

What's Hot in Animal Biosafety?

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Abstract

In recent years, the emergence or re-emergence of critical issues in infectious disease and public health has presented new challenges and opportunities for laboratory animal care professionals. The re-emergence of bioterrorism as a threat activity of individuals or small groups has caused a heightened awareness of biosecurity and improved biosafety. The need for animal work involving high-risk or high-consequence pathogens and for arthropod-borne diseases has stimulated renewed interest in animal biosafety matters, particularly for work in containment. Application of these principles to animals retained in outdoor environments has been a consequence of disease eradication programs. The anticipated global eradication of wild poliovirus has prompted the promulgation of new biosafety guidelines for future laboratory and animal work. Increased concern regarding the use of biologically derived toxins and hazardous chemicals has stimulated a new categorization of facility containment based on risk assessment. Recognition that prion disease agents and other high-consequence pathogens require safe handling and thorough destruction during terminal decontamination treatment has led to the development of new biosafety guidelines and technologies. The implementation of these guidelines and technologies will promote state-of-the-art research while minimizing risk to laboratory animals, researchers, and the environment.

Key Words: biosafety; biosecurity; infectious waste; polio; prions

Introduction

Although the fundamental concepts that are the underpinnings of “biosafety” are well founded, the field continues to evolve as new diseases emerge and older ones re-emerge. The Office of Health and Safety at the Centers for Disease Control and Prevention (CDC¹), in conjunction with the Division of Safety at the National Institutes of Health, publishes the guidelines *Biosafety in*

Microbiological and Biomedical Laboratories (BMBL¹) (Richmond and McKinney 1999). In 1997, with the enactment of the Laboratory Resistration Select Agent Transfer Program regulation (Federal Register 1996), BMBL guidelines were incorporated into the regulation by reference, thereby requiring facilities intending to ship or receive select agents or toxins to be registered with the CDC and to comply with all aspects of the BMBL. New changes to this regulation expand the number of registered facilities to include those that possess these agents and others identified by the US Department of Agriculture (USDA¹) (Federal Register 2002) (also see below).

CDC began hosting biannual biosafety symposia as a means of assisting biosafety professionals, researchers, veterinarians, facility designers, and others to stay current with emerging issues in the field. The American Biological Safety Association (ABSA¹) web site offers access to the Anthologies of Biosafety series, with volumes dedicated to perspectives on laboratory and facility design considerations, application of principles such as prudent practices and procedures (including decontamination), working safely with animals in research, matters of public health, and BSL-4 laboratories (www.absa.org).

We have selected eight biosafety subjects that we consider to be “hot topics” of particular interest to veterinarians and others who work with research animals. We have provided current references that will help the reader focus on these and related biosafety areas.

Biosecurity

Recent incidents of bioterrorism in the United States resulted in significant enhancement of existing regulations and the creation of new regulations governing laboratory security (CDC 2001a). The USA Patriot Act of 2001 places specific restrictions on individuals registered to work with certain highly infectious biological agents and toxins (Select Agents) (PL 107-56). The Public Health Security and Bioterrorism Act of 2002 (PL 107-188) requires all possessors of Select Agents, certain plant pathogens, and organisms

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¹Abbreviations used in this article: ABSA, American Biological Safety Association; ABSL, animal biosafety level; BMBL, *Biosafety in Microbiological and Biomedical Laboratories*; BSL, biosafety level; CDC, Centers for Disease Control and Prevention; CJD, Creutzfeldt-Jakob disease; CLS, chemical safety level; TSE, transmissible degenerative encephalopathy; USDA, US Department of Agriculture; WHO, World Health Organization.

defined by USDA as “high consequence pathogens” (Federal Register 2002) (Agents) to be registered in a classified national database. This Act extends current regulatory controls to dangerous animal pathogens and toxins, and it provides protection of site-specific information regarding the identification of individuals who work with Agents, the nature of the Agents present in a facility, and the local security mechanisms in use.

Appendix F of the CDC publication *Biosafety in Microbiological and Biomedical Laboratories* (Richmond and McKinney 1999) is being revised to address the following topics:

- Establishing policies and procedures to ensure security of laboratories containing Agents;
- Controlling access to areas where Agents are used and stored;
- Knowing who is in the laboratory area (see www.state.gov) for a list of reasons individuals are restricted from Agent areas;
- Knowing which Agents are brought into the laboratory or animal area;
- Developing procedures to ensure appropriate accounting for and use of Agents;
- Knowing what materials are removed from the laboratory area;
- Having an emergency response plan; and
- Having a protocol for reporting incidents.

The Biosecurity Taskforce of the ABSA has prepared guidelines for developing a biosecurity plan (www.absa.org); an example developed by USDA is included in these Taskforce guidelines. One recent publication (Johnson and Royse 2002) provides an excellent strategy for conducting facility risk and vulnerability assessments. Information on biosecurity initiatives related to agricultural crops is available at the American Phytopathological Society’s web site (www.apsnet.org).

Arthropod-borne Diseases

Concern about the rapid spread of West Nile virus across the United States has stimulated greater interest in safe work conditions for persons handling infected vectors or animals. Prudent practices, including awareness, use of personal protective equipment, and insect control measures, should be followed when birds, horses, and nonhuman primates are housed outside.

The Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-borne Viruses has classified laboratory work with West Nile Virus to biosafety level (BSL¹)-3. The 4th edition of the BMBL (Richmond and McKinney 1999) and a subsequent CDC publication (CDC 2001b) recommend BSL-3 practices, safety equipment, and facilities for activities using potentially infectious clinical materials and infected tissue cultures, animals, or

arthropods. Routine diagnostic procedures may be performed in BSL-2 facilities provided the laboratory exhaust air is discharged outdoors and ventilation to the laboratory is balanced to provide directional airflow into the laboratory. All specimen handling, and any other procedure with potential for aerosol production, should be performed in a certified class II biological safety cabinet. Procedures should be in place so that in the event of a failure in the heating-ventilation-air conditioning systems, the air supply will stop before the exhaust so that the rooms remain under negative (or neutral) air pressure.

Of particular interest to the veterinary community are the new Arthropod Containment guidelines proposed by the American Society of Tropical Medicine and Hygiene, which have been under development for the past few years (Benedict 2002). The proposed guidelines define four arthropod containment levels (ACL-1 through -4), characterized by increasingly restrictive containment facilities, equipment, and practices. Factors to be considered in determining the level of containment to be deployed include the biosafety level of the agent of interest, the effect of genetic alterations in the vector, and whether the vector is indigenous to the surrounding environment. It is anticipated that these Guidelines will be published as a supplement to the *Morbidity and Mortality Weekly Report* (MMWR) in 2003.

The ABSA plans to publish *Anthology of Biosafety VI: Arthropod-borne Diseases* in early 2003 (see www.absa.org). This new anthology will contain chapters on working with infected arthropods in BSL-3 containment, biosafety practices for field collections, containment issues for animal studies, and a review of related laboratory-acquired infections.

Containment Issues

Bioterrorism has also stimulated a renewed interest in working under BSL-3 and BSL-4 levels of containment in laboratories and associated animal facilities. Most animal facilities in the United States are built to meet American Biological Safety Association level (ABSL¹)-1 or ABSL-2 criteria. The basic biosafety guidelines for working safely with research animals are provided in the BMBL and in *Occupational Health and Safety in the Care and Use of Research Animals* (NRC 1997). Appropriate levels of containment are determined by first conducting a risk assessment for the work to be performed. The focus should be on the potential for animal-to-human spread (e.g., amplification, aerosol generation) of human or zoonotic pathogens. Risk management involves the various steps taken to mitigate the risk of exposure (e.g., engineering controls, written practices and procedures, use of containment devices and personal protective equipment).

Work with genetically modified animals may involve unique containment considerations. For example, animals with deficient immune systems should be housed under “reverse containment” to ensure isolation from environmental

microorganisms. When reconstituted animals are infected with human or zoonotic pathogens, additional containment is needed.

Readers interested in learning more about design criteria and work practice requirements for higher levels of containment have a number of new sources available to them (e.g., Richmond 2002b). Several excellent papers, including a discussion of safe performance of animal necropsy procedures, are included in *Anthology of Biosafety, V. BSL-4 Laboratories* (Richmond 2002a). Many containment issues for smaller animal species can be addressed through use of improved ventilated caging systems (Detrich 2002). Additional references describe safe work practices in animal containment facilities (Richmond 1996; Richmond and Quimby 1999; Richmond et al. 1997).

Occupational Hazards Associated with Wildlife and Livestock Research

Personnel safety can be exceptionally challenging when wildlife or livestock are held in nontraditional vivaria or field conditions for the purpose of scientific research. Considerable forethought and planning must occur early in the protocol development process to minimize occupational risk. A hazard risk assessment will assist in the selection of appropriate safeguards to protect employees. The following assessment outline, which is generally applicable to planning of any animal research protocol, will assist in the development of safe practices for handling wildlife and large domestic farm animals.

1. Know the animal (behavior and environment).

Employees must be familiar with the animal species, their behavioral characteristics, and physical abilities. This knowledge will enhance the safety of both the research staff and animals. Personnel should anticipate defensive behaviors when approaching or confining wild animals. These behaviors often pose physical risks to the animal and attending staff (Fowler 1995; Fowler and Miller 1999).

Employees also must understand the hazards associated with the work environment. Wildlife research is often conducted in the animal's natural habitat. The outdoor field environment presents unique challenges associated with weather, difficult terrain, and delayed access to emergency care.

2. Know the diseases normally associated with the animal species.

Many animal species harbor zoonotic diseases, even when the animals appear healthy. To reduce the risk of exposure to zoonotic pathogens, the research staff should be familiar with diseases associated with the animal species, especially if the research involves close contact or direct manipulation of the animals. Assessing and controlling the occupational risks presented by zoonotic agents requires knowledge of reservoirs, modes of transmission, and epidemiology, and should be appro-

priately addressed in health and safety plans (Chin 2000; Fowler 1999; NCR 1997; Strickland 2000).

3. Develop an Occupational Health Program.

An employee occupational health program, discussed in detail later in this issue (Wald and Stave 2003), should be developed to address, among other things, all biosafety issues (including biocontainment) relative to the work environment. This occupational medicine program should include consideration, during the design phase of the research protocol, for early implementation of pre-exposure programs (e.g., prophylactic vaccines and medications), routine tuberculin testing, and medical evaluation for respirator use and also provide an opportunity for frank health-related discussions with the employee. Such a program also should address emergency response and postexposure contingencies, such as bite kit preparation and communication of risk (e.g., consequences of infection) to employees before work begins (NRC 1997).

4. Implement restraint and engineering controls.

"Restraint" usually involves physical restraint, chemical restraint, or a combination of the two. Engineering controls include architectural and environmental features that enhance the safety of personnel during approach or manipulation of the animals, such as gated stalls, fenced chutes for corralling the animals, squeeze cages for large animals, and nets for fish and birds. Specialized face and eye protection, respiratory protection, and protective clothing should be required, as appropriate, for employees that have close or direct contact with animals. Appropriate decontamination or disposal of these materials also should be considered during the planning stages of the project (Fowler 1995; Fowler and Miller 1999; NCR 1997).

5. Perform advance training of personnel.

The importance of employee training as a cornerstone of every safety program cannot be overly emphasized. The institution is responsible for ensuring that employees are aware of potential hazards associated with the work environment before the work begins. Employee training should familiarize personnel with potential risks and the use of safeguards and techniques to minimize injury or disease exposure, an especially important consideration when handling nontraditional species in nontraditional work environments. The Occupational Safety and Health Administration requires that institutions develop safety programs that include hazardous risk assessments, employee training, and the development of standard operating procedures to minimize risk and ensure the correct performance of duties (PL 91-596).

Working with Toxins of Biological Origin

Most researchers working with biological agents are familiar with the guidelines found in the BMBL. The BMBL describes the use of four biological safety levels

(BSLs¹)—1 through 4—each having specific guidelines for practices, equipment, and facilities. Similarly, Animal BSLs (1 through 4) have been developed to control the unique hazards presented during biohazard research with animals. But what if the work involves a biological toxin, with chemical properties? How does the BSL concept apply?

Although individuals have used or tried to use or assign a given BSL to work with chemical toxins, it has usually been unsuccessful because chemicals and biological agents have different properties. Procedures, equipment, or facilities used to contain biological agents may not provide the desired level of protection from toxins and hazardous chemicals. Recognizing this, a system was proposed for four chemical safety levels (CSLs¹)—1 through 4—which describe the practices, equipment, and facilities for each CSL (Hill et al. 1999). Risk is managed at each CSL by limiting or restricting usage or type of work. This system could be used in animal procedure rooms to provide appropriate protection from biological toxins and other chemicals. A pilot project is scheduled at CDC laboratories to evaluate this system.

The CDC regulates the use and possession of Select Agent toxins (e.g., *Clostridium botulinum* and *Clostridium perfringens*). Bioterrorism and the safe handling of these toxins are discussed in a recent paper (Hill et al. 2001), including security considerations and requirements for biological agents and toxins (which were the major impetus for regulation of Select Agents). As Thomann (2003) discusses, all personnel who work with chemicals in the research environment should have a good understanding of chemical safety. Another outstanding reference is the National Research Council's *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals* (NRC 1995).

It is not unusual, however, to find that many people who work in laboratories are not knowledgeable in chemical safety. A short commentary points out that many college curricula do not include safety education, and, as a result, many graduates start their careers lacking knowledge and a strong ethic in research safety (Hill 2002). We strongly urge making an effort to include chemical safety in the undergraduate and graduate curricula and in appropriate textbooks.

Considerations for Prion Research Involving Animals

Prions are small proteinaceous particles that are associated with various neurological diseases of animals and humans, referred to as transmissible spongiform encephalopathies (TSEs¹). Specific TSEs have been described in deer, elk, mink (transmissible mink encephalopathy), sheep and goats (scrapie), and cattle (bovine spongiform encephalopathy [BSE]). Human TSEs include Kuru, Creutzfeldt-Jacob disease (CJD¹) (which may be associated with sporadic, familial, or iatrogenic transmission patterns), and a variant of CJD associated with the consumption of BSE-contaminated foods (Pedersen and Smith 2002).

Unlike viruses or other traditional infectious agents, prions contain no detectable nucleic acid and resist standard procedures that denature nucleic acids. The major prion subunit is an abnormal isoform of a host cellular membrane protein. The normal form of the protein is designated PrP^C and the pathogenic isoform is designated PrP^{Sc}, in recognition of scrapie, the earliest described prion-associated disease. The PrP^{Sc} isoform results from conformational changes in PrP^C that render the protein resistant to protease activity (Horwich and Weissman 1997; Prusiner 2001).

The unusual biology of prions is reflected in the epidemiology of prion-associated diseases. These diseases may occur as a result of either genetic events or from exposure to contaminated tissues. Kuru, a fatal neurodegenerative disease observed in the Fore people of New Guinea, was transmitted through the ingestion of human brain tissue during traditional mourning ceremonies for dead kinsmen (Pedersen and Smith 2002). CJD, the most commonly encountered prion-associated disease in humans, may result from spontaneous or inherited autosomal dominant mutations in the gene encoding for PrP (sporadic or familial CJD) or in recipients of contaminated cadaveric pituitary human growth hormone, corneal grafts, or dura mater (iatrogenic CJD) (Pedersen and Smith 2002). Prion transmission is generally believed to be species specific; however, a growing body of evidence indicates that ingestion of the agent of bovine spongiform encephalopathy results in neurodegenerative disease in a variety of mammals, including humans (variant CJD) (Blattler 2002).

An understanding of the unique biology and epidemiology of prion-associated disease is the foundation of an effective biosafety program for prion research in an animal facility. Section VII-D of the current edition of the BMBL provides guidelines for assessment of risk and containment level implementation for prion research (Richmond and McKinney 1999). This section of the BMBL contains facility, administrative, and personal protective equipment recommendations for laboratories and animal facilities working with prions. In addition, recent publications describing animal necropsy procedures at ABSL-3 and -4 (Wilhelmsen et al. 2002) and autopsies on humans (Knolte et al. 2002) provide useful information for development of a biosafety plan for prion research.

Researchers using prions for the first time should carefully plan and review previous studies (Baron and Prusiner 2000). Work involving prions from human and bovine spongiform encephalopathy sources should be conducted at ABSL-2 or -3, depending on the study being performed. ABSL-2 and -3 should be used when inoculating nonhuman primates. The absence of any known effective treatment for prion disease demands caution by researchers.

All other animal prions should be considered BSL-2 pathogens. The properties of prions change dramatically when they are passaged from one species to another. Therefore, human prions that have been passaged in mice may be handled as mouse prions (BSL-2) (NRC 1997). An exception to this guidance is mice expressing human or chimeric

human/mouse transgenes. Transgenic mice, which produce human prions when infected with human prions, have been widely used in animal prion disease research (Prusiner 2001) and should be handled at BSL- 2 or -3 in accord with the guidelines described above.

Procedures for the safe handling and disposal of contaminated tissues are fundamentally important for an effective prion biosafety program (Richmond and McKinney 1999). The highest concentrations of prions are in the central nervous system. Based on animal studies, it is likely that high concentrations of prions are also found in the spleen, thymus, lymph nodes, and lung. The main precaution to take when working with prion-infected or -contaminated material is to avoid puncture of the skin (Richmond and McKinney 1999). The prosector should wear cut-resistant gloves if possible. If accidental contamination of skin occurs, the area should be disinfected by swabbing with 1 N sodium hydroxide for 5 min and then washed with copious amounts of water. Work surfaces should be decontaminated after spills and with repeated wetting for at least 1 hr with 2 N sodium hydroxide.

The risk of skin punctures, aerosol production, and contamination of operating room, morgue surfaces, and instruments should be kept at an absolute minimum. Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care at BSL-3. Formaldehyde-fixed, as well as paraffin-embedded, tissues (especially brain) remain infectious. The BMBL lists the following recommendations:

- Avoid using sharp objects whenever possible. Exercise extreme caution when inoculating animals with syringe and needles.
- Use cut-resistant gloves under two pairs of surgical gloves.
- Minimize aerosols by opening skulls with a Stryker saw inside a plastic bag.
- Wear suitable waterproof protective laboratory clothing for necropsy procedures.
- Use secondary leakproof containers to store tissue specimens and body fluids (NRC 1997).

Special consideration also should be given to waste disposal when performing prion research. These agents are extremely resistant to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals such as formalin and alcohols. The BMBL recommends inactivation by immersion in either 1 N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for at least 1 hr before autoclaving at 134°C for 4.5 hr, followed by incineration. Disposable instruments incinerated after use are preferable to reusable instruments. Specific guidelines for waste disposal and decontamination are available from the World Health Organization (WHO¹) (WHO 1999a).

Prion research has exploded in the past decade, and continued exponential growth should be anticipated. The prion hypothesis is being applied to numerous diseases

ranging from schizophrenia, bipolar disorders, autism, and narcolepsy to ulcerated colitis, Crohn's disease, rheumatoid arthritis, type I diabetes, and systemic lupus erythematosus (Prusiner 2001). Recognizing the hazards and safely working with prions will remain a priority at biomedical research facilities as they continue to plan an important role in this emerging and exciting field.

Animal Carcass Disposal by Hot Alkaline Hydrolysis: An Emerging Alternative to Traditional Methods

The disposal of animal carcasses is a problem faced by many animal facilities, despite efforts to reduce the number of protocols involving euthanasia. Carcasses traditionally have been disposed of by incineration, either locally or off site by a licensed disposal contractor. In recent years, however, these practices have come under increased local, state, and federal environmental regulations. The disposal of carcasses in an environmentally responsible manner is a significant concern for every animal facility.

One recently described alternative for carcass disposal is hot alkaline hydrolysis, a technique shown to inactivate effectively the agents of TSEs (Ernst and Race 1993; Taguchi 1991; Taylor et al. 1999). These agents are characteristically resistant to commonly utilized methods of decontamination (Taylor 2000). WHO guidelines recommend hot alkaline hydrolysis, in addition to incineration, as preferred methods for decontamination of TSEs (Nolte et al. 2002; WHO 1999b).

Hot alkaline hydrolysis uses temperature, pressure, and high pH in combination to reduce complex biological samples to their constituent sugars, amino acids, and soaps. A typical process involves adding the biological sample, primer water (usually 1.5 times the weight of the tissue to be processed), and 50% NaOH or KOH to a final concentration of 15% of the sample tissue weight. The reaction vessel is then sealed, and high-temperature steam is introduced to produce temperatures of at least 285°F and pressures of at least 38 psi. The reaction mixture is continuously circulated in the reaction vessel until a sterile hydrolysate is produced. After the hydrolysate cools, it is diluted with potable water and disposed of either in the sanitary sewer system or by transporting to an off-site landfill. Only calcium-based bone fragments and undigested cellulose remain in the reaction vessel after the hydrolysate is removed. These materials are sterile and can be easily compacted. The weight is usually no more than 10% of the original sample and may also be disposed of as noninfectious waste in a landfill.

Personnel working with systems involving heated and pressurized alkaline hydrolysis solutions must be aware of potential physical hazards. Exposure to hot concentrated bases (alkali) would have serious harmful consequences due to its highly corrosive properties. Personnel should always wear personal protective equipment (including gloves, face shields and goggles, and protective clothing) when investi-

Table 1 Wild poliovirus containment after polio eradication (samples possibly containing wild poliovirus)

Activity	Risk	BSL ^a
Manipulation of samples in which wild poliovirus may be present, e.g., Bacterial isolation RNA extraction Biochemical assays Microscope slide smears	No potential for virus amplification Greatest risks are exposure by ingestion or inoculation Risk of exposure during aerosol-generating activities (e.g., vortexing, centrifugation) of primary sample	BSL-2/polio Wear gloves and eye protection; precautions necessary for sharp objects Use primary containment devices (e.g., BSC, ^a biosealed centrifuge tubes) Vaccinate personnel Store samples in locked freezer Post biohazard warning signs on freezer and laboratory doors Train all personnel regarding risk

^aBSC, biosafety cabinet; BSL, biosafety level.

gating any potential leak or malfunction of the equipment during operation. Pressurized systems may fail and spray the material on nearby personnel or equipment. An emergency safety shower and eyewash must be located in the immediate vicinity.

The decision to implement hot alkaline digestion in an animal facility is complex and involves many factors. The installation and start-up costs (which can be significant) must be weighed against the reduced per batch cost of hydrolysis when compared with incineration. Although hot alkaline digestion produces significantly fewer air pollutants than incineration, it also consumes substantially more water to dilute the hydrolysate to prevent coagulation and the resulting potential blockage of effluent lines. For facilities that process large volumes of animal carcass material or that support TSE research, this technology represents a realistic alternative to traditional waste disposal methods.

Working Safely with Wild Poliovirus

Polio (or poliomyelitis) is a highly infectious disease caused by wild poliovirus. The virus is a member of the genus *Enterovirus* of the family Picornaviridae (small single-stranded RNA virus). There are three wild poliovirus sero-

types: 1, 2, and 3. In humans, the virus is transmitted by person-to-person contact through fecal-oral and oral-oral (upper respiratory droplets) routes. The virus primarily infects cells of the oropharynx, the tonsils, lymph nodes of the neck, and small intestine. Viral replication occurs at the site of implantation. Once infection is established, the virus can enter the blood stream and invade the central nervous system. Human infections have four forms of manifestations: inapparent infection (without symptoms); minor illness (fever, achiness, headache, sore throat, nausea, vomiting, constipation); nonparalytic poliomyelitis (meningitis with severe headache, pain to limbs, back, and neck, stiffness of neck and back); and paralytic poliomyelitis (minor to severe muscle paralysis) (Strickland 2000).

Treatment and Eradication

Other than supportive therapy, there is no specific treatment for poliomyelitis (Strickland 2000). Medical efforts have focused on interrupting transmission through the use of prophylactic vaccines (WHO 1999a, 2002). Because humans are the only natural reservoirs for the virus, eradication efforts appear to be an attainable goal. There is no evidence of a persistent wild poliovirus carrier state or animal or insect reservoirs. Higher nonhuman primates are susceptible

Table 2 Wild poliovirus containment after polio eradication (wild polio stocks)

Activity	Risk	BSL ^a
Manipulation of wild poliovirus cultures	Accidental exposure of laboratory workers Potential release from laboratory through liquid effluent, aerosol, fomite, or inapparent infection	BSL-3/polio Vaccinate personnel
Culturing/virus isolation with samples suspected of containing wild poliovirus	Potential for wild poliovirus amplification in permissive cells	BSL-3/polio Vaccinate personnel

^aBSL, biosafety level.

to infection and disease, but these populations are not large enough to sustain wild poliovirus transmission sufficiently in the absence of human infection (Dowdle et al. 2000). The virus can survive only for finite periods of time in the environment (Dowdle et al. 2000).

In 1988, the WHO established a global polio eradication initiative. The program's current objectives include the containment of wild poliovirus, global polio-free certification, and the development of a posteradication immunization policy (WHO 2002). Once eradication is achieved, the likely sources of wild poliovirus will be in medical and research laboratories. A worldwide inventory of biomedical facilities is currently under way to establish the location of existing stocks as efforts proceed toward limiting all remaining stores of wild poliovirus.

When this issue went to press, the Department of Health and Human Services, in collaboration with other departments of the Executive Branch, had announced a nationwide poliovirus survey of more than 15,000 biomedical laboratories to begin in October 2002. Details that included the survey and inventory timeline were available on the ABSA web page (www.absa.org/Polioviruses.htm). Information is also available at the National Vaccine Program Office, www.cdc.gov/od/nvpo/polio.

Biosafety Considerations

Surveillance and diagnosis will play a major role in the eradication process. Global eradication will rely heavily on the identification of geographical outbreaks and appropriate diagnosis. Research efforts continue to improve diagnostic and treatment capabilities. Once the disease is eradicated within a geographical population, containment of existing laboratory stores is paramount. The adoption of safe laboratory policies will assist with these efforts.

We have outlined the recommended laboratory biosafety precautions for working with wild poliovirus in the laboratory and with laboratory animal models (Tables 1-3). These precautions have been divided into three phases to reflect the need for increased laboratory containment as the virus is reduced in the human population (Dowdle et al. 2000; WHO 1999a). Note in particular the differing requirements for working safely with rodents and susceptible non-human primates (Table 3).

The initial phase is known as Pre-eradication or Phase I—BSL-2/polio and ABSL-2/polio. This phase embraces BSL-2 laboratory practices, which consist of good laboratory and microbiological technique as outlined in the *WHO Laboratory Biosafety Manual* and the *BMBL* (Richmond and McKinney 1999; WHO 1993). Post-eradication or Phase II—BSL-3/polio and ABSL-3/polio—incorporates all laboratory safety practices of BSL-2/polio and ABSL-2/polio in addition to facility requirements that increase containment and facilitate appropriate decontamination. The final phase is Post-immunization or Phase III—BSL-4. When polio viral vaccines are no longer given to personnel who work with wild poliovirus, maximum laboratory containment efforts (BSL-4) are required to ensure that laboratory stocks of wild poliovirus are not reintroduced into the general population. Laboratories that already meet the BSL-3/polio and ABSL-3/polio requirements can achieve BSL-4 by installing a class III BSC (Richmond and McKinney 2000). Safe laboratory practices will play a critical role in containing any remaining stocks of wild poliovirus once global eradication is achieved.

Summary

Investigators should be alert to newly identified hazards that may emerge in the vivarium in an effort to work safely in

Table 3 Wild poliovirus containment after polio eradication (animal work)

Activity	Risk	BSL ^a
Inject permissive mice (e.g., transgenics, reconstituted knock-outs)	Minimal risk of wild poliovirus excretion Fecal-oral transmission	ABSL ^a -2/polio ABSL-2 PPE used Primary containment caging (i.e., microisolators, bioclean units) Use of BSC ^a for cage-changing, necropsy, other aerosol-generating activities Cages are decontaminated before dumping Individual water bottles used Appropriate rack decontamination
Injection of susceptible nonhuman primates with wild poliovirus	Fecal-oral transmission, including bites and scratches Potential aerosol transmission	ABSL-3/polio Full ABSL-3 PPE (e.g., Tyvek suit with head hood, gloves, N99 filter fitted to a powered air purifying respirator) Change in, shower out Room under negative air pressure and HEPA ^a filtration Solid waste and liquid effluent decontamination

^aABSL, animal biosafety level; BSC, biosafety cabinet; BSL, biosafety level; HEPA, high-efficiency particulate air.

animal research. Awareness is a first step in the risk management process, which results in safer conditions for the animal workers and the animals. We have identified a number of hazardous activities that require particular attention, along with references providing guidance for mitigating these hazards. Implementation of these work practices through protocol development, education, and continued vigilance will improve the quality and safety in biohazard research.

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