and maturation, reduced numbers of memory T cells, limited immunoglobulin isotype class switching with IgM predominating, and reduced mucosal immunity organization.[2,44] Maturation and functionality is improved with the BLT humanized model, which utilizes fetal thymic and liver tissue to promote human-specific T cell education and maturation processes, yet additional approaches to reduce murine innate immunity, improve human innate immunity, and promote human adaptive immunity are needed and warranted.[40] These methodologies have been previously reviewed.[2,3,32,38,40,45,46]

In addition to deficiencies in complete immune system functionality and maturation, humanized immunodeficient mouse models are prone to wasting disease or development of xenogenic GVHD, most likely due to reactivity to murine MHC class I and II molecules and residual murine innate immunity. The rate to develop xenogenic GVHD varies between the NSG and BRG immunodeficient models used for creating a humanized murine model, with NSG mice experiencing xenogenic GVHD at a faster rate than BRG mice.[47] One solution is the use of targeted mutations in murine MHC I and II genes in immunodeficient mouse models that may reduce the incidence or development of xenogenic GVHD.[40]

Non-Mouse Immunodeficient Models

In addition to humanized mouse models, immunodeficient rabbits and swine have recently been produced through genomic editing or natural breeding as research models for allografts, xenotransplantation, and regenerative medicine. The need for nonmurine or larger immunodeficient animal models that may support human cellular or tissue engraftment is warranted due to research that may require device implant, tissue-engineered constructs, transplantation, or collection of larger tissue, blood, or target sample volumes.

Utilizing CRISPR/Cas9 technology for single-gene and multigene editing, Yan et al.[48] and Song et al.[49] produced immunodeficient rabbits with deletions in *FOXN1*, *RAG2*, *IL2RG*, and *PKRDC* genes, including multigene combinations. Additional research with these models is needed to demonstrate if immunodeficient rabbits can be successfully engrafted with human hematopoietic stem cells or fetal tissues. If necessary, additional genetic editing to enhance engraftment, improve immune system functionality, and reduce rejection rates of human immune systems in rabbits and in other species should be feasible and efficient with the use of CRISPR/Cas9 technology.

Pigs have genetic, anatomical, and physiologic similarities to humans. Recently, severe combined immune-deficient pigs were reported in a line of Yorkshire pigs secondary to a natural mutation in the Artemis gene necessary for DNA repair during somatic mutation. *SCID* piglets were severely deficient in T and B cells, yet possessed granulocytes, monocytes, and natural killer cells compared with non-*SCID* littermates.[50] Meanwhile, researchers have targeted gene mutations in *RAG1*, *RAG2*, and *IL2RG* in pigs to advance xenotransplanation and regenerative and transplantation medicine.[51–54]

Applications of Humanized Animal Models

Reviews of humanized animal models, especially mouse models and their role in biomedical research, have been published.[2,38,55,56] In summary, humanized animal models have been utilized in cancer biology to research the growth of tumor xenografts and patient derived xenografts (PDX), to elucidate tumor heterogeneity and surrounding stroma with PDX, to study tumor-immune system interactions, and to develop targeted cancer immunotherapy.[57–60] Humanized mouse models have been utilized to study viral diseases such as Epstein Barr virus (EBV),[55] HIV,[55,61–63] dengue,[55] herpes simplex virus,[55] and hepatitis B (HBV),[64] and bacterial diseases[55] like *Mycobacterium tuberculosis* and *Salmonella enterica* Typhi, parasite infection[56] like

malaria, and sepsis.[56] Humanized mouse models have also been utilized in transplantation research regarding human allografts and rejection[65] and auto-immune research including Type 1 diabetes, systemic lupus erythematosus, arthritis, chronic inflammation, and allergic reactions including anaphylaxis.[38]

PDXs are freshly implanted human tissues or cellular suspensions (most commonly human neoplasms or fragments of a primary tumor) that have not originated from prior in vitro culture and are implanted into an immunodeficient animal. Immunodeficient mice have traditionally been utilized to study effects of tumor biology and metastasis via heterotopic or orthotopic implantation of human immortalized in vitro cancer cell lines. However, immortalized in vitro tumor cell lines do not directly recapitulate a patient's distinct histological and molecular tumor-derived architecture, including the tumor's stromal and microenvironment, as genomic and phenotypic characteristics may become increasingly homogeneous with repeated passaging.[66] The development of humanized mouse models provides researchers with an in vivo model to evaluate the interactions and functions of the human immune system (activation or suppression) directly on a patient's tumor and stromal microenvironment. These models can be utilized to predict a personalized and targeted response to traditional chemotherapy or novel immune-modulatory therapy. Lai et al.[58] describe the methodology of patient-derived xenograft models and their role in preclinical cancer biology and cancer research.

Animal Care and Humanized Animal Models

Due to the complexity involved with the creation of humanized animal models, veterinary, husbandry, and Institutional Animal Care and Use Committee (IACUC), oversight is of utmost importance to achieve scientific integrity and optimize animal welfare. Humanized animal models, especially those utilizing mice, generally require preconditioning with ionizing radiation followed by engraftment of the host with human hematopoietic stem cells or tissues. As a result, pain, distress, and morbidity should be minimized as adverse effects secondary to ionizing radiation, xenogenic GVHD, or opportunistic infection may develop. Duran-Struuck and Dysko provide a thorough overview of bone marrow transplantation protocols in mice outlining methods to optimize veterinary care, husbandry, and research practices, including suggested IACUC guidance when reviewing bone marrow transplantation protocols.[67] These concepts may also be applied to humanized mouse models. In summary, animal housing and husbandry care for these animals (humanized mouse models) should be based upon maintaining a strict specific pathogen-free barrier facility utilizing HEPA filtered ventilation (ventilated cages or flexible film isolator) with restricted access and appropriate PPE requirements. Housing rooms should be maintained at positive pressure to the corridor unless the animals are inoculated or implanted with hazardous biological materials. Animals should be provided autoclaved or irradiated food with acidified (pH 2.5–3), reverse osmosis, or autoclaved water. Fluid and hydration maintenance requirements (especially postirradiation), dietary hydration gel supplements, and/or subcutaneous isotonic fluids may be administered in addition to the primary water source.[67]

From an animal welfare perspective, the IACUC, in partnership with the veterinary team, should review each protocol involving humanized models to ensure pain and distress are minimized and humane endpoints are established. For surgical procedures or procedures that generate more than momentary or slight pain, analgesics should be administered (unless withheld as indicated by scientific justification), and environmental enrichment should be provided. Toth and Wallace described and outlined guidelines for defining experimental endpoints for animal research, including humane endpoints for cancer research, which can be applied to humanized mouse models.[68,69] The goal of humane endpoints should be to promote the scientific validity of data while minimizing harm to the animal. When predicting a moribund or humane endpoint, considerations should include monitoring for hypothermia, ability to ambulate, days of consecutive weight loss, and research-specific biomarkers as predictors of imminent death.[68] Similar to bone marrow transplantation, wasting disease, and GVHD as manifested by weight loss, skin alterations and hepatic dysfunction may develop in humanized mouse models. Thus, weight loss of 15% to 20% from baseline without a rebound in weight or a decreased body condition score should be monitored and considered as parameters for humane endpoints.[67,70] Monitoring frequency for signs of humane endpoints should correlate with the expected severity of the experimental procedure and clinical condition of the animal.

Occupational Health and Safety Regulatory Framework

Animal care and use program leadership should carefully consider proposed in vivo activities, including the ethical, philosophical, and legal aspects associated with research incorporating human-derived substances (HDS). Similar to the scientific breakthroughs that are occurring rapidly in stem cell-based research, the guidelines and regulations surrounding this work are also quickly changing. While the institution needs to meet federal, state, and local rules and regulations generally applicable to animal research, certain guidelines, especially in the production and maintenance of humanized animals as they relate to occupational health and safety, must be considered and implemented accordingly. A discussion of the institutional risk assessment process and factors to consider when implementing policies that exceed regulatory standards is included in a following section.

The 2 general laws that govern animal research in the United States are the *Animal Welfare Act* (AWA, 9 CFR)[71] and the *Health Research Extension Act of 1985* (Public Law 99-158).[72] Although neither specifically address personnel safety when working with animals, it is important to abide by these laws as they apply to the institutional animal care and use program. AWA is the only federal law in the United States that requires that minimum standards of care and treatment be provided for certain animals bred for commercial sale, used in research, transported commercially, or exhibited to the public. For AWA, the term *animal* excludes rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research.[71] Since most humanized animals are mice specifically bred for research, they do not fall under the AWA jurisdiction. Meanwhile, the *Health Research Extension Act of 1985* provides the legislative mandate for the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (PHS Policy), which is implemented by the Office of Laboratory Animal Welfare.[73] In contrast with the AWA, the PHS policy applies to any live, vertebrate animal, including mice and rats, used or intended for use in research, research training, experimentation, biological testing, or for related purposes.[73] The PHS requires that institutions that receive federal funds for animal research provide an occupational health program for employees with substantial animal contact.[74]

There are 2 regulatory documents pertinent to OHSPs in laboratory animal research. One document is the *Guide for the Care and Use of Laboratory Animals*[75] by the National Research Council. The *Guide* is an internationally accepted primary reference on animal care and use, and its implementation is required in the United States by the PHS policy. AAALAC International, a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs, uses the *Guide* as one of the 3 primary standards for evaluating animal care and use programs.[76] The *Guide* indicates that each institution must establish and maintain an OHSP as an essential part of the overall animal care and use program, encouraging institutions to tailor needs to its specific program.[75] OHSP deficiencies have consistently ranked in the top 3 AAALAC mandatory findings for correction.[77] The second document that contributes to the OHSP regulatory framework related to the animal care and use program is the *Occupational Health and Safety in the Care and Use of Research Animals*, published in 1997 by the Committee on Occupational Safety and Health in Research Animal Facilities, Institute of Laboratory Animal Resources.[74] This remains the authoritative guidance on the occupational health and safety of personnel in the animal care and use program.

The *Guide* indicates that the OHSP must be consistent with federal, state, and local regulations.[75] The federal law *Occupational Safety and Health (OSH) Act of 1970* (29 CFR Chapter 15) was promulgated to protect employees from hazards in the workplace (CFR 1970).[78] It applies to most private sector employers and their workers and some public sector employers and their workers in the United States. Compliance with regulations and standards under this law can be enforced either directly through the OSH Administration (OSHA) or through an OSHA-approved state plan.[78] State laws on OSH and those pertaining to the use of human cells and tissues must also be followed. For example, human stem cell research in California must abide by state guidelines from the California Department of Public Health Human Stem Cell Research Program. Projects funded through the California Institute for Regenerative Medicine must adhere to their regulations. For more information on allowable human stem cell research within individual states, please refer to the National Conference of State Legislatures summary of State Stem Cell Research regulations (http://www.ncsl.org/research/health/embryonic-and-fetal-research-laws.aspx).

There are 3 regulatory documents that have direct impact on the use of humanized animals in research: the *OSHA Bloodborne Pathogen Standard* (BPS) 29 CFR § 1910.1030,[79] the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL, 5th ed.),[80] and the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*).[81] The BPS is intended to protect workers in diverse settings from occupational exposure to blood and other potentially infectious materials (OPIM). BPS defines *blood* to include "human blood, human blood components, and products made from human blood",[79] including plasma derivatives,[82] while *OPIM* includes human body fluids like semen, vaginal secretions, cerebrospinal fluid, saliva in dental procedures, any bodily fluid that is visibly contaminated with blood, all body fluids in situations where it is difficult or impossible to differentiate between body fluids, and any unfixed tissue or organ (other than intact skin) from a human (living or dead).[79] *Bloodborne pathogens* means pathogenic microorganisms that are present in human blood and can cause disease in humans and include, but are not limited to, HBV, hepatitis C virus, and HIV.[79] The BPS requires employers to provide and ensure employees use appropriate PPE such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks, and eye protection when handling human blood or OPIMs.[79]

OSHA exempts certain human cell lines from the BPS, like those that are procured from commercial vendors or other sources with documented testing to be free of human bloodborne pathogens and which have been protected by the employer from environmental contamination.[83] Screening of the cell lines or "strains" will be for viruses characterized as bloodborne pathogens by the BPS, if the cells are capable of propagating such viruses. A human cell line is defined as in vitro or animal passaged (eg, nude mouse) cultures or human cells that fulfill traditional requirements of a cell line designation, while human cell strains are defined as cells propagated in vitro from primary explants of human tissue or body fluids that have finite lifetime (nontransformed) in tissue culture for 20 to 70 passages.[83]

The BMBL, issued by the Department of Health and Human Services, is considered to be the minimum standard of practice for all US laboratories that handle infectious microorganisms and hazardous biological materials. It provides information on good work practices, proper PPE, safety equipment, and laboratory facility design for each biosafety level (BSL). BMBL's Section V (Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities)[80] presents a summary table with recommended practices, PPE, and primary and secondary barrier characteristics for containment housing of animals administered biohazards. Appendix H describes potential laboratory hazards and recommended practices when working with human, nonhuman primate, and other mammalian cells and tissues.[80] It indicates that all laboratory staff working with human cells and tissues be enrolled in an occupational medicine program specific for bloodborne pathogens, and all staff should work under the policies and guidelines established by the institution's Exposure Control Plan.[80] Besides HIV and the hepatitis viruses specifically mentioned as bloodborne pathogens in the BPS, the BMBL lists the following pathogens to be potentially harbored in human cells and tissues: human T-lymphotropic virus, EBV, human papilloma virus (HPV), human cytomegalovirus, and *Mycobacterium tuberculosis* (lung tissue).[80]

The *NIH Guidelines* describes the practices for constructing and handling recombinant and synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and cells, organisms, and viruses containing such molecules.[81] Thus, the generation and use of transgenic animals in research is subject to the *NIH Guidelines* at any entity in receipt of NIH funds for research involving recombinant or synthetic nucleic acid molecules. Transgenic animal creation falls under various sections of the *NIH Guidelines* depending on the animal and the BSL required, but in most cases requires registration with, and approval from, the institutional biosafety committee (IBC). While the IBC's primary duty is to review research using recombinant and synthetic nucleic acid molecules, most institutions expand the IBC's authority to cover infectious disease research. Thus, the IBC can determine containment housing for such animals. The IBC should be contacted for additional information about the institutional approval process.

Occupational Health and Safety Program

General Components

As mentioned above, OHSP is a requirement for any animal care and use program and for the use of human biologics. The

Guide describes a 3-fold management approach for a robust OHSP that is a shared responsibility of several groups in the institution.[75] First, engineering controls entail appropriate safety equipment provision and facility design and operation. Second, administrative controls need to be implemented to clearly describe processes and standard operating procedures. Finally, when exposure to hazards cannot be engineered completely out of normal operations and when safe work practices and other forms of administrative controls cannot provide sufficient additional protection, the use of personal PPE provides a supplementary means of control and serves as the last line of defense for risk exposure. The reader is directed to the *Guide*[75] and chapter 6 of the *Occupational Health and Safety in the Care and Use of Research Animals*[74] for more detailed information on the principal components of an OHSP. The review article by Dyson et al.[84] also provides additional information on the institutional oversight of occupational health and safety for research programs involving biohazards. Programmatic components like exposure control, disaster plans, health surveillance, training and education, and information management are discussed in the Dyson paper. Lastly, the review article of Villano et al.[85] on PPE gives readers a knowledge base for evaluating the adequacy and effectiveness of institutional PPE requirements by providing a comprehensive review of risk assessment, common PPE used in laboratory animal research, and PPE standards and regulations.

Training and education is an integral component of OHSP. One training specifically needed for the use of HDS should be focused on bloodborne pathogens. This should be given for all personnel who may reasonably anticipate contact with human blood, blood products, tissues, fluids, or OPIM including human cell lines. The BPS requires employers to ensure that workers receive regular training that covers all elements of the standard, including but not limited to information on bloodborne pathogens and diseases, methods used to control occupational exposure like safe handling of sharps and wastes, HBV vaccinations, and medical evaluation, including postexposure follow-up procedures like injury reporting.[79] Such training must be provided on initial assignment, at least annually thereafter, and when new or modified tasks or procedures affect a worker's risk of occupational exposure.[79] Other topics for training that are strongly recommended include donning and doffing procedures of PPE, spill management, and working safely with relevant animal species. Training on these topics can particularly be effective in a practical setting and in conjunction with or as prerequisites for a facility orientation.

It is important to emphasize that OHSP is a shared responsibility of several groups in the institution, including the IACUC, the IBC, veterinary and husbandry staff, researchers, the environmental health and safety (EHS) unit, and OHS healthcare workers such as physicians and nurses (Figure 1). A close collaboration among these groups is necessary for creation and implementation of policies, guidelines, and standard operating procedures that cover risk assessment, provision of engineering controls and PPE, practices, medical treatment and intervention, and education and training.

This shared responsibility can present contemporary challenges such as the formation of departmental silos or independent strategies that can prevent these entities from working efficiently. Successful programs should employ multiple measures by which the IBC, IACUC, AV, and EHS and OHSP personnel can foster effective communication, especially with researchers. An animal containment expert, typically the AV or his/her designee, must be a member of the IBC, per the *NIH Guidelines*.[81] This

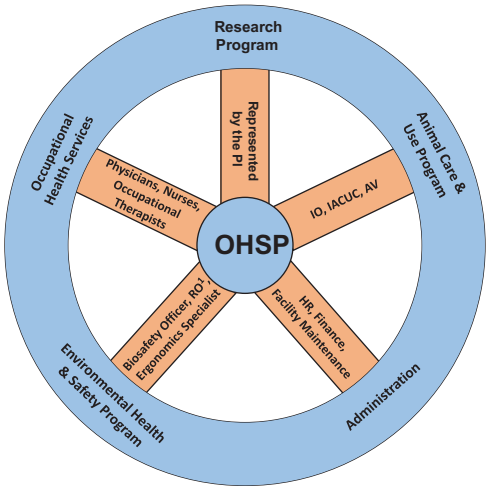

Figure 1 According to the *Guide for the Care and Use of Laboratory Animals* (NCR 2011), coordination among 5 groups in the institution is needed for an effective OHSP. [1]RO = responsible official. This is the individual at the entity who is accountable for entity compliance with the select agent regulations. Adopted from Villano and Ogden[86] with permission.

allows for the animal care program and the IBC to be abreast of current and new challenges that face each group together and independently. Some IACUCs also incorporate an EHS personnel as a committee member or a nonvoting consultant so that she or he can provide valuable occupational health insight to committee members in real time. Finally, IACUCs can integrate the abovementioned groups by having representatives of the IBC, animal care and use program, and EHS/OHSP provide IACUC member training exercises. Management of research applications, that is, IACUC protocol and corresponding IBC application, by the IACUC and IBC, respectively, with input and involvement of EHS personnel may also be discussed and improved. It is important to ensure congruency in the description of animal experiments as described in an IBC application and an IACUC protocol. Although one does not need to be contingent upon the other, having the IBC application approved prior to IACUC protocol approval ensures that risk assessment has been performed and risk mitigation is in place prior to commencement of animal experiments.

Risk Assessment, Guidelines, and Recommendations

Risk assessment is the first step to align the institutional OHSP to personnel safety. This evaluates the workplace to identify hazards and the risks associated with those hazards and determines the appropriate measures that should be in place to effectively eliminate or control the hazard.[85] It carefully evaluates the facility and its equipment and bridges the gap between engineering and administrative controls.[85] Additionally, personnel should be medically evaluated based on several factors, including special conditions like pregnancy and immune status. The nature of activities, especially the potential for aerosolization, is a significant consideration for risk assessment. For example, procedures that entail direct handling of primary human cells or tissues, such as their implantation into animals, can pose higher risk than routine husbandry procedures of animals administered such substances. The final outcome of risk assessment is the provision of engineering standards and PPE and establishment of safety practices based on the appropriate containment level as dictated by hazard identification. This

outcome is to be made collectively by a team that includes safety professionals, occupational health professionals, and veterinary and husbandry personnel and should include input from research personnel.[85] A strong hazard analysis program is dependent on not just identifying and mitigating the risks, but communicating and training the staff of the hazards identified and the controls implemented.[85]

Institutional risk assessment processes pertaining to humanized animals should include the regulatory guidelines produced by the *Guide*, OSHA BPS, BMBL, and the *NIH Guidelines*. While the minimum regulatory requirements must be met, some institutions implement policies that extend beyond these requirements. An example of this relates to the exemption of some human cell lines from the BPS. A concern with perpetual exemption of these cell lines occurs when cells are obtained from a source other than the original vendor or proprietor. Over time, cell lines can become contaminated either by infectious agents or with other cell lines. Institutions that follow the OSHA exemptions for established cell lines should consider best practices to verify or test incoming cell lines or perform periodic validation on current cell lines. In contrast, some institutions include the use of all primary and established cell lines within the scope of their BPS oversight, and some institutions also include all nonhuman primate cells, blood, blood products, tissues, etc., as they pose both a zoonotic risk relating to Macacine herpersvirus 1 (herpes B virus) but also are capable of supporting replication of some common human bloodborne pathogens. The risk assessment for this may incorporate the idea that the list of commonly tested bloodborne pathogens is not comprehensive, there are not reliable tests for all known agents, and not all potential agents have been identified.

The institutional inclusion of all HDS within BPS oversight leads to a second example of regulatory extension: the determination of BSL for use of human cells and tissues. Some institutions require that all human cells, tissues, blood, and blood products not known or suspected to contain biohazardous agents be handled under the approach termed *universal precautions*. Universal precautions employs infection control practices by handling these materials as if they were potential positive for bloodborne pathogens. Alternatively, some institutions require that all human materials be handled at BSL2, with or without the possibility of downgrading to BSL1 upon official review of necessary documentation. There is a lot of overlap between these 2 types of policies (eg, BBP training, PPE usage, aerosol precautions), but incorporation and oversight of users pose different challenges. A risk-based assessment like universal precautions and the elevation of some cells, such as liver carcinoma cells that have a high prevalence of hepatitis, to a higher BSL is a flexible and customizable approach but requires more effort to ensure compliance across the board if there is no formal IBC or institutional oversight. A blanket approach, such as categorical BSL2 assignment, allows for improved oversight by an IBC or other group but requires more effort for full review and may result in push-back from researchers requiring justification.

The risk associated with humanizing animals and the use of such animals should be carefully evaluated by the institution. The BMBL specifically states that each institution should conduct this assessment based on the origin of the cells or tissues (species and tissue type) as well as the source (recently isolated or well characterized).[80] Understanding the human materials these animals have and how these affect the animal's physiology will help determine the risks involved. However, experimental manipulations, especially inoculating these humanized animals with infectious agents, may increase the risk and will thus likely be the final determinant of containment level for animal handling and housing. Therefore, the institution needs to perform risk assessment on 2 stages when working with HDS:

Handling (in vitro)

HDS can either be primary (direct patient-derived) or secondary (commercial vendors). All laboratory work with primary human tissues or body fluids is covered by the BPS,[83] and these may be considered high risk especially if obtained from patients infected with bloodborne pathogens (eg, liver tumor samples from patients with HBV). As such, engineering and work practice controls should be used to eliminate or minimize employee exposure, and where occupational exposure remains after institution of these controls, PPE shall also be used.[79] As mentioned above, however, HDS that are characterized by documented, reasonable laboratory testing to be free of bloodborne pathogens may be exempted from the BPS. This documentation is also necessary for human cervical carcinoma cells or other transformed human cell lines like HeLa cells as they are sometimes adulterated with laboratory pathogens accidentally introduced by cultivation with other cell cultures or physically contaminated by other cell cultures handled in the same laboratory.[83] Recent reports from 2 diagnostic laboratories indicate that EBV[86,87] and HPV16[87] were the most common among a wide variety of pathogens in human samples submitted. The documentation that such cell lines are not OPIM should be a matter of written record and on file with the employer for OSHA review,[83] though institutional risk assessment and best practice may still result in some of these cell lines remaining included in the BPS. Regardless of inclusion under the BPS umbrella, best practice should dictate that all HDS be handled with appropriate precautions due to the unknown potential for bloodborne pathogens not tested for.

It is helpful to review available information for any HDS obtained from commercial vendors, especially the appropriate BSL that may differ from material to material. For example, BSL1 is typically sufficient for handling cell lines derived from normal tissue of healthy patients, while cells that are transformed by natural or laboratory infection with an immortalizating agent such as EBV would require BSL2 containment. The American Type Culture Collection (ATCC), the premier global biological materials resource and standards organization, has all human cell lines accessioned in its general collection tested for HIV, HBV, hepatitis C virus (until August 2012), HPV, EBV, and human cytomegalovirus.[88] The decision to remove hepatitis C from the ATCC virus panel test was based on the reason that there are no known culture cell lines that support the replication of this virus.[88] The institution can choose to use a virus screen panel similar to what ATCC uses to permit BSL-1 conditions. It is of note that the BMBL indicates that human and other primate cells should be handled using BSL-2 practices and containment, and that all work should be performed in a biosafety cabinet and all material decontaminated by autoclaving or disinfection before discarding.[80] Also of note, the correct BSL for human pathogens is determined by the CDC and/or NIH if human pathogens or rDNA is involved, and the commercial designations are not always in agreement with the CDC or with each other, particularly if commercial vendors are located in different countries. For example, while the ATCC lists Raji cells as BSL-2[89] due to the presence of EBV (a risk group [RG] 2 agent per CDC standard), the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures lists Raji cells as BSL-1.[90] The final judgement for making the determination

that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a biosafety professional or other qualified scientist with the background and experience to review such potential contamination and risk, in accordance with the requirements of the BPS.[83]

Administration to animals and animal housing (in vivo)

The containment level for handling an HDS also determines practices for its administration to animals but does not necessarily dictate animal housing requirements. There are several risk assessment factors to consider in determining the appropriate containment level for administration to animals and animal housing. These include the animal's pathogen status, species-specific behavior, route of administration, personnel training, and the use of other hazards, all of which will determine necessity for additional practices to ensure personnel safety. For example, a subcutaneous injection of an HDS to a manually restrained, specific-pathogen free mouse may be performed by staff proficient in the procedure, but administration of a human pathogen to a humanized mouse may necessitate the use of restraint devices or anesthesia. It is important to secure the animal during the HDS administration, as any animal movement may cause a spill and aerosolization or equipment movement and a sharps injury, possibly exposing personnel. Certain surgical procedures that involve implantation of human cells or tissues into an animal's living system and that require extensive manipulation like those involving sharps or the bones (orthopedic surgery) may pose a high risk for aerosolization or punctures/lacerations. PPE requirements and the use of engineering standards like biosafety cabinets and safety sharps should be evaluated based on personnel exposure risk.

Animals administered unmodified and established human cell lines, especially those documented to be free of bloodborne pathogens, may be housed under animal biosafety level (ABSL)-1.[91] As stated above, the institution, through the IBC, may define a human pathogen screening panel like the ATCC's. Meanwhile, ABSL-2 housing is appropriate for humanized animals like the hu-CD34 mice, for animals that carry primary human tissues or body fluids,[36,91] or those administered human materials known to be infected with human pathogens, and if the planned host animal is transgenic for receptors or other genetic loci that could enable infection, replication, or shedding of human pathogens inoculated onto the animal host. Some institutions include in BSL-2/ABSL-2 the category of human material suspected to be infected with human pathogens, with examples including hepatocellular or cervical carcinoma cells based on the high prevalence of hepatitis virus or HPV in these samples.

The use of human cells transduced with viral vectors can also impact containment level for administration and animal housing requirements, and this needs to be reviewed by the IBC with the help of an animal expert[81] and using the *NIH Guidelines*. IBC considerations should, at a minimum, include review of the tropism of the virus for human or animal cells (eg, adenoviral vector administered to an animal with human cells), pseudotyping of the viral vector to expand cell tropism or host range (eg, use of the VSV-g envelope on a nonhuman viral vector), and the transgene of interest (eg, oncogene or toxin). Collins et al.[92] reviewed the most commonly used viral vectors in animal research. NIH classifies most viral vectors as either RG1 (not associated with disease in healthy human adults) or RG2 (associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available); BSL containment requirements are stipulated for these groups as BSL-1 (BL1) or BSL-2 (BL2), respectively.[81] Viral vectors containing less than two-thirds of a eukaryotic viral genome may be handled under BL1 conditions.[92] Most viral vectors used in animal research are either RG2 agents or do not meet this size requirement and therefore require BL2 containment and procedures during preparation, manipulation, and injection.[92] These precautions include restricted access, an appropriate laboratory set-up and signage, staff training, sharps safety, decontamination of waste prior to disposal, and PPE to prevent skin and mucous membrane exposure.[93] All these safety practices are generally recommended for HDS administration to animals. Following these practices for humanized animals may often have additional benefits in that maintaining occupational health-related housing and precautions can aid general animal health upkeep, particularly for viral vectors that are zoonotic or are based on animal pathogens.

For animal experiments involving most viral vectors used in research, the *NIH Guidelines* recommend ABSL-2 containment practices.[81] Both lentiviral and adenoviral vectors can be found on tail swabs for as long as 72 hours after injection, although this positivity was localized and no vector was recovered from the bedding.[94] Although viral vectors are rapidly cleared (ie, within 24 hours) from the blood, the injection site should then be wiped with a disinfectant to further minimize the risk of environmental contamination.[94] The route of delivery of the transduced cells can also impact housing requirements. For example, intracranial delivery methods are designed as closed injection systems that use small volumes such that the potential of superficial contamination is minimal when properly performed.[92] Further clarification from Department of Health and Human Services indicates that ABSL2 containment housing for 1 to 7 days may be permissible for lentiviral vectors.[95] This system is also applicable for standard replication-deficient adenoviral and third-generation herpesviral vectors.[92]

Experimental manipulations of HDS-administered animals may ultimately dictate containment level housing. This is especially true when humanized animals are used as models of infectious disease, in which case BMBL animal housing requirements need to be followed and, depending on the IBC charge, an IBC application is needed. Animal tissues known to be contaminated by deliberate infection with HIV or HBV are also subject to the BPS.[83]

Conclusions

The use of humanized animals provides unique challenges for the institutional animal care and use program and OHSP. Their use comes with the potential for personnel exposure to hazards within the human material, such as bloodborne pathogens, or other hazards like infectious agents and viral vectors as a result of further experimental manipulation of these animal models. Cornerstones for mitigating risks involve thorough risk assessment, appropriate practices, PPE, engineering standards appropriate for the containment level, and personnel education and training, especially relating to bloodborne pathogens. The institution must have a mechanism in place to ensure safety of all personnel working with humanized animals, one that addresses both animal health and welfare issues and safety requirements for working with animals as well as HDS.

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Review Article

Agricultural Animals as Biomedical Models: Occupational Health and Safety Considerations

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Abstract

The use of agricultural animals in biomedical research is increasing. Their overall size and metabolic rate, organ size, longer gestation period, and other physiological similarities make them good candidates for animal models of human disease. There are a number of special considerations for use of traditional farm animals for biomedical research. Differences in physical plant infrastructure, handling equipment, training of personnel, and potential zoonoses are some of the important considerations when traditional farm animals are used in biomedical research. This article provides an overview of some of the special considerations for using traditional agricultural animals in biomedical research. With the growing need for improved translational research, it is reasonable to predict significant growth in these animal models.

Key words: animal husbandry; biomedical research; livestock; safety biosafety; review

Introduction

The use of traditional agricultural animals such as cattle, sheep, goats, and swine in biomedical research is longstanding.[1] Many groundbreaking studies were conducted in these species.[1] For example, major breakthroughs in organ transplantation, reproductive physiology, pulmonary disease, and cardiac support have come about because of animal models using cattle, goats, and sheep.[1–8] Swine are important models of gastrointestinal disease,[9] cardiac disease,[10] and stem cell therapy,[11] and horses have contributed to many studies in the area of exercise physiology.[6,12] The utility of these species in contemporary biomedical research is growing, and the need to improve translation to human medicine will enhance this growth.[13–15]

Although rodents remain the primary animal model in biomedical research,[16] there are a number of drawbacks to their use.[6,17–19] A major one is their size. Their small size and high metabolic rate limits the use of rodents in studies that translate directly to human medicine.[20] Also, rodents do have limitations in the study of some organ systems that confound the ability to translate findings to human medicine. These differences include dissimilarities in the immune system,[21] cardiovascular system,[22] gastrointestinal system,[23] and respiratory system.[24] It is clear that rodent models offer advantages in genetic modeling, low cost of husbandry, short gestation length, and a wealth of previously published literature.[16] The use of rodents in discovery of basic biological processes will continue and is clearly needed.

Rodents, particularly mice, are often utilized because of the ease with which their genome can be manipulated and their short gestation period.[16] The similarities of the mouse and human genome have been studied and these studies found numerous similarities.[6] However, there are important differences not just in sequence of genes, but in how the genes are expressed. Similarly, rats have a short gestation period and have been utilized for many seminal biomedical studies, but

important differences also exist between rat physiology and human physiology.[25] It is increasingly clear that there is a growing need for biomedical studies in larger animals to improve translation to human medicine.[26]

Traditional agricultural animals have longer gestation periods similar to human beings. This allows biomedical modeling of reproductive biology similar to the human condition; however, this does increase the cost of research when these species are used, as they must be housed longer and produce fewer offspring. Moreover, the ability to manipulate the genome has limited studies in large species. These issues are being addressed with modern technology that has allowed the generation of transgenic pigs.[27] A better understanding of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR associated proteins has led to CRISPR-CRISPR associated proteins technology that promises to allow more genetically modified models to be generated.[28] The advantages of larger size, longer gestation period, and other similarities to human beings suggest that traditional agricultural animals will increase in their significance as experimental models.

Although the larger size and longer gestation period offer advantages to producing results that may more readily translate to human medicine,[6] these characteristics also present challenges that must be considered when using traditional agricultural species. These factors add significantly to the cost of conducting research, as the expense associated with the husbandry requirements and veterinary care of these species is significant. Moreover, there is often a requirement for special handling techniques that need careful attention when using traditional agricultural animals as experimental models. Finally, disposal of large animal carcasses at the end of the study can impose a significant cost.[29]

The availability and cost of housing larger species often limits the numbers of animals that can be included in studies. This limitation can result in underpowered studies that lack rigor and are not reproducible. Careful attention to experimental design, such as using a Latin Square model to generate sufficient statistical power and provide appropriate experimental control, is an important consideration when using these species.

Husbandry Issues

Physical Plant

The large size of traditional agricultural animals compared with rodents and smaller mammals is of significant concern to biomedical scientists. According to the Guide for the Care and Use of Laboratory Animals (the Guide), swine and sheep, 2 of the more commonly used species, require approximately 1.5 square meters per animal for a normal-sized, adult animal.[30] This is often difficult to achieve in traditional laboratory animal facilities that are configured to house rodents. Beyond the square footage considerations, the infrastructure of traditional laboratory animal rooms may not be conducive to housing larger species. Floors are often smooth concrete, room drains may be too small, and removal and disposal of large amounts of bedding required for larger species may be problematic for many programs developed to house rodents or even those designed to house slightly larger animals like dogs and cats. Moreover, housing domestic species such as swine and sheep in space designed even for dogs and cats may place a significant burden on the equipment used in this space.[8] Panels and walls built to hold companion animals may not withstand the physical stress placed on them by larger farm animals.[8] This is compounded in urban settings where space to "turn out" large animal species may not be available as an option for enrichment and socialization. Also, as noted above, the disposal of large animal carcasses is much more difficult and expensive than disposal of rodent carcasses.[29]

New construction of research space for large domestic species requires careful consideration of the special needs of each species. This can also be problematic when trying to repurpose existing space. It is also important to understand that space appropriate for swine may not be appropriate for cattle and vice versa. Trying to make efficient use of available space can be hampered by these species differences as the use of each species waxes and wanes over time in the animal research program. This can result in space going unused, which results in increased overall cost to the research institution.

Nonphysical Plant

Consideration of social needs of domestic species can also add to the cost of research with these species.[30] Most agricultural animals are social species, and attention to conditions that allow appropriate social interaction to occur are imperative.[31,32] Designing space to house multiple large animals that is in compliance with the Guide can be challenging. In addition, social interaction includes human interaction as well as interaction with conspecifics.[31,33] Acclimatization to handling by husbandry personnel and research staff is important for generating reliable data.[34] It is clear that animals with familiar pen-mates and that are well acclimated to humans are much less stressed.[35] It follows that the results from studies with these animals would produce more robust results. Clearly, providing conspecific interaction is very important to the well-being of farm animals. When considering the well-being of the animals, it may be worthwhile to consider higher housing densities that are more in line with those used in agricultural settings. If this is done, it is important that an oversight body, such as an IACUC, reviews and approves this plan.[30,36]

Beyond social interaction, it is important that consideration of enrichment, beyond just having a pen-mate, is included in the husbandry of these animals. According to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (the Ag Guide), proper enrichment is "a significant component of refinement effort for animals used in research and teaching."[36] Enrichment is reported to reduce stress in animals and, importantly, allows for species-specific behaviors to be expressed.[36] Animals with no social or physical enrichment often express abnormal behaviors described as stereotypy.[37,38] The types and quality of enrichment vary greatly and should be carefully considered when conducting research with large animals.[39] Numerous articles describe enrichment techniques that benefit farm animal species.[31,33–35,38–45] The benefits of enrichment are well supported, but the benefits of different types of enrichment are still debated.[46] For example, Backus et al found that while enrichment could promote more positive social interaction among pigs, there appeared to be enhanced anxiety among enriched pigs exposed to a novel person introduced into the environment.[47]

Enrichment includes physical objects placed in the pen with animals to provide a varied environment and allow the animals to express behaviors such as nest building or manipulation of objects to reduce stress.[39,46] Nutritional enrichment by providing treats or variations in diet are also reported to be

beneficial as are some sensory enrichment techniques such as playing music.[46,48] Although the benefits of each type of enrichment are still debated, it is clear that application of enrichment must be uniform. Variation of enrichment within a study group can have significant effects on the results of a study and should be avoided.[46] For example, providing nutritional enrichment to a subgroup of animals and not all animals may invalidate the results.

Husbandry of agricultural animals is often complicated by the disease status of the animals.[8] Sourcing of animals varies from program to program. Some institutions purchase animals from sources where disease status is clear and health records are carefully maintained and readily available. These sources include commercial suppliers of laboratory animals who recognize the importance of disease status to the research enterprise, or from land grant institutions who maintain herds with known disease status. Others purchase animals from alternate sources, such as local private farms, where disease status of the animals is not as carefully followed. In the latter case, there is a concern for spread of disease among other laboratory animals as well as a concern for zoonotic diseases that may impact husbandry staff (discussed below).

The contrast in biosecurity at research institutions with many pork producers is also noteworthy. Most producers will not let a person on their farm who has been in contact with other pigs during the past week, and many require shower in and shower out. This precludes employees from having contact with livestock outside of work such as their own farm or with 4-H or FFA activities.[49]

Finally, the concern over disease status can result in extended periods of time in quarantine with the attendant increase in cost of the research. Moreover, agricultural animals typically have a standard schedule of vaccinations and parasite control that may be necessary to control disease in these species.

Safety Concerns Related to Handling of Animals

The size of agricultural animals is a major concern when animals must be handled. The sheer size of the animals presents a potential hazard to staff improperly trained in how to handle these species. The single greatest hazard to humans is being physically run over or kicked by an agricultural animal.[50,51] This hazard includes being injured by equipment used to handle farm animals when the equipment is hit by the animal, for example, the panel on a squeeze chute being kicked and striking personnel.[51] It is also important to remember that improper handling can result in injury to the animal as well as humans trying to manipulate the animal.[50]

Appropriate handling of different species is described in numerous publications.[52,53] Reviewing all these publications is beyond the scope of this overview. However, one of the best sources of training is often working with professionals in the agriculture industry that have worked with specific types of animals for many years. These professionals often have important tips and techniques that allow safe and easy handling of otherwise difficult animals. Finally, it is important to be aware that handling techniques of farm animals has evolved over the past decades. Design of corrals and holding pens and chutes has changed so that animals move through the handling chutes much more easily and with much less stress and trauma. In addition, behaviorists such as Temple Grandin have introduced techniques that reduce the handling stress associated with moving animals.[53] These techniques entail more attention to slow, deliberate movements, attention to the flight path of animals, and a greatly minimized use of physical force. Considering the impact of stress on the physiology and response to disease of animals, these are very important considerations. For example, multiple publications have shown that use of electric prods, used extensively 30 or 40 years ago, on cattle or swine greatly elevates the level of stress hormones in these animals.[43,53–56] Reducing the use of these prods can greatly improve the behavior of the animals and subsequently improve experimental results. It is also important to recognize that research has shown farm animals are very amenable to the use of positive reinforcement to reduce the stress of penning or holding to collect samples.[53] Finally, as noted above, acclimatization of the animals to the experimental environment is very important in reducing the stress the animals experience. Abruptly changing the animal's environment can result in signs of stress, beyond the well-documented changes in the hypothalamo-pituitary-adrenal axis. For example, it has been demonstrated that swine exposed to stressful handling at a slaughterhouse have altered levels of biogenic amines such as serotonin and dopamine in the amygdala and hippocampus.[41] These neurotransmitters have significant effects on behavior and learning.[57] Given the clear effect of stress on the results of a study, these considerations are critical to collection of robust and reliable data.

An additional consideration relative to stress and behavior is the importance of breed on the temperament of the animal.[35,43,53,54] Different breeds of agricultural animals have different temperaments. This is well documented and should not be overlooked when the experimental model is being selected.[58,59] Excitable animals have reduced reproductive performance and overall productivity compared with animals that are less excitable.[60] Additionally, the age of the animal will affect the temperament of the animal.[59] Older animals tend to be less responsive to changes in environment than young animals. This likely relates to the older animal's past experiences and learning that all environmental changes are not necessarily threatening. Finally, it is important to remember that excitable animals present an increased risk to animal workers.

The handling equipment itself is an important safety consideration.[50,61] Squeeze chutes for handling of cattle, particularly the more contemporary hydraulic chutes, present a significant hazard to personnel who are not properly trained. Entrapment in a hydraulic head gate can cause significant injury to personnel. Additionally, a lack of knowledge in how to position oneself when placing holding bars or gates can result in injury to personnel as well as animals and, as noted above, being unaware of the flight path of the animal can result in injury from being run over. Proper training on equipment is essential. The use of contemporary corrals and runways is useful in minimizing stress to animals. Avoiding sharp turns and corners where animals become trapped, keeping the visual fields clean and free of unexpected stimuli, and using flooring that provides adequate footing can all improve the movement of animals during handling.[54] Finally, the equipment must be well maintained and in good working order.

Zoonotic Diseases

As noted above, diseases of animals can be communicated to humans and are thus viewed as zoonotic. The Centers for Disease Control (CDC) reports dozens of diseases that

potentially can be transmitted between animals and people. Excellent resources and fact sheets are available from the CDC (https://www.cdc.gov/ncezid/stories-features/browse/subjects/zoonotic-diseases.html) or sites such as the Center for Food Safety and Public Health at Iowa State University (http://www.cfsph.iastate.edu) that cover the multiple zoonotic diseases transmitted from animals to humans. The agents responsible for this wide variety of diseases vary from prions to external parasites. The breadth of this topic is not the aim of this review. In the research setting, husbandry controls often mitigate exposure to these agents as the research animals are often serotested or vaccinated, and the disease status of the animal is known. Additionally, the laboratory also allows proper parasite control through regularly scheduled treatments with antiparasiticals that can mitigate much of the risk. For these reasons, laboratory-acquired infections seem to be rare,[62,63] but it must be noted that reporting of these incidences is haphazard. Those that are reported focus on issues occurring in the laboratory and not in animal holding areas. Thus, the number of infections from laboratory animal exposure is a small percentage of the total number of zoonotic diseases that are reported. Nonetheless, it is important to recognize potential routes of transmission and take appropriate safety measures to prevent exposure. For example, diseases such as brucellosis or Q fever are often transmitted from fetal tissues and placentae to humans. Assuring appropriate use of personal protective equipment (PPE) when assisting with delivery from ruminants can minimize the risk of infection. Additionally, proper occupational health programs will inform persons most at risk of disease of instances when they might be exposed.

It is also important to remember that infectious agents can go from humans to animals. A good example is the transmission of influenza from people to swine. It is just as important to keep personnel with the flu away from the experimental animals as it is to keep them away from other humans. It is very important to ensure that personnel are vaccinated and free from disease when handling animals (https://www.cdc.gov/flu/swineflu/people-raise-pigs-flu.htm).

Other diseases that often receive prominent attention as important when considering potential infection of humans include Q Fever (*Coxiella burnettii*) and brucellosis (*Brucella melitensis* and *Brucella abortus*) from exposure to placental tissues or amniotic fluid, tetanus (*Clostridium tetani*) from puncture wounds, enteric bacteria (*E coli* and *Salmonella spp.*), and parasites (*Cryptosporidium parvum*) from exposure to feces, leptospirosis from exposure to urine, and external parasites or fungi (*Trichophyton* spp.) that infect the skin.[62] Rarely diseases such as rabies may need consideration other than when animals are procured from sources with unclear disease status. Proper vetting of disease status and training on animal handling can mitigate many of the risks associated with zoonotic diseases. Appropriate considerations for use of PPE can also mitigate potential infections from these agents.

Knowledge of the risk associated with zoonotic diseases and how they are transmitted is an essential part of an effective occupational health program. This includes identifying the susceptible population. For example, Q fever presents a high risk to pregnant women, persons with heart valve disease or vascular disease, and persons with a weakened immune system. However, the risk to a young, healthy male is much less and can be easily mitigated with PPE. Also, sheep are often considered the primary source of the bacteria *Coxiella burnettii*, but other species such as cattle and goats are significant sources of the bacteria. Interestingly, according to a CDC report from 2013, the largest outbreak of Q fever reported was associated with a goat farm and the infection route was windborne transmission of the infectious agent to people downwind from the farm.[64] Nonetheless, the risk among research personnel is likely exposure to reproductive tissues (placenta and amniotic fluid) from cattle, goats, and sheep.[62,64]

It is important in the risk assessment that appropriate PPE be considered. The need for protection should be balanced with the need to properly handle large animals. Too much PPE can present a hazard in that personnel may not be able to properly manage the animals or are hindered in performing techniques critical to the biomedical experiments such as tissue collection.

Future Directions for Use of Farm Animals in Biomedical Research

In recent years the concept of One Medicine, promoted through the One Health Initiative, has been championed by many in the biomedical community.[65–68] This concept focuses on the idea that disease processes in humans are similar to those in domestic species and that environmental factors affect both. Therefore, the study and treatment of disease in one species can benefit the treatment of diseases in other species. Diseases of populations of domestic species that are studied by people in the veterinary community could easily benefit the health of the human population. Multiple examples of this mutual benefit exist in the fields of cancer research, environmental toxicology, and zoonotic diseases.[67,68]

This concept can be extended to other conditions such as genetic disorders and traits that appear in domestic animals. Thousands of disorders or traits have been identified in agricultural animals and many of these are potential models for human disease.[69] These are naturally occurring genetic disorders with possible human counterparts.

Extension of this concept to studying the use of veterinary clinical cases to understand human disease is clearly something that can be employed in biomedical studies. This would entail better incorporation of veterinary medical records into disease databases and a closer working relationship between veterinarians and the human biomedical community. Unfortunately, the use of veterinary clinical cases to inform biomedical research has not grown as much as many have hoped.

Beyond the One Medicine concept there are numerous examples of agricultural animal models being important to the study of human disease. These examples include the study of female reproduction in cattle.[70] The long gestation period, diminished reproductive efficiency with aging, and similarities in ovarian function and embryo-maternal communication make cattle a good model for pathologies arising in women.[6,71,72] Additionally, the high rate of assisted reproductive technologies used in cattle provides a model for the increasing number of women utilizing these techniques and the problems that arise with these techniques.[6] The use of techniques like nuclear transfer and in vitro fertilization often result in abnormally large offspring in both cattle and humans.[6] The study of the mechanisms underlying this phenomenon will be valuable to preventing complications in humans in the future. Similar observations in the paradoxical immune response to the “foreign” fetus in cattle and humans will provide important information in reproductive immunology studies. The longer period of gestation is a critical factor in these studies.

Studies of cardiovascular function in agricultural animals has also contributed greatly to human medicine. The study of atrial fibrillation in goats is an excellent example.[73] Atrial fibrillation is a significant disease in older people. This arrhythmia

causes significant remodeling in the heart. Goats are an excellent model for this disease, and use of this species in future atrial fibrillation work will contribute to therapies to prevent remodeling and help prevent the need for long-term treatment of atrial fibrillation patients.

Goats are also an important orthopedic model. The joints of goats closely resemble the joints of humans.[74,75] Thus, the study of joint pathology in goats can lead to better therapy for diseases such as arthritis in humans.

Sheep are invaluable in the study of respiratory disease.[76,77] Their size and similar pulmonary physiology to humans make them an excellent model for diseases such as cystic fibrosis, respiratory distress syndrome in preterm infants, and infectious diseases such as respiratory syncytial virus infection.[77] Sheep have also contributed greatly to cardiovascular research, particularly in the development of devices to assist the cardiovascular system or replace components of the cardiovascular system.[78]

Swine may be the fastest-growing farm animal model for biomedical research.[27] Their size, reproductive characteristics, and the fact that they can be bred year-round make them an optimal model.[79] They have proven to be invaluable in cardiovascular research. Diseases such as atherosclerosis, aortic aneurysm, and heart failure are modeled in pigs.[80] They are becoming an important model for stroke research and are increasing used in neuroscience research, as they are amenable to learning behavioral tasks and protocols for imaging this species in contemporary instruments are becoming more refined.[81,82] Finally, facilities such as the National Swine Resource and Research Center at the University of Missouri have demonstrated that the genome of this species can be manipulated to produce transgenic animals for research (see http://nsrrc.missouri.edu/).

It is clear that traditional farm animals will gain a more significant role in studies of diseases that affect humans.[7,11,14,15,18,79] Their larger size, longer gestation period, and similar metabolism are only a few of the characteristics that suggest them as excellent models for translational studies to complement the basic science studies conducted in rodents. As the use of these species increases, it is imperative that infrastructure and training be in place to ensure appropriate housing and handling of these animals. These species are going to be a significant component of biomedical research in the future.

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Review Article

Nontraditional Laboratory Animal Species (Cephalopods, Fish, Amphibians, Reptiles, and Birds)

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Abstract

Aquatic vertebrates and cephalopods, amphibians, reptiles, and birds offer unique safety and occupational health challenges for laboratory animal personnel. This paper discusses environmental, handling, and zoonotic concerns associated with these species.

Key words: amphibians; birds; cephalopods; environmental hazards; fish; handling; reptiles; zoonoses

Introduction

Contemporary research facilities commonly include nontraditional laboratory animal species such as birds, reptiles, amphibians, fish, and cephalopods. While application of general safety principles and practices are sufficient in some areas, housing and caring for these animals can pose rather unique challenges. The following sections provide some specific items to consider when developing comprehensive occupational health and safety programs involving nontraditional species.

Environmental Hazards Associated with Housing Nontraditional Species

Wet Environment

Many of the nontraditional laboratory animal species are housed in environments that are high in moisture; these aquatic facilities are associated with potential hazards such as slips, dermatitis, electric shock, and increased exposure to sharp surfaces due to glass enclosures and wet surfaces.

Personal protective equipment (PPE) should include wearing of closed-toe shoes with nonskid soles to prevent slips and falls. Floors in facilities should be pitched to promote drainage and avoid accumulation of stagnant water along with the formation of puddles. Effort should be made to avoid salt and algae build-up on floors, as deposits are slippery.[1,2]

While wearing gloves is imperative in most laboratory settings, they can also trap water against the skin, exacerbating contact. Prolonged and/or frequent water immersion may macerate skin and cause xerosis from the desiccant effects of water. Irritant contact dermatitis from washing gravel and exposure to sea salt crusts has been reported in the marine aquarium industry.[3] Goggles or eye protection should be worn to avoid splash from water when netting fish or amphibians and when handling and cleaning soiled tanks.

Electrical systems in aquatic facilities present a serious occupational hazard. Electric hazards can cause burns, shocks, and electrocutions. Systems should be professionally installed for operation in wet environments. Equipment should be

checked periodically to ensure it is in good condition and free of defects. Frayed or damaged cords must not be used. All circuits in damp locations must have ground fault interrupters,[4,5] nonmetallic conduits should be used, and lighting fixtures should be watertight. Operational procedures such as the use of lockout or tag out procedures should be implemented to control energy sources during repair and maintenance.[6]

A common occupational hazard noted in aquatic facilities is the extensive use of extension cords. All electric cords and wires should be fixed away from water and personnel traffic to prevent falls and electrocution. Electrical equipment should be placed away from splash zones and not under water pipes or tanks. Extra care must be taken if seawater is used (either natural or synthetic) due to the extreme corrosiveness and high electrical conductivity of salt water.[1]

Sharps

Tanks made of polycarbonate are used for high-density housing of zebrafish; however, many smaller populations of fish, amphibians, and reptiles are often housed in glass tanks. While the transparency of glass permits easy observations, daily handling of the tanks can lead to contact with sharp surfaces from broken glass or glass without rounded corners. Dried salt crusts that form along the edges of aquariums and lids from splash and evaporation of salt water cause an abrasive surface. When working in research facilities, regardless of species, care must always be taken when handling needles and scalpels.

Light

Artificial lighting commonly used in aquariums generates ultraviolet light (both UV-A and UV-B). If the light is suspended above the aquaria it should be shielded. Unshielded lights can lead to the development of acute erythema and be a long-term potential for photocarcinogenesis and other UV-induced skin changes in personnel.[3] Ultraviolet sterilizers are the most frequently used method to disinfect water in zebrafish housing systems.[7] Sterilizers must be encased in a protective shield during operation to protect personnel from UV exposure that can damage their eyes and skin.

Chemicals

Disinfectant footbaths are commonly used in aquatic facilities.[8,9] Appropriate PPE should be worn during the preparation of the footbaths as the disinfectant may cause acute inhalation toxicity, skin corrosion, and eye damage during preparation.[10]

Water chemistry test kits are frequently used in aquatic facilities to test ammonia and nitrate levels in water tanks. Sodium hydroxide and sodium hypochlorite may be present in ammonia testing kits; these substances can cause chemical burns and irritation, while nitrate test kits may contain hydrazine, a contact sensitizer.[3,11]

One of the most widely used anesthetic agents in aquatic species is tricaine methane sulfonate. In its powdered form, it can easily be airborne.[12] The compound has been reported to be retinotoxic as well as a mucous membrane irritator.[13,14] The powder should be used only in a well-ventilated area such as outdoors or in a fume hood.

Large Enclosures

Research may necessitate the use of large enclosures to either mimic industry production, such as aquaculture, or to promote species-specific behavior such as flight for songbirds or shoaling and schooling behavior for fish. While these enclosures offer a clear benefit to meet both research and/or animal welfare needs, they present distinct physical hazards.

Approximately 1% of occupational fatalities in the United States result from working with animals, with the majority (67%) related to large animal work.[15] Aquaculture fatalities include drowning, electrocutions, crushing-related injuries, and fatal head injuries. Nonfatal injuries are associated with slips, falls from heights, falls overboard, strains, sprains, and chemicals.[2] Flight cages and tanks for large aquatic species present hazards associated with the potential for falls from high ladders and scaffolding.

Allergens

Allergy to laboratory animals is a well-published occupational hazard; the reported incident rate varies between 10% and 56% of exposed individuals.[16–19] While most of the clinical symptoms reported are from personnel handling rabbit and rodent cages, approximately 10% of individuals exhibit animal-induced asthma to dander, scales, fur, saliva, and body waste.[17,18]

Birds

Bird allergens are an important cause of occupational allergic disease. Reports of Farmer's Lung, Pigeon Breeder's Lung, and Breeder's Lung describe severe respiratory symptoms associated with inhaled antigens and date back to the mid-twentieth century.[20] Allergic symptoms have been described in individuals with exposure to parrots, pheasants, canaries, geese, and owls.[21] The principal causative agents are avian proteins from serum and feathers.[22,23] The incidence rate was reported to be 8% among pigeon breeders and zookeepers with exposure to birds.[24,25] In the zoo study, clinical symptoms included rhinitis, asthma, conjunctivitis, and some dermatitis; exposure to canary serum and/or feathers was found to be most allergenic, followed by parrots and then pigeons.

Hypersensitivity pneumonitis, also known as the Bird's Fancier Lungs, mimics pneumonia and usually occurs several hours after exposure. Occupational hypersensitivity pneumonitis can be acute for those with intermittent high level of exposure to antigens such as when cleaning pens. Chronic disease can occur with daily low level of exposure, such as with bird breeders, and can lead to fibrosis and emphysema.[22]

As with all allergens, exposure must be minimized. Staff should be provided with appropriate respiratory PPE during periods of exposure to high levels of antigens such as when cleaning out pens or when birds molt and shed feathers. For those with clinical symptoms, it is important to be aware that avian antigens can persist in the environment. Despite extensive environmental controls, high levels of antigens can be still detected after 18 months.[22]

Fish

Fish allergies are most often associated with ingestion; however, occupational allergies have been documented in fishermen and seafood-processing workers. The first report involved a fisherman who handled codfish.[26] The processing of seafood has been associated with respiratory allergic symptoms due to aerosolization of fish antigens.[27] Occupational prevalence rates are estimated to be between 7% and 8% for asthma and between 3% and 11% for contact dermatitis.[28–32] As these

occupational allergies involve both contact dermatitis and inhalation of antigens, their consideration in laboratory settings should not be dismissed. In research settings, the processing of fish tissue, particularly at the end of large studies, may lead to the aerosolization of fish antigens.

Reptiles

As the prevalence of reptiles as pets has increased over the last several years, so has the documentation of allergic reactions from exposures. While few research facilities house reptiles, exposure of personnel during field studies may be an occupational hazard consideration.

The first report of an allergic reaction to snake venom was published in 1930. The case involved an individual with a history of a bite from a copperhead and subsequently, he was injected with experimental intradermal injections of a variety of venoms including *Crotalus*. He then developed allergic symptoms when handling dried venom, confirmed through a positive skin test to *Crotalus* venom.[33] Respiratory allergic reactions occurred in a snake handler, with no history of bites, when exposed to rinkhals (*Hemachatus haemachatus*) venom. It was suspected that the sensitivity developed from inhalation or contact with venom present on the snake's skin and mucus membranes.[34] Other reports involve anaphylactic shock secondary to snake bites from a rattlesnake (species not identified) and a king cobra (*Ophiophagus hannah*).[35,36]

A few reports have been documented on allergies to iguanas. One patient complained that respiratory symptoms were accentuated when handling his pet iguana; IgE antibody to protein from scale extracts from both his iguana and a local zoo's iguanas were identified.[37] Other allergic respiratory symptoms have been reported from exposure to iguanas.[38] Symptoms are reported to be more intense when exposed to male iguanas, who have larger femoral pores/glands. The pores' secretions are primarily made of proteins and used to mark their territories. It was presumed that some material shed by lizards become airborne and caused sensitization.[39] Additional reports involved reactions to bites; one involved a dermal hypersensitivity consistent with the pattern seen in arthropod-bite reaction,[40] and a second was an anaphylactic reaction to a Gila monster bite.[41]

Amphibians

There are limited reports of allergy to amphibians in the literature. As with fish, the majority involves food allergies. The earliest publication concerning research animals involved a laboratory technician who experienced asthmatic attacks when handling frogs (*Rana esculenta*).[42] Another report involved asthmatic symptoms and contact dermatitis in a laboratory technician from handling bullfrogs (*Lithobates catesbeianus*) and extracting brain tissue. Years later, that same individual accidentally injected herself with extracts from frog brain tissue, and she developed swelling in her right hand, stridor, and dyspnea; IgE antibody to frog extracts were identified.[43] A third patient developed allergic symptoms two years after he began handling frogs. Specific IgE antibody to frog venom was demonstrated, and his symptoms remitted after he changed occupation.[44]

Feed (crickets, mealworms)

Cricket (Gryllidae) and mealworm beetle (*Tenebrio molitor*) colonies are often maintained in animal facilities to produce feed for frogs, reptiles, and birds; they can also be used as a source of environmental enrichment for nonhuman primates. These animals are not usually considered as part of an occupational hazard program; however, they can be a cause of occupational allergy based on the following reports.

A research facility produced two hundred thousand crickets (*Acheta domesticus*) per week as a feed source for amphibians. Allergy symptoms of ocular pruritis, rhinitis, and bronchial asthma were reported in two animal care personnel. Specific IgE antibodies to cricket extract were isolated. Three of the eleven other workers in the facility also had a positive skin prick test to the cricket extract.[45] Another occupational exposure also described respiratory symptoms. The employee had direct contact with three different species of crickets (*Gryllus campestris*, *G. bimaculatus*, and *A. domesticus*), and specific IgE for each species of crickets was identified.[46] A third report included contact urticaria in addition to respiratory symptoms in an employee where crickets were bred.[47] A subacute hypersensitivity pneumonitis was also reported in a man who previously owned an avian pet shop.[48]

Finally, sensitivity to mealworm beetles (*Tenebrio molitor*) was reported in workers at a specialty insect breeding facility and among personnel in an entomology laboratory.[49,50]

Hazards Associated with Handling Nontraditional Species

Trauma

Knowledge and practice in proper restraint techniques along with well-designed holding facilities that facilitate safe access to the animals are the mainstays of avoiding animal-inflicted trauma. Relevant literature is available about restraint and immobilization approaches.[51–53] Well-developed restraint techniques take advantage of knowing which defensive/offensive attributes of the animal are most likely to inflict injury and working the animal in ways that neutralize those threats. In many species bites or damage from hard bills or beaks are the most probable cause of trauma, making restraint of or avoidance of the head a primary objective. However, in many species other appendages, either armed with claws, talons, venomous spines, or simply just massive and powerful, can be even more dangerous than the head.

Bites, particularly from larger species, can be dangerous. Clearly this is well recognized for the crocodilians and their many-toothed jaws. It is relatively uncommon to find these species in research facilities, but they do occur. Similarly, snapping turtles and sea turtles can inflict very painful and debilitating bites if not handled properly, taking care to keep hands away from their mouths and respecting their considerable rapid reach with their long flexible neck. Their jaw closing pressures are less than those of a human using molars when scaled for head size, but their mouth anatomy and tendency to bite and hold make their bites a formidable risk to avoid.[54,55]

Considerable literature is written about the risks of various nonvenomous snake and lizard bites, though concerns are generally related to avoiding damage to the teeth of the animal and preventing sepsis from the bite would. It is incumbent on facility managers to recognize the potential hazards and to solicit input from experts experienced working with these species to help develop safe husbandry and handling SOPs. The recognition of potential for harm from smaller creatures with less obviously powerful oral armament is equally important. Lizards and larger amphibians can inflict sometimes painful

bites on unwary husbandry or restraint personnel. Much of the challenge with these situations is avoiding being mistaken for food. Washing hands and avoiding hand movements that mimic prey are key preventative measures that can help in this regard.

Overall, with few exceptions such as large amphiumas, amphibian bites are generally considered inconsequential. The lack of dentition on the lower jaw, relatively small weakly affixed teeth, and lack of jaw manipulation after the bite for most species means bites usually barely break the skin if that. There is more concern for injuring the mouth of these species than the risk of damage to the bitten human. This is true even for the large African bullfrogs (*Pixicephalus spp*) that are occasionally found in research settings. Their bite can be quite painful because of their powerful jaw and grip. The key is avoid pulling the hand away. To break the grip of an African Bullfrog, it is suggested that the frog be held under cold running water until it voluntarily releases its grip.

There is one important exception among the amphibians with regard to bites. Members of the genus *Ceratophrys*, sometimes referred to as the Pac Man Frogs, have a combination of unusually short, relatively highly ossified jaws with an ossified mandibular symphysis.[56] Those jaws provide greater leverage than most amphibian jaws. This, combined with a very unusual recurving tooth structure where teeth are also strongly attached to the jaws, allows the horned frogs to inflict serious bites on unsuspecting handlers.[57,58] Recent research has shown that one of these species, *Ceratophrys cranwelli*, has a bite force similar to those of mammalian predators and approaching that of crocodilians when scaled for head width.[57] This work has led to speculation that ancient giant amphibians (*Beelzebufo spp*) may have preyed upon dinosaurs.

There is a wide array of bite risk across the broad range of bony fishes, elasmobranchs, and invertebrates. Most individuals are aware that the incredibly sharp edges of the modified placoid scales that serve as teeth for many sharks can inflict major trauma in a very short encounter. Less well known perhaps are the painful bites that may be inflicted by beaks of large cephalopods. For the most part the larger species will not be found in research facilities, but the bite of the giant Pacific octopus (*Octopus doefleini*) can cause significant tissue damage and is painful.[59] Bites of smaller species of octopus may be complicated by secondary bacterial infections and development of nonhealing granulomatous wounds.[60]

The beaks of many birds are capable of inflicting pain and damage to unwary people. The bite force and beak strength of many parrots can inflict severe wounds, and physical head restraint is a key to safe handling. Secondary infections including those caused by introduction of rickettsial and mycobacterial organisms should be considered in the management of parrot bites.[61] Many raptors can inflict serious wounds with their beaks, particularly the scavenging birds that are adapted to working large carcasses and crushing bones with their beaks. Again, management of secondary infections should be a component of the trauma management.

Trauma from other than bite wounds can and does occur across the spectrum of species considered in this large taxonomic group. Long-billed birds, including herons, egrets, cranes, etc., will stab out quickly with their bill, aiming for eyes. Head control is critical when handling these species and some institutions require wearing eye protection. This can be a good thing so long as it does not confer a false sense of security to the bird handlers. Talons of the feet are the most damaging weapon of many of the raptors. In handling these species, it is critical to contain foot movement even prioritized over complete control of the head. The tail of crocodilians is a particularly challenging weapon used to suddenly knock prey or a predator to the ground where the head and mouth can be better brought into play. For larger specimens it is critical that the tail be managed simultaneously with efforts to restrain the head. Larger lizards can similarly inflict damage, including lacerations with their tails. The tails of iguanas, varanid lizards, and other large lizards should be restrained during handling. Large constrictor snakes will use their bodies to wrap and crush prey. Care should be taken to avoid allowing even relatively small constrictors to be in a position to wrap the neck. For larger snakes, multiple handlers will be necessary to avoid the risk of the handler holding the head being wrapped and suffocated by the snake.

Aquatic species come equipped with a variety of spines that may be venomous in addition to well designed for inflicting trauma. Stingrays and other batoids are equipped with rather apparent spines on the dorsum near the base of their tails. If their presence is not required for the research it is common for the spines to be routinely removed to reduce the risk of trauma/envenomation. Smaller fish have a variety of spines, often associated with dorsal or pectoral fins. The channel catfish is a good example. The spines can cause a painful wound. Assumption of spines existing until proven otherwise is a very good policy when handling fish species that have not been maintained previously in a facility.

Similarly, some extant species of *Coleoids* (octopus, squid, cuttlefish) have hooks or hooked suckers that can come as a rude surprise to handlers unaware of the extra armament. Species of octopus and squid more often maintained in research facilities tend to not have hooks, but hooks are found in many members of the *Onychoteuthidae*, *Enoploteuthidae*, *Octopoteuthidae*, *Gonatidae*, and *Cranchiidae*, and when a new species is proposed for management it will be useful to establish whether or not it has hooks or hooked suckers.

Electric Shock (Electric Fish)

Electric shock is a hazard peculiar to fishes. Though many people become very concerned when they learn they may be dealing with an electric fish, actually the vast majority of the 348 known species of electric fishes generate very small fields with their dedicated electric organ, usually 1 volt or less. These fields are not used for immobilizing prey or defense but rather for navigating and exploring their environment, exploring objects, or even communication. Most fishes capable of generating an electric field of this nature also have the ability to sense electric fields.[62] Species of weakly electric fish found commonly in research settings would include several different species of freshwater knife fish from genera in several families and freshwater elephant fish or mormyrids from various genera in the family *Mormyridae*. They pose no electrical hazard to personnel.

Fishes that generate dangerous electrical fields are also found in research settings. These include the well-known freshwater electric eel (*Electriphorus electricus*), electric catfishes, and the marine electric rays. The freshwater species generate high-voltage, low-amperage discharges to overcome the high impedance of freshwater and have been well studied.[63] Electric eels have been documented to generate up to 600-volt discharges[64] but also generate low-voltage signals used similarly to those of the weak signal generators. The electrogenic marine rays (*Torpedo spp*.) and a group of marine perciform fish known as stargazers produce low-voltage but high-amperage

discharges well designed for propagation in their highly conductive environment. Torpedo rays if completely rested have been reported to produce charges as high as 220 volts,[65] but many researchers question this measurement and field measurements are more in the rage of 45 to 60 volts.[66,67] Though these stronger electrical discharges are unlikely to kill a healthy human that has no underlying medical problems, they could easily incapacitate a person sufficiently to cause them to fall or long enough for them to drown.[64,68,69] Caution in handling and working around these species is well advised. Generation of high voltage or current rapidly depletes adenosine triphosphate (ATP) levels in the generating organs of these animals over time. Some individuals advocate stimulating the animal prior to handling to reduce their ability to discharge during manipulation.[69] However, this technique should not be relied upon, and electrical insulating gloves should be used by staff when handling the animal.

Toxins and Venoms

The toxicology of venomous snakes and the relatively few venomous lizards is a well-studied field, and considerable information on the safety procedures appropriate to managing them in captive situations is available.[51,70] It is beyond the scope of this brief review to go into detail. The keys to safe management of venomous snakes in captivity include (1) cage security with cages always locked, (2) handling and husbandry by personnel trained in all SOPs in tandem, (3) emergency security communications and alarms, (4) practiced routine, escape, and bite SOPs that include rapid access to trained health care professionals and a rigorously maintained availability of appropriate antivenin.

Other reptiles and birds generally do not pose toxin or venom risks. However, several amphibians and fishes produce toxic skin secretions of various forms that can be problematic or even lethal for humans not aware of them. Similarly, many invertebrate species produce potent venoms and toxins. Many of these species can be found useful in research and may be maintained in research facilities.

The poison dart frogs of the genus *Dendrobates* are colorful neotropical frogs that produce neuromuscular blocking compounds that have been exploited by natives for creating rapidly acting darts for immobilization of small prey. These curare-like substances are actually generated by the frogs through metabolizing precursors ingested in their native diet, primarily specific species of ants. This explains why captive-bred animals may be relatively if not completely devoid of the toxins. Wild dendrobatid frogs retain metabolites and can produce the skin toxins for years in captivity. Also, these animals are very sensitive to absorbing toxins such as nicotine or disinfectants that might be on the hands of a human. Because of this and the difficulty of being certain of the origin of the animal or how long they have been in captivity, these animals are best not handled directly. Instead, it is best to "handle" these species with clear containers that allow close observation, imaging, and such diagnostic activities as well as facilitate transfer between habitats etc.

In contrast to the dendrobatid frogs, the cardioactive steroids referred to as bufdienolides are synthesized by toads such as the cane toad (*Rhinella marina*, formerly *Bufo marinus*) without dependence on specific precursors from dietary items. The toad bufdienolides are derived from cholesterol and have similar activity to plant cardenolides, being inhibitors of membrane-bound Na+/K+ ATPase.[71] They are referred to as cardiac glycosides though, unlike plant bufdienolides, those from toads do not conjugate with a carbohydrate.[72] The bufdienolides are secreted by skin glands and particularly the parotoid glands of many toads. There is a strong ontogenic relationship to toxicity in amphibians excreting bufdienolides, both in relation to the amount and number of toxin species in tissues. The eggs of cane toads contain at least 28 varieties of bufdienolides in larger quantities than the two to eight compounds found in larvae, with the quantity decreasing throughout development. Juvenile toads generally secrete at most five bufdienolide toxins.[73] Therefore, the greatest care should be placed on avoiding skin contact with eggs and early toad larvae. However, adult toads continue to secrete a limited number of but clinically impactful cardioactive bufdienolides in their parotoid and skin secretions throughout their lives. Handling with disposable gloves is a necessary precaution in laboratory settings.

Recently, two species of South American frogs, Greening's frog (*Corythomantis greening*) and Bruno's casque-headed frog (*Aparasphenodon brunoi*), have been reported as venomous frogs.[74] This is a bit of a stretch because their relatively unique adaptation is the presence of bony spikes on their heads, which they use to abrade the skin of predators to open access for their quite toxic mucous skin secretions. Those secretions are indeed quite toxic, but the claim as venomous stretches the delineation between toxins and venoms.

Most if not all salamanders secrete toxins from skin glands. Several species of salamander produce some very potent toxins, including tetrodotoxin. Tetrodotoxin, also referred to as tarichatoxin, is an amino perhydroquinazoline derivative that is among the most toxic nonprotein substances known.[75] Ironically, this toxin is also found in species of marine pufferfish, suggesting some very interesting convergent evolution. Tetrodotoxin is only found in the true newts in the family Salamandridae. Concentrations are highest in newt species found in western North America followed by newts of eastern North America, Asia, and lowest in European newts. It is in highest concentrations in skin, ovaries, and ova of females and skin and blood of male newts. Weak alkalinity destroys tetrodotoxin. Interestingly, tetrodotoxin content of tissues increases over time in captivity (1 year) in females, suggesting exogenous factors are not involved in the toxin synthesis.[76]

Toxins and venoms of marine animals are well covered in some admittedly hard-to-find reference books that span thousands of pages.[77,78] The complexity of the topic is compounded by the vast diversity of marine vertebrates and invertebrates. The interest in marine toxins for basic and applied investigation means many species may be maintained in laboratory animal facilities, including species whose toxicology is not well characterized. Some species of interest are reasonably well known, including venomous fishes such as the stone fishes and lion fishes, the infamously toxic cone shells, and the blue ringed octopus. The best approach to any marine species being held for research is to investigate the literature for any indication of associated toxins and then, if finding none, assume that it may not yet be reported.

Zoonoses Associated with Nontraditional Species

Bacterial Zoonoses

Bacterial pathogens are the most commonly described zoonotic agents associated with nontraditional research species. *Chlamydia* (*Chlamydiophila*) *psittaci* is a bacterium most commonly found in birds; however, horses, pigs, and dogs have

been identified as occasional hosts.[79] In 2014, several cases of human psittacosis in a veterinary school in Australia were linked to exposure to equine fetal tissues.[80,81] It was concluded that the horse was most likely infected by wild birds.[80] Avian species most commonly infected with *Chlamydia psittaci* are parrots, cockatiels, budgerigars, and other psittacines. Pigeons are also an important reservoir. Outbreaks in turkeys, ducks, and chickens have been described, and infections have been documented in songbirds, sea birds, and over 460 avian species worldwide.[82] Transmission occurs by inhalation of infected nasal discharge or aerosolized dried feces. Disease in birds is variable and can range from acute systemic illness to mild conjunctivitis. Inapparent carriers have also been documented.[83] In humans, *Chlamydia psittaci* symptoms can include fever, chills, headache, and pneumonia. Psittacosis is treated with antibiotics. Proper quarantine, diagnostic testing, and appropriate PPE will help minimize personnel risk.

Mycobacterium marinum and related species (*M. fortuitum*, *M. ulcerans*, *M. chelonae*, and other "atypical mycobacteria") are zoonotic bacteria associated with aquatic species.[84] Mycobacteria are found in both fresh and salt water environments. Fish infected with *M. marinum* can develop visceral granulomas and skin ulceration.[85] Humans typically contract the disease through contamination of a preexisting wound when they are conducting activities such as handling fish or cleaning tanks.[86,87] Disease in humans usually manifests as self-limiting granulomas on extremities and has been called fish tank granulomas, fish handlers' disease, and fish fanciers' finger. Infection can progress and become more invasive, particularly in immunocompromised patients.[86,88,89] Recently, a novel clinical presentation of eczema-like scaling and crusting was described in three patients.[90] Mycobacteriosis is treated with combination antibiotic therapy for a prolonged duration. Surgical excision may also be indicated.[86] Effective colony management and use of PPE will mitigate risk of transmission of atypical mycobacteriosis.

Salmonella is a gram-negative bacterium with two species and thousands of serovars. This organism may be present as part of the normal gut flora in some species. Crowding, stress, and poor husbandry can be contributing factors in disease outbreaks. *Salmonella* has long been associated with reptiles, particularly turtles. Bearded dragons, iguanas, corn snakes, boa constrictors, frogs, and salamanders have also been implicated in transmission of *Salmonella* to humans.[91,92] Birds are susceptible to *Salmonella* infections, with poultry, pigeons, and even psittacines linked to human cases.[93] Animals that are positive for *Salmonella* may be asymptomatic or may exhibit a variety of signs, ranging from diarrhea and dehydration to visceral granulomas, arthritis, and sepsis. Animal-to-human transmission occurs primarily through contact with feces or contaminated surfaces. In humans, *Salmonella* typically causes headache, fever, and gastrointestinal signs. Frequent hand-washing, good sanitation and husbandry practices, and use of appropriate PPE will diminish likelihood of transmission of *Salmonella*.

Vibrio vulnificus is a bacterium found in marine environments and has an affinity for warmer temperature and lower salinity. The organism can cause hemorrhagic and ulcerative disease in fish, including species such as eels and pompano. In humans, infection of a preexisting skin wound can result in painful necrotizing infections and even septicemia.[94–96]

Streptococcus iniae is a gram-positive bacterium that infects fish, including tilapia, catfish, and hybrid striped bass. Infected fish demonstrate clinical signs and lesions of the central nervous system. In humans, the organism can infect wounds and cause cellulitis. Endocarditis and meningitis can occur with systemic infections.[88,96,97]

Erysipelothrix rhusiopathiae is a gram-positive bacterium that is found worldwide in a wide variety of species, including birds, reptiles, fresh and salt water fish, and cephalopods.[98] Turkeys are especially sensitive and develop skin discoloration, diarrhea, depression, and septicemia.[99] The organism can also be found in the protective mucous layer of fish. Recent reports of fish disease include hemorrhagic septicemia in eels and cutaneous hemorrhage and necrosis in ornamental tropical fish.[100,101] Wound contamination during handling infected animals is the primary means of transmission to humans. The disease in humans manifests as a localized cutaneous infection ("erysipeloid"), which can be quite painful; a generalized cutaneous cellulitis; and septicemia, which may have accompanying endocarditis.[102]

Dermatophilus congolensis is a filamentous bacterium that causes exudative skin lesions and has been described in a variety of species, including crocodilians. Humans contract the disease through contact with infected animals. In humans, the disease typically manifests as self-limiting pustules, furuncles, or eczematous lesions.[98]

Viral Zoonoses

The primary zoonotic viral diseases in birds are Newcastle Disease, avian influenza, and West Nile Virus. Newcastle Disease is caused by a paramyxovirus and is of most concern in poultry. Disease in birds is characterized by gastrointestinal, respiratory, and neurologic signs. Humans can be infected by direct contact with infected birds, especially chickens. Newcastle Disease can cause conjunctivitis, headaches, and fever in humans.[98,103]

Avian influenza is an orthomyxovirus that infects birds and can be transmissible to humans. Virus is shed in droppings and respiratory secretions. Free-ranging and migratory waterfowl frequently act as carriers. Clinical signs in affected chickens and turkeys are variable and can include respiratory disease, comb and wattle edema, and neurologic disease.[99] Avian influenza can cause severe respiratory disease in humans.[98]

West Nile Virus is transmitted by mosquitoes and has affected over three hundred bird species in the United States. Crows, hawks, and owls are especially susceptible. Affected birds show various neurologic signs, including ataxia, paresis, and seizures. Infected humans may be asymptomatic or show signs of encephalitis.[104] Treatment is supportive. Practices to prevent avian viral diseases include limiting exposure of captive animals to wild carriers, having effective quarantine and management practices, and proper use of PPE.

Fungal Zoonoses

Histoplasma capsulatum is a fungus commonly associated with dove and pigeon feces and can cause respiratory disease in humans. Good sanitation and husbandry practices will diminish potential transmission to humans.[93]

Microsporum gallinae, a dermatophyte of poultry, causes scaly cutaneous lesions. This disease can be transmitted to humans by direct contact with infected birds.[99] Proper use of PPE will prevent bird to human transmission.

Conclusions

Ensuring personnel safety in animal facilities housing nontraditional species can pose unique challenges. Enlisting help

from construction and safety experts well-versed in the design of aquatic and avian facilities can ensure provision of safe and functional housing units. Understanding basic biology and behavior of the particular species and consultation with specialists in the field to assist with development of current best practices will address concerns related to handling, restraint, and housing. Review of literature regarding zoonoses, particularly recent case reports and population studies, will help in determining proper PPE and other precautions when dealing with unfamiliar species. Attention to these details in the planning stages will result in optimal and safe environments for nontraditional research animals and the personnel caring for them.

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Review Article

Replacement, Refinement, and Reduction in Animal Studies With Biohazardous Agents

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Abstract

Animal models are critical to the advancement of our knowledge of infectious disease pathogenesis, diagnostics, therapeutics, and prevention strategies. The use of animal models requires thoughtful consideration for their well-being, as infections can significantly impact the general health of an animal and impair their welfare. Application of the 3Rs—replacement, refinement, and reduction—to animal models using biohazardous agents can improve the scientific merit and animal welfare. Replacement of animal models can use in vitro techniques such as cell culture systems, mathematical models, and engineered tissues or invertebrate animal hosts such as amoeba, worms, fruit flies, and cockroaches. Refinements can use a variety of techniques to more closely monitor the course of disease. These include the use of biomarkers, body temperature, behavioral observations, and clinical scoring systems. Reduction is possible using advanced technologies such as in vivo telemetry and imaging, allowing longitudinal assessment of animals during the course of disease. While there is no single method to universally replace, refine, or reduce animal models, the alternatives and techniques discussed are broadly applicable and they should be considered when infectious disease animal models are developed.

Key words: alternatives; ethogram; humane end points; imaging; in vitro; infectious disease; reduction; refinement; replacement; score sheet; telemetry; temperature

Biohazards, infectious agents that pose a risk to human health and the environment, have plagued humans for centuries, and the crusade to identify disease progression and pathogenesis, develop diagnostics and therapeutics, and identify preventative strategies has included the use of animal models. The National Institutes of Health has invested millions of dollars in

biocontainment research facilities with animal holding capabilities, including two designated National Biocontainment Laboratories and 12 Regional Biocontainment Laboratories to facilitate research on biodefense and emerging infectious diseases.[1] Research into these agents has steadily increased over the past 16 years and does not appear to be decelerating as emerging agents, antimicrobial resistance, and bioterrorism continue to be threats (Figure 1).

Animal use in biohazard studies requires careful consideration of the well-being of the animals involved, as their health will undoubtedly be compromised, resulting in potential pain or distress. It is incumbent upon the investigators and Institutional Animal Care and Use Committees to properly address animal welfare concerns and consider the 3Rs established by Russell and Burch.[2] The 3Rs outlined in *The Principles of Humane Experimental Technique* are replacement, reduction, and refinement. The aim of these tenets is to improve the treatment of research animals while advancing science.[3] They are based on the belief that humane treatment of animals is required for sound science, as experiments conducted with animals that experience the least possible distress produce better scientific results.[3,4] Applying the 3Rs to infectious disease studies (synonymous with biohazards for our purposes) can be very challenging given that infection will usually result in distress in the animals as clinical disease develops; however, the long-term benefit to human and animal health can be tremendous. Therefore, we are ethically obligated to apply the principles of the 3Rs to minimize the impact the infectious agent may have on the well-being of the research animals.

Both the Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals state that the Institutional Animal Care and Use Committees must consider alternatives to painful and distressful procedures.[5,6] The 3Rs are often used as a basis to identify alternatives. Although not entirely in alignment with Russell and Burch's definition of the 3Rs,[3,4] the *Guide for the Care and Use of Laboratory Animals* (the Guide) defines the 3Rs as follows: "*Replacement* refers to methods that avoid using animals. The term includes absolute replacements (ie, replacing animals with inanimate systems such as computer programs) as well as relative replacements (ie, replacing animals such as vertebrates with animals that are lower on the phylogenetic scale). *Refinement* refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. *Reduction* involves strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information."[7]

Replacement

Perhaps the most challenging application of the 3Rs in infectious disease studies is replacement. Many aspects of the disease processes cannot be fully mimicked without a whole-body response. Nonetheless, there are examples of absolute and relative replacements in infectious disease studies. The examples below show the promise these replacements bring to understanding organismal biology, disease pathogenesis, and propelling drug discovery forward.

In Vitro, Mathematical, and Computer Models

In vitro approaches such as cell cultures, organoids, organs-on-a chip, 3D printed tissues (additive manufacturing), and mathematical and computer modeling expand our ability to study biohazardous agents while minimizing the use of animals. In vitro studies facilitate exploration of cellular, molecular, and genomic mechanisms of disease. They also support investigation of potential therapeutic agents. Since animal models may not adequately replicate responses or pathogenesis of human disease, these approaches play a complimentary role.[8] Garira recently performed an extensive review of multiscale models of infectious disease systems.[9] This review describes the use of a systems approach to categorize infectious disease information into a series of scales based on host, pathogen, environment, and health intervention. Once completed, these multiscale models will pave the way for new alternative applications with increased predictive value for these complex systems.

Citing the lack of suitable animal models, the need for better predictors of human immune responses, and the multitude of pathogens that grow poorly outside the human host, all of which hamper the ability to study infectious agents, genetically modify them, or develop appropriate therapies, Mills and Estes hypothesized that advances in tissue engineering and increased

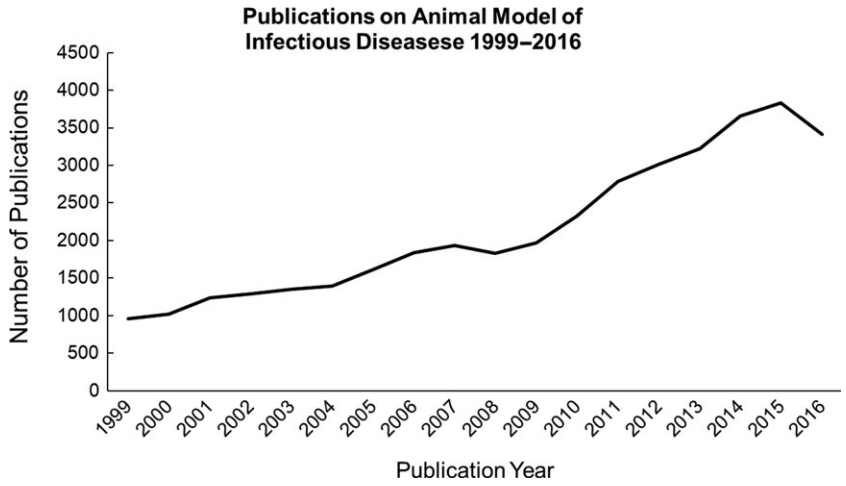

Figure 1. Publications resulting from a PubMed search using the search terms "animal" and "model" and "infectious disease" from 1999 to 2016.

scientific collaboration provide pathways to overcoming these barriers.[10] They describe a variety of in vitro models from different organs useful for studying disease pathogenesis. There have been several recent reviews of in vitro models of infectious diseases, particularly engineered approaches.[11–19] Engineered tissue models range from relatively simple systems with an air-liquid interface with one to two cell types to more complex systems mimicking tissue and organ functionality.[13,14,19] In vitro approaches allow for more control and manipulation than possible in whole animal systems. Use of pluripotent stem cells capable of self-regeneration greatly magnifies the capabilities of these systems.[12] While these systems are enormously useful, there are some limitations to their effectiveness:[13,14] they are not appropriate for long-term studies and they do not contain all the systems in a living organism. Nevertheless, in vitro systems facilitate standardization, a higher throughput, may be less expensive, are more humane, and may allow better understanding of disease progression at the molecular level.[19,20]

Cell Culture

Cell culture systems have played a key role in understanding the pathogenesis of infectious diseases and development of therapeutic agents against those organisms. Such systems have typically involved a single layer of cells in a culture dish although microcapillaries have been used as well.[11,21] Both primary cells and cell lines are employed in studying infectious agents. Cancer cell lines have helped elucidate the mechanism of action of anthrax toxins such as lethal toxin, toxin capillary morphogenesis gene-2, and Tumor Endothelial Marker 8.[22–26] The fundamental steps in the pathogenesis of inhalational anthrax including cellular spore uptake, germination conditions, cellular translocation of spores, and antimicrobial efficacy have been demonstrated using this approach.[21,27–30] New virulence factors for *Yersinia pestis*, which could serve as potential vaccine candidates, have been developed using the RAW 264.7 murine macrophage cell line.[31] Several model systems exist for studying tuberculosis in vivo and in vitro.[11,32,33] Cultured macrophages have been used in *Mycobacterium tuberculosis* studies of pathogenesis and disease progression, escape from phagolysosomes, intracellular multiplication, and gene expression.[34–37] Cell culture systems are critical for rapid screening of new drugs and speeding translation in vivo.[38] Although much progress has been made with in vitro models, it is important to acknowledge the differences in performance between in vitro and in vivo systems; generally, in vitro models are not complete replacements for animal models.[30,39]

Engineered Tissues and 3D Models

A major advance in the cell culture models has been the development of engineered tissues and 3D models, some of which can be printed onto a matrix and used for drug development and disease progression.[19,40,41] Various approaches and the future of tissue engineering with applications to viral infections have been reviewed.[42] In vitro models using intestinal cells for studying host-microbe interactions, including co-culture approaches, tissue explants, bio-reactors constituting and modulating physiological conditions, organoids developed from pluripotent stem cells, and 3D matrices that allow self-assemblage and differentiation into apical lumens and basal environments, have been described.[43]

There are different model systems for studying tuberculosis, and a synopsis of the scientific potential, limitations, costs, infrastructure requirements and skills needed was recently reviewed.[44] 3D organoid models provide a unique means of investigating this devastating disease. A human lung model consisting of lung-derived cell lines and peripheral blood macrophages reportedly displays characteristics of lung tissue, including stratified epithelium, extracellular matrix, and mucus secretion. Infection of this tissue model with *M. tuberculosis* results in formation of what resembles early tuberculosis granuloma.[33] A 3D tissue-specific model consisting of epithelial cells and fibroblasts cultured on a collagen matrix on top of a porous matrix using organotypic culture methods is reputed to stratify and secrete matrix at the air interface.[45] Using primary human macrophages infected with *M. tuberculosis*, migration of immune cells and development of granulomas occurred.

Organotypic cultures of human lung slices have been used to study anthrax spore movement between antigen presenting cells and alveolar epithelial cells, and the influence of anthrax toxin on the process.[27] Entry of spores into the lung is a critical step in the development of the disease, and this approach provides a reproducible way to understand the mechanism. A 3D model of human airway tissues grown on a collagen/fibronectin gel support at an air-liquid interface mimicking in vivo situation has been developed.[46] The system models anthrax inhalation and permits understanding of the role of the constituent cells.

Mathematical Models

Mathematical models can elucidate the behavior of infectious agents under different conditions and are particularly useful in epidemiological studies. This approach has been used to effectively forecast spread and control of several infectious diseases.[47–51] Using hierarchical Bayesian and multilevel mathematical models to incorporate information on disease dynamics of host phylogeny, Banerjee determined that smaller passerine birds are more competent at spreading the disease caused by West Nile Virus compared with larger nonpasserine species.[52] Li used mathematical modelling to develop an in vitro assay to quantify neutralizing activity against anthrax lethal toxin. The assay is valid for human, rhesus, and rabbit sera. It is crucial in the quantification of anthrax lethal toxin, assessment of anthrax vaccines, and avoids the use of animals.[53]

In Silico Approaches

Computer modeling plays a critical role in studying infectious diseases and in the development of new therapies. A computational design for a protein that could be used as a potential therapy for anthrax has been developed.[54] In this study, the *Bacillus anthracis* poly-γ-D-glutamic acid capsule was targeted by modifying CapD, a *B. anthracis* γ-glutamyl transpeptidase. By making circular permutations to CapD using computational protein engineering via a Rossetta software suite,[55,56] the quality and production of the enzyme was improved. The role of CapD was further explored using a Gauss View 5 graphical user interface and to identify the rate-limiting steps of CapD catalysis and inhibition.[57,58] Other approaches to understanding new lead compounds to treat this disease have been proposed using computational methods.[49,59–61]

Invertebrates

Relative replacements for infectious disease research have included the use of less sentient species, including amoeba,

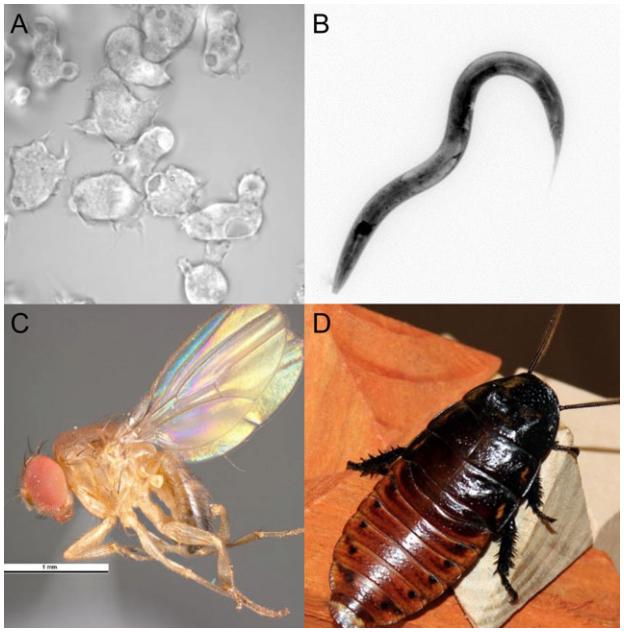

Figure 2. Invertebrate species used to study biohazardous agents. (A) *Dictyostelium discoideum* (image courtesy of Brad Borlee, Colorado State University). (B) *Caenorhabditis elegans* (image courtesy of Tai Montgomery, Colorado State University). (C) *Drosophila melanogaster* (Pest and Diseases Image Library, Bugwood.org). (D) *Gromphadorhina laevigata* (image courtesy of Mathew Camper, Colorado State University).

Caenorhabditis elegans, wax moths, fruit flies, and cockroaches (Figure 2). These models may be desirable because of their ease in cultivation, low maintenance costs, minimal space requirements, and minimal ethical concerns.[62] Several studies using these species involve innate immune responses, which have been demonstrated to be similar to the mammalian innate immune response.[63]

The protozoa, *Dictyostelium discoideum*, can be genetically modified and shares many cellular defense mechanisms present in mammals, particularly related to phagocytosis. Bacterial virulence factors required for survival in mammalian hosts also permit survival in *D. discoideum*.[64] *D. discoideum* has been used as a model for *Legionella pneumophila*, *Mycobacteria* sp., *Klebsiella pneumonia*, *Yersinia pseudotuberculosis*, *Pseudomonas* sp., and others. Studies with *D. discoideum* have provided insight into how *L. pneumophila* evade the host immune response permitting bacterial replication. Mycobacterial studies with *D. discoideum* have provided insight on intracellular survival and cellular spread. *Nramp1* mutants of *D. discoideum* negatively affect Mycobacterial growth, similar to *Nramp1* in mice.[64]

C. elegans has been used to evaluate virulence of *Salmonella enterica* serovar Typhimurium and response to therapy.[65] *C. elegans* mutants are readily available and when combined with bacterial mutants enable investigators to study a specific host response to a virulence factor.[66]

Galleria mellonella, the greater wax moth, has been used to study the virulence of several pathogens including *Burkholderia cepacia* complex,[67] *Pseudomonas* sp.,[68] and *Streptoccous* sp.[69] In addition to possessing an innate immune response similar to mammals, *G. mellonella* experiments can be performed at 37°C, which is the body temperature of the natural hosts.[69] This is in contrast to Drosophila, which is typically reared at 25°C.[70] Studies using *Streptococcus suis*-infected *G. mellonella* larvae demonstrated similar virulence compared with the pig model, and the infection model initiated the larvae's innate immune response. This model was also used to assess the response to antibiotic therapy, and antibiotic treatment following inoculation resulted in larval survival.[69]

Fruit flies (*Drosophila melanogaster*) have been shown to be beneficial to study the virulence and innate immune response to a number of pathogens, including *Burkholderia* sp.,[71] *Pseudomonas aeruginosa*,[72] *S. enterica* serovar Typhimurium,[73] and *Cryptococcus neoformans*.[74] Studies involving *Pseudomonas*-infected Drosophila were able to identify bacterial mutants with reduced virulence.[72] This model could be used for high-throughput screening of bacterial isolates. Further, the authors were able to identify defects in the Drosophila immune response through genetic mutations that revealed insight into the host immune response to *Pseudomonas* infections.

The Madagascar hissing cockroach (*Gromphadorhina laevigata*) has been used as a model to study virulence determinants and immune responses to *Burkholderia* species.[75] The authors found that the relative virulence of several mutant strains of *Burkholderia pseudomallei*, *B. mallei*, and *B. thailandensis* was comparable to the virulence in a Syrian hamster model. They also reported high numbers of *B. pseudomallei* in the cytoplasm of the hemocyte, a component of the insect innate immune response, with multinucleated giant cell formation, providing potential mechanisms for immune evasion. While these replacements of higher animals have limitations, they can be used as an important model for subsequent studies in higher hosts.

Refinement

Refinements to experimental procedures should be used to improve the well-being of animals in infectious disease studies. Many of these refinements take advantage of emerging technologies that allow investigators to identify disease progression in a longitudinal manner. These assessments can help identify humane end points precluding prolonged distress in the animal model. The Guide defines humane end points as "the point at which pain or distress in an experimental animal is prevented, terminated, or relieved."[7] The ability to identify an animal experiencing clinical signs early in the course of disease, and as it is progressing, is crucial to determining a more humane end point during experimentation that avoids unnecessary pain and distress.[76] By determining an earlier end point, we are able to reduce or even avoid unnecessary pain or distress that animals may experience when infected with viral, bacterial, or fungal agents and still obtain the results needed for the experimental design. The refinements discussed can be variable depending on the animal models, agent, route of infection, and housing conditions; a one-size-fits-all approach is unlikely to be effective.[77]

Biomarkers

There are a number of biological mediators released by the body in response to infections that could serve as potential biomarkers for infectious disease models. Cytokine perturbations are frequent during infectious disease progression. In a nonhuman primate model of simian immunodeficiency virus, cytokines and chemokines were measured following infection and a differential expression of various cytokines was identified between progressive and nonprogressive infections.[78] While the aim of this study did not involve these measurements for end point determination, these findings highlight the concept of using biological mediators such as cytokines to assess

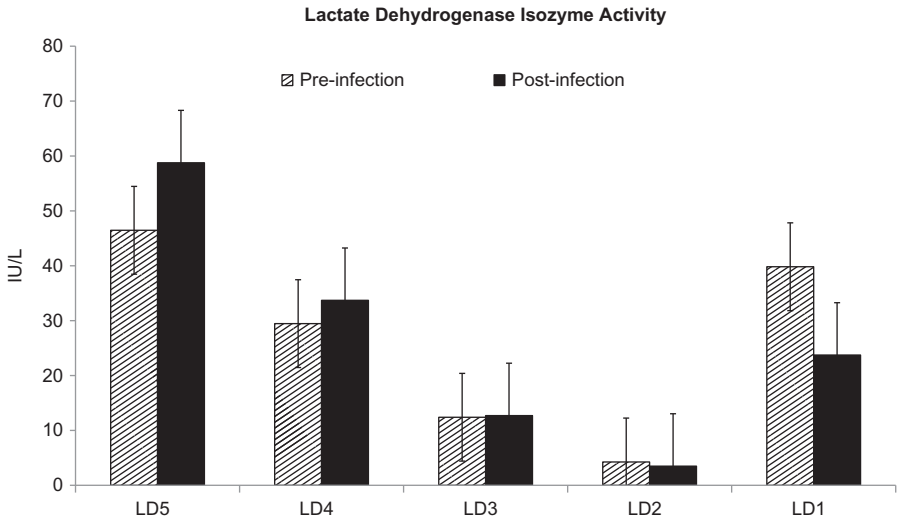

Figure 3. Lactate dehydrogenase isoenzyme activity following infection with *Mycobacterium tuberculosis* in guinea pigs (Murray et al. 2016).

disease progression. These changes will likely be specific to the agent and the model and will need to be developed on a case-by-case basis.

Acute phase proteins, which are nonexistent or minimally present in the blood of healthy animals,[79] are produced in response to a variety of stimuli including trauma, infection, stress, neoplasia, and inflammation and may provide an objective marker for humane end points in infectious disease models.[80] Examples of common acute phase proteins include C- reactive proteins, serum amyloid A, haptoglobin, α2-macroglobulin, and others.[79,80] In a nonhuman primate model of *Bordetella bronchiseptica*, C-reactive proteins were 54 to 96 times the baseline values, suggesting that longitudinal monitoring of C-reactive protein could provide a means of studying disease progression.[81]

Serum chemistry and enzyme activity may also be used as biomarkers. Lactate dehydrogenase (LD) and isozyme levels have been used as diagnostic and prognostic tools for numerous human diseases, including liver disease,[82] myocardial infarction,[83] hematological disease,[84] and malignancies such as lymphoma and leukemia.[85,86] Studies in human infections of tuberculosis have demonstrated that LD may be elevated in the serum and pleural fluid of infected individuals. Moreover, some studies have shown that tuberculosis-infected humans have altered serum LD isozyme profiles, particularly an elevated fraction of LD-3.[87,88] Guinea pigs similarly express all 5 LD isozymes. Lactate dehydrogenase isozymes from guinea pigs prior to inoculation with *M. tuberculosis* and 4 weeks postinoculation were evaluated.[89] Guinea pigs infected with *M. tuberculosis* had elevated LD-5 and attenuated LD-1 (Figure 3), suggesting a correlation between disease advancement and serum LD isozymes and the potential for the future development and use of isozymes as a biomarker for tuberculosis disease progression.[89]

Temperature and Thermography

Body temperature is an objective measurement that has been used in experiments to determine end stages of disease.[90,91] An elevated body temperature can indicate a systemic defense mechanism in response to an infection and is associated with behavioral changes such as lethargy, anorexia, and reduced grooming that can be assessed during the course of disease.[92] A drop in body temperature below a certain point can indicate imminent death.[79,90,93] Using mice as an example, the normal body temperature in a mouse ranges from 37.0°C to 37.2°C (98.8°F–99.3°F), and a decrease of more than 4.0°C to 6.0°C correlates to impending death after multiple types of immune system challenges, including snake venom and fungal inoculations.[90,91] However, this is not always the case, as recovery has been reported even after temperatures dropped to below 28.0°C.[94]

Efficiently and effectively measuring the body temperature of rodents often proves to be a difficult task and depending on how much stress the animal endures during this process, it may lead to a falsely increased temperature. Thermistor probes, surface temperature probes, and implantable transponders, some of which require frequent handling of the animals, have all been used to measure temperature. Infrared cameras have been used more recently as a noninvasive means to determine body temperature in animals. To demonstrate the utility of thermography in assessing body temperature, 3 mice were intranasally inoculated with *B. pseudomallei* and body temperature was recorded from transponders and thermography.[95] Thermography assessed in 3 different regions of the body—eye, ear, and flank—correlated with body temperature acquired by transponder during the 3 days postinfection (Figure 7). Regardless of the means of acquiring temperature, baseline measurements are critical for any temperature monitoring to be successful. This is demonstrated in the following example using body temperature as a means to monitor *Francisella tularensis* infection in mice.[96]

In this study, mice were implanted with temperature transponders and inoculated intradermally with 4 different strains of *F. tularensis*. Temperatures recorded every 1 to 2 hours postinoculation revealed 3 distinct phases: normal, febrile, and hypothermic. The authors found a correlation between the time of death and change in temperature between the phases. They also identified an individual "drop point" (an end point for death based on temperature) for each mouse based on the first temperature after the febrile phase to fall below the mean normal phase and determined the frequency of temperature monitoring to be no less than every 6 hours for their model. While the temperature patterns were similar for all mice, the length of each phase varied based on the bacterial strain; the time to reach a certain phase was also variable. This example demonstrates the ability of temperature monitoring to determine

humane end points. However, it also serves as a reminder of the complexity of infectious disease experiments in which factors such as bacterial strain can impact the utility of a previously used end point. Therefore, it is important to continuously assess the chosen end point information to determine if adjustments are necessary.

Clinical Signs and Score Sheets

The use of score sheets to guide animal observations and record animal conditions as a means of identifying appropriate humane end points is not novel[97] but is an important concept of refinement.[3] It is particularly useful in infectious disease research where animals may experience pain or distress during the course of disease. Score sheets not only assist in minimizing the duration of pain or distress but also may reduce the number of animals used in study as intervention via euthanasia allows greater opportunities for sample collection and yields more clinical and research data over death. Scoring systems should provide clear and concise information that highlights the clinical signs of particular relevance, which in turn increases reproducibility. Retrospective analysis of score sheets from completed experiments permits modification of future score sheets to identify the earliest possible clinical signs as surrogate markers for death.[98]

Score sheets should be dynamic documents, refined based on new observations during the course of the experiments. Score sheets must be adapted for different species, different experimental agents, and other variables that can occur from experiment to experiment. Therefore, there is no universal score sheet, but there are points to consider in the overall design.

Score sheets must focus observers on clinical signs that are unambiguous and have the most influence on animal welfare. The design starts with information about the species and agent or disease process in the study. Ideally, the design should occur in conjunction with protocol and endpoint development. The score sheet must clearly indicate the monitoring schedule, the anticipated clinical signs, and the end points incorporated in the protocol in a manner that facilitates its use by all observers.

The use of score sheets is particularly important for pilot studies if the research staff has limited or no experience with the test agent and no descriptions are available in the literature. The goal of the pilot study is to determine the time course and behavior of the animals after exposure to the agent or induction of disease process. This may require death as an end point to identify surrogate markers predicting death. The observations during pilot studies must be frequent and occur during the light and dark cycles. Four times a day monitoring (every 6 hours) is a suitable starting point for observations for those studies with an unknown outcome. An example pilot observation sheet is provided in Figure 4.

Depending on the overall goals of the project, pilot studies conducted as an LD50 study may be appropriate, especially if recovery after significant illness is expected.[99] It is critical for the research staff to understand that the point of the study is to identify surrogate markers for death. Administering a dose greater than the LD50 will likely result in a rapid death, which might make it difficult to determine what clinical signs or combination of clinical signs predict death. Small numbers of animals, for example 10, for these types of study are usually sufficient as long as an appropriate dose is used.

Once pilot data or literature information determines which clinical signs are predictive, then extraneous clinical signs can be excluded from the scoring, and the predictive clinical signs can be used in the pivotal study. For example, the authors (Dohm) performed a pilot study in preparation for a mouse influenza projective that identified the predictive clinical signs and monitoring frequency that enabled euthanasia 24 to 36 hours before death (Figure 5). Using this score sheet, the investigator was able to collect viral and experimental vaccine titers from nearly every animal, which was not previously possible.

Interpretation of clinical signs is critical and can be variable. For example, “difficult breathing” may seem explicit; however, different observers may have dramatically different perceptions and interpretations. In a mouse influenza study, the protocol listed “difficult breathing” as the primary criteria to euthanize animals before the planned time point or death. Some staff familiar with the agent may perceive this as open-mouth breathing and very close to moribund, while other staff may define it as a more rapid breathing but otherwise normal. Definitions of clinical signs should be unambiguous and clearly understood prior to their inclusion in score sheets and underscore the need for training for observers.

The “score” system does not need to be complex and can be more of a binary function, observed or not observed. This simplifies the scoring to ensure compliance rather than maximize the data from the score sheet. As the research staff uses the sheets, they may continue to refine and reintroduce scales for each observation.

Score sheet can also be used conditionally based on the onset of clinical signs. For example, with some tuberculosis studies, clinical signs may not be present for a year or more, or studies with highly virulent infections can be very short in duration where no clinical signs are expected. However, when or if clinical signs appear, then it is important to institute the score sheet. The frequency of monitoring can also change based on clinical signs. The example provided in Figure 6 indicates that twice daily observations are required and that use of the score sheet becomes mandatory when any animal in the experimental group begins to show signs. The varying needs of the study and welfare of the animals will dictate the best possible configuration of the form.

The information and examples provided here focus on mice, because they are the most commonly used mammalian laboratory animal. However, many references are available that list options for clinical signs in other species, including humans.[98,100–109]

Ethograms

Ethograms are a means of assessing well-being by characterizing species-specific behaviors, recognizing that normal behaviors may be altered when the animals are distressed. There are a number of behaviors that can be evaluated, including general activity, grooming, sleep, postures, social interactions, reproductive behaviors, nesting behavior, and feed and water consumption.[110] Many of these parameters can be incorporated into a score sheet for clinical assessments as described above.

Activity and Appearance

Much information can be gained by observing individual behavior, interactions with cage mates, daily activities, and basic self-preservation. Watching these interactions, or lack thereof, may provide the first indication that an abnormality exists.[97] Mice, like most animals, have decreased activity levels and interactions with cage mates when they are distressed. An abnormal, depressed, or moribund mouse may be less mobile, have

Protocol endpoints: Death as an Endpoint Pilot Protocol – death is required to determine if surrogate markers are detectable prior to that point. Animals will receive supportive care in the form of gel diet placed on the cage floor once clinical signs are noted. The study will end on day 14 after infection.

Morning and Evening: temperature and behavior check. **Noon:** weight and behavior check. **Night:** behavior check, unless otherwise indicated by **Behavior Score.**

Behavior Score: Each check equals one point. Add checks, record in 'Behavior score' column. Totals of 4 or more require additional intervention, i.e. temp and/or wt check.

Date			Cage/Group	

Time	Mouse No		Weight	Rectal Temp	Behavior(✓)											Behavior Score Total	Euth (E)/ Died (D)/ Include time	Initials
					Normal	Ruffled fur/ Hunched	Shaking, Ataxic/ Seizures	Head tilt/ Other neuro. signs	Squinty eyes/ Sunken eyes/ Diarrhea	Discharge– Ocular/ Nasal/ Inside front legs	Abnormal resp.– Rapid shallow/ Slow labored/ Other	Open mouth breathing	Sunken/ Depressed Abdomen	No movement in 30–60s when isolated/ Recumbent	Loss of righting reflex			
	1	Morn.																
		Noon																
		Eve.																
		Night																
	2	Morn.																
		Noon																
		Eve.																
		Night																

Figure 4. Relatively generic score sheet appropriate for pilot studies where the test agent has little to no previous characterization. Note this form tracks individual animals and attempts to collect as much information as possible to identify humane end points and thereby eliminate the need for death as an end point.

Protocol endpoints: mice will be euthanized when the rectal temperature reaches 86°F, >35% weight loss, or if they exhibit loss of righting reflex.

Morning and Evening (at least 8 hours apart): temperature and behavior check. **Daily:** weight check.

Behavior Score: Each check equals one point. Add checks, record in 'Behavior score' column. Totals of 4 or more require increased monitoring, every 6 hours.

Date			Cage/Group	

Time	Mouse No		Weight	Rectal Temp	Behavior(✓)											Behavior Score Total	Euth (E)/ Died (D)/ Include time	Initials
					Normal	Ruffled fur/ Hunched	Shaking, Ataxic/ Seizures	Head tilt/ Other neuro. signs	Squinty eyes/ Sunken eyes/ Diarrhea	Discharge– Ocular/ Nasal/ On inside front legs	Abnormal resp. –Rapid shallow/ Slow labored/ Other	Open mouth breathing	Sunken/ Depressed Abdomen	When isolated no movement within 30–60s/ Recumbent	Loss of righting reflex			
	1	Morn.																
		Noon																
		Eve.																
		Night																
	2	Morn.																
		Noon																
		Eve.																
		Night																
		Eve.																
		Night																

Figure 5. Score sheet for influenza study developed based on a prior pilot study. Note this form tracks individual animals, but for this protocol only requires twice a day monitoring for 5 to 6 days.

Endpoints: Mice euthanized at 0, 24, and 48 hours post infection, or if moribund/lack righting reflex.

Daily: Behavior check starting 24 hours after aerosol infection, twice a day, at least 8 hours apart. No clinical signs are expected, but if any animal in the group exhibits any, use this sheet. Animals with clinical signs have hydration evaluated, +/– given SQ or IP fluids, and provided gel diet on the cage floor.

Behavior Check: For each cage check appropriate behavior column, indicate number of animals, if any, found dead or euthanized for health reasons.

Date of Aerosol Exposure								Experiment/Agent							
Cage #		Behavior ✓		Euth (E)/ Died (D)	Date	Time	Initials	Cage #		Behavior ✓		Euth (E)/ Died (D)	Date	Time	Initials
		Normal	Ruffled fur/ Hunched							Normal	Ruffled fur/ Hunched				
1	Morn.				Comments:			1	Morn.				Comments:		
	Eve.				Comments:				Eve.				Comments:		
2	Morn.				Comments:			2	Morn.				Comments:		
	Eve.				Comments:				Eve.				Comments:		

Figure 6. Score sheet for short study where clinical signs are not expected. Information on the score sheet specifies when to use the sheet based on clinical signs noted in the animals.

an unkempt appearance, or decreased coat sheen due to decreased desire or effort to properly groom itself.[97,111] Decreased or absent grooming can lead to a buildup of ocular secretions around the eyes and nose, such as porphyrin, and excess anal and skin gland secretions, all of which cause a disheveled and unkempt appearance.

In mice, overall appearance is best identified before outside influence such as room entry, cage manipulation, or handling alter behavior. Abnormal behaviors may be masked as mice are a prey species. By the time mice display overt clinical, it is likely that pain or distress may have been overlooked or gone unnoticed.[97]

A pain scale based on facial appearances, known as the "mouse grimace scale," was recently developed in mice. The mouse grimace scale rates orbital tightening, nose bulge, cheek bulge, ear position, and whisker change on a 0 to 2 scale, where higher ratings are more indicative of pain.[112,113] Similar grimace scales have been developed for rats, rabbits, dogs, sheep, and horses.[114–117]

Nest Building

Nest building is a common behavior in rodent species. Functions of a nest include thermoregulation, rearing and maternal activity, avoidance of predators, shelter from harsh lighting, and protection from environmental conditions.[118,119] Nest material is considered part of environmental enrichment, allowing for sensory and motor stimulation. In the laboratory setting, nests may act to shield the mouse from humans and external stimuli as well as aid in thermoregulation by acting as insulation.[119] Nest building is a specific behavior of rodents that may be used as a noninvasive tool to estimate pain, distress, neurological dysfunction, and overall well-being.[119–121]

Decreased nest building is observed in some infections, for example, following hippocampal degeneration caused by prion infection.[122,123] Reduced nest building is also a nonspecific response to illness. This is demonstrated when a cage change occurs and the mouse is given fresh material for nest building. Jirkof developed a scoring system, 0 to 5, based on how and to what extent nesting material was manipulated 9 hours after placement.[119] Most healthy mice will start to manipulate fresh nesting material in under an hour of its introduction, typically at the end of the dark phase.[119,121] There have been many modifications to assessing nest-building performance but most have concluded that healthy mice tend to have the most organized nests, whereas postsurgical, painful, or diseased mice have a decreased nest-building activity.[119–121,124]

Time-to-integrate-to-nest test (TINT) is another noninvasive method to assess well-being in mice. This occurs after a nest has already been established and then an additional piece of nesting material is placed in the cage. The TINT is the time required for a mouse to incorporate this new piece of material into their current nest.[118] Healthy mice are highly motivated and usually integrate new nesting material into their main nest within 10 minutes.[118] The test is more accurate if the initial nest built meets the following criteria for healthy mice: a central nest site, a shredding of at least 80% of the provided material, and a slightly cupped shape of a height less than half of what would be required to cover a mouse.[119,124] TINT performed after painful surgeries has resulted in a failed attempt to integrate the material.[118] Although not specifically evaluated for use in infectious disease studies, TINT provides an additional means of refinement as it is a noninvasive and rapid screening test that can be readily performed with little training.

Weight Loss and Food and Water Intake

Food and water intake can be environmental or species dependent, thus it is good to have a baseline from a healthy animal of the specific species or strain being studied to compare to the one under study.[93,125] Alternatively, baseline observations can be performed on the individual animals to determine their specific, normal weight and food and water intake. Fluid intake may be more challenging in smaller species, such as rodents, since they are typically provided a large volume of water in relation to their daily requirement. While a starting weight of the bottle could be measured, it would not include the loss of water due to jostling of bottle during cage changes. It may be more beneficial to collect urine and test osmolality or specific gravity to determine the concentration of the urine assuming that the kidneys are functioning properly.

Fecal production and consistency as well as skin tent can be used to roughly assess hydration. To decrease the subjectivity of skin tent, the time for the skin to return to normal should be timed. A decrease in food and water intake will inevitably lead to a loss in body condition and weight. Animals that have systemic infections can undergo weight loss because of an increase in inflammatory cytokines (TNF, IL-1, and IL-6) that result in elevated leptin, a protein previously demonstrated to cause anorexia.[126] Serum cytokine concentrations may be correlated with weight loss.[127] Cachexia is a common sequelae of anorexia, from which the animal may be unable to recover.[79,126,128] Based on previous publications, a commonly used end point is loss of 10% to 20% of its body weight during the course of a study.[129]

The following provides an example of integrating behavioral and physiological parameters to monitor disease progression.[95] Mice were implanted subcutaneously with a passive integrated transponder (PIT) tag and inoculated intranasally with *B. pseudomallei*. Mice were observed, and a clinical scoring system was applied twice daily during the course of infection. The clinical scoring system was based on a scale of 1 to 4 and accounted for activity, nest building, posture, hair coat, and facial grimace.[130] A score of 1 was considered a normal healthy rodent; a score of 2 was mild illness characterized by subtle behavioral changes, mild orbital tightening, a transient hunched posture; a score of 3 was a moderate illness characterized by obvious behavioral changes, decreased activity, prolonged hunched posture, and moderate to marked orbital tightening; and a score of 4 was marked illness or morbidity characterized by nonresponsiveness to stimulation, slow breathing, and sunken or closed eyes. Body weights were recorded twice daily. Body temperature was measured daily using PIT tag recordings and thermal imaging. Mice became clinically ill by the second day postinoculation with an average clinical score of 4 and a marked reduction in body temperature as measured by PIT tags and thermal imaging (Figures 7 and 8). This demonstrates that the behavioral findings correlated well with the marked drop in body temperature in this model and provides investigators with a more subjective criteria for assessing well-being (body temperature) compared with the more objective clinical scoring. However, if individuals are properly trained, it also demonstrates that proper clinical observations can provide a timely assessment of distress. In this example, the observations were conducted twice daily, and, given the marked changes within a short time during the daily observation, it would be more prudent to make more frequent observations. Nonetheless, it demonstrates the utility of using a score sheet with components of an ethogram and temperature monitoring to assess disease progression to develop an appropriate endpoint.

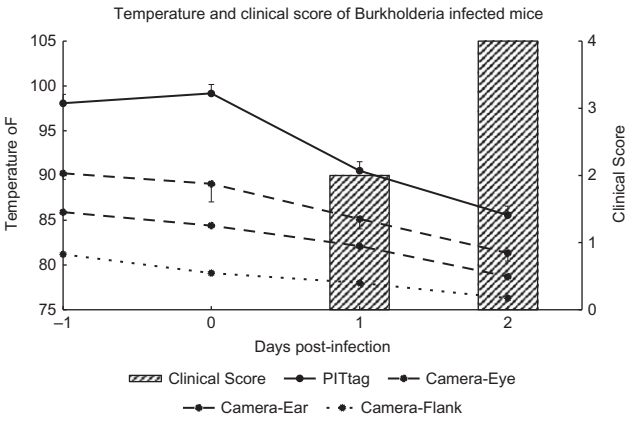

Figure 7. Body temperature and clinical signs associated with *Burkholderia pseudomalli* infection in mice. PIT tag located at the interscapular space. Body temperature acquired using the thermal camera (FLIR) from the eye, ear, and flank of the mouse. Clinical score based on a scale of 0 to 4: 0, normal; 1, potential illness; 2, mild, definitive illness; 3, moderate illness; and 4, severe illness, moribund, requires euthanasia (Lee and Kendall 2015).

Reduction

The ability to reduce the number of animals used in infectious disease models has been substantial given the significant technological advancements in animal research. The most influential of these technologies are telemetry and imaging modalities that

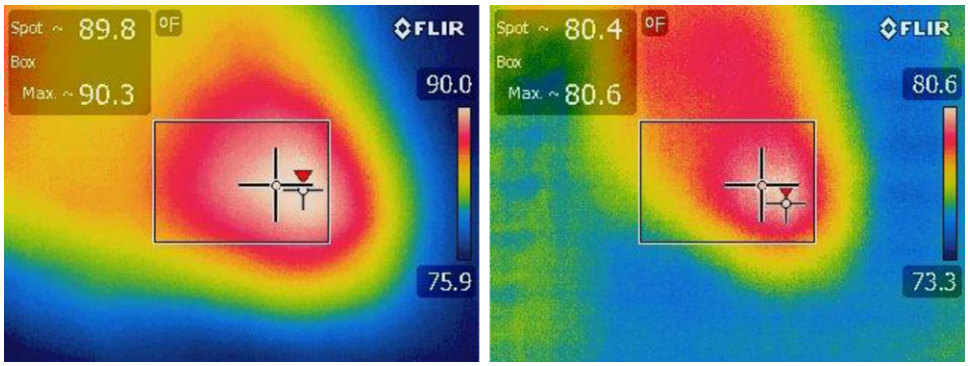

Figure 8. Thermography image of the eye from *Burkholderia psuedomallei*-infected mice demonstrating the drop in body temperature. Left: prior to infection, has a temperature of 90.3°F. Right: 2 days after infection, demonstrates a temperature of 80.6°F (Lee and Kendall 2015).

allow for longitudinal evaluation of animals over time, thereby reducing the need for euthanasia at specific time points in a study. Telemetry offers the ability to continuously monitor an animal's physiological response over time. Implementing the use of imaging techniques into these type of studies allows for a significant reduction in animal numbers in cases where the disease pathology could be followed by imaging at multiple time points in the same animals vs euthanizing at those time points to collect tissues. In some cases, end points may also be adjusted to earlier in the disease process because pathology is monitored in real time permitting for greater precision of the model, a valuable refinement in animal use.[131]

Telemetry

Telemetric monitoring enables researchers to reduce the number of animals used in studies that rely on larger cohorts of animals, varying degrees of monitoring, and moribund or fatal endpoints.[7] It is generally defined as the uninhibited capture and transmission of data to remote locations with computed reception to enable analysis of physiological biomarkers.[132] It has been used by biomedical researchers for over a century and has progressively improved in transmission capabilities, biocompatibility, parameters measured, size, functional lifespan, and ease of implantation.[133–135] A concise historical review of radiotelemetry use in small laboratory animals has been published.[132]

Telemetry can capture a variety of physiologic parameters, longitudinally, in real-time, in unrestrained, conscious animals, depending on the device used. These may include body temperature, heart rate, blood pressure, respiratory rate, and locomotor activity. In addition, biopotential signals such as electrocardiogram, electroencephalogram, and electromyogram can be monitored as required by investigators following challenge. Telemetric monitoring relies on placement of a transponder capable of collecting desired physiologic parameters then transmitting that data to a receiver for subsequent analysis. Data may be obtained noninvasively via external or surface electrodes or through the use of implanted interrogation probes and leads. The frequency of data transmissions can be continuous or intermittent, either at predetermined intervals or as programmed by the investigator. Baseline data collection for study purposes may then be initiated once animals have healed from surgery.[136,137]

Other benefits include noninvasive monitoring during periods of acclimation to gather baseline data as well as activity monitoring during the seldom visualized dark phase, the time at which rodents are the most active.[138] In addition, telemetry allows accurate data collection while reducing animal stress. Brief physical handling, restraint, and rectal probe placement to permit the identification of body temperature has been shown to cause transient iatrogenic elevations in body temperature that may not reflect the true clinical condition of infected animals.[93,139–141] Telemetry was used in a Lassa virus surrogate hamster model to identify postchallenge alterations, including a disruption of the normal diurnal temperature pattern and elevated body temperature not easily and accurately captured through nontelemetric methods.[142]

Refinement and reduction is especially pronounced when the collection of large amounts of physiological data allows for the construction of humane and informative end points in pathogen-induced models of infectious disease. Remote transmission of data with telemetry can be particularly advantageous in studies requiring high containment (animal biosafety level 3 or 4) where multiple animals can be monitored continuously and simultaneously throughout acclimation, challenge, and progression of disease from a relatively convenient location outside of containment.[136,137,142] Depending upon the parameters selected, telemetric monitoring can significantly reduce the number of personnel entries into containment by augmenting the evaluation of animal health through direct observation and minimize the need to handle and disturb cages. Repeated containment entries can be a source of significant financial cost, and direct manipulation of infected animals for collection of data carries some risk to the handler and stress to the animal.[143]

Despite these benefits, it is important to note that although parameters may be identified through telemetric monitoring, each pathogen-induced model exhibits an individual fingerprint of physiological alterations in regards to measurable markers such as time to onset of febrile state and development of terminal hypothermia.[142,144] Thus, each model must be evaluated individually, especially when considering the identification and implementation of humane end points. In fact, routine temperature evaluation has been suggested as a mandatory requirement for monitoring infectious disease studies.[79] Trammell and Toth asked retrospectively if temperature alone or temperature and body weight (T × BW) together could effectively identify humane end points in several mouse models of infectious disease.[145] In some cases, such as DBA/2J mice inoculated with *C. albicans*, temperature and T × BW could be used to identify if mice would live or die. In contrast, these same parameters were found to not be accurate predictors when normal C57BL/6J mice were given the same pathogen. In conclusion, Trammell and Toth found that the application of these end points was dependent upon mouse strain and pathogen interaction, highlighting the requirement for model-specific end points.

Precise monitoring of physiologic parameters permits the implementation of investigational therapeutic strategies or humane euthanasia. Investigators have recently refined a murine cecal ligation and puncture model of sepsis with telemetry by identifying the point of physiologic deterioration by evaluating heart rate, core temperature, and mobility. Independent host response varies, and telemetry allows researchers to include animals at the exact physiologic parameters needed to recapitulate human enrollment criteria rather than relying on predetermined time points. This produces a more uniform group of experimental animals, effectively reducing variability and allowing for appropriate and precise intervention through investigational therapies or timely euthanasia. An added benefit is that the researcher may immediately collect blood and tissues, preventing degradation and loss of data that may occur if impending death is not recognized. Importantly, it also prevents the inclusion of animals that would exhibit spontaneous recovery, regardless of therapeutic intervention.[146]

Telemetric monitoring requires a significant financial investment in implantable transponders, data acquisition systems, and software. Surgical implantation of transponders and associated leads can be technically demanding and postsurgical complications may arise. The applicability of a given transponder can be greatly influenced by the physical size of the device and may preclude implantation in certain anatomic locations, especially in rodents. The batteries powering transponders have a defined lifespan and occasionally malfunction, causing decreased functional capability and limiting the value of collected data. Although uncommon, the loss of transponder functionality postchallenge effectively removes the implanted

animal from the current study. Transponders are frequently single-use rather than repeatedly sterilized for this reason. Despite these apparent drawbacks, the investment of telemetry early can be offset over time through the reduction in animal numbers, a finding exhibited in a sepsis model but likely in alignment with many infectious disease models.[147] Overall, telemetry allows the potential to collect a tremendous amount of clinically relevant data that can inform study design, implement refined end points, and reduce the number of animals required to conduct rodent infectious disease studies.

Imaging

The use of advanced imaging techniques has played an integral part in reducing the number of animals used on projects, and they are increasingly being used to image in vivo disease processes in animals, including infectious disease research models. Infectious disease research invariably requires examining the pathogenesis and distribution of the agent in live systems, and historically this equates to euthanasia of the animals at multiple time points to collect and analyze tissues. This not only leads to a greater number of animals being needed, but it also requires that the disease process is allowed to progress to severe or terminal disease in some animals, which increases the animals' pain and distress. Instead, noninvasive imaging modalities can be used to track disease progression over time. A wide array of imaging modalities are being used for infectious disease studies, including positron emission tomography (PET), computerized tomography (CT), magnetic resonance imaging (MRI), and optical imaging, that benefit researchers and reduce the number of animals used.[131,148,149]

PET is a molecular imaging technique widely used in clinical human and animal medicine to visualize a variety of in vivo biological processes, most commonly neoplastic diseases. Positron-emitting radiotracers are administered, and then the machine detects these emissions and produces 3-dimensional images of their locations within the body. Various radiotracers are used based on the tissue or cell type to be detected. 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) is the most routinely used for cancer studies because malignant cells have an increased uptake of glucose and the radiotracer will concentrate in neoplastic tissues. However, all metabolically active cells will uptake glucose, so this tracer is also useful when tracking cells such as macrophages, neutrophils, and lymphocytes present in infectious disease processes.[149–151] Other radiotracers targeting specific tissues or cells are being developed, which will allow greater specificity in tracking infectious disease processes. For example, Zika virus has been imaged in mice using [^{18}F]DPA-714, which targets a translocator protein that is highly upregulated in central nervous system-activated microglia, reactive astrocytes, and multiple leukocytes.[152] [^{18}F]FDG PET was used to study cerebral malaria in nonhuman primates and demonstrated decreased cerebral metabolic activity.[149] ^{64}Cu-diethylene-triaminepentaacetic acid was administered to mice infected with *F. tularensis* via various routes, and PET imaging demonstrated the rapid dissemination to multiple tissues with different trafficking patterns depending on route of infection.[149]

CT uses sectional x-ray imaging to develop a 3-dimensional image of the anatomy of interest.[150] Attenuation of the x-rays by the various tissue types in the body allows for differentiation and identification of the structures, both normal and abnormal. The administration of contrast agents to the animal increases the visualization of soft tissues and can also have additional molecules added that will attach to specific cells, such as those found in infectious and inflammatory conditions. Influenza virus in ferrets has been imaged via CT based on studies showing that a pulmonary ground-glass opacity corresponds to areas of alveolar edema, which is a major histological lesion early in disease.[153] MicroCT equipment is now available for small animal imaging, which allows for decreased expense and smaller space requirements.[150]

MRI uses a strong magnet and radiofrequency energy to detect atomic nuclei polarization in the body to develop an image of internal soft tissue structures.[150] Although MRI has an extremely weak signal and poor sensitivity, this can be overcome by the administering contrast agents such as ^{1}H, ^{31}P, ^{13}C, $^{17}O_2$, gadolinium, or iron-based agents.[149,150] Various techniques for administering the magnetic waves have also been developed to image different tissues, including dynamic-contrast MRI, diffusion-weighted MRI, and blood oxygen level-dependent MRI.[150] MRI has been used to image the disease pathologies associated with Chagas disease, human African trypanosomiasis, malaria, tuberculosis, schistosomiasis, and HIV-1 in various animal models.[149] For example, a macaque infected with simian/human immunodeficiency virus that presented with HIV-associated dementia was imaged via MRI and demonstrated enhanced signaling in multiple areas of the brain, as well as ventricular dilation and asymmetric atrophy, with procession to death of the animal in a short time frame, demonstrating that MRI may be useful in the early diagnosis of HIV-associated dementia.[154]

CT and MRI are often combined with PET to visualize direct comparison of the anatomical and the functional findings, overlapping the PET image on top of the anatomical image (CT or MRI).[155,156] Influenza in ferrets was imaged using PET-CT and demonstrated spatiotemporal progression of the inflammation in real time.[157] PET-CT was utilized in *M. tuberculosis*-infected macaques to track treatment responses by pulmonary lesion size and to determine that latent infections could be predicted to develop reactivation based on granuloma number, size, or FDG uptake.[151,158]

Optical imaging is a broad term used to describe the detection of visible light that arises from either the excitation of a fluorescent protein/molecule or from an enzyme-catalyzed oxidation reaction known as bioluminescence.[131] Special cameras using sensitive photon detectors within cooled, intensified, charge-coupled devices are able to quantify the spatial and temporal distribution of light within the tissues to track cells, tissues, or organisms labelled with the light-emitting molecule.[131,159] For bioluminescent imaging (BLI), several naturally occurring luciferases have been cloned and produced for regular use, including firefly, jellyfish, sea pansy, and *Vibrio* and *Photorhabdus* bacteria luciferases.[148,159] More recently, click-beetle luciferase was developed to image *Listeria monocytogenes* in murine infection models.[160] The luciferase reporters are inserted into the infectious organism's genome, and the animal model is infected with the tagged organism. These natural luciferase systems (Lux-enzymes) require energy, oxygen, and a substrate (luciferin), which is administered to the animal prior to imaging.[159,160] Luc-systems are an alternative to administering exogenous luciferin using the bioluminescent bacteria *Photorhabdus* or *Xenorhabdus*, which encodes genes to produce luciferase and luciferin.[148] In these ways, the disease progression can be tracked via the optical imaging device.

BLI has been used to study a wide array of infectious diseases including *Salmonella typhimurium*, *P. aeruginosa*, *Y. pestis*, *Staphylococcus aureus*, HSV-1, poxviruses, and many more.[131,148,161] Although pathogenesis and disease trafficking are the most common

uses for the BLI imaging, it is also being used to monitor for the effectiveness of therapeutics and preventatives. In one study, a DNA vaccine for Chikungunya virus was evaluated by creating a pseudovirus system expressing the firefly luciferase reporter protein (pHIV-CHIKV-Fluc) and showed promising protective effects of the vaccine tested.[162]

Fluorescent imaging is accomplished by utilizing molecular probes with a fluorescent reporter group. Near-infrared dyes with emission wavelengths of 650 to 900 nm are often used because they can emit through ≥2 cm of tissue.[150,163,164] The dyed probes are developed to target specific tissue or cell types and are then injected into the animal prior to imaging to track those cells, for example, inflammatory, apoptotic, or bacterial cells.[163,164] An alternative to injection of dyes is the integration of a fluorescent protein molecule into the cell or gene of interest. Green fluorescent protein (GFP) is one of the most well-known of these, with red fluorescent protein also commonly used, although there are a multitude of others now available for specific imaging functions.[150] Fluorescent molecular tomography is a progression of this methodology that allows for 3-dimensional reconstitution of the fluorescent accumulation in the tissues leading to quantification of the processes taking place.[150] Fluorescent imaging has been used to track Schistosomiasis parasitic burdens and *S. aureus* endocarditis and muscle infections in mice.[149,163,164] A GFP-tagged Influenza virus studied in mice allowed for imaging of both viral and immune cell tracking, then was compared to the progression of the disease after viral inhibitor treatments.[165] A double transfected (bioluminescent and fluorescent tagged), virulent *Leishmania donovani* organism was developed to noninvasively perform a longitudinal study of this parasitic disease process in mice.[166] Although BLI appears to be the most prevalent in the literature, most of the imaging equipment that utilizes BLI can image fluorescent proteins and many also include digital radiography co-registration to provide an anatomical overlay, therefore greatly increasing the flexibility and range of use for this type of imaging.[131] Dual labelling of microorganisms is becoming more popular, for example, tagging an organism with luciferase in tandem with GFP, which allows for both counting of organisms via fluorescence and metabolic activity quantification via bioluminescence.[131]

The benefits of these modalities on research and animal use are apparent; however, the special circumstances surrounding working in biohazardous environments must be taken into consideration when deciding to implement their use in projects. Facility space, environmental containment, and ability to sanitize or sterilize the equipment after use and between experiments must be carefully planned. Many high containment facilities are solving these issues in novel and inventive ways and are publishing their outcomes, which will allow others to more easily implement these imaging modalities in their projects.[167–175]

Conclusion

Replacement, reduction, and refinement are basic premises of using animals in research, but the unique needs of studying biohazardous agents in animal models can lead to difficulties in applying these principles. Animals that may become acutely sick from bacterial or viral infections show nonspecific clinical signs that most reliably include: changes in body temperature, decreased grooming, appearance changes, loss of interest for daily activities, reduction in food intake, and decreased socialization. This poses challenges in applying the 3Rs as inducing distress is required for the project to mimic the disease process and clinical symptoms of the disease.[176] While application of the 3Rs may be challenging, there are advancements that make it feasible, and here we summarized many applications in infectious disease research. Most would agree that death as an end point in infectious disease studies is not acceptable. Applying the 3Rs to infectious disease work to reduce the distress animals incur during these studies not only improves animal welfare, it also enhances the science. In vitro model systems provide opportunities to assess cellular interactions using cell culture models, and more advance interactions can be performed with engineered multi-tissue model systems. Simple model systems such as *C. elegans* and Drosophila have been verified in vertebrate model systems as they possess similar cellular biology, including innate immune responses that can be used as replacements to vertebrate animal models. *D. discoideum*, *C. elegans*, and Drosophila are intriguing models because they offer the ability for high-throughput screening of virulence factors and pathogen-host interactions using genetically modified hosts. Score sheets, when properly developed and applied, provide an excellent tool for refining animal use. Evaluation of behaviors, activity, facial grimace, body temperature, food and water intake, and nest building can offer valuable insight into the disease progression and can readily be incorporated into scoring systems. Monitoring these parameters can aide in early end point determination before significant clinical illness is present. While there are challenges to using these parameters, they have been used successfully in specific models and can be applied to other models. Advancements in technology have helped to reduce the number of animals used in infectious disease studies. Telemetry and imaging modalities allow the continuous and longitudinal assessment of animals over the course of disease. This allows assessment of a single animal over time, which was previously done by euthanizing cohorts at various time points during disease. While there is no single method available to universally apply to all infectious disease models, several techniques to replace, refine, and reduce the number of animals used in infectious disease research are available and should be considered when developing animal models.

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Review Article

Disaster Planning for Animals in Hazardous Agent Containment Units

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Abstract

Disaster response planning for laboratory animal facilities is a time- and personnel-intensive undertaking. This article outlines numerous considerations in formulating a plan for disaster response in a high containment animal unit. The planning process is discussed around a set of elements: planning team formation, situational understanding, goal and objective determination, plan development, preparation, and rehearsal or implementation. The importance of an appropriate planning team and personnel development is explored in relationship to exemplary disaster scenarios such as natural disaster and terrorism. Specific risks such as hazardous agent and animal species type serve to delineate goal-setting methods. These goals provide the framework for an institutional disaster plan. The review further uses elements of the planning process to explore the difficulties of euthanasia of animals treated with hazardous agents. Ultimately, the pitfalls of handling media relations following disaster are examined. Proactive measures for preparing to speak to the media and mitigate negative perceptions of research are presented.

Key words: disaster response; containment unit; laboratory animal facility; animal; review

Introduction

Disaster response planning for laboratory animal facilities is a time- and personnel-intensive undertaking. Recent disasters such as Superstorm Sandy and Hurricane Katrina have led to a surge in recommendations and guidance for disaster preparedness. As we have learned, disaster planning is not as easy as putting a plan to paper or hiring a company and putting each animal on a truck cage by cage. Development of personnel at all levels is critical to a successful disaster response, especially when contending with animals in a containment unit. This review focuses predominantly on hypothetical worst-case scenarios in animal biocontainment units. Understanding potential risks associated with biological, chemical, or radiation hazards could prevent chaotic responses. Awareness of the dynamics of emergency response and preparedness can strengthen skills for decision-makers and build a work culture based on risk evaluation.

There are many resources available for developing a plan and preparing facility personnel for disaster response. There are only a few published commentaries directly addressing the intricacies of handling animals in high containment facilities during a disaster, such as the review by Swearengen et al., 2010.[1] The current review expands high containment units to include United States Biosafety Levels BSL-3, BSL-4, and BSL-3Ag and certain chemical or radiation hazards.[2] Containment unit disaster response planning is often a small component of the larger facility plan. It requires the same dedication and thought in planning a response for a conventional animal unit. However, complexities are greater with the additional regulations, safety considerations, personnel

training, and public perception associated with containment level research.

This review examines appropriate disaster response planning, including specific risk considerations, plan and personnel development, humane euthanasia challenges, and handling media relations. Discussions of real-time response and recovery are not included within this article.

Advanced Considerations In Resilience Planning

Disaster planning must be tailored to the individual institution. The planning process for disaster has been outlined in numerous articles, generally focusing around a common set of elements: planning team formation, situational understanding, goal and objective determination, plan development, preparation, and rehearsal or implementation.[3]

Planning Team Formation: Stakeholders

The leadership of institutions with containment facilities must fully appreciate the intricacies that accompany this work, especially as it pertains to the care and development of personnel running a containment unit, expectations of safety, and euthanasia. Institutional leadership may view comprehensive disaster preparedness as extraneous time and effort, leading to increased costs.[4] The most difficult hurdles in disaster planning are the infrequency of disasters and the belief "it won't happen to us" or "we are ready for it."[5,6] The risk of a high-profile disaster in a containment unit often encourages the active support of leadership. Still, the vision for a disaster response for containment animal facilities usually comes from the animal facility team. This vision must earn the backing of institutional leadership, or the plan is unlikely to succeed.[7]

Establishing a guiding coalition to achieve this vision requires careful consideration of members. Greater numbers of participants are required for containment level planning. All groups who may be affected by a containment unit disaster must be included. At any institution or company (either private or government), multiple departments will play important roles in a large-scale emergency situation. Not all entities' planning teams will comprise the same positions or expertise. Planners include subject matter experts (e.g., biological safety officer, veterinarian, principal investigators), first responders (e.g., police, fire department, emergency medical), government (e.g., local, state, federal), institutional administrators (e.g., senior leadership, facility managers, security, media relations), the institutional animal care and use committee, and day-to-day operators (husbandry and veterinary technicians).[8] Teams may further vary depending on a number of factors such as infrastructure, geographical location, biosafety levels, etc. For example, an agricultural institution utilizing biological select agents or toxins (BSATs) and livestock would likely have additional internal and external stakeholders, such as local farmers (Table 1). Facilities in dense, urban areas may face vocal community scrutiny, necessitating additional media relation expertise.[9,10]

More participants increase the potential for conflicts arising from differing objectives or viewpoints. Position in institutional hierarchy may also drive committee dynamics. The hands-on knowledge of personnel involved in day-to-day operations is instrumental in exploring and revealing points of weakness in the disaster response. Their opinions should not be diminished in the planning process due to fear and/or anxiety barriers of perceived judgment by persons above them in a workplace hierarchy.[11] For this group to work smoothly, leadership should consider educating members in techniques such as organizational communication[12] to ensure planning receives input from all groups. This technique facilitates open conversation and listening among all team members, rather than dominance by more senior managers.[12] Employees with a voice in the process are more likely to engage during plan development and implementation, leading to a successful response and recovery.

Situational Understanding

Unfortunately, there can never be full mitigation of risk, especially in a containment facility. Facilities must undertake risk control to bring risk to the lowest tolerable or acceptable level.[13] Situational understanding is recognized by the Federal Emergency Management Association's National Incident Support Manual as a key component in disaster response.[14] It is the ability to identify risks, assess these risks, and further prioritize them regarding potential danger to human health or research in the context of historic catastrophic events, future possibilities, and understanding the capabilities of the organization. Response goals are then based on these situational considerations.[15]

Risk Assessment

One of the first steps in situational understanding is commonly referred to as risk assessment. This assessment establishes a baseline for an institution by identifying and evaluating the potential for risk. Proper procedures must be in place to influence the outcome of a potential, critical situation and protect the health and safety of the personnel working at a given institution or installation. Risk assessment is an ongoing process. As new events unfold, protective measures are reassessed and the proper recourse established.[16] Organizations are encouraged to apply this process to research protocols or emergency response procedures, like chemical spills. Having subject matter experts and institutional committees involved with the risk evaluation and decision-making process at any phase is critical. They are responsible for determining the existing threats and vulnerabilities, thus keeping the institution a safer workplace.

Specific Considerations for Situational Awareness

Situational awareness takes a great deal of creativity to consider all threats and an astute mind to recognize them. Organized methodologies for creative analysis are available, the use of which can facilitate development of containment unit responses. One such example of creative problem solving is the Osborn-Parnes Creative Problem Solving process.[17] Structured problem-solving enables decision-making teams to not miss any risks or ignore subsequent response goals and objectives. These analytic tools can further planning-team cohesiveness and efficacy by giving equal voice to all ideas. The following are examples of risks which may profoundly impact a containment unit in disaster.

Human Risk The most common of risks, human error, cannot be overlooked.[18,19] Human error could lead to release of a pathogen or data. Human error can also worsen the situation during a disaster response. This error may have significantly greater impact on human and animal life if it occurs in a containment unit. Of the many risks to a containment facility, managing this one is likely the most rewarding and will be discussed in detail below in the plan development and review section.

Situational understanding may identify another human risk inherent in many plans. Nearly all disaster plans incorporate

outside agencies as part of the response. In reality, a major disaster will likely require these responders to address human injury first and may delay or prevent actions benefitting the animal facility. When available, outside responders may not even enter a containment area either by design or because of fear of the hazards. Animal facility on-site personnel may have to respond first to disaster in a containment facility while awaiting outside agencies. This may require decisions and actions the animal facility staff consider the responsibility of others, such as fire quenching or medical emergencies. This possibility should serve as a core issue during planning.

Humans may also pose risk due to malicious intent. Information technology poses great opportunity and threat for containment facilities. Technology has greatly increased capabilities in data collection and processing as well as facility operations. Cybercrime may compromise security and safety systems or release classified information. Cyberattacks could potentially confound identification of animals, administered agents, or biosafety elements through malicious data alteration. This could expose personnel to hazards unknowingly. Illegal entry or override of security measures may occur, allowing release of animals in an act of domestic terrorism. Classified information may be hacked, misconstrued, and released with wicked intent. This could incite public fears against the work done in research facilities and lead to public relation disasters or threats upon the facility and/or personnel.

Public perception of high containment research is an inherent risk. Much of this perception focuses on worst-case scenarios such as accidental release of pandemic influenza or Ebola. Others focus on the potential for weaponization of research items like an infectious disease.[20] The public's perception of a potential breach from a hazardous containment suite is a significant risk to the facility's reputation. As discussed in the section on media relations, facilities should understand public perception of the work and proactively mitigate a negative perception of danger. If a risk to the public from containment animals does arise from a disaster, this must also be clearly and appropriately explained before it is misconstrued, intentionally or otherwise.

Species Risks The species type in a containment facility impacts risk. Nonhuman primates and agricultural animals can be among the most difficult species to handle during or following a disaster. Both categories complicate disaster preparations. Nonhuman primates generally will remain in a cage during or following disaster. The most likely risk will be injury to the animal itself and the need to treat it in nonstandard conditions like power outage, flooding, or room damage. Animals at agriculture-specific biosafety level BSL-3Ag are unique. The facility itself serves as the primary biocontainment tool for loose-housed agricultural animals.[16] Agricultural animals may become frightened during a disaster such as a tornado or earthquake and may be behaviorally unpredictable. During or following a disaster, animals may not be contained in pens or stalls. Free-roaming animals increase risks to personnel because of the animals' often large size. Frightened animals are far more likely to injure themselves and may require on-site treatment (if the building is intact), sedation, or euthanasia to contain the biological agents they may harbor. Facilities should prepare personnel to handle these situations. Consequently, considerable emergency preparation is necessary to ensure the safety of personnel, the welfare of the animals, and the biosecurity of infected animals.

One of the major risks to animals in a containment facility is the inability to rapidly move them to safety. Following Super Storm Sandy, animals were transported from the New York University Langone Medical Center to another local facility for housing until permanent housing was repaired or rebuilt. This capability is unlikely for containment level animals, especially larger animals. Suitable containment facilities at alternate locations, or even the ability to safely transport hazard-exposed animals, will likely be limited. Shelter-in-place is also probably not realistic for containment level animals if there is a structural breach or even the risk of one. Facility directors should still have conversations and agreements (even if only verbal) with nearby facilities to understand the capabilities each may have in aiding others during time of disaster. As will be discussed in the humane considerations section, this movement limitation leads to difficult decisions regarding euthanasia in disaster preparation or response.

Another potential sequela of certain disasters may be the escape of containment level animals. No matter the cause, facilities should have some method for locating, trapping, and potentially euthanizing these animals. For example, following an earthquake it might be impossible to determine how many mice are unaccounted for if cages fall off a rack. Accurate mouse census numbers may be unavailable if animals are tracked by cage rather than individual. Transponders might be one method for location. For larger animals, consideration may be given to pre-event communication with a nearby zoo. These facilities regularly practice animal escape drills and often have established response plans with local authorities, such as local law enforcement.[21] These facilities have organized recapture teams with expertise in setting traps and darting or shooting animals to allow for humane live or lethal capture of larger animals such as nonhuman primates or equine species.[21] Zoos may be involved in their own disaster response efforts during an actual emergency. Therefore, consideration should be given to meeting with these organizations prior to a disaster.

Disaster Type Considerations As part of disaster planning, facilities should consider the most likely disaster(s) to strike their containment facility. When considering environmental catastrophes, institutes should engage local and federal response agencies who have calculated these risks.[22] Infrastructural needs will vary depending on location, age of building, zoning, or other requirements. Planning groups must prioritize environmental risks and solutions.[23] Facilities in an area prone to tornado strikes need to decide on structural ratings compatible with the highest predictable local wind speeds or consider placement of facilities within underground locations.[22,24] Conversely, flood zone areas should consider aboveground facility placement to reduce risk of animal drowning or agent release in effluent following flood. Even if buildings are built to withstand structural damage from earthquakes, other repercussions must be considered.[25] As seen in a major earthquake in Japan in 2011, the buildings withstood the earthquake, but vibrations sent cages cascading from racks.[26] Future events like this could result in hundreds or thousands of mice or other rodent species running through the containment unit,

Table 1. Examples of Internal and External Stakeholders Involved in Risk Management

Internal Stakeholders	External Stakeholder
Administration	First responders
Biosafety officer or EHS department	Government (federal, state, local)
Facility managers	External media
Principal investigators	Community members
Public safety/security	
Internal media relations	

requiring capture and euthanasia. Large animal cages or stocks in BSL-3Ag could conceivably open and release animals into larger holding areas or beyond.

Agent Considerations Many facilities house animals that harbor naturally occurring zoonotic diseases, such as *Macacine herpesvirus* (B-virus). With an increase in microbiota research, rodents may be obtained with unclear adventitious agent status (e.g., feral or pet store rodents). These animals carry risks for zoonotic disease such as lymphocytic choriomeningitis, Hantavirus, or enteric pathogens. Individual risks associated with these animals may not be identified due to disease-shedding dynamics or facility biosecurity testing regimes. Disaster may lead to release of these animals or human exposure. Dependent on species and funding, as with conventional animals, losses may or must be reported to the USDA and OLAW respectively postdisaster.[27,28] Animals infected with biological select agents and toxins, such as many in BSL-3Ag environments, are treated as an "agent"-containing vessel. Previous reviews provide guidance on the increased identification and accounting requirements for select agent- inoculated animals euthanized, found dead, or lost after disaster.[1,29,30]

Besides biologic agents, radioactive and chemical agents are also administered to animals to further biomedical aims. Practically speaking, most chemical agents given to animals will not pose serious risk to first responders or facility personnel once administered to the animal. Volumes will vary based on size of animal and may increase risk especially if excreted in feces or urine and pose an aerosol risk. Certain chemicals such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine may pose a human risk, even after administration to animals. Volatile radioactive agents such as Iodine-131 used at therapeutic doses in animals pose small risk to responders or staff. Ventilation failure is a significant contingency as a volatile radioactive material may concentrate to high levels in a closed room. Responders entering an area with a highly-radioactive airborne agent could be exposed to radiation doses in excess of regulatory limits. Simple precautions, such as ensuring that radioactivity detectors are maintained in multiple locations to increase accessibility during or following a disaster, can greatly reduce exposure risk.

Environmental Hazards Although most facilities consider hazards contained within animals in their containment unit disaster response, additional hazards common to many, if not all, facilities need also be considered. Chemical sanitization and disinfection agents are frequently found in large quantities in animal facilities, often in proximity to a containment suite where they are used. Chemicals such as 12.5% sodium hypochlorite (bleach) or chlorine dioxide are capable of skin or respiratory toxicity. Persons rushing into animal containment areas without consideration of the presence of these chemicals or others like isoflurane may be unnecessarily exposed if spills or leaks occurred, especially if ventilation is compromised. Similarly, facilities may have separate gas supply areas for containment units. Displaced gas cylinders can take on torpedo-like properties if manifolds fail. They are also explosion risks in times of fire.

High containment areas are generally separated from conventional housing areas geographically and/or by structural safeguards such as airlocks and increased security measures. Facilities should ensure storage of required materials: specialized personal protective equipment (PPE), caging, food and bedding, flashlights, communication equipment like walkie-talkies or satellite phones in alternate locations beyond animal holding areas. During Super Storm Sandy, the storm rendered inoperable the door to one storage area containing PPE. Fortunately, this was not the only central storage location. If a containment area is breached, it may be necessary to don appropriate PPE in a location other than the usual gowning station to reduce risk at time of entry.

Regulatory Considerations

For all the numerous contingencies encountered during disaster response, laws, regulations, or guidelines set by external agencies such as the USDA, CDC, and US Nuclear Regulatory Commission provide objective guidance in planning. This can be a comprehensive list, especially if select agents, hazardous chemical agents, or radioactive materials are used. The facility must ensure that it is in compliance with all regulations and should maintain as high a standard as feasible in accordance with guidance documents. As there are numerous documents available on these topics, this review will not focus on them.

Determining Goals and Objectives

Appropriate situational awareness underlies the goals and objectives needed to eliminate risk. Each goal aims to reduce a specific risk by completing objectives.[15] A goal of having appropriately trained personnel will likely be one of the most commonly proposed by facilities. An objective to achieve would be to develop personnel's soft or nontechnical skills necessary for disaster response. These include communication capabilities, stress prevention, leadership skills, and situational awareness.[31,32] Nontechnical skills are of particular importance for a containment unit where deliberate actions are required. For example, a containment unit may actually not be harmed during a disaster. If personnel have not developed appropriate nontechnical skills along with plan knowledge, panic may ensue because of uncertainty.[31] A panicked employee may leave the containment unit without appropriate decontamination. An injured employee or one tending to an injured employee may also head straight for the emergency room, turning a contained environment into a potential community exposure.[33] Even if human error occurs, calm decision-making will prevent compound worsening of this error. Panicked decisions will only worsen the situation.[34]

Many decisions during or after a disaster will likely come from an Incident Command System (ICS). An ICS provides a formal hierarchical command structure, often lead by the head of institutional safety.[35] All other institutional departments will fall into roles beneath this lead. Transitioning the guiding coalition into the ICS provides a management structure resulting in better decisions and more effective use of available resources. Having worked closely together to formulate a plan using concepts such as organizational communication, members know the capabilities and strengths of one another. This leads to trust. Many ICS delineate clear roles and responsibilities for participants. If a person within the ICS hierarchy is not available at time of response, another leader can step up to guide the response calmly and appropriately.

Another goal should include appropriate understanding and delineation of the roles first responders may play. The presence of first responders may make a situation worse due to unfamiliarity with site and hazards. Many containment units do not anticipate entry of first responders into the facility in time of disaster or crisis. Instead, plans may prescribe that trained

facility staff respond to situations such as personnel injury, active flooding, or initial area evaluation after earthquake. Prepared facilities should ensure that their employees receive training from responders such as firefighters and paramedics. Part of this training ensures appropriate decontamination of injured personnel or communication to first responders that decontamination was not possible due to injuries. First responders must also understand situational risks if they enter or approach an animal containment area. For example, large agricultural animals may complicate on-site injury reporting during extraction efforts. Personnel exiting the facility could be injured from the cause of the disaster, from a frightened animal, or from potential biological agent exposure. Pre-disaster communication of goals and role delineation is paramount.

Plan Development and Review

Many of the areas of weakness that containment units may identify will involve significant strategic challenges such as ensuring buildings are disaster-proofed or IT configurations are fortified against hackers. Facilities would do well to take the areas for improvement identified and categorize them into strata such as that provided by FEMA. FEMA breaks planning down into 3 levels: strategic, operation, and tactical.[36] Strategic planning examines the larger picture in response, including allocation of resources—financial and other—to ensure appropriate capabilities and capacities for withstanding and rebuilding after disaster.[36] Even careful strategic planning may not mitigate all risk, leaving the possibility for disastrous consequences. As illustrated in the comprehensive examination of the National Bio- and Agro-Defense Facility in Manhattan, Kansas, even one of the most studied and planned animal high containment units may have potential, unpredictable weaknesses in the face of natural disaster.[34] History has shown that proposed upper tolerance limits for anticipated weather or other natural disasters may not be sufficient in events such as Super Storm Sandy and Tropical Storm Allison.[35,37]

While strategic planning looks to attenuate risks through integral capabilities, further operational and tactical planning serve to complete contingency responses. Operational plans aim to identify the roles and responsibilities of people identified in situational awareness and goal setting. These are the paper plans including business continuity, emergency operations, and risk management.[15] Tactical planning focuses on the personnel and auxiliary resources required in the actual response.[15]

Ultimately, tactical planning may be the most critical for success in a disaster response. The development of personnel involved in daily care of animals, and thus most likely to be involved in a response, is paramount. As previously discussed, facilities must develop both the plan and personnel. Without appropriate, disaster-prepared personnel, no paper plan will succeed. Creative evaluation and implementation of plans should follow development.

Rehearsing for the Plan and Learning to Improvise

There are 2 types of rehearsal exercises used: discussion and operation based. Discussion-based exercises include seminars, workshops, tabletop exercises, and games, whereas operation-based exercises include drills as well as functional and full-scale exercises. Discussion-based exercises can be used to familiarize players with or develop new, plans, policies, agreements, and procedures; they focus on strategic, policy-oriented issues.[38] Operation-based exercises are used to validate plans, policies, agreements, and procedures; clarify roles and responsibilities; and identify resource gaps. Furthermore, they rehearse activities such as initiating communications or mobilizing personnel and resources.[38]

An effective way to reduce the impact of an incident is to share practical knowledge through exercises. Institutions utilizing BSATs must follow regulatory requirements dictating the need to execute training or exercises on an annual basis. These include examples such as response to the previous scenario of injury of personnel in high containment areas. Facility personnel should rehearse performing cardiopulmonary resuscitation when unable to access a person's airway due to PPE and ensuring that a responder can safely move injured personnel out of a containment facility without further injury or contamination of others. All personnel who may work in biocontainment areas should have annual training. A key component is a live, scenario-driven exercise to ensure that those who work in this environment can extract both themselves and a colleague if an emergency arises. Institutions that do not work with BSATs, but with pathogens still considered high risk, may not be obligated to conduct training or exercises but nonetheless may benefit from following best practices.

No matter the type of training or drills employed, they should be commensurate with the target audience, whether it be the ICS, cleaning personnel, or external stakeholders. The training should reinforce the roles, responsibilities, and procedures required in the event of an emergency. The implementation of exercises should be continuous, as it is a practical tool for simulating new ideas and concepts and further developing communication methods among all players.

The practice of proposed scenarios should not be done only in controlled, scheduled trainings. Stress induces a significant change in personnel thought processes.[19] Simple actions in a controlled environment become difficult with pressure. The threat of a hazardous agent is such pressure. The authors recommend using development techniques that place employees in challenging situations. Training like this increases participant comfort in handling the pressures of disaster.[31] Examples include discussion-based forums like tactical decision games[31,39] or operation-based ones such as working in a power outage. Many overlooked difficulties are elucidated in these trainings such as trying to find flashlights or realizing that head lamps are better to keep hands free. Without appropriately developed personnel, no amount of planning will lead to a successful disaster response.

Development of both technical and nontechnical skills requires time investment. Beyond the aforementioned nontechnical skill training, containment unit staff need specific technical skills. More than one person should have the appropriate technical skills to handle animals in a biocontainment unit. All essential personnel may not be available depending on the nature of the disaster. Training should include investigative staff that run high containment Animal Biosafety Levels (ABSL) satellites. These personnel and animal facility personnel must be comfortable with responding to disaster or another crisis. Laboratory personnel working in a high containment facility should undergo the same disaster response education as that of the primary animal handling staff. For containment facilities that are managed by laboratory personnel, there should be a backup plan in the event that they are not present or available in a disaster.

Humane Considerations

In or following a disaster, mass euthanasia techniques for containment animals should be considered. Personnel may have to

euthanize animals if they cannot be appropriately treated or if their well-being is affected by worsening conditions. Considering the timing of euthanasia for these animals requires a great deal of thought and preparation. Guidelines for the order of euthanasia of animals should be developed such as that described in the Rockefeller University, NY disaster response plan.[8] Euthanasia starts with containment level animals.

Containment facilities require specialized and time-intensive care for the animals that may not be feasible during or after a disaster. Leadership of a containment facility must be prepared to activate or support the decision to depopulate animals in a high containment facility when the magnitude of disaster is uncertain such as for a predicted pandemic, storm, or other foreseeable potential catastrophe. Preemptive euthanasia may prevent animal suffering if sufficient staff cannot report to work due to disaster and care for animals or carry out euthanasia post-event.[8] Events that occur without warning may also require euthanasia. When staff are present, euthanasia may be rapidly completed. If staff are delayed or unable to enter a facility, animal welfare may be significantly impacted.

Euthanasia should be a focus area for plan development. Situational awareness, planning, and rehearsal of plan should include ensuring adequate, accessible supplies for euthanasia. For rodents and other small animals, planning must ensure that adequate CO_2 is available and calculations of time required to euthanize all rodents in a containment facility. Rodent mass euthanasia can be a time-consuming endeavor in a standard facility.[8] Large animals require sufficient euthanasia solution and a review of the time necessary to find and don appropriate PPE, enter the facility, draw up solution (performed with saline or appropriate substitute), and mock-administer it. Some animals may require sedation prior to administration of euthanasia, adding additional time to the process. In high containment facilities, it may take upwards of 30 to 40 minutes just to gown up to enter the facility.[40] During or after an event people may rush to provide care for the animals. Mistakes may affect human life if staff rush or do not respond calmly. Employees must understand that their lives come first. In their dedication to the animals, this is something that animal facility personnel may not always immediately consider. When rapid euthanasia is required, facilities may need to look to alternate methods beyond conventional laboratory animal methodologies.

The American Veterinary Medical Association is developing guidelines on depopulation of animals, including laboratory animals.[41] Critical to this document is the distinction between depopulation and culling. Depopulation is done in response to a disaster or crisis situation and culling is done for pest or stray control or prophylactically to prevent disease spread.[41] Depopulation will only be done in extraordinary circumstances in which leadership predicts that animals will eventually die as a result of the disaster. This death may be prolonged and potentially stressful or painful to the animals. The AVMA emphasizes that the choice of termination method should adhere to ethical standards and guidelines as well as state and federal laws.[41]

The guidelines reinforce the role of the attending veterinarian in deciding the best method of depopulation. The method required may not adhere to euthanasia guidelines in the time of emergency. For both ethical and humane reasons, disaster depopulation planning should not be the attending veterinarian's alone. Rather, it should be a group effort to determine depopulation options. Various scenarios should be considered depending on time, resources, and personnel available. For example, in the face of a rising pandemic illness, cage-by-cage CO_2 euthanasia of rodents may not be feasible. When a rapid response is required, placement of large numbers of mice in a large container prefilled with CO_2 may be necessary. CO_2 canisters may be limited or in inaccessible areas with more unlikely to arrive soon. Although these do not conform to the AVMA guidelines on euthanasia, in the event of disaster, routine, time-consuming methods of euthanasia may result in prolonged animal suffering.

High containment facilities provide an opportunity for technologic advances, which may aid in emergency euthanasia. Current technology includes the capability to introduce CO_2 into rodent microisolator racks in situ. High containment units for large animals could consider retrofit or new construction of areas to allow room-level euthanasia with CO_2, argon, or nitrogen. These considerations could reduce the need for personnel to enter rooms in times of disaster and allow for rapid, humane killing of animals. Technology such as CO_2 or nitrogen foam has been developed for mass depopulation of poultry farms.[42] This technology may be adaptable to laboratory animal use in time of emergency. These techniques require numerous safeguards against human imperilment, loss of function in disasters, or accidental use. Remote euthanasia capabilities warrant further studies to reduce personnel exposure to toxic agents and emotional burdens. By reducing personnel time and effort through remote euthanasia, a greater number of noncontainment level animals may be saved or treated.

Following mass euthanasia, carcasses must be disposed of properly. Redundant systems for disposal of high containment carcasses must exist in the forms of autoclaves, incinerators, or tissue digesters. Depending on the disaster type, these may not be functional during the recovery period. Alternate disposal methods appropriate to state and federal guidelines must be found. This may require contracting services with outside vendors capable of legally transporting these carcasses to a functional disposal system. If no transport available, carcasses may have to be placed in sealed bags and held until disposal possible. Housing rooms may serve as temporary morgues if they can be secured.

Considerations for the people involved in the disaster response is needed. These are trying times and the loss of animal life can severely affect those who were their caretakers. Discussions and mental preparation for the possibility for mass euthanasia must be made in advance. Following a disaster, arrangements should be made to provide counseling to all persons involved in the response. Personal experience from the authors underscores the importance of this service.

Media Response

In the immediate and continued wake of a disaster to a containment facility, the public will want to know what happened to the animals and has anything dangerous escaped. Most animal facility personnel, leadership included, will state that they are not to speak to the press and that their institution's public relations office will handle everything in the event of a disaster. The harsh reality, as experienced by the authors (GR and JP), is that it is unrealistic to expect PR staff members to know the animal facility and its operations in the level of detail often required of the press. To illustrate this point, try an experiment. Give your PR staff a briefing about everything you think they would ever need to know about the animal research facility. Then have a different person in the laboratory animal field ask them rapid fire questions, as a reporter would do. Even if senior PR staff have been given a presentation about animal research at the institution, they likely will not know the details of the disaster plan, precisely how animals are maintained, what hazardous agents are in use, etc. Given the 24-hour news cycle and prevalence of social media outlets, bad news travels

faster than ever before and there simply is not enough time for multiple layers of leadership to ponder and prepare curated statements to the press. Animal facility leadership, who know their operation better than anyone, should be capable of presenting a statement to the press, likely with PR staff also present to provide a sense of security to the institution. While the PR office may control the institution's social media accounts, animal facility leadership should be prepared to provide statements to the PR office for use on social media.

Most of the fear of talking to the press comes from historical issues related to those who oppose animal research. While those individuals still exist, in the current political and socio-economic environment, the public as a whole is much more accepting of science and the need for biomedical research.[43] The public does, however, have an understandable expectation of honesty, and the laboratory animal community needs to start being more open and transparent.[44] If the public sees the people involved in animal research as the competent, caring individuals that we are, they are more likely to give us the benefit of the doubt and be a receptive audience in the event of a disaster.

General Summary

In this review, we focus on the challenges of developing a response plan for high containment animal research units. Many of these challenges reflect the need to adequately prepare personnel with skills beyond their everyday technical knowledge. This may not be a high priority for many facilities. Daily operations consume available time for development. Often only the bare minimum training is completed. This will not be sufficient when disaster strikes. Prior preparation can even reduce the stress of speaking to the media. Unclear communication with press may result in misspoken words or unclear responses. Theoretical written responses, such as plans for euthanasia, may not suffice. Close review and operational drills should be used to validate these. Institutes should work to incorporate stress in their training. This review emphasizes the importance of at least annual training and review for a high containment unit disaster plan.

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