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Pathobiology and Management of Laboratory Rodents Administered CDC Category A Agents

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The Centers for Disease Control and Prevention Category A infectious agents include *Bacillus anthracis* (anthrax), *Clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), variola major virus (smallpox), *Francisella tularensis* (tularemia), and the filoviruses and arenaviruses that induce viral hemorrhagic fevers. These agents are regarded as having the greatest potential for adverse impact on public health and therefore are a focus of renewed attention in infectious disease research. Frequently rodent models are used to study the pathobiology of these agents. Although much is known regarding naturally occurring infections in humans, less is documented on the sources of exposures and potential risks of infection to researchers and animal care personnel after the administration of these hazardous substances to laboratory animals. Failure to appropriately manage the animals can result both in the creation of workplace hazards if human exposures occur and in disruption of the research if unintended animal exposures occur. Here we review representative Category A agents, with a focus on comparing the biologic effects in naturally infected humans and rodent models and on considerations specific to the management of infected rodent subjects. The information reviewed for each agent has been curated manually and stored in a unique Internet-based database system called HazARD (Hazards in Animal Research Database, http://helab.bioinformatics.med.umich.edu/hazard/) that is designed to assist researchers, administrators, safety officials, Institutional Biosafety Committees, and veterinary personnel seeking information on the management of risks associated with animal studies involving hazardous substances.

Abbreviations: ASBL, Animal Biosafety Level; CDC, Centers for Disease Control and Prevention

In June 1999, a meeting of national experts was convened to review and comment on the threat potential to civilian populations of various biological agents that could be used for biowarfare.[110] Numerous characteristics of the agents were considered, including: 1) the potential impact on public health (based on expected morbidity and mortality rates) in the event of a disease outbreak; 2) the ease with which the agent could be mass-produced, dispersed across a population, and transmitted between individuals; 3) the predicted public response to an outbreak, including incitement of public fear and civil disruption; and 4) the specialized resources anticipated to be needed during an outbreak, including drug or vaccine stockpile requirements and enhanced disease surveillance efforts and materials. Agents considered were classified into 3 priority categories (A, B, and C). When evaluated by the above criteria, Category A agents were designated as having the greatest potential to induce significant negative impact on public health.[110] The Centers for Disease Control and Prevention (CDC) Category A agents include *Bacillus anthracis* (anthrax), *Clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), variola major virus (smallpox), *Francisella tularensis* (tularemia), and the filoviruses (for example, ebola virus) and arenaviruses (for example, lassa virus) that cause viral hemorrhagic fevers (Table 1).

All of the CDC Category A agents are also agents that could pose a threat to public and animal and plant health and safety (http://www.cdc.gov/od/sap/). The United States PATRIOT Act of 2001 (PL 107-56) and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (PL 107-188), along with its implementing Department of Health and Human Services regulation Possession, Use, and Transfer of Select Agents and Toxins, contain specific provisions and regulations that considerably affect the eligibility and performance of researchers working on these select agents.[69] The CDC and the US Department of Agriculture are responsible for regulating the possession, use, and transfer of these biologic agents.

Most noteworthy advances in human infectious disease research have developed through research using animals. Laboratory animals serve multiple roles in biomedical research: they are frequently used in the development of novel technologies; they often serve as models of human and animal disease processes; and they are used to screen the safety and efficacy of potential diagnostic and treatment methods. The laboratory animal species used most frequently include the mouse (*Mus musculus*), rat (*Rattus norvegicus*), guinea pig (*Cavia porcellus*), and hamster (*Mesocricetus* spp). Here we compare the pathobiology induced in rodents given Category A agents with that described for naturally infected humans. We also address considerations related to conducting risk assessment and managing infected rodents.

The CDC Category A agents include 3 bacteria, 1 bacterial toxin, and 3 types of viruses. Their general physical and biologic characteristics are shown in Table 2. Because variola major virus is no longer used in research, we will discuss vaccinia virus, a closely related virus previously used as the vaccine strain against smallpox disease and now used instead of variola major when modeling the disease. The specific physical and biologic characteristics of the Category A agents must be considered when developing a management system to support research involving, and

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Table 1. Criteria and weighting[a] used to evaluate the potential biologic threat of CDC Category A agents

Disease	Public health impact		Dissemination potential		Public perception	Special preparation
	Disease	Death	Production and dissemination[b]	Person-to-person transmissibility		
Smallpox	+	++	+	+++	+++	+++
Anthrax	++	+++	+++	0	+++	+++
Pneumonic plague	++	+++	++	++	++	+++
Botulism	++	+++	++	0	++	+++
Tularemia	++	++	++	0	+	+++
Viral hemorrhagic fever[c]	++	+++	+	+	+++	++

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[a]Agents were ranked from highest threat (+++) to lowest (0).
[b]Potential for production and dissemination in quantities that would affect a large population, based on availability, BSL requirements, most effective route of infection, and environmental stability.
[c]Viral hemorrhagic fevers due to Filoviruses (ebola, marburg) or Arenaviruses (lassa, machupo).

provide care for, infected rodent subjects. Because of the diversity of the agents in Category A, no single management plan is suitable for all agents; a research program involving each agent must be examined individually.

Rodent Model Selection and Comparative Pathobiology

Many rodent species have been used in the study of the Category A agents. Table 3 lists some common rodent models and their primary use in related research. As in other areas of biomedical research, the mouse has become the primary mammalian model system used for infectious disease research both when studying the natural disease process and when developing drug treatments and vaccine candidates. The mouse model is frequently preferred over other animal models because of their genetic similarity to humans, the relative ease of manipulating and analyzing the mouse genome, and the availability of thousands of unique inbred and genetically modified strains. Because the immune defense and physiologic mechanisms of mice and humans are largely similar, mice are an indispensable tool for studying host–pathogen interactions that determine the balance between microbial and host survival.[14,78] In addition, mice are small and inexpensive to house compared with many other species, and many mouse-specific reagents are commercially available.

Selection of the appropriate strain of mouse is vitally important, because strains of mice often exhibit markedly different responses to infection. The most widely used model system in tularemia research involves infection of various strains of mice with either the live human-attenuated *F. tularensis* vaccine strain (LVS strain) or the fully virulent SCHU S4 strain.[40,41] Mice of the AKR, BALB/cBy, C57BL/10, and SJL strains are most resistant to the proliferation of LVS; whereas DBA/2, CBA, 129, C3H/HeJ, and A strains exhibit intermediate resistance, and SM mice are most susceptible.[2] The gender of the mice used also can affect experimental results. For example, a vaccine protection study showed that male CBA and CB6F1 mice were less protected against *Y. pestis* challenge by a fully recombinant subunit vaccine than were female mice, but no difference was noted between sexes of the BALB/c and C57BL6 strains.[75] In both male and female mice of all these 4 strains, antigen-specific antibody titers peaked at days 28 and 35, but these titers were maintained for longer than 1 y only in female mice. Strains of mice that are especially suited for use in the study of category A agents have been described. [127]

Although mouse models frequently are preferred, other rodent models may more accurately reflect the human–pathogen interaction with some agents.[36] For example, the Brown Norway rat is an excellent model of *Y. pestis* in humans because it demonstrates many of the clinical and pathologic changes seen in humans, including the development of buboes, which do not develop in mice.[100,115] The Fischer 344 inbred strain of rat is susceptible to tularemia infection and can be rendered immune by the use of the LVS strain. Fischer 344 rats are a more useful model than mice for further investigation of tularemia infection because their induced immune responses to *F. tularensis* infection more accurately model the disease in humans.[74]

An appropriate model should either simulate or impede a target process or characteristic in humans. In selection of an animal model, the pathogenesis and clinical signs induced in humans and in multiple animal species are important considerations. Although an infection model need not precisely mimic the human condition in every way, it must reflect the specific feature of interest. For example, guinea pigs frequently are used to model human infection with ebola virus. However, this species does not exhibit the hemorrhagic syndrome commonly associated with human infection and therefore is unsuitable for examining many aspects of this syndrome.[30]

Each infectious agent induces a unique pathogenesis and may cause different clinical symptoms in human or rodent hosts (Table 4). The clinical symptoms manifested are largely a direct result of the pathologic mechanism induced in the species. For example, botulism toxin is a potent zinc proteinase that cleaves 1 or more of the fusion proteins by which neuronal vesicles release acetylcholine into the neuromuscular junction;[3] associated clinical symptoms are related mainly to nerve control and muscle movement. In contrast, in anthrax infection, vegetative *B. anthracis* organisms release many toxins, leading to macrophage death, the strong inflammatory response that is responsible for edema, and hemorrhage.[59] Pathogenesis is influenced not only by the disease agent and model species but also by experimental procedures, such as route of administration. For example, *F. tularensis* LVS strain is fully virulent when delivered intraperitoneally to mice but is attenuated when delivered intradermally.[41]

Management of CDC Category A agents

Federal regulations and national standards increasingly are influencing the conduct of research. The associated mandatory reviews are necessary to safeguard the welfare of human and animal research subjects, ensure the scientific validity of the study,

Table 2. General characteristics of CDC Category A agents

Agent	Taxonomy	Physical and biologic characteristics
B. anthracis (anthrax)	Class: Bacilli Family: Bacillaceae NCBI Taxonomy ID: 1392	• Morphology: Gram-positive, nonmotile coccobacilli. Spores within the cell cause no swelling. A capsule forms in vivo or under appropriate condition in vitro. • Growth: facultative anaerobe and grows on common media between 15 and 40 °C. Sporulation occurs under abundant oxygen but not in vivo. • Toxins and virulence factors: Protective antigen, lethal factor, edema toxin, Inh, MprF, anthrolysins, and Dls[60] • Stability: Vegetative bacteria have low survival rates outside of animal or human host. Spores may persist for a prolonged time period. Spores resist drying, heat, ultraviolet light, gamma radiation, boiling for 10 min, and most disinfectants.[67,117]
C. botulinum toxin (botulism)	Class: Clostridiales Family: Clostridiaceae NCBI Taxonomy ID: 1491	• Morphology: *C. botulinum* is anaerobic, gram-positive, spore-forming bacillus (0.2–4 × ≤20 μm). • Toxins: 7 types of botulism toxin (A–G) exist. Types A, B, E, and F cause illness in humans. These toxins are all zinc endopeptidases. • Stability: Despite its extreme potency, botulism toxin is easily destroyed by heat. Low pH and high salinity enhance heat sterilization. Salt, nitrates, and nitrites suppress germination of spores in food.[61]
F. tularensis (tularemia)	Class: Gammaproteobacteria Family: Francisellaceae NCBI Taxonomy ID: 263	• Morphology: Small (0.2 × 0.2–0.7 μm), pleomorphic, poorly staining, gram-negative coccobacillus.[33] • Growth: Grows best in cysteine-enriched broth, thioglycollate broth, cysteine heart blood agar, buffered charcoal-yeast agar, and chocolate agar.[33] • Virulence factors: None characterized. Potential genomic pathogenicity island.[121] • Stability: Can survive for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses. There is little information about survival of intentionally dispersed particles; however, experts predict a short half-life due to desiccation, solar radiation, oxidation, and other factors.[33]
Y. pestis (plague)	Class: Gammaproteobacteria Family: Enterobacteriaceae NCBI Taxonomy ID: 632	• Morphology: Nonmotile, gram-negative bacillus, sometimes coccobacillus, that shows bipolar (also termed 'safety pin') staining with Wright, Giemsa, or Wayson stain • Growth: Grows optimally at 28 °C on blood agar or MacConkey agar, typically requiring 48 h for observable growth • Virulence factors: Several well characterized virulence factors including toxin production, immune inhibitors, and antiphagocytic factors. • Stability: Susceptible to environmental conditions such as sunlight and heating and does not survive for a prolonged time period outside of host. Some research proposes that *Y. pestis* may persist in soil for some time, although there is no conclusive evidence of environmental risk to humans. Plague aerosol is estimated to be infectious for approximately 1 h postdissemination.[66]
Ebola virus (hemorrhagic fever)	Family: Filoviridae Genus: Ebola NCBI Taxonomy ID: 186536	• Four recognized subtypes: Ebola Zaire (EBO-Z), Reston (EBO-R), Sudan (EBO-S), and Ivory Coast (Côte d'Ivoire; EBO-CI)[20] • Morphology: Filamentous, enveloped, negative-sense single-stranded RNA virus.[10] • Replication: Similar to rhabdomyxoviruses and paramyxoviruses.[10] Can be cultured in macrophages and Vero E6 cells.[125] • Stability: Virus may persist in a liquid or dried material in the environment at room temperature for several days.[10]
Lassa virus (hemorrhagic fever)	Family: Arenaviridae Genus: Arenavirus NCBI Taxonomy ID: 11620	• Morphology: Negative-sense single-stranded RNA virus with 2 RNA segments.[27] • Replication: Can be cultured in Vero cells, 4–5 d are sufficient for plaque development.[101] • Stability: Virus may be present in pharyngeal secretions and urine for 3–4 wk postinfection. Virus is also stable in aerosol form at relatively low levels of humidity for 10 min to 1 h and can be dispersed considerable distances to cause infection.[124]
Vaccinia virus[a] (smallpox vaccine)	Family: Poxvirus Genus: Orthopoxvirus NCBI Taxonomy ID: 1491	• Morphology: Enveloped double-stranded DNA virus • Replication: Poxviruses are unique in their ability to replicate entirely within the cytoplasm of a cell[91] • Stability: Vaccinia virus can persist in the environment even when dried.[131] Additional research is needed to determine the viability of the vaccine strains of vaccinia virus in the environment.[123]

[a]Vaccinia virus is used in place of variola major virus in smallpox research.

and identify and evaluate any associated risks to human health. Animal research must be reviewed and approved by an institutional animal care and use committee. In an effort to further protect human health, institutional biosafety committees are becoming more involved in aspects of animal use protocols that use hazardous agents, even though their mandate applies specifically to research involving recombinant DNA.[94] For research using hazardous substances, appropriate risk assessment is essential so that all potential risks to personnel can be identified and subsequently managed effectively. In addition, to maintain the integrity of the research, inadvertent transmission of an organism between animal subjects or experimental groups must be prevented.

Issues to consider when performing risk assessment for activities performed in a traditional laboratory setting using infec-

Table 3. Use of rodent models in CDC Category A agent research

Agent	Rodent models	Research use
B. anthracis (anthrax)	Mouse	*B. anthracis* strain Sterne reduces virulence for humans. Subcutaneous infection of A/J mice with strain Sterne induces systemic disease with markedly similar pathologies to those observed during human anthrax.[37] Mouse models are widely used for anthrax research.[8,59,135] For example, *B. anthracis* edema toxin causes extensive tissue lesions and rapid lethality in mice.[47]
	Rat	Fisher 344 rat is often used as a challenge model for prophylaxis research[113] or to study anthrax sepsis.[32]
	Guinea pig	Guinea pigs are often used in *B. anthracis* virulence and vaccine study.[55,102,128]
	Hamster	Golden Syrian hamster is well protected by vaccination with the STI1 Russian human live vaccine against challenge by virulent *B. anthracis* H7 isolate.[104] However, the licensed Anthrax Vaccine Adsorbed human vaccine fails to protect golden Syrian hamsters.[46]
C. botulinum toxin (botulism)	Mouse	The mouse bioassay is the most sensitive and specific measurement of toxin activity for botulism toxin detection and standardization.[63] Mouse models have been used to study the pathogenesis of intestinal colonization of *C. botulinum* spores,[88] immune responses induced by toxin components,[35] and vaccine efficacy analysis.[77]
	Rat	Rat models are used to study the pathophysiology and pathogenesis of botulism toxins.[25,38,119]
	Guinea pig	Guinea pig models are used to study the pathophysiology and pathogenesis of botulism toxins.[73,108,109]
F. tularensis (tularemia)	Mouse	The most widely used model involves infection of various strains of mice with either the attenuated (for humans) live vaccine strain (LVS) or fully virulent SCHU S4 strain of *F. tularensis*.[40,41] Specific strains of mice used depend on the parameters being studied but include T cell deficient mice (such as *nu/nu* and α/β or double α/β–γ/δ T cell knockout mice), severe combined immunodeficient (*scid*) mice, gamma interferon knockout mice, and various inbred strains of mice.[2,40] Mice of the AKR, BALB/cBy, C57BL/10, and SJL strains are most resistant; DBA/2, CBA, 129, C3H/HeJ, and A strains have intermediate resistance; while SM mice are most susceptible to the proliferation of LVS.[2] In another study, Fortier and colleagues found A/J, BALB/cHSD, C3H/HeNHSD, and SWR/J to be much more susceptible to infection with the LVS strain than C3H/HeJ and C57BL/6J.[50]
	Other rodents	Other species of rodents including rats, hamsters, guinea pigs, and prairie dogs have occasionally been used as models but are rarely used now.
Y. pestis (plague)	Mouse	Mice were used as models during the 1950s for molecular pathogenesis and vaccine studies.[115,116] In the recent literature, a number of inbred strains and outbred stocks of mice are used, including BALB/c, C57BL/6, and Swiss-Webster.[1,75,79,100]
	Rat	Brown Norway rats are models of plague and demonstrate the pathogenesis and host response to infection. The disease in rats closely resembles the disease in humans.[115]
	Guinea pig	Guinea pigs are used in vaccine efficacy studies.[76]
Ebola virus (hemorrhagic fever)	Mouse	Mouse models (predominantly BALB/c) are used to evaluate the efficacy of ebola vaccines, antiviral drugs, and monoclonal antibodies[10,56] and to model the pathology of disease of Zaire strain-infected primates.[11] Severe combined immunodeficiency (*scid*) mice are used to investigate the role of human antibodies in the development and treatment of ebola virus infection.[57]
	Guinea pig	Strain 13 guinea pigs frequently are used as models of human disease progression and to assess viral virulence.[11,30,99]
Lassa virus (hemorrhagic fever)	Mouse	CBA/calac (H-2k) mice are used as a model for vaccine and immune mechanism studies.[64] Although mice do not effectively model human lassa virus infection, they are useful economical models to examine the capacity to elicit an immune system response.[83]
	Guinea pig	Inbred (strain 13) guinea pigs infected with lassa virus are a model of human infection.[83] They are preferential for the study of vaccine and treatment routines. Outbred (Hartley) guinea pigs are more resistant to lassa virus infection and thus are useful in generating antibodies for vaccines and for study of the natural basis of the nonfatal course of infection.[72] Outbred (Hartley) strain guinea pigs are also used in studying aerosol infectivity of lassa virus.[124]
Vaccinia virus (smallpox vaccine)	Mouse	Mouse models are used to study the complications associated with human smallpox vaccination[6,120] and to evaluate compounds and immunomodulators for antiviral activity against orthopox viral diseases.[10,106] Two of the most frequently employed models are the mouse tail lesion models (MTLM) and the mouse lethality model (MLM). The MTLM involves a nonlethal, self-limiting infection in which vaccinia virus is injected in the tail vein. The number and severity of lesions are used as an indication of the virulence of the virus.[9] The MLM evaluates the antiviral activity of substances as evidenced by a prolonged survival time in severe combined immunodeficient (*scid*) mice that are administered a lethal dose of virus.[120] Mice also are used to model the progressive vaccinia virus infection of immunocompromised persons. For this model, hairless mice are immunosuppressed with cyclophosphamide and then inoculated with vaccinia virus through a scarified skin lesion.[122]
	Guinea pig	Among guinea pigs, strain 13 guinea pigs are used most frequently as a model of human infection. They are also used in the evaluation of new vaccines.[62]
	Other rodents	Recently, vaccinia virus has been used as a viral vector in gene therapy owing partially to the virus's ability to infect multiple animal species and to accept large inserts of foreign DNA.[91,114]

Table 4. Pathogenesis and clinical symptoms associated with infection

Agent	Pathogenesis	Clinical symptoms
B. anthracis (anthrax)	Spores are phagocytosed by macrophages and germinate within phagolysosomes. Vegetative bacteria release many toxins leading to macrophage death. Lethal toxin act on host macrophages and induce the release of proinflammatory cytokines responsible for inducing sudden and fatal shock.[59] Edema toxin causes localized edema and systemic shock.[60] Other virulence factors involve survival within phagolysosomes and on mucosal surfaces (Inh and MprF), escape from phagolysosomes and phagocytic cells (anthrolysins), iron-acquiring products (Dlp), and regulation of cellular products (AtxA and AcpA).[60]	Mouse: A/J mice infected with Sterne strain induced similar pathologies observed during human disease, such as edema, hemorrhage, secondary pneumonia, and lymphocytolysis.[37] Human: Percutaneous introduction of spores causes a local ulcerative inflammatory lesion (pustule) covered by a black scab (eschar). Subcutaneous edema and septicemia may occur with fatality rate of 10%–20%. Inhalation anthrax causes pulmonary edema, hemorrhagic pneumonia, and probably meningitis with mortality nearing 100%.[60]
C. botulinum toxin (botulism)	Three forms of botulism exist: foodborne, wound, and intestinal. Once absorbed, botulism toxin is transferred in the bloodstream to peripheral cholinergic synapses. It binds irreversibly to the neuromuscular junction and enzymatically blocks neurotransmitter release.[3] Botulism toxin consists of a light chain with zinc endopeptidase activity, a heavy chain for forming a pore allowing the light chain to pass, and a binding domain for binding to nerve cells. Various types of botulism toxin hydrolyze different docking proteins required by neurotransmitter-containing vesicles to fuse with the presynaptic membrane. Once hydrolyzed, the synapse degenerates.	Mouse and rat: Signs of wound botulism include labored abdominal breathing, constriction of the abdomen, limb paresis, and total paralysis. These symptoms last for 2–4 h, followed by death if mice are not treated.[34] Intragastic injection of *C. botulinum* type A spores also induces symptoms due to botulism toxin in mice 8–9 d old.[126] The response in rats to colonization by *C. botulinum* is similar to that of mice.[89] Human: Regardless of route of administration, common symptoms include weakness, paralysis, fatigue, dry mouth, and difficulty swallowing. Prominent neurologic symptoms include ptosis, diplopia, blurred vision, often enlarged or sluggishly reactive pupils, dysarthria, dysphonia, and dysphagia. As paralysis extends beyond bulbar musculature, loss of head control, hypotonia, and generalized weakness become prominent.[3]
F. tularensis (tularemia)	Infection with *F. tularensis* is usually through the skin although infection via the mucous membranes, gastrointestinal tract, and lungs can also occur. Bacteria multiply in the primary lesion and then spread to the regional lymph nodes causing a transient bacteremia, spreading organisms throughout the body. The principal target organs are the lymph nodes, lungs and pleura, spleen, liver, and kidney. Within the tissues, the typical lesion is characterized by focal, suppurative necrosis. Early lesions are comprised primarily of polymorphonuclear leukocytes. As the lesion matures, there is an accumulation of macrophages, epithelioid cells, and lymphocytes and the development of granulomatous inflammation.[33,41]	Mouse: Mice infected intranasally with virulent bacilli exhibited ruffled fur and hunched gait by 2 days post infection At day 5 post infection surviving mice were lethargic, hunched, huddled together, exhibited piloerection, weight loss, and were hypothermic.[29,132] BALB/c mice inoculated intradermally with *F. tularensis* LVS (vaccine strain) developed skin lesions characterized by necrotic dermatitis at 4 d postinfection.[130] Human: Ulceroglandular tularemia is the most common form of disease. Typical incubation is 3–6 d. Clinical signs consist of flu-like symptoms including fever, chills, headache, and generalized aches. Chronic ulceration develops at the site of infection and the regional lymph nodes enlarge. A transient bacteremia can ensue leading to bacterial dissemination to a variety of tissues. The course of the disease is protracted but rarely fatal.[41] Typhoidal tularemia is an acute septicemic form of the disease that occurs without dermal ulceration or lymphadenopathy. Mortality is considerable (30% to 60%).[41] Oropharyngeal tularemia causes a sore throat, enlarged tonsils and cervical lymph nodes, and an oropharyngeal pseudomembrane. Gastrointestinal tularemia is characterized by symptoms such as chronic, mild diarrhea to widespread gastrointestinal ulceration and death. [41] Pneumonic tularemia is the most acute form of the disease. [41]
Y. pestis (plague)	Plague bacilli move via the lymphatics from the skin to the regional lymph nodes. In the lymph nodes they are ingested and most are killed by polymorphonuclear leukocytes. A small number of bacteria are ingested by macrophages but are not killed and instead multiply intracellularly where they produce various virulence factors, including antiphagocytic factors. Once released from the macrophages, they resist ingestion and multiply rapidly in the lymph nodes. Cellulitis and occasionally abscessation occur in association with the presence of buboes. Bacteria eventually enter the bloodstream leading to seeding of visceral organs. Severe systemic disease develops (termed septicemic plague) as bacteria multiply peripherally. Gram-negative septicemia, shock, disseminated intravascular coagulopathy, gangrene, and purpura may develop. Hematogenous spread of bacteria to the lungs results in the development of pneumonic plague, bronchopneumonia, pulmonary cavitation or consolidation, and bloody or purulent sputum.[138]	Mouse: C57BL/6 mice inoculated intranasally with 10^5 *Y. pestis* huddled together and individually were listless, tachypneic, unresponsive to handling, and hunched up at 36 h. Mice began to die at 60 h postinfection;[79] 90% of intradermally injected mice developed fatal bubonic plague in 2–5 d. The earliest sign of infection was lameness in the limb nearest the injection site and regional lymphadenopathy.[116] These infected mice did not develop typical buboes despite infected lymph nodes.[115] Rat: Brown Norway, Sprague Dawley, or Wistar rats inoculated intradermally with 500 colony-forming units of plague bacilli developed a red papule at the site of inoculation 1–2 d postinfection. All rats developed rough haircoat, limping, and regional lymphadenopathy at 2–12 d postinoculation. Terminal plague developed 12–18 h after lameness was observed. Prior to death or euthanasia rats exhibited polydipsia, watery eyes, poorly groomed coat, hunched posture, and reluctance or inability to move.[115] Guinea pig: Guinea pigs were anorexic and lethargic 5 d postinfection.[76] Human (bubonic plague): Fever, chills, headache, myalgia, arthralgia, and lethargy are common 2 to 6 d after infection. Pain and tenderness in the regional lymph nodes follows, along with redness and swelling of the overlying skin (buboes). Bubonic plague may progress to systemic toxemia, tachycardia, prostration, agitation, confusion, convulsions, delirium, and death. Without intensive therapy, septicemic plague can rapidly progress to disseminated intravascular coagulation, refractory hypotension, renal shutdown, and acute respiratory distress syndrome, shock, and death.[84]

Table 4. Pathogenesis and clinical symptoms associated with infection (cont.)

Agent	Pathogenesis	Clinical symptoms
Ebola virus (hemorrhagic fever)	In rodents, the severity of infection is influenced by the animal's age and immune status as well as the virulence of the virus. Human isolates do not reliably cause fatal disease in adult, immunocompetent rodents whereas infection with specific species-adapted viruses may be lethal. In guinea pigs, mice and humans, cells of the mononuclear phagocytic system are the first targets of infection followed by infection of associated connective tissues and parenchymal cells.[10,16,56] The amount and location of fibin deposits varies with animal species.[11,30] Species-adapted strains show differing levels of virulence both across species and by route of administration.[12,30,85] In humans, microvascular damage and activation of the clotting cascade also occurs.[71] Death often occurs secondary to massive cell death, fluid shift, hemorrhage, and vascular abnormalities.[10]	Mouse: Clinical signs most frequently observed in rodents include fever, ruffled fur, lethargy, and progressive weight loss. Mice may develop coagulation abnormalities, which can result in spontaneous bleeding from the orbit, bladder, gastrointestinal tract, or other structures within the abdominal cavity.[11] Guinea pig: Guinea pigs do not develop a hemorrhagic syndrome similar to that seen in humans.[112] Human: Incubation period is 2 to 21 d.[137] Signs and symptoms of infection may include fever, chills, headache, muscle and joint pain, anorexia, nausea, vomiting, abdominal pain, hypotension, tachypnea, bradycardia, conjunctivitis, conjunctival injection, diarrhea (with or without blood), and pharyngitis.[16,137] Later in the infection, a rash and blood clotting abnormalities may develop.[10] Disseminated intravascular coagulation and central nervous system involvement may develop.[137]
Lassa virus (hemorrhagic fever)	In the adult-infected LCM mouse, disease is associated with destruction of antigen specific cytotoxic T lymphocytes.[101] Strain 13 guinea pigs develop leucopenia, decrease of hemoglobin concentration, elevation of liver aminotransferases, and liver lipidosis.[83] In humans, pathogenesis of lassa virus involves initial replication at the site of infection. The lymph nodes are an important site of virus growth, as are the lung, and later, other parenchymal organs. Interstitial infiltrates and edema may occur during the course of infection. In infections by any route, the macrophage is usually identified as an early and prominent cell involved. Many epithelial structures are readily identified as containing antigen and nucleic acids. Widespread infection of the marginal zone and necrosis of lymphoid follicles of the spleen and lymph nodes is a common lesion. [101]	Guinea pig: Strain 13 guinea pigs show signs of fever and weight loss; develop interstitial pneumonia, septal and alveolar edema, and liver lesions; and die within 2 wk postinfection.[83] Human: Disease presents with fever, muscle aches, sore throat, nausea, vomiting, and chest and abdominal pain; however, approximately 80% of human infections are mild or asymptomatic.[83] Generalized weakness as well as facial and neck swelling are common symptoms. Diarrhea, shock, vomiting, hemorrhage, and sometimes multisystem organ failure are typical symptoms of late stages of infection.[4] Fatal disease is characterized by irreversible shock.[48] Incubation period ranges from 2 d to 3 wk.[4,19]
Vaccinia virus (smallpox vaccine)	In humans, after vaccination of healthy, immunocompetent adults with Dryvax (Wyeth Laboratories, Philadelphia, PA) vaccine, the virus invades keratinocytes, causing areas of necrosis and vesicle formation. Viremia and involvement of other organs is rarely observed. Additional, severe, adverse effects may occur.[10,49] In rodents, the pathogenicity of the virus is dependent on the strain and concentration of vaccinia virus, the route of inoculation, and the species and strain of animal infected.[13] Virus may be recovered from the site of cutaneous inoculation in mice[106] and possibly guinea pigs.[53] Infection was fatal in all athymic and some rhino mice.[58,106,120] Intranasal inoculation of BALB/c mice resulted in a localized infection of the lungs, followed by dissemination to visceral organs and the brain.[107]	Mouse: Local swelling, edema, and necrosis may develop in mice after intradermal scarification of the tail. Human: Dermal inoculations generally cause localized skin infection in immunocompetent individuals but may spread and cause severe disease in immunocompromised humans. Potential outcomes after immunization of immunocompetent adults include papule development at the vaccination site that may progress to a pustule, scab, and then scar. Lymph node swelling, mild fever, lethargy, and a dermal rash may develop. Extensive skin lesions and central nervous system deficits[10] may develop in immunocompromised humans.

tious agents have been described and include the agent's induced pathogenicity in human hosts, routes of transmission, stability, infectious dose, concentration, and origin as well as the availability of effective prophylaxis and data from animal studies, the presence of an effective medical surveillance system, and the experience and skill of at-risk personnel.[131] Although the basic method for conducting an assessment is very similar to that used to evaluate any use of live animal subjects, multiple additional factors must be considered, largely because of the inherent variability induced by a living organism.

Risk assessment in infectious disease research using animal subjects. Simply speaking, risk assessment is a systematic method of identifying sources of hazards (and the potential magnitude of their negative effects) inherent in a situation, identifying personnel who may be exposed to the hazard, and determining what equipment and practices are needed to adequately contain the hazard.

In infectious disease research using animal subjects, risk assessment is performed to help determine practices that must be instituted and equipment that must be used to protect personnel from unacceptable levels of risk. A separate risk assessment may be conducted to examine issues related to maintaining the health of animals within the colony that may be exposed inadvertently to purposefully infected animals. The expertise of multiple personnel should be used when conducting risk assessment, including occupational safety specialists, Institutional Biosafety Committee members (if recombinant agents will be used), researchers most familiar with the hazards involved, and employees familiar with the institution's animal care-related procedures. These individuals must review many aspects of the animal use protocol because investigators, especially those new to an area of research, may not recognize the importance of some details (that is, routes of administration, collection and processing of pre- or postmortem tissue samples) and therefore not describe them sufficiently in general

occupational health-related documents.

Although formal risk assessment may be conducted concurrent with or after review by the institutional animal care and use committee, researchers should consider relevant information during study design. If the published literature lacks information relevant to risk assessment, the potential for human exposure may be estimated inaccurately. As a result, unnecessary precautions may be used, which can hinder research and markedly increase cost. Alternatively, the degree of risk may be underestimated, and inadequate precautions may be instituted and potentially result in disease. When sufficient information is unavailable, a preliminary study should be conducted to explore the issues specifically needed for risk assessment.

One aspect of risk assessment is identifying personnel potentially exposed to the hazards. Although research staff who work directly with the study animals are at obvious risk, additional personnel associated with the animal facility must also be considered. These include animal health technicians who may observe and handle the animals daily, cagewash personnel who may handle soiled caging and equipment prior to or after disinfection, and maintenance personnel who must service the biocontainment facilities. In addition, potential exposure of the public and the environment must also be considered and managed. The degree of contact of each of these groups with the research animals and their immediate environment will vary based upon the design of the facility and regulatory or security restrictions associated with the agents.

Multiple factors regarding the hazardous agent itself must be evaluated when determining the containment procedures appropriate for each study. Many of the factors are similar to those considered when determining the containment practices required for benchtop research. These include the magnitude of the negative consequences associated with inadvertent human exposure to the substance given the availability of safe and effective vaccines and postexposure treatment options. For example, the inherent risks of working with ebola virus, for which neither vaccines nor highly effective treatment options exist, differ markedly from those associated with the less-virulent *F. tularensis*, which often can be treated reliably. In addition, the biologic and physical characteristics of the hazardous substance must be determined, because appropriate containment procedures will vary depending on the associated stability of the substance within the environment. For example, accumulation of infectious organisms within animal caging may be greater with an agent highly resistant to environmental exposure than an agent highly susceptible to it. The environmental stability of an agent is less of a consideration in studies using highly virulent substances, because the exposure of personnel to these organisms is strictly controlled.

Compared with procedures performed in bench research, additional issues must be considered when performing a risk assessment related to animal studies. The evaluation must consider all aspects of the study related to animal subjects. Although much is currently known regarding the basic properties of hazardous substances used in animal research, information on the potential risks and sources of exposures after administration of the substance to the animal often is not readily available. Researchers and occupational safety professionals frequently are required to perform lengthy literature reviews for the necessary information or contact colleagues in the field of study who may have, but not have published, the information.

An additional factor to consider includes the route of administration of the hazardous substance to the animal subject. A substance administered topically to an animal may induce risks not associated with parental administration. For instance, substances applied topically are likely to contaminate the animals' environment and serve as a source of exposure. In addition, any risks related to animals' ingestion of topically applied substances, which can occur during normal grooming activities, must be considered. These complications may be reduced if an occlusive covering is applied over the exposure site on the animal.

Exposure of the animal's immediate environment also may occur after substance administration by other routes. For instance, the surface of the animal may become contaminated if injected inoculum leaks from the injection site or if the inoculum is inadvertently delivered to an unintended body cavity (such as the intestines) and then is excreted into the environment.

Researchers and animal facility personnel must be aware of the known or suspected biologic activity of a substance after its administration to animal subjects, but this information is frequently not available in the published literature. Will administration result in a rapid proliferation and dissemination of the substance throughout the body, or will the agent remain localized to one body region? Will hazardous toxins or spores be produced as may occur following anthrax administration? Will a hazardous substance be excreted or shed by the animal, such as through tears, saliva, urine, or feces? If so, these substances then may contaminate the surface of the infected animal or its cagemate(s), caging, laboratory areas, and equipment. If such contamination is expected, then the quantity of the contaminant must be estimated to determine whether it is sufficient to induce infection or disease in exposed personnel.

In addition, the potential routes of exposure to personnel in contact with animals, their tissues, or their environment must be evaluated. The most obvious route of exposure is accidental self-inoculation during administration of the substance to the animal. However, other routes of exposure that involve direct or indirect contact with the animal or its tissues should be considered. The risk inherent to each form of exposure depends largely on the route of excretion or shedding of any hazardous substances by the animal and the effective routes of transmission to humans. Direct routes of exposure include physical trauma inflicted by the animal, such as bites or scratches, and direct contact with the hazardous substance after administration to the animal, as detailed earlier. Human exposure to organisms aerosolized in the animal's respirations also must be considered. Indirect exposure may occur through contact with contaminated equipment or caging. In addition, experimental procedures must be assessed for the risk that they may introduce to personnel. For instance, when exposing animals to an aerosolized hazardous substance, special precautions must be instituted to protect personnel at the time of substance administration and to minimize contamination of the animal's skin and fur, which then could serve as sources of future exposure.

Management of rodent subjects exposed to infectious agents. After the degree of induced risk has been estimated through the risk assessment process, the methods of managing the risks to an acceptable level must be devised. The CDC has described 4 Animal Biosafety Levels (ABSL; numbered 1 through 4, in order of increasing risk) intended to minimize the health risks to individuals working with live animals infected with agents that induce or may induce disease in humans.[131] The appropriate containment level should be selected according to the agents and procedures that

will be used. Although risk assessment will help to identify some of the specific precautions needed to minimize or eliminate risks associated with a hazard, this process is useful only if a well-structured and managed animal care program exists. The infrastructure for such programs includes staff training, occupational health and safety programs, standard operating procedures, and so forth.

The 3 basic elements of containing risk associated with hazardous agents are facility design, laboratory practice and technique, and safety equipment.[131] Facility design incorporates engineering controls into the building construction. The specific design criteria will depend on the agents being used. For ABSL2 agents, few specialized facility design elements are needed. At ABSL3 and ABSL4, specific features are required, such as ventilation controls, integral autoclaves, and others.[52] For security purposes, biocontainment facilities for Category A agents (even ones that can be managed at ABSL2) often are designed to include a small laboratory, a procedure room, and an animal holding area. Each area must be designed to ensure that the agent is contained and prevented from entering the surrounding areas. Examples of engineering controls include ventilation systems, controlled wall penetrations, plumbing systems, and decontamination systems. Ventilation systems in biocontainment facilities must be designed to maintain the facility under negative pressure. The use of redundant exhaust fans in the ventilation system is one approach that can be used to ensure that the air pressure remains negative. Another approach uses supply and exhaust fan interlocks in the control system so that a failure of one side of the ventilation system will cause a response in the other side.[52] High-efficiency particulate air filter treatment of exhaust air generally is used to prevent dispersal of infectious agents to the surrounding area. Another facility design criterion is to engineer tight seals at all wall penetrations and wall–ceiling and wall–floor junctions. Facility plumbing, especially waste water lines, must be designed to prevent escape of pathogens. Personal showers and toilets often are not included in ABSL2, 3, or 4 biocontainment facilities. If they are designed into the facility, then the wastewater from these systems may require collection and decontamination before it is delivered to the general building waste system.

Laboratory practice and technique comprise the next element of containment to reduce risk in biocontainment animal housing facilities. These operational controls are the array of standard operating procedures developed to protect employees and ensure containment of the agents being studied. For example, procedures must be instituted to process and dispose of soiled bedding from animal cages. For some pathogens, the soiled cages and dirty bedding should be autoclaved within the biocontainment facility to completely eliminate the risk. However, with ABSL2 agents, securely transporting the dirty cages and bedding to another location for autoclaving is acceptable, if necessary. The selection and use of appropriate disinfectants and disinfecting procedures is an important operational control. The susceptibility of Category A agents to disinfection varies considerably. The selection of the appropriate agent is determined by the physical and biologic characteristics of the agent (Tables 2 and 5). *B. anthracis* spores must be autoclaved, and contaminated surfaces must be cleaned by a 3-step process in order to ensure proper decontamination. In contrast, *Y. pestis* is highly susceptible to disinfection (Table 5). In general, high-level disinfectants should be used on hard surfaces, such as biosafety cabinets and work surfaces. An autoclave or ethylene oxide sterilizer can be used to disinfect equipment and supplies, whereas rooms can be sterilized by use of vaporized hydrogen peroxide or chlorine dioxide gas. Some guidelines have been developed.[7,80,111] In addition, a monitoring program should be established to ensure the effectiveness of disinfection procedures.

Appropriate animal handling procedures will reduce risk by reducing personnel injury and cross-contamination between experimental groups. Depending on the behavior of the animal species and the skill of the handler, some procedures may best be performed with anesthetized animals, thus facilitating safe handling and reducing the risk of self-inoculation.

Another set of important laboratory practices relates to the management of animal carcasses and biologic samples. Carcasses and tissues can contain viable infectious organisms and must be disposed of in a manner that ensures that pathogens are not released into the environment. Necropsies must be performed with the utmost care because of the use of sharp instruments and the risk of lacerations and punctures. Carcasses must be transported in leakproof containers for incineration.[52,131] Incineration is one method of decontamination of carcasses and tissues, but other methods (including alkaline hydrolysis and exposure to high temperatures) may be acceptable.[52]

The third element of containment is the use of safety equipment such as personal protective equipment and nonstructural equipment. Selection of appropriate personal protective equipment depends on the characteristics of the agent and the tasks being performed. For example, if the agent must be administered by the aerosol route, if the necessary procedures are likely to create aerosols, or if the agent is naturally shed by the aerosol route, then respiratory protection must be used. Several types of respiratory protection devices could be used alone or in combination, including fit-tested respirators, biologic safety cabinets, and specialized inhalation exposure systems (for example, Middlebrook airborne infection apparatus [Glas-Col, Terre Haute, IN]). Laminar flow hoods commonly used in specific pathogen-free rodent barriers are not acceptable for containment of infectious agents. Only biologic safety cabinets should be used in biocontainment facilities.

The selection of personnel to work within biocontainment facilities is becoming an important issue that must be addressed by animal resource managers. Working within biocontainment facilities can be physically challenging, especially when respirators or specialized personal protective equipment is required. In addition, the technical nature of the job and need to follow detailed protocols precisely require technicians with high levels of training, experience, and skill. Factors such as these may require establishment of special job classifications to ensure hiring of qualified personnel. Because of the security issues associated with the use of Category A agents, managers must determine the number of personnel who will be allowed access to the facility. Another issue that can arise is whether personnel who work with Category A agents should receive a pay differential to compensate for the higher level of technical duties. However, such additional pay may incorrectly be interpreted as 'hazard pay,' with the perception of greater risk than actually may be present. Indeed, some technicians may see work in a biocontainment facility as a desirable assignment and may actually volunteer for such a position.

An ongoing program of monitoring and evaluating the implemented procedures is useful for allowing prompt resolution of problems that arise after the initiation of an experiment. Open lines of communication between animal care staff, researchers, and occupational safety personnel must be maintained so that issues that arise can be identified and corrected.

Table 5. Factors related to managing animals given Category A agents

Agent	Disinfection	Infectious dose	Excretion and transmission
B. anthracis (anthrax)	Spores are killed by autoclaving (120 °C for 15 min) and dry heat (150 °C for 60 min).[60,117] Disinfection of contaminated surfaces involves a 3-step approach aimed at (i) preliminary disinfection by select disinfectants including 10% formaldehyde (approximately 30% formalin) and 4% glutaraldehyde (pH 8.0–8.5); (ii) cleaning of all surfaces by straightforward washing and scrubbing using ample hot water; and (iii) final disinfection using 10% formaldehyde, 4% glutaraldehyde (pH 8.0–8.5), 3% hydrogen peroxide, or 1% peracetic acid.[129]	Inhalational human anthrax LD_{50} is 2500 to 55,000 spores. LD_{10} is as low as 100 spores. Unknown doses for human cutaneous and gastrointestinal anthrax.[65] Syrian hamster LD_{50} of subcutaneous *B. anthracis* Ames and H7 isolate spores is approximately 10 spores. Oral challenge of guinea pigs with 10^8 spores failed to induce infection.[5]	The endospore is the infectious unit and can proliferate in soil rich in calcium and nitrate at temperature above 15.5 °C, especially after flooding. Infection occurs by ingestion of contaminated feed or water, or via wound infection and arthropod bites. Excretion and postmortem discharges from a few infected animals can contaminate the environment and cause outbreak. Human infection takes place via skin wounds, inhalation, and ingestion, typically in occupations dealing with animals and animal-derived material such as imported hides, wool, and bone. Anthrax occurring under industrial conditions often occurs in the lethal airborne version.[60]
C. botulinum toxin (botulism)	Heating to an internal temperature of 85 °C for at least 5 min will detoxify contaminated food or drink. Spills of cultures or toxin can be decontaminated using sodium hypochlorite (0.1%).[3]	The mouse LD_{50} values for botulotoxins range from 1 ng/kg to 5 ng/kg, and similar or lower values have been estimated for humans.[90] It is estimated that the lethal amounts of crystalline type A toxin for a 70-kg human is about 0.09–0.15 μg intravenously or intramuscularly, 0.70–0.90 μg inhalationally, and 70 μg orally.[3]	*C. botulinum* can colonize in the gastrointestinal tract with in vivo production of toxin. It results in prolonged excretion of toxin and *C. botulinum* in the stool.[22] When animals die, spores germinate and generate toxin, and further contaminate the environment. Toxin ingestion, spore ingestion and wound contamination may lead to botulism. Dead rodents in feed can be the source of outbreaks.[61]
F. tularensis (tularemia)	Decontamination can be achieved by spraying contaminated area with 10% bleach solution and, after 10 minutes, cleaning with a 70% solution of alcohol. Soap water can be used to clear area of less hazardous contaminants. Waterborne infection should be prevented by city water chlorine levels.[33]	In BALB/c and C57BL/6 mice, <10 CFU of type A or type B inhalation or intradermal inoculation is sufficient to cause infection.[28] Intraperitoneal infection in mice requires 1 CFU to cause infection.[50] More than 90% of Fischer 344 rats died 5–13 d postexposure to 5 log10 cells of aerosolized tularemia.[74] Inhalation of as few as 10 CFU of virulent type A *F. turalensis* bacilli is sufficient for human infection.[26] Oral dosage of human tularemia is about 10^8 organisms.[31]	*F. tularensis* is transmitted largely through bites by ticks, flies, and mosquitoes and by contact with contaminated environments. Humans are commonly infected by contact (percutaneous, conjunctival, inhalation, ingestion). Its transmission from person to person has not been documented.[33]
Y. pestis (plague)	There is no evidence prompting a need for environmental disinfection of an area exposed to aerosolized plague bacteria because the organism is susceptible to environmental conditions and does not survive for long outside the host.[66]	C57BL/6 mice are infected with 1×10^4 colony-forming units of aerosol *Y. pestis* bacteria intranasally; the lowest dose showing 100% mortality rate.[79] In Swiss Webster mice, subcutaneous doses of 10^6 or 10^7 CFU of C092 Pgm^- strain of *Y. pestis* is sufficient to cause infection.[134] Infectious dose in human blood ranges from <10 to 4×10^7 CFU/ml.[100]	Fleas acquire *Y. pestis* from an infected blood meal. Infected fleas transmit the bacteria by bites in rodents, such as rats and ground rats. Humans are attacked by infected fleas when more susceptible rodents die. Infected mammals (including humans) may spread plague by the respiratory route.[66,100]
Ebola virus (hemorrhagic fever)	Steam sterilization is the most effective form of decontamination. A 1:100 dilution of household bleach or treatment with any standard hospital disinfectant registered with the US Environmental Protection Agency (such as quaternary ammonium compounds or phenols) should be used to disinfect surfaces and objects contaminated by blood or other bodily fluids.[10] Virus also can be inactivated by exposure to ultraviolet or gamma irradiation, 1% formaldehyde, and lipid solvents.[133]	In BALB/c mice, the LD_{50} of a mouse-adapted strain of ebola Zaire is approximately 1 virion or $1/30^{th}$ of a plaque-forming unit on Vero cells. Limited data exists regarding human infections due to difficulties in obtaining appropriate samples from the limited number of confirmed human cases, which often occur in geographically and politically isolated areas.[10]	Transmission in rodents can occur through parental inoculation. Strain 13 guinea pigs can be infected with a guinea pig-adapted strain of ebola Zaire through purposeful conjunctival or oral exposure.[70] Ebola can be transmitted between humans by direct contact with virus-containing bodily excretions.[10] There is no direct evidence of aerosol transmission in a clinical setting,[16] although the possibility of this route cannot be ruled out.[70]

Table 5. Factors related to managing animals given Category A agents (cont.)

Agent	Disinfection	Infectious dose	Excretion and transmission
Lassa virus (hemorrhagic fever)	Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, and ultraviolet light. Surfaces contaminated with blood or other bodily fluids should be disinfected with a registered hospital disinfectant or 10% bleach solution.[19]	LD_{50} for strain 13 guinea pigs is 0.3 plaque-forming units.[83] LD_{50} for outbred Hartley strain guinea pigs of aerosol virus is 15 plaque-forming units.[124] In humans, 1–10 organisms are sufficient to cause human infection.[51]	Lassa virus is excreted in high amounts via *Mastomys* spp urine.[17] Virus may be transmitted via small particle aerosol among outbred guinea pigs.[124] The most common mode of transmission in humans is via used needles and syringes as well as exposure to virus-containing blood or bodily fluids such as vomit, urine, or stool.[19] Direct contact of skin or mucous membranes with virus-containing soil, litter, inanimate objects, or surfaces as well as ingestion of uncooked rodents or other contaminated food or drink also can cause disease.[27] Transmission via aerosol is unlikely from person to person;[19] however, inhalation of infected rodent excreta could cause human infection.[4]
Vaccinia virus (smallpox vaccine)	1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde,[105] 5% Virkon (50% potassium peroxomonosulfate 5% sulfamic acid and 15% sodium alkylbenzene sulfonate), Dettol (4%–8% chloroxylenol, isopropanol, and castor oil soap).[15]	In rodents, 0.05 ml of a 10^{-3} dilution of IHD virus in nutrient broth was sufficient to cause dermal lesions of CD1 mice.[9] The LD_{50} dose of Levaditi strain in 8 strains of mice after intradermal scarification of the tail was found to be 1×10^3 to $1 \times 10^{4.8}$ pox-forming units.[13] In humans, the percutaneous dose for vaccinia immunization is approximately 5×10^{10} plaque-forming units.[86]	Vaccinia virus can be transmitted between infected and uninfected mice. Exposure may occur through direct contact or contact with fomites.[54] The rate of transmission varies with the strain of virus[81] and mice.[13] It is questioned if guinea pigs with vaccinia-induced skin lesions may transmit the virus to cagemates.[53,62] In humans, infectious organisms have been detected in exudates, crusts, respiratory secretions, and tissues. Transmission to humans can occur through mucous membrane exposure, dermal exposure (especially in areas where skin integrity is compromised), ingestion, and parental administration.[131]

CFU, colony-forming units; LD_X, dose lethal to X% of the test population.

Occupational health and safety programs for personnel who work with animals are required by national standards.[95,96] When Category A agents are used in animals, the health program should be extended to ensure that personnel are protected sufficiently and that accidental exposures or infections are identified rapidly. In evaluating the potential negative health consequences induced by an agent, personnel are assumed to be healthy and immunecompetent. The risks and appropriate containment practices and equipment will be influenced by the health of the employee (for example, immune impairment due to existing illness, immunosuppressant medication, or pregnancy). Personnel should be encouraged to disclose this information to the designated institutional health professional so that necessary accommodations can be instituted or the personnel can be reassigned to other duties. Confidentiality must be maintained throughout the process for the employee. Technicians and healthcare workers should be trained to recognize clinical signs associated with agents in use. Specialized medical surveillance programs may have to be instituted to monitor personnel.

HazARD: Hazards in Animal Research Database

Information directly related to the CDC Category A agents, summarized in Tables 2 to 6, is detailed in a web-based HazARD (Hazards in Animal Research Database) database system (http://helab.bioinformatics.med.umich.edu/hazard/). HazARD serves as a central repository of pertinent information used when performing risk assessment for rodent studies involving the administration of hazardous substances. The hazardous substances addressed in HazARD are infectious agents (bacterial, viral, and recombinant agents), toxins, and chemicals. The database is unique in its design to facilitate comparison of the pathobiology induced by a hazardous substance across humans, rodents, and select other animal species as well as the relevant comparative biology of these animals. All the data within the database is manually curated from peer-reviewed literature and reliable websites and books, is stored in a MySQL database, and can be queried through the website available at the University of Michigan Medical School, which uses the PHP scripting language and is powered by an Apache server.[87] An interactive data submission and review system has been developed to allow users to submit data to the site. The information is posted publicly only after critical review and approval by an internal expert. The HazARD database system will assist biomedical researchers, administrators, safety officials, Institutional Biosafety Committees, and veterinary personnel to find well-referenced information on hazard-associated pathobiology and risk management in laboratory animal studies. The sections in this article that address the pathobiology and management of rodents given CDC Category A agents illustrate many features available in HazARD that are useful in both basic research and clinical laboratory animal management.

Conclusion

The similarities between humans and animals are the basis for the use of animals to study the pathogenesis induced by infectious agents. Such comparative studies have made key contributions to our understanding of human diseases and the development of rational prevention and treatment strategies for disease. However, an organism may display markedly different pathogenesis in humans and animals. These differences often are not emphasized when the research is reported. Comparison of human and animal pathobiology (for example, pathogenesis and clinical symptoms) is important in preventing misinterpretation of data from these

Table 6. Factors related to preventing human diseases contracted within the laboratory environment

Agent	Documented occupationally acquired infections	Vaccine availability	Recommended biosafety containment level (BSL)
B. anthracis (anthrax)	Numerous cases of laboratory-associated anthrax occurred primarily at facilities conducting anthrax research. However, no laboratory-associated cases of anthrax have been reported in the United States since a human anthrax vaccine was introduced in the late 1950s.[131]	Anthrax Vaccine Adsorbed (BioThrax) is the only vaccine approved by the US Food and Drug Administration for the prevention of anthrax infection in individuals at high risk of exposure to *B. anthracis*. [42]	BSL2 for clinical materials and diagnostic quantities of infectious cultures. ABSL2 for experimentally infected laboratory rodents. BSL3 for activities with production quantities or concentrations of cultures, and a high potential for aerosol production.[131]
C. botulinum toxin (botulism)	There was only 1 report of botulism associated with the handling of the agent or toxin in the laboratory or working with naturally or experimentally infected animals.[131]	No commercially available vaccine in the United States. Experimental vaccines are under development.[98]	BSL2 for materials known or potentially containing the toxin; BSL3 for activities with a high potential for aerosols, production quantities of toxin, and purified toxins.[131]
F. tularensis (tularemia)	Tularemia is a commonly reported laboratory-associated bacterial infection. Almost all cases occurred at facilities involved in tularemia research. Although not reported, cases have occurred in clinical laboratories.[131] Laboratory workers exposed to tularemia cultures are most susceptible to contracting the pneumonic form.[31] Recently, laboratory workers in Boston were exposed to infection while subculturing tularemia-positive blood cultures, preparing gram stains, examining agar plates, and making suspensions for X- and V-factor assays.[118]	No commercially available vaccine in the United States. Experimental vaccines are under development.[93] A live attenuated vaccine strain (LVS) has been used for personnel in laboratories working with live bacteria. It is in clinical trials but is available only for at-risk military personnel. Because of problems in its production, it may never be licensed by the US Food and Drug Association. Consequently, several new vaccines are under active development. [93]	BSL2 practices and containment should be used for routine diagnostic activities with clinical materials, BSL3 for all manipulations of cultures and for experimental studies involving infectious materials with a potential for aerosol and droplet production (centrifuging, grinding, vigorous shaking, growing cultures in volume, and animal studies).[33]
Y. pestis (plague)	Historically, a number of fatal cases of plague infection have been reported. A particular case involved 2 laboratory workers in Johannesburg, South Africa who had worked with the bacteria for several years; both died of the disease despite several plague vaccinations.[103] Naturally occurring cases of plague have been reported in the United States. It is a proven but rare laboratory hazard.[131]	No commercially available vaccine in the United States. Experimental vaccines are under development.[66] A formalin-inactivated vaccine was used for many years but is no longer available in the United States.[21]	Microbiology laboratories should use BSL2 precautions when processing simple clinical materials and BSL3 precautions for activities posing a risk of aerosol or droplet production (for example, centrifuging, grinding, vigorous shaking, and animal studies). Bone-sawing procedures associated with surgery or postmortem examination should be avoided.[66]
Ebola virus (hemorrhagic fever)	Infection has been documented in animal care personnel attending to Reston-strain infected cynomolgus monkeys (*Macaca fascicularis*)[18] and in a laboratory worker processing infected human samples.[43]	No commercially available vaccine in the United States. Experimental vaccines are under development.[45]	BSL4 practices, safety equipment, and facilities for all activities using known or potentially infectious materials of human, animal, or arthropod origin.[131]
Lassa virus (hemorrhagic fever)	Laboratory infection can result from direct contact with virus-containing blood, urine, or pharyngeal secretions.[4] In 1970, a female laboratory worker was infected with lassa virus during an autopsy of a subject from Nigeria. After the arrival of lassa virus in the United States, 2 other laboratory workers were infected, 1 with live virus and the other via an unknown route; 1 died whereas the other displayed severe signs of disease.[17,103]	No commercially available vaccine available in the United States. Experimental vaccines are under development.[23]	BSL4 practices and facilities are recommended for all activities using known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with lassa virus should be submitted to a BSL4 containment facility.[131]
Vaccinia virus (smallpox vaccine)	Multiple cases of accidental infection of laboratory workers have been reported.[82,92,97,136] Infections also have occurred in persons in physical contact with recent vaccinees.[24,39]	Dryvax (Wyeth Laboratories, Philadelphia, PA) is the only approved vaccinia vaccine in the United States.[49] It is recommended that individuals working with animals infected with virulent strains of vaccinia virus be prophylactically vaccinated against the disease.[24] However, risk assessment should be conducted to examine the potential exposure level of personnel.[68] Vaccination is generally contraindicated for persons with a history of eczema, atopic dermatitis, immunodeficiency, or heart disease.[49]	BSL2 practices and facilities recommended for handling all infectious poxviruses (excluding variola) within a laboratory setting.[68,131] Vaccinia virus vectors also should be handled at BSL2.[44]

2 systems. Knowledge of the correct animal pathobiology due to infectious organisms facilitates performance of a complete and accurate risk assessment and the development of appropriate animal management techniques.

During the past 5 y, infectious disease research has undergone considerable changes and entered a new era. Funding for research on agents that could be used as weapons has increased dramatically. Many new and renovated facilities are being built at research institutions to expand the infrastructure needed to study biowarfare agents. Parallel to these changes, the role and responsibilities of veterinarians in infectious disease research has and will continue to change. Many of the target agents are zoonotic animal pathogens. As a result, many veterinarians are becoming important members of interdisciplinary research teams working on biodefense issues, either as principal investigators directing the research or as clinicians providing medical services. As comparative medical scientists, veterinarians have much to offer the research community in the study of infectious disease. Those of us who work in biomedical research are obligated to become informed about the animal models used in biodefense research and to contribute, through our expertise as whole-animal biologists, to the growing effort in infectious disease research needed to protect our citizens and our food supply.

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