Section VIII-C: Parasitic Agents

General Issues

This section focuses on potential hazards of working in settings in which exposures to viable parasites could occur, and approaches to decrease the likelihood of accidental exposures. Available data are limited; the perspective provided is based on review of the literature regarding reported cases of occupationally-acquired parasitic infections, available information for selected parasites regarding potential intervention measures (e.g., disinfection approaches), and knowledge about parasite biology and about the epidemiology and clinical aspects of parasitic infections. Additional details regarding occupationally-acquired cases of parasitic infections and recommendations for post-exposure management are available elsewhere,[1–3] as is further perspective about zoonoses of occupational health importance in laboratory animal research.[4] Information about diagnosing and treating parasitic infections and perspective regarding special considerations for persons who are immunocompromised or pregnant can be obtained from various reference materials, including the website of CDC's Division of Parasitic Diseases and Malaria, and are available at https://www.cdc.gov/parasites. Diagnostic resources and information about parasitic life cycles, including routes of transmission, are available through CDC's DPDx website at https://www.cdc.gov/dpdx.

Note: Microsporidia historically were considered parasites but are now recognized by most experts as fungi. However, because of their traditional association with parasitology, microsporidia are discussed in Section VIII-C: Parasitic Agents.

Blood and Tissue Protozoal Parasites

In descending order of total number of reported cases of infection reported in the literature, the blood and tissue protozoal parasites that have been associated with documented cases of occupationally acquired infection are: *Trypanosoma cruzi*, *Plasmodium* spp., *Toxoplasma gondii*, *Leishmania* spp., and *Trypanosoma brucei* subspp.[1] Other blood/tissue protozoa of potential concern include *Babesia* spp., the free-living amebae, including *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia pedata*; and the *Sarcocystis* spp. that can cause intramuscular sarcocystosis. In addition, various genera/species of microsporidia (now classified as fungi) may pose an occupational risk for extraintestinal infection; see below regarding an occupationally-acquired case of microsporidiosis.

In alphabetical order: *Leishmania* spp. cause various syndromes, including visceral, cutaneous, and mucosal leishmaniasis (clinical presentation is in part species dependent); *Plasmodium* spp. cause malaria; *T. gondii* causes toxoplasmosis; *T. cruzi* causes American trypanosomiasis (Chagas disease); and *T. brucei* subsp. *gambiense* and subsp. *rhodesiense* cause human African trypanosomiasis

(sleeping sickness). Depending in part on parasite and host factors, infective stages of these parasites may be found in the bloodstream, either briefly (e.g., during a particular phase of the infection), intermittently, or during all or most of the course of the infection. Among these parasites, tissue tropisms vary by genus and species, including which, if any, tissues/organs may become infected and whether the tissue and blood stages of the parasite differ. Some of these pathogens have been reported to be transmitted via blood transfusion, organ/tissue transplantation, and congenitally.[5–7]

Occupational Infections

Occupationally-acquired cases of infection with *Leishmania* spp., *Plasmodium* spp., *T. gondii*, and *Trypanosoma* spp. have been reported. The most commonly reported modes of transmission have included sharps (e.g., needlestick) injuries and other percutaneous exposures (e.g., through preexisting cuts, breaks, or microabrasions).[1,2] Vector-borne transmission to laboratorians has been reported, particularly for *Plasmodium* spp. (*P. falciparum*, *P. vivax*, and the simian parasite *P. cynomolgi*) but also for *T. cruzi* and *Leishmania* major.[1] Other reported laboratory routes of transmission have included mucous membrane exposures (*T. gondii*, *Leishmania* spp., and *T. cruzi*) and ingestion (*T. gondii*).[1,2] Laboratory-associated cases of infection with *Leishmania* spp., *T. gondii*, and *T. cruzi* have also been reported in persons who were working with these organisms but did not recall a discrete accident or exposure.[1,2]

Laboratory-associated cases of infection with blood/tissue protozoa may range from asymptomatic to severe. One individual with a reported case of laboratory-associated *Leishmania* infection developed clinical manifestations consistent with visceral involvement (e.g., fever, splenomegaly, leukopenia);[1,2] this case was caused either by *L. donovani* or by *L. infantum*, which is in the *L. donovani* species complex. The other laboratorians with reported cases of occupationally-acquired *Leishmania* infection (including, but not limited to, the other persons infected with parasites in the *L. donovani* species complex) developed skin lesions (cutaneous leishmaniasis), with or without associated lymphadenopathy.[1,2] One of the individuals who developed cutaneous leishmaniasis ultimately developed mucosal leishmaniasis as a sequela. In this instance, the etiologic agent was *L. amazonensis*, a species found in parts of South America. Overall, the exposure routes for the reported laboratory-associated cases of *Leishmania* infection have included accidental needlestick injuries, preexisting non-intact skin, mucosal contact, and the bite of an infected sand fly in an insectary.[1]

Occupationally-acquired *Plasmodium* infection may be associated with clinical manifestations such as fever, chills, fatigue, and hemolytic anemia. Malaria may be severe and life-threatening, particularly if caused by *P. falciparum*. Mosquito-transmitted (sporozoite-induced) *Plasmodium* infections have been

documented repeatedly in laboratory settings.[1] The other reported cases of occupationally-acquired *Plasmodium* infection have occurred in persons (including healthcare workers) who had accidental sharps injuries or exposures of non-intact skin.[1,2]

Laboratory-associated *T. gondii* infection may range from asymptomatic to relatively mild (e.g., flu-like symptoms, rash, lymphadenopathy) to life-threatening (e.g., myocarditis and encephalitis). Laboratorians have become infected with *T. gondii* via ingestion of sporulated oocysts from feline fecal specimens, as well as via percutaneous (e.g., through needlestick injuries or non-intact skin) or mucosal contact with tachyzoites or bradyzoites from human or animal specimens (e.g., peritoneal fluid from experimentally infected rodents) or cultures.[1,2]

The clinical manifestations of the acute phase of *T. cruzi* infection may include swelling and redness at the site of exposure, fever, rash, and lymphadenopathy. Life-threatening myocarditis and meningoencephalitis may develop. Approximately 20% to 30% of chronically infected persons ultimately develop clinical manifestations, typically cardiac and less often gastrointestinal (megaesophagus or megacolon). Laboratorians have become infected with *T. cruzi* via percutaneous or mucosal exposures, such as to blood from experimentally infected animals or to feces from infected triatomine bugs.

Infection with *T. b. rhodesiense* (East African) and *T. b. gambiense* (West African), which are vector-borne in nature (see below), may cause swelling and redness at the site of exposure, as well as various clinical manifestations during the hemolymphatic stage of the infection. East African trypanosomiasis typically is associated with a more acute course than the West African form, with early invasion of the central nervous system (CNS). After the parasite (of either subspecies) invades the CNS, the infection typically is fatal unless treated. Laboratorians have become infected with *T. brucei* subspp. through sharps injuries or non-intact skin.[1,2]

Various genera/species of microsporidia found naturally in non-human animals can cause extraintestinal infection in humans. Tissue tropisms vary by genus/species and also may be affected by host factors. Spores (i.e., the infective form) of microsporidia are hardy and can survive for long periods in the environment; ingestion is the primary route of transmission in nature, whereas other exposure routes could cause infection in laboratory settings. The one reported laboratory-associated case of microsporidiosis—a case of keratoconjunctivitis without systemic symptoms—occurred in an immunocompetent laboratorian who was accidentally exposed to *Encephalitozoon cuniculi* “when several drops of culture supernatant containing several million spores were spilled into both eyes.”[8]

No laboratory-associated cases of intramuscular sarcocystosis have been reported. However, humans who ingest fecally shed oocysts or sporocysts of

Sarcocystis nesbitti or of various unidentified *Sarcocystis* spp. with unknown carnivorous definitive hosts may develop intramuscular cysts.[9]

Babesia microti and other *Babesia* spp., which can cause human babesiosis (piroplasmosis), are transmitted in nature by the bite of an infected tick. Although no laboratory-associated cases of *Babesia* infection have been reported, such cases could be acquired through percutaneous contact with contaminated blood from infected persons or animals or, for culturable *Babesia* spp., with cultured parasites. Bites from naturally or experimentally infected ticks may also pose a risk.

Among the free-living amebae (FLA), *Naegleria fowleri* causes primary amebic meningitis, which typically progresses rapidly and causes death, whereas *Acanthamoeba* spp., *B. mandrillaris*, and *S. pedata* may cause granulomatous amebic encephalitis, which typically is more subacute or chronic. FLA may also cause disfiguring skin lesions (*Acanthamoeba* spp. and *B. mandrillaris*) and potentially blinding keratoconjunctivitis, particularly in association with the use of contact lenses or the presence of corneal abrasions (*Acanthamoeba* spp.). No laboratory-associated cases of infection with FLA have been reported. However, potentially infective stages of FLA may be found in tissue, cerebrospinal fluid, and other types of specimens from infected persons and in laboratory cultures of the organisms.

Natural Modes of Infection

Leishmania spp., *Plasmodium* spp., and American and African trypanosomes are transmitted in nature by blood-sucking insects. Sandflies in the genera *Phlebotomus* and *Lutzomyia* transmit *Leishmania* spp.; mosquitoes in the genus Anopheles transmit *Plasmodium* spp.; triatomine bugs, including *Triatoma*, *Rhodnius*, and *Panstrongylus* spp., transmit *T. cruzi*, which is found in the feces rather than the saliva of the bugs; tsetse flies in the genus *Glossina* transmit African trypanosomes; and ixodid (hard) ticks transmit *Babesia* spp.

Malaria is widely distributed in the tropics, although the prevalence and incidence rates of *Plasmodium* infection vary in and among areas of endemicity. In aggregate, seven *Plasmodium* spp. have been documented to infect humans in nature, primarily *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* but also the simian species *P. knowlesi*, *P. cynomolgi*, and *P. simium*.

Leishmaniasis is endemic in parts of the tropics, subtropics, and southern Europe. Many *Leishmania* spp. are zoonotic (e.g., have rodent or canine reservoir hosts); however, infected humans serve as epidemiologically important reservoir hosts in some settings for some species, including *L. donovani* and *L. tropica*. Only cats and other felines can serve as definitive hosts for *T. gondii*, which is found worldwide. Birds and mammals, including sheep, pigs, rodents, cattle, deer, and humans, can become infected via ingestion of tissue cysts or mature

(sporulated) fecal oocysts and subsequently develop tissue cysts (e.g., in skeletal muscle, myocardium, brain, eyes). Chagas disease is endemic in Mexico, Central America, and South America; sporadic vector-borne cases also occur in focal areas of the southern United States. Various domestic and wild mammals are found naturally infected with *T. cruzi*. African trypanosomiasis is endemic in sub-Saharan Africa but is highly focal in its distribution. *T. b. gambiense* occurs in parts of western and central Africa, whereas *T. b. rhodesiense* occurs in parts of eastern and southern Africa. *T. b. rhodesiense* is a zoonotic infection with cattle or, in a more limited role, game animals serving as reservoir hosts, whereas humans are the only epidemiologically important hosts for *T. b. gambiense*. *Babesia* infections are found worldwide in animals, and multiple *Babesia* spp. have been documented to infect humans; examples of animal reservoir hosts include white-footed mice (*Peromyscus leucopus*) and other small mammals for *B. microti* and cattle for *B. divergens*.

Laboratory Safety and Containment Recommendations

BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended for activities involving infective stages of the parasites discussed in this section.

Depending in part on the parasite and the phase of the infection, infective stages of blood and tissue protozoa may be present in blood and various body fluids and tissue specimens, including in cultures and homogenates, from infected humans and from experimentally or naturally infected animals, including arthropod vectors if pertinent. See above regarding the primary laboratory hazards. The risks for accidental exposures and occupationally-acquired infections in persons working with cultures, tissue homogenates, blood, or other specimens that contain any of the organisms discussed here, including during procedures that might create aerosols or droplets, should be reduced by use of PPE (e.g., long-sleeved laboratory coat/gown, gloves, face shield, sturdy closed footwear, clothing that covers exposed legs), in conjunction with containment in a biosafety cabinet (BSC). For work with infected arthropod vectors, the prevention measures include using the relevant PPE, as well as maintaining and transporting vectors in facilities or transport containers that reasonably preclude the exposure of personnel or the escape of the arthropods. See Appendix E for additional information.

Special Issues

Transfer of Agent Importation of any of these agents requires CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of these agents to another country. See Appendix C for additional information.

Intestinal Protozoal Parasites

Intestinal protozoal parasites that pose an occupational risk include *Cryptosporidium* spp., which cause cryptosporidiosis; *Cyclospora cayetanensis*, which causes cyclosporiasis; *Cystoisospora belli*, which causes cystoisosporiasis; *Entamoeba histolytica*, which causes intestinal and extraintestinal (e.g., liver abscess) amebiasis; *Giardia duodenalis*, which causes giardiasis; and *Sarcocystis* hominis (from beef) and *S. suihominis* (from pork), which cause intestinal sarcocystosis[9] (see above regarding *Sarcocystis* spp. that can cause intramuscular sarcocystosis). *Dientamoeba fragilis* (for which a cyst stage recently was identified)[10] and *Blastocystis* spp.[11] are additional intestinal protozoal parasites that may pose risk to laboratory workers, although their pathogenic potentials in humans continue to be debated.[10,12] Multiple genera/species of microsporidia (now classified as fungi) can cause intestinal microsporidiosis in humans.

Occupational Infections

Laboratory-associated infections with *Cryptosporidium* spp., *E. histolytica*, *G. duodenalis*, and *C. belli* have been reported.[1–3] The reported cases typically have been associated with ingestion of the parasite and, if symptomatic, with gastrointestinal symptoms. Laboratory work that does or may entail exposure to *Cryptosporidium* oocysts warrants special care. Occupationally-acquired infections have occurred quite commonly in personnel working with this agent, especially if infected calves were the source of the oocysts.[1,2] Other infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts via droplets of this small organism (i.e., 4–6 µm in diameter) might occur.[1,2] Rigid adherence to protocol (see below) should reduce the risks for accidental exposures and occupationally-acquired infections in laboratory and animal care personnel.

Natural Modes of Infection

All of these intestinal protozoa have cosmopolitan distributions. In nature, the primary route of transmission is ingestion of an environmentally hardy oocyst (for the coccidia), cyst (for *E. histolytica* and *G. duodenalis*), or spore for the microsporidia. The ID50 has been best established for the zoonotic species *Cryptosporidium parvum*: the reported ID50 has ranged from 12 to 2,066 ingested oocysts, depending on the strain tested;[13] and the ID50 for one strain of *C. hominis* ranged from 10 to 83 oocysts.[14] Because intestinal protozoa multiply in the host, ingestion of even small inocula could cause infection and illness. The role, if any, for non-human reservoir hosts differs among the intestinal protozoa. Cattle, other mammals, and birds can be infected with various *Cryptosporidium* spp.

Humans are the primary hosts for *E. histolytica* and *C. belli* and are the only established hosts for *C. cayetanensis*. Most human cases of *G. duodenalis* infection likely are acquired via direct or indirect human-to-human transmission,

although zoonotic transmission may rarely occur, particularly from companion cats and dogs. The parasites discussed in this paragraph do not require more than one host to complete their life cycle.

Laboratory Safety and Containment Recommendations

BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended for activities involving infective stages of the parasites discussed in this section.

Depending on the organism, infective stages of these parasites and of microsporidia may be present in the feces and/or in other body fluids (e.g., bile) and tissues. Appropriate standard precautions are recommended, with special attention to personal hygiene (e.g., handwashing), the use of PPE, and laboratory practices that reduce the risk for accidental ingestion of these organisms. Use of a BSC and/or face shield should also reduce the possibility of airborne transmission via contaminated droplets (e.g., when working with liquid suspensions of *Cryptosporidium* oocysts). *Cryptosporidium* oocysts are infectious when shed in stool because they have already fully sporulated and do not require further development outside the host; the oocysts are often present in high numbers in stool and are environmentally hardy. In contrast, the oocysts of *Cystoisospora belli* and *Cyclospora cayetanensis* require an extrinsic maturation period to become infective, which, under favorable environmental conditions, may be relatively short (potentially, <24 hours) for *C. belli* but is quite long (typically, at least 1–2 weeks) for *C. cayetanensis*.

For disinfection of contaminated surfaces (e.g., benchtops and equipment), commercially available iodine-containing disinfectants are effective against *E. histolytica* and *G. duodenalis*, when used as directed, as are high concentrations of chlorine (one cup of full-strength commercial bleach [~5% chlorine] per gallon of water [1:16, vol/vol]).[1,2] Because undiluted 3% (10 volumes) commercial hydrogen peroxide is known to kill *Cryptosporidium* oocysts after a sufficiently long contact time (data for *Cystoisospora* and *Cyclospora* oocysts are not available), the following approach can be used to decontaminate a surface affected by a laboratory spill containing *Cryptosporidium* oocysts.[1] After removing organic material from the contaminated surface (e.g., by using a conventional laboratory detergent/cleaner) and absorbing the bulk of the spill with disposable paper towels, flood and completely cover the surface with undiluted hydrogen peroxide. Dispense hydrogen peroxide repeatedly, as needed, to keep affected surfaces covered and wet/moist for approximately 30 minutes. Absorb residual hydrogen peroxide with disposable paper towels, and allow surfaces to dry thoroughly (10 to 30 minutes) before use. Care should be taken to autoclave or similarly disinfect all paper towel litter and other disposable materials before disposal. Reusable laboratory items can be disinfected and washed in a laboratory dishwasher by using the sanitize cycle and a detergent containing

chlorine. Alternatively, contaminated items may be immersed for approximately one hour in a water bath preheated to 50ºC and washed thereafter in a detergent/disinfectant solution.

Special Issues

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Cestode Parasites

Cestode parasites that pose an occupational risk include *Echinococcus* spp., *Hymenolepis (Rodentolepis) nana*, and *Taenia solium*. Echinococcosis is caused by cestodes in the genus *Echinococcus*: *E. granulosus* causes cystic echinococcosis, *E. multilocularis* causes alveolar echinococcosis, and *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis. Humans serve as intermediate hosts and harbor the metacestode or larval stage, which produces a hydatid cyst. *Hymenolepis nana*, the dwarf tapeworm, is cosmopolitan in distribution and causes hymenolepiasis, which is intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes *taenia*sis, which is the infection of the intestinal tract with the adult worm, and cysticercosis, which is the development of larval/tissue cysts (i.e., cysticerci) in various parts of the body, such as brain and subcutaneous tissue.

Occupational Infections

No Laboratory-associated infections with any cestode parasite have been reported.

Natural Modes of Infection

H. nana may act as a one-host parasite and does not require maturation in an intermediate host. *H. nana* is directly transmissible by ingestion of eggs shed in the feces of definitive hosts (i.e., infected humans or rodents). The life cycles of *Echinococcus* and *Taenia* spp. require two hosts. Canids, including dogs, wolves, foxes, coyotes, and jackals, serve as definitive hosts for *E. granulosus*; and various herbivores, such as sheep, cattle, deer, and horses, serve as intermediate hosts. Foxes and coyotes are the principal definitive hosts for *E. multilocularis*, although various canids and felids also can become infected. Rodents serve as intermediate hosts. Bush dogs and pacas serve as the definitive and intermediate hosts, respectively, for *E. vogeli*. Dogs also may be infected. Wild felines, including cougars, jaguarondi, jaguars, ocelots, and pampas cats, are the definitive hosts for *E. oligarthrus*. Various rodents, such as agoutis, pacas, spiny rats, and rabbits, serve as intermediate hosts. Humans become infected with

Echinococcus spp. when eggs shed by definitive hosts are accidentally ingested. For *T. solium*, humans serve as definitive hosts (i.e., harbor the adult tapeworm) but also may serve as accidental intermediate hosts (i.e., harbor cysticerci, larval/tissue cysts). Pigs, which are the usual intermediate hosts, become infected as they scavenge human stool that contains *T. solium* eggs.

Laboratory Safety and Containment Recommendations

Infective eggs of *Echinococcus* spp. may be present in the feces of carnivore definitive hosts.[4] *E. granulosus* poses the greatest risk because it is the most common and widely distributed *Echinococcus* sp. and because dogs are the primary definitive hosts. For *T. solium*, infective eggs in the feces of humans serve as the source of infection; accidental ingestion of infective eggs is the primary laboratory hazard. Ingestion of cysticerci of *T. solium* or *Taenia* asiatica in pork and *T. saginata* in beef could cause human intestinal infection with the adult tapeworm. Ingestion of the eggs of *H. nana* shed in the feces of definitive hosts (humans or rodents) could result in intestinal infection.

Although no Laboratory-associated infections with *Echinococcus* spp. or *T. solium* have been reported, the consequences of such infections could be serious. For echinococcal infections, the severity and nature of the signs and symptoms, if any, depend in part on the location of the cysts, their size, and condition (alive vs. dead). Clinical manifestations associated with a liver cyst could include hepatosplenomegaly, abdominal pain, and nausea, whereas a lung cyst may cause chest pain, dyspnea, and hemoptysis. For *T. solium*, ingestion of eggs from human feces can result in cysticercosis. Subcutaneous or intramuscular *T. solium* cysts may be asymptomatic; although cysts in the CNS also may be asymptomatic, they may cause seizures and other neurologic manifestations.

For laboratory work with infective stages of the cestode parasites discussed here, BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended, with special attention to personal hygiene (e.g., handwashing), the use of PPE, and laboratory practices that reduce the risk for accidental ingestion of infective eggs. For example, gloves should be worn when there may be direct contact with feces or with surfaces contaminated with fresh feces either from carnivores potentially infected with *Echinococcus* spp., humans potentially infected with *T. solium*, or humans or rodents potentially infected with *H. nana*.

Special Issues

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Trematode Parasites

The trematode parasites that pose the greatest occupational risk are the *Schistosoma* spp., although others, including *Fasciola* spp., are of concern. *Schistosoma mansoni* causes intestinal schistosomiasis. The adult flukes typically reside in the venules of the bowel and rectum. *Fasciola hepatica*, the sheep liver fluke, causes fascioliasis, in which the adult flukes live in the bile ducts of the human or animal host.

Occupational Infections

Laboratory-associated infections with *S. mansoni* and *F. hepatica* (one possible such case) have been reported, but accidental infections with other *Schistosoma* spp. could also occur.[1,2] Laboratory-associated infections with *F. hepatica* may be asymptomatic or associated with various clinical manifestations, such as right upper quadrant pain, depending in part on the phase of the infection. Most laboratory exposures to schistosomes would result in low worm and egg burdens, with low-risk for long-term morbidity, although acute infection may be associated with clinical manifestations (e.g., dermatitis, fever, cough, hepatosplenomegaly, lymphadenopathy).

Natural Modes of Infection

F. hepatica has a cosmopolitan distribution and is most common in sheep-raising areas; other natural hosts include goats, cattle, hogs, deer, and rodents. Snails in the family *Lymnaeidae*, primarily species of *Lymnaea*, serve as intermediate hosts for *F. hepatica* and release cercariae that encyst on vegetation. Humans become infected with *F. hepatica* by eating raw or inadequately cooked vegetation, especially green leafy plants, such as watercress, on which metacercariae have encysted. The same route of transmission is applicable to *Fasciola gigantica* (giant liver fluke) and *Fasciolopsis buski* (an intestinal fluke). Infection with other trematodes requires consumption of the infected intermediate host (mainly fish or crustaceans); therefore, the laboratory risk posed by these pathogens is minimal if appropriate standard precautions are followed, including the use of PPE.

S. mansoni is endemic in parts of Africa, South America, and the Caribbean. Free-swimming cercariae in contaminated bodies of water infect humans via skin penetration. The natural snail hosts capable of supporting development of *S. mansoni* are various species of *Biomphalaria*.

Laboratory Safety and Containment Recommendations

Infective stages of *F. hepatica* (metacercariae) and *S. mansoni* (cercariae) may be found, respectively, encysted on aquatic plants or free-living in the water in laboratory aquaria used to maintain snail intermediate hosts. Ingestion of fluke metacercariae and skin penetration by schistosome cercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may

also result in exposure of skin or mucous membranes to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or aquaria.

All of the reported cases of laboratory-associated schistosomiasis have been caused by *S. mansoni*, which probably in part reflects the fact that a laboratory life cycle for *S. mansoni* can be maintained using mice, which is not possible for the other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, or *S. guineensis* could easily occur via transdermal penetration if infected snail intermediate hosts are kept in aquaria or if laboratorians work with water samples that contain infective cercariae. In addition, exposure to cercariae of non-human (e.g., avian) species of schistosomes may cause mild-to-severe dermatitis (i.e., swimmer's itch).

BSL-2 and ABSL-2 practices, including appropriate PPE and containment equipment/facilities, are recommended for laboratory work with infective stages of the trematode parasites discussed here (i.e., when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally infected snail intermediate hosts). For example, in addition to gloves, long-sleeved laboratory coats and face shields or other protective garb should be worn when working in the immediate area of aquaria or other water sources that may contain schistosome cercariae. Cercariae can be killed on contact with 70% ethanol.[15] Therefore, precautionary measures include having squirt bottles that contain 70% ethanol as well as bottles that contain hand sanitizers for which alcohol is the active ingredient strategically placed around the laboratory to facilitate immediate access after accidental spills/exposures.[15] Various approaches (e.g., ethanol, bleach, heat) can be used to kill snails and cercariae in the water of laboratory aquaria before discharge to sanitary sewers. For example, heating the water to ≥50°C will kill the cercariae within seconds.[15]

Special Issues

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Nematode Parasites

Nematode parasites that pose an occupational risk include the ascarids; *Strongyloides stercoralis*; hookworms (both human and animal); *Enterobius vermicularis* (human pinworm); and the human filariae, primarily *Wuchereria bancrofti* and *Brugia* spp. Three hookworm species cause patent disease in humans: *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* (which also

causes patent disease in cats and dogs). *Ancylostoma braziliense*, *A. caninum*, and *Uncinaria stenocephala* cause hookworm infection in cats and dogs and can also cause cutaneous larva migrans in humans. *Ascaris lumbricoides* causes ascariasis in humans and pigs. *Baylisascaris procyonis* (a parasite of raccoons), *Toxocara canis* (dog reservoir), and *Toxocara cati* (cat reservoir) cause visceral, ocular, and neural larva migrans in humans. Larval anisakid nematodes (in fish and squid) cause anisakiasis. *Trichuris trichiura* (whipworm) causes trichuriasis in humans. *E. vermicularis* (pinworm; humans only) causes enterobiasis (oxyuriasis). *S. stercoralis* (humans and dogs) causes strongyloidiasis; animal *Strongyloides* spp. may cause cutaneous larva migrans. *Angiostrongylus cantonensis* causes eosinophilic meningitis, and *Trichinella* spp. cause trichinellosis.

Occupational Infections

Laboratory-associated infections with human hookworms, *A. lumbricoides*, *E. vermicularis*, and *Strongyloides stercoralis* have been reported.[1–3] Laboratory infections with hookworm and *Strongyloides* spp. presumptively acquired from infected animals have also been reported.[1–3] Allergic reactions to various antigenic components of human and animal ascarids and anisakids from fish (e.g., aerosolized antigens) may pose risk to sensitized persons.

Laboratory-associated infections with these nematodes may be asymptomatic or associated with a range of clinical manifestations, depending in part on the parasite species and the location(s) of the parasite in the host. The clinical manifestations of infection with *A. lumbricoides* may include cough, fever, and pneumonitis as larvae migrate through the lungs; the larvae develop into adult worms in the small intestine. Infection with *E. vermicularis* usually causes perianal pruritus, with intense itching.

Natural Modes of Infection

Human hookworm and *S. stercoralis* infections are acquired via transdermal penetration of the skin by infective filariform larvae. These nematodes are commonly found in tropical and subtropical regions of the world and cause infection in the small intestine. In contrast to hookworms, *S. stercoralis* is autoinfective and infection may be lifelong if untreated. Intradermal migration of *S. stercoralis* larvae can be associated with a rapidly moving, serpiginous, pruritic eruption referred to as larva currens ("racing" or "running" larva). The time required for *Strongyloides* larvae passed in stool to develop into infective filariform larvae may be as short as approximately two days (i.e., 48 hours); the time required for hookworm larvae to become infective may be as short as three days.

Human cutaneous larva migrans (creeping eruption) occurs when infective larvae of animal hookworms (typically dog and cat hookworms) or of animal *Strongyloides* spp. penetrate the skin and begin wandering. Hookworm infections in dogs and cats and *Strongyloides* spp. infections in animals are endemic worldwide.

A. caninum larvae can also cause infection if ingested. On rare occasions, ingested *A. caninum* larvae have developed into non-gravid adult worms in the human gut, leading to eosinophilic enteritis.

A. lumbricoides and *T. trichiura* infections are endemic in tropical and subtropical regions of the world. *T. canis* and *T. cati* are found worldwide in dogs and cats, respectively. *B. procyonis* is found primarily in raccoons but may also infect dogs. All of these parasites are transmitted via ingestion of embryonated (larvated) eggs. Unembryonated eggs passed in the stool require 2–3 weeks to larvate and become infectious. The eggs are very hardy in the environment and are resistant to most disinfectants (see below).

E. vermicularis is found worldwide, but pinworm infection tends to be more common in school-age children than adults and in temperate than tropical regions. Pinworm infection is acquired by ingestion of eggs (e.g., eggs on contaminated fingers after scratching the perianal skin). Eggs passed by female worms are not immediately infective but require only several hours to become fully infectious. Pinworm infection is of relatively short duration (approximately 60 days on average) unless reinfection occurs.

Some anisakid larvae (*Anisakis* spp., *Pseudoterranova decipiens*, and *Contracecum* spp.) are infective to humans via ingestion. The larvae may be coughed up, be vomited, or form eosinophilic granulomas in the gastrointestinal tract. These nematodes also are antigenic and may cause immediate hypersensitivity reactions (e.g., urticaria, anaphylaxis) when infected fish are ingested.

Laboratory Safety and Containment Recommendations

Eggs and larvae of most nematodes are not infective in freshly passed feces; development to the infective stages may require from less than one day to several weeks, depending in part on the genus/species and the environmental conditions. Ingestion of infective eggs or transdermal penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel.

To minimize the risk for transdermal penetration when working with cultures or fecal specimens that may contain infective hookworm or *Strongyloides* spp. larvae, PPE should be used to cover exposed skin. In an investigation in which *S. stercoralis*–positive stool specimens were reexamined after they had been stored at 4°C for 24, 48, and 72 hours, 23% of the 74 specimens examined still had viable larvae after refrigeration for 72 hours.[16] The following iodine concentrations have been shown to kill infective larvae immersed in an aqueous iodine solution for one to five minutes: 50 ppm iodine for *S. stercoralis* larvae, 60 ppm for *N. americanus* (hookworm) larvae, and 70 ppm for *A. caninum* (hookworm) larvae.[17] In vitro exposure to 70% ethanol has been shown to kill infective *S. stercoralis* larvae within 4.3 ± 1 minutes (mean ± standard deviation).[18] In vitro exposure to 70% ethanol has been shown to kill 95.6% of 45 infective

N. americanus larvae within five minutes and to kill all such larvae within 10 minutes.[19] Taking into consideration the data summarized in this paragraph, Lugol's iodine (1% povidine iodine; 10,000 ppm) may be used to kill *N. americanus* and *S. stercoralis* infective larvae on exposed skin and 70% ethanol (which leaves far less residue on surfaces) may be used to disinfect contaminated laboratory surfaces and equipment.

Ascarid (*A. lumbricoides*, *Toxocara* spp., *B. procyonis*) and *E. vermicularis* eggs are sticky; special care is warranted to ensure that contaminated surfaces and equipment are thoroughly cleaned. Precautions are warranted even when working with formalin-fixed stool specimens. Ascarid eggs, which are exceptionally environmentally resistant, may continue to develop to the infective stage in formalin;[20] they also may continue to develop despite exposure to high concentrations of disinfectants for long periods. However, ascarid eggs can be deactivated by the use of heat at or above 60ºC for more than 15 minutes.

Accidental ingestion of larvated (infectious) eggs of *Toxocara* and *B. procyonis* could lead to visceral migration of larvae, including invasion of the eyes and CNS. The larvae of *Trichinella* in fresh or digested animal tissue, or of *A. cantonensis* in fresh or digested mollusk tissue, could cause infection if accidentally ingested. Vector arthropods infected with filarial parasites pose a potential hazard to laboratory personnel. The prevention measures include using the relevant PPE (e.g., gowns, gloves, closed shoes); maintaining and transporting vectors in facilities or transport containers that reasonably preclude the exposure of personnel or the escape of infected arthropods are also essential. See Appendix E for additional information.

The use of primary containment (e.g., BSC) during work that may be associated with aerosolization should reduce the potential for exposure to aerosolized antigens of ascarids and anisakids, which can cause allergic reactions in sensitized persons. Special attention to use of PPE and to personal hygiene (e.g., handwashing) is warranted when working with any of the nematode pathogens discussed here.

Special Issues

Transfer of Agent Importation of any of these agents requires CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of these agents to another country. See Appendix C for additional information.

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Section VIII-D: Rickettsial Agents

Coxiella burnetii

Coxiella burnetii is a bacterial obligate intracellular pathogen that is the etiologic agent of Q (query) fever. It undergoes its developmental cycle within an acidic vacuolar compartment, exhibiting many characteristics of a phagolysosome. The biphasic developmental cycle consists of a small cell variant (SCV) and a large cell variant (LCV). The SCV is the more structurally-stable cell variant, persisting for extended periods of time outside of host cells and exhibiting resistance to extracellular stresses (drying, extreme temperatures, environmental conditions). The LCV is the larger, metabolically-active variant, which facilitates replication of the agent.[1–4] The organism undergoes a virulent (phase I) to avirulent (phase II) transition upon serial laboratory passage in eggs or tissue culture.

The ID of phase I organisms in laboratory animals has been calculated to be as small as a single organism.[5] The estimated human ID for development of Q fever by inhalation is approximately 10 organisms.[6] Typically, the disease manifests with flu-like symptoms including fever, headache, and myalgia, but can also present with pneumonia and hepatomegaly. Infections range from subclinical to severe, and primary/acute infections respond readily to antibiotic treatment. Although rare, *C. burnetii* can cause chronic infections such as endocarditis, granulomatous hepatitis, or vascular infections.[7]

Occupational Infections

Q fever is the second most commonly reported Laboratory-associated infection (LAI) in Pike's compilation with outbreaks involving 15 or more persons recorded in several institutions.[8,9] Infectious aerosols are the most likely route of LAI. Experimentally infected animals may also serve as potential sources of infection for laboratory and animal care personnel. Exposure to naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.[10,11]

Natural Modes of Infection

Q fever occurs worldwide. A broad range of domestic and wild mammals are natural hosts for Q fever and may serve as potential sources of infection. Parturient animals and their birth products are common sources of infection. The placenta of infected sheep may contain as many as 10^9 organisms per gram of tissue[12] and milk may contain 10^5 organisms per gram. The resistance of the organism to drying and its low infectious dose can lead to dispersal from contaminated sites. The agent may also be present in infected arthropods, and it may be present in the blood, urine, feces, milk, and tissues of infected animals or human hosts.

Laboratory Safety and Containment Recommendations

Recent advances leading to cell-free media supporting the growth of *C. burnetii*[13] have greatly reduced the necessity of using embryonated eggs or cell culture techniques for propagation and accompanying extensive purification procedures. Exposure to infectious aerosols and parenteral inoculation remain the most likely sources of infection to laboratory and animal care personnel.[8,9]

BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of *C. burnetii*, the necropsy of infected animals, and the manipulation of infected tissues. Because infected rodents may shed the organisms in urine or feces,[8] experimentally infected animals should be maintained under ABSL-3. A specific plaque-purified clonal isolate of an avirulent (phase II, Nine Mile Strain, plaque purified clone 4) strain is exempt from the Select Agent Regulations and may be safely handled under BSL-2 conditions.[14] BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears.

Special Issues

C. burnetii is among the most environmentally stable of non-spore forming bacteria with a known capacity for extended survival in soil or other contaminated materials, such as animal products, for years.[4] The ID approaches a single organism,[5] thus the capacity for airborne or aerosol transmission is high. Infections are frequently asymptomatic, or cause relatively mild, flu-like symptoms, but can be severe. Chronic infections (i.e., endocarditis) are possible, particularly in those with pre-existing valvular damage or immunocompromised individuals. Q fever is a known hazard during pregnancy.[15]

Exposure to naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.[10,11] Recommended precautions for facilities using sheep as experimental animals are described by Spinelli and Bernard.[10,16]

Vaccines Q fever vaccines are not commercially available in the United States. Individuals with valvular heart disease should not work with *C. burnetii*. Work with *C. burnetii* should be avoided during pregnancy. See Section VII for additional information.

Select Agent *C. burnetii* is considered a Select Agent under the Code of Federal Regulations (42 CFR Part 73). All rules concerning the possession, storage, use, and transfer of Select Agents apply. Appendix F contains additional information on Select Agents, including contact information for registration and obtaining appropriate permits for importing, exporting, or transporting this agent.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

Rickettsia species **and** ***Orientia tsutsugamushi***

Rickettsia prowazekii, *Rickettsia typhi*, the Spotted Fever Group agents of human disease (*Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia akari*, *Rickettsia australis*, *Rickettsia sibirica*, and *Rickettsia japonica*), *Orientia tsutsugamushi*, *Rickettsia philipii* (*Rickettsia* 364D), *Rickettsia parkeri*, and various other *Rickettsia* spp. either known as or suspected to be human pathogens of varying pathogenicity are the respective etiologic agents of epidemic typhus, endemic (murine) typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsialpox, Queensland tick typhus, North Asian spotted fever, Japanese spotted fever, scrub typhus, Pacific Coast tick fever (PCTF), and *Rickettsia parkeri* rickettsiosis.

Rickettsia spp. are bacterial obligate intracellular pathogens that are transmitted by arthropod vectors and replicate within the cytoplasm of eukaryotic host cells. *Rickettsia* spp. are broken into four groups within the genus: the typhus group, the Spotted Fever Group, a transitional group, and an ancestral group.[17] The more distantly related scrub typhus group is now considered a distinct genus, *Orientia*. *Rickettsia*e are primarily associated with arthropod vectors in which they may exist as endosymbionts that infect mammals, including humans, through the bite of infected ticks, lice, fleas, or mites.

Occupational Infections

Although not a natural route of infection, some *Rickettsia* spp. can be infectious by an aerosol route, thus adherence to BSL-3 practices is essential. Parenteral inoculation/needlestick injuries are also among the more common routes of laboratory infection. Infections can also be acquired by conjunctival inoculation.

Pike reported 56 cases of epidemic typhus with three deaths, 68 cases of murine typhus, and 57 cases of laboratory-associated typhus (type not specified).[8] Three cases of murine typhus were reported from a research facility.[18] Two of these three cases were associated with the handling of infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation.

Rocky Mountain spotted fever (RMSF) is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal and occurred prior to 1940.[8] Since that time, two fatalities occurred, in the same facility and presumably from the same exposure, among a laboratory worker and a custodian in 1977. These illnesses were presumed to be employment-related.[19]

Oster reported nine cases occurring from 1971 to 1976 in one laboratory, which were believed to have been acquired as a result of exposure to infectious aerosols.[20]

Natural Modes of Infection

The epidemiology of rickettsial infections is a reflection of the prevalence of the *rickettsia*e in the vector population and the interactions of the arthropod vector with humans. Epidemic typhus is unusual among *rickettsia*e in that humans are considered the primary host. Transmission is by the human body louse, and outbreaks are now associated with breakdowns of social conditions.[21] Under these conditions, even with appropriate treatment, mortality averaged about 4%.[22] Endemic typhus is maintained in rodents and transmitted to humans by fleas. The various spotted fever group *rickettsia*e are limited geographically, probably by the distribution of the arthropod vector (usually ticks), although specific spotted fever group *rickettsia*e are found on all continents.[23]

Laboratory Safety and Containment Requirements

Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of Laboratory-associated infection.[24] Aerosol transmission of *R. rickettsii* has been experimentally documented in non-human primates.[25] Five cases of rickettsialpox recorded by Pike were associated with exposure to bites of infected mites.[8]

The tissues of naturally and experimentally infected mammals and their ectoparasites are potential sources of human infection. The organisms are relatively unstable under ambient environmental conditions.

BSL-3 practices and containment equipment are recommended for activities involving culture propagation or specimen preparation and propagation of clinical isolates known to contain or potentially containing *Rickettsia* spp. pathogenic to humans.

Arthropod Containment Level 3 (ACL-3) practices and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease.[26]

Laboratory work with *Rickettsia* spp. may be conducted in a BSL-2 facility with enhanced special practices including strict access control, competency, and adherence to BSL-3 practices. Laboratories should be locked and access to non-essential personnel should be prohibited. BSL-3 practices include, but are not limited to, appropriate personal protective equipment (e.g., rear-closing gowns, gloves, eye protection, and respiratory protection such as N95 respirators or PAPRs), use of BSCs when handling any open container with potentially infectious material, and primary containment, such as sealed centrifuge rotors

and other means of containment outside the BSC. Disruption of infected cells or yolk sacs should be accomplished within the BSC using an enclosed chamber to minimize the potential for aerosols. If eggs are used for propagation, the site of inoculation should be sealed with an appropriate sealant prior to transfer to an incubator. BSL-2 facilities with BSL-3 practices are recommended for all manipulations of known or potentially infectious materials, including the necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell cultures. Use of sharps should be minimized. When use of sharps is necessary, they should be disposed of and decontaminated appropriately. All contaminated materials should be effectively decontaminated before removal from the laboratory. If transport to an autoclave is necessary, materials should be double-bagged.

BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures with inactivated samples, including serological and fluorescent antibody procedures, nucleic acid amplification, and for the staining of impression smears after fixation.

ABSL-2 practices and facilities are recommended for the holding of experimentally infected mammals other than arthropods. Several species including *R. montanensis*, *R. rhipicephali*, *R. bellii*, *R. amblyommatis*, and *R. canadensis* are not known to cause human disease and may be handled under BSL-2 conditions. New species are frequently described and should be evaluated for appropriate containment on a case-by-case basis.

Because of the proven value of antibiotic therapy in the early stages of infection, it is essential that laboratories working with *rickettsia*e have an effective system for reporting febrile illnesses in the laboratory, animal facility, and support personnel; medical evaluation of potential cases; and the institution of appropriate antibiotic therapy when indicated. Prophylactic antibiotic treatment following a potential exposure is discouraged in the absence of clinically compatible signs and symptoms and could delay onset of disease. Vaccines are not currently available for use in humans.

Laboratory Surveillance

Since 1940, only two laboratory fatalities have occurred due to *R. rickettsii*.[19,27,28] This incident emphasizes the necessity of controlling access to the laboratory and expeditious reporting of any exposure or unexplained illness.

Special Issues

Occupational Health Recommendations Under natural circumstances, the severity of disease caused by rickettsial agents varies considerably.[23,29] In the laboratory, very large inocula are possible, which might produce unusual and very serious responses. Surveillance of personnel for Laboratory-associated

infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease. See Section VII for additional information.

Infections adequately treated with specific anti-rickettsial chemotherapy on the first day of disease do not generally present serious problems. However, delay in instituting appropriate chemotherapy may result in debilitating or severe acute disease ranging from increased periods of convalescence in typhus and scrub typhus to death in *R. rickettsii* infections. The key to reducing the severity of disease from LAIs is a reliable surveillance system, which includes:

1. Round-the-clock availability of an experienced medical officer knowledgeable about infectious disease;
2. Education of all personnel on signs and symptoms of disease and the advantages of early therapy;
3. A non-punitive, anonymous reporting system for all recognized accidents; and
4. The reporting of all febrile illnesses, especially those associated with headache, malaise, and prostration when no other certain cause exists.

Select Agent *R. prowazekii* is considered a Select Agent under the Code of Federal Regulations (42 CFR Part 73). All rules concerning the possession, storage, use, and transfer of Select Agents apply. Appendix F contains additional information on Select Agents, including contact information for registration and obtaining appropriate permits for importing, exporting or transporting this agent.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-E: Viral Agents

Hantaviruses

Hantaviruses are negative-sense RNA viruses belonging to the genus *Hantavirus* within the family *Bunyaviridae*. The natural hosts of hantaviruses are rodent species and they occur worldwide. Hantavirus pulmonary syndrome (HPS) is a severe disease caused by hantaviruses such as Sin Nombre virus or Andes virus whose hosts are rodents in the subfamily *Sigmodontinae*. This subfamily only occurs in the New World, so HPS is not seen outside North and South America. Hantaviruses in Europe and Asia frequently cause kidney disease, called nephropathica epidemica in Europe, and hemorrhagic fever with renal syndrome (HFRS) in Asia. HFRS caused by Seoul or Seoul-like viruses originating from *Rattus* sp. has been described worldwide. Hantaviruses have been recently described worldwide in shrews, but no human disease has been described yet from these viruses.

Occupational Infections

Documented Laboratory-associated infections have occurred in individuals working with hantaviruses.[1–4] Extreme caution must be used in performing any laboratory operation that may create aerosols (e.g., centrifugation, vortex-mixing). Operations involving rats, voles, and other laboratory rodents should be conducted with special caution because of the extreme hazard of aerosol infection, especially from infected rodent urine.

Natural Modes of Infection

HPS is a severe, often fatal disease that is caused by Sin Nombre and Andes or related viruses.[5,6] Most cases of human illness have resulted from exposures to naturally infected wild rodents or to their excreta. Human infections and illness (caused by Seoul-like virus) have been reported in Europe and the U.S. in people raising and trading pet rats.[7,8] Person-to-person transmission does not occur, with the exception of a few rare instances documented, for Andes virus.[9,10] Arthropod vectors are not known to transmit hantaviruses.

Laboratory Safety and Containment Recommendations

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented.[4–6,10] Exposures to rodent excreta, especially aerosolized infectious urine, fresh necropsy material, and animal bedding are presumed to be associated with risk. Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin and, in particular, animal bites. Viral RNA has been detected in necropsy specimens and in patient blood and plasma obtained early in the course of HPS;[11,12] however, the infectivity of blood or tissues is unknown.

All work involving inoculation of virus-containing material into rodent species permissive for chronic infection should be conducted at ABSL-4. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility using BSL-3 practices, containment equipment, and procedures. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment, and procedures. Potentially infected tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures.

BSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a BSC is recommended for all handling of human body fluids when potential exists for splatter or aerosol. Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures.

Special Issues

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Hendra Virus (formerly known as Equine Morbillivirus) and Nipah Virus

Hendra virus (HeV) and Nipah virus (NiV) are members of the genus called *Henipavirus*, within the family *Paramyxoviridae*.[13] Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named Hendra virus, occurred in horses in Australia in 1994 and 1995. From 1994 to 2017, there have been more than 90 confirmed cases of Hendra virus infection in horses in Queensland and in northeast New South Wales. Following contacts with infected horses, four out of the seven human cases described were fatal and associated with encephalitis or respiratory disease. During 1998–1999, an outbreak of illness caused by a similar but distinct virus, now known as Nipah virus, occurred in Malaysia and Singapore. Human illness, characterized by fever, severe headache, myalgia, and signs of encephalitis occurred, in individuals in close contact with infected pigs (i.e., pig farmers and abattoir workers).[14–16] A few patients developed a respiratory disease. Approximately 40% of cases resulted in fatalities. Following the 1998–1999 outbreak in Malaysia, the WHO Regional Office for South-East Asia reported 16 outbreaks in Bangladesh and India between 2001 and 2012, totaling 263 cases. Person-to-person transmission of Nipah virus in Bangladesh and India are reported regularly. Transmission also occurs from direct exposure to infected bats and through consumption of raw date palm sap contaminated with infectious bat excretions. In 2014, an outbreak of Nipah virus occurred in the Philippines that resulted in deaths of horses and humans. Outbreaks of Nipah in South-East Asia have a strong seasonal pattern,

occurring between December and May, possibly due to bat breeding season or the date palm sap harvesting season.[17–19] A new henipavirus, Cedar virus, has been isolated from pteropid bats and has significantly reduced virulence in several animal models. The reduced virulence is likely related to alterations found in the P gene, which ablates the production of innate immune antagonist proteins.

Occupational Infections

No Laboratory-associated infections are known to have occurred because of Hendra or Nipah virus exposure. However, people in close contact with Hendra virus-infected horses, especially veterinary professionals (i.e., four cases with two fatalities), are at high risk of contracting the disease.[20–24]

Natural Modes of Infection

The natural reservoir hosts for the Hendra and Nipah viruses appear to be fruit bats of the genus *Pteropus*.[25–27] Studies suggest that a locally occurring member of the genus, *Pteropus giganteus*, is the reservoir for the virus in Bangladesh.[28] Individuals who had regular contact with bats had no evidence of infection (i.e., antibody) in one study in Australia.[29] Human-to-human transmission has been described in familial clusters and associated with close care of severely ill patients.[30]

Laboratory Safety and Containment Recommendations

The exact mode of transmission of these viruses has not been established. Most clinical cases to date have been associated with close contact with horses, equine blood or body fluids (Australia), or pigs (Malaysia/Singapore), but presumed transmission from *Pteropus* bats to humans via palm date juice has been recorded in Bangladesh. Live virus has been detected in bat urine, implying the important role of urine in transmitting henipaviruses to spillover hosts. Hendra and Nipah viruses have been isolated from tissues of infected animals. In the outbreaks in Malaysia and Singapore, viral antigen was found in central nervous system, kidney, and lung tissues of fatal human cases, and virus was present in secretions of patients, albeit at low levels.[31,32] Active surveillance for infection of healthcare workers in Malaysia has not detected evidence of Laboratory-associated infections in this setting.[33]

Because of the unknown risks to laboratory workers and the potential impact on indigenous livestock, should the virus escape a diagnostic or research laboratory, health officials and laboratory managers should evaluate the need to work with the virus and the containment capability of the facility before undertaking any work with Hendra, Nipah, or suspected related viruses. BSL-4 is required for all work with these viruses. Once a diagnosis of Nipah or Hendra virus is suspected, all diagnostic specimens also must be handled at BSL-4. ABSL-4 is required for any work with infected animals.

Work with Cedar virus in a new animal model should be performed at ABSL-3 until it is demonstrated that the virus does not result in observable illness. Work with Cedar virus in susceptible animal hosts can be performed at ABSL-2 if it has been demonstrated that the virus is avirulent/non-pathogenic and following a risk assessment of the proposed work.

Special Issues

Vaccines Vaccines are not available for use in humans, but Hendra vaccine is available in Australia for horses.

Select Agent Hendra and Nipah virus are Select Agents requiring registration with CDC or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Hepatitis A Virus, Hepatitis E Virus

Hepatitis A virus (HAV) is a positive-sense single-stranded RNA virus, the type species of the Hepatovirus genus in the family *Picornaviridae*. Hepatitis E virus (HEV) is a positive-sense single-stranded RNA virus of the genus Orthohepevirus in the family *Hepeviridae*. There are four major hepatitis E genotypes that infect humans: genotypes 1, 2, 3, and 4.

Occupational Infections

Laboratory-associated infections with hepatitis A or E viruses do not appear to be an important occupational risk among laboratory personnel. However, hepatitis A is a documented hazard in animal handlers and others working with naturally or experimentally infected chimpanzees and other non-human primates.[34] Workers handling other susceptible primates (e.g., owl monkeys, marmosets) also may be at risk for hepatitis A infection. Hepatitis E virus appears to be less of a risk to laboratory personnel than hepatitis A virus, except during pregnancy, when infection with HEV genotype 1 can result in increased maternal and fetal morbidity or mortality. Exposure to HEV-infected pigs, the primary animal reservoir for hepatitis E virus, rabbits, or macaques may pose an occupational hazard to animal handlers, but the extent of this risk is unknown.

Natural Modes of Infection

Most infections with hepatitis A are foodborne and occasionally waterborne. The virus has, on rare occasions, been transmitted through blood, blood-derived products, and other potentially infectious materials. Usually, infectious virus is

present in feces and blood during the incubation period, prodromal phase of the disease, and one week after jaundice onset, but it is not transmitted later in infection and the convalescence period. Hepatitis E virus genotypes 1 and 2 are transmitted via the fecal-oral route primarily by contaminated water in developing countries resulting in sporadic cases and occasionally large outbreaks. Hepatitis E virus genotypes 3 and 4 are associated with zoonotic hepatitis E infections transmitted to humans mainly through consumption of raw or undercooked pork and game meat or by contact with infected animals. This occurs in developed countries and results in sporadic cases. Transmission through blood and blood-derived products has been reported. Infection generally causes an acute self-limiting disease after an incubation period of two to six weeks but chronic infection with genotype 3 has been reported in immunocompromised individuals.

Laboratory Safety and Containment Recommendations

These agents may be present in feces and blood of infected humans and non-human primates. Feces, stool suspensions, and other contaminated materials are the primary hazards to laboratory personnel. Care should be taken to avoid puncture wounds when handling contaminated blood from humans or non-human primates. There is no evidence that aerosol exposure results in infection. Although hepatitis A virus is known to be one of the most stable viruses in the environment, hepatitis E virus is also very stable.

BSL-2 practices, containment equipment, and facilities are recommended for the manipulation of hepatitis A and E viruses, infected feces, blood, or other tissues. ABSL-2 practices and facilities are recommended for activities using naturally or experimentally-infected non-human primates or other animal models that may shed the virus.

Special Issues

Vaccines FDA-licensed inactivated vaccines against hepatitis A are available. There are no FDA-licensed vaccines against hepatitis E in the U.S., but a vaccine is currently available in China.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus

Hepatitis B virus (HBV) is the type species of the *Orthohepadnavirus* genus in the family *Hepadnaviridae*. Hepatitis C virus (HCV), with six genotypes, is the type species of the *Hepacivirus* genus in the family *Flaviviridae*. Hepatitis D virus (HDV) is the only member of the genus *Deltavirus*.

Occupational Infections

Hepatitis B has been one of the most frequently occurring Laboratory-associated infections, and laboratory workers are recognized as a high-risk group for acquiring such infections.[35,36,38]

Hepatitis C virus infection can occur in the laboratory as well.[37] The prevalence of the antibody to hepatitis C (anti-HCV) is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that HCV is spread predominantly by the parenteral route.[39]

Natural Modes of Infection

These viruses are naturally acquired from a carrier during blood transfusion, injection, tattooing, or body piercing with inadequately sterilized instruments. Non-parenteral routes, such as domestic contact and unprotected (heterosexual and homosexual) intercourse, are potential modes of transmission. Vertical transmission (i.e., mother to child) is also possible.

Individuals who are infected with the HBV are at risk of infection with HDV, a defective RNA virus that requires the presence of HBV for replication. Infection with HDV usually exacerbates the symptoms caused by HBV infection.

Laboratory Safety and Containment Recommendations

HBV may be present in blood and blood products of human origin, in urine, semen, CSF, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards.[40] The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains have not been identified.

HCV has been detected primarily in blood and serum, less frequently in saliva, and rarely or not at all in urine or semen. It appears to be somewhat stable at room temperature on surfaces or equipment.[41,42] Infectivity of the virus is sensitive to repeated freezing and thawing.

BSL-2 facilities with additional primary containment and personnel precautions, such as those described for BSL-3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. ABSL-2 practices, containment equipment, and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other non-human primates (NHPs). Gloves should be worn when working with infected animals and when there is the likelihood of skin contact with infectious materials. In addition to these

recommended precautions, persons working with HBV, HCV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.[43]

Special Issues

Vaccines Licensed recombinant vaccines against hepatitis B are available and are highly recommended for laboratory personnel.[35,36,38] Vaccines against hepatitis C and D are not yet available for use in humans, but vaccination against HBV will also prevent HDV infection.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Macacine alphaherpevirus **1 (Herpesvirus Simiae, Cerocopithecine herpesvirus I, Herpes B Virus)**

B virus is a member of the *Alphaherpesvirus* genus (simplexvirus) in the family *Herpesviridae*. It occurs naturally in macaque monkeys, of which there are nine distinct species. Macaques may have primary, recurrent, and latent infections, often with no apparent symptoms or lesions. B virus is the only member of the family of simplex herpesviruses that can cause zoonotic infections. Human infections have been identified in at least 50 instances, with approximately 80% mortality when untreated.[44] There have been no reported fatal cases where prompt first aid with wound or exposure site cleansing was performed within minutes after the exposure and post-exposure prophylaxis was given. Reactivated ocular disease has occurred in one individual,[45] and three infections resulting in seroconversion to B virus have occurred in the last decade. Cases prior to 1970 were not treated with antiviral agents because none were available. Morbidity and mortality associated with zoonotic infection result from invasion of the central nervous system, resulting in ascending paralysis ultimately with loss of ability to sustain respiration in the absence of mechanical ventilation. From 1987–2016, five additional fatal infections brought the number of lethal infections to 21 since the discovery of B virus in 1932.[46]

Occupational Infections

B virus is a hazard in facilities where macaque monkeys are present. Mucosal secretions (i.e., saliva, genital secretions, and conjunctival secretions) are the primary body fluids associated with the risk of B virus transmission. However, it is possible for other materials to become contaminated. For instance, in 1997 a research assistant at the Yerkes Primate Center suffered a mucosal splash without injury while transporting a caged macaque; the individual subsequently died.[47] Based on the work being performed, the activity was considered

low-risk at that time. However, feces, urine, or other fluids and surfaces may be contaminated with virus shed from mucosal fluids. Zoonoses have been reported following virus transmission through a bite, scratch, or splash accident, but in at least two cases, no recognized exposure could be recalled. In one such case, fatality occurred. Multiple cases of B virus have also been reported after exposure to monkey cell cultures and to central nervous system tissue. There is often no apparent evidence of B virus infection in the animals or their cells and tissues, making it imperative that all suspect exposures be treated according to recommended standards.[44] However, the risks associated with this hazard are readily reduced by practicing barrier precautions and by rapid and thorough cleansing immediately following possible site contamination. Precautions should be taken when work requires the use of any macaque species, even antibody-negative animals. Animals that are seronegative may be acutely infected and shedding virus but not yet antibody positive. In most documented cases of B virus zoonosis, the virus was not recovered from potential sources except in four cases, making speculations that some macaque species may be safer than others unfounded. The loss of five lives in the past three decades underscores that B virus infections have a low probability of occurrence, but when they do occur there are high consequences.

Specific, regular training for B virus hazards, including understanding the modes of exposure and transmission, should be provided to individuals encountering B virus hazards. Training should also include proper use of engineering controls and personal protective equipment, which is essential to prevention. Immediate and thorough cleansing following bites, scratches, splashes, or contact with potential fomites in high-risk areas appears to be helpful in prevention of B virus infections.[47] First aid and emergency medical assistance procedures are most effective when institutions set the standard to be practiced by all individuals encountering B virus hazards.

Natural Modes of Infection

B virus occurs as a natural infection of Asiatic macaque monkeys and approximately 10% of newly caught rhesus monkeys have antibodies against the virus, which is frequently present in kidney cell cultures of this animal. In macaque species, the virus can cause vesicular lesions on the tongue and lips and sometimes of the skin. B virus is not present in blood or serum in healthy infected macaques. Transmission of B virus appears to increase when macaques reach sexual maturity.

Laboratory Safety and Containment Recommendations

The National Academies Press published the Institute for Laboratory Animal Research's (ILAR) guidelines for working with non-human primates.[48] The guidelines provide additional information regarding risks and mitigation strategies when handling non-human primates.

Asymptomatic B virus shedding accounts for most transmission among monkeys and human workers, but those working in the laboratory with potentially infected cells or tissues from macaques are also at risk. Exposure via mucous membranes or skin breaks provides this agent access to a new host, whether the virus is being shed from a macaque or human, or is present in or on contaminated cells, tissues, or surfaces.[44] B virus is not generally found in serum or blood, but these products obtained through venipuncture should be handled carefully because contamination of needles via skin can occur. When working with macaques directly, the virus can be transmitted through bites, scratches, or splashes only when the animal is shedding virus from mucosal sites. Fomites or contaminated surfaces (e.g., cages, surgical equipment, tables) should always be considered sources of B virus unless verified as decontaminated or sterilized. Zoonotically infected humans should be cautioned about autoinoculation of other susceptible sites when shedding virus during acute infection.

BSL-4 facilities are recommended for the propagation of viruses obtained from diagnostic samples or stocks. Experimental infections of macaques as well as small animal models with B virus are recommended to be restricted to ABSL-4 containment. BSL-3 practices are recommended for handling diagnostic materials with possible B virus. BSL-2 practices and facilities are suitable for all activities involving the use or manipulation of tissues, cells, blood, or serum from macaques with appropriate personal protective equipment.

All macaques regardless of their origin should be considered potentially infected. Animals with no detectable antibody are not necessarily B virus-free. Macaques should be handled with strict barrier precaution protocols and injuries should be tended immediately according to the recommendations of the B Virus Working Group led by NIH and CDC.[44]

Barrier precautions and appropriate first aid are the keys to prevention of severe morbidity and mortality often associated with B virus zoonoses. These prevention tools were not implemented in each of the five B virus fatalities during the past three decades. Guidelines are available for safely working with macaques and should be consulted.[44,49] The correct use of gloves, masks, and protective coats, gowns, aprons, or overalls is recommended for all personnel while working with non-human primates, especially macaques and other Old World species; this is inclusive for all persons entering animal rooms where non-human primates are housed. To minimize the potential for mucous membrane exposure, some form of barrier is required to prevent droplet splashes to eyes, mouth, and nasal passages. Types and use of personal protective equipment (e.g., goggles or glasses with solid side shields and masks, or wrap-around face shields) should be determined with reference to the institutional risk assessment. Specifications of protective equipment must be balanced with the work to be performed so that

the barriers selected do not increase workplace risk by obscuring vision and contributing to increased risk of bites, needlesticks, scratches, or splashes.

Special Issues

Post-exposure prophylaxis with oral acyclovir or valacyclovir should be considered when exposures are thought to have occurred. Even a slight scratch can result in transmission. Therapy with intravenous acyclovir and/or ganciclovir in documented B virus infections is also important in the reduction of morbidity following B virus zoonotic infection.[44] Ganciclovir is generally reserved for symptomatic cases confirmed by CSF evaluation. Because of the seriousness of B virus infection, experienced medical and laboratory personnel should be consulted to develop individual case management. Barrier precautions should be observed with confirmed cases. B virus infection, as with all alphaherpesviruses, is lifelong in macaques.[50] There are no effective vaccines available and no curative therapeutics for humans.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Human Herpes Virus

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. Thus far, nine herpesviruses have been isolated from humans: herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) 6A, 6B, 7, and 8.51

Because these viruses establish lifelong latency in human tissues, they may manifest either as primary or recurrent infections. HSV primary and recurrent infections are usually characterized by localized vesicular lesions at or near the site of the initial infection. Primary infection with HSV-1 often occurs in early childhood and may be mild and unapparent. Symptoms such as fever or malaise can sometimes occur. HSV-1 is a frequent cause of viral meningoencephalitis. Genital infections, usually caused by HSV-2, generally occur in adults and are sexually transmissible.

Disseminated disease and encephalitis that may occur in neonatal infections can be fatal. EBV is the most frequent cause of infectious mononucleosis and is also associated with the pathogenesis of several lymphomas and nasopharyngeal cancer.[52,53] EBV-associated cancers normally have viral genomes integrated into the transformed cells. HCMV is often undiagnosed, presenting as a nonspecific

febrile illness with features of infectious mononucleosis. HCMV can cause severe congenital syndrome, which may manifest as mental retardation, microcephaly, motor disabilities, and chronic liver disease in infants who were exposed to the virus in utero.[51] Congenital HCMV is also a frequent cause of deafness in children who were exposed to the virus in utero.

Primary infection with VZV causes chickenpox, while recurrences of this viral infection cause herpes zoster (shingles). Primary infection with HHV-6B or HHV-7 can cause exanthem subitum (roseola), a common childhood rash-associated illness and can also be a cause of infectious mononucleosis syndrome.[53,54] Other clinical manifestations of roseola include nonspecific febrile illness and febrile seizures. Reactivation of HHV-6 is usually identified only in the severely immunocompromised, when it may be associated with encephalitis or other manifestations. Disease caused by HHV-6A, which is a less common infection that usually occurs after early childhood, is less well-understood. HHV-8 is the causative agent of Kaposi's sarcoma and of primary effusion lymphoma.[55] High-risk groups for HHV-8 include HIV-infected men who have sex with men and individuals from areas of high endemicity, such as Africa or the Mediterranean.[56] The prevalence of HHV-8 is also higher among intravenous drug users than in the general population.[56] At least one report has provided evidence that, in African children, HHV-8 infection may be transmitted from mother to child.[57]

While few of the human herpesviruses have been demonstrated to cause Laboratory-associated infections, they are both primary and opportunistic pathogens, especially in immunocompromised hosts, in whom recurrent infections can be particularly severe and even life-threatening. Macacine alphaherpesvirus 1 (B-virus, Monkey B virus) is not a human herpesvirus and is discussed separately in the preceding agent summary statement.

Occupational Infections

Few of the human herpesviruses have been documented as sources of Laboratory-associated infections. Although this diverse group of viral agents has not demonstrated a high potential hazard for Laboratory-associated infection, frequent presence in clinical materials and common use in research warrant the application of appropriate laboratory containment and safe practices.

Natural Modes of Infection

Given the wide array of viruses included in this family, the natural modes of infection vary greatly, as does the pathogenesis of the various viruses. These viruses both infect and establish latency in different types of cells leading to some of the major clinical differences in the disease that they cause. Transmission of human herpesviruses in nature is generally associated with close, intimate

contact with a person excreting the virus in their saliva, urine, or other bodily fluids.[57] For example, VZV is transmitted person-to-person through direct contact, aerosolized vesicular fluids, and respiratory secretions. HHV-8 and CMV can be transmitted through organ transplantation[58,59] and blood transfusion.[60] The ability of HHV-6 to integrate into the human genome allows vertical transmission in a small percentage of cases.

Laboratory Safety and Containment Recommendations

Clinical materials, including blood, urine, and saliva, and isolates of human herpesviruses may pose a risk of infection following ingestion, parenteral inoculation, and droplet exposure of the mucous membranes of the eyes, nose, or mouth, exposure to non-intact skin, or inhalation of concentrated aerosolized materials. Clinical specimens containing the more virulent Macacine alphaherpesvirus 1 (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection, though the combination of a suspected herpes simplex infection with exposure to a rhesus macaque should trigger serious concern in the treating physician, and ideally would involve special labelling and consultation with the microbiology laboratory. HCMV may pose a special risk to pregnant women because of potential infection of the fetus. All human herpesviruses pose an increased risk to persons who are immunocompromised and are not previously immune to these viruses.

BSL-2 facilities with additional containment and procedures, such as those described for BSL-3, should be considered when producing, purifying, and concentrating human herpesviruses, based on risk assessment. BSL-2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAIs, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates or during the necropsy of animals.

Autologous transformation of B cells using EBV should not be performed.

Containment recommendations for Macacine alphaherpesvirus 1 (B-virus, Monkey B virus) are described in the preceding agent summary statement.

Special Issues

Vaccines Vaccines for varicella-zoster are licensed and available in the United States. In the event of a laboratory exposure to a non-immune individual, varicella vaccine is likely to prevent or at least reduce the severity of disease.[61]

Treatment Antiviral medications are available for treatment or prevention of infections with several of the human herpesviruses.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Influenza Viruses

Influenza is an acute viral disease of the respiratory tract. The most common clinical manifestations are fever, headache, malaise, sore throat, cough, and muscle aches. GI tract manifestations (e.g., nausea, vomiting, diarrhea) are rare but may accompany the respiratory phase in children. The two most important features of influenza are the epidemic nature of illness and the mortality that arises from pulmonary complications of the disease.[62]

Influenza virus infection may be associated with extrapulmonary complications, including viral myocarditis and viral encephalitis. Cardiovascular deaths during influenza epidemics have increased indicating that cardiovascular complications, including exacerbation of chronic underlying conditions, are important contributors to influenza-related morbidity and mortality.[63,64]

Influenza viruses are enveloped RNA viruses belonging to the family *Orthomyxoviridae*. There are four serotypes of influenza viruses—A, B, C, and D, of which human infections have been virologically confirmed for all except influenza D viruses. Influenza A viruses are further classified into subtypes by the surface glycoproteins hemagglutinin (H) and neuraminidase (N). Emergence of new subtypes (antigenic shift) in humans occurs at irregular intervals with Type A viruses. New subtypes can result from reassortment of human, swine, and avian influenza A virus genes. If there is little or no population immunity and the viruses are able to spread in a sustained manner from human-to-human, they can be responsible for rare pandemics. Minor antigenic changes within a circulating seasonal influenza A virus subtype or influenza B virus lineage (antigenic drift) are ongoing processes that are responsible for annual epidemics that make the annual reformulation of influenza vaccines necessary.

Influenza A viruses of different antigenic subtypes occur naturally in many domestic and wild avian species and have formed sustained lineages in swine, equine, and canine species. Avian origin influenza A viruses also sporadically infect multiple other mammalian species. Two influenza A virus subtypes have only been detected in bats. Novel influenza A virus infections of humans (zoonotic transmission of avian or variant [swine-origin] influenza A viruses) occur sporadically.[65] Limited, non-sustained human-to-human transmission of some novel influenza A viruses has been reported following prolonged unprotected exposures to an ill index case.[66–68] Interspecies transmission and reassortment of influenza A viruses have been reported to occur among humans, pigs, and wild and domestic fowl. The influenza A viruses responsible for the 1918, 1957, 1968, and 2009

pandemics contained gene segments closely related to those of avian or swine influenza A viruses.[69–71] Control of influenza is a continuing human and veterinary public health concern.

Occupational Infections

LAIs, in the absence of animals, have not been well documented in the literature. However, it is believed that there is a risk of possible exposure to infectious influenza virus in the laboratory, especially through work with high concentrations of virus and/or experimental operations that generate aerosols (e.g., centrifugation, vortex-mixing). Animal-associated infections in the laboratory or the field have been reported.[72–74] LAIs may result from inoculation of mucous membranes including the upper respiratory tract through fomite transmission (e.g., touching virus-contaminated gloves to one's face following handling of tissues, feces, or secretions from infected animals; touching contaminated door handles or computer keyboards and then touching mucous membranes).

Natural Modes of Infection

Near-range inhalation through droplet/airborne spread is the predominant mode of influenza virus transmission among humans. Transmission may also theoretically occur through direct contact of contaminated surfaces and subsequent inoculation of mucous membranes including the upper respiratory tract since influenza viruses may persist for hours on surfaces particularly in the cold and under conditions of low humidity.[69] The incubation period is from one to four days. Recommendations for antiviral treatment and chemoprophylaxis of influenza are available.[75]

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tissues or secretions of humans and infected animals and birds. In addition, the agent may be present in the intestines and cloacae of many infected avian species. Influenza viruses may be disseminated in multiple organs in some infected animal species. The primary laboratory hazard is inhalation of the virus from aerosols generated by infecting animals or by aspirating, dispensing, mixing, centrifuging, or otherwise manipulating virus-infected materials. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. The potential for introducing influenza viruses with novel genetic composition into humans is unknown.

Seasonal Human Influenza Viruses BSL-2 facilities, practices, and procedures are recommended for diagnostic research and production activities utilizing contemporary influenza A, B, and C viruses circulating among humans (e.g., H1/H3/B). ABSL-2 is appropriate for work with these viruses in animal models.

Zoonotic and Animal Influenza A Viruses BSL-3 or ABSL-3 containment, with enhancements directed by regulatory authorities, should be used for laboratory work with low pathogenicity avian influenza (LPAI) A viruses that have caused zoonotic infections, particularly those with fatal outcomes (e.g., H7N4, H10N8). Work with Asian lineage A(H7N9) and non-U.S.LPAI A viruses should also be conducted in BSL-3 or ABSL-3 laboratories with practices, procedures, and facilities enhancements, as directed by regulatory authorities.

BSL-2 with enhanced facilities, practices, and procedures, as directed by regulatory authorities, should be used for working with domestic LPAI A viruses (e.g., H1–4, H6, H8–16) and equine, canine, and swine influenza A viruses. ABSL-2 with enhancements directed by regulatory authorities is appropriate for work with these viruses in animal models. Asian lineage A(H7N9) LPAI viruses have caused sporadic zoonotic infections with high mortality in humans since 2013.[76]

Non-Contemporary Human Influenza Viruses Non-contemporary, wild-type human influenza A(H2N2) viruses or reassortants containing the H2 or N2 RNA segments should be handled with increased caution. Important considerations in working with these viruses are the number of years since an antigenically related virus last circulated and the potential presence of a susceptible population. BSL-3 and ABSL-3 practices, procedures, and facilities are recommended with rigorous adherence to respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators and eye protection, or positive air-purifying respirators (PAPRs) are recommended for use. Cold-adapted, live attenuated A(H2N2) vaccine viruses may be worked with at BSL-2, but it is recommended that a risk assessment be performed before working with such viruses, and attention should be paid to prevent generation of reassortants that have H2 and/or N2 RNA segments and lack attenuating features of the parental attenuated viruses.

Historical, wild-type human influenza A(H1N1) and A(H3N2) viruses that have not circulated among humans in many years should be handled with increased precaution since younger adult workers and children have little or no immunity against such viruses. It is recommended that a risk assessment be performed before working with such viruses; this would include consideration of the number of years since a closely related virus last circulated among humans. For example, pre-2009 A(H1N1) viruses have not circulated in humans since the 2009–2010 season and there is little antigenic similarity between these viruses and the A(H1N1)pdm09 viruses that were responsible for the 2009 influenza pandemic. Other examples may arise in the future. In such cases, a more cautious approach to containment utilizing elevated Biosafety Levels and practices is warranted (e.g., BSL-2 with enhanced practices, procedures, and facilities).

1918 Influenza A(H1N1) Pandemic Virus Any research involving reverse genetics of the 1918 influenza A(H1N1) pandemic virus should proceed with extreme caution. Research findings suggest that exposure to A(H1N1)pdm09 virus through immunization or infection would provide protection against the reconstructed 1918 A(H1N1) virus.[77] Moreover, several serological studies of the A(H1N1)pdm09 virus have provided evidence for the presence of preexisting, cross-reactive antibodies to a 1918-like H1N1 virus from previous vaccinations or infections.[78,79] However, the 1918 A(H1N1) virus is still considered to pose both biosafety and biosecurity threats. The following practices and conditions are recommended for manipulation of reconstructed 1918 influenza A(H1N1) viruses and laboratory animals infected with the viruses. These following practices and procedures are considered minimum standards for work with the fully reconstructed virus.

- BSL-3 and ABSL-3 practices, procedures, and facilities;
- Animals, including non-human primates (NHPs), should be housed in primary barrier systems in ABSL-3 facilities;
- Use of negative pressure, HEPA-filtered respirators, or PAPRs;
- Rigorous adherence to respiratory protection and clothing change protocols;
- HEPA filtration for treatment of exhaust air; and
- Personal showers prior to exiting the laboratory.

Highly Pathogenic Avian Inluenza (HPAI) A Viruses Manipulating HPAI A viruses (e.g., H5, H7) in biomedical research laboratories also requires additional precautions because some viruses may pose increased risk to laboratory workers and have significant agricultural and economic implications. BSL-3 and ABSL-3 with enhanced practices, procedures, and facilities, as directed by regulatory authorities, are required, including clothing change and personal showering protocols. Loose-housed animals infected with HPAI A viruses must be contained within ABSL-3Ag facilities. See Appendix D for additional information. Negative pressure, HEPA-filtered respirators and eye protection, or positive air-purifying respirators are recommended for HPAI A viruses with potential to infect humans.

Other Influenza Recombinant or Reassortant Viruses When considering the biocontainment level and attendant practices and procedures for work with other influenza recombinant or reassortant viruses, the IBC, or equivalent resource, should consider but not limit consideration to the following in the conduct of protocol-driven risk assessment.

- The gene constellation used;
- Any mutations that are introduced and may result in enhancement of a pathogen's transmissibility and/or virulence;[80]
- Clear evidence of reduced virus replication in the respiratory tract of appropriate animal models, compared with the level of replication of the wild-type parent virus from which it was derived;

- Evidence of clonal purity and phenotypic stability; and
- The number of years since a virus that was antigenically related to the donor of the hemagglutinin and neuraminidase genes last circulated.

If adequate risk assessment data are not available, a more cautious approach to containment, utilizing elevated Biosafety Levels and practices, is warranted.

Special Issues

Occupational Health Considerations Institutions performing work with HPAI and LPAI A viruses that have infected humans; non-contemporary wild-type human influenza A viruses, including recombinants and reassortants; and viruses created by reverse genetics of extinct virus strains (e.g., 1918 strain) should develop and implement a specific medical surveillance and response plan. At a minimum, these plans should: 1) strongly recommend annual vaccination with a currently licensed influenza vaccine for such individuals; 2) provide employee counseling regarding disease signs and symptoms including fever, conjunctivitis, and respiratory symptoms; 3) establish a protocol for monitoring personnel for these symptoms; 4) include collection of acute and convalescent serum samples in the event of a possible LAI; and 5) establish a clear medical protocol for responding to suspected Laboratory-associated infections. Antiviral drugs (e.g., oseltamivir, zanamivir) should be available for treatment of illness or post-exposure treatment/chemoprophylaxis, as necessary.[75] It is recommended that the virus under study be tested for susceptibility to antiviral drugs. All personnel should be enrolled in an appropriately constituted respiratory protection program.

Select Agent The reconstructed 1918 influenza A(H1N1) virus and HPAI viruses are Select Agents requiring registration with CDC or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation and transfer of animal-origin viruses and diagnostic specimens obtained from animals require APHIS importation permits. CDC/PHS import permits are required for importation of seasonal influenza A, B, and C viruses and specimens obtained from humans. CDC/PHS permits may also be required for importation of animal-origin influenza viruses of known zoonotic potential. Importation and transfer of Select Agent viruses require APHIS/CDC importation permits. APHIS permit-driven containment, facility requirements, and personnel practices and/or restrictions may be applied for the possession and handling of animal-origin and zoonotic viruses. This may also include laboratory data/results to exclude the possibility of contamination with HPAI Select Agent viruses in specimens. A DoC export license or license exemption may be required for the export of Select Agent viruses to another country. See Appendix C for additional information.

Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis (LCM) is a rodent-borne viral infectious disease that presents as aseptic meningitis, encephalitis, or meningoencephalitis. The causative agent is the LCM virus (LCMV) that was initially isolated in 1933. The virus is the prototypical member of the family *Arenaviridae*.

Occupational Infections

LAIs with LCM virus are well documented. Most infections occur when chronic viral infection exists in laboratory or pet rodents, especially mice, hamsters, and guinea pigs.[81–83] Nude and severe combined immune deficient (SCID) mice may pose a special risk of harboring silent chronic infections. Mice shedding the virus may be asymptomatic. Inadvertently infected cell cultures also present a potential source of infection and dissemination of the agent.

Natural Modes of Infection

LCMV infections have been reported in Europe, the Americas, Australia, and Japan, and may occur wherever infected rodent hosts are found. Several serologic studies conducted in urban areas have shown that the prevalence of LCMV infection among humans ranges from 2% to 10%. Seroprevalence of 37.5% has been reported in humans in the Slovak Republic.[84]

The common house mouse, *Mus musculus*, naturally spreads LCMV. Once infected, these mice can become chronically infected as demonstrated by the presence of virus in blood and/or by persistently shedding virus in urine. Infections by *Callitrichid* hepatitis virus, a strain of LCMV, have also occurred in NHPs in zoos, including macaques and marmosets.

Humans become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva; by ingesting food contaminated with the virus; by contamination of mucous membranes with infected body fluids; or by directly exposing cuts or other open wounds to virus-infected blood. Several clusters of organ recipients from donors with unrecognized acute LCMV infection have been described with poor survival rates in the immunosuppressed recipients.[85–89] The source of donors' infection is usually untraceable except in one case where a pet hamster that was not overtly ill was incriminated.[89] Pregnant women infected with LCMV have transmitted the virus to their fetuses that resulted in death or serious central nervous system malformation as a consequence.[90]

Laboratory Safety and Containment Recommendations

The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces, and tissues of infected animal hosts and humans. Parenteral inoculation, inhalation, contamination of mucous membranes or broken skin with infectious tissues or fluids from infected animals are common hazards. Aerosol transmission is well documented.[81]

Of special note, tumors may acquire LCMV as an adventitious virus without obvious effects on the tumor. The virus may survive freezing and storage in liquid nitrogen for long periods. When infected tumor cells are transplanted, subsequent infection of the host and virus excretion may occur.

Women of childbearing age should be made aware of risks posed by LCMV or rodents potentially infected by LCMV. Women who are pregnant or planning to become pregnant should be provided medical counseling that informs them of these risks with LCMV or animals potentially infected with LCMV.

Strains of LCMV that are shown to be lethal in non-human primates should be handled at BSL-3. BSL-3 is also required for activities with high potential for aerosol production, work with production quantities or high concentrations of infectious materials, and for manipulation of infected transplantable tumors, field isolates, and clinical materials from human cases. Work with infected hamsters should be done at ABSL-3.

BSL-2 practices, containment equipment, and facilities are suitable for activities utilizing known or potentially infectious body fluids and for cell culture passage of laboratory-adapted strains. ABSL-2 practices, containment equipment, and facilities are suitable for studies in adult mice with mouse brain-passaged strains requiring BSL-2 containment.

Special Issues

Vaccines Vaccines are not available for use in humans.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Poliovirus

Poliovirus is the type species of the *Enterovirus* genus in the family *Picornaviridae*. Picornaviruses are small viruses with an RNA genome. Enteroviruses are likely transient inhabitants of the gastrointestinal tract and are stable at acid pH. There are three poliovirus serotypes: PV1, PV2, and PV3. Immunity to one serotype does not produce significant immunity to the other two.

Occupational Infections

Laboratory-associated poliomyelitis is uncommon. Twelve cases, including two deaths, were reported between 1941 and 1976.[91,92] Several instances of asymptomatic laboratory infections with poliovirus have been reported, but no laboratory-associated poliomyelitis has been reported for over 40 years. Both inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV) are

highly effective in preventing disease. OPV alone induces mucosal immunity, which gradually fades over subsequent years. Poliovirus infections among immunized laboratory workers remain undetermined in the absence of laboratory confirmation. An immunized laboratory worker may unknowingly be a source of poliovirus transmission to susceptible persons in the community.[93] In April 2017, a spill of WPV2 in a production facility in the Netherlands infected one operator whose stool tested positive for poliovirus. This incident highlights the risk of containment breach and emphasizes the need for appropriate incident response planning and government oversight.[94]

Natural Modes of Infection

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with inapparent infections. Person-to-person spread of poliovirus via the fecal-oral route is the most common route of transmission, although the oral-oral route may account for some cases. Only one in several hundred infections of unimmunized persons with wild poliovirus leads to paralytic disease, with the vast majority of infections being asymptomatic or accompanied by minor, flu-like symptoms.

At one time, poliovirus infection occurred throughout the world. Transmission of wild poliovirus ceased in the United States by 1979. A polio eradication program conducted by the Pan American Health Organization led to elimination of polio from the Western Hemisphere in 1991. The Global Polio Eradication Program, led by the World Health Organization, has dramatically reduced the number of paralytic cases.

The last case of wild PV2 (WPV2) was detected in 1999, and certification of WPV2 eradication occurred in 2015. Since WPV2 was eradicated, all polio cases associated with PV2 have been caused by oral polio vaccine (OPV) directly (vaccine-associated paralytic polio [VAPP]) or by vaccine-derived polio type 2 virus (VDPV2). Due to continued occurrence of VAPP and outbreaks and chronic infections associated with VDPV2, WHO discontinued all routine OPV2 use as of May 1, 2016 by coordinating a global switch from trivalent OPV to bivalent OPV, containing only OPV1 and 3, along with the introduction of a single dose of inactivated polio vaccine (IPV). The last case of WPV3 occurred in Nigeria in 2012 and certification of WPV3 eradication occurred in 2019. As of 2019, only three countries (Pakistan, Afghanistan, and Nigeria) are considered to be endemic for WPV1. Complete polio eradication is expected in the near future.

Laboratory Safety and Containment Recommendations

Poliovirus is present in stool and in throat secretions of infected persons and in lymph nodes, brain tissue, and spinal cord tissue in fatal cases. In addition, poliovirus may be present in environmental samples (e.g., sewage).

Ingestion and parenteral inoculation are the primary routes of infection for laboratory workers. For immunized persons parenteral inoculation likely presents a lower risk. The potential for aerosol exposure is unknown. Laboratory animal-associated infections have not been reported, but infected non-human primates should be considered to present a risk.

Laboratory personnel working with and visitors with access to known poliovirus or infectious materials potentially containing poliovirus must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased occupational risk should receive another dose of IPV. Available data do not indicate the need for more than a single lifetime IPV booster dose for adults.[95]

Type 2 and WPV3 Declaration of WPV2 eradication and the termination of routine OPV2 use initiated the containment of PV2 under the WHO Global Action Plan III (GAPIII).[96] GAPIII seeks to decrease the risk of reintroduction of eradicated polioviruses from laboratories and other facilities by calling for the destruction of non-essential poliovirus materials and containment of retained poliovirus material in certified poliovirus-essential facilities that adhere to the containment measures specified in GAP III. These measures include a biorisk management system, biosafety, security, and physical laboratory features and, at the time of this writing, apply to WPV2 and VDPV types 2 and 3, VDPV2, and OPV2 infectious materials as well as WPV and VDPV potentially infectious materials (e.g., fecal, respiratory secretion, and environmental samples collected at a time and in a place where WPV or VDPV was present). The U.S. National Authority for Containment (NAC) of Poliovirus at the CDC is responsible for working with poliovirus facilities to achieve certification. At the time of final eradication of all poliovirus types, additional GAPIII physical laboratory containment measures will be required for WPV and VDPV materials.

OPV2 potentially infectious materials are subject to the Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses.[97,98] This document assigns risk categories based on the material and work performed and outlines specific risk mitigation measures that are much less stringent than GAPIII.

Type 1 and OPV3 When final eradication is declared, GAPIII containment will also apply to types 1 and OPV3. Laboratories and other facilities are encouraged to destroy all PV1 and OPV3 materials not essential for research or other work.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for all activities using poliovirus infectious and potentially infectious materials, including environmental and clinical samples. Contact the U.S. NAC for enhanced measures for work with eradicated poliovirus types and strains.

Laboratories should work with attenuated Sabin OPV strains unless there are strong scientific reasons for working with wild polioviruses. Contact the NAC for additional measures for work with WPV and VDPV types 2 and 3, and OPV2 infectious materials.

Special Issues

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information. Contact the NAC prior to transfers of polioviruses.

Poxviruses

Four genera within the *Chordopoxvirinae* subfamily (family *Poxviridae*) contain species that can cause human disease: *Orthopoxvirus, Parapoxvirus, Yatapoxvirus,* and *Molluscipoxvirus*.[99] Most species in these genera are zoonotic with the exception of variola virus (*Orthopoxvirus*) and molluscum contagiosum virus (*Molluscipoxvirus*), which are solely human pathogens.[100,101] As most Laboratory-associated infections involve accidents associated with orthopoxviruses, only species of this genus will be discussed further.

Occupational Infections

Vaccinia virus is the prototypical orthopoxvirus, and its well-studied characteristics make it commonly used in both general and biomedical research.[102] Thus, vaccinia virus is the leading agent of laboratory-associated poxvirus infections. LAIs with replication-competent species, including wild-type and modified strains of vaccinia virus, have occurred even in previously vaccinated laboratorians. Other persons at risk for occupational exposure include animal care personnel having direct contact with vaccinated or infected animals or their secretions, or healthcare personnel who care for vaccinated or infected patients or administer a live vaccinia virus.[102,103]

The manifestation of infection is dependent upon factors such as virus species, route of entry, and host immune status. Infection results in the development of one to several lesions (localized) or a generalized rash (systemic) on the skin and/or mucous membranes. Infection with variola or monkeypox virus causes a febrile prodrome that is preceded by a distinct systemic rash illness. Vaccinia virus and cowpox virus typically cause a single lesion at the site of infection; however, multiple lesions and a generalized rash may also take place. Uncomplicated disease typically resolves within several weeks.[99,100]

Natural Modes of Infection

The most well-known orthopoxvirus is variola virus, which causes smallpox. After an extensive vaccination campaign, smallpox was declared eradicated in 1980. Monkeypox occurs sporadically in several West and Central African countries but remains endemic in the Democratic Republic of Congo. The importation of wild-caught animals from Ghana into the United States resulted in a 2003 monkeypox outbreak that affected multiple states. Vaccinia virus is used to make the current smallpox vaccine. Naturally-acquired infections with vaccinia virus exist outside of the United States.[104] Cases of human cowpox occur in Europe and Asia. Rodents are known or suspected to play a part in the transmission of monkeypox, cowpox, and vaccinia viruses.[99–101]

Laboratory Safety and Containment Recommendations

Vaccination with vaccinia virus can afford protection against infection from other species of orthopoxviruses. Smallpox vaccination occurs via scarification using a multi-puncture method with a bifurcated needle. The current U.S.-licensed smallpox vaccine, ACAM2000, uses a replication-competent vaccinia virus strain. Symptoms such as fever, headache, and swollen lymph nodes are prevalent following vaccination. Adverse events include localized reactions (e.g., robust take), unintentional transfer of virus (e.g., self-inoculation, ocular vaccinia), diffuse dermatologic complications (e.g., eczema vaccinatum, non-specific post-vaccination rash), progressive vaccinia, cardiac complications, fetal vaccinia, and postvaccinial central nervous system disease. Due to the severity of complications that can arise from vaccination, the vaccine is not recommended for persons with certain contraindications.[99,103,105,106]

Orthopoxviruses are stable in a wide range of environmental temperatures and humidity. Virus may enter the body through the mucous membranes (e.g., eye splashes, inhalation of droplets or fine-particle aerosols), broken skin (e.g., needlesticks, scalpel cut), ingestion, or by parenteral inoculation. Sources of exposure include fomites, infected human or animal tissue, excretions or respiratory secretions, or infectious cultures.[106]

Routine vaccination with ACAM2000 is recommended for laboratory personnel who directly handle cultures or animals contaminated or infected with replication-competent vaccinia virus, recombinant vaccinia viruses derived from replication-competent vaccinia strains (i.e., those that are capable of causing clinical infection and producing infectious virus in humans), or other orthopoxviruses that infect humans (e.g., monkeypox, cowpox, and variola).[106] Vaccination is advised every three years for work with monkeypox and variola viruses, and every 10 years for cowpox and vaccinia viruses. Vaccination is not required for individuals working in laboratories that only manipulate replication-deficient strains of vaccinia virus (modified virus Ankara [MVA], NYVAC, TROVAC,

and ALVAC). Vaccination may be offered to healthcare workers, animal care personnel, and vaccinators who have contact with contaminated materials. Vaccination does not protect against non-Orthopoxvirus species.[103,106]

Research with variola virus is restricted to two WHO-approved BSL-4 and ABSL-4 facilities; one is the CDC in Atlanta, GA, and the other is the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia. ABSL-3 practices, containment equipment, and facilities are recommended for monkeypox work in experimentally or naturally infected animals. BSL-2 facilities with BSL-3 practices are advised if vaccinated personnel perform laboratory work with monkeypox virus. BSL-2 and ABSL-2 containment plus vaccination are recommended for work with vaccinia and other human pathogenic poxviruses. The lowering of containment to BSL-1 for the manipulation of attenuated poxviruses and vectors (e.g., modified virus Ankara [MVA], NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used may be considered. However, higher levels of containment are recommended if these strains are used in work areas where other orthopoxviruses are manipulated. Vaccination is not required for individuals working only in laboratories where no other orthopoxviruses or recombinants are handled. BSL-2 and ABSL-2 plus vaccination are recommended for work with most other poxviruses. Note that for research subject to the *NIH Guidelines*, approval to lower containment from BSL-2 must be requested from NIH Office of Science Policy.[107]

Special Issues

The CDC provides information on a variety of topics relating to variola, monkeypox, and vaccinia viruses online at https://www.cdc.gov. For non-emergency information on potential human infections, smallpox vaccination, or treatment options, the CDC Poxvirus Inquiry Line can be contacted at 404-639-4129 or CDC-Info can be reached at 800-232-4636. To obtain smallpox vaccine, CDC Drug Services can be reached by phone at 404-639-3670 or by email at drugservice@cdc.gov. Clinicians or health departments may contact the CDC Emergency Operations Center in critical circumstances.

Select Agent Congo Basin monkeypox, Variola major, and Variola minor are Select Agents requiring registration with CDC for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent The importation of poxviruses into the United States and/or their interstate transport may be subject to the rules and regulations of the CDC Import Permit Program, CDC Division of Select Agents and Toxins, and/or the USDA Animal and Plant Health Inspection Service. The exportation of poxviruses may require a DoC permit.

Rabies Virus and related lyssaviruses

Rabies is an acute, progressive, fatal encephalitis caused by negative-stranded RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae*.[108,109] *Rabies lyssavirus* (formerly Rabies virus) is the representative member (type species) of the genus and is responsible for the majority of human and animal cases of rabies worldwide. Currently, there are 14 recognized viral species within the genus Lyssavirus, which can be found in Table 1.

Occupational Infections

Rabies LAIs are extremely rare; two cases have been documented. Both cases resulted from presumed exposure to high concentrations of infectious aerosols—one generated in a vaccine production facility[110] and the other in a research facility.[111] Naturally or experimentally-infected animals, their tissues, and their excretions are also a potential source of exposure for laboratory and animal care personnel.

Natural Modes of Infection

The natural hosts of rabies virus are many bat species and terrestrial carnivores, but any mammal can be infected. The saliva of infected animals is highly infectious, and bites are the usual means of transmission, although infection through superficial skin lesions or mucosa is possible.

Laboratory Safety and Containment Recommendations

When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, saliva, and lacrimal secretions, but rabies viral antigens may be detected in all innervated tissues. The most likely sources for exposure of laboratory and animal care personnel are accidental parenteral inoculation, cuts, or needlesticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids. Infectious aerosols have not been a demonstrated hazard to personnel working with routine clinical materials or conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the two recorded cases of laboratory-associated rabies resulted from presumed exposure to the fixed Challenge Virus Standard and Street Alabama Dufferin strains, respectively.[110,111]

Additional precautions (such as BSL-2 with BSL-3 practices) should be considered when working with lyssaviruses other than rabies virus; refer to Table 1. BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals. Pre-exposure rabies vaccination is recommended for all individuals prior to working with lyssaviruses or infected animals or engaging in diagnostic, production, or research activities with these viruses.[112] Rabies

vaccination is also recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. The presence of virus-neutralizing antibodies in vaccinated individuals should be ascertained.[112,113] Prompt administration of post-exposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.[112,113]

In cases where it is not possible to open the skull or remove the brain within a BSC, such as an autopsy or routine diagnostics, use appropriate methods and personal protective equipment (PPE), including dedicated laboratory clothing, heavy or chainmail gloves to avoid cuts or sticks from cutting instruments or bone fragments, and an N95 respirator combined with a face shield or a PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets. Ample coverage of a 10% bleach solution should be used during and after the procedure for decontamination of exposed or contaminated surfaces and equipment.[114]

To prevent the generation of aerosols, a handsaw is recommended instead of an oscillating saw and contact of the saw with brain tissue is avoided. Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.

Table 1. Viruses currently included in the genus *Lyssavirus*

Species	Acronym	Recommended Biosafety Level
Aravan lyssavirus*	ARAV	2
Australian bat lyssavirus	ABLV	2
Bokeloh bat lyssavirus*	BBLV	2
Duvenhage lyssavirus	DUVV	2
European bat 1 lyssavirus	EBLV-1	2
European bat 2 lyssavirus	EBLV-2	2
Ikoma lyssavirus*	IKOV	3
Irkut lyssavirus	IRKV	2
Khujand lyssavirus*	KHUV	2
Lagos bat lyssavirus*	LBV	3
Mokola lyssavirus	MOKV	3
Rabies lyssavirus	RABV	2
Shimoni bat lyssavirus*	SHIBV	3
West Caucasian bat lyssavirus*	WCBV	3

*No human cases have been documented

Notes: This table is final as of publication, but it will be updated in future editions of BMBL to reflect the discovery of new, divergent lyssaviruses. When handled in a BSL-2 laboratory, BSL-3 pracitices and procedures should be used.

Special Issues

The CDC provides information on a variety of topics relating to Rabies virus, lyssaviruses, and pre/post-exposure prophylaxis online at https://www.cdc.gov. For non-emergency information on potential human infections, or treatment options, the CDC Rabies Duty Officer can be contacted at 404-639-1050 or CDC-Info can be reached at 800-232-4636.

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)

The family *Retroviridae* is divided into two subfamilies: 1) the *Orthoretrovirinae* with six genera including the genus *Lentivirus*, which includes HIV-1, HIV-2, and SIVs; the genus *Deltaretrovirus*, which includes human and simian T-lymphotropic viruses (HTLV-1, HTLV-2, HTLV-3, HTLV-4, and STLVs); and the genus *Betaretrovirus*, which includes simian type D retrovirus (SRV); and 2) the *Spumaretrovirinae*, which has recently been updated to contain five genera,[115] including the genus *Simiispumavirus*, which includes simian foamy viruses (SFVs) that can occasionally infect humans in close contact with infected non-human primates (NHPs). Of these, only HIV and HTLV are pathogenic in humans and are now classified as known human carcinogens in the National Toxicology Program's Report on Carcinogens.[53] SIV/HIV genetic recombinants, known as SHIVs, are used in NHPs as models of HIV infection. The composition of SHIVs can vary but generally consist of an SIV genetic backbone containing specific HIV genes or gene regions.

Occupational Infections

Since 1991, data on occupational HIV transmission in health care workers (HCW) have been collected through a CDC-supported National HIV Surveillance system following a standardized case investigation protocol by state health department HIV staff with help from CDC.[116,117] For surveillance purposes, laboratory workers are defined as those persons, including students and trainees, who have worked in a clinical or HIV laboratory setting anytime since 1978. Cases reported in this system are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (i.e., a negative HIV-antibody test at the time of the exposure that converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of 2013, confirmed HIV infections among 58 HCWs were reported, including 20 laboratory workers, of

which only one involved a laboratory worker who sustained a needle exposure while working with an HIV-infected culture. There were another 49 HCWs exposed to HIV-infected blood, including four persons exposed to concentrated virus in a laboratory.[116,117]

Workers have been reported to develop antibodies to simian immunodeficiency virus (SIV) following exposures.[118–120] One case was associated with a needlestick that occurred while the worker was manipulating a contaminated needle after bleeding an SIV-infected macaque monkey.[121] Another case involved a laboratory worker who handled macaque SIV-infected blood specimens without gloves. Though no specific incident was recalled, this worker had dermatitis on the forearms and hands while working with the infected blood specimens.[118] A third worker was exposed to SIV-infected primate blood through a needlestick and subsequently developed antibodies to SIV.[118] Of these three persons, only the worker exposed via dermatitis showed evidence of a persistent infection. To date, there is no evidence of illness or immunological incompetence in any of these workers. However, workers who have been occupationally exposed to HIV/SIV are recommended to immediately start an antiretroviral regimen. SFV infections in humans have occurred due to cross-species transmission following a variety of NHP exposures (e.g., working with NHPs, hunting and butchering NHPs) resulting in life-long, persistent infection but without any evidence for disease. Higher prevalences have been reported in individuals exposed to NHPs by bites, especially those reporting severe bite wounds. There has been a report of a laboratory infection while handling SFV.[119] Laboratory infection with SRV has been reported in two workers but without molecular evidence of persistent infection or disease.[122] SRV infection was also reported in one AIDS patient with lymphoma but without a history of NHP contact. Dual infection of a laboratory worker with SFV and SRV has also been reported but without evidence of secondary transmission of disease.[122] STLV infection of laboratory workers has not been reported but is known to occur in persons who hunt NHPs.[123,124]

Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates, including NHPs. Within the human population, the spread of HIV and HTLV is by close sexual contact, parenteral exposure through blood, blood-derived products, or other potentially infectious materials and from mother to child. Transmission of SFV and SRV from infected persons has not been reported.[122,124,125]

SIV infection of NHPs rarely causes disease but can lead to immunodeficiency and AIDS-like illness similar to that seen in HIV-infected humans.[123] STLV infection of NHPs has been reported to cause T-cell lymphomas and leukemia, generalized skin lesions, and splenomegaly.[123] SRV-infected macaques can show symptoms similar to AIDS in humans, and this presentation is called simian AIDS (SAIDS).[123] SRV-infected macaques have also displayed retroperitoneal

fibromatosis, necrotizing stomatitis with osteomyelitis, acute death, splenomegaly, lymphadenopathy, and fibroproliferative disorders. Disease has not been associated with NHPs naturally infected with SFV.[123]

Laboratory Safety and Containment Recommendations

HIV and HTLV have been isolated from blood, semen, saliva, urine, CSF, amniotic fluid, breast milk, cervical secretions, and tissues of infected persons and experimentally infected NHPs. Additionally, HIV has been isolated from tears of infected persons.

SIV, SHIV, and STLV have been isolated from blood, CSF, and a variety of tissues of infected NHPs.[123] Limited data exist on the concentration of virus in semen, saliva, cervical secretions, urine, breast milk, and amniotic fluid. Virus should be presumed to be present in all primate-derived tissue cultures, in animals experimentally infected or inoculated with SIV, SHIV, or STLV, in all materials derived from SIV, SHIV, and STLV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.[126]

SFV and SRV have been isolated from NHP blood and a variety of other tissues and can be cultured in vitro. Virus should be presumed to be present in all NHP-derived tissue cultures, in animals experimentally infected or inoculated with SFV or SRV, in all materials derived from SFV or SRV cultures, and in/on all equipment and devices coming into direct contact with any of these materials, similar to the handling of human clinical materials.[123]

Although the risk of occupationally-acquired infection with retroviruses is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus, and human breast milk.

In the laboratory, retroviruses should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other than intact skin) from a human (living or dead), in retrovirus cultures, in all materials derived from retrovirus cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

The skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. It is unknown whether infection can occur via the respiratory tract. The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.

Activities involving large-scale volumes or preparation of concentrated retroviruses, including HIV, SIV, or SHIV, should be conducted at BSL-3. Activities, such as producing research-laboratory-scale quantities of retroviruses, including HIV, SIV or SHIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, can be performed in a BSL-2 facility using BSL-3 practices.

Standard Precautions and personal protective equipment should be used when working with all body fluids even if the infection status of the individual or animal is unknown.[126] BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids, and tissues from NHPs and humans infected with retroviruses. ABSL-2 is appropriate for NHPs and other animals infected with retroviruses, including HIV, SIV, or SHIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2. Since 1996, post-exposure prophylaxis with antiretrovirals has been recommended to prevent infection following occupational exposures.[127]

In addition to the aforementioned recommendations, persons working with any retrovirus, including HIV, SIV, or SHIV, or other bloodborne pathogens, should consult the OSHA Bloodborne Pathogen Standard.[43]

Special Issues

It is recommended that all institutions establish written policies (e.g., treatment, prophylaxis protocols) regarding the management of laboratory exposure to retroviruses (HIV, SIV). See Section VII for additional information.

The risk associated with retroviral vector systems can vary significantly, especially lentiviral vectors. Because the risk associated with each gene transfer system can vary, it is recommended that all gene transfer protocols be reviewed by the institution's biosafety review committee or IBC.

Transfer of Agent Importation of this agent or materials containing this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) Coronaviruses

Note: the 6th edition of the BMBL had already undergone final clearance at the time of the 2019 coronavirus pandemic. For the latest biosafety recommendations regarding work with SARS Coronavirus 2 (SARS-CoV-2) please consult the CDC COVID-19 website at (https://www.cdc.gov/coronavirus/2019-nCoV/index.html).

Several human coronaviruses have been identified that can be broadly classified into low and high pathogenicity. Low pathogenic human coronaviruses include 229E, HKU1, OC43, and NL63. High pathogenic coronaviruses include SARS and MERS-CoV. SARS is a viral respiratory illness caused by SARS-associated coronavirus (SARS-CoV) within the family *Coronaviridae*. SARS was retrospectively recognized in China in November 2002. Over the next few months, the illness spread to other Southeast Asian countries, North America, South America, and Europe following major airline routes.[128] The majority of disease-spread occurred in hospitals, among family members, and contacts of hospital workers. From November 2002 through July 2003, when the global outbreak was contained, a total of 8,098 probable cases of SARS were reported to the WHO from 29 countries.

In general, SARS patients present with fever (temperature greater than 100.4°F [>38.0°C]), malaise, and myalgia quickly followed by respiratory symptoms including shortness of breath and cough. Ten to 20% of patients may have diarrhea. Review of probable cases indicates that the shortness of breath sometimes rapidly progresses to respiratory failure requiring ventilation. The case fatality rate is about 11%.

A second human coronavirus that causes severe disease, Middle East Respiratory Syndrome coronavirus (MERS-CoV), was first identified in Saudi Arabia in September 2012.[128–130] Between 2012 and mid-2017, the WHO confirmed 1,952 cases with 693 deaths.[131] Cases have been confirmed in 27 countries, though all cases have been linked to residents of the Arabian Peninsula.[131] A wide clinical spectrum of MERS-CoV infections has been reported with asymptomatic infection identified during outbreaks, acute respiratory illness in most symptomatic patients, or severe presentation including rapidly progressive pneumonitis, respiratory failure, septic shock, or multi-organ failure resulting in death.[132] Globally, 35–40% of cases reported to WHO have resulted in fatality. Common signs and symptoms at hospital admission include fever, chills/rigors, headache, non-productive cough, dyspnea, and myalgia.

Occupational Infections

Three different episodes of SARS-CoV transmission to laboratory workers occurred in 2003 and 2004 in research laboratories in Singapore, Taiwan, and Beijing.[133–135] The events in 2004 involved two different laboratory personnel, with one case resulting in secondary and tertiary transmission of the virus to close contacts and healthcare providers.[133] Each occurrence was linked to a deviation from protocol or established laboratory practices.[134,135] Additionally, no laboratory-associated cases have been associated with the routine processing of SARS or MERS diagnostic specimens for detection of virus; however, both coronaviruses represent an emerging infectious disease for which risk to the medical and laboratory community is not fully understood; therefore, caution

should be exercised when handling specimens that could potentially contain SARS or MERS-CoV.

Natural Modes of Infection

The mode of transmission in nature is not well understood. It appears that SARS is transmitted from person-to-person through close contact such as caring for, living with, or having direct contact with respiratory secretions or body fluids of a suspected or probable case.[136] SARS is thought to be spread primarily through droplets, aerosols, and possibly fomites. The natural reservoir for SARS-CoV is unknown.

MERS-CoV transmission can occur in hospital settings through close contact. In the community, transmission can occur between ill people and others through close contact. Transmission may also occur in the community through close contact with infected dromedary camels who may be a reservoir for the virus. The incubation period of MERS-CoV is usually two to five days; however, it can range from two to 14 days.[131]

Healthcare workers are at increased risk of acquiring SARS or MERS from an infected patient, especially if involved in pulmonary/respiratory procedures such as endotracheal intubation, nebulization of medications, diagnostic specimen collection, sputum induction, airway suctioning, positive-pressure ventilation, and high-frequency oscillatory ventilation.

Laboratory Safety and Containment Recommendations

SARS and MERS coronaviruses may be detected in respiratory, blood, urine, or stool specimens. The exact mode of transmission of coronavirus Laboratory-associated infections have not been established, but in clinical settings, the primary mode of transmission appears to be through direct or indirect contact of mucous membranes with infectious respiratory droplets.[136,137]

SARS and MERS coronavirus propagation in cell culture and the initial characterization of viral agents recovered in cultures of clinical specimens must be performed at BSL-3. Respiratory protection should be used by all personnel.

Inoculation of animals for potential recovery of SARS- or MERS-CoV for characterization of putative SARS or MERS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used.

Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities using BSL-3 practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection should be used.

In clinical laboratories, respiratory specimens, whole blood, serum, plasma, and urine specimens should be handled using Standard Precautions at BSL-2.[138] Work using intact, full-length genomic RNA should be conducted at BSL-2.

In the event of any break in laboratory procedure or accident (e.g., accidental spillage of material suspected of containing SARS- or MERS-CoV), procedures for emergency exposure management and environmental decontamination should be immediately implemented and the supervisor should be notified. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred. See Special Issues below.

Special Issues

Occupational Health Considerations Personnel working with the virus or samples containing or potentially containing the virus should be trained regarding the symptoms of SARS- and MERS-CoV infection and counseled to report any fever or respiratory symptoms to their supervisor immediately. Post-exposure baseline serum samples should be taken following any potential exposures. Personnel should be evaluated for possible exposure and the clinical features and course of their illness should be closely monitored for any signs or symptoms of disease. Institutions performing work with SARS- or MERS-CoV or handling specimens likely to contain the agent should develop and implement a specific occupational medical plan with respect to this agent. The plan, at a minimum, should contain procedures for managing:

- Deviation from protocol or established laboratory procedures;
- Exposed workers without symptoms;
- Exposed workers who develop symptoms within ten days of an exposure; and
- Symptomatic laboratory workers with no recognized exposure.

Further information and guidance regarding the development of a personnel exposure response plan are available from the CDC.[139] Laboratory workers who are believed to have had a laboratory exposure to SARS- or MERS-CoV should be evaluated, counseled about the risk of SARS- and MERS-CoV transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, and diarrhea. The verification code for this document is 717418

Local and/or state public health departments should be promptly notified of laboratory exposures and illness in exposed laboratory workers.

Select Agent SARS-CoV is a Select Agent requiring registration with CDC or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent The importation of SARS- and MERS-CoV into the United States and/or its interstate transport may be subject to the rules and regulations of the CDC Import Permit Program, CDC Division of Select Agents and Toxins, and/or the USDA Animal and Plant Health Inspection Service. The exportation of SARS-CoV may require a DoC permit.

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