Section VIII—Agent Summary Statements

Section VIII-A: Bacterial Agents

Bacillus anthracis

Bacillus anthracis, a gram-positive, non-hemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease of mammals, including humans. Like all members of the genus *Bacillus*, under adverse conditions *B. anthracis* has the ability to produce spores that allow the organism to persist for long periods until the return of more favorable conditions. Reports of suspected anthrax outbreaks date back to as early as 1250 BC. The study of anthrax and *B. anthracis* in the 1800s contributed greatly to our general understanding of infectious diseases. Much of Koch's postulates were derived from work on identifying the etiologic agent of anthrax. Louis Pasteur developed the first attenuated live vaccine for anthrax.

Most mammals are susceptible to anthrax; it mostly affects herbivores that ingest spores from contaminated soil and, to a lesser extent, carnivores that scavenge on the carcasses of diseased animals. Anthrax still occurs frequently in parts of central Asia and Africa. In the United States, it occurs sporadically in animals in parts of the West, Midwest and Southwest.

The infectious dose varies greatly from species to species and is route-dependent. The inhalation anthrax infectious dose (ID) for humans primarily has been extrapolated from inhalation challenges of nonhuman primates (NHP) or studies done in contaminated mills. Estimates vary greatly but the medium lethal dose (LD_{50}) is likely within the range of 2,500-55,000 spores.[1] It is believed that very few spores (10 or less) are required for cutaneous anthrax.[2]

Occupational Infections

Occupational infections are possible when in contact with contaminated animals, animal products or pure cultures of *B. anthracis*, and may include ranchers, veterinarians and laboratory workers. Numerous cases of laboratory-associated anthrax (primarily cutaneous) have been reported.[3,4] Recent cases include suspected cutaneous anthrax in a laboratory worker in Texas and a cutaneous case in a North Dakota male who disposed of five cows that died of anthrax.[5,6]

Natural Modes of Infection

The clinical forms of anthrax in humans that result from different routes of infection are: 1) cutaneous (via broken skin); 2) gastrointestinal (via ingestion); and 3) inhalation anthrax. Cutaneous anthrax is the most common and readily treatable form of the disease. Inhalation anthrax used to be known as "Woolsorter disease" due to its prevalence in textile mill workers handling wool and other contaminated animal products. While naturally occurring disease is no longer a

significant public health problem in the United States, anthrax has become a bioterrorism concern. In 2001, 22 people were diagnosed with anthrax acquired from spores sent through the mail, including 11 cases of inhalation anthrax with five deaths and 11 cutaneous cases.[7]

Laboratory Safety and Containment Recommendations

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. The primary hazards to laboratory personnel are: direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Efforts should be made to avoid production of aerosols by working with infectious organisms in a BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. ABSL-2 practices, containment equipment and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.

Special Issues

Vaccines A licensed vaccine for anthrax is available. Guidelines for its use in occupational settings are available from the ACIP.[8,9] Worker vaccination is recommended for activities that present an increased risk for repeated exposures to *B. anthracis* spores including: 1) work involving production quantities with a high potential for aerosol production; 2) handling environmental specimens, especially powders associated with anthrax investigations; 3) performing confirmatory testing for *B. anthracis*, with purified cultures; 4) making repeated entries into known *B. anthracis*-spore-contaminated areas after a terrorist attack; 5) work in other settings in which repeated exposure to aerosolized *B. anthracis* spores might occur. Vaccination is not recommended for workers involved in routine processing of clinical specimens or environmental swabs in general diagnostic laboratories.

Select Agent *B. anthracis* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require 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.

Bordetella pertussis

Bordetella pertussis, an exclusively human respiratory pathogen of worldwide distribution, is the etiologic agent of whooping cough or pertussis. The organism is a fastidious, small gram-negative coccobacillus that requires highly specialized culture and transport media for cultivation in the laboratory. Its natural habitat is the human respiratory tract.

Occupational Infections

Occupational transmission of pertussis has been reported, primarily among healthcare workers.[10-16] Outbreaks, including secondary transmission, among workers have been documented in hospitals, long-term care institutions, and laboratories. Nosocomial transmissions have been reported in healthcare settings. Laboratory-acquired pertussis has been documented.[17,18]

Natural Modes of Infection

Pertussis is highly communicable, with person-to-person transmission occurring via aerosolized respiratory secretions containing the organism. The attack rate among susceptible hosts is affected by the frequency, proximity, and time of exposure to infected individuals. Although the number of reported pertussis cases declined by over 99% following the introduction of vaccination programs in the 1940s, the 3- to 4-year cycles of cases have continued into the post-vaccination era.[19-21]

Laboratory Safety and Containment Recommendations

The agent may be present in high levels in respiratory secretions, and may be found in other clinical material, such as blood and lung tissue in its infrequent manifestation of septicemia and pneumonia, respectively.[22,23] Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to

generate potentially infectious aerosols. BSL-3 practices, containment equipment, and facilities are appropriate for production operations.

Special Issues

Vaccines Pertussis vaccines are available but are not currently approved or recommended for use in persons over six years of age. Because this recommendation may change in the near future, the reader is advised to review the current recommendations of the ACIP published in the Morbidity and Mortality Weekly Report (MMWR) and at the CDC Vaccines and Immunizations Web site for the latest recommendations for adolescents and adults.

Transfer of Agent Importation of 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.

Brucella species

The genus *Brucella* consists of slow-growing, very small gram-negative coccobacilli whose natural hosts are mammals. Seven *Brucella* species have been described using epidemiologic and biological characteristics, although at the genetic level all brucellae are closely related. *B. melitensis* (natural host: sheep/goats), *B suis* (natural host: swine), *B. abortus* (natural host: cattle), *B. canis* (natural host: dogs), and *B.* "*maris*" (natural host: marine mammals) have caused illness in humans exposed to the organism including laboratory personnel.[24,25] Hypersensitivity to *Brucella* antigens is a potential but rare hazard to laboratory personnel. Occasional hypersensitivity reactions to *Brucella* antigens occur in workers exposed to experimentally and naturally infected animals or their tissues.

Occupational Infections

Brucellosis has been one of the most frequently reported laboratory infections in the past and cases continue to occur.[4,26-28] Airborne and mucocutaneous exposures can produce LAI. Accidental self-inoculation with vaccine strains is an occupational hazard for veterinarians.

Natural Modes of Infection

Brucellosis (Undulant fever, Malta fever, Mediterranean fever) is a zoonotic disease of worldwide occurrence. Mammals, particularly cattle, goats, swine, and sheep act as reservoirs for brucellae. Multiple routes of transmission have been identified, including direct contact with infected animal tissues or products, ingestion of contaminated milk, and airborne exposure in pens and stables.

Laboratory Safety and Containment Recommendations

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological cultures[29] or working on open bench tops.[30] Aerosols from, or direct skin contact with, cultures or with infectious clinical specimens from animals (e.g., blood, body fluids, tissues) are commonly implicated in human infections. Aerosols generated during laboratory procedures have caused multiple cases per exposure.[30,31] Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose and mouth result in infection. The infectious dose of *Brucella* is 10-100 organisms by aerosol route and subcutaneous route in laboratory animals.[32,33]

BSL-2 practices, containment equipment, and facilities are recommended for routine clinical specimens of human or animal origin. Products of conception containing or believed to contain pathogenic *Brucella* should be handled with BSL-3 practices due to the high concentration of organisms per gram of tissue. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended, for all manipulations of cultures of pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies.

Special Issues

Vaccines Human *Brucella* vaccines have been developed and tested in other countries with limited success. A human vaccine is not available in the United States.[34]

Select Agent *Brucella abortus, Brucella melitensis, and Brucella suis* are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of 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.

Burkholderia mallei

Burkholderia mallei (formerly *Pseudomonas mallei*) is a non-motile gram-negative rod associated with glanders, a rare disease of equine species and humans. While endemic foci of infection exist in some areas of the world, glanders due to natural infection is extremely rare in he United States.

Occupational Infections

Glanders occurs almost exclusively among individuals who work with equine species and/or handle *B. mallei* cultures in the laboratory. *B. mallei* can be very infectious in the laboratory setting. The only reported case of human glanders in the United States over the past 50 years resulted from a laboratory exposure.[35] Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Mode of Infection

Glanders is a highly communicable disease of horses, goats, and donkeys. Zoonotic transmission occurs to humans, but person-to-person transmission is rare. Clinical glanders no longer occurs in the Western Hemisphere or in most other areas of the world, although enzootic foci are thought to exist in Asia and the eastern Mediterranean.[36] Clinical infections in humans are characterized by tissue abscesses and tend to be very serious.

Laboratory Safety and Containment Recommendations

B. mallei can be very hazardous in a laboratory setting. In a pre-biosafety era report, one-half of the workers in a *B. mallei* research laboratory were infected within a year of working with the organism.[37] Laboratory-acquired infections have resulted from aerosol and cutaneous exposure.[37,38] Laboratory infections usually are caused by exposure to bacterial cultures rather than to clinical specimens. Workers should take precautions to avoid exposure to aerosols from bacterial cultures, and to tissues and purulent drainage from victims of this disease.

Primary isolations from patient fluids or tissues may be performed with BSL-2 practices, containment equipment, and facilities in a BSC. Procedures must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals, or when large quantities of the agent are produced. Procedures conducted outside of a BSC (centrifugation, animal manipulation, etc.) that generate infectious aerosols require respiratory protection. Sealed cups should be used with all centrifuges and these should be opened only inside a BSC. Gloves should be worn when working with potentially infectious material or animals. Animal work with *B. mallei* should be done with ABSL-3 practices, containment equipment, and facilities.

Special Issues

Select Agent *B. mallei* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of 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.

Burkholderia pseudomallei

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*) is a motile gram-negative, oxidase-positive rod that is found in soil and water environments of equatorial regions, including Southeast Asia, Northern Australia, Central America and South America. This organism is the causative agent of melioidosis, an unusual bacterial disease characterized by abscesses in tissues and organs. Victims of the disease frequently exhibit recrudescence months or years after the initial infection.

Occupational Infections

Melioidosis is generally considered to be a disease associated with agriculture; however, *B. pseudomallei* can be hazardous for laboratory workers. There are two reports of melioidosis in laboratory workers who were infected by aerosols or via skin exposure.[39,40] Laboratory workers with diabetes are at increased risk of contracting melioidosis.[41]

Natural Modes of Infection

While primarily a disease found in Southeast Asia and Northern Australia, melioidosis can occasionally be found in the Americas.[42] Natural modes of transmission include the exposure of mucous membranes or damaged skin to soil or water containing the organism, the aspiration or ingestion of contaminated water, or the inhalation of dust from contaminated soil. In endemic areas, 5-20% of agricultural workers have antibody titers to *B. pseudomallei*, in the absence of overt disease.[43]

Laboratory Safety and Containment Recommendations

B. pseudomallei can cause a systemic disease in human patients. Infected tissues and purulent drainage from cutaneous or tissue abscesses can be sources of infection. Blood and sputum also are potential sources of infection.

Work with clinical specimens from patients suspected of having melioidosis and of *B. pseudomallei* cultures may be performed with BSL-2 practices, containment equipment, and facilities. Work should be done in a BSC. Gloves always should be worn when manipulating the microorganism. In cases where infectious aerosols or droplets could be produced, or where production quantities of the organism are generated, these procedures should be confined to BSL-3 facilities with all pertinent primary containment against escape of aerosols. Respiratory protection must be used if the microorganism is manipulated outside of a BSC, such as during centrifugation or handling infected animals. Sealed

cups should be used in all centrifuges and these should be opened only in a BSC. Animal studies with this agent should be done at ABSL-3.

Special Issues

Select Agent *B. pseudomallei* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of 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.

Campylobacter (C. jejuni subsp. jejuni, C. coli, C. fetus subsp. fetus, C. upsaliensis)

Campylobacters are curved, S-shaped, or spiral rods associated with gastrointestinal infections (primarily *C. jejuni* subsp. *jejuni* and *C. coli*), bacteremia, and sepsis (primarily *C. fetus* subsp. *fetus* and *C. upsaliensis*). Organisms are isolated from stool specimens using selective media, reduced oxygen tension, and elevated incubation temperature (43°C).

Occupational Infections

These organisms rarely cause LAI, although laboratory-associated cases have been documented.[44-47] Experimentally infected animals also are a potential source of infection.[48]

Natural Modes of Infection

Numerous domestic and wild animals, including poultry, pets, farm animals, laboratory animals, and wild birds are known reservoirs and are a potential source of infection for laboratory and animal care personnel. While the infective dose is not firmly established, ingestion of as few as 500-800 organisms has caused symptomatic infection.[49-51] Natural transmission usually occurs from ingestion of organisms in contaminated food or water and from direct contact with infected pets, farm animals, or infants.[52]

Laboratory Safety and Containment Recommendations

Pathogenic *Campylobacter sp.* may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. The primary laboratory hazards are ingestion and parenteral inoculation of *C. jejuni*. The significance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Transfer of Agent Importation of 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.

Chlamydia psittaci (Chlamydophila psittaci), C. trachomatis, C. pneumoniae (Chlamydophila pneumoniae)

Chlamydia psittaci, *C. pneumoniae* (sometimes called *Chlamydophila psittaci* and *Chlamydophila pneumoniae*) and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Chlamydiae are nonmotile, gram-negative bacterial pathogens with obligate intracellular life cycles. These three species of *Chlamydia* vary in host spectrum, pathogenicity, and in the clinical spectrum of disease. *C. psittaci* is a zoonotic agent that commonly infects psittacine birds and is highly pathogenic for humans. *C. trachomatis* is historically considered an exclusively human pathogen and is the most commonly reported bacterial infection in the United States. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans.

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the most commonly reported laboratory-associated bacterial infections.[26] In cases reported before 1955[4], the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of laboratory-acquired infections with *C. trachomatis* attribute the infection to inhalation of large quantities of aerosolized organisms during purification or sonification procedures. Early reports commonly attributed infections to exposure to aerosols formed during nasal inoculation of mice or inoculation of egg yolk sacs and harvest of chlamydial elementary bodies. Infections are associated with fever, chills, malaise, and headache; a dry cough is also associated with *C. psittaci* infection. Some workers exposed to *C. trachomatis*

have developed conditions including mediastinal and supraclavicular lymphadenitis, pneumonitis, conjunctivitis, and keratitis.[53] Seroconversion to chlamydial antigens is common and often striking although early antibiotic treatment may prevent an antibody response.

Laboratory-associated infections with *C. pneumoniae* have been reported.[54] Exposed workers were asymptomatic and infection was diagnosed by serology. The route of infection was attributed to inhalation of droplet aerosols created during procedures associated with culture and harvest of the agent from cell culture.

With all species of *Chlamydia*, mucosal tissues in the eyes, nose, and respiratory tract are most often affected by occupational exposures that can lead to infection.

Natural Modes of Infection

C. psittaci is the cause of psittacosis, a respiratory infection that can lead to severe pneumonia requiring intensive care support and possible death. Sequelae include endocarditis, hepatitis, and neurologic complications. Natural infections are acquired by inhaling dried secretions from infected birds. Psittacine birds commonly kept as pets (parrots, parakeets, cockatiels, etc.) and poultry are most frequently involved in transmission. *C. trachomatis* can cause a spectrum of clinical manifestations including genital tract infections, inclusion conjunctivitis, trachoma, pneumonia in infants, and LGV. The LGV strains cause more severe and systemic disease than do genital strains. *C. trachomatis* genital tract infections are sexually transmitted and ocular infections (trachoma) are transmitted by exposure to secretions from infected persons through contact or fomite transmission. *C. pneumoniae* is a common cause of respiratory infection; up to 50% of adults have serologic evidence of previous exposure. Infections with *C. pneumoniae* are transmitted by droplet aerosolization and are most often mild or asymptomatic, although there is a body of evidence associating this agent with chronic diseases such as atherosclerosis and asthma.

Laboratory Safety and Containment Recommendations

C. psittaci may be present in the tissues, feces, nasal secretions and blood of infected birds, and in blood, sputum, and tissues of infected humans. *C. trachomatis* may be present in genital, bubo, and conjunctival fluids of infected humans. Exposure to infectious aerosols and droplets, created during the handling of infected birds and tissues, are the primary hazards to laboratory personnel working with *C. psittaci*. The primary laboratory hazards of *C. trachomatis* and *C. pneumoniae* are accidental parenteral inoculation and direct and indirect exposure of mucous membranes of the eyes, nose, and mouth to genital, bubo, or conjunctival fluids, cell culture materials, and fluids from infected cell cultures or eggs. Infectious aerosols, including those that may be created as a result of centrifuge malfunctions, also pose a risk for infection.

BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular or genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feathers of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical isolates known to contain or be potentially infected with the LGV serovars (L_1 through L_3) of *C. trachomatis*. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials, including but not limited to use of primary containment equipment such as BSCs.

Gloves are recommended for the necropsy of birds and mice, the opening of inoculated eggs, and when there is the likelihood of direct skin contact with infected tissues, bubo fluids, and other clinical materials.

ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are indicated for activities involving any of these species with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

Special Issues

Transfer of Agent Importation of 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.

Neurotoxin-producing Clostridia species

Clostridium botulinum, and rare strains of *C. baratii* and *C. butyricum,* are anaerobic spore-forming species that cause botulism, a life-threatening food-borne illness. The pathogenicity of these organisms results from the production of botulinum toxin, one of the most highly potent neurotoxins currently recognized. Purified botulinum neurotoxin is a 150 kDa protein that acts selectively on peripheral cholinergic nerve endings to block neurotransmitter release.[55] The principal site of action is the neuromuscular junction, where blockade of transmission produces muscle weakness or paralysis. The toxin also acts on autonomic nerve endings where blockade of transmission can produce a variety of adverse effects. The toxin may also contain associated proteins that may increase its size to as high as 900 kDA.

Occupational Infections

There has been only one report of botulism associated with handling of the toxin in a laboratory setting.[56] However, concerns about potential use of the toxin as an agent of bioterrorism or biological warfare have led to increased handling of the substance by investigators studying mechanism of action and/or developing countermeasures to poisoning.[57]

Natural Modes of Infection

Botulinum toxin occurs in seven different serotypes (A to G), but almost all naturally-occurring human illness is due to serotypes A, B, E, and F.[58] Botulism occurs when botulinum toxin is released into circulation following ingestion of preformed toxin. However, animal studies have shown that botulism may occur through inhalation of preformed toxin. Use of appropriate personal protective equipment should prevent potential exposure through mucus membranes. Symptoms and even death are possible by accidental injection of botulinum toxin. Risk to toxin exposure is dependent on both route of exposure and toxin molecular weight size. Exposure to neurotoxin producing Clostridia species does not cause infection; however, in certain rare circumstances (Infant Botulism, Wound Botulism, and Adult colonization), the organism can colonize the intestinal tract and other sites and produce toxin. In Wound Botulism, exposure to toxin is caused by introduction of spores into puncture wounds and *in situ* production by the organism. Infants less than 1 year of age may be susceptible to intestinal colonization and develop the syndrome of Infant Botulism as a result of *in situ* production of toxin. Similarly to Infant Botulism, ingestion of spores by adults with a compromised gastrointestinal tract (GI), such as following GI surgery or long-term administration of antibiotics, may increase risk for intestinal infection and *in situ* production of toxin. See the *C. botulinum* Toxin Agent Summary Statement and Appendix I for additional information.

Laboratory Safety and Containment Recommendations

Neurotoxin producing *Clostridia* species or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water).[59] In addition, bacterial cultures may produce very high levels of toxin.[60] In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is due to the presence of the toxin and not due to a potential of infection from the organisms that produce the toxin. Although spore-forming, there is no known risk to spore exposure except for the potential for the presence of residual toxin associated with pure spore preparations. Laboratory safety protocols should be developed with the focus on prevention of accidental exposure to the toxin produced by these *Clostridia* species.

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin[61] including the handling of potentially contaminated food. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

Special Issues

Vaccines A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an Investigational New Drug (IND). Vaccination is recommended for all personnel working in direct contact with cultures of neurotoxin producing Clostridia species or stock solutions of Botulinum neurotoxin. Due to a possible decline in the immunogenicity of available PBT stocks for some toxin serotypes, the immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks, followed by a booster at 12 months and annual boosters thereafter. Since there is a possible decline in vaccine efficacy, the current vaccine contains toxoid for only 5 of the 7 toxin types, this vaccine should not be considered as the sole means of protection and should not replace other worker protection measures.

Post-Exposure Treatment An equine antitoxin product is available for treatment of patients with symptoms consistent with botulism. However, due to the risks inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present.

Select Agent Neurotoxin producing *Clostridia* species are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of 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.

Clostridium tetani and Tetanus toxin

Clostridium tetani is an anaerobic endospore-forming gram-positive rod found in the soil and an intestinal tract commensal. It produces a potent neurotoxin, tetanospasmin, which causes tetanus, an acute neurologic condition characterized by painful muscular contractions. Tetanospasmin is an exceedingly potent, high molecular weight protein toxin, consisting of a heavy chain (100kD) subunit that binds the toxin to receptors on neuronal cells and a light chain (50kD) subunit that blocks the release of inhibitory neural transmitter molecules within the central nervous system. The incidence of tetanus in the United States has declined steadily since the introduction of tetanus toxoid vaccines in the 1940's.[62]

Occupational Infections

Although the risk of infection to laboratory personnel is low, there have been five incidents of laboratory personnel exposure recorded.[4]

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 130 cases of tetanus reported to CDC from 1998 through 2000, acute injury (puncture, laceration, abrasion) was the most frequent predisposing condition. Elevated incidence rates also were observed for persons aged over 60 years, diabetics, and intravenous drug users.[63] When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate and produce tetanospasmin. The incubation period ranges from 3 to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.[64]

Laboratory Safety and Containment Recommendations

The organism may be found in soil, intestinal, or fecal samples. Accidental parenteral inoculation of the toxin is the primary hazard to laboratory personnel. Because it is uncertain if tetanus toxin can be absorbed through mucous membranes, the hazards associated with aerosols and droplets remain unclear.

BSL-2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxin. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies.

Special Issues

Vaccines The vaccination status of workers should be considered in a risk assessment for workers with this organism and/or toxin. While the risk of laboratory-associated tetanus is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals further reduces the risk to laboratory and animal care personnel of toxin exposures and wound contamination, and is therefore highly recommended.[62] The reader is advised to consult the current recommendations of the ACIP. [65]

Transfer of Agent Importation of 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.

Corynebacterium diphtheriae

Corynebacterium diphtheriae is a pleomorphic gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism is easily grown in the laboratory on media containing 5% sheep blood. *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most widespread bacterial diseases in the pre-vaccine era.

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported.[4,66] Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Natural Modes of Infection

The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds, in blood, and on the skin. Travel to endemic areas or close contact with persons who have returned recently from such areas, increases risk.[67] Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles contaminated with secretions from infected people. Naturally occurring diphtheria is characterized by the development of grayish-white membranous lesions involving the tonsils, pharynx, larynx, or nasal mucosa. Systemic sequelae are associated with the production of diphtheria toxin. An effective vaccine has been developed for diphtheria and this disease has become a rarity in countries with vaccination programs.

Laboratory Safety and Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

Special Issues

Vaccines A licensed vaccine is available. The reader is advised to consult the current recommendations of the CIP.[65] While the risk of laboratory-associated diphtheriai is low, the administration of an adult diphtheria-tentanus toxoid at 10-year intervals may further reduce the risk of illness to laboratory and animal care personnel.[62]

Transfer of Agent Importation of 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.

Francisella tularensis

Francisella tularensis is a small gram-negative coccobacillus that is carried in numerous animal species, especially rabbits, and is the causal agent of tularemia (Rabbit fever, Deer fly fever, Ohara disease, or Francis disease) in humans. *F. tularensis* can be divided into three subspecies, *F. tularensis* (Type A), *F. holarctica* (Type B) and *F. novicida*, based on virulence testing, 16S sequence, biochemical reactions and epidemiologic features. Type A and Type B strains are highly infectious, requiring only 10-50 organisms to cause disease. Subspecies *F. novicida* is infrequently identified as the cause of human disease. Person-to-person transmission of tularemia has not been documented. The incubation period varies with the virulence of the strain, dose and route of introduction but ranges from 1-4 days with most cases exhibiting symptoms in 3-5 days.[68]

Occupational Infections

Tularemia has been a commonly reported laboratory-associated bacterial infection.[4] Most cases have occurred at facilities involved in tularemia research; however, cases have been reported in diagnostic laboratories as well. Occasional cases were linked to work with naturally or experimentally infected animals or their ectoparasites.

Natural Modes of Infection

Tick bites, handling or ingesting infectious animal tissues or fluids, ingestion of contaminated water or food and inhalation of infective aerosols are the primary transmission modes in nature. Occasionally, infections have occurred from bites or scratches by carnivores with contaminated mouthparts or claws.

Laboratory Safety and Containment Recommendations

The agent may be present in lesion exudates, respiratory secretions, cerebrospinal fluid (CSF), blood, urine, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets has resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.[69]

BSL-2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Laboratory personnel should be informed of the possibility of tularemia as a differential diagnosis when samples are submitted for diagnostic tests. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies and for experimental animal studies. Preparatory work on cultures or contaminated materials for automated identification systems should be performed at BSL-3. Characterized strains of reduced virulence such as *F. tularensis* Type B (strain LVS) and *F. tularensis* subsp *novicida* (strain U112) can be manipulated in BSL-2. Manipulation of reduced virulence strains at high concentrations should be conducted using BSL-3 practices.

Special Issues

Select Agent *F. tularensis* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of 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.

Helicobacter species

Helicobacters are spiral or curved gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 20 recognized species, including at least nine isolated from humans. Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.[70] The main habitat of *H. pylori* is the human gastric mucosa. Other *Helicobacter* spp. (*H. cinaedi*, *H. canadensis*, *H. canis*, *H. pullorum*, and *H. fennelliae*) may cause asymptomatic infection as well as proctitis, proctocolitis, enteritis and extraintestinal infections in humans.[71,72] *H. cinaedi* has been isolated from dogs, cats and Syrian hamsters.

Occupational Infections

Both experimental and accidental LAI with *H. pylori* have been reported.[73,74] Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Natural Modes of Infection

Chronic gastritis and duodenal ulcers are associated with *H. pylori* infection. Epidemiologic associations have also been made with gastric adenocarcinoma. Human infection with *H. pylori* may be long in duration with few or no symptoms, or may present as an acute gastric illness. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

Laboratory Safety and Containment Recommendations

H. pylori may be present in gastric and oral secretions and stool.[75] The enterohepatic helicobacters (e.g., *H. canadensis*, *H. canis*, *H, cinaedi*, *H. fennelliae*, *H. pullorum*, and *H. winghamensis*) may be isolated from stool specimens, rectal swabs, and blood cultures.[72] Protocols involving homogenization or vortexing of gastric specimens have been described for the isolation of *H. pylori*.[76] Containment of potential aerosols or droplets should be incorporated in these procedures.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the agents. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

Transfer of Agent Importation of 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.

Legionella pneumophila and other Legionella-like Agents

Legionella are small, faintly staining gram-negative bacteria. They are obligately aerobic, slow-growing, nonfermentative organisms that have a unique requirement for L-cysteine and iron salts for *in vitro* growth. Legionellae are readily found in natural aquatic bodies and some species (*L. longbeachae*) have been recovered from soil.[77,78] They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 48 known *Legionella* species, 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently encountered in human infections.[79-81]

Occupational Infections

Although laboratory-associated cases of legionellosis have not been reported in the literature, at least one case, due to presumed aerosol or droplet exposure during animal challenge studies with *L. pneumophila*, has been recorded.[82] Experimental infections have been produced in guinea pigs, mice, rats, embryonated chicken eggs, and human or animal cell lines.[83] A fatal case of pneumonia due to *L. pneumophila* was diagnosed in a calf, but only 1.7% (2/112) of the other cattle in the herd had serological evidence of exposure to *Legionella*.[84] The disease was linked to exposure to a hot water system colonized with *Legionella*. Animal-to-animal transmission has not been demonstrated.

Natural Modes of Infection

Legionella is commonly found in environmental sources, typically in man-made warm water systems. The mode of transmission from these reservoirs is aerosolization, aspiration or direct inoculation into the airway.[85] Direct person-to-person transmission does not occur. The spectrum of illness caused by *Legionella* species ranges from a mild, self-limited flu-like illness (Pontiac fever) to a disseminated and often fatal disease characterized by pneumonia and respiratory failure (Legionnaires disease). Although rare, *Legionella* has been implicated in cases of sinusitis, cellulitis, pericarditis, and endocarditis.[86] Legionellosis may be either community-acquired or nosocomial. Risk factors include smoking, chronic lung disease, and immunosuppression. Surgery, especially involving transplantation, has been implicated as a risk factor for nosocomial transmission.

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tract specimens (sputum, pleural fluid, bronchoscopy specimens, lung tissue), and in extrapulmonary sites. A potential hazard may exist for generation of aerosols containing high concentrations of the agent.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of potentially infectious materials, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. ABSL-2 practices, containment equipment and facilities are recommended for activities with experimentally-infected animals. Routine processing of environmental water samples for *Legionella* may be performed with standard BSL-2 practices. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organisms are manipulated, BSL-2 with BSL-3 practices is recommended.

Special Issues

Transfer of Agent Importation of 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.

Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospires are ubiquitous in nature, either free-living in fresh water or associated with renal infection in animals. Historically, these organisms have been classified into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*) groups, but recent studies have identified more than 12 species based on genetic analysis. These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified as of 2003.[87] These organisms are the cause of leptospirosis, a zoonotic disease of worldwide distribution. Growth of leptospires in the laboratory requires specialized media and culture techniques, and cases of leptospirosis are usually diagnosed by serology.

Occupational Infections

Leptospirosis is a well-documented laboratory hazard. Approximately, 70 LAI and 10 deaths have been reported.[4,26] Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.[88-90] It is important to remember that rodents are natural carriers of leptospires. Animals with chronic renal infection shed large numbers of leptospires in the urine continuously or intermittently, for long periods of time. Rarely, infection may be transmitted by bites of infected animals.[88]

Natural Modes of Infection

Human leptospirosis typically results from direct contact with infected animals, contaminated animal products, or contaminated water sources. Common routes of infection include abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection observed in agricultural workers and other occupations associated with animal contact.

Laboratory Safety and Containment Recommendations

The organism may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental parenteral inoculation, and direct and indirect contact of skin or mucous membranes, particularly the conjunctiva, with cultures or infected tissues or body fluids are the primary laboratory hazards. The importance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed at ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

Special Issues

Transfer of Agent Importation of 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.

Listeria monocytogenes

Listeria monocytogenes is a gram-positive, non-spore-forming, aerobic bacillus; that is weakly beta-hemolytic on sheep blood agar and catalase-positive.[91] The organism has been isolated from soil, animal feed (silage) and a wide range of human foods and food processing environments. It may also be isolated from symptomatic/asymptomatic animals (particularly ruminants) and humans.[91,92] This organism is the causative agent of listeriosis, a food-borne disease of humans and animals.

Occupational Infections

Cutaneous listeriosis, characterized by pustular or papular lesions on the arms and hands, has been described in veterinarians and farmers.[93] Asymptomatic carriage has been reported in laboratorians.[94]

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (hot dogs, luncheon meats), paté and smoked fish/seafood.[95] Listeriosis can present in healthy adults with symptoms of fever and gastroenteritis, pregnant women and their fetuses, newborns, and persons with impaired immune function are at greatest risk of developing severe infections including sepsis, meningitis, and fetal demise. In pregnant women, *Listeria monocytogenes* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.[92]

Laboratory Safety and Containment Recommendations

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.[91,92,96,97] Naturally or experimentally infected animals are a source of exposure to laboratory workers, animal care

personnel and other animals. While ingestion is the most common route of exposure, *Listeria* can also cause eye and skin infections following direct contact with the organism.

BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected or potentially infected materials. ABSL-2 practices, containment equipment and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes*.

Special Issues

Transfer of Agent Importation of 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.

Mycobacterium leprae

Mycobacterium leprae is the causative agent of leprosy (Hansen disease). The organism has not been cultivated in laboratory medium but can be maintained in a metabolically active state for some period. Organisms are recovered from infected tissue and can be propagated in laboratory animals, specifically armadillos and the footpads of mice. The infectious dose in humans is unknown. Although naturally occurring leprosy or leprosy-like diseases have been reported in armadillos[98] and in NHP,[99,100] humans are the only known important reservoir of this disease.

Occupational Infections

There are no cases reported as a result of working in a laboratory with biopsy or other clinical materials of human or animal origin. However, inadvertent human-to-human transmissions following an accidental needle stick by a surgeon and after use of a presumably contaminated tattoo needle were reported prior to 1950.[101,102]

Natural Modes of Infection

Leprosy is transmitted from person-to-person following prolonged exposure, presumably via contact with secretions from infected individuals.

Laboratory Safety and Containment Recommendations

The infectious agent may be present in tissues and exudates from lesions of infected humans and experimentally or naturally infected animals. Direct contact of the skin and mucous membranes with infectious materials and accidental parenteral

inoculation are the primary laboratory hazards associated with handling infectious clinical materials. See Appendix B for appropriate tuberculocidal disinfectant.

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and NHP, because coughing with dissemination of infectious droplets does not occur in these species.

Special Issues

Transfer of Agent Importation of 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.

Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis*, *M. africanum,* and *M. microti* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.[4,26,103-105] The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent.[106] Naturally or experimentally infected NHP are a proven source of human infection.[107] Experimentally infected guinea pigs or mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

Natural Modes of Infection

M. tuberculosis is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop

disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. Infectious aerosols produced by coughing spread tuberculosis. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Human-to-human transmission via aerosols also is possible.

Laboratory Safety and Containment Recommendations

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears[108] and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID_{50} <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Liquifaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.[109,110]

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2.[111] BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) of previously skin-test-negative personnel can be used as a surveillance procedure.

Vaccines The attenuated live BCG, is available and used in other countries but is not used in the United States for immunization.

Transfer of Agent Importation of 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.

Mycobacterium spp. other than M. tuberculosis complex and M. leprae

More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. In the past, mycobacterial isolates that were not identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria or mycobacteria other than tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. The most common types of infections and causes are:

1. pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii, M. avium*, and *M. intracellulare;*
2. lymphadenitis associated with *M. avium* and *M. scrofulaceum;*
3. disseminated infections in immunocompromised individuals caused by *M. avium*;
4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans,* swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum, M. chelonei*, and *M. abscesens*.

Occupational Infections

Laboratory-acquired infections with *Mycobacterium* spp. other than *M. tuberculosis* complex have not been reported.

Natural Modes of Infection

Person-to-person transmission has not been demonstrated. Presumably, pulmonary infections are the result of inhalation of aerosolized bacilli, most likely from the surface of contaminated water. Mycobacteria are widely distributed in the environment and in animals. They are also common in potable water supplies, perhaps as the result of the formation of biofilms. The source of *M. avium* infections in immunocompromised persons has not been established.

Laboratory Safety and Containment Recommendations

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental parenteral inoculation are the primary laboratory hazards associated with clinical materials and cultures. Aerosols created during the manipulation of broth cultures or tissue homogenates of these organisms also pose a potential infection hazard.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacteria* spp. other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to ensure the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* to avoid skin exposure. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Transfer of Agent Importation of 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.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a gram-negative, oxidase-positive diplococcus associated with gonorrhea, a sexually transmitted disease of humans. The organism may be isolated from clinical specimens and cultivated in the laboratory using specialized growth media.[112]

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.[113-116] These infections have presented as conjunctivitis, with either direct finger-to-eye contact or exposure to splashes of either liquid cultures or contaminated solutions proposed as the most likely means of transmission.

Natural Modes of Infection

Gonorrhea is a sexually transmitted disease of worldwide importance. The 2004 rate of reported infections for this disease in the United States was 112 per 100,000 population.[117] The natural mode of infection is through direct contact with exudates from mucous membranes of infected individuals. This usually occurs by sexual activity, although newborns may also become infected during birth.[112]

Laboratory Safety and Containment Recommendations

The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, and CSF. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are known primary laboratory hazards. Laboratory-acquired illness due to aerosol transmission has not been documented.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions such as those described for BSL-3 may be indicated when there is high risk of aerosol or droplet production, and for activities involving production quantities or high concentrations of infectious materials. Animal studies may be performed at ABSL-2.

Special Issues

Transfer of Agent Importation of 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.

Neisseria meningitidis

Neisseria meningitidis is a gram-negative coccus responsible for serious acute meningitis and septicemia in humans. Virulence is associated with the expression of a polysaccharide capsule. Thirteen different capsular serotypes have been identified, with types A, B, C, Y, and W135 associated with the highest incidence

of disease. The handling of invasive *N. meningitidis* isolates from blood or CSF represents an increased risk to microbiologists.[118,119]

Occupational Infections

Recent studies of LAI and exposures have indicated that manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.[119] Investigations of potential laboratory-acquired cases of meningococcal diseases in the United States showed a many-fold higher attack rate for microbiologists compared to that of the United States general population age 30-59 years, and a case fatality rate of 50%, substantially higher than the 12-15% associated with disease among the general population. Almost all the microbiologists had manipulated sterile site isolates on an open laboratory bench.[120] While isolates obtained from respiratory sources are generally less pathogenic and consequently represent lower risk for microbiologists, rigorous protection from droplets or aerosols is mandated when microbiological procedures are performed on all *N. meningitidis* isolates, especially on those from sterile sites.

Natural Modes of Infection

The human upper respiratory tract is the natural reservoir for *N. meningitidis.* Invasion of organisms from the respiratory mucosa into the circulatory system causes infection that can range in severity from subclinical to fulminant fatal disease. Transmission is person-to-person and is usually mediated by direct contact with respiratory droplets from infected individuals.

Laboratory Safety and Containment Recommendations

N. meningitidis may be present in pharyngeal exudates, CSF, blood, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with handling of isolates on an open laboratory bench, exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for infection in the laboratory.

Specimens for *N. meningitidis* analysis and cultures of *N. meningitidis* not associated with invasive disease may be handled in BSL-2 facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. Isolates of unknown source should be treated as sterile-site isolates.

If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing a laboratory coat, gloves, and safety glasses or full-face splash shield. BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production

quantities or high concentrations of infectious materials. Animal studies should be performed under ABSL-2 conditions.

Special Issues

Vaccines The quadrivalent meningococcal polysaccharide vaccine, which includes serogroups A, C, Y, and W-135, will decrease but not eliminate the risk of infection, because it is less than 100% effective and does not provide protection against serogroup B, which caused one-half of the laboratory-acquired cases in the United States in 2000.[118,120] Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination.[118,121,122]

Transfer of Agent Importation of 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.

Salmonella serotypes, other than S. Typhi

Salmonellae are gram-negative enteric bacteria associated with diarrheal illness in humans. They are motile oxidase-negative organisms that are easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation from clinical materials. Recent taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotypes Typhimurium and Enteritidis (commonly designated *S. Typhimurium* and *S. Enteritidis*) are the serotypes most frequently encountered in the United States. This summary statement covers all pathogenic serotypes except *S. Typhi*.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.[4,26,124-125] Primary reservoir hosts include a broad spectrum of domestic and wild animals, including birds, mammals, and reptiles, all of which may serve as a source of infection to laboratory personnel. Case reports of laboratory-acquired infections indicate a presentation of symptoms (fever, severe diarrhea, abdominal cramping) similar to those of naturally-acquired infections, although one case also developed erythema nodosum and reactive arthritis.[126,127]

Natural Modes of Infection

Salmonellosis is a food borne disease of worldwide distribution. An estimated 5 million cases of salmonellosis occur annually in the United States. A wide range of domestic and feral animals (poultry, swine, rodents, cattle, iguanas, turtles,

chicks, dogs, cats) may serve as reservoirs for this disease, as well as humans.[128] The most common mode of transmission is by ingestion of food from contaminated animals or contaminated during processing. The disease usually presents as an acute enterocolitis, with an incubation period ranging from 6 to 72 hours.

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, urine, and in food, feed, and environmental materials. Ingestion or parenteral inoculation are the primary laboratory hazards. The importance of aerosol exposure is not known. Naturally or experimentally infected animals are a potential source of infection for laboratory and animal care personnel, and for other animals

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as a BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.

Special Issues

Transfer of Agent Importation of 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.

Salmonella Typhi

Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotype Typhi, commonly designated *S. Typhi*, is the causative agent of typhoid fever. *S. Typhi* is a motile gram-negative enteric bacterium that is easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation of this organism from clinical materials.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel.[4,129,130] Ingestion and less frequently, parenteral inoculation are the most significant modes of transmission in the laboratory. Secondary transmission to other individuals outside of the laboratory is also a concern.[131] Laboratory-acquired *S. Typhi* infections usually present with symptoms of septicemia, headache, abdominal pain, and high fever.[129]

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal bloodstream infection of worldwide distribution. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low (<103 organisms) and the incubation period may vary from one to six weeks, depending upon the dose of the organism. The natural mode of transmission is by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers.[123]

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, gallbladder (bile), and urine. Humans are the only known reservoir of infection. Ingestion and parenteral inoculation of the organism represent the primary laboratory hazards. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. ABSL-2 facilities, practices and equipment are recommended for activities with experimentally infected animals. ABSL-3 conditions may be considered for protocols involving aerosols.

Special Issues

Vaccines Vaccines for *S. Typhi* are available and should be considered for personnel regularly working with potentially infectious materials. The reader is advised to consult the current recommendations of the Advisory Committee on

Immunization Practices (ACIP) published in the CDC Morbidity and Mortality Weekly Report for recommendations for vaccination against *S. Typhi*.[132]

Transfer of Agent Importation of 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.

Shiga toxin (Verocytotoxin)-producing Escherichia coli

Escherichi coli is one of five species in the gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most intensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.

The diarrheagenic *E. coli* strains have been characterized into at least four basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset of which are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*.[123] In addition to clinical significance, *E. coli* strains are commonly-used hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains. Procedures for safely handling laboratory derivatives of *E. coli* or other pathotypes of *E. coli* should be based upon a thorough risk assessment.

Occupational Infections

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel.[133-138] The infectious dose is estimated to be low—similar to that reported for *Shigella* spp., 10-100 organisms.[136] Domestic farm animals (particularly bovines) are significant reservoirs of the organisms; however, experimentally infected small animals are also sources of infection in the laboratory.[139] Verocytotoxin-producing *Escherichia coli* have also been in wild birds and rodents in close proximity to farms.[140]

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli.* Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been observed in families, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks

associated with swimming in a crowded lake and drinking unchlorinated municipal water.[139] In a small proportion of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

Laboratory Safety and Containment Recommendations

Shiga toxin-producing *E. coli* are usually isolated from feces. However, a variety of food specimens contaminated with the organisms including uncooked ground beef, unpasteurized dairy products and contaminated produce may present laboratory hazards. This agent may be found in blood or urine specimens from infected humans or animals. Accidental ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment or in devices such as a BSC or safety centrifuge cups. Personal protective equipment, such as splash shields, face protection, gowns, and gloves should be used in accordance with a risk assessment. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

Transfer of Agent Importation of 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.

Shigella

The genus *Shigella* is composed of nonmotile gram-negative bacteria in the family Enterobacteriaceae. There are four subgroups that have been historically treated as separate species, even though more recent genetic analysis indicates that they are members of the same species. These include subgroup A (*Shigella dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Members of the genus *Shigella* have been recognized since the late 19th century as causative agents of bacillary dysentery, or shigellosis.[123]

Occupational Infections

Shigellosis is one of the most frequently reported laboratory-acquired infections in the United States.[131,141] A survey of 397 laboratories in the United Kingdom revealed that in 1994-1995, four of nine reported laboratory-acquired infections were caused by *Shigella*.[142] Experimentally infected guinea pigs, other rodents, and NHP are proven sources of laboratory-acquired infection.[143,144]

Natural Modes of Infection

Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. Most transmission is by fecal-oral route; infection also is caused by ingestion of contaminated food or water.[123] Infection with *Shigella dysenteriae* type 1 causes more severe, prolonged, and frequently fatal illness than does infection with other *Shigella*. Complications of shigellosis include hemolytic uremic syndrome, which is associated with *S. dysenteriae* 1 infection, and Reiter chronic arthritis syndrome, which is associated with *S. flexneri* infection.

Laboratory Safety and Containment Recommendations

The agent may be present in feces and, rarely, in the blood of infected humans or animals. Accidental ingestion and parenteral inoculation of the agent are the primary laboratory hazards. The 50% infectious dose (oral) of *Shigella* for humans is only a few hundred organisms.[143] The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment such as a BSC or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of 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.

Treponema pallidum

Treponema pallidum is a species of extremely fastidious spirochetes that die readily upon desiccation or exposure to atmospheric levels of oxygen, and have not been cultured continuously *in vitro*.[145] *T. pallidum* cells have lipid-rich outer membranes and are highly susceptible to disinfection with common alcohols (i.e., 70% isopropanol). This species contains three subspecies including *T. pallidum* spp. *pallidum* (associated with venereal syphilis), *T. pallidum* spp. *endemicum* (associated with endemic syphilis), and *T. pallidum* spp. *pertenue* (associated with Yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel. Pike lists 20 cases of LAI.[4] Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.[146] *T. pallidum* is present in the circulation during primary and secondary syphilis. The ID_{50} of *T. pallidum* needed to infect rabbits by subcutaneous injection has been reported to be as low as 23 organisms.[147] The concentration of *T. pallidum* in patients' blood during early syphilis, however, has not been determined. No cases of laboratory animal-associated infections are reported; however, rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans.

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum* and transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (Yaws), or direct mucous contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs in many areas of the world, whereas Yaws occurs in tropical areas of Africa, South America, the Caribbean, and Indonesia. Endemic syphilis is limited to arid areas of Africa and the Middle East.[145]

Laboratory Safety and Containment Recommendations

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. Accidental parenteral inoculation, contact with mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical samples from humans or infected rabbits. Gloves should be worn when there is a likelihood

of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of 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.

Vibrio enteritis species (V. cholerae, V. parahaemolyticus)

Vibrio species are straight or curved motile gram-negative rods. Growth of *Vibrio* species is stimulated by sodium and the natural habitats of these organisms are primarily aquatic environments. Although 12 different *Vibrio* species have been isolated from clinical specimens, *V. cholerae* and *V. parahaemolyticus* are the best-documented causes of human disease.[148] Vibrios may cause either diarrhea or extraintestinal infections.

Occupational Infections

Rare cases of bacterial enteritis due to LAI with either *V. cholerae* or *V. parahaemolyticus* have been reported from around the world.[4] Naturally and experimentally infected animals[149] and shellfish[150,151] are potential sources for such illnesses.

Natural Modes of Infection

The most common natural mode of infection is the ingestion of contaminated food or water. The human oral infecting dose of *V. cholerae* in healthy non-achlorhydric individuals is approximately 10^6-10^{11} colony forming units,[152] while that of *V. parahaemolyticus* ranges from 10^5-10^7 cells.[153] The importance of aerosol exposure is unknown although it has been implicated in at least one instance.[149] The risk of infection following oral exposure is increased in persons with abnormal gastrointestinal physiology including individuals on antacids, with achlorhydria, or with partial or complete gastrectomies.[154]

Laboratory Safety and Containment Recommendations

Pathogenic vibrios can be present in human fecal samples, or in the meats and the exterior surfaces of marine invertebrates such as shellfish. Other clinical specimens from which vibrios may be isolated include blood, arm or leg wounds,

eye, ear, and gallbladder.[148] Accidental oral ingestion of *V. cholerae* or *V. parahaemolyticus* principally results from hands contaminated from the use of syringes or the handling of naturally contaminated marine samples without gloves.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Vaccines The reader is advised to consult the current recommendations of the ACIP published in the MMWR for vaccination recommendations against *V. cholera*. There are currently no human vaccines against *V. parahaemolyticus*.

Transfer of Agent Importation of 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.

Yersinia pestis

Yersinia pestis, the causative agent of plague, is a gram-negative, microaerophilic coccobacillus frequently characterized by a “safety pin” appearance on stained preparations from specimens. It is nonmotile and nonsporulating. There are three biotypes of *Y. pestis,* differentiated by their ability to ferment glycerol and reduce nitrate. All three biotypes are virulent. The incubation period for bubonic plague ranges from two to six days while the incubation period for pneumonic plague is one to six days. Pneumonic plague is transmissible person-to-person;[155] whereas bubonic plague is not. Laboratory animal studies have shown the lethal and infectious doses of *Y. pestis* to be quite low (less than 100 colony forming units).[156]

Occupational Infections

Y. pestis is a documented laboratory hazard. Prior to 1950, at least 10 laboratory-acquired cases were reported in the United States, four of which were fatal.[4,157] Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Natural Modes of Infection

Infective fleabites are the most common mode of transmission, but direct human contact with infected tissues or body fluids of animals and humans also may serve as sources of infection.

Primary pneumonic plague arises from the inhalation of infectious respiratory droplets or other airborne materials from infected animals or humans. This form of plague has a high case fatality rate if not treated and poses the risk of person-to-person transmission.

Laboratory Safety and Containment Recommendations

The agent has been isolated, in order of frequency of recovery, from bubo aspirate, blood, liver, spleen, sputum, lung, bone marrow, CSF, and infrequently from feces and urine, depending on the clinical form and stage of the disease. Primary hazards to laboratory personnel include direct contact with cultures and infectious materials from humans or animal hosts and inhalation of infectious aerosols or droplets generated during their manipulation. Laboratory and field personnel should be counseled on methods to avoid fleabites and accidental autoinoculation when handling potentially infected live or dead animals.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. In addition, because the infectious dose is so small, all work, including necropsies of potentially infected animals should be performed in a BSC. Special care should be taken to avoid generating aerosols or airborne droplets while handling infectious materials or when performing necropsies on naturally or experimentally infected animals. Gloves should be worn when handling potentially infectious materials including field or laboratory infected animals. BSL-3 is recommended for activities with high potential for droplet or aerosol production, and for activities involving large-scale production or high concentrations of infectious materials. Resistance of *Y. pestis* strains to antibiotics used in the treatment of plague should be considered in a thorough risk assessment and may require additional containment for personal protective equipment. For animal studies, a risk assessment that takes into account the animal species, infective strain, and proposed procedures should be performed in order to determine if ABSL-2 or ABSL-3 practices, containment equipment, and facilities should be employed. BSL-3 facilities and arthropod containment level 3 practices are recommended for all laboratory work involving infected arthropods.[157] See Appendix G for additional information on arthropod containment guidelines.

Special Issues

Select Agent *Yersinia pestis* is an HHS select agent requiring registration with CDC for the possession, use, storage and transfer. See Appendix F for further information.

Transfer of Agent Importation of 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.

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